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Diary

Online publication date: 24-Jul-2005
This month sees the arrival of another pioneering BJU International initiative, the first in a series of articles entitled ‘Great Drug Classes.’

Over the last two decades, in urology and sexual health we have seen the arrival of many new major drug classes that have revolutionized patient management. Although the characteristics of individual drugs are well described (often in relation to competitors) in individual papers and reviews, the editorial board felt that there was a void in the availability and dissemination of easily readable information. This has culminated, after several iterations, in the first of the series of Great Drug Classes, i.e. that on phosphodiesterase inhibitors.

In this prototype and all subsequent articles in the series, two eminent authors in the field (generally one scientist and one clinician) have been asked to follow a distinct template covering: Introduction explaining why the drug class is important to healthcare professionals; historical perspective; background science; clinical data covering efficacy, therapeutic ratio, PK-PD relationships and including an algorithm on how this fits into the contemporary management of the disease; and finally future prospects, but only as it relates to the primary indication.

Is this just an excuse for another unreadable lengthy review, giving the authors a chance to cite their favourite references (often their own) or for the pharmaceutical industry to present the most appropriate ‘representative’ data? The guidelines established by the BJU International should minimize both of these possibilities. The authors are invited by the Journal and they must construct the whole article in the context of the above template in ~10,000 words, and using an absolute maximum of 100 references. In general, statements will be made about the class as a whole and only key features of individual drugs will be presented. Hopefully this will be a good way to focus the mind and the pen, and yet create an easily digestible article.

The editorial team would like to thank Tom Lue and Culley Carson for being the willing guinea-pigs in establishing this new venture. As you might imagine, this was made particularly difficult due to the wealth of data and publications available for discussion, dissection and eventual inclusion.

We at the BJU International look forward to your comments on this style of article and suggestions for the future. It is anticipated that the series will on average appear bi-annually. To celebrate the launch of the Great Drug Classes, one member of the drug series is featured on the outside cover.

MICHAEL G. WYLLIE
Associate Editor
Prostate cancer represents in many ways an ideal candidate for chemoprevention, because of its high incidence and long latency to clinically significant disease [1]. Because of this, increasingly many patients are asking their urologist directly what steps they can take to reduce their risk of being affected by the disease. If we as clinicians do not provide appropriate evidenced-based advice, then our patients are likely to end up taking an expensive cocktail of ‘natural’ preparations, often purchased at considerable expense from their local health-food store.

So what is the current evidence that there is anything now available that can safely and effectively reduce the risk of prostate cancer? This is an especially pertinent issue, as ever-increasing numbers of prostate biopsies are being taken, and urologists are seeing more men who are deemed ‘high-risk’, either as result of a raised PSA level, prostatic intraepithelial neoplasia, or a positive family history of prostate malignancy.

Selenium is a trace nutrient essential for the activity of glutathione peroxidase, which may reduce oxidative damage to DNA. Several studies suggest a useful effect, but the best (and still indirect) evidence for its chemopreventive activity comes from the Nutritional Prevention of Cancer Study Group’s randomized trial of selenium to reduce the recurrence of skin cancer. After 10 years of follow-up (mean time on treatment 4.5 years), men taking selenium at a dose of 200 µg/day had a 63–74% reduction in the risk of prostate cancer [2].

Vitamin E is the other supplement for which there is reasonable, but again indirect, evidence for a genuine chemopreventative effect in this context. In the Alpha-Tocopherol Beta-Carotene Cancer Prevention Trial [3] there was a statistically significant reduction of both prostate cancer incidence and mortality of ≈40% in men receiving 50 IU of α-tocopherol daily.

Many clinicians have been in the habit of advising higher doses of vitamin E, often 400 IU/day, but recently published evidence suggests that the recommended dose should be ≤150 IU/day. Miller et al. [4] reported a meta-analysis of 19 trials, recruiting 135 967 participants; nine of 11 trials testing high-dosage (<400 IU) vitamin E showed a greater risk for all-cause mortality for those on vitamin E than in controls. The difference in mortality risk in high-dosage vitamin E trials was 39 per 10 000 persons (95 CI, 3–74; \( P = 0.035 \)). For low-dosage vitamin E trials, the risk difference was −16 per 10 000 persons (CI −41 to −10; \( P > 0.2 \)). A dose–response analysis showed a statistically significant relationship between vitamin E dosage and all-cause mortality, with increased risk for dosages of >150 IU/day (Fig. 1).

The true safety and effectiveness of selenium and vitamin E should become clearer when the results of the SELECT study become available. This trial, which is sponsored by the USA National Cancer Institute, is a randomized, double-blind, placebo-controlled, population-based clinical trial designed to test the efficacy of selenium and vitamin E either alone or combined [5]. The target accrual is 32 400 individuals and the study duration is planned for 12 years. Unfortunately results are not expected until 2013 (SELECT details available at http://www.crab.org/SELECT/).

In theory, some of the most logical chemopreventative agents for prostate cancer are the 5α-reductase inhibitors. Finasteride, the first compound developed in this class, which inhibits isoenzyme type 2, has been evaluated in the Prostate Cancer Prevention Trial [6]. In that study 18 882 men with a normal DRE and a PSA level of <3.0 ng/mL were randomized to either finasteride 5 mg/day or placebo, for 7 years. Prostate biopsy was advised if the PSA was >4.0 ng/mL or the DRE became abnormal. Prostate cancer was detected in 18.4% of men in the finasteride group and 24.4% in the placebo group, a 24.8% reduction (\( P < 0.001 \)). However, tumours were of Gleason score 7–10 in 6.4% of the finasteride-treated men, compared with 5.1% of the placebo group (\( P = 0.005 \)), and sexual side-effects were more common in the finasteride arm. The explanation for the slight preponderance of less well-differentiated tumours in the men treated with finasteride so far remains elusive. Although the result could be artefactual, because of the known effect of finasteride on prostatic epithelial architecture, there remains the worrying possibility that the effect could be real. Until the position becomes clearer, finasteride should probably not be recommended as a chemopreventive agent for prostate cancer.

Dutasteride is a dual inhibitor of both 5α-reductase types 1 and 2. As such it results in suppression of dihydrotestosterone by >90%, compared with a suppression of ≈70% with finasteride. The Reduction of Prostate Cancer Events trial has just completed recruiting 8000 men to receive either 0.5 mg of dutasteride or placebo for 4 years [7]. Biopsies must be negative within 6 months of accrual and repeat biopsies will be taken at 2 and 4 years. The results will not be available for some time yet, but should throw new light on the issue.
Encouragingly, it was recently proposed that the consumption of red wine might be protective against prostate cancer [8]. Schoonen et al. interviewed 753 middle-aged patients newly diagnosed with prostate cancer, and 703 age-matched controls. Their lifelong alcohol habits, choice of beverage and prostate cancer history were assessed using an elaborate scoring process. Overall, total alcohol, beer, liquor and white wine consumption were not associated with the risk of prostate cancer. However, with red wine, every additional glass drunk per week showed a statistically significant 6% decrease in relative risk. Men drinking 4–7 glasses/week were almost 25% less likely to have the disease (a relative risk reduction of 48%).

So how should we advise patients while awaiting more data? A combination of selenium 200 µg and vitamin E at ≤150 IU per day may be effective, and seems unlikely to cause significant side-effects, provided appropriate doses are used. A glass or two of red wine may be helpful, and tastes good! A myriad of other remedies are promoted as being effective [9], but in the absence of firm evidence from randomized studies or adequate safety data, patients should be discouraged from using compounds that may do more harm than good, and that are also likely to damage the wallet!

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A PROPOSAL FOR A NEW CLASSIFICATION FOR OPERATIVE PROCEDURES FOR STRESS URINARY INCONTINENCE

PAUL ABRAMS, PAUL HILTON*, MALCOLM LUCAS† and TONY SMITH‡ –
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A newcomer to the field of stress incontinence surgery might be bewildered by the spectrum of surgery offered for this condition, extending from injectables through so-called ‘minimally invasive’ procedures and conventional open procedures, to the artificial urinary sphincter. Over the last 20 years many new procedures, some involving implanted devices, have come and gone at regular intervals. All have promised much, but regrettably most have failed to fulfil their promise. In many instances there has been little evidence to support new causal theories on which the procedures are said to be based. The two reasonably well accepted theories for female stress incontinence are bladder neck/urethral hypermobility and intrinsic sphincter deficiency. In truth, most women have an element of both, although pure intrinsic sphincter deficiency can be seen in neurological conditions, such as meningomyelocele, or after cauda equina trauma.

In recent years, the ‘tenison-free’ concept and the insistence of a ‘mid-urethral position’ for slings has been much discussed. However, it seems that there is neither evidence for an effective sling being tension-free, nor for the mandatory position to be mid-urethral. What is undoubtedly true is that almost all urologists and gynaecologists have recognized the value of ensuring that the urethra is restored to a normal anatomical position, so that the principal aims are to prevent descent and to avoid over-elevation.
Therefore, all procedures, be they needle suspension procedures (now largely defunct), retropubic bladder neck suspensions, rectus sheath slings or the new synthetic tapes, are presently performed at low tension. The misnomer of ‘tension-free’ has come about from the way synthetic tapes are implanted with the patient supine. However, stress incontinence episodes do not occur when a woman lies flat, unless the urethral function is truly dreadful. When the patient stands, then the anatomical relationships between the procedure (be that suture, tape, etc.) and its body anchoring points will change for every procedure, and the hammock behind the posterior wall of the proximal urethra will tighten to prevent the caudal and posterior movement of the posterior wall of the bladder neck and proximal urethra. This will apply however the support is created, with the theoretical exception of injectables and the artificial sphincter. We believe the simple hammock theory of Delancey to be a proper explanation of why female stress incontinence occurs and how a wide range of procedures cure the condition. Perhaps we should call it ‘the unifying theory of female stress incontinence’. This theory cannot apply to men in the same way and perhaps here the concept of intrinsic sphincter deficiency is more relevant. We think that most procedures act in two basic ways and would like to propose the following classification for discussion.

**Urethral/bladder neck-stabilizing procedures**

- Vaginal wall suspension procedure; needle procedures (Peyrera, Stamey, Raz, Gittes);
- suprapubic procedures, open or laparoscopic (Marshall Marchetti, Burch, vagino-obturator shelf procedure);
- vaginal procedures (anterol colporraphy).
- Suburethral retropubic space slings biological (autologous, allograft, xenograft);
- synthetic (e.g. tension-free vaginal tape, SPARC);
- Suburethral obturator foramen procedures biological tapes synthetic tapes (e.g. TOT, Monarch)

**Urethral sphincter augmentation**

- Intramural urethral injectables
- bulking agents (e.g. collagen, hydroxyapatite)
- devices
- Extra-urethral retropubic devices (e.g. ACT balloon);
- Extra-urethral, fixed-resistance perineal devices in men;
- Extra-urethral circumferential variable-resistance devices (e.g. the American Medical System artificial urinary sphincter).

We hope that this classification allows established procedures and new techniques to be viewed according to their proposed mode of action. If new modes of action emerge they could be added to this classification. The classification should prevent the frequent claims for new theories and mechanisms by which new procedures will be more effective; claims which so often have turned out to be further examples of ‘the Emperor’s New Clothes’.

**RENEAL TRANSPLANTATION AND MANPOWER ISSUES**

**DLER BESARANI and DAVID CRANSTON** – Oxford Radcliffe Hospitals NHS Trust, Churchill Hospital, Oxford, UK

Accepted for publication 29 March 2005

**INTRODUCTION**

Renal transplantation is the treatment of choice for patients with end-stage renal failure. In the UK, >5000 patients are waiting for a kidney transplant, but because of the shortage of donor organs and surgeons to undertake the surgery, patients have to wait longer for their operation. This is an internationally recognized problem. Last year there were 1330 kidney transplant operations in the UK (with 47 kidney-pancreas operations). Therefore, there is a need to double or triple the number of transplant operations, to reduce the waiting list. To do this we have to both encourage organ donation and train more transplant surgeons. Recently, the speciality has not been attractive for most junior surgical trainees [1]. The unattractiveness of the speciality might be a result of several factors, e.g. the excessive unsociable working hours, low potential for financial progress and lack of private work. Nevertheless, there are good opportunities for trainees across the speciality and many opportunities for research are available.

Traditionally, the renal transplant operation has been undertaken by surgeons trained in general, vascular or urological surgery. Relatively few renal transplants are done by urologists in the UK, in contrast to Europe and North America, which have a much higher urological input. Indeed the AUA has a vascular and transplant subsection. The reason for the lack of interest in transplantation by most UK urologists is entirely clear, but may relate to some of the factors outlined above.

The proposal for changes to training in urology as part of the modernization of medical careers will have a further impact on transplantation. Clearly, the changes in urology to create more generalist and office urologists to look after the speciality may further decrease the number of those who have an interest in renal transplantation. However, with the disappearance of much open surgery, it may create an opportunity for those who still enjoy the challenge of complex urological problems sometimes seen after renal transplant surgery. In addition to this, the laparoscopic urologist is arguably the best-trained person to undertake laparoscopic donor nephrectomy. Many potential living donors are now requesting laparoscopic surgery, which has several advantages over open nephrectomy in terms of postoperative pain, early return to work and better cosmetic results [2]. However, adequate training and monitoring is essential to prevent complications to the donor or damage to the kidney.

The Morris report [3] supported the involvement of urologists with a major commitment to renal transplantation. The combination of specialities would provide
career development and access to private practice. Such surgeons would be able to take on other operations, such as nephron-sparing surgery for RCC, and retroperitoneal work. They would also be able to provide specialist support in the transplant team for urological problems in the transplant population. In one audit study in which the Carrel Club (an association of trainee surgeons in transplantation) database was used, 110 trainees were identified in the UK between 1997 and 1998. Interestingly, only 45% intended to apply for a consultancy in transplantation and most trainees (27 of 31) wanted their transplantation commitments to be combined with a second speciality [4].

To perform more organ transplants and care for these patients, two or three times more transplant trainees than are at present will need to be enrolled in the national training programmes. Transplantation has put a heavy demand on surgical services, which has not been matched by adequate manpower and facilities. The transplant surgeons are often also responsible for creating vascular or peritoneal access. Overall, resources, manpower and facilities for transplantation in general are inadequate across the country. To solve the manpower problem, strategic planning is required. There are several ways to tackle this issue. One approach would be to have a team which involves urologists and vascular surgeons in every renal transplant unit in the country to provide a comprehensive input into the management of patients with renal failure.

Renal transplantation is very cost effective compared with dialysis; in 2002–03, UK Transplant recorded 17 110 people in the UK with a functioning kidney transplant. In the present year, these patients will save the National Health Service £363 million in the dialysis costs that they would have needed if they did not have a functioning kidney transplant [5].

CONCLUSIONS

Currently the discrepancy between the workload and the number of transplant surgeons is large, and will become even greater in future if no action is taken. However, the new training programme in urology may offer a way forward. Subspecialist training could include renal transplantation and a fellowship could be offered (as indeed already exists in some centres). The number of fellowships available in any subspeciality will depend on national requirements’ [6]. Entry would be by open competition but the opportunities for a urologist to become involved in transplantation are available and would be welcomed by many units, and those trainees who still find fulfilment in solving complex problems and who enjoy open surgery would find it very rewarding.

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IS PREMATURE EJACULATION ALL IN THE MIND? JOHN DEAN, IAN EARDLEY, GEOFF HACKETT, JEREMY HEATON and ROGER KIRBY

INTRODUCTION

It is generally accepted that premature ejaculation (PE) is related to psychological factors and erectile dysfunction is linked with organic causes. Clinically, the two conditions can be differentiated by physical examination, penile Doppler ultrasonography, penile tumescence testing and psychological assessment [1]. However, this differential diagnosis is still insufficient in some cases, and there are other examples whereby current definitions and prejudices must be questioned (e.g. psychogenic erectile dysfunction or injury-related PE). So this leaves an unanswered question; is PE a condition of perception, or organic, or both?

To answer this question we examine the historical and current definitions of PE. According to Kaplan [2], PE is caused by poor voluntary control over the timing of ejaculation. Current definitions, including those proposed by the American Psychiatric Association [3], continue with this ‘line of thinking’, by emphasising the emotional and interpersonal nature of the condition. There is also greater promotion of the couples’ perception of PE, i.e. what may be normal for one couple may be regarded as PE by another. If this is the case then how can PE be defined other than as a condition of perception involving poor confidence, low sexual satisfaction or unrealistic expectations?

Even when assessing the current ‘branching classifications’, which take into account the variable causes of PE, it could be psychogenic, arteriogenic, neurogenic, endocrinological and/or cavernosal in origin. Thus perhaps it is fairer to say that PE is ‘all in the mind’ of some patients, reflecting a single sector of the spectrum of the condition, which incidentally is probably a better representation of the argument.

Looking also at the neurophysiology of ejaculation, whereby neurological control of the sexual response occurs at the supra-spinal, spinal and infra-spinal levels, primary PE (i.e. PE since puberty) can be caused by developmental delay of this control mechanism. There is also evidence to suggest that it is a conditioned response relating to this process, and those with an early onset may be unable to regain ‘normal’ ejaculatory control later in life. In these patients, the neurological control may be no different to
In the light of this, perhaps the argument has become one of classification. Having identified an inorganic form of PE, when can this type of PE be distinguished from performance anxiety or psychogenic erectile dysfunction? These conditions are acknowledged in the medical literature, and the high, worldwide prevalence of these conditions supports their existence. There is also a significant association between psychopathological conditions and other sexual dysfunctions. For example, several psychopathological conditions have been identified with erectile dysfunction (and the organic causes are not always established). These include depressive disorders (18–35%), anxiety disorders (37%), or psychotic disorders (46–47%) [5]; these findings also lend weight to the existence of ‘all in the mind’ conditions relating to sexual function.

Further evidence for the ‘all in the mind’ argument comes from studies in which patients continue to improve after therapy has stopped. In a study of 65 men with psychogenic erectile dysfunction who were treated with sildenafil for 6 weeks, a significant proportion (89%) showed an improvement. After treatment was stopped, 66% still maintained their gains 6 weeks later [6]. Does this represent the introduction of a positive-behavioural cycle (i.e. improved sexual functioning = improved self-confidence = improved sexual functioning) or does it indicate a previously unknown organic cause? Indeed, how did sildenafil induce such a significant response in patients with a primary condition, is one of cognition not perception, and this in itself represents an organic control mechanism. In patients with PE this mechanism has not adapted (or responded) normally to external stimuli, and it is this that separates PE from normal ejaculation.

Furthermore, there is evidence to support several organic causes of PE, including illness, injury (spinal), surgery and medication use (side-effects or abuse). This breaks the myth that PE has psychological causes. However, existing evidence still lends itself to distinguishing between primary premature and secondary (late-onset) ejaculation, largely in terms of ‘organic’ vs ‘psychogenic’. The two are characterized by differences in bulbocavernous reflex, latency time, history and demonstrable organic illness [8]. PE can ‘run in families’, which is suggestive of an inherited condition where the underlying cause has not been identified, and these patients could be those wrongly considered to have an ‘all in the mind’ condition.

The comorbid nature of PE (i.e. it is evident with many other conditions such as depression or hypogonadotrophic hypogonadism) is also indicative of a causal link, albeit one that has not been readily identified. There are other outstanding problems, e.g. many patients have been successfully treated for hypogonadism but they retain their PE. In one study of 10 patients treated by aromatase inhibition (anastrozole), hormone levels (testosterone and oestradiol) returned to normal after 2 weeks but the PE remained [9]. We could argue that this supports a mind-based condition, but it suggests that the link between the conditions has not yet been identified. Not dissimilar is the case with penile hypersensitivity; recent studies suggest that it is not a major contributing factor to PE, e.g. in one study of 18 patients with lifelong PE there was no significant ($P < 0.05$) difference in sensitivity of the glans penis, dorsum or frenulum compared with controls [10]. However, topical anaesthetics have been very effective in this context; in one study of 42 men the intravaginal ejaculatory latency time increased from 1.49 to 8.45 min ($P < 0.001$) after treatment with topical lidocaine-prilocaine [11]. These anomalies suggest that larger studies are needed to assess the impact of organic causes on PE, and diagnostic techniques may also need to come under closer scrutiny.

However, further condemnation for the mind theory comes from the effectiveness of treatment with antidepressants, particularly selective serotonin re-uptake inhibitors. This suggests that PE may be a result of a neurotransmitter imbalance (e.g. serotonin), which may also account for the high association between the condition and psychological disorders. A recent pilot study showed that fluoxetine was effective at treating both panic disorder and comorbid PE in the same patients. In this open-label study, 10 patients were given fluoxetine 20 mg for 8 weeks; there were significant improvements in PE at 2 weeks, and in panic and sexual satisfaction at 4 weeks [12]. It is assumed that other agents (e.g. tricyclic antidepressants) will have similar properties.

In summary, PE is a complex condition. If the view that it is ‘all in the mind’ refers to its link with psychological disorders, emotional disturbances or situations, then there is evidence to show that, in some cases, there are established underlying causes (e.g. neurotransmitter imbalances). Even considering the differences between primary and secondary PE, a multifactorial approach is needed to effectively diagnose and differentiate the two, and this is also the case for distinguishing between PE and erectile dysfunction. In addition, pharmacological treatments are effective at treating PE, including that of a psychogenic nature (as shown by the success with sildenafil). However, patients still fare better if they are given a more ‘holistic’ treatment strategy, often involving experimentation with behavioural therapy, partner involvement, counselling and pharmacological intervention. Tailoring the diagnostic expertise and treatment strategies will greatly
benefit from large-scale trials based on some of findings described above.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST


REFERENCES

5 Farre JM, Fora F, Lasheras MG. Specific aspects of erectile dysfunction on psychiatry. Int J Impot Res 2004; 16 (Suppl. 2): 546–9
11 Busato W, Galindo CC. Topical anaesthetic use for treating premature ejaculation: a double-blind, randomized, placebo-controlled study. BJU Int 2004; 93: 1018–21
Mini-reviews

In the first of these mini-reviews the selection of therapy for the maintenance of sexual function in patients with BPH is outlined, along with an explanation of how altered regulation of neurotransmitters, especially noradrenaline, may underlie the syndrome of LUTS and sexual dysfunction.

Other mini-reviews outline the current status of robotic surgery to treat renal and adrenal disorders, and its future applications, and the potential use of the nitric oxide/cGMP pathway as a potential target to treat BOO associated with benign prostatic enlargement.

Finally, the capacity to be creative in academic departments is extolled as a core property of academicians, and its surfacing described as having the potential to revitalize individuals and departments.

Selecting therapy for maintaining sexual function in patients with benign prostatic hyperplasia

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KEYWORDS
BPH, LUTS, adrenoceptors, α-blocker, 5α-reductase inhibition, sexual function

INTRODUCTION
In patients with BPH, LUTS can diminish quality of life (QoL) by interfering with sexual function [1–3], which has been shown to be an important component of QoL among men even in their later years [4,5]. As QoL becomes more of a consideration in managing BPH, the effects of BPH treatment on sexual function need to be included in patient management decisions.

EPIEDEMOLOGY OF LUTS, BPH AND SEXUAL DYSFUNCTION
In a cohort of 80 774 Dutch men aged ≥45 years [6], the incidence rate of LUTS was 5 per 1000 man-years and increased with age, while the overall prevalence rate was 10.3%. It was reported that more than half of men aged >60 years have BPH, with 15–30% of such men experiencing LUTS [7].

The prevalence of sexual dysfunction, including erectile dysfunction (ED) and ejaculatory disorders (EjDs), also increases with age [8]. ED has a reported global prevalence of 18.9–69.2% [9]. In a community-based, longitudinal study of 3924 Dutch men, of whom 9–20% were determined to have BPH, ED prevalence rates were 3% in men aged 50–54 years and 26% in men aged 70–78 years, and EjD prevalence was 3–35% [10,11]. A multinational study involving both community and clinic cohorts showed ED prevalence rates of 53% and 60%, respectively, and EjD prevalence rates of 47% and 62%, respectively, among patients with LUTS [12]. A study in 1274 European men with LUTS showed prevalence rates for ED of 62% and EjDs of 63%, with both being highly bothersome to patients, even in advanced age [12].

PATHOPHYSIOLOGY OF BPH, LUTS AND SEXUAL DYSFUNCTION

BPH AND LUTS
The development of BPH requires the elaboration of testosterone by the testes. Men castrated before puberty do not develop BPH, and BPH is rare in men castrated in adulthood. Within the prostate, testosterone is converted to 5α-dihydrotestosterone (DHT) by 5α-reductase. DHT is important both for the development of the prostate and for its enlargement later in life.

The prostate has a large complement of α-adrenoceptors, particularly in the prostatic capsule, with varying concentrations in the bladder neck region and maximum concentrations in the trigone. The two classes...
of adrenoceptors, α-1 and α-2, are also selectively distributed: α-1s are more abundant in the lower urinary tract and in the blood vessels. (The α-1 and α-2 also have differing effects on male sexual function, as discussed below). Three subtypes of α1-adrenoceptors, 1A, 1B and 1D, are also selectively distributed in the prostate, penis and urinary tract, and these distributions vary with age.

The preponderance of adrenoceptors in the smooth muscle of the prostate suggests that stimulating the receptors could cause an increase in smooth muscle tone in the prostate, thereby increasing pressure on the urethra and resulting in BOO or acute urinary retention (Fig. 1) [13]. In fact, α-blockers have been shown to relax prostatic smooth muscle, with improvements in both irritative and obstructive symptoms.

**SEXUAL DYSFUNCTION (ED AND EJD)**

Penile erection is a complex neurovascular event involving the sympathetic, parasympathetic and somatic nervous systems, which mediate psychogenic and reflexogenic erections via the spinal cord. This process involves a balance of pro-erectile and anti-erectile neurotransmitters, e.g. noradrenaline, serotonin, dopamine and γ-aminobutyric acid.

The normal process of ejaculation proceeds initially with stimulation by the sympathetic nervous system, which results in contraction of the prostate, vas deferens, epididymis and seminal vesicles, and ends with the flow of seminal fluid into the urethra. EjD may occur in the presence of any pathological disorder that involves the lower urinary tract structures in the ejaculation pathway, including the prostate. In addition, neurological and psychological factors may also be implicated in the development of EjD [14].

**LUTS, BPH AND SEXUAL DYSFUNCTION**

A link between LUTS/BPH and sexual function [15] is emerging despite the perception that BPH per se does not adversely affect sexual function. Sexual dysfunction can profoundly affect older men [16], as many still engage in sexual activity [3]; in one survey, 42% of men aged >50 years considered sex ‘important’ or ‘very important’ [2]. Both voiding and storage symptoms have been associated with sexual dysfunction [17].

Other age-related changes that may influence the relationship between LUTS/BPH and sexual dysfunction include declines in circulating androgen levels and the effects of some medications likely to be used in elderly patients. The bothersome effect of obstructive and irritative symptoms, and negative expectations related to sexual performance, can impair sexual performance and QoL among patients with LUTS/BPH [18].

Sexual dysfunction and incontinence often occur in conjunction with LUTS [19], and this raises the possibility of a shared mechanism involving similar noradrenergic and/or other neurotransmitter pathways. BPH can be marked by increases in the concentrations and distribution patterns of α1 receptor subtypes in the prostate, and these same receptor subtypes have been located within penile tissue, where they play an anti-erectile role. It is possible that alterations in these receptor populations may occur in the penis and contribute to the development of sexual dysfunction. Autonomic modulation of α1-receptors and their subtypes at sites outside the genitourinary tract, including the human spinal cord and in both sympathetic and parasympathetic tracts, may also be involved in LUTS as well as in sexual function and dysfunction [20].

**MEASURES TO EVALUATE SYMPTOMS AND SEXUAL FUNCTION IN PATIENTS WITH LUTS AND BPH**

Several well-validated symptom scoring scales have been developed for evaluating LUTS/BPH, and there are generic or disease-specific QoL instruments. With the recognition that treatments for BPH may affect sexual function, several instruments have been used to evaluate sexual function among patients with LUTS/BPH, including the International Index of Erectile Function, the Brief Sexual Function Inventory, the BPH-Health-related Quality of Life scale, the ICSsex questionnaire and the Danish Prostatic Symptom Score questionnaire.

Although evaluations of sexual dysfunction have traditionally focused on ED, sexual functioning encompasses many domains, including satisfaction with intercourse, ejaculation, sexual desire and overall satisfaction. Clinical experience with sexual function scales shows a strong relationship between LUTS and sexual difficulties [14,21]; in addition, sexual satisfaction progressively
decreases as the severity of LUTS advances from mild to severe [22–25]. Even when age-adjusted, patients who score higher on the IPSS have poorer sexual function, as defined by the Brief Sexual Function Inventory [23] or other sexual function scales [24]. When patients and their partners are asked about the history of urinary symptoms and sexual dysfunction, they generally recall both problems as starting concurrently [25]. Studies using these measurement instruments show that men with ED are twice as likely to have LUTS as are men without ED [3].

Not all men will be bothered to the same degree by the same symptoms. Both the prevalence and the bothersomeness of sexual disorders have been shown to be strongly associated with the severity of LUTS, even when age and comorbidities are taken into account [1]. This was the finding from the Multinational Survey of the Aging Male [1], which evaluated >12,000 men aged 50–80 years, in six European countries and the USA. In this survey, 83% of men reported frequent sexual activity, although the frequency decreased with age and was inversely associated with age and the severity of LUTS. Of the patients evaluated, 49% reported ED, 48% had EjD, and 7% had pain during sex. Both ED and EjD were reported as bothersome by most men who experienced them. Problems in each domain of sexual function were strongly associated with the severity of LUTS, independent of age and other comorbidities. Overall, LUTS were present in 90% of the men, but only 11% were being treated medically.

These data raise two significant issues that affect clinical practice: (i) the possible underestimation of the effects of LUTS on patients and therefore of a corresponding need for therapy; and (ii) the need for more thorough assessment of patients with LUTS, including evaluation of sexual function, via the use of validated scales that assess all sexual domains.

**BPH THERAPIES: IMPACT ON SEXUAL FUNCTION**

While the nonssexual side-effects of medical treatments for BPH, especially the vasodilatation-related symptoms of dizziness, asthenia and postural hypotension are well-documented [26–29], the effects of BPH treatments on sexual function are less so. The following represents a summary of clinical data.

**WATCHFUL WAITING AND SURGERY**

Watchful waiting is often used in patients with mild symptoms or symptoms that are not particularly bothersome. For the many patients who eventually require treatment, TURP is the most common surgical procedure. Although highly effective, it is associated with significant morbidity and sexual dysfunction (EjD in 25–55% of cases; ED in 13%) [30–32]. Open prostatectomy, used especially in men with large prostates (>60 g), also has a high success rate but is associated with frequent complications, including deleterious effects on sexual function. Prospective studies evaluating the impact of minimally invasive surgery on sexual function have been few, but have assessed outcomes over periods of 1–4 years [33–35]. There have been some reports suggesting possible pain or discomfort with ejaculation [35] but also emergence of retrograde ejaculation (18%) [33], decreases in ejaculate volume, and reduced erectile strength [34].

**5α-REDUCTASE INHIBITOR (5-ARI)**

Originally medical treatment for BPH was focused on androgen blockade, either by surgical castration or with medication such as the androgen-receptor blocker flutamide. Inhibition of androgens can reduce the size of the prostate but can also cause ED and reduced libido. The focus of pharmacotherapy shifted to with the discovery that men who were deficient in 5ARI due to a homozygous mutation had feminized urogenital structures and prostates only 10% of the normal size.

Conversion of testosterone to DHT by 5α-reductase increases the potency of androgens in target tissues, including the prostate. DHT has a role in the normal differentiation and growth of the prostate, as men with enlarged prostates have higher levels of DHT. Two 5ARIs are used in the treatment of BPH, i.e. finasteride and dutasteride.

**FINASTERIDE**

Treatment with finasteride, a competitive SARI acting on one isozyme that does not bind to the androgen receptor, effectively reduces prostate size, by 19% after 1 year [36] and by 27% after 3 years [37]. The greatest reductions appear to occur in men with larger prostates at the initiation of therapy. Finasteride has also yielded an improvement in urinary flow rates and in symptom relief. Finasteride is associated with significant adverse effects on sexual function in = 10% of subjects [30,36,38], which has led to discontinuation of patients from the drug in several studies [36,38]. In a 2-year, prospective, double-blind trial of finasteride 5 mg/day, 15.8% of finasteride-treated subjects developed ED, 10% reported decreased libido and 7.7% developed EjD.

**DUTASTERIDE**

Dutasteride is an inhibitor of both 5α-reductase isozymes and has been shown to reduce the risk of acute urinary retention and the need for surgery in men with BPH [39]. Similar sexual side-effects to those with finasteride are expected with dutasteride, given that these effects are directly related to the drug’s therapeutic mechanism of action [39]. In a review of safety and tolerability data from several 2-year blinded trials and safety studies of dutasteride, sexual adverse events (including decreased libido, abnormal ejaculation, gynaecomastia and impotence, which occurred more often with dutasteride than with placebo) were those most frequently reported [40].

**α-BLOCKERS**

With the identification of α1-adrenoceptors as the predominant mediators of contraction of prostate smooth muscle, α-blockers have become first-line treatment for BPH. The first-and second-generation drugs include prazosin, doxazosin and terazosin. More recently, two agents claimed to be selective for one or more α1-adrenoceptor subtypes, tamsulosin and alfuzosin, were introduced. Both of these have been claimed to show clinical uroselectivity (i.e. eliciting desired effects on obstruction and LUTS relative to adverse events) and better tolerability than the traditional α-blockers [27,41–44].

**NON-SUBTYPE SELECTIVE AGENTS: TERAZOSIN AND DOXAZOSIN**

Terazosin and doxazosin are non-subtype selective and were originally developed for their antihypertensive properties. Both agents are effective in relieving the symptoms of BPH, but can be associated with
Tamsulosin and alfuzosin are 'uroselective' agents: Tamsulosin and alfuzosin are effective in treating symptoms and urinary flow rates, and the symptoms of BPH progression by 66% compared with observed disease progression in the placebo group [65]. In a recent long-term study of combined therapy and finasteride showed that during an average of 4.5 years of treatment, combined therapy reduced the risk of acute urinary retention by 81%, the need for invasive therapy by 67%, and symptom progression by 66% compared with observed disease progression in the placebo group [65]. These changes were significantly better than those seen with either drug alone.

Safety data reporting the effects on sexual function are available from several trials that have evaluated the use of an α-blocker (e.g. doxazosin, terazosin, alfuzosin) combined with the 5ARI, finasteride, in the treatment of BPH [63,64,66]. In the 1-year Veterans Affairs Cooperative Study, the combination of terazosin and finasteride was associated with the highest reported rates of ED (combination, 10.5%; terazosin, 6%; placebo, 5%; P = 0.05) and ED (combination, 7%; terazosin, 0.3%; finasteride, 2%; placebo, 1%; P < 0.001). In another 1-year study, again the combined therapy was associated with the highest rates of ED (combination, 10.5%; doxazosin, 5.8%; finasteride, 4.9%; placebo, 27% over 1 year N/A EjD, ED, decreased libido N/A [37,61,62].

**COMBINED α-BLOCKADE AND 5-ARI**

In contrast to α-blockers, the 5ARIs do not act rapidly and often take 0.5–1 year to be effective. Because the 5ARIs and α-blockers have different modes and onset of action, studies have examined combinations of these agents. Studies of up to 1 year in duration failed to show that combined therapy was more effective in treating symptoms than α-blocker therapy alone [63,64]. However, a recent long-term study of combined therapy with doxazosin and finasteride suggested that during an average of 4.5 years of treatment, combined therapy reduced the risk of acute urinary retention by 81%, the need for invasive therapy by 67%, and symptom progression by 66% compared with observed disease progression in the placebo group [65]. These changes were significantly better than those seen with either drug alone.

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<td>Decrease =27% over 1 year</td>
<td>N/A EjD, ED, decreased libido</td>
<td>N/A</td>
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<td>Dutasteride</td>
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<td>[62]</td>
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<td>Terazosin</td>
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*A long-term study on sexual function in hypertensive patients [45] suggests that ED may occur with doxazosin, although the difference with placebo was not statistically significant (11.6% vs 16.7%; P = 0.32); †EjD rates were 4–26% [27,48,49]; ‡EjD rates were <1% [29,50]; N/A, not applicable.

**TABLE 1 Clinical characteristics of 5ARIs and α1-adrenoceptor antagonists in the treatment of BPH**

In a recent long-term study of combined therapy with doxazosin and finasteride showed that during an average of 4.5 years of treatment, combined therapy reduced the risk of acute urinary retention by 81%, the need for invasive therapy by 67%, and symptom progression by 66% compared with observed disease progression in the placebo group [65]. These changes were significantly better than those seen with either drug alone.

Increased with alfuzosin treatment; in the 3-month ALFORTI study, no EjD was reported in any of the three treatment groups [50]. In a 12-month extension of the same study, alfuzosin once daily produced sustained improvements in symptoms and urinary flow rates [50,51]. In the placebo-controlled 3-month ALFUS study [29] treatment with alfuzosin 10 mg once daily induced a 3.6-point mean reduction in the IPPS from baseline in the absence of any deleterious effect on sexual function. Temporary EjD was reported in one patient (0.6%) in each treatment group, with the cases being considered not related to the study drug, as there was spontaneous resolution with no need to discontinue therapy.

Unlike the non-subtype selective α-blockers (e.g. prazosin, terazosin and doxazosin), tamsulosin and alfuzosin are associated with a low incidence of postural symptoms, similar to that seen with placebo [26,27,52]. Although alfuzosin shows no subtype specificity on in vitro tests, it does appear to be clinically uroselective [44,53–56].

**‘UROSELECTIVE’ AGENTS: TAMSULOSIN AND ALFUZOSIN**

Tamsulosin and alfuzosin are α-blockers that are claimed to be uroselective or act preferentially on the lower urinary tract, i.e. they are clinically and physiologically uroselective agents. Both effectively improve urinary flow rates and the symptoms of BPH without affecting blood pressure at the doses used. Although tamsulosin has shown little effect on blood pressure, findings from several clinical trials indicate that use of this drug may be associated with EjD in some patients; in these studies, EjD was reported in 4–26% of patients treated with tamsulosin [27,48,49].

Alfuzosin is used extensively in Europe as a treatment for BPH and was recently approved by the USA Food and Drug Administration. Sexual dysfunction does not appear to be increased with alfuzosin treatment; in the 3-month ALFORTI study, no EjD was reported in any of the three treatment groups [50]. In a 12-month extension of the same study, alfuzosin once daily produced sustained improvements in symptoms and urinary flow rates [50,51]. In the placebo-controlled 3-month ALFUS study [29] treatment with alfuzosin 10 mg once daily induced a 3.6-point mean reduction in the IPPS from baseline in the absence of any deleterious effect on sexual function. Temporary EjD was reported in one patient (0.6%) in each treatment group, with the cases being considered not related to the study drug, as there was spontaneous resolution with no need to discontinue therapy.

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Alfuzosin is associated with a much lower incidence of EjD than is tamsulosin [57]. EjD has occurred in 10–11% of subjects taking tamsulosin 0.4 mg/day and 18–26% of those taking 0.8 mg/day [48,58]. In a 1-year extension study, 30% of patients had EjD during treatment with tamsulosin, causing 2% to discontinue treatment; 6% had ED [59]. In contrast, the incidence of EjD was <1% in clinical studies of alfuzosin once-daily, and other sexual adverse events did not occur at incidences significantly greater than those reported with placebo [29,50]. These findings are supported by long-term trials with other formulations of alfuzosin [54,60]. A summary of the clinical characteristics of α-blockers and 5ARIs is shown in Table 1 [37,61,62].

Cardiovascular or vasodilatory side-effects, e.g. dizziness, asthenia and postural hypotension. In a 48-month study evaluating the long-term effects of antihypertensive agents, including doxazosin, on sexual function, rates of ED at study endpoint were 11.6% with doxazosin and 16.7% with placebo, although the difference was not statistically significant (P = 0.32) [45]. In addition, a 1.6% incidence of ED was reported with terazosin [46].

In a more recent prospective study, the new extended-release formulation of doxazosin was shown, using the International Index of Erectile Function, to produce a substantial improvement in sexual function in patients with BPH and comorbid ED [47].

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The 6-month ALFIN study evaluated the sustained-release formulation of alfuzosin 5 mg twice daily with finasteride 5 mg once daily and the combination of the two in the treatment of BPH [66]. Here too, combined therapy was associated with higher rates of ED than was either of the drugs when used as monotherapy (combination, 7.4%; alfuzosin, 2.2%; finasteride, 6.7%; P < 0.002). No episodes of EjD occurred with alfuzosin, and lower rates of EjD were reported with combined therapy than with finasteride alone (0.9% vs. 1.5%; P = 0.04).

CONCLUSIONS

The effect of α-blockers on sexual function merits closer attention because recent clinical experience suggests that these agents may differentially affect certain aspects of sexual dysfunction, particularly ED. Overall, the use of α-blockers not only effectively relieves the symptoms of LUTS/BPH but also preserves, and in some cases may improve, erectile function. Although some tolerability differences may be evident among individual α-blockers, the effectiveness and safety of BPH therapy must always be assured. Given the increasing evidence of comorbid sexual dysfunction. Although some tolerability differences may be evident among individual α-blockers, the effectiveness and safety of BPH therapy must always be assured. Given the increasing evidence of comorbid sexual symptoms of LUTS/BPH but also preserves, and in some cases may improve, erectile function. Although some tolerability differences may be evident among individual α-blockers, the effectiveness and safety of BPH therapy must always be assured. Given the increasing evidence of comorbid sexual dysfunction.

CONFLICT OF INTEREST

None declared.

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THERAPY FOR MAINTAINING SEXUAL FUNCTION IN BPH

57 Schulman CC, Cortvriend J, Jonas U et al. Tamsulosin, the first prostate-selective α1A-adrenoceptor antagonist. Analysis of a multinational, multicentre, open-label study assessing the long-term efficacy and safety in patients with benign prostatic obstruction (symptomatic BPH). Eur Urol 1996; 29: 145–54


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Abbreviations: ED, erectile dysfunction; EjD, ejaculatory disorder; QoL, quality of life; 5ARI, 5α-reductase inhibitor; DHT, dihydrotestosterone.
Surgical procedures are performed through the robotic arm, allowing the surgeon to control the camera and instruments. The surgery is coordinated through a procedure that requires no reconstruction and can be performed well with pure laparoscopy. However, robotic nephrectomy, radical nephrectomy, and urothelial surgery are difficult for inexperienced surgeons. The robot provides an excellent solution to these issues, allowing relatively less experienced laparoscopists the ability to offer minimally invasive surgery to their patients which is learned more rapidly than pure laparoscopy, with improved technical performance.

In urology, the main use for the robot has been in radical prostatectomy for prostate cancer. The reasons for this include the small and deep working space, need for precise dissection of the urethra, division of the bladder neck, preservation of the neurovascular bundle, and reconstruction of the urethrovaginal junction, and often simply the sheer numbers of prostatectomies.

The use of the robot for renal and adrenal surgery is less well explored, possibly because simple nephrectomy is a standardized procedure requiring no reconstruction and can be performed well with pure laparoscopy. However, radical nephrectomy, nephroureterectomy, reconstructive renal and ureteric surgery are difficult for inexperienced laparoscopic surgeons. In open surgery, the surgeon’s actions are coordinated through a complex, highly integrated and controlled interaction of visual and tactile feedback. However, in laparoscopic urological surgery this fundamental coordinated feedback is significantly minimized or lost. In addition, the surgeon’s actions are further compromised by limitations in the movement of the instruments, known as degrees of freedom. Furthermore, the two-dimensional vision present in standard laparoscopy results in loss of the perception of depth, as well as the need for a human assistant to control the camera. The impediment to the widespread use of laparoscopy in urology has been the complexity and technical demands of urological procedures, which is why it is limited to relatively few experts and centres worldwide. However, with laparoscopic nephrectomy becoming the standard of care for benign nonfunctioning kidneys, laparoscopically inexperienced surgeons will feel the need to offer this to their patients and may benefit from the more rapid learning allowed by the robot. We review published reports on the use of robotic assistance for renal and adrenal surgery and discuss its potential benefits.

RENAL SURGERY

Patient positioning and port placement for robotic renal and adrenal surgery have been described previously. For simple and radical nephrectomy, Table 1 lists relevant reports. Kavoussi et al. described the initial use of a robot for nephrectomy in a laboratory model where an experienced surgeon controlled the camera through a robotic arm, while the surgery was performed laparoscopically by another surgeon. Partin et al. expanded this use, with the surgeon controlling up to two robotic arms that held the laparoscope and a retractor, in four patients undergoing nephrectomy. Gill et al. described the first completely robotic, telepresent nephrectomy in a porcine model using an AESOP arm to control the camera and two Zeus robotic arms to perform the surgery on five kidneys in pigs. They were technically successful in all the pigs, with longer operative times than conventional laparoscopy. Sung and Gill went on to compare the two available robot systems for nephrectomy in 11 procedures and concluded that the da Vinci system (Intuitive Surgical, Sunnyvale, CA) was more intuitive and allowed shorter operating times than the Zeus system.

In July 2001, Guillonneau et al. reported the first telerobotic nephrectomy in a human. They used a Zeus robotic surgical system with two arms for surgical manipulation and an AESOP robotic arm to control the camera. The robotic instruments were used to complete the dissection of the hilum and the kidney, while the patient-side assistant applied laparoscopic clips using a standard 12 mm port. They noted technical problems in instrument availability for the robot and relied heavily on the patient-side assistant. The surgery was successfully completed in 200 min with no mortality. Marella et al. presented a series of 18 robot-assisted laparoscopic nephrectomies at the annual meeting of the AUA in 2004 and compared their results with 23 cases of hand-assisted laparoscopic nephrectomy. The operative duration was longer with the robot, with no immediate advantage to its use. Hubert et al. also presented data on 16 nephrectomies in 10 patients (six bilateral simple, two radical and two donor nephrectomies) using the da Vinci robot. They had one conversion to open surgery with a mean operative time of 110 min and no significant blood loss. They considered that robotics helped their team with moderate experience in laparoscopy to expand the possibilities of minimally invasive surgery.

Moore et al. assessed the feasibility of a telementored radical nephrectomy and reported failure caused by poorly positioned robotic arms. A successful telementored radical nephrectomy between the USA and Singapore was subsequently reported in 2000 by Lee et al., who concluded that these systems may help less experienced laparoscopic surgeons perform complex tasks.
TABLE 1 Robot-assisted nephrectomy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>N cases</th>
<th>Type</th>
<th>Technical success</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sung and Gill [12]</td>
<td>Swine</td>
<td>11</td>
<td>Simple</td>
<td>All</td>
<td>Long operative time; learning curve</td>
</tr>
<tr>
<td>Guillonneau et al. [13]</td>
<td>Human</td>
<td>1</td>
<td>Simple</td>
<td>All</td>
<td>Comparison of Zeus and da Vinci systems</td>
</tr>
<tr>
<td>Marella et al. [14]</td>
<td>Human</td>
<td>18</td>
<td>Simple</td>
<td></td>
<td>Zeus and AESOP systems</td>
</tr>
<tr>
<td>Hubert et al. [15]</td>
<td>Human</td>
<td>12</td>
<td>Simple</td>
<td>11/12</td>
<td>Compared with hand assisted laparoscopy</td>
</tr>
<tr>
<td>Pedraza et al. [16]</td>
<td>Human</td>
<td>1</td>
<td>Nephroureterectomy</td>
<td>All</td>
<td>Moderate laparoscopy experience</td>
</tr>
<tr>
<td>Horgan et al. [17]</td>
<td>Human</td>
<td>12</td>
<td>Donor</td>
<td>All</td>
<td>Longer vessels retrieved, shorter hospitalization</td>
</tr>
</tbody>
</table>

with the help of remote experienced surgeons.

Apart from these sporadic reports, there have been no consistent series of cases reported. One of the principal reasons for this is probably the absence of benefit perceived with purely ablative procedures, which have become standardized in pure laparoscopy.

NEPHROURETERECTOMY AND PARTIAL NEPHRECTOMY

Nephroureterectomy, particularly with removal of a cuff of bladder in cases of TCC of the bladder, provides a more likely application for the reconstructive skills of the robot-assisted technique. Dissection of the lower end of the ureter also requires greater precision to avoid injury to the pelvic viscera. Currently used techniques for managing the lower end of the ureter during laparoscopic radical nephroureterectomy include endoscopic resection, pluck removal, stapling and freehand suturing [22]. Established oncological principles and replication of the open surgical techniques can be achieved through a two-layered suture closure of the bladder wall. Similarly, significant reconstruction is required in managing an open pelvicalyceal system and bleeding parenchyma after a partial nephrectomy. Laparoscopy has become an established option for partial nephrectomy, with minimal morbidity and with results similar to those for open surgery [23].

While this seems to be a potentially useful area for robotic application, the only reported nephroureterectomy using a robot was by Pedraza et al. [16] who performed a heminephroureterectomy bilaterally in a girl with duplicated ureters and nonfunctional upper poles. The robot was used for hilar dissection and isolation of the renal pole, while the remaining procedure was completed through pure laparoscopy. During the Third International Robotic Urology Symposium (IrUS 2004) at Detroit in October 2004, surgeons from Guy’s Hospital, London, presented a case of nonfunctioning kidney with megaureter that was operated through an extraperitoneoscopic approach. Nephroureterectomy was performed using four ports in 3 h with an entirely retroperitoneal approach, and the patient was discharged 4 days after surgery. This case showed the feasibility of robot-assisted surgery through an entirely retroperitoneal approach, similar to that in pure laparoscopy.

DONOR NEPHRECTOMY

Donor nephrectomy for renal transplantation requires more meticulous dissection than simple nephrectomy. The procedure also requires that patient morbidity is minimised, particularly because it is being performed on an otherwise healthy individual. Laparoscopic donor nephrectomy was the first step in achieving this goal and has now become established in over 100 centres worldwide, with minimal morbidity [24–26]. The establishment of this technique has also contributed to an increase in the number of donor nephrectomies, thus helping to minimize the gap between donors and recipients [24]. Horgan et al. [17,27] reported the largest experience in robot-assisted donor nephrectomy. In their 12 cases reported in 2002, they used the da Vinci robotic system to procure the left kidney, in all cases using four ports and a pre-placed hand-port for specimen retrieval [17]. They found the robotic approach, similar to that in pure laparoscopy, to be especially useful in harvesting right-sided kidneys, where the renal vein is shorter and sometimes needs to be divided as it enters the vena cava. This necessitates suture closure of the caval defect, which could be more dextrously accomplished with robotic assistance. Indeed, Sung and Gill [12] reported one such repair of the vena cava during an adrenalectomy in a swine model.

PYELOPLASTY

Laparoscopic pyeloplasty (Table 2) [1–3,5,12,28–30] for PU obstruction has become a standardized procedure with success rates equivalent to open pyeloplasty, and minimal morbidity because it is less invasive [31–33]. The technique is versatile, allowing the management of patients with all types of pathology, including crossing vessels, high ureteric insertion and redundant pelvis. Laparoscopic pyeloplasty requires a significant amount of surgical dexterity because of the precise suturing. This technical difficulty has been cited as the main reason for its limited widespread application [34]. Robotic technology is ideally suited to decrease the technical difficulty in such cases. It permits a greater degree of freedom at the wristed instruments, with three-dimensional vision, allowing precise placement of fine sutures.
Sung et al. [28] showed the technical feasibility of robot-assisted pyeloplasty in a study on pigs, where they performed six robotic and four conventional laparoscopic pyeloplasties, the robotic procedures being conducted using a Zeus operating system. They also reported similar results when comparing the Zeus and da Vinci robotic systems, with faster surgery using the latter [12]. Guillonneau et al. [29] confirmed the technical feasibility and safety of the robotic approach in a study on 10 farm pigs. These studies suggested the technical superiority of the da Vinci system in achieving successful outcomes, and the initial human cases were reported in 2002 by Gettman et al. [30]. They reported their experience of robot-assisted dismembered Anderson-Hynes pyeloplasty in nine patients, with no intraoperative complications or conversion. Their mean operative duration was 138 min and one patient required a re-operative closure of a renal pelvis defect, away from the anastomotic site. The same authors went on to compare their results with conventional laparoscopic dismembered pyeloplasty and reported shorter operative and anastomotic times with the robot [3]. In a recent update of their series of 49 patients, including 10 with failed previous endopyelotomies, 40 had a robot-assisted dismembered Anderson-Hynes pyeloplasty with a mean suturing time of 43 min. There were no intraoperative complications or conversion to open surgery. At a mean follow-up of 7.4 months, they reported complete success in all their procedures [35].

The major impact of robotic assistance in this otherwise complex laparoscopic procedure has been that surgeons claiming lower laparoscopy skills have also produced similar results when comparing the Zeus and da Vinci systems. Yohannes and Burjonrappa [5] also reported complete success in all their procedures.

TABLE 2 Robot assisted pyeloplasty

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>N cases</th>
<th>Technical success</th>
<th>Complications</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sung et al. [28]</td>
<td>Swine</td>
<td>6</td>
<td>All</td>
<td>0</td>
<td>Technical feasibility of robotic pyeloplasty</td>
</tr>
<tr>
<td>Sung and Gill [12]</td>
<td>Swine</td>
<td>12</td>
<td>All</td>
<td>0</td>
<td>Comparison of Zeus and da Vinci systems</td>
</tr>
<tr>
<td>Guillonneau et al. [29]</td>
<td>Swine</td>
<td>10</td>
<td>All</td>
<td>0</td>
<td>Chronic porcine model</td>
</tr>
<tr>
<td>Gettman et al. [30]</td>
<td>Human</td>
<td>9</td>
<td>All</td>
<td>11% (late)</td>
<td>138 min operative duration</td>
</tr>
<tr>
<td>Gettman et al. [3]</td>
<td>Human</td>
<td>49</td>
<td>All</td>
<td>2% (late)</td>
<td>43 min suturing time</td>
</tr>
<tr>
<td>Bentas et al. [1]</td>
<td>Human</td>
<td>11</td>
<td>All</td>
<td>0</td>
<td>No laparoscopic pyeloplasty experience</td>
</tr>
<tr>
<td>Hubert et al. [2]</td>
<td>Swine</td>
<td>14</td>
<td>All</td>
<td>0</td>
<td>Limited laparoscopy experience</td>
</tr>
<tr>
<td>Yohannes and Bur jonrappa [5]</td>
<td>Human</td>
<td>1</td>
<td>All</td>
<td>0</td>
<td>Lower surgeon fatigue</td>
</tr>
</tbody>
</table>

ADRENALECTOMY

The adrenal gland is another organ which seems particularly well suited for the laparoscopic approach (Table 3) [12,36–42]. Most tumours are small and require a large incision for open surgical access. Sung et al. [12] reported the initial porcine study comparing the Zeus and da Vinci robotic systems for adrenalectomy. They found more rapid operations with the robot [3]. Lower surgeon fatigue.

TABLE 3 Robot assisted adrenalectomy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>N cases</th>
<th>Technical success</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sung and Gill [12]</td>
<td>Porcine</td>
<td>10</td>
<td>All</td>
<td>Compared Zeus and da Vinci systems</td>
</tr>
<tr>
<td>Young et al. [36]</td>
<td>Human</td>
<td>1</td>
<td>All</td>
<td>Incidentaloma</td>
</tr>
<tr>
<td>Bentas et al. [37]</td>
<td>Human</td>
<td>4</td>
<td>All</td>
<td>Phaeochromocytoma</td>
</tr>
<tr>
<td>Desai et al. [38]</td>
<td>Human</td>
<td>2</td>
<td>All</td>
<td>High conversion rate due to technical difficulties</td>
</tr>
<tr>
<td>Beninca et al. [39]</td>
<td>Human</td>
<td>9</td>
<td>5</td>
<td>Compared with standard laparoscopy, similar outcomes</td>
</tr>
<tr>
<td>Brunaud et al. [40]</td>
<td>Human</td>
<td>14</td>
<td>All</td>
<td>199 min</td>
</tr>
<tr>
<td>Undre et al. [41]</td>
<td>Human</td>
<td>2</td>
<td>All</td>
<td>No intraoperative complication</td>
</tr>
<tr>
<td>D’Annibale et al. [42]</td>
<td>Human</td>
<td>1</td>
<td>All</td>
<td>Five ports</td>
</tr>
</tbody>
</table>

complex surgery easily and quickly. They also considered that robot assistance could provide faster training for reconstructive laparoscopy, with less fatigue for the surgeon. Peschel et al. [3] also reported a subjective decrease in operative difficulty and surgeon fatigue with the use of a robot in their series of 49 cases. The more rapid learning after using robotic assistance has been confirmed by others [4,5].
Horgan et al. [43] reported the first human adrenalectomy using the robot in 2001. While they do not describe their technique, they also performed 32 other surgical procedures using this device. Young et al. [36] performed a robot-assisted adrenalectomy for an incidental left adrenal mass in a patient being evaluated for mediastinal widening. Pathology showed a rare adrenal oncocytoma. Later that year, Bentas et al. [37] reported four adrenalectomies by a transperitoneal approach with no complications or conversions, and Desai et al. [38] reported two adrenalectomies, including one for a phaeochromocytoma. Their two patients had a mean hospital stay of 2.5 days and no complications during or after surgery.

Beninca et al. [39] reported their experience of nine robot-assisted adrenalectomies (six for an adenoma, two for phaeochromocytoma and one for an incidentaloma) and compared them with nine laparoscopic adrenalectomies. Surgery was significantly longer in the robotic group (mean 133 vs 82 min) but there were no intraoperative complications. However, they had to convert to traditional laparoscopy because of technical difficulties in four robotic cases. In another comparative study, Brunaud et al. [40] evaluated their results of 14 robotic- (da Vinci) assisted adrenalectomies with another 14 patients undergoing standard laparoscopic adrenalectomy. They reported longer operations for robotic surgery but a progressive decrease with increasing experience. They found no significant advantage of robotics but noted that unlike standard laparoscopy, there was no effect of body mass index on the technical outcome with robotic surgery, suggesting a possible benefit of this technique.

PERCUTANEOUS ACCESS AND RENAL TRANSPLANTATION

Percutaneous renal surgery for managing calculi, PUJ anomalies or upper tract tumours requires the placing of a carefully positioned needle into the pelvi-calyceal system. This is usually done by the surgeon or radiologist under ultrasonographic or fluoroscopic guidance, and can be difficult in patients with minimal dilatation. The possibility of stereotactic-robotic assistance using an interface was first reported in 1997 [44]. The authors successfully punctured the desired calyx in 10 of 12 procedures using a robotic system. The following year, the same authors described their robotic system, ‘PAKY’, which permitted the insertion of a needle in both an in-vitro porcine model and patients, using fluoroscopic guidance [45]. The device was successful in each of its attempts within a mean access time of 8.2 min. While this approach appears promising, there are no reports of its incorporation into a regular clinical programme.

Robots are also being used in conjunction with advanced imaging methods to develop virtual models that may help to enhance surgical skills. Hoznek et al. [46] describe the development of a surgical model that can assist the surgeon in choosing the best possible port position for the robot to reach the target organ. It also considers data such as optimal tissue handling, ergonomics, visibility and instrument manoeuvrability. Such models are also being used for training surgeons.

In 2002, Hoznek et al. [47] described a cadaveric renal transplantation with the entire vascular and uretero-vesical anastomosis being performed by a remote surgeon using a robot. The authors consider that such telerobotic surgery may help to prevent transmission of infection between patient and recipient, apart from providing greater dexterity during the fine vascular anastomosis, an argument similar to that for its use in microsurgical vasectomy reversal.

CONCLUSIONS

As with every new technology, robot-assisted surgery has to pass through various stages of evaluation before it can be accepted as a standard option of therapy. Currently, it often seems to be a procedure looking for an indication. However, this was also the case with laparoscopy before it became accepted as the standard of care for several procedures. The initial reports cited here attest more to its safe applicability in surgery that is otherwise feasible through pure laparoscopy. The main proposed advantages for this technology are its enhanced dexterity, precision and ergonomics. These issues are undoubtedly still present with laparoscopy, which is difficult to learn, with often suboptimal results by surgeons not dextrous enough in suturing.

The widespread common knowledge about the advantages of laparoscopy has made it imperative for most practising urologists to offer the option to their patients. However, the lack of significant experience and training opportunities makes it imperative that easier learning tools are made available to these physicians. Robot-assisted surgery has opened these options for several laparoscopically naive or minimally qualified surgeons.

The major problem with robot-assisted surgery is the cost of equipment and surgery. The argument brings a sense of deja vu of similar problems faced by laparoscopy when it was initially introduced. While there is no doubt that it may be difficult to envision a
robotic revolution if the costs remain as they currently are, the expansion of indications coupled with lowering of equipment costs as more machines are sold will undoubtedly help to balance the books.

CONFLICT OF INTEREST

None declared.

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Targeting bladder outlet obstruction from benign prostatic enlargement via the nitric oxide/cGMP pathway?

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KEYWORDS

nitric oxide, cyclic GMP, phosphodiesterase, BPH

INTRODUCTION

The high age-related incidence of benign prostatic enlargement (BPE) often associated with BOO and LUTS is of considerable significance for public health. Although rarely life-threatening, BPE has a severe impact on the quality of life of patients. Surgical therapy has been considered the reference standard for treating BPE, but pharmacological therapy is increasingly common, as reflected in the fewer surgical interventions for BPE over the last 20 years. Current medical therapies rely basically on three approaches: (i) α-adrenoceptor antagonists reduce prostatic smooth muscle tone by blocking the effects of noradrenaline released from sympathetic nerve terminals; (ii) 5α-reductase inhibitors reduce prostate volume by blocking the conversion of testosterone to dihydrotestosterone; and (iii) phytotherapeutic drugs seem to be able to improve LUTS by a more or less unknown mechanism.

During the last 25 years nitric oxide (NO) has been recognized as a unique biological signalling molecule involved in numerous physiological processes and body functions. NO was originally known as ‘endothelium-derived relaxing factor’, which if released by endothelium cells can induce the relaxation of vascular smooth muscle [1]. Furthermore, NO is also involved in immunological responses [2] and acts as an important noncholinergic, noradrenergic neurotransmitter in both the central and peripheral nervous systems [3,4].

Also within the genitourinary system, NO appears to be an important messenger. Both NO-based mechanisms, the endothelium-derived and the neurogenically mediated relaxation of cavernosal smooth muscle cells in the penis, have been reported [5,6]. Sildenafil, an inhibitor of phosphodiesterase (PDE) type 5, revolutionized the treatment of erectile dysfunction and highlighted the NO/cGMP pathway as a focus of attention for the urological community to further understand the role of NO in the genitourinary system. Thus, NO-based processes have also been implicated elsewhere in the genitourinary system, e.g. within the detrusor, the urethra and the striated external urethral sphincter. Currently there is growing evidence that the NO/cGMP pathway is involved in regulating the prostatic smooth muscle tone. This review considers the available knowledge about the significance of the NO/cGMP pathway for treating BOO and concomitant LUTS related to BPE. A hypothesis is developed and possible strategies to prove or disprove the suggested approach discussed.

NEUROANATOMY

The human prostate receives autonomic innervation from the pelvic plexus travelling through the cavernosal nerves. Basically, parasympathetic fibres innervate the glandular structures and control prostatic secretion via a cholinergic mechanism; sympathetic nerves innervate the capsule and stroma of the prostate, and control the smooth muscle tone via adrenergic receptors.

In both noradrenergic and non-noradrenergic nerves innervating the human prostate the enzyme NO synthase (NOS) has been found [7]. In a morphological study using immunohistochemistry, NOS activity was found in both the transition and the peripheral zone of the human prostate [8]. NO-containing neurons appeared to originate in the neurovascular bundles later penetrating the prostate capsule and dispersing in the glandular tissue. The level of NOS activity differs in both zones of the prostate, indicating that there is not a uniform distribution within the gland; NOS activity was higher in the peripheral than in the transitional zone. In both zones NOS was localized to nerve fibres and ganglia within the smooth muscles of the prostatic stroma and the subepithelial plexus, as well as in the glandular epithelium [8].

Another morphological study on human prostatic tissue could not confirm these results and found no difference in nitrimergic innervation density among different parts of the prostate [9]. Using histochemical NADPH-diaphorase staining, NOS immunohistochemistry and ultrastructural NADPH examination, there was a dense nitrimergic innervation of the glandular epithelium, fibromuscular stroma and blood vessels [9].

In both studies NOS-containing nerves were found in close relationship to prostatic smooth muscle cells and it was speculated that NO promotes prostatic smooth muscle relaxation [8,9]. Furthermore, the rich nitrimergic innervation around the prostatic glands found in both morphological studies generated the assumption that NO is also involved in regulating prostatic secretion [8,9].

NO NEUROPHARMACOLOGY

Nerves containing NOS have the capacity to synthesise NO that can serve as a neurotransmitter. NO catalyses the reaction of the amino acid L-arginine to NO and L-citrulline in the presence of oxygen and NADPH. Currently, three isoforms of the NOS are known, neuronal (first detected in neuronal tissues), endothelial (first found in vascular endothelium) and so-called inducible NOS (first detected in macrophages). After being released from the site of synthesis the NO diffuses freely to the target tissue where the molecule is thought to act as a neurotransmitter on nonadrenergic, ---

noncholinergic nerves. Throughout the lower urinary tract a nitrinergic innervation has been identified at different densities; almost all NO-induced effects are inhibitory. The NO-mediated responses are thought to act through an intracellular increase in the second messenger cGMP via stimulation of the enzyme guanylate cyclase [10–12]. NO-dependent relaxation of urethral smooth muscle was reported in various species, including rabbit [13] and men [14]. While the nitrinergic innervation and NOS enzyme activity is rich in the urethra, it seems to be sparse within the detrusor muscle. A NO-mediated detrusor smooth muscle relaxation is still controversial; although recent results suggest that detrusor relaxation and contractility might be modulated by NO levels and that NO released from the urothelium may be a mediator of detrusor relaxation during the storage phase of bladder function [15].

NO AND PROSTATIC PHYSIOLOGY

Prostatic specimens from various species including humans have been studied in vitro. Takeda et al. [16] were the first to find that NO is involved in the control of prostatic smooth muscle function. In their study, NO donors caused a relaxation of human and canine prostatic tissue, with the relaxing effect being significantly greater in the human than in the canine prostate. Heglund et al. [17] confirmed these observations in human prostatic tissue and reported a relaxing effect of NO on noradrenaline-contracted prostatic preparations, while the inhibition of NOS effectively counteracted the relaxing effects. Morphological and functional results in that study suggested that neuronally derived NO contributes to the inhibitory control of tension in the prostatic stroma.

Another important issue is the effect of age and prostatic volume on the nitrinergic innervation of the prostatic gland. Aikawa et al. [18] studied the effect of age on the endogenous NO-mediated prostatic smooth muscle relaxation and the nitrinergic innervation in the rabbit prostate, and found that both are reduced with ageing. In canine hyperplastic prostates the level of neuronal NO was reduced, suggesting that neuronal NOS expression is down-regulated in the prostate with benign cellular proliferation [19]. In human hyperplastic prostate tissue the nitrinergic innervation was lower than in normal prostates [9]. Thus, a NO donor had an antiproliferative effect on human hyperplastic prostatic smooth muscle cells [20]. Gradini et al. [21] studied prostatic tissue from men with and without hyperplasia for different isoforms of NOS. While neuronal and endothelial NOS were expressed in both normal and hyperplastic glands, inducible NOS was expressed only in hyperplastic glands. The appearance of inducible NOS has been linked to the influence of sex hormones, which have been considered to be involved in the development of prostatic hyperplasia. From this study it was concluded that NO might have a potential role in the pathogenesis of BPE.

These finding may implicate a possible involvement of NO in the pathogenesis of BPE, because a reduced nitrinergic innervation or a relative NO deficiency may increase the tone of the prostatic smooth muscle, which potentially leads to the BOO associated with clinical BPE.

TREATING BPE VIA THE NO/cGMP PATHWAY

From currently available knowledge there is evidence that drugs acting on the NO/cGMP pathway might have a potential role in treating subvesical obstruction caused by BPE. The hypothesis relies on the relaxing effect of NO on prostatic smooth muscle cells that potentially decrease subvesical obstruction and improve both voiding and bothersome LUTS. Considering the pathophysiology of LUTS, the focus has shifted from the prostate to the bladder [22], and recent results suggest that detrusor relaxation and contractility may be modulated by NO levels [15]. NO augmented or released from the urothelium may be a mediator of detrusor relaxation during the storage phase of micturition and therefore may have favourable effects on LUTS.

Basically, two classes of drugs might be relevant for the suggested approach; first, oral NO donors, and second PDE-inhibiting drugs. As heavy bleeding is often reported during prostatic surgery, the gland is considered to have a rich blood supply. After oral intake of sildenafil there was a relevant increase in periurethral blood flow, using colour Doppler TRUS measurements [23], suggesting that oral administration is a feasible approach.

In vitro the NO donor sodium nitroprusside relaxed prostatic smooth muscle strips isolated from the transition zone of the prostate [24]. Currently, oral NO donors are widely used for treating coronary artery disease. Several advantages of NO donors make the further evaluation of their effect on infravesical resistance worthwhile. Many NO donors are well known drugs with good tolerability and a long established safety record, and their variable pharmacokinetic properties could be an advantage. Especially the fast-acting formulations with an onset of action within minutes could allow new treatment strategies with intermittent drug use alone or combined with a classical medical BPE therapy.

BPE and coronary artery disease occur in the same age groups and the coincidence of both problems in elderly people is supposedly high. Klötz et al. [25] studied 32 patients who had a urological evaluation before starting nitrate medication for cardiovascular disease. All patients underwent uroflowmetry with a determination of the postvoid residual urine volume, TRUS and PSA screening. According to prostatic symptom scores the authors found that 15 patients had obstructive voiding symptoms, while 17 reported no subjective voiding complaints. At 2 weeks and 3 months after starting nitrate medication the patients were re-evaluated; those who had reported obstructive symptoms before nitrate medication improved significantly as assessed by peak urinary flow rates, symptom scores and postvoid residual urine volume, while asymptomatic patients did not change. PSA values and prostate volumes remained unchanged in both groups. The authors concluded that NO medication influence voiding variables in patients with obstructive BPE and explained this by a potential smooth muscle relaxation within the prostate.

PDEs have been identified in different regions of the human prostate [24,26,27]. In vitro, the functional relevance is supported in that the adrenergically induced tension of prostatic smooth muscle strips could be relaxed by inhibitors of PDE-4 and -5 [24]. Furthermore, sildenafil inhibited the proliferation of prostatic hyperplastic tissue [28]. As sildenafil has revolutionized the treatment of erectile dysfunction, many men worldwide take PDE-inhibiting drugs regularly. The prevalence of both erectile dysfunction and LUTS increases with age, and a close relationship of sexual function and voiding function has been
recognized in several studies [29–31]. Medical treatment of lower urinary tract dysfunction is known to influence a patient's sexual function [32] but almost nothing is known of how treating erectile dysfunction with PDE-inhibiting drugs influences voiding dysfunction from BPE. Sairam et al. [33] assessed the effect of sildenafil on lower urinary tract function; the coincidence of erectile dysfunction and voiding difficulties associated with BPE in older men is well known, and indeed treatment with sildenafil appeared to improve urinary symptom scores in that study, suggesting a possible role of PDE-inhibiting drugs in treating BPE in the future.

Functional studies in vivo assessing the direct effect of NO on the human lower urinary tract are rare. However, after oral administration in healthy humans, a NO donor had a functionally relevant effect on the resting tone and contractile behaviour of the human external urethral sphincter in vivo [34]. In a functional study in humans with spinal cord injury, subvesical obstruction caused by detrusor-sphincter dyssynergia was successfully reduced by oral administration of a NO donor [35]. Recently, the immediate influence of systemic NO augmentation on bladder outlet resistance was investigated in healthy men using pressure-flow studies. Relative to the mean average flow rate, the average intravesical pressure during micturition, the ratio of mean average intravesical pressure to mean average flow rate, and the mean intravesical pressure at maximum flow rate, there was a significant reduction in bladder outlet resistance in healthy men within 20 min of sublingual administration of an NO-donor [36].

To confirm the suggested new approach, both urodynamics experiments and chronic clinical studies in men with BPH might be of value. As a first step, uroflowmetry is suggested in the absence and presence of NO using standardized nomograms.

The safety and efficacy of the suggested approach need to be assessed in clinical trials. For clinical long-term trials NO donors or PDE-inhibiting drugs with long half-lives are preferred, to maintain a sufficient drug level over several hours and to offer the opportunity to use a once-daily administration scheme. As BPE in older men causes obstruction and bothersome LUTS, clinical studies must address both changes in obstruction and LUTS, which can be assessed by frequency-volume charts, voiding diaries and the standardized IPSS questionnaire.

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CONFLICT OF INTEREST
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Abbreviations: BPE, benign prostatic enlargement; NO(S), nitric oxide (synthase); PDE, phosphodiesterase.
The vital role of creativity in academic departments

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KEYWORDS
creativity, organization, surgery, academic department

INTRODUCTION
Organizations such as academic departments and universities have long had a tacit understanding of the need for creativity. However, the practice of creativity has usually been allowed to develop through individual efforts and nurturing environments, with no formal encouragement or realisation of the true benefits. Creativity has been an implicit expectation of the new appointment as well as a criterion for advancement in a somewhat stylised form (university promotion criteria). However, the disciplined recognition and implementation of original, untried and potentially risky new thought has the potential to confer significant competitive advantage [1]. Particularly in chaotic environments, where there is little to differentiate competitors, creativity may provide the flexibility of output, process or structure that enables survival and growth. A university department with creativity potentially improves its capacity to compete for funding, people, reputation and, eventually, postgraduate students.

CREATIVITY AND THE DEPARTMENT
If creativity is recognisable on an individual level without much difficulty it is a small step to grasping the concept of organizational creativity: ‘the creation of a valuable, useful new product, service, idea, procedure or process by individuals working together in a complex social system’ [2]. Individual creativity, innovation and organizational change and creativity are closely linked and hierarchically related, as shown in Fig. 1a.

All creativity, innovation and organizational change can be traced ultimately to human beings, most often aided by the environment, other humans and technology [2]. Creativity arises as a result of human thinking and can be seen as a particular form of knowledge or understanding that makes a transition from being internal, or tacit, to being revealed or explicit [3]. Without communication in some form it remains unrecognized. Individuals working in an organization contribute their individual aliquots of creativity to the benefit of themselves as individuals and the organization. However, it is clear that only an individual can truly be said to be creative [4] and organizational creativity flows from that. So Fig. 1a might be redrawn as Fig. 1b, where the sum of creativity in an organization comes from contributions from individuals while the total creative impact may be organizational. The recognition and measurement of creativity and innovation in an academic department is most clearly embodied in the criteria for promotion, and comes from peer recognition in scholarly publications, research grants or innovative educational programmes. Enlightened universities have recently recognized patenting as evidence of scholarly creative activity. Publication count, impact factor, grant duration, grant value and standing of the granting agency are the common metrics of merit in academia. Usually administrative effort and administrative or financial innovation are defined as service and, unless they extend into the realm of scholarly publication, would not be considered for the individual in terms of promotion or merit, although the organization may benefit and change as a result. Thus creativity in managing the placement of sick elderly patients through an 11-day power cut (January 1998) was not recognized for the physicians involved (they did not publish); nonetheless, their creative management of the crisis changed the organizations (department, hospital and faculty-adopted crisis planning processes). The narrow university measures of academic achievement can overlook real organizational change.

Creativity is vital to the development of new therapies, and evidence-based medicine [5] is at the pinnacle of quality care. Yet intrinsically, evidence-based medicine can only support a concept once it has been created and reaffirmed many times, making the major creative contribution a relatively distant historical footnote. Nevertheless, iterative medical advance remains a necessary goal in an academic department. The creative contribution of a better way of doing surgery or medicine probably occurs in small measure almost daily, is very difficult to communicate except to trainees, is very difficult to evaluate and would rarely rise to the level of influencing a department or its environment. The adoption of creative steps pioneered elsewhere becomes a proxy for innovation and may be encouraged. Keeping up with a standard of care is a necessary informal and unrecognized manifestation of creativity. The development and implementation of new therapies and procedures help patients, may bring financial reward and may also bring kudos to a department, faculty and university, boosting academic standing and improving the quality of the incoming students.

CREATIVITY IN AN ACADEMIC MEDICAL DEPARTMENT
It is easily seen that, within this framework of creativity, an increase in publication quantity and quality should further the career of the individuals and enhance the standing of the department. Publications and creative research planning underlie applications for research funding, which is commonly the second major measure of creative output. Patenting activity, often in tandem with technology transfer units, has become better established and recognized in universities. Increased patent and intellectual property activity is now more commonly recognized as a valid contribution. That patenting is a measure central to increased national competitiveness is not widely appreciated. Patenting is incorporated in global measures of national competitiveness and universities are integral to that measure [6].

It has been indicated that motivation, expertise and creative thinking underpin organizational creativity [7]. Improvements in
Creativity as:

a subset of innovation and change, and b, as a product of individual effort.

Creativity in Academic Departments

Creativity Blocks

The potentially 'dyscreative' forces in a department can be paralleled to those identified in business organizations, even though the product is different, i.e. time pressure, administrative pressures, culture, leadership, short-term thinking, resources (money, time and skilled personnel), unclear goals, poor communication, fear of change and a failure to initiate it [9].

There are some common errors that contribute to creative dullness: Defining the problem incorrectly (a time and perspective issue); judging ideas too quickly (common); stopping with the first good idea (often relates to time pressure); and failing to get the politics right [10]. The potential barriers to innovation are:

- **Culture:** the fixed mindset; the established assumptions, beliefs and values ('nothing fails like success').
- **Technological or structural:** inertia based on technology that has been committed to; rules, regulations and procedures that are self-justifying and punitive of failure.
- **Management:** styles that kill ideas, discourage risk-taking and inhibit feedback.
- **People:** resistance to change, complacency, conflicts and incompetence.

In an academic medical department the assumption that creativity is given free rein and is self-sufficient is not necessarily justified. The lack of infrastructure funding in medicine and surgery is common; it is the rare department with the present generation of surgical robot and it is even rarer to have two generations of surgical robots. Physicians are notoriously resistant to even inspired leadership, and the culture of independence in medicine decreases the likelihood of team activity. Teams incorporate a redundancy of thinking that favours new ideas and creative solutions [4]. Furthermore, in medicine and surgery the overarching need for safety often quenches progress in the name of caution ('It's too risky', 'better to be a fast follower, than a first mover') [11]. In addition, managerial practices have a greater capacity to stifle creativity when there are many streams of management, as is the case in many academic medical departments (hospital, departmental, faculty, university).

Making Creativity Work for an Academic Department

It has been said that 'Creativity is as much about believing in new ideas and bringing them into form, as it is about generating the ideas in the first place' [12]. Several factors have been identified that encourage creativity: challenge, freedom, resources, intellectual working environment, encouragement, and organizational support [7].

In revitalizing an organization the organization should be viewed as a whole and not as a series of disconnected problems. Think of an organization as a homeostatic system; the changes have to be system-wide to gain lasting effect, embody feedback and be internal (not external consultant-based).

In shaping a creative department, attributes (human traits) that favour innovation can be better selected than made; so when recruiting, select a creative 'right-brained' surgeon. Conceptual skills, with the emphasis on cognition and the ability to reassemble old learning in new ways, are more amenable to improvement by training; seminars and retreats may usefully be directed to this. Behaviours can be influenced by leadership and culture (especially in academia) [13]. Processes can be reshaped and simplified to emphasize the creative areas and limit the dulling of people who should flourish. A categorized table of revitalizing steps is:

- **Culture:** redesign duties and responsibilities and reward ideas, reward learning even if it does not progress to creativity.
- **Technological or structural:** improve communications, reward technical creativity, discourage inactivity, mitigate the effects of time pressure.
- **Management:** introduce formal training in organizational theory, creativity and lateral thinking, use of protected time.
- **People:** avoid unhelpful reactions to change, (cursory evaluation and rejection of ideas, evaluations biased by status or insecurity), admit imperfections and mistakes.

These are some of the elements that will support an increase in creativity. The design of an action plan will vary significantly with the departmental context, but includes identifying the need for change, establishing current views, agreeing a course of action and a timeline, setting some measurable outcomes and planning for review and
feedback. A department can reinforce this internally by guaranteeing participatory safety, encouraging the challenging of assumptions, overtly deciding to target excellence, incorporating people who ‘don’t fit’, supporting ‘favourite’ projects and encouraging fun [14]. Individuals should be prepared to give ideas away, challenge others to act on their ideas, and be creative connectors (critical but not corrosive).

In a department the criteria for success and contribution should be lowered (to less than those required for promotion), there should be broader support for other dimensions of creativity (administration, consulting) and some concepts relating to quality (not just quantity) should be introduced. Resources should be directed to where they will increase creativity (e.g. grant and report writing, in the short term, and new staff and new types of staff in the long term). A department should increase its willingness to recognize and harness success in a wider scope of activities; and then it should nudge its environment in a similar direction.

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Phosphodiesterase type 5 inhibitors for erectile dysfunction

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The cyclic nucleotide signalling pathway mediates the smooth-muscle relaxing effects of nitric oxide necessary for normal erectile function. Down-regulation of this pathway is central to the pathophysiology of many forms of erectile dysfunction (ED), which is often associated with other chronic diseases (e.g. hypertension, type 2 diabetes mellitus) and treatments (e.g. certain drugs, radical prostatectomy). Conversely, selective inhibition of the enzyme that catalyses the degradation of cGMP (phosphodiesterase type 5, PDE-5) promotes erectile responses to sexual stimulation. The successful launch and commercialization of the selective PDE5 inhibitor (PDE5I) sildenafil transformed the treatment of ED, not only by providing an effective, well tolerated oral ED therapy, but also by fostering greater candour about the problem among men. Sildenafil is highly effective in promoting erectile responses across a wide spectrum of severity and causes of ED, including patients with ED that is often refractory to treatment. The recent advent of vardenafil, which has the highest in vitro potency of all available PDE5Is, and tadalafil, which has a prolonged half-life that may enable couples to have sexual activity with less planning, represent further advances. Other PDE5Is offering further potential improvements are under active investigation.

KEYWORDS
cGMP, efficacy, erectile dysfunction, pharmacokinetics, phosphodiesterase type 5, safety, sildenafil, vardenafil, tadalafil

INTRODUCTION
The diagnosis and treatment of erectile dysfunction (ED) provide opportunities to enhance patient health and well-being. This condition affects >150 million men across the world [1], including ~17% of Europeans [2]. Despite effective ED therapies, 70–90% of men with ED do not receive treatment. ED erodes the quality of life for many patients and their partners, and often signals the presence of other treatable diseases, including hypertension and type 2 diabetes mellitus (DM2). In this article we survey the aetiology and pathophysiology of ED, and consider at greater length the treatment of this condition using selective inhibitors of phosphodiesterase type 5 (PDE5Is).

HISTORICAL PERSPECTIVE
CYCLIC NUCLEOTIDE SIGNALLING PATHWAY

Although the availability of oral medications that inhibit PDE5 dates only to 1998, the basic science underlying their development can be
traced back 50 years. In 1958, Sutherland and Rall [3] discovered an enzyme (PDE) that abolished the biological actions of the second messenger cAMP in mammalian tissue extracts. This magnesium-dependent PDE cleaved the 3',5'-cyclic phosphate moiety, hydrolysing the phosphodiester bond to form the inactive linear 5'-AMP. Further investigation also showed that this PDE was inhibited by methylxanthines and stimulated by imidazole [3,4].

Physiological functions are highly sensitive to intracellular levels of cyclic nucleotides, including cAMP and cGMP. These cyclic nucleotides are tightly regulated, such that maximum physiological responses are produced by transient, two- to three-fold increases in intracellular concentrations [5]. Cyclic nucleotide levels are determined by: (i) synthesis through the activities of adenylate cyclase and guanylate cyclase on ATP and GTP, respectively; and (ii) enzymatic degradation (inactivation) through the activity of PDEs.

cGMP is a second messenger for the smooth-muscle relaxing effects of nitric oxide (NO); endothelium-derived relaxing factor) within the penis. The cascade of events linking NO to sGC activates this enzyme, with increased formation of cGMP; cGMP then triggers a biochemical cascade culminating in penile vasodilatation, increased blood flow, expansion of erectile tissues, and trapping of blood through compression of emissary veins.

Because PDE5 is the predominant PDE in the penis [6], selective inhibition of this iso enzyme increases intracellular cGMP levels in vascular and corporal SMCs and thus potentiates the NO-mediated vasorelaxant (erectile) response to sexual arousal. The first in vivo demonstration of this effect was reported in 1992 at the AUA meeting and published in 1994 [7]. The researchers reported that cavernosal nerve-stimulated penile erections were markedly enhanced when monkeys were given the PDE5 inhibitor zaprinast [7]. In 1998, Louis Ignarro, Robert Furchgott and Ferid Murad were awarded the Nobel Prize in Physiology/Medicine for their work on NO-cyclic nucleotide signal-transduction mechanisms, which supported the development of PDE5Is.

PDEs

Enzymes that regulate diverse physiological functions in a wide range of organs and tissues, PDEs are divided into 11 families encoded by distinct genes. Members of each family have >65% amino-acid homology [8] and substrate (e.g. cAMP, cGMP) specificity. Conservation of the catalytic domain suggests that PDEs pre-date the evolutionary divergence of eumetazoans from fresh-water sponges. A conserved catalytic domain consisting of ~300 amino acids shows 20–45% amino-acid sequence homology across PDE family members, suggesting that the three-dimensional structures of the catalytic domains are similar.

PDE5 is widely distributed, with animal studies having located the enzyme in vascular, pulmonary and visceral smooth muscle, as well as kidney, platelets and the cerebellum (Table 1). Based largely on mammalian in vivo studies, PDEs are considered putative regulators or modulators of olfaction (by PDE1), visual transduction (PDE6) and other neural activities; insulin action (PDE3); and platelet aggregation, vascular tone and other cardiovascular effects (PDE3, PDE5).

Agents that inhibit PDEs have been evaluated as treatments for a wide range of diseases. Among the earliest recognized PDE inhibitors were caffeine and theophylline [4]. The PDE3 inhibitors milrinone and amrinone were investigated in the 1980s as positive inotropic agents for patients with heart failure.

The PDE4 inhibitor cilostazol was used as a treatment for intermittent claudication. Another PDE4 inhibitor, rolipram, was investigated for treating depression, and to reduce coronary artery smooth-muscle proliferation, thus preventing re-stenosis.
after percutaneous intervention. Another PDE4 inhibitor, roflumilast, is under late-stage clinical investigation as a treatment for chronic obstructive pulmonary disease. The antiplatelet treatment diprydamole blocks PDE8 and PDE9 as well as PDE5.

OVERVIEW OF THE PDE5I CLASS

The first PDEI developed for treating ED was papaverine; this opium poppy alkaloid is a relatively nonselective PDEI, promoting cavernosal smooth-muscle relaxation by inhibiting both PDE5 and PDE3, and thus increasing intracellular levels of cGMP and cAMP, respectively. Treatment with papaverine may further promote smooth-muscle relaxation through blockade of voltage-dependent Ca\(^{2+}\) channels. Papaverine is administered via injection into the corpora cavernosa of the penis, either alone or together with other vasoactive substances (e.g. alprostadil).

The first oral PDE5I approved for treating ED, sildenafil citrate, was initially investigated as a treatment for angina pectoris. Before sildenafil, oral ED therapy was confined to largely ineffective treatments, including off-label trazodone and the natural remedy yohimbine. Zaprinast is also a selective PDE5I that has been evaluated in studies of sexual dysfunction. Sildenafil transformed the treatment of ED; the success of this ED therapy and attendant public awareness helped to usher in a new era of candour about sexual dysfunction. In the USA, presentation rates for ED more than doubled after the regulatory approval of sildenafil.

There are now three oral medications (sildenafil, vardenafil and tadalafil) that inhibit PDE5 and are indicated for treating ED. Each of the three PDE5I registration programmes involved >2000 patients. In the USA, sildenafil was approved in 1998 and both vardenafil and tadalafil in 2003. As of 2001, sildenafil had been approved for use in >110 countries; of this writing, >20 million patients across the world have received sildenafil and >4 million have received tadalafil for ED.

Although the PDE5Is offer effective therapy for ED, the recent Men’s Attitudes to Life Events and Sexuality study showed that only 16% of men with ED were receiving a PDE5I [9]. Among commercially insured adult Americans, the prevalence of sildenafil use among pharmacy beneficiaries increased by 84% from 1998 to 2002, but this still only represented 1.4% (15 246/1 077 318) of pharmacy beneficiaries at the end of this interval [10].

BASIC SCIENCE

PHYSIOLOGY OF ERECTION

The erectile response to sexual arousal depends on a complex series of neural and vascular events that transform the penile vasculature and erectile tissues from a contracted, minimally perfused state to a relaxed, blood-engorged state. The urogenital reflex underlying erection consists of sensory afferents from the penis and autonomic efferents from parasympathetic neurones with cell bodies in the sacral spine. This reflex is influenced by supraspinal structures, including the medial preoptic area (MPOA) and paraventricular nucleus of the hypothalamus, which assimilate sexual sensations (images, smells, touch, tastes) with cognitions, fantasies, memories and other input.

Various neurotransmitters, including central NO, oxytocin and dopamine, are recognized as pro-erectile neurotransmitters [11]. The observation that dopamine exerted central pro-erectile effects helped to trigger the development of a sublingual dopamine agonist (Uprima\textsuperscript{®}, Ixense\textsuperscript{TM}). Although Ixense is available in Western Europe for treating ED, Uprima has not been approved by the USA Food and Drug Administration.

The sacrospinal ‘sex centre’ also receives descending inhibitory input from serotonergic centres within the brain. In vivo work suggested that serotonin (5-hydroxytryptamine) receptors inhibit certain sexual activities or processes and facilitate others. In animals, selective agonists of 5-hydroxytryptamine-2 receptors inhibit erectile responses but facilitate ejaculation [11]. Other central mediators with inhibitory effects on sexual behaviours and/or erectile function [EF] include enkephalins, prolactin, and \(\gamma\)-aminobutyric acid [11].

During the erectile response, the penis acts as a capacitor, filling with and retaining blood. The major anatomical structures involved in male sexual function are the three corpora: a pair of dorsally located corpora cavernosa, and a more ventrally situated corpus spongiosum, which houses the urethra and is more involved in ejaculation than erection. Each corpus (or body) is a longitudinal cylindrical structure containing a mass or bundle (trabecula) of smooth muscle.

Within this meshwork of smooth muscle/connective tissues are endothelial-lined vessels and spaces (also termed sinusoids or lacunae). Vascular supply is from the pudendal-cavernous-helicine arterial tree. Each corpus is encapsulated by a fibrous sheath, the tunica albuginea, which also confers structural support.

The smooth-muscle tone of the corpora cavernosa determines the erectile state of the penis. Vascular tone is maintained in a dynamic equilibrium consisting of contractile (anti-erectile) and relaxant (pro-erectile) factors. In the absence of sexual arousal, both trabecular and vascular smooth muscle is tonically contracted through sympathetic neural input. Release of endothelin-1 and prostaglandin F\(_2\alpha\) (PGF\(_{2\alpha}\)) from endothelial cells, as well as other mediators (e.g. angiotensin II, neuropeptide Y), exerts vasoconstrictor effects, further limiting blood flow into the penis (Fig. 1) [12].

The contractile effects of noradrenaline and endothelin-1 are partly mediated by the RhoA/Rho-kinase signalling pathway (Fig. 2) [13,14]. When noradrenaline or endothelin-1 binds to SMC receptors, RhoA is converted from an inactive, cytosolic GDP complex into an active, membrane-bound GTP complex.

RhoA-GTP in turn activates Rho-kinase, which phosphorylates the myosin-binding subunit of the regulatory enzyme myosin light-chain (MLC) phosphatase, thus inhibiting this enzyme. With MLC phosphatase inactive, MLCs remain phosphorylated, are sensitized to intracellular Ca\(^{2+}\), and bind to actin filaments, thus promoting smooth-muscle contraction.

Release of NO from nonadrenergic, noncholinergic neurones in response to sexual arousal triggers the shift toward vascular and smooth-muscle relaxation (Fig. 3) [12]. This shift is amplified by enhanced parasympathetic input, with increased acetylcholine release and further NO output from endothelial cells lining helicine arterioles. Although several vasodilators have been implicated in the
erectile response, including vasoactive intestinal peptide and PGs, as well as acetylcholine, NO is considered the pivotal physiological stimulus for cavernosal vasorelaxation.

cGMP is a second messenger for the vasorelaxant effects of NO (Fig. 3) [12]. Through several downstream mediators, including protein kinase G, cGMP triggers a cascade of events leading to reduced intracellular availability of Ca^{2+} ions. These events include phosphorylation of Ca^{2+} channels by a cGMP-dependent protein kinase, with reduced influx of Ca^{2+} across the plasma membrane; membrane hyperpolarization through increased Na^+/K^+-ATPase activity, with further reduced influx of Ca^{2+} and Ca^{2+} sequestration by cellular organelles, including the sarcoplasmic reticulum. Under conditions of low cytosolic Ca^{2+}, myosin heads detach from actin filaments and smooth muscles relax. With vasodilatation of helicine arterioles, there is a rapid, \( \approx 10 \)-fold rise in penile perfusion with engorgement of the corporal sinuses. Shear forces ascribed to this increased blood flow activate eNOS within sinusoidal endothelial cells, amplifying NO release and potentiating smooth-muscle relaxation and the erectile response.

Expansion of trabecular erectile tissues leads to compression by the noncompliant tunica albuginea of thin-walled subtunical veins draining the penis, restricting venous outflow. As blood is retained within the penis by this veno-occlusive mechanism, intracorporal pressure approaches mean arterial pressure (full erection phase). Contraction of
perineal skeletal muscles (ischio cavernosus, bulbocavernosus) is necessary to achieve the rigid erection phase, which produces supra-systolic pressure within the corpora cavernosa.

Enzymatic degradation of cGMP by PDE5 is the key event terminating the above cascade. Consequently, selective inhibition of PDE5 by sildenafil, vardenafil or tadalafil potentiates the erectile response. Because this response depends on NO synthesis and release upon sexual stimulation, PDE5Is are contingent agonists of the sexual response; they are effective only with sufficient sexual arousal.

Although the NOS-NO-sGC-cGMP and Rho-kinase signalling pathways are largely independent, in vivo studies [15] suggest that there is cross-talk between them. Protein kinase G, a downstream mediator in the cyclic-nucleotide signalling pathway, inhibits RhoA activation, biasing the Rho-kinase pathway toward vasorelaxation and penile erection.

Rho-kinase inhibition represents a promising pharmacological approach to treating ED. Topical administration of the Rho-kinase inhibitor Y-27632 to the tunica albuginea induced penile erection in rats, elevating intracorporal pressure within a few minutes, with maximal effects at 10–15 min [16]. These beneficial effects were accompanied by only marginal changes in mean arterial pressure.

CAUSES OF ED

ED has many causes (Fig. 4) [17], with most (75–80%) cases being attributed to vascular or neural disorders. Other causes include endocrine imbalances, anatomical disorders (Peyronie's disease) and psychological conditions (and/or their treatments). In many cases, more than one of these factors contribute to ED.
Vascular

Blood flow into the penis is a vascular process dependent on a functioning endothelium. Thus, endothelial pathology often leads to ED. The degree of ED varies directly with the number of arterial risk factors, including hypertension, hyperlipidaemia, DM2 and smoking [18]. Several factors, including atherosclerosis, DM2 and ageing itself, are construed as NO-deficient processes.

According to the basic science, DM2 and advanced age are associated with down-regulation of the NO–cGMP signal-transduction mechanism, partly via increased formation of advanced glycation end products. These compounds, such as pentosidine, can glycate or inactivate ('quench') NO and/or inhibit eNOS [19]. In addition, corporal smooth-muscle strips from men with ED and DM undergoing penile implantation showed impaired endothelium-dependent and autonomically mediated smooth-muscle relaxation [20]. This impaired smooth-muscle relaxation was significantly correlated with the duration of DM.

**In vivo** work in senescent rats showed that the presence of advanced age and/or vascular risk factors (e.g. hypertension) may be associated with decreases in: (i) NOS activity per gram of tissue; and/or (ii) NO-stimulated sGC levels, as well as increases in inducible NOS (iNOS) activity with elevated SMC apoptosis [21–24]. Apoptosis, with fewer SMCs in human corporal tissues, may also contribute to ED by compromising the veno-occlusive mechanism [25].

Hypertension is also a well-documented risk factor for ED [26]. In the human microvasculature, hypertension attenuates endothelium-dependent NO-mediated SMC relaxation. Hypertension also represents a broader dysregulation of vascular relaxant and contractile factors. In the presence of hypertension, NO may be unable to offset the anti-erectile influences of increased peripheral sympathetic neural activity and other pro-contractile mediators (e.g. endothelin-1, prostanoids, neuropeptide Y, angiotensin II) [13].

**Neural**

CNS disorders associated with ED include Parkinson’s disease, Alzheimer’s disease, stroke, multiple sclerosis and major depressive disorder. The estimated prevalence of ED is 70% in men with multiple sclerosis, 60% in those with Parkinson’s disease and 30% in those with stroke [17]. Given sacrospinal involvement in the urogenital reflex, it is not surprising that ED occurs in up to 80% of men with sacrospinal-cord lesions, compared with 10% with suprasacral lesions. Other central causes of ED include head trauma, tumour and temporal-lobe epilepsy [17].

Peripheral nervous system disorders contributing to the development of ED include autonomic insufficiency and diabetic (somatic and autonomic) neuropathy. Peripheral neuropathies may also be secondary to vitamin B deficiencies, chronic alcoholism, scleroderma, systemic lupus erythematosus, amyloidosis or AIDS. Sensory penile thresholds often increase significantly as a result of peripheral nervous system...
disorders. These thresholds are critical to the ability of men to achieve and maintain erections. Because of elevated sensory thresholds in elderly men with ED and/or DM, increased tactile stimulation is often necessary to ensure an adequate erectile response with or without PDE5Is.

Local-tissue/anatomical. The erectile response to sexual stimulation is critically dependent on: (i) adequate compliance of the corpora cavernosa, which enables erectile tissues to expand during the ‘filling’ phase; and (ii) the structural integrity of the tunica albuginea, which supports the corpora as they become engorged with blood and mediate the veno-occlusive mechanism during the ‘storage’ phase.

Cavernosal. Causes of veno-occlusive dysfunction (i.e. ‘venous leak’) include DM, Peyronie’s disease and atherosclerosis, which can lead to degeneration of the tunica albuginea and/or alter endothelial function or gap junctions; chronic ischaemia, which can alter collagen content (i.e. corporal fibrosis); and psychogenic inhibition, which can result in inadequate neurotransmitter release.

Endocrine

Apart from DM, endocrine abnormalities are considered to be less frequent precipitants of ED than either neural, vascular or anatomical factors. However, a large endocrine screening programme conducted by Buvat and Lemaire [27] showed that 9% of men with ED and aged >50 years met diagnostic criteria for hypogonadism. In the First International Consultation on Erectile Dysfunction, the WHO recommended that testosterone be assayed in men with low sexual desire and atrophic testes (≤19 mL) because ED secondary to hypogonadism is potentially reversible using testosterone-replacement therapy [28].

Animal studies indicate that androgens may promote EF by suppressing the responsiveness of cavernous vascular SMCs to the pro-contractile (i.e. anti-erectile) effects of α-adrenergic input [29]. In vivo work also suggests that reductions in testosterone may accompany, as well as cause, ED; increased NOS activity in the corpora cavernosa occurs with normalization of testosterone in castrated animals. Under oxidative and nitrosative stresses, increased iNOS expression with formation of toxic peroxynitrites may also reduce both MPOA neurones needed for central processing of sexual input and neurones generating GnRH.

Prolactin levels are elevated in ~5% of men complaining of ED [30]. Hyperprolactinaemia is often characterized by low libido and other forms of sexual dysfunction, gynaecomastia, galactorrhoea, and/or exceedingly low testosterone levels (<150 ng/dL). Hyperprolactinaemia can occur in association with DM, chronic renal failure and cirrhosis of the liver, among other conditions.

Obesity and/or DM can also negatively affect circulating androgens by raising sex hormone-binding globulin [31]. Both hyperthyroidism and hypothyroidism have also been cited as contributors to the development of ED [17]. Chronic alcoholism can reduce EF in part through effects on the androgen/oestrogen balance [17,31].

DM can compromise erectile responses via all four mechanisms (i.e. vascular, neural, local-tissue, endocrine). This fact may help to explain why: (i) the prevalence of ED is up to 75% in men with DM2; and (ii) men with ED and DM2 often present with a worse quality of life and ED that is refractory to therapy.

Many medical and surgical treatments can also cause ED. Treatment with certain vasodilators, antihypertensives, and diuretics (e.g. thiazides, spironolactone) increase the risk of ED. For instance, α-adrenergic blockers (e.g. propranolol) can impair EF by enhancing α-adrenoceptor activity and/or suppressing libido [11,17].

Other iatrogenic causes of ED include treatment with psychotropic agents and medications associated with hyperprolactinaemia. These include medications with effects on central dopamine, such as α-methylidopa and reserpine, which deplete central dopamine stores; dopamine-receptor blockers, such as chlorpromazine, butyrophenones (e.g. metoclopramide); and agents that block the effects of endogenous dopamine, such as amoxapine, cimetidine and verapamil.

Cocaine abuse can also elicit ED through effects on endogenous dopamine, as well as through increased α-adrenergic activity and endothelial dysfunction.
Several pelvic, perineal and other surgical procedures can induce ED through adverse effects on neural and/or vascular structures. The prevalence of ED is estimated at 40–70% in men after radical prostatectomy (RP, nerve-sparing), 20–60% after abdominoperineal resection, 30% after aorto-iliac vascular bypass, and 4% after TURP [17]. Pelvic irradiation may also contribute to the development of ED.

In addition to spinal-cord injury, other forms of trauma, such as pelvic fractures, may give rise to ED through injury to vascular and/or neural structures. Habitual bicycling for long distances can impair EF, possibly via perineal nerve entrapment or penile arterial injury [32,33].

BIOCHEMISTRY AND PHARMACOLOGY

STRUCTURES

The molecular structures of sildenafil, vardenafil, tadalafil and the natural substrate cGMP are depicted in Fig. 5 [34]. Although the structure of tadalafil differs from the structures of sildenafil and vardenafil, all three PDE5Is share a heterocyclic nitrogen-containing double-ring system. This core ring structure mimics the purine base of cGMP and interacts with the same PDE5 catalytic site.

The chief structural difference between sildenafil and vardenafil is in these core ring systems; N\textsuperscript{1} of the purine ring in cGMP binds to Gln-817 of the catalytic site. Slight differences in the core ring system of vardenafil may enable stronger interactions with one or more amino acids that are important for binding to the PDE5 catalytic site, including Gln-817, Tyr-612, Val-782, Phe-820, and Leu-785. Tadalafil is one of a series of \(\alpha\)-carboline–based PDE5Is and has a piperazinidine ring rather than the hydantoindione ring found in sildenafil.

THREE-DIMENSIONAL STRUCTURE OF PDE5 AND BINDING INTERACTIONS BY INHIBITORS

Three isoforms (splice variants) of PDE5 (PDE5A1-A3) have been identified in animal and/or human tissues [35]; the predominant isoform is PDE5A2. These isoforms differ in their N-terminal regions, which correspond to different 5′ regions of mRNA. The human PDE5 gene is located on chromosome 4q26 and comprises 23 exons encompassing >100 kb. A PDE5A promoter preceding these exons includes a 139-bp core, as well as a 156-bp downstream enhancer and a 308-bp upstream enhancer. The PDE5A promoter is inducible by cGMP, such that increased cGMP levels lead to increased PDE5 gene expression in a negative-feedback loop [36].

All three PDE5 isoforms share similar substrate specificity and cGMP catalytic activity. PDE5A3 expression in males is limited to smooth muscle in the penis, bladder, urethra, prostate gland and aorta, whereas both PDE5A1 and PDE5A2 are more widely distributed.

PDE5 is a homodimeric, cytosolic enzyme consisting of two identical \(\approx99\)-kDa subunits. Each subunit is a chimeric protein comprising both a carboxy-terminal metal-binding catalytic domain and an amino-terminal regulatory (allosteric) domain [37]. Within the regulatory domain are two identical tandem homologous sequences of 110 amino acids (GAF \(\alpha\) and \(\beta\) domains). PDE5 is a zinc-dependent enzyme with high substrate specificity for cGMP (\(K_m\) \(\approx1–5\) \(\mu\)mol) over cAMP (\(K_m\) \(\approx300\) \(\mu\)mol). The three PDE5Is bind exclusively to the catalytic domain, not the regulatory domain. Binding of either a PDE5I or cGMP to one catalytic domain does not appear to kinetically influence the activity of the catalytic domain on the other monomer.

A Korean group elucidated the three-dimensional structure of the PDE5 catalytic site (residues 537–860) using X-ray crystallographic analyses (Fig. 7) [38]. The PDE5 catalytic site comprises three helical subdomains: a C-terminal helical bundle (residues 726–860), a linker region (residues 679–725), and an N-terminal cyclin-fold domain (residues 537–678). The PDE5Is bind to the catalytic site (Fig. 7) with a stoichiometry of \(\approx1\) mole of PDE5I per PDE5 subunit, according to tritiated-substrate binding studies [34]. The PDE5 active site is accessed via a narrow (1 nm) cleft housed within a larger 33 nm substrate pocket containing four subdomains. These include a metal-binding region (M site), a core pocket (Q pocket) and two other regions less...
PHOSPHODIESTERASE TYPE 5 INHIBITORS FOR ERECTILE DYSFUNCTION

PHOSPHODIESTERASE TYPE 5 INHIBITORS FOR ERECTILE DYSFUNCTION

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FIG. 6. Schematic representation of PDE5, Reproduced with permission from [37]. Copyright 2004 Nature Publishing Group. All rights reserved.

An ethyloxyphenyl structure in sildenafil and vardenafil fits into the H pocket, which includes the amino acids Phe-786, Ala-783 and Leu-804. The L region then forms a lid over sildenafil and vardenafil through residues 662–664, narrowing the passage and sterically limiting access to the PDE5 active site [38].

Tadalafil does not interact with the L region and forms a single hydrogen bond to Gln-817, rather than the bidentate hydrogen bond between this amino acid and sildenafil, within the Q pocket. On the other hand, the cyclic methylenedioxyphenyl substituent of tadalafil undergoes more extensive interactions with the H pocket than does either sildenafil or vardenafil. These more extensive hydrophobic interactions may help to explain how tadalafil maintains high affinity to the catalytic site without binding to the L region [38].

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BINDING POTENCY

The concentration of each agent needed to inhibit PDE5 activity by 50% (IC_{50}) is 3.7 nmol/L for sildenafil, 0.091 nmol/L for vardenafil, and 1.8 nmol/L for tadalafil; thus, the sildenafil : vardenafil : tadalafil in vitro potency ratio is 1 : 41 : 2 [34]. PDE5-specific dissociation constant (K_d) values, which are more direct indices of enzyme binding, indicate similar potency relations among the PDE5Is [34].

PDE SELECTIVITY

As shown in Table 2 [39], all three PDE5Is are highly selective for PDE5 over other PDEs, although there are differences among the agents. The PDE5 : PDE6 selectivity ratio is 6.8 : 1 for sildenafil, 2.9 : 1 for vardenafil, and 780 : 1 for tadalafil. PDE6 is localized to photoreceptor cells in the rods and cones, and is activated following phototransduction by binding of the G-protein visual pigment transducin to PDE6. The difference in the selectivity ratio of tadalafil compared with sildenafil or vardenafil may account for the lower frequency of visual side-effects with tadalafil (<0.1%) compared with sildenafil, i.e. colour tinge to vision (chromatopsia), increased sensitivity to light or blurred vision in ≤3% of patients, or vardenafil (e.g. blurred vision, chromatopsia in <2% of patients) at therapeutic doses [40–42]. Imbalances in cGMP levels can interfere with visual signalling and culminate in photoreceptor cell death and retinal degeneration. Insufficient numbers of patients with retinitis pigmentosa have been studied to safely recommend administration of any PDE5Is to patients with this condition [40–42].

Sildenafil and vardenafil are one to two orders of magnitude more selective for PDE5 than PDE11 compared with tadalafil. Tadalafil has been shown to inhibit human recombinant PDE11A1 activity at therapeutic concentrations [41]. However, the function of PDE11, as well as the roles of other PDEs (PDEs 7–10), have not been completely elucidated. PDE7 has been localized in skeletal muscle and T lymphocytes; PDE8 in the small and large intestines, testis and ovary; PDE9 in the brain, intestine and spleen; PDE10 in the brain, testis and thyroid gland; and PDE11/PDE11A in the prostate, pituitary and salivary glands, as well as the kidney, liver, testis and skeletal muscle. Although PDE11 is expressed in the testis and pituitary gland as well as in smooth muscle [43], daily tadalafil treatment did not alter spermato genesis or reproductive-hormone secretion in healthy men with mild or no ED [44].

CLINICAL DATA

PHARMACOKINETICS

All three PDE5Is are rapidly absorbed after oral administration; the absolute bioavailability is 40% for sildenafil and 15% for vardenafil. The median time to maximum concentration (t_{max}) is 1 h for sildenafil and vardenafil, and 2 h for tadalafil. Median t_{max} values as low as ≤40 min have been reported with vardenafil at the 20 mg dose and a supra-therapeutic dose of 40 mg [45,46].

High-fat meal intake influences the absorption profiles of both sildenafil and vardenafil. Sildenafil’s maximum plasma concentration (C_{max}) is reduced by 29% and t_{max} delayed by 1 h when the agent is administered with a high-fat meal. When vardenafil was administered with a high-fat breakfast including 910 calories, with 57% of total calories derived from fat (58 g), the C_{max}}
was reduced by ∼18% compared with the value after an overnight fast [47]. In addition, the \( t_{\text{max}} \) was delayed from 1 to 2 h, whereas the extent of absorption, as indicated by area under the concentration : time curve (AUC) was essentially unaltered. On the other hand, meal intake has no effect on the absorption of tadalafil.

The three PDE5Is are distributed widely throughout tissues and extensively (≥ 94%) protein bound. Volumes of distributions (\( \text{V}_d \)) are 105 L for...
Both sildenafil and vardenafil have terminal half-life \( t_{1/2} \) values of 4–5 h [40,45,46]. Tadalafil has a \( t_{1/2} \) of 17.5 h, which is consistent with a broad window of clinical responsiveness [48,49]. Excretion of all three PDE5Is is principally as fecal metabolites [40–42].

In men aged ≥65 years, the AUC (i.e. systemic exposure) of sildenafil increases by 40% [40]. The AUC and \( C_{\text{max}} \) of vardenafil are increased by 52% and 34%, respectively, in elderly men [42]. Hence, lower starting doses are recommended for older men, i.e. 25 mg for sildenafil dosing [50].

**TABLE 2**

Relative selectivity profiles of PDE5Is

<table>
<thead>
<tr>
<th>Selectivity ratio vs PDE5</th>
<th>Sildenafil</th>
<th>Vardenafil</th>
<th>Tadalafil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PDE5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDE5a</td>
<td>290</td>
<td>630</td>
<td>20 000</td>
</tr>
<tr>
<td>PDE5b</td>
<td>1 100</td>
<td>5 000</td>
<td>21 000</td>
</tr>
<tr>
<td>PDE5c</td>
<td>110</td>
<td>460</td>
<td>11 000</td>
</tr>
<tr>
<td>PDE5d</td>
<td>19 000</td>
<td>7 200</td>
<td>49 000</td>
</tr>
<tr>
<td><strong>PDE3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDE3a</td>
<td>12 000</td>
<td>7 700</td>
<td>38 000</td>
</tr>
<tr>
<td>PDE3b</td>
<td>17 000</td>
<td>15 000</td>
<td>18 000</td>
</tr>
<tr>
<td>PDE4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PDE4a</td>
<td>6 000</td>
<td>46 000</td>
<td>30 000</td>
</tr>
<tr>
<td>PDE4b</td>
<td>5 800</td>
<td>33 000</td>
<td>22 000</td>
</tr>
<tr>
<td>PDE4c</td>
<td>5 200</td>
<td>34 000</td>
<td>23 000</td>
</tr>
<tr>
<td>PDE4d</td>
<td>3 600</td>
<td>16 000</td>
<td>13 000</td>
</tr>
<tr>
<td>PDE5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PDE6</td>
<td>6.8</td>
<td>2.9</td>
<td>780</td>
</tr>
<tr>
<td>PDE7a</td>
<td>22 000</td>
<td>200 000</td>
<td>47 000</td>
</tr>
<tr>
<td>PDE7b</td>
<td>19 000</td>
<td>310 000</td>
<td>30 000</td>
</tr>
<tr>
<td>PDE9a</td>
<td>540</td>
<td>3 600</td>
<td>19 000</td>
</tr>
<tr>
<td>PDE10a</td>
<td>3 100</td>
<td>12 000</td>
<td>9 000</td>
</tr>
<tr>
<td>PDE11a</td>
<td>1 500</td>
<td>640</td>
<td>14</td>
</tr>
</tbody>
</table>

Selectivity ratios are based on IC50 values for each enzyme relative to the IC50 for PDE5. IC50 denotes the concentration of substrate (PDE5I) needed to inhibit half the enzymatic activity; lower values denote more potent enzymatic inhibition, e.g., the PDE5 : PDE2 selectivity ratio of 19 000 for sildenafil indicates that the concentration of sildenafil needed to inhibit half the PDE2 enzymatic activity is 19 000 times higher than the concentration needed to inhibit half that of PDE5. From [39].
with alcohol alone. Only at a higher alcohol dose (0.7 g/kg) equivalent to 180 mL of vodka (40% alcohol) did tadalafil significantly lower blood pressure and/or induce postural effects [41]. None of the PDE5I USA package labels recommends co-administration with alcohol.

Potent inhibitors of cytochrome P3A4, including HIV protease inhibitors (e.g. indinavir, ritonavir), azole antifungals, and erythromycin, can increase systemic exposure (AUC) of the PDE5Is by 2- to 16-fold. For example, the protease inhibitor ritonavir increases the systemic exposure (AUC) of sildenafil 11-fold, and caution should be exercised when prescribing the two medications concurrently [40]. Vardenafil therapy should not exceed a single 2.5 mg dose per 24 h in patients taking indinavir, ketoconazole 400 mg, or itraconazole 400 mg, or per 72 h in those taking ritonavir [42]. Tadalafil treatment should not exceed a single 10 mg dose, and should not be taken more than once per 72 h, in patients taking potent cytochrome P3A4 inhibitors [41].

Cytochrome P3A4 inducers such as rifampin can reduce circulating PDE5I levels.

Grapefruit juice inhibits first-pass cytochrome P450 metabolism in the gastrointestinal tract and may thus increase oral bioavailability of the PDE5Is. A randomized crossover trial [58] evaluated the effects of taking a single sildenafil 50 mg dose 1 h before, or at the same time as, 250 mL of grapefruit juice in 24 healthy male volunteers. Compared with water (reference period), grapefruit juice consumption increased the AUC values for sildenafil and N-desmethylsildenafil by 23–24% and slightly prolonged the tmax (by \(\approx 15\) min). Grapefruit juice also rendered sildenafil pharmacokinetics more variable, and the authors concluded that the combination should be avoided, especially in patients who might be prone to more marked haemodynamic effects [58].

**EFFICACY**

**BACKGROUND ON OUTCOME MEASURES**

The PDE5Is are effective across a wide range of outcomes in promoting erectile responses to sexual stimulation. However, as articulated by a recent multidisciplinary expert panel [59], the efficacy of ED treatments should not be equated with their therapeutic effectiveness when either evaluating or predicting treatment outcomes. As shown in Fig. 8 [59], the concept of therapeutic effectiveness integrates the treatment response of the patient with the treatment satisfaction of both the patient and his partner.

As the gatekeeper to consensual sexual intercourse, the partner needs to be considered when planning, assessing expectations and motivations, and evaluating the outcomes of treatment. A recent European survey [60] showed that lack of sexual interest on the part of the partner contributed to the 31% rate of patient noncompliance with effective sildenafil treatment.

Treatment response is further subdivided into efficacy and safety/tolerability; the last two need to be factored under treatment response, in part because adverse events (AEs) or concerns about long-term health may compromise adherence to the regimen and thus reduce treatment response. Efficacy outcome measures with potential utility in randomized controlled trials (RCTs) and clinical practice are further subdivided into objective and subjective measures.

Penile plethysmography using the RigiScan® device (Timm Medical, Eden Prairie, MN) enables objective evaluations of penile rigidity based on changes in circumference at different anatomical loci of the penis. The two major assessment paradigms include responses to visual sexual stimulation and nocturnal penile tumescence testing, the latter of which is physiologically sound in part because the rigidity of nocturnal erections correlates significantly with corporal SMC content [61].

On the other hand, the ability to engage in and complete intercourse is a more conservative outcome measure. These variables are typically reported by the patient and/or partner and are thus subjective endpoints. Assessments using event logs or diaries include the Sexual Encounter Profile (SEP), which helps to delineate the proportion of responders at specific drug doses. The third question of the SEP (SEP-Q3) diary is, "Did your erection last long enough for you to have successful intercourse?" The International Index of Erectile Function (IIEF) developed by Rosen et al. [62] is a validated multidimensional (15–item) questionnaire that has been translated into several languages (and cross-validated). The EF domain is a highly sensitive and reliable barometer of treatment efficacy. On the basis of an analysis of 1151 subjects, IIEF EF domain scores of \(\geq 26\) are correlated with ‘no ED’ [63].

Abridged versions of the IIEF, including the IIEF-5 (containing only five items) or Sexual Health Inventory for Men, are also available. Although of limited value to characterize efficacy, global (overall) assessments, such as the Global Assessment Question (GAQ; ‘Did the treatment improve your erections?’), play a role in many RCTs. The use of these and other overlapping efficacy measures enables highly reliable and consistent evaluations of treatment efficacy.

To capture data from the other chief domain of therapeutic effectiveness (treatment satisfaction), the Erectile Dysfunction Inventory of Treatment Satisfaction (EDITS) developed by Althof et al. [64] is useful. The EDITS enables the investigator to reliably assess both patient and partner satisfaction with therapy, and probe the effects of

**FIG. 8. Therapeutic effectiveness: the emerging conceptual framework for evaluating and predicting outcomes of therapies for ED. Based on data from [59].**

- Treatment effectiveness
  - Treatment response
    - Complete
    - Partial
    - Non-response
  - Efficacy
    - Objective (Rigiscan)
    - Subjective
    - IIEF, IIEF-5 (SHIM), SEP, GAQ
  - Safety/tolerability
  - Patient Complete
    - Partial
    - Dissatisfied
  - Partner Complete
    - Partial
    - Dissatisfied
satisfaction on treatment continuation. A score of ≥50 (on a scale of 0–100) is indicative of treatment satisfaction.

SHARED EFFECTS/OUTCOMES

Sildenafil, vardenafil and tadalafil are highly effective in enhancing ED across a wide range of outcome measures, causes of ED, patient subgroups and regional populations [65–69]. Because of differences in trial designs, comparisons among the three PDE5Is across studies are not feasible. Therefore, the clinical data below cannot be presented in an entirely parallel manner.

The effects of sildenafil, vardenafil and tadalafil have been evaluated with penile plethysmography in individual studies [45,70–73]. Sildenafil improved nocturnal penile erections in men with and without ED, and increased nocturnal penile erection frequency in healthy volunteers [71]. In men with ED, vardenafil 20 mg significantly improved penile rigidity compared with placebo [45]. Similarly, in a study involving men with ED, tadalafil significantly improved penile rigidity compared with placebo, and this effect persisted 24 h after dosing [72].

RCTs show similar efficacy and tolerability of sildenafil, vardenafil and tadalafil in men with ED of varying severity and aetiology. Sildenafil phase III trials were reported in 1998 [74]. In a dose-escalation (50–100 mg) study involving 329 men (mean age 59 years) with ED for ≥5 years (organic ED in ≥55%), the mean score for the IIEF EF domain at the end of 12 weeks of treatment was 22.1 in the sildenafil group and 12.2 in the placebo group (P < 0.001).

Scores on the orgasmic function, intercourse satisfaction, and overall satisfaction domains (but not the sexual desire domain) also significantly improved. Pivotal RCTs with each of the PDE5Is have shown no significant improvement in libido among patients receiving active treatment compared with placebo [48,74,75].

The efficacy of vardenafil was evaluated in 805 men (age 57 years) with a duration of ED of ≥2.9 years [75]. At least 54% of men in each treatment group had organic ED. The mean IIEF EF score increased (improved) from 12.8 at baseline to 21 at week 12 of vardenafil 20 mg treatment compared with an increase from 13.6 to only 15.0 in the placebo group [75]. In addition, ≥73% of patients randomized to vardenafil 10 mg and 81% of those randomized to the 20 mg dose reported that treatment improved their erections, in contrast to 39% of placebo-treated patients (P < 0.001 for each comparison vs placebo).

About 40% of patients with severe ED had an IIEF EF domain score of ≥26 after 12 weeks of treatment with vardenafil 20 mg, as did half of those with moderate ED and ≥79% of those with mild ED at baseline (Fig. 9). The vardenafil pivotal trials excluded sildenafil nonresponders [75].

Similarly, integrated phase III tadalafil studies [48] involving 1112 patients showed that the IIEF EF domain score at the end of 12 weeks of treatment (or greater) was ≥24 in men receiving tadalafil 20 mg, compared with ≥15 in men receiving placebo (P < 0.001). Patients were ≥59 years old, 61% had organic ED, and 90% had ED for >1 year. Treatment with tadalafil 20 mg also significantly enhanced the intercourse satisfaction and overall satisfaction domains of the IIEF. A total of 81% of responses to the GAQ indicated improved erections in the tadalafil 20 mg group, in contrast to 35% in the placebo group (P < 0.001) [48]. More than 70% of intercourse attempts were successfully completed with tadalafil 20 mg from ≥30 min to 36 h after dosing (Fig. 10) [48].

DIFFICULT-TO-TREAT ED

Men with DM are at greater risk of ED; in a European survey [76] involving 1460 patients with DM (34% with severe ED), DM was responsible for a 3- to 4-fold increase in the risk of ED, particularly in men with neuropathy, severe depressive symptoms, and/or current or former nicotine use. Men with both ED and DM also report significantly worse disease-specific health-related quality of life [77].

The Sildenafil Diabetes Study Group [78] showed that 56% of men with ED and DM who received sildenafil (25–100 mg) treatment for 12 weeks reported improved erections, in contrast to 10% of patients receiving placebo (P < 0.001). In that study, 61% of men randomized to sildenafil reported at least one successful attempt at sexual intercourse, compared with 22% of controls (P < 0.001). Similarly, 452 men with ED and DM treated with vardenafil for 12 weeks had significant increases in the EF domain score of 5.9 for vardenafil 10 mg and 7.8 for vardenafil 20 mg compared with 1.4 for placebo (P < 0.001 for each comparison) [79]. Based on positive responses to the GAQ after 12 weeks of treatment, 54% and 72% of patients receiving vardenafil 10 mg and 20 mg, respectively, reported that their erections were improved, in contrast to 13% of placebo

FIG. 9. The percentage of patients returning to an EF domain score (≥26) associated with ‘no ED’, stratified by baseline ED severity (intent-to-treatment population, last observation carried forward). Reproduced with permission from [75]. Copyright 2003 American Society of Andrology. All rights reserved.
controls (P < 0.001 for each comparison vs placebo) [79]. At the endpoint, 54% of intercourse attempts were successful in men receiving vardenafil 20 mg and 49% in those receiving vardenafil 10 mg, compared with 23% in placebo controls (P < 0.001 for each comparison vs placebo). Among men with severe ED, the intercourse success rate was 40% in patients receiving vardenafil 20 mg compared with 11% in placebo controls (P < 0.001).

In the study of men with ED and DM reported by Sáenz de Tejada et al. [80] tadalafil 10 mg or 20 mg significantly improved the IIEF EF domain score by 6.4 and 7.3, respectively, compared with 0.1 for placebo (P < 0.001 for each comparison). The EF domain scores were similar to those reported in a retrospective analysis of 12 RCTs involving 637 men with ED and DM [81].

Sildenafil, vardenafil and tadalafil have all been shown to be effective therapies for men with ED and DM. In addition, tadalafil was an effective treatment for ED in men with a history of microvascular complications associated with their DM; 22% of patients randomized had a history of diabetic retinopathy, laser treatment for diabetic eye disease, or a urine microalbumin : creatinine ratio >3.0 [80]. On the other hand, sildenafil and vardenafil RCTs excluded patients with proliferative diabetic neuropathy or autonomic neuropathy [78,79].

Despite the favourable data for PDE5I therapy in patients with DM, longer-term RCTs are needed. In the longitudinal Exploratory Comprehensive Evaluation of Erectile Dysfunction study [77], men in an observational disease registry showed substantial improvements in the EF and intercourse satisfaction domains of the IIEF after 6 months of treatment, followed by relapse to nearly pre-treatment levels at 12 months. There was a similar relapsing trend in the Emotional Life domain of the Psychological Impact of Erectile Dysfunction scale.

**PROSTATE CANCER**

Because intact innervation of the penis is necessary for physiological erectile responses, substantial proportions of patients with prostate cancer have ED after either nerve-sparing retropubic RP or radiation therapy. Among such patients, treatment with each of the PDE5Is results in significant improvements in EF. In an open-label study of sildenafil involving 84 men (mean age 62 years) with ED 2.1 years after RP, 53% receiving sildenafil at 50–100 mg reported improved erections and 40% reported an enhanced ability to achieve and maintain erections [82]. EF was directly related to the degree of nerve sparing, with men after bilateral nerve-sparing tending to respond better than those receiving unilateral or non-nerve-sparing RP. A lower pathological stage and greater age were also predictive of improved outcomes.

Therapy with vardenafil 10 and 20 mg for 12 weeks also significantly enhanced EF in 440 men with ED associated with either bilateral or unilateral nerve-sparing RP [83]. Patients were >60 years old and >2 years (20 months) after RP at randomization. On the basis of the responses to the GAQ, 65% of patients who completed treatment with vardenafil 20 mg reported improved erections, in contrast to 13% of placebo controls (P < 0.001). At study completion, successful penetration was reported in 48% of attempts with vardenafil 20 mg compared with 22% with placebo (P < 0.001), and successful intercourse in 34% of attempts with vardenafil 20 mg (vs 10% with placebo; P < 0.001).

A double-blind RCT involving 303 men (mean age 60 years) with ED seen 12–48 months (mean = 25 months) after bilateral nerve-sparing RP showed that treatment with tadalafil 20 mg for 12 weeks significantly enhanced EF [84]. Among all tadalafil patients, 62% reported improved erections at the completion of study compared with 23% of controls (P < 0.001). About 54% of intercourse attempts resulted in successful penetration among men randomized to tadalafil, compared with 32% in controls (P < 0.001); 41% of intercourse attempts were successfully completed among patients on tadalafil compared with 19% among controls (P < 0.001) [84].

Treatment benefits were significant and more pronounced in the about two-thirds of patients capable of penile tumescence after RP; 71% of men on tadalafil 20 mg with tumescence after RP reported improved erections at the endpoint, compared with 24% of controls (P < 0.001). In these same groups, 69% of intercourse attempts resulted in successful penetration, and 52% of attempts resulted in successful intercourse, at endpoint (P < 0.001 for each comparison vs placebo) [84].

Based on data presented at the AUA meeting in 2003, early or prophylactic
treatment of ED may promote normalization of EF after nerve-sparing RP; 67% of patients having bilateral nerve-sparing RP randomized to intracavernosal alprostadil injections (three times weekly for 6 months) recovered erections sufficient for sexual intercourse, compared with 20% in those with no such treatment. Further, treatment of patients after RP with sildenafil was associated with a significant increase in the return of spontaneous erections compared with placebo. This improvement may be attributable to neuronal regeneration secondary to enhanced nocturnal erection oxygenation, although further studies are required to test this hypothesis [85].

ONSET OF ACTION

Considerable attention has turned to the onset of treatment effects in men with ED receiving PDE5Is, even though all three medications have an onset of activity within 20 min. The time of onset of activity is 14 min with sildenafil, 10 min with vardenafil and 16 min with tadalafil, using similar methods [72,73,86]. Under the study design, patients receive four doses of a PDE5I or placebo for at-home use as needed over a 4-week interval. Patients use a stopwatch and record the earliest time to an erection judged to be sufficient for sexual intercourse, after which they record the time spent engaging in sexual activity.

In the Onset Time of Vardenafil in Men With ED trial [87], 21% of intercourse attempts using vardenafil 10 mg were successfully completed at 10 min, compared with 14% for placebo (P = 0.025). Setting aside the somewhat artificial design involving couples using stopwatches before intercourse, the meaning of these studies is open to question, especially their use to differentiate PDE5Is. For instance, in an RCT involving tadalafil treatment, 15.7% of intercourse attempts were successful within 16 min after tadalafil 20 mg, compared with 7.7% after placebo, or 16 successes with tadalafil and eight with placebo from 100 attempts [72].

However, by conservative estimates the true frequency per number of total doses is four (16/4) with tadalafil and two (8/4) with placebo, because patients qualified with a single successful attempt from four doses. Thus, the difference between tadalafil and placebo at 16 min is only two successes per 100 attempts. Similarly, based on the reported study [87], there were 21 successes from 100 attempts with vardenafil 10 mg within 10 min of dosing, compared with 14 for placebo. Dividing the numerator and denominator by 4, the difference between vardenafil and placebo at this sample time is once again about two successes per 100. Because only a few more intercourse attempts are successful within 1 h after PDE5I administration compared with placebo, it is prudent to recommend PDE5I dosing ≥ 1 h or more before attempted intercourse.

EFFICACY OF PDE5I: POSSIBLE WITHIN-CLASS DIFFERENCES

Sildenafil has confirmed efficacy across a wide spectrum of causes of ED (particularly organic) and patient nationality (Fig. 11). As reviewed by Sadovsky et al. [65], sildenafil is effective in patients with: cardiovascular disease; prostate cancer (including men receiving radiation or androgen deprivation therapy); spinal-cord injury; end-stage renal failure; major depressive disorder and iatrogenic (selective serotonin reuptake inhibitor-induced) ED; multiple sclerosis; Parkinson’s disease; and spina bifida. Treatment with tadalafil or vardenafil is also effective in men with ED with a broad spectrum of causes and patients from diverse cultures [48,67–69,88].

Patient and partner satisfaction, two critical components of therapeutic effectiveness, have also been well documented with sildenafil. In a recent meta-analysis of 14 RCTs, Montorsi and Althof [89] reported that 74% of female partners of men aged <65 years who were receiving sildenafil were satisfied with treatment (i.e. EDITS score 50) in contrast to 25% of partners of men receiving placebo (P < 0.001). Similar findings were reported among partners of elderly men. Correlations between patient and partner EDITS scores were significant (P < 0.001) in both placebo (r = 0.80) and sildenafil treatment (r = 0.86) groups.

In a review of sildenafil data [90], Seftel reported that some patients (14–47%) have suboptimal acceptance or lack of long-term adherence to therapy with sildenafil. These findings reflect the fact that drug adherence is a complex matter, with potential psychosocial and physiological underpinnings. From the psychosocial perspective, restoration of EF can reveal other difficulties in a relationship, acting as a ‘therapeutic probe’ for other sources of discord.

From the physiological standpoint, genetic polymorphisms may influence the response to sildenafil therapy [91,92]. Recent work by a German group [91] suggested that therapeutic responses to this PDE5I were affected by polymorphisms in genes for angiotensin-converting enzyme and NOS. Lin [35] also suggested that increased cGMP levels from PDE5I treatment may increase the expression of PDE5. As this feedback loop ensues, increasing dosages of PDE5 inhibitors may be required to produce the desired therapeutic effect over the long-term. However, clinical experience does not support this concept because studies show that PDE5I efficacy is maintained during long-term therapy [88,93,94].

For men with low testosterone levels who did not respond to sildenafil, adjunctive testosterone ‘rescue’ therapy (testosterone gel) has been used successfully. In a RCT, 75 hypogonadal men with ED and testosterone levels of <4 μg/L who had not responded to sildenafil therapy alone received placebo or a 1% testosterone gel 5 mg daily together with sildenafil 100 mg [95]. After 12 weeks, men receiving testosterone and sildenafil 100 mg had superior increases (improvements) in the EF domain of the IIEF (4.4 units) compared with sildenafil-placebo (2.1 units; P = 0.029). Adjunctive testosterone-sildenafil regimens also significantly improved overall satisfaction and orgasmic satisfaction domains, as well as other efficacy outcome measures, compared with sildenafil alone [95].

Sexual stimulation is vital because PDE5Is potentiate NO-mediated vasorelaxation only in the presence of adequate sexual arousal. Patients should also be counselled to use a PDE5 inhibitor several times before declaring it ‘ineffective’; for instance, the cumulative probability of success with sildenafil increases up to 9–10 attempts, after which it stabilizes [96].

The long-term efficacy and tolerability of vardenafil have been reported in recent clinical trials ranging from 26 weeks in a USA multicentre study [97], to 2 years in a European multicentre trial [88]. In the latter, 566 men age ≥18 years (mean = 55; range 22–89) who had ED for ≤6 months in a stable heterosexual relationship were eligible, provided that they had no history...
of treatment with vardenafil or either poor responsiveness or intolerance to sildenafil therapy [88]. The mean IIEF EF domain score in patients randomized to vardenafil 20 mg increased from 13.8 at baseline, which is consistent with moderate ED, to 25.7 at up to 2 years. The latter score approaches the threshold of 26, an EF domain score defined as ‘no ED’ [63].

Treatment with vardenafil 20 mg more than doubled the proportion of sexual intercourse attempts resulting in vaginal penetration (SEP-Q2) or successful intercourse (SEP-Q3), to 90–94% after 2 years of treatment (Fig. 12) [88]. Interestingly, 65% of patients’ sexual partners reported that erections were maintained after penetration when the patient took vardenafil 20 mg, and 53% reported that intercourse was either always or almost always satisfactory. On the other hand, patients reported no substantial changes in either the ‘partnership relation’ or ‘general satisfaction’ items of the checklist [88]. This finding underscores the fact that the effects of ED treatments on erectile responses do not necessarily confer benefits to patient-partner relationships.

Tadalafil therapy has a broader window of clinical responsiveness than either sildenafil or vardenafil because of its longer half-life (17.5 vs 4–5 h for sildenafil or vardenafil) [40–42]. Tadalafil enhances EF in men with ED for up to 36 h. Thus, tadalafil may be associated with less planning or pressure to have sexual intercourse after dosing. Dissociation of the sexual encounter from the time of taking the medication may be associated with greater patient and/or partner convenience.

Active comparator (crossover) trials evaluating preferences for one PDE5I over another among patients with ED receiving sildenafil or tadalafil have been conducted. In prospective randomized crossover studies involving ~600 men with ED, patients preferred tadalafil over sildenafil by statistically significant margins of 7 : 3 to 9 : 1, including two European populations [98–100]. These ratios were stable and statistically significant irrespective of comorbidity or previous sildenafil use (Fig. 13) [98].

Despite patient preference for tadalafil over sildenafil in these prospective clinical trials, comparing the efficacy of either vardenafil or tadalafil against sildenafil cannot readily control for the relative novelty of the newer agents. Moreover, it is intrinsically difficult to compare the treatment effects of tadalafil, which is designed for use at a dose of either 10 or 20 mg, with those of sildenafil, whose three dose levels are more conducive to titration.

**TOLERABILITY AND SAFETY**

Most AEs associated with PDE5I therapy can be ascribed to inhibition of PDE5 in nonpenile tissues. Untoward events observed with sildenafil, vardenafil and tadalafil include headache, dyspepsia, flushing, myalgia/back pain and rhinitis. In RCTs, flushing was more common in patients receiving sildenafil or vardenafil, and back pain/myalgia was more common in those receiving tadalafil. However, these events were typically mild and transient, abated with time, and prompted treatment discontinuation in small proportions (≤3%) of patients [48,69,74,97].

Based on USA prescribing information for sildenafil [40], AEs occurring in ≥3% of patients comprise headache in 16% of patients taking sildenafil compared with 4% of men receiving placebo, flushing (10% vs 1%), dyspepsia (7% vs 2%), and nasal

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**FIG. 11. Efficacy of sildenafil treatment in patients participating in double-blind placebo-controlled trials.**

Panel A: percentage of patients responding yes to the question, ‘Did treatment improve your erections?’ Panel B: patient-reported percentage of attempts at sexual intercourse that were successful during the last 4 weeks of treatment. *P < 0.001; †P < 0.0001. Reproduced with permission from [65]. Copyright 2001. International Journal of Clinical Practice. All rights reserved.
congestion (4% vs 2%). Discontinuations due to AEs were infrequent, occurring in 2.5% of patients receiving vardenafil, compared with 2.3% of patients receiving placebo [40].

Based on USA prescribing information for vardenafil [42], AEs reported by ≥3% of patients comprised of headache in 15% compared with 4% of men receiving placebo, flushing (11% vs 1%), rhinitis (9% vs 3%) and dyspepsia (4% vs 1%).

According to USA prescribing information for tadalafil [41], AEs occurring in ≥3% of patients comprised headache in 11–19% of patients taking tadalafil at as-needed doses of 5, 10 or 20 mg, compared with 5% of men receiving placebo; dyspepsia (4–10% vs 1%); back pain (3–6% vs 3%); and myalgia (1–4% vs 1%).

Long-term studies of sildenafil [101], vardenafil [88], and tadalafil [94] found similar AE profiles compared with those described above. Because tadalafil and vardenafil have been available for a shorter time than sildenafil, these agents may not have been on the market long enough to discern differences (vs sildenafil) in their safety profiles, particularly for infrequent AEs.

In the long-term study by Stief et al. [88], the incidence of treatment-emergent AEs was highest within the first few weeks of vardenafil treatment, then rapidly declined with further use up to 2 years.

Similar findings were recorded in an analysis of 4348 patients (age 55 years) with mostly moderate-severe ED in 17 double-blind flexible-dose (25–100 mg) sildenafil RCTs ranging from 8 to 26 weeks in duration. Treatment-related AEs, including headache, dizziness, dyspepsia, rhinitis and abnormal vision, peaked at ~15% during the first 2 weeks of treatment and declined to ~4% at treatment weeks 6–8. The AE rate × time plots showed a bimodal distribution, with a second peak (AE rate ~8%) at treatment weeks 8–10. Maximum AE rates with sildenafil were 1.2% for abnormal vision and 1.8% for dyspepsia over treatment weeks 0–2, 0.8–1.0% at weeks 8–10, and <1% at weeks 12–14. In all, 1.4% of patients discontinued sildenafil or placebo because of AEs [102].

SAFETY

Although clinical trials of sildenafil, vardenafil, and tadalafil included many patients with cardiovascular disease and DM, they excluded patients with unstable cardiovascular disease [40–42]. Accordingly, PDE5Is are either not recommended or are to be used with caution in men with unstable angina, recent myocardial infarction (MI), cardiac failure, life-threatening/uncontrolled arrhythmia, poorly controlled blood pressure (resting blood pressure <90/50 mmHg or >170/110–110 mmHg) and/or heart failure [40–42]. In addition, patients with left-ventricular outflow obstruction secondary to aortic stenosis or idiopathic hypertrophic subaortic stenosis, as well as men with severe autonomic insufficiency, may be especially sensitive to the vasodilator effects of PDE5Is.

Consensus guidelines have been issued for risk-stratifying and counselling patients with concomitant sexual dysfunction and cardiovascular disease [103]. Men with ED,
particularly those with concomitant cardiovascular disorders, need to be counselled that there is a transient increase in the relative risk of cardiovascular events during and within \( \approx 2 \) h after sexual intercourse. However, the absolute risk of having a MI within each hour after sexual activity is considered to be low in otherwise healthy men with ED. Patients experiencing cardiac symptoms when initiating sexual activity should be counselled to seek medical attention immediately and abstain from further sexual activity.

Exercise echocardiographic studies show that treatment with sildenafil 50–100 mg, vardenafil 10 mg or tadalafil 10 mg did not adversely affect haemodynamic variables, exercise capacity (treadmill time), and/or time to cardiac ischaemia (or first awareness of angina) in patients with stable coronary artery disease [41,104,105].

In sildenafil and tadalafil clinical trials reported to date, the incidence rates of MI were low and similar to those with placebo, i.e. 0.53/100 patient-years for patients taking sildenafil in open-label studies and 0.80/100 patient-years for those receiving sildenafil in RCTs \( (P = 0.88 \text{ vs } 0.84/100 \text{ patient-years with placebo}) \) [106]. The overall incidence rate of MI across 43 clinical trials in tadalafil-treated patients was 0.33/100 patient-years: 0.36/100 patient-years in open-label studies and 0.26/100 patient-years in RCTs \( (P = 0.41/100 \text{ patient-years for placebo controls}) \) [107]. The slight disparities between MI incidence rates across sildenafil and tadalafil trials may reflect differences in age or other patient characteristics at baseline.

There is a first-dose haemodynamic effect in patients taking vardenafil 10 mg; in a randomized crossover multiple-dose comparison, 35 men with ED also received three doses of sildenafil 50 mg weekly [108]. Changes in blood pressure and heart rate after the first dose of vardenafil were greater than after the first dose of sildenafil. Heart rate increased by 3.1 beats/min, while systolic blood pressure decreased by 8.02 mmHg and diastolic blood pressure by 6.6 mmHg. Syncpe was reported in three (8.6%) patients after the first dose of vardenafil [108]. However, two of these three patients received concomitant therapy with doxazosin for BPH. In the USA, vardenafil treatment is contraindicated in patients treated with α-adrenergic blockers [42].

Even at supra-therapeutic doses consistent with concomitant treatment using cytochrome P450 inhibitors or the presence of renal impairment, none of the PDE5Is increases the QTc interval in a clinically significant manner [41,109,110]. Neither sildenafil 50–400 mg nor vardenafil 10–80 mg increased the absolute QT interval, and each agent modestly prolonged the QTcF (Fridérica-corrected) interval \( 1 \) h after dosing in healthy men aged 45–60 years \( (\text{mean } 53) \) [109]. At 1 h after a 50-mg dose of sildenafil, the uncorrected QT interval changed by a mean of \(-2\) ms, the linear QT interval (QTci) by 4 ms, and the QTcF interval by 6 ms compared with placebo; corresponding values for a supra-therapeutic 400 mg dose were \(-1, 6\) and 9 ms. At 1 h after a 10-mg dose of vardenafil, the uncorrected QT interval changed by \(-2\) ms, the QTci interval by 4 ms and the QTcF interval by 8 ms compared with placebo; corresponding values for a supra-therapy 80 mg dose were \(-2, 6\) and 10 ms [109]. In a separate study of tadalafil at a single oral dose of 100 mg, the mean change in QTcF for tadalafil relative to placebo was 3.5 ms, and the mean change in the individually corrected QT interval was 2.8 ms relative to placebo. In addition, torsade de pointes has not been reported in studies of sildenafil, vardenafil or tadalafil [41,109,110].

The systemic exposures of PDE5Is may be increased in patients with renal insufficiency and/or hepatic impairment. Starting or other doses of PDE5Is may need to be limited to sildenafil 25 mg, vardenafil 5 mg or tadalafil 5–10 mg in certain patients with these conditions [40–42].

**DIAGNOSIS AND TREATMENT OPTIONS FOR MEN WITH ED**

The first step in evaluating and treating ED is to conduct a thorough history and focused physical examination (Fig. 14A) [111]; the latter should be used in every patient with ED, and should include an assessment of body habitus (secondary sexual characteristics) and an evaluation of the cardiovascular, neurological and genitourinary systems, focusing on penile, testicular and rectal examinations. Blood pressure and heart rate should be measured if not assessed in the previous 3–6 months. In conducting the physical examination, special attention should be given to signs of penile abnormality (e.g. Peyronie's disease); prostatic enlargement or other abnormalities; and signs or symptoms indicative of hypogonadism. In such cases, further diagnostic evaluation should be undertaken.

Whether to screen all patients with ED for hypogonadism, as well as the threshold testosterone level below which exogenous testosterone should be administered, are complex and controversial matters. Certain investigators contend that circulating testosterone should be determined in all men aged > 50 years, but only in the presence of low libido or abnormal physical examinations (e.g. atrophic testes <19 mL in younger men. The presence of low testosterone (<300 ng/dL) may warrant further endocrine assessments, including prolactin and LH levels, and other testing.

Investigators have voiced concerns that long-term testosterone replacement may unmask occult neoplasms, particularly prostate carcinomas. Cancer of the prostate or breast contraindicates testosterone replacement therapy, and both baseline and on-treatment monitoring of haemoglobin, haematocrit, and prostate changes by a DRE, PSA assay, voiding changes and/or prostate biopsy, are recommended.

The clinician should attempt to involve both the patient and his partner (if available) in treatment planning. When considering the range of treatment options [Fig. 14B] [111], it is important to assess the couple's treatment goals, expectations, and preferences.

Focus-group research shows that patients opt for different ED treatments based on a wide range of lifestyle considerations that are not strictly medical. These include the relative costs, reversibility, discretion, simplicity of the regimen, long-term risk and partner acceptability of treatment. The assessment and treatment planning phases offer opportunities for patient and partner counselling, including the necessity for adequate sexual stimulation to promote PDE5I efficacy. Counselling should be tailored to the individual cultural, religious and lifestyle patterns and needs of each couple.

Lifestyle modification may be advised for patients who smoke, abuse alcohol, or lead sedentary lifestyles. However, in large-scale longitudinal epidemiological studies involving men in middle age at baseline, only increased
physical activity has been associated with the reversal of ED. In a recent clinical study, weight loss improved EF in about a third of obese patients [112]. On multivariate analysis, increased physical activity and reduced body mass index were significant independent predictors of increases (improvements) in the IIEF EF domain.

Patients or partners who are dissatisfied with the outcome of PDE5I treatment, or in whom such therapy is contraindicated, are typically referred to a urologist. Such specialists may recommend the use of a vacuum erection or constriction device, or more invasive medical therapies such as cavernosal injections or transurethral suppositories of vasoactive agents (e.g. PGE1, alprostadil). For the small minority of patients with ED that is refractory to the foregoing treatments, penile vascular surgery or implantation of a penile prosthesis is a potential option.

FUTURE PROSPECTS

Other PDE5Is with potentially enhanced pharmacological properties are under clinical and experimental investigation for treating ED. Successful introduction and commercialization of these agents may assist in further bridging the gap in treating ED. Enhanced therapeutic options will probably bring more patients to their physician’s offices, while enhanced supportive care will probably remain important to facilitate couples’ transitions from avoidance and/or abstinence to greater intimacy [113].

Investigational PDE5Is for ED include Pfizer’s UK-343664, which is more selective for PDE5 over PDE6 compared with sildenafil, and UK-357903, which has been well tolerated in human studies [114]; Bristol-Myers Squibb’s BMS-341400; FR229934, which has been in-licensed from Fujisawa Pharmaceutical by TAP Pharmaceuticals (Abbott Laboratories and Takeda Chemical Industries Ltd); and E-8010, which is under late-stage clinical development by Eisai.

Oral and transurethral TA-1790, an experimental PDE5I, is also under investigation by Vivus. Oral TA-1790, which is in phase II development, has high PDE5 : PDE6 selectivity and less pronounced declines in systemic blood pressure (vs sildenafil) when co-administered with organic nitrates in preclinical studies. Vivus also manufactures the Medicated Urethral System for Erection (MUSE®) for transurethral
delivery of PGE1, as well as the over-the-counter Actis® adjustable constriction loop for patients with ED secondary to venous leak.

As with the clinical management of other chronic conditions, including DM2, coronary artery disease and hypertension, the future of ED therapies will probably involve greater use of combined regimens addressing more than one pathophysiological process. For instance, preclinical work showed that concomitant use of the PDE5I zaprinast together with the Rho-kinase inhibitor Y-27632 synergistically enhanced erectile responses of spontaneously hypertensive rats, with increased intracorporal pressures in response to electrical field stimulation [15]. Also promising are potentially 'curative' treatments, such as gene or growth factor therapies, including vascular endothelial growth factor for vasculogenic ED, brain-derived neurotrophic factor for neurogenic ED, as well as eNOS, calcitonin-gene related peptide, and intracavernosal potassium-channel openers for general ED.

CONCLUSIONS

The assessment and treatment of ED confer unique clinical opportunities to enhance health and well-being. Because a dysregulated NO-cyclic nucleotide signalling pathway is a pivotal pathophysiological factor in many forms of ED, treatments that up-regulate this pathway represent promising pharmacological avenues. Of all therapies, none has met with greater clinical success, or transformed the treatment landscape in more fundamental ways, than the PDE5I sildenafil. However, patient acceptance of and adherence to even the most effective and well-tolerated therapies for ED are largely influenced by other than medical (lifestyle) factors. The recent advent of the highly effective and well-tolerated PDE5Is vardenafil and tadalafil, and potential agents now in clinical development, may assist clinicians in tailoring treatment regimens to the unique needs of each couple.

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Abbreviations: ED, erectile dysfunction; EF, erectile function; DM(2), diabetes mellitus (type 2); RP, radical prostatectomy; PDE(n)(I), phosphodiesterase (type n) (inhibitor); (e)(n)(I)NO(S), (endothelial) (neuronal) (inducible) nitric oxide (synthase); SMC, smooth muscle cell; sGC, soluble guanylate cyclase; MLC, myosin light chain; MPOA, medial preoptic area; PG, prostaglandin; AUC, area under the concentration : time curve; AEs, adverse events; RCT, randomized controlled trial; II(EF), International Index of (Erectile Function); GAQ, Global Assessment Question; SEP, Sexual Encounter Profile; EDITS, Erectile Dysfunction Inventory of Treatment Satisfaction; MI, myocardial infarction.
Carbonic anhydrase IX and the future of molecular markers in renal cell carcinoma

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kidney cancer, molecular markers, carbonic anhydrase IX, staging, targeted therapy

INTRODUCTION
Cancer of the kidney and renal pelvis is estimated to account for 36 160 new cases and 12 660 deaths in the USA in 2005 [1]. RCC is a highly aggressive tumour; a third of patients will have evidence of metastasis at the time of diagnosis [2] and >40% of patients with RCC will die from their disease [3]. Advances in imaging, staging and treatment have resulted in improved survival for a selected group of patients and an overall change in the natural history of RCC [4]. Future advances will require a deeper understanding of the genetic and protein-expression profile of each histological type of RCC. A molecular profile of each tumour will probably improve treatment and guide patient selection for targeted therapies. Carbonic anhydrase IX (CA IX) is the most significant molecular marker for RCC to date. CA IX is located downstream of the von Hippel-Lindau (VHL) tumour-suppressor gene and is activated by the hypoxia-inducible pathway [5]. The highly specific expression pattern of CA IX has affected the diagnosis, staging and treatment selection for patients with RCC. We present a comprehensive review of CA IX and discuss the promise that molecular markers hold to revolutionise the diagnosis, staging, treatment, surveillance and survival of patients with RCC.

THE DEVELOPMENT OF STAGING SYSTEMS: FROM MACRO TO MICRO TO MOLECULAR
RCC staging systems have developed in parallel with the rapid increase in understanding of the tumour biology of kidney cancer. The first formal staging systems, proposed by Flocks and Kadesky [6] and later modified by Robson [7], used the anatomical information available to clinicians at the time. Numerous refinements have led to the current TNM system proposed by the Union Internationale Contre le Cancer [8]. Integrated staging systems were then developed from numerous non-anatomical variables that were shown to be prognostic indicators for RCC. For example, the University of California Los Angeles (UCLA) Integrated Staging System (UISS) supplements the anatomical TNM staging with the Eastern Cooperative Oncology Group (ECOG) performance status and the Fuhrman grade [9] of the tumour [10]. The UISS is a powerful tool for assessing patients with localized and metastatic RCC [11]. Inclusion of important genetic and protein molecular markers, such as CA IX,
represents the next advance in RCC staging systems [12].

CA IX

The CA family comprises enzymes that catalyse the reversible conversion of carbon dioxide and water to carbonic acid. CA IX is a transmembrane enzyme that is thought to play a role in the adaptation of tumours to hypoxic conditions, by regulating the pH of the intracellular and extracellular compartment [13]. CA IX expression by normal tissues is limited to the gastrointestinal tract, gallbladder and pancreatic ducts [14]. There is over-expression of CA IX in tumours of the kidney, cervix, breast, lung, oesophagus, stomach, biliary tree, colon, bladder and skin [13,14]. Furthermore, it is also over-expressed in RCC specimens [15]. CA IX is not expressed by normal fetal or adult kidney specimens, suggesting that it has no role in organogenesis, but rather is a product of tumour biology [16]. CA IX expression varies among different kidney tumours. There is positive immunohistochemical staining in clear cell, granular, spindle and papillary RCC, but not in chromophobe RCC and oncocytomas [17]. The expression of CA IX is regulated by hypoxia [18] through hypoxia-inducible factor 1-α (HIF-1α) [19]. Loss of function of the VHL tumour suppressor gene, either by mutation or hypermethylation, can also lead to HIF-1α accumulation [20], and up-regulation of CA IX expression (Fig. 1). Restoring VHL function in RCC cell lines down-regulates CA IX expression to normal levels [21]. The clear cell subtype of RCC is genetically linked with the loss of VHL function and is commonly associated with a hypoxic tumour microenvironment. CA IX expression serves as a strong biomarker for kidney cancer and is particularly important for clear cell RCC.

CA IX AND RCC STAGING

The first report of CA IX immunohistochemistry in RCC found positive expression in 46 of 47 primary and seven of eight metastatic lesions [22]. A study at UCLA confirmed the high specificity of CA IX staining, with 94% of clear cell RCC specimens staining positively; CA IX expression was uniformly negative in normal

FIG. 1. The ras/raf and AKT pathways are activated through tyrosine kinase receptors. These pathways up-regulate the expression of HIF-1α. In hypoxia, or through loss of VHL function, HIF-1α can translocate into the nucleus and act as a master transcription regulator, leading to the production of CA IX.
kidney tissue [23]. Recursive partitioning survival-tree analysis [24] defined a threshold of 85% expression as the optimum to differentiate disease-free survival (Fig. 2). Patients with metastatic RCC and <85% CA IX expression had a significantly worse disease-free survival (hazard ratio 3.1, 95% CI 1.99–4.83) in a multivariate analysis controlling for tumour T stage, Fuhrman grade, nodal involvement and performance status. Low CA IX expression was also associated with a worse prognosis for patients with clinically localized high-risk tumours [23].

**MULTIPLE-MARKER STAGING SYSTEMS INCORPORATING CA IX**

High-throughput tissue-array analysis has ultimately allowed for the simultaneous study of multiple molecular markers in hundreds of tissue specimens. A natural extension of this technology is the creation of molecular staging systems that integrate multiple markers with traditional prognostic variables. CA IX expression has been combined with Ki67, a marker of cellular proliferation, to further stratify patients into low- (high CA IX/low Ki67), intermediate- (high Ki67 or low CA IX) and high-risk (high Ki67/low CA IX) groups [25]. A multimarker model was created, incorporating a host of markers associated with the development of malignancies. Immunohistochemical analysis of Ki-67, p53, gelsolin, CA IX, CA XII, PTEN, EpCAM [26], and vimentin was performed on a custom tissue microarray using clear cell RCC specimens from 318 patients [12]. Increased staining for Ki-67, p53, vimentin and gelsolin correlated with worse survival, while the inverse was true for CA IX, PTEN, CA XII and EpCAM. In multivariate analysis, CA IX, vimentin and p53 were statistically significant predictors of survival, independent of the tumour T stage, the presence of metastasis, ECOG performance status and Fuhrman grade. A prognostic model was then constructed using a combination of clinical variables and marker data. This nomogram was calibrated, using bootstrap bias-corrected estimates, to be accurate to within 10% of the actual 2- and 4-year survival rates. This integrated molecular model provides more accurate prognostic information than standard clinical predictors such as TNM stage, histological grade and ECOG performance status. The ability to predict survival based entirely on protein expression and to improve traditional staging systems with protein expression information are dramatic examples of the potential of molecular marker technology and its important future role in RCC staging systems.

**CA IX AND RESPONSE TO INTERLEUKIN-2**

CA IX expression also predicts the response to interleukin-2-based immunotherapy. In a cohort of patients from UCLA, all patients with a complete response to interleukin-2-based regimens had high CA IX expression (>85%) [23]. Using an identical threshold of 85%, Atkins et al. [27] showed that tumour specimens with high CA IX expression were significantly more likely in patients who had a complete or partial response to high-dose interleukin-2-based therapy (odds ratio 3.3). This higher response rate was associated with a significant survival benefit and survival for >5 years was limited to patients with high CA IX-expressing tumours. Importantly, the association between CA IX expression and patient response to interleukin-2-based therapy is maintained across the various histological subtypes of RCC, e.g. papillary and chromophobe subtypes respond poorly to interleukin-2 and have little to no CA IX expression. Response to interleukin-2-based therapy is modest and treatment with interleukin-2 is associated with significant morbidity [28]. The ability to select a patient group with higher response rates to interleukin-2 will decrease unnecessary exposure of patients to treatment toxicity. This approach will also maximize the benefit of interleukin-2-based immunotherapy, increasing response rates among patients selected for treatment. Molecular markers will play a significant role in selecting patients for interleukin-2-based immunotherapy and for the numerous emerging targeted therapies.

**CA IX AS A POTENTIAL THERAPEUTIC TARGET**

The highly specific expression of CA IX by RCC makes it an attractive candidate for vaccine development. Being important in RCC tumour biology, CA IX provides a rationale, beyond functioning merely as a tumour-associated antigen (TAA), for its use as a target for immunotherapy [5]. CA IX-derived CD8+ and CD4+ T cell epitopes have been reported, which are immunogenic and can induce CA IX–specific T cells in vitro [29,30]. CA IX-transduced peripheral blood monocytes have generated cytotoxic T cell lymphocytes capable of lysing CA IX expressing cancer cells [31]. To enhance immunogenicity, a granulocyte macrophage colony-stimulating factor (GM-CSF)-CA IX...
fusion molecule has been created [31–33]. This dual-delivery strategy provides the TAA with an immunomodulating agent for antigen-presenting cells. The GM-CSF-CA IX molecule was delivered using plasmid or adenovirus vectors. Interestingly, CA IX was the only TAA common to all the tumour lysates used to generate antitumour response using this fusion-protein strategy [32]. Other targeted strategies involve direct administration of anti-CA IX antibodies. The first CA IX antibody (G250) was generated from the immunisation of mice with human RCC homogenates [22], WX-G250, or Rencarex® (Wilex, Germany), is a humanized chimeric version of this antibody. Early studies showed WX-G250 treatment to be well tolerated in 36 patients, resulting in one complete response, one partial regression and 11 patients with stable disease [34]. A phase III clinical trial, the Adjuvant Rencarex (WX-G250) Immunotherapy Phase III trial to Study Efficacy in nonmetastatic RCC (ARISER), is currently enrolling patients investigating this approach in an adjuvant setting.

CA IX TARGETED IMAGING AND RADIOIMMUNOTHERAPY

RCC lesions are also being targeted by coupling radioisotopes to antibodies directed against CA IX (131I-mG250) [35]. Early phase I/II clinical trials confirmed the accurate imaging of lesions of >2 cm. There were several minor tumour responses, with an apparent improvement in survival compared with historical trials [36]. These trials were limited by the development of human antimouse antibodies targeting the murine G250 antibody. A humanized chimeric version of the antibody (cgG250) was created to avoid this immune response and to increase the maximum tolerated dose of the antibody [37]. 131I-labelled cgG250 also has excellent imaging characteristics with no evidence of host response, and a phase I/II clinical trial is currently enrolling patients [38]. The techniques used to harness the selective expression of CA IX for imaging and the delivery of therapeutic agents could also be applied to any emerging molecular marker.

FUTURE DIRECTIONS IN MOLECULAR MARKERS

The hypoxia-inducible pathway contains many potential targets for developing drug therapy, including markers of angiogenesis, the AKT/mTOR pathway and NFκB [5]. Agents targeting angiogenesis pathways, vascular endothelial growth factor (VEGF)-A and the family of tyrosine kinase receptors are currently undergoing clinical trials. Antibody therapy against VEGF-A has already shown a longer time to progression for patients with metastatic clear cell RCC [39]. As new therapies are discovered and tested in clinical trials, it will become increasingly important to understand the protein expression of these drug targets to guide the selection of patients most likely to benefit from these promising agents.

CONCLUSION

High-throughput tissue arrays have facilitated the rapid analysis of potential molecular markers. CA IX can be used to predict survival in patients with metastatic clear cell RCC and response to interleukin-2-based immunotherapy. Staging systems incorporating CA IX with Ki67 and other molecular markers are demonstrably better than traditional clinical nomograms. Combining tumour anatomy, pathology, histology and molecular profiling has allowed for further refinement of staging constructs. Molecular markers, e.g. CA IX, represent attractive targets for directed imaging, immunotherapy, and the development of novel vaccines and pharmaceuticals. The understanding of tumour biology gleaned from molecular marker research will be critical to the future treatment of patients with RCC and for the development of a cure for kidney cancer.

CONFLICT OF INTEREST

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Abbreviations: CA IX, carbonic anhydrase IX; VHL, von Hippel-Lindau; UCLA, University of California Los Angeles; UISS, UCLA Integrated Staging System; ECOG, Eastern Cooperative Oncology Group; HIF-1α, hypoxia-inducible factor 1α; TAA, tumour-associated antigen; GM-CSF, granulocyte macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor.
Therapy targeted at vascular endothelial growth factor in metastatic renal cell carcinoma: biology, clinical results and future development

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KEYWORDS
RCC, VEGF, VHL, bevacizumab, SU11248, BAY 43–9006

INTRODUCTION
A limited subset of patients with metastatic RCC obtain clinically meaningful benefit from standard interleukin-2 and/or interferon-α therapy [1]. A growing understanding of the underlying biology of RCC has identified vascular endothelial growth factor (VEGF) as a logical therapeutic target. Therapy directed against the biological activity of VEGF has undergone initial clinical testing in metastatic RCC, with evidence of a substantial antitumour effect; further investigation is ongoing.

VEGF
VEGF is a glycoprotein that is critically important in both normal and tumour-associated angiogenesis through several mechanisms: increased microvascular permeability to plasma proteins [2], induction of endothelial cell division and migration [3,4], promotion of endothelial cell survival through protection from apoptosis [5] and reversal of endothelial cell senescence [6]. VEGF exerts its biological effect through interaction with receptors present on the cell surface. These transmembrane tyrosine kinase receptors include VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), selectively expressed on vascular endothelial cells [7]. Upon binding of VEGF to the extracellular domain of its receptor, dimerization and autophosphorylation of the intracellular receptor tyrosine kinases occurs, and a cascade of downstream proteins are activated. These protein cascades promote the pro-angiogenic endothelial cell effects noted above.

VEGF expression is regulated by several factors. Pertinent to RCC, VEGF expression results from inactivation of the von Hippel-Lindau (VHL) tumour-suppressor gene observed in most RCC (see below), thus identifying VEGF as a particularly relevant therapeutic target in RCC.

BIOLOGY OF VEGF EXPRESSION IN RCC
Patients with an inherited mutation of the VHL gene often develop clear-cell RCC (CCRCC) tumours. VHL syndrome kindreds were subsequently investigated and the VHL gene mapped to chromosome 3p25–26 [8]. In sporadic (not inherited) CCRCC, there is also VHL gene inactivation through mutation or methylation in most tumours [9]. Non-clear cell RCC tumours do not have VHL gene abnormalities in structure or expression.

BIOLGICAL CONSEQUENCES OF VHL GENE INACTIVATION IN RCC
The VHL gene encodes a 213 amino acid protein (pVHL); in conditions of normoxia and normal VHL gene function, pVHL is the substrate-recognition component of a ubiquitin-ligase complex that targets a protein transcription factor, hypoxia-inducible factor (HIF), for proteolysis [10–12]. In conditions of hypoxia or with defective pVHL function, HIF is constitutively activated and not broken down through the proteosome, leading to increased transcription of hypoxia-inducible genes [13,14]. Several hypoxia-inducible genes are activated by this process, including VEGF [13,14], platelet-derived growth factor (PDGF) [15], TGF, erythropoietin, chemokine receptor 4 and carbonic anhydrase 9, which could play a role in the pathogenesis and clinical features of CCRCC. Among these VHL-mediated genes, VEGF has emerged as an initial target in CCRCC. Examination of RCC tumours for VEGF (mRNA transcripts and/or VEGF protein) show VEGF overexpression in the vast majority of samples [9].

Taken together, these data provide compelling evidence for VHL inactivation in most CCRCC tumours, leading to VEGF overexpression that drives tumour angiogenesis. Thus, inhibiting VEGF has been pursued as a therapeutic target in RCC.

CLINICAL RESULTS OF VEGF-TARGETED THERAPY IN RCC
Strategies to inhibit VEGF in RCC, including binding of the VEGF protein, blockade of the VEGF receptor, or inhibiting VEGF receptor signalling through their tyrosine kinases, have recently been tested clinically in metastatic RCC. Table 1 summarizes the clinical data on selected anti-VEGF agents in RCC. Comparison of anti-VEGF agents is not currently possible because separate studies have used different patient selection, methods and outcome criteria. Nonetheless, there is significant antitumour activity (both objective responses, and tumour regression not meeting criteria for response, recorded as stable disease), placing VEGF blockade strategies at the forefront of the clinical investigation of RCC.

ANTI-VEGF ANTIBODY (BEVACIZUMAB)
A recombinant human monoclonal antibody against VEGF (rhuMAb VEGF, bevacizumab; Avastin®, Genentech, South San Francisco, CA) binds and neutralises all biologically active isoforms of VEGF [16]. This humanized antibody inhibits bovine capillary endothelial cell proliferation in response to VEGF, and has antitumour effects in sarcoma and breast cancer cell lines [16].
against the EGFR showed limited antitumour activity with small molecules or antibodies directed against the biological effect through interaction with the VHL-regulated growth factor for RCC, with a factor receptor (EGFR) strategy. TGF-β, combined with an anti-epidermal growth factor receptor (ER) inhibitor, showed benefit in the clinical trial. Bevacizumab was further investigated in a randomized trial; all patients pre-treated with bevacizumab had a 33% response rate (RECIST criteria). An intent-to-treat analysis showed a significantly longer time to progression in the high-dose than the placebo arm (4.8 vs 2.9 months; P < 0.001, log-rank test). There were no life-threatening toxicities or deaths attributable to bevacizumab. In the high-dose bevacizumab arm, hypertension of any grade occurred in 36% of patients, and grade 3 hypertension (not controlled by one standard antihypertensive medication) in 21%. There was asymptomatic proteinuria with no renal insufficiency in 62% of patients, and grade 3 proteinuria in 36% of patients. The median duration of response of 26%, including two patients treated with bevacizumab and erlotinib. No thromboembolic events were reported in any arm.

Bevacizumab was further investigated combined with an anti-epidermal growth factor receptor (EGFR) strategy. TGF-β is a VHL-regulated growth factor for RCC, with a biological effect through interaction with the EGFR [19–21]. However, single-agent studies with small molecules or antibodies directed against the EGFR showed limited antitumour effect [22]. Nonetheless, preclinical investigation in human RCC xenograft models of bevacizumab and erlotinib, a small molecule EGFR inhibitor, showed the potential benefit of combined therapy on tumour growth inhibition, perhaps because EGFR resistance is mediated through VEGF [23]. A clinical trial in metastatic RCC with bevacizumab 10 mg/kg i.v. daily for 2 weeks combined with erlotinib 150 mg oral every day reported a 25% partial response rate [24]. A recently completed placebo-controlled, randomized phase II trial of bevacizumab with or without erlotinib in untreated, metastatic RCC may provide further insight into potential additive or synergistic clinical effect of this combined therapy. To enhance the blockade of proteins that may play a role in the pathogenesis of CCRCC downstream of HIF-1α, imatinib (Gleevec, Novartis), a known PDGFR receptor inhibitor, has been added to this two-drug regimen for additional horizontal blockade. Results of these phase III studies are pending.

**SMALL-MOLECULE VEGFR INHIBITORS**

An alternative approach to VEGF inhibition involves small molecule tyrosine-kinase inhibitors. These agents inhibit not only VEGF, but also other receptors in the split kinase domain superfamily of receptor tyrosine kinases, including the PDGFR, which is expressed in pericytes, and serve as structural supporting cells for endothelial cells; thus class effects of these drugs on PDGFR may have therapeutic relevance.

**SU11248**

SU11248 (Sutent Pfizer, Inc. La Jolla, CA) is an orally bioavailable oxindole small-molecule tyrosine-kinase inhibitor of VEGFR-2 and PDGFR-B. In vitro assays showed inhibition of VEGF-induced proliferation of endothelial cells and PDGF-induced proliferation of mouse fibroblast cells. Investigation in mouse xenograft models showed growth inhibition of various implanted solid tumours and eradication of larger, established tumours [25].

SU11248 was investigated in a single-arm, multi-institutional phase II study in 63 patients with advanced RCC in whom initial cytokine treatment had failed [26]. Patients were treated with 50 mg daily of oral SU11248 on a ‘4-weeks on’, ‘2-weeks off’ cycle. Fifteen patients (24%) had a partial response, as defined by the Response Evaluation Criteria In Solid Tumours (RECIST) criteria. An additional five patients (8%) had a partial response but await confirmation of response status. Of the 15 patients who had a partial response, one progressed at 5 months and two receiving placebo. No thromboembolic events were reported in any arm.

**TABLE 1 A summary of clinical results with VEGF-targeting agents in metastatic RCC**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial design</th>
<th>Clinical activity</th>
<th>Common toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>Randomized, placebo-controlled trial; all patients pre-treated</td>
<td>10% response rate (WHO criteria) Delay of time to progression vs placebo (2.5 vs 4.9 months)</td>
<td>Hypertension, proteinuria</td>
</tr>
<tr>
<td>Bevacizumab + erlotinib</td>
<td>Single-arm phase II, 32% pre-treated</td>
<td>21% response rate (RECIST criteria)</td>
<td>Rash, hypertension, proteinuria</td>
</tr>
<tr>
<td>SU11248</td>
<td>Single-arm phase II, all pre-treated</td>
<td>33% response rate (RECIST criteria)</td>
<td>Fatigue/asthenia, nausea, diarrhoea, stomatitis</td>
</tr>
<tr>
<td>BAY 43–9006</td>
<td>Randomized discontinuation design, 86% pretreated</td>
<td>15% response rate (WHO criteria) 4% response rate (RECIST criteria)</td>
<td>Hand–foot syndrome, rash, fatigue, diarrhoea, hypertension</td>
</tr>
</tbody>
</table>

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taken off the study for asymptomatic decreases in left ventricular ejection fraction of > 20% compared to baseline. A confirmatory single-arm phase II trial in 100 patients with cytokine-refractory metastatic RCC recently completed accrual.

**BAY 43–9006**

BAY 43–9006 (Sorafenib Bayer Pharmaceuticals, West Haven, CT and Onyx Pharmaceuticals, Richmond, CA) is an orally bioavailable bi-aryl urea Raf kinase inhibitor, which inhibits Ras-dependent human tumour xenograft models [27]. Activated Ras promotes cell proliferation through the Raf/MEK/ERK pathway by binding to and activating Raf kinase. BAY 43–9006 also directly inhibits VEGFR-2, VEGFR-3 and PDGFR-B [28]. Xenograft models treated with daily BAY 43–9006 had significant inhibition of tumour angiogenesis, as measured by anti-CD31 immunostaining. A major question, still unanswered, is what, if any, role the Raf kinase inhibitory activity plays in its clinical activity. Based on its multi-targeted profile (VEGFR and PDGFR), similar to SU11248, the Raf kinase inhibition may not be of clinical significance in CCRCC.

A phase II randomized discontinuation study with BAY 43–9006 was reported in refractory solid tumours, including the initial 106 patients with metastatic RCC [29]. All patients received oral BAY 43–9006 400 mg twice daily, and patients with stable disease after 12 weeks of treatment were randomized to continue drug or receive placebo. Patients with ≥25% tumour shrinkage by the sum of the bidimensional measurement of tumours at 12 weeks (defined as ‘responders’) continued open-label BAY 43–9006. Of the first 89 patients with RCC who had reached the initial 12-week assessment, 71% had stable disease and 28% progressed, and were removed from the trial. Using the RECIST criteria for tumour measurements, only 4% of patients had objective responses, but many had ≥25% regression using the WHO (bidimensional) criteria. More recently, Bayer released data from the randomized cohort of 65 patients who received either placebo or continued on BAY 43–9006 after stable disease at 12 weeks. Those randomized to placebo had a median progression-free survival of only 6 weeks, and after 24 weeks only 18% of patients were progression-free. However, those randomized to continue BAY 43–9006 had a median progression-free survival of 23 weeks, with half progression-free at 24 weeks.

**ONGOING CLINICAL TRIALS OF VEGF-TARGETED THERAPY IN RCC**

Given the promising data showing an effect of bevacizumab on time to progression in RCC, an Intergroup phase III trial investigating the addition of bevacizumab to initial systemic therapy in RCC is underway [30]. Patients with metastatic CCRCC and no previous systemic therapy are randomized to either low-dose interferon-α2b (Intron A, Schering-Plough, Kenilworth, NJ) 9 MU three times weekly, or the same dose and schedule of interferon-α2b combined with bevacizumab 10 mg/kg i.v. every 2 weeks. Patients are stratified by nephrectomy status and established prognostic factors to ensure balanced randomization [18,31,32]. The primary endpoint of the trial is overall survival. A similarly designed phase III trial is underway in Europe using interferon-α2a (Roferon, Hoffmann-LaRoche, Grenzach-Wyhlen, Germany) instead of interferon-α2b.

Clinical trials of another approach to initial therapy in metastatic RCC, high-dose interleukin-2 combined with bevacizumab, are also soon to begin. The rationale for this combination includes that inhibition of VEGF may prevent much of the tumour-induced immunosuppression attributed to VEGF and thereby enhance the immune-enhancing effects of interleukin-2 [33,34]. In addition, interleukin-2 toxicity may be reduced by the vascular effects of bevacizumab. Bevacizumab may decrease the significant vascular leak syndrome associated with interleukin-2 and allow more doses of interleukin-2 to be administered, with less toxicity. Finally, the response subpopulation may be different for the different agents. Bevacizumab 10 mg/kg i.v. daily for 2 weeks will be integrated with standard high-dose interleukin-2 regimens, with both progression-free and overall survival as primary endpoints. Bevacizumab combined with low-dose interleukin-2 will also be evaluated in a separate trial.

The role for SU11248 in the treatment of metastatic RCC is being further investigated in a confirmatory single-arm trial of second-line therapy, and in a randomized phase III trial of first-line therapy compared with interferon-α. Future investigations will test the efficacy and tolerability of combining SU11248 with additional agents that target pathways implicated in RCC carcinogenesis (e.g. gefitinib, a small molecule EGFR inhibitor).

Further, the clinical cross-resistance of this class of agents is unknown. Given the distinct anti-VEGF mechanism of the small molecule agents such as SU11248 and the extended spectrum against VHL-mediated targets relevant to RCC (e.g. PDGF), there is a biological rationale for investigating SU11248 in bevacizumab-refractory patients. A multicentre trial of SU11248 in patients with metastatic RCC who have disease progression despite previous bevacizumab therapy is ongoing, with a primary endpoint of overall response rate.

A phase III, randomized controlled trial of BAY 43–9006 vs placebo in patients who had received and failed one previous systemic biological therapy (interferon or interleukin-2) has recently completed accrual. The trial was intended to enrol 884 patients, with overall survival the primary endpoint and progression-free survival the secondary endpoint. Other trials are using BAY 43–9006 combined with additional inhibitors of the VEGF pathway (bevacizumab), inhibitors of the TGFα-EGFR pathway (gefitinib or erlotinib) or mTOR inhibitors (tesirinolimus, CCI-779, Wyeth Pharmaceuticals) that may decrease translation of HIF-1α. These trials are in their early phases of dose-finding and toxicity at both the National Cancer Institute-intramural and an extramural group of centres (Vanderbilt-Penn-Harvard). Following these trials will be a large randomized phase II trial of doublets of bevacizumb, BAY 43–9006, erlotinib and CCI-779 through the Eastern Cooperative Oncology Group. There will be six arms with 50 patients enrolled per arm, with time to progression as the primary endpoint. Finally, a phase I/II trial of BAY 43–9006 with interleukin-2 is planned within the Cytokine Working Group. Because of concerns over the effects that a Raf kinase inhibitor (BAY 43–9006) may have on proliferation of lymphocytes stimulated by interleukin-2, the two treatments will be given in sequence, with no overlap in their periods (3–6 weeks) of administration.

Lastly, a placebo-controlled, randomized study of BAY 43–9006 in the neoadjuvant setting is planned through the Eastern Cooperative Oncology Group, with a progression-free survival endpoint.
Comparison of renal biopsy material before treatment and nephrectomy specimens afterward will provide further insight into the molecular mechanisms of this agent. Further, the safety of administering this agent before surgery will be evaluated. Although activity of this class of agents was reported in the metastatic setting, patients with earlier stage RCC should be treated in a clinical trial until safety and efficacy in this setting is confirmed.

FUTURE DIRECTIONS

The exciting clinical response data with VEGF inhibition in RCC has provided an opportunity for treatment advances in this historically resistant malignancy. As the clinical activity of existing agents is further defined in ongoing trials, several questions on optimizing their utility remain. Furthermore enrichment of patients susceptible to VEGF blockade, beyond restriction to clear cell histology, is needed. Examination of RCC tumour tissue for the predictive value of VHL mutation status, HIFα, VEGF expression or other markers is warranted. Investigation of clinical predictive/prognostic factors, as have been developed for patients with RCC undergoing immunotherapy, is warranted with this class of agents.

CONFLICT OF INTEREST

Brian Rini received research funding from Genentech and Pfizer; and is a paid consultant to Bayer. Jeffrey Sosman received research funding from Genentech, Chiron, Pfizer and Wyeth.

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Abbreviations: VEGF(R), vascular endothelial growth factor (receptor); VHL, von Hippel-Lindau; CCRC, clear cell RCC; HIF, hypoxia-inducible factor; PDGF, platelet-derived growth factor; EGFR, epidermal growth factor receptor; RECIST, Response Evaluation Criteria in Solid Tumours.
Multidetector computed tomography vs magnetic resonance imaging for defining the upper limit of tumour thrombus in renal cell carcinoma: a study and review

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OBJECTIVE

To compare the findings of multidetector computed tomography (CT) with surgical pathology and magnetic resonance imaging (MRI), to determine the accuracy of delineating the superior extent of inferior vena cava (IVC) thrombotic involvement in renal cell cancer (RCC).

PATIENTS AND METHODS

A prospective database was examined of 11 patients (median age 65 years, range 45–77) being assessed for suspected IVC extension of RCC tumour thrombus with both multidetector CT and MRI. All had pathology confirming RCC, and eight of those undergoing surgery had pathological confirmation of tumour thrombus extent. All images were analysed originally, then reanalysed by two independent radiologists, an experienced urologist and a urological trainee unaware of the original reports and other imaging results, with a final determination on tumour thrombus level by consensus.

RESULTS

The multidetector CT results were completely accurate when compared with surgical specimens and were in agreement with MRI on all but one occasion, where MRI determined the renal vein to be clear when it was involved on CT and at surgery, giving MRI an accuracy of seven of eight samples.

CONCLUSIONS

Whilst there were few patients and further studies are needed, multidetector CT was comparable with MRI in determining tumour thrombus level. More importantly, in the eight patients with surgical pathological confirmation, multidetector CT was accurate in all. Ultimately, it may replace MRI as the ‘gold standard’ for imaging to delineate the upper limit of tumour thrombosis in RCC.

KEYWORDS

renal cell carcinoma, RCC, spiral CT, MRI, venous thrombosis, inferior vena cava, surgery

INTRODUCTION

RCC often invades the renal vein and may extend into the inferior vena cava (IVC) or right atrium [1]. Current staging incorporates such invasion and aids in prognosis. Accurate staging is paramount when assessing patients and planning surgical resection. Of patients undergoing radical nephrectomy for RCC, 4–10% have IVC involvement. The surgical approach and assistance of other specialist surgeons is often crucial the further the tumour thrombus extends [2]. The superior extent of the thrombus determines the operative approach [3,4]. Traditionally, venacavography was the ‘gold standard’ investigation to delineate thrombus level, with a reported sensitivity of 100% [5–7]. Since the advent of CT and MRI, venacavography is now used rarely. In particular, multiple-plane imaging and accuracy has made MRI the ‘gold standard’ investigation of RCC with suspected thrombus [8–10]. Nevertheless, CT remains the investigation of choice for most RCCs, as it is often clear that there is no IVC invasion. More than 10 years ago, MRI was compared with CT to delineate IVC involvement, but the quality of CT imaging then was poorer, with reconstructed images unavailable. CT was unable to delineate the exact level of the upper limit of thrombus, the major factor in planning surgery [11,12]. However, with advances in CT technology and multiple-plane reconstructions now available, MRI may not be necessary. In a review of renal imaging, Israel and Bosniak [8] commented that ‘with the advent of multidetector CT scanning, it is unclear whether any proposed advantage of MRI still holds true’.

The purpose of the present study was to compare the findings of multidetector CT, providing images in many planes, with surgical pathology and MRI to determine the accuracy of delineating the superior extent of IVC thrombosis involvement in RCC.

PATIENTS AND METHODS

A prospective database of multidetector CT and MRI information was maintained from 2001; additional data were specifically collected on patients with RCC and tumour thrombus, including demographics, surgery, pathology and follow-up. Eleven patients (median age 65 years, range 45–77) were assessed for suspected IVC extension of RCC with both methods (Table 1). Patients were staged according to the 1997 TNM classification; all had pathology confirming RCC, and eight had surgery, with operative and pathological confirmation of thrombus extent. There were no exclusions and ethics committee approval was not required, as MRI is current best practice.

All CT was done on multidetector (-row) machines (Multidetector GE Lightspeed plus, eight-slice, General Electric Medical Systems, Milwaukee, USA). The protocol was identical, with four phases: non-contrast phase images were obtained from the aortic arch to the
symphysis pubis at 2.5 mm intervals; an arterial phase with 100 mL of intravenous non-ionic contrast medium (iohexol), injected at 3 mL/s (timed bolus injection), with scanning starting once the contrast agent was apparent in the aorta (usually 20 s); a delayed phase taken 90 s from the injection with contrast agent, and scanning from the aortic arch to the symphysis pubis; and finally an extra delayed phase, at 10 min after injection with contrast agent, scanning the kidneys and IVC only. From all scans coronal reconstructions of the kidneys and IVC were produced. A thrombus was diagnosed in the IVC when a low-attenuation filling defect was apparent within the lumen [13]. Injection with contrast medium enhanced the thrombus, and when there was incomplete obstruction of the blood flow, the intraluminal enhancement was peripheral and ring-like (‘doughnut’ appearance). Focal enhancement of the vena caval wall, or infiltration of adjacent soft tissue, indicated vena caval wall invasion, as described previously [12].

For MRI, the same machine was used (GE Echospeed, Software version 9). The protocol consisted of six phases: axial T1 breath-hold; axial T2 breath-hold; coronal T1 breath-hold; dynamic axial T1 with intravenous contrast agent (20 mL of gadolinium); coronal gadolinium venogram; and delayed axial T1 with fat suppression. Three-dimensional reconstructions were used for gadolinium images, with 2.5 mm slices.

RCCs had a varied MRI signal, the most common appearance being a mass with an intensity intermediate between the renal cortex and the medulla on T1-weighted images, and hyperintense on T2-weighted images. A thrombus was diagnosed in the venous system when there was a filling defect and several planes were consulted where necessary. A tumour thrombus was diagnosed when the signal intensity and contrast enhancement matched the primary tumour. For a bland or pure clot thrombus to be diagnosed there had to be no enhancement after giving the contrast agent.

There have been attempts to grade RCC IVC thrombus by superior extent at two, three or four levels [1,14,15]. We chose five levels, distinguishing suprahepatic IVC extent from right atrial involvement, and described those at the junction of the renal vein and IVC as ‘renal vein only’ (Fig. 1) which is more practical from a surgical planning perspective.

All images were analysed originally by two experienced radiologists at the time of scanning, with data recorded. Images were then re-analysed by two independent radiologists unaware of the original reports and other imaging results, with a final determination on tumour thrombus level by consensus. An experienced urologist and urological trainee, also unaware of the reports, assessed the CT scans and recorded their superior level of tumour thrombus. Two pathologists reported all pathology specimens, and the operative level of the thrombus was confirmed by two surgeons before recording, using the levels of thrombus defined.

### RESULTS

Overall, CT accurately delineated the tumour thrombus level in the IVC in all eight patients, compared to MRI which was correct in seven of the eight (Table 1). The CT results for thrombus level matched those of MRI (Fig. 2) on all but one occasion where MRI determined the renal vein to be clear when it was involved on CT and at surgery. This was a difficult case, as tumour was encasing the vein (noted on MRI) as well as being in the

<table>
<thead>
<tr>
<th>Age, years/sex</th>
<th>CT Level</th>
<th>MRI Level</th>
<th>Surgery</th>
<th>Stage</th>
<th>Grade</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>69/F</td>
<td>RV</td>
<td>RV</td>
<td>RV</td>
<td>T3a</td>
<td>2</td>
<td>Clear</td>
</tr>
<tr>
<td>64/M</td>
<td>RV</td>
<td>Nil</td>
<td>RV</td>
<td>T3b</td>
<td>2</td>
<td>Clear</td>
</tr>
<tr>
<td>65/M</td>
<td>RV</td>
<td>RV</td>
<td>RV</td>
<td>T3b</td>
<td>3</td>
<td>Clear</td>
</tr>
<tr>
<td>45/F</td>
<td>INFRA</td>
<td>INFRA</td>
<td>INFRA</td>
<td>T3b</td>
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<td>Sarcomatoid</td>
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<tr>
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<td>INFRA</td>
<td>INFRA</td>
<td>T3a</td>
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<td>Clear</td>
</tr>
<tr>
<td>69/M</td>
<td>INTRA</td>
<td>INTRA</td>
<td>INFRA</td>
<td>T3a</td>
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</tr>
<tr>
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<td>INTRA</td>
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<td>T3b</td>
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</tr>
<tr>
<td>73/F</td>
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<td>INTRA</td>
<td>INTRA</td>
<td>T3b</td>
<td>4</td>
<td>Clear</td>
</tr>
<tr>
<td>65/M</td>
<td>SUPRA</td>
<td>SUPRA</td>
<td>Nil</td>
<td>T3c</td>
<td>2</td>
<td>Clear</td>
</tr>
<tr>
<td>51/F</td>
<td>RV</td>
<td>RV</td>
<td>Nil</td>
<td>T4</td>
<td>High</td>
<td>RCC (metastasis)</td>
</tr>
<tr>
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<td>INTRA</td>
<td>INTRA</td>
<td>Nil</td>
<td>T4</td>
<td>High</td>
<td>RCC (metastasis)</td>
</tr>
</tbody>
</table>

RV, renal vein; INFRA, infrahepatic vena cava; INTRA, intrahepatic vena cava; SUPRA, suprahepatic vena cava.

**FIG. 1.** Diagram depicting the delineation of the upper limit of tumour thrombus extension in RCC as used in the study: RV, renal vein; INFRA, infrahepatic vena cava; INTRA, intrahepatic vena cava; SUPRA, suprahepatic vena cava; RA, right atrium.
lumen, making interpretation difficult. Of the remaining seven patients having surgery, there was concordance of opinion as to thrombus level on MRI and CT. One patient was upstaged from T3a to T4 because of disease extension, not detected on either CT or MRI, for tumour extension beyond Gerota's fascia, but the thrombus level remained unchanged. Only tumour thrombi were diagnosed on MRI.

Three patients had no surgical intervention but CT and MRI were in agreement as to tumour thrombus level in the IVC. Two patients had significant widespread metastatic disease and were considered inappropriate for nephrectomy and immunotherapy. Finally, one patient refused surgery who had previously had a nephrectomy for T1 disease; he was undergoing tumour surveillance when at 18 months after surgery he developed a tumour vein thrombus with extension into the suprahepatic vena cava.

When the opinion of the experienced urologist was compared with that of the radiologists for CT, the delineation of thrombus from tumour thrombus [9,10]. MRI may also sometimes delineate bland thrombus versus tumour thrombus [21]. The sensitivity for tumour thrombus extent, combining CT with ultrasonography improved the sensitivity of tumour thrombus extent, but remained much less sensitive than MRI [22].

The CT level of RCC thrombus and pathological specimens has been compared using conventional CT, with an accuracy of 95% on axial scanning alone [1], but few other studies have reached such precision (Table 2) [11,12,19,23,24]. Studies have also assessed MRI and CT in parallel, but not directly against each other, with MRI having a greater accuracy (complete) at diagnosing the superior extent of thrombus compared to CT (76%) [2,7]. MRI has also been correlated with CT for overall staging accuracy (74–88% MRI vs 67–100% CT), but the superior extent of thrombus involvement has not been specifically highlighted [13,21]. To date, only five studies have directly compared conventional CT to MRI in delineating the thrombus level of extension into the IVC in RCC (Table 2) and these were undertaken >10 years ago in fewer than 50 patients [11,12,19,23,24].

Overall, studies directly comparing MRI with CT found MRI to be completely accurate for the superior extent of tumour thrombus, whereas CT had a mean diagnostic accuracy of only 65% (Table 2). Of more concern for CT was that it completely missed tumour thrombus in four patients in three of the studies [11,12,24]. These CT images were only axial, and they would not compare to current multiplanar imaging. Other reasons for the inaccuracy of CT include incorrect timing and an insufficient amount of intravenous contrast agent in the IVC. These areas have been improved, with better software and high-powered contrast bolus injectors. In support of this, a recent study comparing multidetector CT and MRI for overall RCC staging had similar accuracy with both methods, but tumour thrombus extension into the IVC was not specifically assessed [15].

Although in general MRI is completely accurate in delineating the level of tumour thrombus in the vena cava in RCC, some inaccuracies were reported. In two cases the extent of tumour thrombus was underestimated at the level of the hepatic veins [25,26]. In three other studies the use of preoperative MRI in a total of 57 patients was
90–96% accurate at determining thrombus level, which was similar to the present accuracy [21,27]. In the study with 10 patients, only six from eight venacavography scans were accurate; clearly, no imaging method is always completely accurate, and even ‘gold standards’ of imaging may be incorrect.

MRI technology has also developed with time; previously, MRI using spin-echo sequences was unable to overcome flow-related intraluminal signals from thrombus and external compression of the vena cava, creating artefacts that made assessing the signal difficult [18]. Also, respiratory and cardiac motion artefacts compromised the delineation of tumour thrombus extent and may explain the cases discussed above. To overcome these issues, gradient-recalled echo sequences were introduced for MRI of vascular structures, including tumour thrombus in RCC, with success [26]. Furthermore, MR image acquisition has become faster, providing more images in a single breath-hold, reducing movement artefact. Further developments will result in even greater imaging capabilities of MRI, but access and cost remain strong impediments to its widespread use.

Other imaging methods have been investigated to delineate tumour thrombus, e.g. ultrasonography and transoesophageal echocardiography. Ultrasonography is not appropriate, as many studies are technically indeterminate because they rely on operator and patient characteristics [7]. There are limited data for transoesophageal echocardiography but no study has shown that it adds any diagnostic advantage, and it may only have a small role during surgery in patients having a cardiopulmonary bypass [28].

Multidetector CT allows faster data acquisition than single-detector CT, with no loss of image quality because of short gantry rotation intervals combined with multiple detectors at each level, providing increased coverage [29]. This, along with short interscan delays, allows image acquisitions in multiple phases of renal parenchymal enhancement and contrast agent excretion in the collecting system after giving one bolus of intravenous contrast agent [30]. Another advantage of CT is improved spatial resolution, providing high-quality three-dimensional datasets of the renal vessels, comparable with angiography and conventional urography [31]. The benefits outlined above also pose significant challenges, including selecting the optimal imaging sequences, controlling radiation exposure to the patient, and efficiently managing the increased data.

Currently, MRI will remain the ‘gold standard’ for delineating the level and extent of tumour thrombus in the IVC in the staging of patients with RCC. While our experience is limited, multidetector CT was accurate when compared with the surgical specimens and is probably at least the equivalent of, if not better than, MRI in determining thrombus level. Whilst encouraged by these early results, the accuracy of multidetector CT in defining tumour thrombus in RCC must await further analysis from other centres, and so we will continue to use both methods until we are satisfied that it is equal to or better than MRI. Finally, with CT developing rapidly, the challenge as clinicians will be to evaluate new imaging sequences, controlling radiation exposure to the patient, and efficiently managing the increased data.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contribution of Dr Greg Fitt FRANZCR, University of Melbourne, Department of Radiology, Austin Hospital, in helping to analyse the images for this study.

TABLE 2 A summary of studies directly comparing CT with MRI and surgery to ascertain the accuracy of delineating the superior extent of RCC tumour thrombus. Studies apart from the present used conventional CT

<table>
<thead>
<tr>
<th>Study</th>
<th>N patients</th>
<th>Method</th>
<th>Level of thrombus</th>
<th>Accuracy, n/N</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>11</td>
<td>CT</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[23]</td>
<td>8</td>
<td>CT</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[11]</td>
<td>5</td>
<td>CT</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[24]</td>
<td>5</td>
<td>CT</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[12]</td>
<td>16</td>
<td>CT</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[13]</td>
<td>15</td>
<td>CT</td>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>MRI</td>
<td></td>
<td></td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Two patients had thrombus extent confirmed at autopsy; †Extent of thrombus not specified, but all patients had surgery or biopsy to confirm RCC.

CONFLICT OF INTEREST

None declared.

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Abbreviations: IVC, inferior vena cava.
Epothilones and the next generation of phase III trials for prostate cancer

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INTRODUCTION

During 2005, an estimated 232 090 men will have prostate cancer diagnosed (one in six men), while 30 350 men will die from the disease in the USA [1]. Because of PSA screening, most patients present with localized prostate cancer and are candidates for definitive local therapy. Despite local therapy for localized disease, the actuarial 10-year likelihood of biochemical disease recurrence is ~25% [2,3]. For patients who progress to systemic disease, or less commonly for those who initially present with advanced disease, androgen deprivation is regarded as the optimum first-line treatment [4,5]. Unfortunately, androgen- ablative therapy is only palliative, with a median duration of response of 12–24 months [4,5]. Second-line hormonal manipulation in men who progress on androgen deprivation results mostly in a biochemical response [6–8], which is generally transient and has no demonstrable impact on survival. Hormone-refractory prostate cancer (HRPC) is a progressive morbid disease, leading to eventual death over a median of 12–18 months. Chemotherapy in this setting has been actively investigated over the last two to three decades, and until recently was only palliative. The recent studies of docetaxel-based chemotherapy in men with androgen-independent prostate cancer showed a survival benefit for the first time in this disease state [9,10] and lifted the burden of HRPC as a chemoresistant disease [11].

These studies also provided proof-of-principle that targeting the tubulins is a fruitful strategy for effective therapy in HRPC. Based on this optimism, investigations of epothilones are rapidly advancing in HRPC, and this is the only new class of chemotherapeutic agents which have provided phase II activity in HRPC to date.

The broad spectrum of anti-neoplastic activity and the diverse clinical applications of taxanes have engendered significant interest in identifying mechanistically similar but structurally distinct compounds. Epothilones emerged as a new class of putative anti-neoplastic drugs based on in vitro assays designed to competitively inhibit the binding of paclitaxel to microtubules [12]. Epothilones are macrolides extracted from a variety of myxobacteria including Myxococcus xanthus or Sorangium cellulare [13]. Like paclitaxel and docetaxel, the epothilones function by stabilizing the polymerized microtubule [14]; however, the epothilones are structurally distinct.

While an excellent contemporary review discusses in detail the mechanistic and cell-culture-based observations in the development of epothilones [15], we briefly discuss here their target (tubulins) and biological observations that provide insights to the anti-neoplastic activity of epothilones.

Active anticancer drugs (Vinca alkaloids and taxanes) work by perturbing the dynamic equilibrium of microtubule polymerization and depolymerization [16]. The formation of microtubules is essential for normal mitosis and cell division. This involves polymerization of heterodimeric α/β tubulin subunits, with multiple isoforms of both α and β tubulin present in proliferating human cells, and is regulated by several microtubule-associated proteins. Intact microtubule function is required for the formation and functioning of the mitotic spindle, and cells treated with agents that interfere with polymerization or depolymerization show changes in spindle formation, as well as arrest at the G2/M phase of cell cycle, which via poorly understood mechanisms is associated with induction of apoptosis [17–19]. Also, recent data suggest that an important component of the useful anticancer activity of these types of drugs involves anti-angiogenic effects on tumour-associated endothelial cells [20]. Moreover, certain alterations, e.g. loss of p53 function, which is common in many cancer cells, may confer hypersensitivity to taxanes as a result of altered expression of genes that are regulated by p53 [19].

Epothilones also induce microtubule bundling, formation of multipolar spindles and mitotic arrest [12]. Epothilones compete with paclitaxel for binding to microtubules and suppress microtubule dynamics in a manner similar to paclitaxel [21]; cell lines selected for resistance to epothilones contain mutations in β-tubulin that map near the taxane-binding site identified in a crystal structure of a docetaxel-β-tubulin complex [22]. However, recent studies in yeast reveal differences in the interactions between taxanes and epothilones with microtubules; epothilones stabilize Saccharomyces cerevisiae microtubules whereas paclitaxel does not, presumably as a result of differences of their individual binding interactions on tubulin function [23].

Preclinical studies also show important differences between epothilones and taxanes in drug-resistance mechanisms, both at the target site and in the drug-efflux pump, P-glycoprotein. Epothilone cytotoxicity is unaffected by an alanine-to-threonine substitution at residue 364 in β tubulin that confers resistance to paclitaxel [21]. This has led to a hypothesis that clinically, tumour cells resistant to taxanes will retain sensitivity to epothilones and hence provide a role for these class of compounds in the setting of clinical progression after taxane therapy. However, resistance to epothilones may also result from β tubulin mutations [24,25] and these cell lines were also found to be cross-resistant to paclitaxel. Another well established mechanism of taxane resistance to values in the sub- to nanomolar concentration range, and comparison of the inhibitory concentrations, involves over-expression of the multidrug efflux pump, the
P-glycoprotein. Epothilones are more cytotoxic than paclitaxel in cell culture, with the concentration for 50% inhibition by various epothilones being slightly higher than those of paclitaxel in P-glycoprotein-expressing cell lines [15,26]. These results have led to hypothesis that epothilones may be more active than taxanes in patients with malignancies characterized by high levels of P-glycoprotein expression.

Epothilones exist in at least four forms (A-D) [15]. Four epothilone analogues are currently in human clinical trials in various phases of development, including aza-epothilone B (BMS-247550), a water-soluble semisynthetic analogue of epothilone B (BMS-310705), epothilone B (EPO906), and epothilone D (KOS-862). In the following sections we discuss the early clinical results and observations on the future development of these agents.

CLINICAL DEVELOPMENT OF EPOTHILONES

AZA-EPOTHILONE B (BMS-247550; IXABEPILONE)

This agent has shown potent cytotoxic effects on paclitaxel-sensitive and -insensitive cells, and in taxane-resistant tumour cell lines over-expressing the P-glycoprotein [14]. Phase I trials of BMS-247550 have been conducted for a cremophor-based formulation in a variety of schedules, including a single 60-min infusion every 21 days, a weekly schedule, five-times daily every 21 days and three times daily every 21 days. There were anti-tumour responses in patients with melanoma, ovarian, nonsmall cell lung cancer and breast cancer, many previously treated with paclitaxel- or docetaxel-containing regimens [15]. Phase I evaluations of this agent in cytotoxic combinations (e.g. with carboplatin) are also ongoing. A dosing schedule of 40 mg/m\(^2\) once every 3 weeks as a single agent was most prominently recommended and subsequently adopted for phase II testing.

SINGLE-AGENT PHASE II TRIAL IN HRPC

The most mature study reported for front-line activity in phase II settings for any epothilone was for BMS-247550 via two presentations at the annual American Society of Clinical Oncology (ASCO) meeting, 2004. A phase II single-agent trial (South-West Oncology Group, SWOG, 0111) was reported by Hussain et al. [27]. The primary objective of this study was to assess the PSA response. Eligible patients were those who had metastatic prostate cancer and in whom androgen-deprivation therapy and antiandrogen withdrawal had failed; previous chemotherapy was an exclusion criterion. Patients were treatment at 40 mg/m\(^2\) i.v. over 3 h every 3 weeks. Premedication with 50 mg of diphendydramine and 150 mg ranitidine was administered 1 h before treatment. Forty-one patients (median age 73.1 years; median PSA 126.5 ng/mL) were enrolled. There was anti-tumour activity in 16 patients (39%) with a ≥50% PSA decline, and 14 of the responding patients (34%) had a confirmed PSA decrease (Table 1). Of these 14 patients, 10 had a decrease in PSA of >80%.

Randomized Phase II Trial of Ixabepilone Alone or Combined with Estramustine

After earlier data showing that adding oral estramustine to microtubule stabilizers is associated with apparently greater activity in prostate cancer, the combination of ixabepilone and estramustine in HRPC was investigated in another phase II multicentre trial by Kelly et al. [28]. Eligible patients were chemotherapy-naïve with progressive disease. Treatment was with ixabepilone at 35 mg/m\(^2\) i.v. on day 2 with or without estramustine 280 mg orally three times daily on days 1 to 5 every 3 weeks. Low-dose prophylactic warfarin (2 mg/day) was given orally to patients receiving estramustine. There were 45 patients treated in the combination arm and 47 in the ixabepilone arm (92 in all). There was an objective response in eight of 25 patients (32%) treated with ixabepilone alone and in 11 of 23 (48%) in the combined arm (Table 2).

\[\text{TABLE 1 Ixabepilone in front-line HRPC. From [26].}\]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ixabepilone</th>
<th>Estramustine</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA response (41)</td>
<td>14 (34)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Confirmed, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unconfirmed, n (%)</td>
<td>2 (5)</td>
<td></td>
</tr>
<tr>
<td>Objective response (19), n (%)</td>
<td>3 (16)</td>
<td></td>
</tr>
<tr>
<td>Time to treatment failure, months</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>PFS, months</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Toxicity (grade 3 or 4), n (%)</td>
<td>7 (17)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>5 (12)</td>
<td></td>
</tr>
<tr>
<td>Sensory neuropathy (grade 3)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Motor</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
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</tr>
<tr>
<td>Infection</td>
<td>5 (12)</td>
<td></td>
</tr>
</tbody>
</table>

\[\text{TABLE 2 The efficacy of ixabepilone/estramustine vs ixabepilone, and the prominent adverse events. From [27].}\]

**Variable** | **Ixabepilone/estramustine** | **Ixabepilone** |
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>N</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>Efficacy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective response</td>
<td>11/23 (48)</td>
<td>8/25 (32)</td>
</tr>
<tr>
<td>Bone scan stable</td>
<td>28/36 (78)</td>
<td>24/40 (60)</td>
</tr>
<tr>
<td>≥50% PSA decline</td>
<td>31/45 (69)</td>
<td>21/44 (48)</td>
</tr>
<tr>
<td>Days to PSA progression</td>
<td>141</td>
<td>145</td>
</tr>
<tr>
<td>Adverse events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3/4, %</td>
<td>18/12 (92)</td>
<td>13/9</td>
</tr>
<tr>
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<td>Febrile neutropenia</td>
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</tr>
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<td>Thrombosis</td>
<td>7/0 (12)</td>
<td>13/0</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>7 (17)</td>
<td>13/0</td>
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</table>

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occurred in 48% of patients treated with ixabepilone alone and in 69% in the combination arm. The time to PSA progression was similar in both arms (141 days in the combined arm and 145 days in the ixabepilone-only arm). Neutropenia and neuropathy were also the main adverse events in this study (Table 2). Neutropenia occurred in 84% of patients but was tolerable (grade 1 or 2); grade 3 neutropenia occurred in 7–13% of patients. The severity of neuropathy improved over time and after a median follow-up of 413 days, grade 2 or 3 neuropathy had improved to grade 0 or 1 in 18 of 19 patients; 9% of patients in the estramustine arm had a grade 3 or 4 thrombotic event.

RESPONSE TO TAXANES AFTER IXABEPILON THERAPY IN HRPC

Because preclinical data showed no cross-resistance between epothilones and taxanes, patients in the phase II study by Kelly et al. [28] who went on to receive second-line taxane therapy were analysed retrospectively [29]. Of the 49 patients evaluated, those who had been treated with either ixabepilone alone (23 men) or with combined estramustine (28 men) benefited from taxane therapy. There were PSA responses from second-line taxane therapy in 51% of patients (95% CI 33–66%), with a median time to PSA progression of 4.6 months. There were PSA responses in 61% of first-line responding patients, but significantly there were PSA responses also in a third of those who did not respond to first-line ixabepilone therapy.

The median survival in this cohort was 10.7 months from the initiation of second-line taxane-based therapy. Hence, this analysis supports the hypothesis that epothilones and taxanes are not cross-resistant, and may be useful in tandem. A multicentre National Cancer Institute-sponsored phase 2 trial currently recruiting is examining the use of second-line ixabepilone vs mitoxantrone and prednisolone in patients with metastatic disease and progressive disease after taxane therapy.

These preliminary phase II results are consistent with preclinical data and suggest that BMS-247550 is a broadly active anticancer drug. A schedule involving daily administration for 5 days every 3 weeks in second-line nonsmall cell lung cancer reported less neurotoxicity (6% grade III/IV toxicity) than a single dose given every 21 days [30]; however, whether these schedules differ in terms of anticancer efficacy in HRPC remains to be determined.

BMS-310705 (WATER-SOLUBLE EPOTHILONE B ANALOGUE)

BMS-310705 is a water soluble, semisynthetic analogue of epothilone B and hence does not require a cremophor-based formulation. It has been evaluated in phase I trials with two different schedules, involving a 15-min infusion given every 3 weeks [31] or weekly for 3 consecutive weeks every 28 days [32]. No premedications were used and there were no hypersensitivity reactions. For the every-3-week schedule, neuropathy was dose-limiting and led to a recommendation of 40 mg/m² as the phase II dose. When administered weekly for 3 consecutive weeks every 28 days, grade 3 diarrhoea was dose-limiting at 30 mg/m². At the 20 mg/m² dose using this schedule, 25% of patients missed the third weekly dose because of diarrhoea.

Also, at this dose sensory neuropathy occurred during the fourth course in two-thirds of the patients. Based on these results, evaluation of a 2-weeks on, 1-week off schedule for BMS-310705 is ongoing. Responses were documented with both schedules, including partial responses in patients with ovarian, bladder, stomach and breast cancer, and a complete response in a patient with nonsmall cell lung cancer. Based on the encouraging activity of BMS-247550, further evaluation of BMS-310705 is also planned in HRPC.

EPOPHILONE B (EPO906; PATUPILONE)

Patupilone (EPO 906; epothilone B) is a more potent microtubule stabilizer than paclitaxel and in preclinical studies was found to accumulate in intracellular concentrations several hundred times greater than in the extracellular medium [33]. It is formulated in polyethylene glycol-300, minimizing the potential for carrier-associated adverse reactions. Despite being structurally very similar to ixabepilone, patupilone is associated primarily with diarrhoea, whereas ixabepilone is associated with neuropathy as its primary dose-limiting toxicity. This important distinction may favourably affect the further development of this agent, given that its toxicity does not overlap with that of other taxanes.

Hussain et al. [34] reported the results of a multicentre phase II study of weekly patupilone in patients with HRPC. A maximum of one previous chemotherapy regimen was allowed in this trial. Patients were treated with six cycles of patupilone 2.5 mg/m² per week for 3 of 4 weeks. Forty-five patients (median age 69 years) were enrolled and 29 (64%) had received previous chemotherapy. Patupilone was associated with grade 3 diarrhoea in 22% of patients, resulting in grade 3 or 4 dehydration in 11%. No grade 3 or 4 neuropathy was reported. Weekly treatment was associated with a 50% PSA response in seven of 28 patients (25%; Table 3). Importantly, three of the seven responders had received previous taxane-based chemotherapy. The median duration of PSA response was 2.2 months. Also, the preliminary results of several phase II trials of EPO906 in refractory solid tumours were reported at the 20th Chemotherapy Foundation Symposium (http://www.mssm.edu.proxy/lib.umich.edu/tcf/archives/symposiumnotes/index.shtml). These early phase II results suggest that EPO906 is a broadly active drug and is able to induce responses in at least some patients with taxane-resistant disease. Further clinical development has not been publicly disclosed, but is anticipated.

EPOPHILONE D (KOS-862)

The phase I evaluation of KOS-862 included several dosing schedules: a single dose every
3 weeks, a daily dose three times every 3 weeks, a fixed-rate dose every 3 weeks, and a weekly dose for 3 weeks with a 1-week rest [15]. There was significant toxicity in patients treated with the single-dose every 3 weeks, which included impaired gait and cognitive/perceptual abnormalities, sensory neuropathies, and fatigue. There were responses observed in heavily pre-treated patients with testicular, ovarian, pancreatic and breast cancers. Dose-limiting toxicities have not been reported yet. Phase II studies in front-line HRPC settings are planned.

**WHERE DO WE GO FROM HERE?**
**NEXT-GENERATION (PHASE III) TRIALS IN HRPC**

A new generation of clinical trials will evaluate a variety of newer agents against traditional targets (e.g. epothilones against the mitotic spindle), and against entirely new targets in validated prostate-cancer pathways (angiogenesis and endothelin pathway, among others) based on a deeper understanding of the biology of androgen-independent prostate cancer. This area of ‘rational therapy development’ based on an understanding of the basic biology of prostate cancer, rather than empirical evaluation of chemotherapeutic agents, is the new frontier which holds the most promise in advancing the systemic treatment of HRPC. This next generation of phase III trials in HRPC are described, along with their rationale and study designs.

**TESTING TARGETED THERAPY IN PHASE III SETTINGS: THE SWOG 0421 TRIAL**

The endothelin pathway is particularly important in several phases of prostate cancer development and progression, but appears to be especially important in the progression of bone metastases [35–38]. In the normal prostate gland, mature endothelin (endothelin-1) is produced by epithelial cells. The highest concentrations of endothelin-1 in the body are found in seminal fluid. In prostate cancer, key components of endothelin-1 clearance, endothelin-B receptor binding [39] and neutral endopeptidase activity are diminished [40], resulting in an increase in local endothelin-1 concentrations. There is also increased endothelin-A-receptor expression with advancing tumour stage and grade in both primary and metastatic prostate cancer [35,41]. By contrast, endothelin-B tends not to be expressed, probably due to gene silencing through methylation of the promoter [36,42,43]. Hence, the endothelin axis is hyperactive in prostate cancer, while the pathway has an important and perhaps essential role in the progression of bone metastases from prostate cancer [37,38].

Atrasentan (ABT-627) is an orally bioavailable inhibitor of the endothelin-A receptor [44]. Atrasentan inhibits prostate cancer cell-related paracrine mitogenic stimulation of co-cultured osteoblasts mediated in part through the insulin growth factor pathway and is thought to be important in the initiation of bone metastases [45,46]. Atrasentan also inhibits cascading self-stimulatory autocrine effects of endothelin-1 during the metastatic process seen in model systems [45].

Atrasentan has completed randomized, placebo-controlled phase 2 and 3 studies in men with HRPC, with time to progression as the clinical endpoint. The phase 2 randomized, controlled trial evaluated the activity of 2.5 mg or 10 mg of atrasentan in patients with metastatic HRPC. In that study of 288 patients, there was a significantly longer median time to disease progression (196 days vs 129 days, P = 0.021) and to PSA progression (155 days vs 71 days; P = 0.002) in the 84 evaluable patients enrolled in the 10-mg arm and the placebo arm [104 men], respectively [47]. Both measures were also longer in the 10-mg group, although the median time to PSA progression was not statistically significant in this arm. Atrasentan was well tolerated, with the most common and significant treatment-related adverse events being headache, rhinitis and peripheral oedema.

Results from the recently reported phase III trial evaluating the 10-mg dose of atrasentan (408 men) vs placebo (401) in patients with metastatic HRPC continued to show beneficial results in favour of atrasentan, although the primary endpoint of disease progression (i.e. new lesions, clinical symptoms, skeletal complications, or pain) were not statistically significant in the intent-to-treat analysis [48]. Nevertheless, increases in bone alkaline phosphatase, total alkaline phosphatase and PSA were significantly reduced in patients treated with atrasentan, suggesting that this agent delays disease progression.

Quality-of-life variables, as measured by the Functional Assessment of Cancer Therapy – Prostate, were also significantly improved with atrasentan, most notably in the pain component of the prostate cancer subscore. As with the earlier trial, the most common adverse events were rhinitis, headache and peripheral oedema. A pooled intent-to-treat meta-analysis of all 1097 patients randomized to receive either atrasentan or placebo in the two trials [47,48] was conducted to more precisely estimate the treatment effect of the agent and to increase the power to detect a modest but clinically meaningful effect [49].

Results of the meta-analysis showed a significant increase in the time to disease progression with atrasentan (P = 0.013), which translated into a 19% reduction (hazard ratio 1.19) in the risk of disease progression. Of note, the improvement was detected by 3 months and was sustained throughout the study period.

There were also significant decreases in the incidence of and the onset to pain in the atrasentan vs placebo groups (P = 0.003). The median pain-free duration in the atrasentan arm was 7 months, which was 97 days longer than in the placebo arm. Patients receiving atrasentan had a lower incidence of pain and remained pain-free longer, for a median of 224 vs 127 days in the placebo arm (Table 3).

There is good preclinical evidence for an additive effect of atrasentan and taxanes. In ovarian cell-line models pretreatment with atrasentan sensitizes the cells to paclitaxel-induced apoptosis [50]. In xenograft models, the combination has additive effects on tumour detumescence, apoptotic indices and angiogenesis [51]. Based on this, and the independent activity of both agents in HRPC, the SWOG designed a protocol (SWOG 0421) to evaluate, in a randomized, placebo-controlled and direct comparison, treatment with docetaxel with or without atrasentan (Fig. 1). With the PFS as the primary outcome and median survival as the main secondary outcome, this trial with 706 patients is powered at 96% to detect a 33% increase in PFS (from 6 to 8 months) and powered at 85% to detect a 30% increase in median survival with the addition of atrasentan to docetaxel-based chemotherapy. Additional outcomes, such as improvement in pain, quality of life, PSA response and its surrogates for survival, objective tumour response and bone turn-over markers, will also be ascertained.
TESTING BIO-CHEMOTHERAPY IN PHASE III SETTINGS: THE CANCER AND LEUKAEMIA GROUP B (CALGB) 9040 TRIAL

An essential step in the metastasis of solid tumours is the growth of new blood vessels, which must be generated for metastases to grow. Vascular growth factors, including vascular endothelial growth factor (VEGF), matrix metalloproteins and integrins, regulate the process of angiogenesis. Inhibiting these targets can arrest tumour growth and inhibit metastatic spread. These vascular growth factors are expressed in both the tissue and serum of patients with prostate cancer [52]. Elevated VEGF levels portend a poor prognosis in HRPC. Bevacizumab, a humanized monoclonal antibody directed against VEGF, is active in combination with chemotherapeutic agents in advanced colorectal carcinoma. A similar therapeutic approach has been undertaken with bevacizumab in prostate cancer. A trial by the CALGB found promising activity in HRPC with the combination of docetaxel 70 mg/m\(^2\) every 3 weeks, estramustine 280 mg oral three times daily on days 1–5 and bevacizumab 16 mg/kg every 21 days [53]; 79 patients were enrolled and nine of 17 evaluable patients had a partial radiographic response. Of 20 patients evaluable for PSA decline, 13 (65%) had a confirmed PSA decline by half. At the time that this trial was reported, the trial had yet to mature and the median survival was not reported. Encouraged by this early indication of significant activity, the CALGB initiated the CALGB 9040 phase III trial (Fig. 2) to evaluate the first bio-chemotherapy combination in phase III settings in HRPC. In cooperation with the Eastern Oncology Cooperative Group and the National Cancer Institute of Canada, the trial is designed to enrol 1020 patients, stratified by the Halabi nomogram [54]. The primary outcome is overall survival with a 95% power to detect a 25% increase in median survival (from 19 to 24 months). Secondary outcomes include PSA response, PFS and response rate. This trial is designed to recruit over 36 months and have data on follow-up for at least 24 months.

SUMMARY

While the phase II trials of epothilones are currently ongoing, ixabepilone (BMS-247550) and patupilone (EP0906) have provided the most convincing phase II data for activity in HRPC, including no cross-resistance with taxanes. This class of compounds is the only new chemotherapeutic to have provided the most advanced data in phase II settings in HRPC. Therefore, the logical next step will be to pursue definitive phase III trials to confirm the activity of epothilones in tandem with docetaxel, given the experience to date. Such trials will lay the foundation for defining the role of epothilones in the first- and second-line settings in HRPC. The distinct toxicity profiles of each of these drugs will probably influence their future development and combination therapy with existing chemotherapy regimens.

Also, for the first time, phase III trials in rationally designed combinations of targeted therapeutics (SWOG 0421 and CALGB 9040) are being undertaken in the USA for patients with HRPC, under the auspices of the major cooperative groups. However, we will not be able to rapidly develop and provide these agents for patients with cancer unless there is a concerted and serious effort by all involved in the care of these patients to enrol them into key clinical trials investigating exciting new classes of compounds.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

Maha Hussein received research support from BMS.

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Abbreviations: HRPC, hormone-refractory prostate cancer; SWOG, South–West Oncology Group; CALGB, Cancer and Leukemia Group B; PFS, progression-free survival; VEGF, vascular endothelial growth factor.
Tumour markers for managing men who present with metastatic prostate cancer and serum prostate-specific antigen levels of <10 ng/mL

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OBJECTIVE

To define immunohistochemical features of the primary cancers that might help in the differential diagnosis and monitoring of treatment in men presenting with metastatic prostate cancer and low serum levels of prostate-specific antigen (PSA), who can be difficult to diagnose and manage.

PATIENTS AND METHODS

Paraffin blocks of prostate biopsies were obtained for 33 patients presenting with untreated metastatic prostate cancer and serum PSA levels of <10 ng/mL. Sections were immunostained for PSA, prostatic acid phosphatase (PAP), prostate-specific membrane antigen (PSMA), androgen receptor (AR), chromogranin A and CD 56.

RESULTS

The combined Gleason scores were 8–10 in 25 men (76%) and 6 or 7 in the other eight (24%). Morphologically, there were no neuroendocrine features. PSA immunostaining was equivocal in 12 (36%) cases and in a further 19 (58%) was strong but focal and could be missed on biopsy sampling. PSMA was expressed in 90% of cases, and staining was widely distributed in nine of the 12 in which PSA staining was equivocal. There was strong AR expression in 30 (91%) cases and it was present in areas where PSA was absent.

CONCLUSION

In this patient group, immunohistochemical assessments of PSMA and AR are potentially useful as diagnostic markers.

KEYWORDS

PSA-negative prostate cancer, prostate-specific membrane antigen, androgen receptor

INTRODUCTION

PSA provides a reliable serum marker for most men with metastatic prostate cancer [1–3], but there is a small group who present with low serum levels of PSA in the presence of metastatic disease [4,5]. The clinical features of the ‘PSA-negative group’ have been described and include men presenting with untreated metastatic disease with serum PSA levels of <10 ng/mL [6]. These cancers have a worse prognosis because they tend to be aggressive and to respond poorly to hormone ablation, and can be difficult to manage because of the lack of a reliable serum marker [6]. The series of cases presented here is the largest group of these patients yet studied [6].

The primary aim of this study was to use PSA and androgen receptor (AR) immunostaining to determine to what extent ‘PSA-negative cancers’ are typical of prostate adenocarcinoma. We then investigated whether immunohistochemical staining of alternative prostate proteins and makers of neuroendocrine differentiation might be useful in patients in whom the serum PSA levels are misleadingly low. The two primary candidates as alternative markers were prostatic acid phosphatase (PAP) and prostate-specific membrane antigen (PSMA), neither of which has been evaluated in the context of low serum levels of PSA. PAP was in widespread clinical use before the advent of PSA testing, but is less sensitive and specific [7] and was superseded. PSMA is more highly expressed in malignant than in benign prostatic tissue [8], but is not used routinely as a tissue or serum marker.

PATIENTS AND METHODS

Patients presenting with untreated histopathologically confirmed metastatic prostate cancer and a serum PSA level of <10 ng/mL were identified from the BAUS Cancer Registry 2000 and 2001 databases, and from local referrals to the Middlesex Hospital, providing a combined total of 33 men. Paraffin-embedded formalin-fixed archival prostatic tissue specimens were obtained. Serial sections of each case were cut and slides stained with haematoxylin and eosin (H&E) and reviewed by a pathologist with a special interest in urological pathology, to confirm the diagnosis and grade of carcinoma. The Local Ethics Committee of the University College of London National Health Service Trust and the South-East Multi-Centre Research Ethics Committee approved the study.

For immunohistochemical staining, 3 μm serial tissue sections from each case were mounted onto Vectabond-coated slides and stained using antibodies to PSA, AR, PSMA, PAP, chromogranin A and CD56 (Table 1). In addition, cytokeratin-7 and -20 and 34βE12 antibodies were used to exclude TCC in three cases with no classical morphological features of prostate cancer [9]. Appropriate positive and negative controls were included for each antibody, and standard methods of antigen
retrieval and previously validated antibody concentrations for all antibodies other than PSMA were used. The optimum dilution of and preferred method of antigen retrieval for PSMA were determined by titrating the antibody using a high-grade radical prostatectomy sample as a positive control.

In brief, the tissue sections were dewaxed in xylene and taken through a series of graded alcohols to water. The method of antigen retrieval, primary antibody concentration and method of staining are also summarised in Table 1. After antigen retrieval, endogenous peroxidase activity was quenched by incubation with peroxidase blocking solution (DAKO K5006). Then 200 μL of each primary antibody was incubated at the required concentration in antibody diluent (DAKO S2022) for 60 min at room temperature. The EnVision system (DAKO), using a one-step system for the secondary antibody, and streptavidin–biotin complex and reducing background staining from endogenous biotin [10], was used for all immunohistochemical staining except PSMA. For the EnVision technique, the EnVision horseradish peroxidase rabbit/mouse reagent (DAKO K5007), containing dextran coupled to peroxidase and goat secondary antibody molecules against rabbit and mouse, was incubated at room temperature for 60 min. The immunoperoxidase antigen-antibody reaction products were visualised by incubation in diaminobenzidine for 10 min at room temperature. The tissue was counterstained with Harris’ haematoxylin for 2 min, dehydrated in alcohols and cleared in xylene.

PSMA was visualised using a goat peroxidase-antiperoxidase system [11]. After antigen retrieval and washing as described above, slides were incubated with normal rabbit serum 1 : 10 for 10 min at room temperature. Primary antibody (goat) was then added for 60 min followed by a 1 : 200 dilution of rabbit antigoat secondary antibody in excess. Then 200 μL of 1 : 100 goat-peroxidase-antiperoxidase was added for 60 min at room temperature with diaminobenzidine detection as before.

Two of the authors (A.F. and A.J.B.) independently assessed the immunohistochemical staining while unaware of sample origin, using a semiquantitative assessment and considering the intensity (0, negative; +, low intensity, just above level of background staining; ++, moderate intensity; ++++, high intensity) and extent (focal/diffuse) of staining. Focal areas containing <10 cells with low intensity staining were classified as negative (Fig. 1).

### RESULTS

In 19 (58%) cases archival tissue was obtained from TRUS-guided prostatic biopsy specimens, with a further 13 specimens derived from TURP and one from a bladder neck resection; 27 of 33 (81%) patients had bone metastases and four presented with soft-tissue metastases. All patients received primary hormonal treatment [6].

Twenty-five men (76%) had a combined Gleason score of 8–10 with the remaining eight (24%) having a score of 6 or 7. No case had morphological features of neuroendocrine differentiation. One case initially classified as a prostatic carcinoma with a combined Gleason score of 10 was subsequently re-classified as a urothelial carcinoma on the basis of immunohistochemical expression of cytokeratin-7 and 34βE12.

Of the 33 cases, nine (27%) were completely negative for PSA and a further three were classified as focal + (Table 2). In a further 19 (58%) cases there was strong staining (++, ++ or +++), but it was focal and could easily have been missed by a needle biopsy sampling. In only two cases was there widespread staining for PSA in the malignant cells. The association of PSA staining with AR, PSMA, PAP, chromogranin A and Gleason score is summarised in Table 2.

There was strong staining for AR in most of the nuclei in 29 (88%) cases, with moderate or strong staining in nine that were negative or focally + for PSA (Table 2). In the three cases that were focally + for PSA, the AR staining was present in areas that were negative for PSA. Only three cases were negative for both PSA and AR, and one was re-classified as a urothelial tumour on the basis of staining for cytokeratin-7 and 34βE12 staining.

Twenty-four (73%) cases were positive for PAP, although the staining was classified as focal in all of these. There was an association between PSA and PAP staining in 19 cases, with 16 cancers showing moderate or strong focal staining for both PSA and PAP and three being negative for both markers.

There was PSMA staining in 30 (90%) of the cases, and it was widespread in 21 of these positive cases. In the 12 cases that were negative or focal + for PSA, PSMA was diffusely positive in 10. The only case that was negative for both PSA and PSMA was re-classified as a urothelial cancer on the basis of cytokeratin staining.

<table>
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*High pH, 25 min microwaving at full power, stand 10 min, in Dako high pH retrieval solution (S3007); NR, no antigen retrieval used; TE, 20 min microwave at full power in Tris-EDTA buffer, pH 9.0; MWD, 25 min microwave at full power, 10 min stand, in Dako Retrieval Solution (S1699); PC, 2 min pressure cooking at full pressure in citrate buffer pH 6.0.

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Chromogranin A immunostaining was negative in 24 cases (72%), eight of which were also negative for PSA (Table 2). In six cases (18%) there was strong but focal immunostaining for the neuroendocrine marker. However, the extent of staining was not considered sufficient to classify these tumours as neuroendocrine. CD56 was focally positive in only three (9%) cases.

Three cases were examined with urothelial markers, as they had no classical morphological features of prostate cancer. Two cases were cytokeratin-7 positive and cytokeratin-20 negative, the other cytokeratin-7 negative and cytokeratin-20 positive. 34BE12 was positive in one cytokeratin-7 positive case, which was reclassified as a urothelial tumour, as it also lacked expression of PSA, PSMA, PAP and AR.

**DISCUSSION**

All 12 of the 33 tumours in which PSA immunostaining was classified as negative or focal + were of high-grade. Although PSA-negative prostate cancers are rare, they are more likely to be found amongst cancers that are high-grade [12]. It has been suggested that such cases undergo ‘de-differentiation’, losing characteristics of prostate epithelial cells and hence altering the relationship between serum PSA levels and tumour volume [13]. This ‘functional de-differentiation’ [14] has been associated with a poor hormonal response and more aggressive biological behaviour. Similarly, first-line hormonal responses and overall survival are shorter in this group of patients [6].

Despite the low serum PSA levels, there was at least some evidence of staining for PSA in >70% the cases, although in many of these the staining was focal and could be missed on prostatic biopsy sampling. Although PSA and PAP are usually concordant in advanced prostate cancer [1], occasional discordance in immunohistochemical studies has been described [15]. The results were concordant for PSA and PAP in most cases, although in only six (18%) was there widespread PAP immunostaining. Thus, PAP staining could also be subject to sampling error on prostate biopsy.

In contrast to PAP and PSA, there was staining for PSMA in all but three cases, and it was...
diffuse in the vast majority, in agreement with previous studies [8,16]. In tumours where there were both PSA-negative and -positive areas PSMA staining occurred in both regions. Focally positive areas of PSA staining might be missed on biopsy, so the present results suggest that PSMA staining may be of value in this patient group to aid diagnosis and confirm prostatic origin despite the low serum PSA level. Alterations in the AR by mutation, deletion or over-expression have all been implicated in the development of hormone-refractory prostate cancer [17]. Given that PSA is an androgen-dependent gene [18], we postulated that AR deletion is a potential mechanism to explain the low levels of serum PSA in untreated metastatic prostate cancer. However, there was strong and widespread staining of nuclei for AR in 88% of cases and it was present in all but three of the cases that were negative or weakly positive for PSA. The widespread AR staining might, like that for PSMA, be of value in the diagnosis of prostate cancer in patients with low serum levels of PSA.

It was postulated that neuroendocrine differentiation is one mechanism responsible for low PSA production in poorly differentiated prostate tumours [19]. In a recent study, tissue neuroendocrine markers, including chromogranin A and neuroendocrine-specific enolase, were identified in 18 patients with clinically progressive androgen-independent prostate cancer and low serum PSA levels [20], suggesting that a proportion of low serum PSA and metastatic prostate cancers were neuroendocrine in origin. In contrast, in the present men with untreated metastatic prostate cancers and low serum levels of PSA, no tumour had a predominantly neuroendocrine phenotype.

CD56, which has been used to detect neuroendocrine differentiation [21], was focally positive in only three (9%) cases. Thus, whilst neuroendocrine differentiation may be one mechanism by which prostate cancers develop resistance after androgen ablation, it does not appear to be a significant factor explaining the low serum levels of PSA in this group of untreated patients.

In conclusion, men presenting with untreated metastatic prostate cancer and inappropriately low levels of serum PSA (<10 ng/mL) are rare, difficult to manage and have a poor prognosis. We identified 33 cancers in this category, the largest series yet described, and used immunohistochemistry on the primary tumours. The results show that PSMA and AR immunostaining are widespread in most of these tumours, including cases in which PSA and PAP staining is negative or weak and focal. Thus, these markers may be of value in the diagnosis of prostate cancer in patients with clinical suspicion of disease but low serum levels of PSA. In addition, the potential value of PSMA as a serum biomarker of prostate cancer merits further investigation.

ACKNOWLEDGEMENTS

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Abbreviations: PSMA, prostate-specific membrane antigen; AR, androgen receptor; PAP, prostatic acid phosphatase; H&E, haematoxylin and eosin.
Age-specific reference levels of serum prostate-specific antigen and prostate volume in healthy Arab men

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OBJECTIVE

To determine age-specific reference ranges for serum prostate-specific antigen (PSA) concentration and prostate volumes in a population of healthy Arab men.

SUBJECTS AND METHODS

Blood samples were taken from 396 healthy Arab men (from Kuwait and Oman) aged 15–79 years and from across the social spectrum. Men aged >40 years had a digital rectal examination and transrectal ultrasonography of the prostate to determine prostate volume. The serum PSA level was measured using commercial kits, and age-specific ranges for PSA levels and prostate volume determined.

RESULTS

The serum PSA ranges (ng/mL) for each age range in Arab men were: 40–49 years, 0–0.9; 50–69, 0–2.7; 70–79, 0–5.5 ng/mL; the respective prostate volumes were 8–22, 9–30 and 10–33 mL. The serum PSA level and prostate volume correlated with age (P < 0.001). Arab men had lower serum PSA levels and prostate volumes than those reported for Caucasians, but similar to those reported for Asians (Japanese and Chinese).

CONCLUSION

These results indicate that Arab men have lower PSA levels and prostate volumes than Caucasians. The levels are slightly lower than those reported in the Japanese and, as in the Japanese, low PSA levels and small prostate volumes might be related to the low incidence of clinical prostate cancer in Arab men.

KEYWORDS

prostate-specific antigen, prostate volume, Arab, Caucasian, Asian

INTRODUCTION

Factors responsible for differences in the incidence of prostate cancer in different parts of the world remain unclear. The incidence is low in Asia (3–8 per 100 000 men per year), intermediate in Africa and Eastern Europe, and high in Western Europe and North America (Fig. 1) [1–4]. PSA remains the most useful clinical tumour marker in the diagnosis of prostate cancer and strategies to enhance its performance include age- and race-specific ranges, measurement of free to total PSA ratio (f/tPSA), PSA density and PSA velocity [5–7]. The aim of the present study was to determine age-specific reference ranges for serum PSA levels and prostate volumes in apparently healthy Arab men aged 15–79 years.

SUBJECTS AND METHODS

Venous blood samples were taken from 396 healthy indigenous Arab men from Kuwait and Oman, aged 15–79 years. Most were selected from healthy men attending the Central Blood Banks in Kuwait and Oman to donate blood. We selected volunteers from all governorates or districts in both countries and representative samples from across different social classes. To be included in the study, men aged >40 years did not have LUTS and had normal urinary flow rates (maximum urinary flow rate >15 mL/s with a voided volume of >150 mL). Men aged >40 years had a DRE and TRUS to determine prostate volume using the formula for a prolate ellipsoid (width × length × height × 0.52). All TRUS examinations were by one radiologist (M.S.) using a Logic 500 Scanner (GE Medical Systems, Milwaukee, WI, USA) with a 7.5 MHz endocavity transducer (model E721), scanning the gland in sagittal and axial planes. The DRE was performed by three experienced urologists (E.O.K., K.A.A. and A.A.). None of the volunteers had any known serious systemic disease or were on any medications known to affect normal hormone production or serum PSA levels, in accordance with previously established standards [8]. Other demographic data collected from each volunteer included social habits, particularly smoking, marital status and whether they had problems with fertility. Each patient’s weight and height were also recorded.

Serum total and free PSA levels were measured using third-generation kits (Immulate, Diagnostic Products Corp. Inc. Webster, TX, USA). Serum samples were aliquoted in 2.5-mL batches and stored at −80 °C until analysis within 6 months of sample collection. The study was approved by the Local Ethics Committee and informed consent was obtained from all volunteers. The volunteers were further informed that, should a disease be diagnosed as a result of these tests, they would be informed and additional tests conducted before recommending further treatment.

Volunteers found to have an abnormal DRE irrespective of PSA value, or a PSA level of >10 ng/mL, had a sextant prostate biopsy with TRUS guidance. Similarly, volunteers with an abnormal echo pattern at TRUS had a prostate biopsy. The biopsies were taken from the apex, middle and base of the right and left lobes in the parasagittal plane. If a hypoechoic lesion was detected in the peripheral or
FIG. 1. Incidence (age standardized rate) of prostate cancer by world region (using data from [1]).

USA Blacks
USA Whites
Australia
Germany
England Wales
Zimbabwe (White)
Zimbabwe (Black)
Kuwait
China (Shanghai)

0 50 100 150
Number of cases per 100 000 men/year

FIG. 2. Frequency distribution of total PSA profile in normal Arab men. Median 0.47, 396 men, reference range 0.05–5.51.

TABLE 1 Mean (sd) serum levels of total PSA, free PSA, and f/tPSA, and prostate volumes in normal Arab men*

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>N patients</th>
<th>Total PSA, ng/mL</th>
<th>Free PSA, ng/mL</th>
<th>f/tPSA, %</th>
<th>Prostate volume, mL</th>
<th>Range, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–19</td>
<td>52</td>
<td>0.32 (0.25)</td>
<td>0.07 (0.05)</td>
<td>25 (6.12)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>20–29</td>
<td>68</td>
<td>0.56 (0.32)</td>
<td>0.12 (0.11)</td>
<td>21.1 (3.11)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>30–39</td>
<td>64</td>
<td>0.49 (0.33)</td>
<td>0.11 (0.08)</td>
<td>21.2 (4.22)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>40–49</td>
<td>62</td>
<td>0.55 (0.68)</td>
<td>0.13 (0.25)</td>
<td>23.2 (5.22)</td>
<td>14.4 (2.3)</td>
<td>8–22</td>
</tr>
<tr>
<td>50–59</td>
<td>78</td>
<td>1.12 (2.09)</td>
<td>0.18 (0.17)</td>
<td>14.2 (2.67)</td>
<td>17.8 (3.3)</td>
<td>9–27</td>
</tr>
<tr>
<td>60–69</td>
<td>60</td>
<td>2.76 (4.26)</td>
<td>0.43 (0.43)</td>
<td>14.8 (3.11)</td>
<td>22.4 (6.1)</td>
<td>9–30</td>
</tr>
<tr>
<td>70–79</td>
<td>12</td>
<td>4.14 (3.49)</td>
<td>0.68 (0.58)</td>
<td>14.2 (2.31)</td>
<td>22.9 (4.1)</td>
<td>10–33</td>
</tr>
</tbody>
</table>

*Arab men (Kuwaitis and Omans), none had symptoms of prostatism, all had normal urinary flow rates.

daily for 3 days. Men found to have prostate cancer were excluded from the analysis.

All assays were carried out while unaware of sample origin. Quality control samples were included within assay runs, as specified by the kit’s manufacturers. The number of serum samples analysed for PSA or free PSA was determined when a statistically significant trend was established with age from regression plots. The interassay coefficients of variation for PSA and free PSA were 2.1% and 5.1% respectively, and the intra-assay coefficients of variation were 3.1% and 3.2%, respectively.

Descriptive statistical methods were applied for the entire study cohort. For normally distributed variables the reference range was the mean ± 2 SD, while for those with non-normal distribution the reference range was the median [2.5–97.5 percentile]. Pearson product-correlation coefficients were calculated to measure the association between serum PSA levels and age, prostate volume and age, and PSA levels and prostate volume. For all analyses, P < 0.05 was considered to indicate significance.

Nomograms showing the distribution of serum PSA levels and prostate volumes as a function of age were generated from least-squares regression models. A nomogram of the distribution of serum PSA levels as a function of prostatic volume was also constructed similarly.

RESULTS

Table 1 shows the mean (sd) total PSA, free PSA and f/tPSA levels in normal healthy Arab men aged 15–79 years, and the number of men sampled in each age group, which ranged from 52 in those 15–19 years old to 12 in those aged 70–79 years. The few men in the latter group reflects the scarcity of this age group in Kuwait and Oman, where the population distribution is skewed, with more than half the population aged <25 years, and thus finding volunteers aged >60 years who were suitable for inclusion in this study proved difficult. The mean (sd) PSA level was 0.56 (0.70) ng/mL for men aged 40–49 years and 4.79 (3.65) ng/mL for men aged 70–79 years. Figure 2 shows the frequency distribution of total PSA in these Arab men; it is not a normal distribution, hence the median PSA of 0.47 ng/mL and reference range of 0.05–5.51 (2.5 and 97.5 percentiles) is more appropriate than using the mean. Figure 3 shows the good correlation (r = 0.520) between total PSA level and age in Arab men, with the total PSA value for age derivable as 0.076 (age) + 1.754 ng/mL (P < 0.001). Free PSA values closely mirrored those of total PSA (Figs 4 and 5); like total PSA, free PSA increased with age (P < 0.001), but f/tPSA declined with age (Table 1).

Table 1 also shows that the mean prostate volume and range increased with age in Arab men; the mean (sd) volume was 14.4 (2.3) mL for men aged 40–49 and 22.9 (4.1) mL for men aged 70–79 years.

The relationships between serum levels of PSA and fPSA and age were similar to the trends previously reported for Caucasians. The
numbers of serum samples analysed for PSA and free PSA were determined when a statistical significant trend \((P < 0.001)\) with age was established, as shown in Figs 3 and 5. Thus total and free PSA levels showed progressive increases with age (Figs 3 and 5).

**DISCUSSION**

The present study shows that, as in other communities, the reference range for total PSA levels and prostate volume must be established for each community, as there are substantial differences in normal reference values. For clarity, the values determined in the present study are tabulated with values reported previously for White men from the USA, Japanese, and Chinese (Table 2). In all four populations, PSA levels and prostate volumes increased with age. For Arab men, the normal median total PSA level of 0.45 ng/mL is lower than the 0.8 ng/mL reported for Japanese men \([5]\). Similarly Arab men aged >59 years have smaller prostates than Japanese men \((P < 0.06)\) and White men \((P < 0.001)\). As shown by studies in other populations, the present study also confirmed that there is a significant \((P < 0.05)\) correlation between patient age, total PSA level \((r = 0.387)\) and prostate volume \((r = 0.29)\) \([5,9–11]\).

In 1993, Oesterling et al. \([9]\) and Dalkins et al. \([10]\) developed age-specific reference ranges for serum PSA levels for White men from the USA; the reference ranges are higher than for Japanese men, which in turn are higher than for Arab men (Table 2). Chinese men appear to have the lowest age-specific reference ranges for PSA levels, as reported by He et al. \([12]\) (Table 2). For each age group, from 40–79 years, the upper limit of normal for serum PSA levels for Arab men is lower than in Japanese or in White men. The clinical implication of this finding is that the serum PSA value for an Arab man has a different clinical meaning than the same value for a similarly aged Japanese or a White man. Hence, whereas a PSA level of 2 ng/mL will be considered normal for a White man or a Japanese man aged 40–49 years, for an Arab man of the same age and PSA value, the possibility of prostate cancer needs to be excluded. The Japanese PSA values were measured with the IMx PSA assay (Abbott Laboratories, Abbott Park, IL, USA) and those of the White men reported by Oesterling et al. \([9]\) by the Tandem-R PSA assay (Hybritech Inc. San Diego, CA, USA), whereas we used third-generation Immulite kits. However, it is unlikely that the lower values in Arab men can be attributed solely to the diagnostic kits used \([5,9,13,14]\); as Jacobsen et al. \([13]\) showed, PSA is fairly stable, especially if the serum has been separated early after blood sample collection and stored appropriately. This implies that the assay technique should not substantially affect the values of PSA measured, and this was confirmed by Junker...
et al. [14], who compared four different total and free PSA assays and found no significant difference in the mean values obtained, especially when the total PSA level was < 25 ng/mL. Furthermore, as prostate glands in Arab men are smaller than in White or Japanese men, and as it is known that the larger the prostate the higher the PSA level, the smaller prostate in Arab men is the most likely explanation for the low PSA levels [15].

Prostate volume was measured with the formula used by Oesterling et al. [9] for White men and by Oesterling et al. [5] for Japanese men, so the differences in PSA values and prostate volumes are probably real. After adjusting for prostatic size and patient’s age, there is still a significant difference in PSA values between White and Arab men (P < 0.001). The higher levels of serum PSA beyond that which can be accounted for by prostate size alone might also reflect differences in the cellular composition of the prostate glands in the three communities. This has clinical implications for prostate pathology; e.g. while the autopsy prevalence of latent prostate cancer shows little racial or geographical variation, the autopsy prevalence of ‘proliferative’ (more extensive and less well differentiated) latent prostate cancer shows racial and geographical variations similar to those seen for clinically diagnosed prostate cancer [16,17]. Similarly, from local experience of TURP for BPH in Arab men over a 10-year period, the prostate causing symptoms of BOO is smaller than in Caucasians, and histology tends to show that the prostate has more stromal elements and fewer glandular elements than found in Caucasians [18]. Finally, a recent prospective report indicated that, in men with no prostate cancer at initial screening, the risk of developing prostate cancer in any given 4-year period is greater for Dutch men aged 55–69 years than for their Japanese counterparts, because the former have higher normal PSA levels [19].

The f/tPSA in the Arab men studied also declined with age, from 25% in those 15–19 years old to 14.2% in those aged 70–79 years. There is considerable disagreement about the effect of age on f/tPSA in healthy men; some have reported an increase with age [20,21], some a decrease [22], and others claim that f/tPSA is independent of age [23]. Reasons given for these differences include that in some of the studies [23] prostate cancer was not excluded in men whose sera were used for the analysis. The present study included only men who did not have clinically detectable prostate cancer, so the present finding that age independently influences f/tPSA is likely to be more accurate, and is consistent with the findings of Lein et al. [22].

However, it would appear that the only reason to determine the f/tPSA is as a guide as to whether to take a prostate biopsy, to exclude prostate cancer for men with PSA levels in the ‘grey zone’ of 4–9.9 ng/mL. Thus, it is used to enhance the specificity of total PSA values in the grey zone [24,25].

The value of estimating f/tPSA in normal men is at present unclear. However, Carter et al. [26] argued that men with no prostate cancer but with very low f/tPSA levels are at greater risk of developing prostate cancer. They further stated that the lower the value, the more aggressive the prostate cancer that the patient develops [26]. The threshold for suspicion of prostate cancer is a f/tPSA of <14.5% [22]. In the present study, the mean f/tPSA in normal men in the ‘prostate cancer years’ (60 years) was 14.2%, about the same as the threshold for the diagnosis of suspicion of prostate cancer. This high mean f/tPSA in Arab men might be another reason for their low incidence of prostate cancer. Alternatively, it might reflect an underlying biological difference between Arab and Caucasian prostates.

In conclusion, the present study confirms earlier reports that serum PSA levels and prostate volume are age- and race-dependent, so it is appropriate to have age-specific reference ranges for these variables in various communities around the world. This will enhance the positive predictive value of PSA estimation in the diagnosis of prostate cancer in each community. The present results indicate that Arab men have lower PSA levels and prostate volumes than reported previously for White men. The levels are only slightly lower than those found in Japanese men. As in Japanese and Chinese men, low PSA levels and small prostate volumes might partly explain the low incidence of prostate cancer in Arab men.

**CONFLICT OF INTEREST**

None declared. Source of funding: Kuwait University Research Grant MS 01/99.

**ACKNOWLEDGEMENTS**

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**TABLE 2** Comparison of serum PSA levels and prostate volumes as a function of age in healthy White men from the USA [8], Japanese [5], Chinese [12] and Arab men

<table>
<thead>
<tr>
<th>Age range, years</th>
<th>Serum PSA range, ng/mL</th>
<th>Prostate volume range, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USA White*</td>
<td>Japanese*</td>
</tr>
<tr>
<td>40–49</td>
<td>0–2.5</td>
<td>0–2–2</td>
</tr>
<tr>
<td>50–59</td>
<td>0–3.5</td>
<td>0–3</td>
</tr>
<tr>
<td>60–69</td>
<td>0–4.5</td>
<td>0–4</td>
</tr>
<tr>
<td>70–79</td>
<td>0–6.5</td>
<td>0–5</td>
</tr>
</tbody>
</table>

* Data from [5,8]; † Data from [12]; P < 0.001 (Arab vs USA White); P < 0.01 (Arab vs Japanese); P calculated using Student’s t-test.
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Abbreviations: f/PSA, free/total PSA.
Analysis of peripheral blood for prostate cells after autologous transfusion given during radical prostatectomy

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Accepted for publication 29 March 2005

OBJECTIVES
To determine if cells expressing prostate-specific antigen (PSA) can be detected in blood collected by a cell-saver during radical prostatectomy (RP) or in the peripheral blood after intraoperative autotransfusion (IAT).

PATIENTS AND METHODS
In all, 112 men with clinical T1c–T2 prostate cancer undergoing RP were prospectively assessed. A cell-saver system was used in each to collect blood from the surgical field after prostate manipulation. IAT was given based on clinical indications. Standardized peripheral blood samples were collected from patients before RP, in the recovery room afterward, and at 3–5 weeks after surgery. A reverse-transcriptase-polymerase chain reaction assay for PSA mRNA was used to detect prostate cells in cell-saver and peripheral blood samples. Patients were followed after surgery with PSA measurements to assess biochemical failure.

RESULTS
PSA-expressing cells were detected in 88% of cell-saver reservoir and 13% of preoperative blood samples. No PSA-expressing prostate cells were detected in any peripheral blood samples collected 3–5 weeks after surgery. Analysis of data with 40 months of follow-up showed IAT was not an independent predictor of biochemical failure in multivariate analysis.

CONCLUSIONS
Although IAT blood contains PSA-expressing cells, none could be detected 3–5 weeks after surgery. IAT during RP was not associated with a greater risk of biochemical failure.

KEYWORDS
prostate cancer, surgery, transfusion, PSA, cells

INTRODUCTION
Substantial blood loss during radical prostatectomy (RP) frequently necessitates immediate resuscitation. Intraoperative autotransfusion (IAT) of blood collected from the surgical field has been proposed as an alternative method of volume support, but its use has been limited because of the theoretical possibility of haematogenous tumour dissemination [1]. Long-term retrospective studies support the safety of IAT use during RP [2–4] but prospective analysis of possible haematogenous dissemination from IAT during the perioperative and immediate postoperative state has not been explored. RT-PCR offers a unique tool for studying this question, as it can be used to identify prostate tumour cells within the peripheral circulation before, during and after prostate cancer surgery [5–7]. The goal of the present study was to use RT-PCR to determine if PSA-expressing prostate cells could be detected in the peripheral blood immediately after IAT or after RP.

PATIENTS AND METHODS
Men with clinical T1c–T2 prostate cancer undergoing RP were assessed prospectively between 1994 and 1997; enrolment was based on informed patient consent and patient availability after RP. Exclusion criteria included previous pelvic external beam radiation therapy and radiological evidence of metastasis on CT or bone scan.

All patients had a retropubic RP using the standardized technique defined by Walsh et al. [8]. A Brat2 Cell Saver (Cobe Cardiovascular, Arvadawa, CO) was used to collect blood from the surgical field after dividing the dorsal venous complex. All IAT blood was processed with centrifugation and saline washes by the device before repackaging; no additional filters were used. Indications for transfusion were based on clinical assessment by the anaesthesiologist using intraoperative haematocrit, blood pressure and heart rate as guidelines. If immediate transfusion was required, IAT was used preferentially over allotransfusions or pre-donated units if >200 mL of intraoperatively collected blood was available. Standardized 5 mL samples from the Cell Saver of each patient were collected in EDTA-dosed specimen tubes after the blood was processed and centrifuged. Standardized 5 mL samples of blood were also collected from each patient’s peripheral venous circulation 1 h before surgery, in the recovery room after completing RP, and 3–5 weeks after surgery.

All samples were maintained at 4 °C and analysed within 24 h of collection using an RT-PCR assay for PSA mRNA, previously described by Katz et al. [5]. Briefly, samples were diluted with PBS, layered and centrifuged. The nucleated cell layer was then recovered and washed again with PBS. RNA was extracted from the cells using the guanidinium thiocyanate/phenol/chloroform extraction technique described by Chomezynski et al. [9]. A sample of 1 μg of RNA was used for the reverse transcription, with PCR then used for 35 cycles, using the PSA-specific oligonucleotide primers: PSA 3′-5′-CACAGACACCCCATCCTATC-3′ and PSA 3′-5′-GAGATGACAGACCCATTATC-3′.
5′-5′-GATGACTCCAGCCACGACT-3′. Samples of β2-microglobulin were used as internal controls for the reaction. Aliquots of the reaction were then separated with electrophoresis on 2.5% agarose gels, stained with ethidium bromide and the 710 bp target identified under ultraviolet light. The identity of the amplified product was established through radiolabelled PSA oligonucleotide probes. A positive assay was defined as any detectable PSA-expressing cells in the sample. Additional blood samples from 11 patients, including five men and six women undergoing surgery unrelated to prostate cancer, were also analysed for PSA before surgery.

In all, 112 men were assessed prospectively; 48 received IAT (group 1) and 64 were not transfused (group 2). Age, PSA level before RP, operative blood loss, volume of IAT used, pathological cancer grade and stage, and PSA level after RP were recorded for each patient. Patients were assessed with serum PSA for biochemical failure at the first visit after RP, 6 months after surgery and then yearly. Biochemical failure was defined as a PSA level of >0.2 ng/mL or initiation of adjuvant therapy with radiation or hormonal manipulation.

Continuous and categorical variables were analysed statistically using Student’s t-test, Fisher’s exact tests, or the chi-square test. Multivariate analysis used Cox proportional hazard models to determine hazard ratios associated with any increased risk of biochemical failure, with P < 0.05 considered to indicate significance in all tests.

RESULTS

There was no statistically significant difference in age, preoperative PSA level, Gleason score and tumour stage after RP between the groups. Patients in group 1 receiving IAT had a larger mean estimated blood loss (1645 mL) than those in group 2 (1205 mL, P < 0.007), with those in group 1 receiving a mean IAT transfusion volume of 566 mL. Table 1 compares the demographics of the two groups.

Molecular analysis was available on 97 preoperative peripheral and Cell Saver blood samples. PSA-producing cells were identified in 13% and 88% of the respective samples. Peripheral blood taken immediately after and 3–5 weeks after surgery was analysed for 19 and 28 patients in groups 1 and 2, respectively; samples could not be obtained from the remaining patients because there was no follow-up by the primary surgeon within the study period. Immediately after surgery, three patients (16%) in group 1 had PSA-producing cells in their peripheral blood, vs only one (4%) of those in group 2 (P = 0.29). However, at 3–5 weeks after surgery, no PSA-producing cells were detected in the peripheral blood from either group. Two of the 40 control samples from 11 different patients with no known prostate cancer (5%) tested positively for PSA.

Both groups of patients were followed for a mean of 43 and 46 months, respectively, and data were collected for 47 and 53 patients in each respective group. There were nine biochemical failures in group 1 and 17 in group 2. Data were unavailable for three patients in each group. Cox adjusted hazard models showed that IAT was not an independent predictor of biochemical failure, with an adjusted hazard ratio of 0.766.

To explain these findings we suggest that PSA-producing cells identified in the Cell Saver samples sustained structural damage during IAT processing that ultimately made them nonviable in an in vivo environment. Karczewski et al. [15] examined this premise and noted that 62% of tumour cells suffered lethal trauma after processing by an autotransfusion device, and the remaining cells had morphological changes. The damaged PSA-expressing cells in the present study may also have been rapidly cleared after transfusion by the patient’s cellular immune system [16]. More studies are needed to confirm these findings and discover possible
mechanisms by which PSA cells are cleared from the peripheral circulation after IAT.

A theoretical limitation in the present study was the use of RT-PCR to identify PSA-producing cells in the peripheral circulation. In other studies, RT-PCR assays have been shown to have inconsistent detection rates of PSA-producing cells [17]. However, we could reproducibly detect 10 PSA-producing cells per 10^6 human B-lymphocytes with only a 5% false-positive rate. Consequently, we are confident in our ability to detect relevant levels of circulating PSA-producing cells.

Although we found that IAT was not an independent predictor of biochemical failure after RP, the few patients and relatively short follow-up prevented the detection of small changes in survival rate between the groups. However, interestingly, tumour stage was an independent predictor of biochemical failure, and tumour Gleason score approached significance as an independent predictor. As these variables are well known as independent predictors of biochemical failure after RP [18], we suggest that the study could identify strong predictors of failure.

In conclusion, although IAT samples contain PSA-expressing cells, few cells can be detected in the peripheral circulation hours after transfusion. No cells can be detected by the first visit after RP. In the present patients, the use of IAT during RP was not associated with a greater risk of biochemical failure.

CONFLICT OF INTEREST

None declared.

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Abbreviations: RP, radical prostatectomy; IAT, intraoperative autotransfusion.
New perioperative management reduces bleeding in radical retropubic prostatectomy

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OBJECTIVE
To describe the effect of modifications to radical retropubic prostatectomy (RRP, known to be associated with severe bleeding) on blood loss in a retrospective analysis comparing RRP by one experienced surgeon before and after the changes.

PATIENTS AND METHODS
The new method comprised reducing the intravenously applied volume, using a peridural catheter and maintaining a 25–30° Trendelenburg position. The difference in haemoglobin before and after RRP was analysed before the changes (group 1) and after (group 2). If transfusions were required the haemoglobin value was corrected, whereby 1 mL of erythrocyte concentrate increased the patient’s haemoglobin by 0.03 g/L.

RESULTS
Assessment was possible in 201 of 234 patients, 110 from group 1 and 91 from group 2. The mean transfusion-corrected difference in haemoglobin was 53 g/L in group 1 (20% transfusion rate) and 35.2 g/L in group 2 (1.09% transfusion rate; \( P > 0.001 \)). The median intravenous volume applied was 5.96 L in group 1 and 3.49 L in group 2 (\( P < 0.001 \)). The complication rate did not differ between the groups.

CONCLUSION
This new method minimizes the intraoperative blood loss during RRP; transfusions are only necessary in rare cases and the complication rate remained unaltered.

KEYWORDS
prostate cancer, radical retropubic prostatectomy, bloodless surgical technique, blood transfusions

INTRODUCTION
Radical retropubic prostatectomy (RRP) is known to involve severe bleeding; even experienced surgeons report a blood transfusion rate of up to 20% [1–5]. Santorini’s plexus and other venous plexuses of the pelvic floor have to be severed before the intervention. Moreover, potency-sparing surgery requires keeping coagulation or any mechanical haemostasis at a minimum in the area of the fragile neurovascular bundles that must be preserved [6].

We modified our perioperative method in various ways in January 2001, with the aim of minimizing vessel-filling in the minor pelvis and thus reducing the probability of bleeding. We describe the effect of these modifications on blood loss in a retrospective analysis comparing \( \approx 100 \) RRP by one experienced surgeon before the change (group 1) with \( \approx 100 \) thereafter (group 2).

PATIENTS AND METHODS
In January 2001, the perioperative routine for RRP was modified as follows: (i) we reduced the intraoperative fluid volume, particularly up to the time of resection; (ii) we inserted a peridural catheter (T12–L2) and administered bupivacaine; (iii) we used 25–30° of Trendelenburg positioning. The analysis included 234 patients who had RRP between 1998 and 2003 (120 before and 114 after the change), all by one very experienced urologist.

No basic changes were made in the surgical technique that could have affected the results. An extensive lymphadenectomy was used routinely in all patients. Patients were excluded from further analysis if they had a haemorrhagic disease or idiopathic anaemia, or if they had additional interventions potentially involving severe bleeding or long operating times under the same anaesthesia (e.g. hemicolectomy). Another exclusion criterion was previous radiotherapy or TURP. Patients were included only if their written anaesthetic protocol permitted clear assignment to one of the groups and whose documented laboratory values enabled a follow-up, particularly of their haemoglobin level. The examination covered the haemoglobin loss calculated from the difference between that before RRP and that on the evening after RRP. The value after RRP of patients with blood transfusions during surgery was corrected by 0.03 g/L per mL of transfused erythrocyte concentrate, considering that 1000 mL of erythrocyte concentrate contains 15 g of haemoglobin, and that a 63-year-old man has a mean intravascular volume of 5 L; e.g. the transfusion of 250 mL raises the haemoglobin level by 7.5 g/L. In addition, during the follow-up, thrombocytes and serum creatinine levels were analysed.

The retrospective morbidity analysis of the intervention was based on the complications listed in the patient’s records. Preoperative comorbidity was documented using the Charlson Comorbidity Scores [7].
RESULTS

After applying inclusion and exclusion criteria, 201 patients were assessable, 110 in group 1 and 91 in group 2; most patients excluded from the analysis had surgery in the transitional phase and could not be clearly assigned to a specific group. A few had no laboratory values to document the change in haemoglobin, or had additional major interventions under the same anaesthesia.

The epidemiological variables did not differ between the groups; the patients had a median age of 63 years, a preoperative PSA level of 9 ng/mL and a Charlson Comorbidity Score of 0 (maximum of 2 points in all patients). Patients of both groups mostly had clinical stage T1c (half in each group); the highest stage being cT2c (three patients in each group).

No basic changes were made in the surgical technique during the observation period, although the fraction of nerve-preserving operations was increased from 51% in group 1 to 75% in group 2.

Table 1 shows the blood loss as the difference in haemoglobin; there was a very significant difference in the transfusion-corrected haemoglobin difference (53 vs 35.2 g/L; \( P < 0.001 \)) and in the transfusion rate, at 20% in group 1 and 1.09% in group 2 (\( P < 0.001 \)). The applied volume during RRP was 5.96 L in group 1 and only 3.49 L in group 2 (\( P < 0.001 \)). The remaining variables did not differ significantly between the groups, e.g. epidural catheter period (median 4 days, SD 1.4), other laboratory variables, number of complications, etc. In particular, in group 2 with a restricted intraoperative volume, there was no increase in acute pre-renal failure. The serum creatinine level did not differ significantly between the groups at the time of discharge, at 90.2 \( \mu \text{mol/L} \) in group 1 and 85.1 \( \mu \text{mol/L} \) in group 2 (\( P = 0.1 \)). Epidural anaesthesia reduced the mean arterial pressure, but not to a degree that required therapeutic intervention.

The complications are listed in Table 2; there were major complications in 4.5% of group 1 and 5.5% of group 2, and the difference was not statistically significance.

DISCUSSION

Large series of open RRP have reported very good oncosurgical results, but even highly experienced surgeons have an average transfusion rate of 4–50% [8–13]. The present technique might be a first step to solve this problem. The combination of reduced intraoperative fluid, use of an epidural catheter and of the Trendelenburg position resulted in significantly less blood loss and transfusions, while complications did not differ. The novel aspect of the present compared with previous studies is the evaluation of the effect of combining commonly used techniques, e.g. reduced intraoperative fluid, epidural anaesthesia and Trendelenburg position. In a study by Shir et al. [14], the influence of epidural anaesthesia combined with general anaesthesia during RRP gave no significant difference in blood loss from general anaesthesia alone, but when epidural anaesthesia only was used there was a positive influence. Unfortunately, that study had no additional information about possible changes in blood pressure during surgery. The reduction in blood pressure in the present study underscores the effectiveness of epidural anaesthesia and is thus an indirect sign of effective sympathicolysis. However, it did not result in hypotension that required therapeutic intervention.

The failure to detect positive effects when combining general and epidural anaesthesia [14] might be a result of various factors, e.g. insufficient epidural anaesthesia, the absence of Trendelenburg positioning or reduced intraoperative fluid. The combination of these three factors appears to contribute the most to the present findings. Earlier studies in the 1960s, examining the effect of a peridural catheter and/or relative hypotension during open prostate enucleation, showed less blood loss during surgery [15,16].

Findings reported by Barré et al. [17] show that Trendelenburg positioning alone likewise markedly reduced the probability of bleeding. They determined the absolute blood loss and that through drainage, while in the present study we assessed haemoglobin loss, and thus the results are not directly comparable. In the present study we also reduced fluid volume and used an epidural catheter to enhance the effect of low vessel filling in the minor pelvis.

The study by Barré et al. [17] also describes the use of Trendelenburg positioning with no leg-
down tilt. In the present study we used a 10° of leg-down tilt to improve visualization of the minor pelvis, without reducing the effect of the head-down position. The abdomen and upper body were tilted downward by 25–35°. The result was always a head-down position, and in relation to the legs. This position initially impairs visibility in the minor pelvis, but the disadvantage can easily be overcome by using a xenon head-lamp, by which none of the interventions in group 2 required a temporary or permanent change of Trendelenburg position to improve visibility.

Even patients who have laparoscopic surgery are placed in a (sometimes extreme) Trendelenburg position. The low blood loss reported [18,19] may also be attributable to this positioning and not only to the high CO₂ pressure. Another option to achieve effective sympatholysis is a combination of a higher dose of inhalative narcotics and opiates. Our department is currently performing a prospective randomized study to determine which of the individual factors is crucial and which can be omitted. In a first approach, the influence of general anaesthesia in combination with a peridural catheter is compared to general anaesthesia alone. Patients in both groups are in a Trendelenburg position and have relative hypovolaemia.

In the present study, the haemoglobin difference measured was corrected for any transfusion [as 1 L of erythrocyte concentrate contains 15 g of haemoglobin and the intravascular volume of a 63-year-old man is ~5 L]. However, the variability of the latter factor could reduce the accuracy of the present retrospective analysis. Thus, the recently initiated prospective study will analyse not only the laboratory values but also the volume in the aspirator, the weight of the abdominal pads and the volume, as well as the haemoglobin fraction in the drainage. However, analysis of the change in haemoglobin alone appears to be valid, as the blood loss is known to be frequently underestimated with the other variables (volume in the aspirator, etc.) [20]. Patients in group 1 received significantly more intravenous fluids, which may lead to dilution and misinterpretation of the intraoperative haemoglobin levels. As the effect from intravenous dilution will disappear 24 h after surgery, the difference in haemoglobin level was evaluated by comparing those before and 24 h after surgery.

The present study detected the relative sympatholytic effect of the epidural catheter by a decrease in arterial pressure. A markedly reduced arterial pressure can impair ‘sensitive’ organs like the brain, heart and kidneys [21,22], but the incidence of acute renal failure and myocardial infarction was no greater, possibly because cardiovascular patients were not included. Brain function was not objectively assessed either before or after surgery; there were no reported possible adverse effects of Trendelenburg positioning, e.g. oedema in the upper extremities or face. The complication rate was in the range reported by others [23,24].

Theoretically, as more nerve-sparing RRs are conducted an equal increase of blood loss subsequent to such surgery might be expected. In contrast, blood loss was significantly lower than in former, non-nerve sparing procedures, underlining the effectiveness of the new perioperative management of patients undergoing RRP.

There were three intraoperative rectal lesions in group 1 but only one in group 2. All cases had local spread of a tumour with extracapsular extension. Better selection helps to minimize such major complications.

In conclusion, blood loss during open RRP is minimized by using the present method, involving both urologists and anaesthesiologists, and comprising reduced intraoperative fluids, a peridural catheter and Trendelenburg positioning; with this combination, blood transfusions are required only in rare cases.

CONFLICT OF INTEREST

None declared.

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Abbreviations: RRP, radical retropubic prostatectomy.
Do all patients with high-grade prostatic intraepithelial neoplasia on initial prostatic biopsy eventually progress to clinical prostate cancer?

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OBJECTIVE
To assess the clinical outcome of patients with a diagnosis of high-grade prostatic intraepithelial neoplasia (PIN) on initial prostatic biopsy, with a minimum of 5 years of follow-up, as such patients are at greater risk of having prostate cancer on subsequent biopsy.

PATIENTS AND METHODS
Between November 1992 and October 1998, 21 patients were identified as having PIN on their initial transrectal ultrasonography-guided prostate biopsy. None of these patients had a focus of cancer on the initial biopsy. Their medical data were reviewed retrospectively to determine the natural history of PIN in these patients. Patients who were not identified as having cancer were followed every 6–12 months with prostate-specific antigen (PSA) testing and digital rectal examinations (DRE).

RESULTS
A mean (range) of 7 (2–8) cores were taken at initial biopsy; the mean age of the patients was 63 (53–77) years and mean PSA level 9.1 (4.9–17.6) ng/mL. Six patients had an abnormal DRE at presentation. A mean of 8 (7–10) cores were obtained on the second biopsy; six patients were diagnosed with cancer, with a mean Gleason score of 6 (5–7), while three were diagnosed with persistent PIN. These three patients had a third prostate biopsy which showed cancer of Gleason score 6 in one and benign prostatic hyperplasia in two. After a mean follow-up of 72.2 (60–84) months, none of the remaining 12 patients was diagnosed with clinically significant cancer. Five of these patients went on to a third prostate biopsy, with no evidence of cancer. One patient died from unrelated causes during this period.

CONCLUSION
This study affirms our current practice of following patients with PIN conservatively if a second or third subsequent prostate biopsy is negative. Whether PIN is a premalignant lesion or merely a lesion associated with cancer needs to be addressed in multicentre studies with a follow-up of >10 years.

KEYWORDS
prostatic intraepithelial neoplasia, carcinogenesis, prostate neoplasm

INTRODUCTION
Patients with high-grade prostatic intraepithelial neoplasia (PIN) on initial prostate biopsy are at greater risk of having prostate cancer on subsequent biopsy. The mean (range) incidence of PIN is 9 (4–16)% of prostate biopsies [1]. When PIN is found on initial sextant prostate biopsy, there is a 22–58% risk of finding cancer on subsequent biopsies [2–4]. With extended prostate biopsies, the chance of finding cancer on immediate repeat prostate biopsies appears to be significantly less [5,6]. PIN has been estimated to precede the onset of cancer by 5 to >10 years [1]. It is unknown if men will significantly benefit from therapy for PIN. To our knowledge there are no published long-term reports studying the outcome of men diagnosed with PIN in initial TRUS-guided prostate biopsy. Therefore, we assessed the clinical outcome of patients with a diagnosis of PIN on initial prostate biopsy and with ≥5 years of follow-up.

PATIENTS AND METHODS
To obtain data with ≥5 years of follow-up, all patients who, between November 1992 and October 1998, had a TRUS-guided prostate biopsy by one radiologist (D.D.) were evaluated. All patients with a focus of cancer on their initial biopsy were excluded. Twenty-one patients were identified to have PIN on their initial prostate biopsy; none of these patients had a focus of cancer on the initial biopsy. Their medical data were reviewed retrospectively to determine the natural history of PIN in these patients; they were followed every 6–12 months with PSA testing and a DRE. Patients and their family physicians were contacted to ascertain and confirm the most up-to-date clinical follow-up data. The decision to proceed with a third prostate biopsy was based on latest PSA level, change in PSA level, DRE, age, comorbidities and patient preference. There was no specific or consistent rate of PSA change that prompted another biopsy.

RESULTS
A mean (range) of 7 (2–8) cores were taken at the initial prostate biopsy. The mean age of the patients was 63.5 (53–77) years and the mean PSA level 9.1 (4.9–17.6) ng/mL. Six patients had an abnormal DRE at presentation. All 21 patients had a second prostate biopsy within 18 months of their initial biopsy, at which a mean of 8 (7–10) cores were obtained. Six patients were diagnosed with cancer, with a mean Gleason score of 6 (5–7). One of these patients was the only man to also have atypical small acinar...
proliferation on his initial biopsy. Three patients were diagnosed with persistent PIN; they went on to have a third prostate biopsy, which revealed cancer. Gleason score 6 in one and BPH only in the other two. All seven patients who were diagnosed with cancer during the study period had a radical prostatectomy and all had organ-confined (pT2a–pT2c) disease. After a mean follow-up of 72.2 (60–84) months, none of the remaining 14 patients was diagnosed with clinically significant cancer. Five of these patients went on to have a third prostate biopsy, with no evidence of cancer. One patient died from unrelated causes during this period; the patients’ characteristics and results are summarized in Table 1.

**DISCUSSION**

There is significant evidence that implicates PIN as the most likely histologically identifiable precursor lesion of prostate cancer [1]. Abnormalities in the genotype of PIN lesions appear to be an intermediate between normal prostate epithelium and cancer, as many genetic alterations in PIN are also present in prostate cancer [7–10]. In addition, progressive abnormalities in phenotype in PIN also appear to be an intermediate stage before the onset of cancer, in terms of impairment of cell differentiation and regulatory control towards prostate carcinogenesis [11]. Despite this evidence, it is not definitive whether PIN is a premalignant lesion or merely a lesion associated with cancer. This question needs to be addressed in multicentre studies with a long-term follow-up. Furthermore, it is unknown if PIN is a precursor to a subsequent or occult concomitant prostate cancer that will become clinically significant and cause morbidity or mortality.

Despite the association of prostate cancer with PIN, none of the present patients who had a negative second or third prostate biopsy progressed to a diagnosis of clinically significant cancer. Over ≥5 years of follow-up. Indeed, most patients (14 of 21) did not have significantly significant cancer with the relatively long follow-up of >5 years. The biopsies taken in these patients cannot definitively exclude the presence of microscopic foci of disease, and therefore these patients will require continuing follow-up for their PIN until their life expectancy is such that they may not benefit from any of the present therapies for prostate cancer. Nonetheless, following patients conservatively with routine biannual or annual history, physical examination (including a DRE), and PSA determinations appears to be a safe strategy in the management of men found to have PIN on initial prostate biopsy with a negative subsequent biopsy.

There is emerging data that with the current use of extended prostate biopsy, even immediately repeating the prostate biopsy may be unnecessary [5,6]. This differs from the sextant biopsy, in which repeat biopsies for the initial biopsy finding of PIN was recommended because of the 22–58% chance of identifying cancer on a subsequent biopsy [2–4]. It may also be possible to follow conservatively men who have had an extended TRUS prostate biopsy, with no immediate second biopsy. The history, DRE and PSA levels, as well as the rate of PSA change, can then direct when to take a repeat prostate biopsy in these patients. Saturation biopsies have been reported, but the true biological potential of cancer diagnosed by such biopsy is not known [12].

There is no definitive data that correlates PIN with the development of lethal prostate cancer to support therapy for isolated PIN [13,14]. In addition, if PIN is truly a precursor lesion to cancer, and if the latency period to develop overt malignancy is 5 to >10 years [1], men can potentially be followed with conservative management and may never require any therapy for cancer that they might develop. This would also depend upon the length of follow-up, age and other medical comorbidities developed during the follow-up, in addition to the predictors of the biology of prostate cancer, e.g. Gleason score, PSA level and DRE findings [15].

Lefkowitz et al. [16] reported that eight of 31 men with PIN were subsequently identified as having prostate cancer, using a 3-year follow-up interval for prostate biopsy, regardless of PSA level or DRE findings. The clinical outcome was not reported after radical prostatectomy in four men, and radiotherapy in the remaining four. It is unknown if detecting and treating prostate cancer at this particular time in the natural history of their

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**TABLE 1** The patients’ characteristics at the time of the initial prostate biopsy, the pathological results of subsequent prostate biopsies and the length of follow-up

<table>
<thead>
<tr>
<th>Patient/age, years</th>
<th>DRE</th>
<th>PSA, ng/mL</th>
<th>Biopsy</th>
<th>Gleason score</th>
<th>Follow-up, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/59</td>
<td>nodule</td>
<td>6.7</td>
<td>PIN cancer</td>
<td>6</td>
<td>116</td>
</tr>
<tr>
<td>2/58</td>
<td>BPH</td>
<td>7.5</td>
<td>PIN cancer</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>3/69</td>
<td>BPH</td>
<td>9.2</td>
<td>PIN cancer</td>
<td>6</td>
<td>62</td>
</tr>
<tr>
<td>4/53</td>
<td>BPH</td>
<td>9.5</td>
<td>PIN* cancer</td>
<td>7</td>
<td>75</td>
</tr>
<tr>
<td>5/64</td>
<td>BPH</td>
<td>9.6</td>
<td>PIN cancer</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>6/71</td>
<td>BPH</td>
<td>15.0</td>
<td>PIN BPH BPH</td>
<td>n/a</td>
<td>77</td>
</tr>
<tr>
<td>7/71</td>
<td>indurated</td>
<td>8.5</td>
<td>PIN BPH BPH</td>
<td>n/a</td>
<td>65</td>
</tr>
<tr>
<td>8/77</td>
<td>indurated</td>
<td>6.8</td>
<td>PIN BPH BPH</td>
<td>n/a</td>
<td>63</td>
</tr>
<tr>
<td>9/70</td>
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<td>8.2</td>
<td>PIN BPH n/a</td>
<td>n/a</td>
<td>80</td>
</tr>
<tr>
<td>10/57</td>
<td>BPH</td>
<td>17.0</td>
<td>PIN BPH BPH</td>
<td>n/a</td>
<td>71</td>
</tr>
<tr>
<td>11/65</td>
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<td>4.9</td>
<td>PIN BPH BPH</td>
<td>n/a</td>
<td>64</td>
</tr>
<tr>
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<td>BPH</td>
<td>17.6</td>
<td>PIN BPH BPH</td>
<td>n/a</td>
<td>84</td>
</tr>
<tr>
<td>13/60</td>
<td>BPH</td>
<td>6.6</td>
<td>PIN cancer</td>
<td>6</td>
<td>62</td>
</tr>
<tr>
<td>14/56</td>
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<td>6.7</td>
<td>PIN BPH BPH</td>
<td>n/a</td>
<td>60</td>
</tr>
<tr>
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<tr>
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<td>PIN BPH n/a</td>
<td>n/a</td>
<td>76</td>
</tr>
<tr>
<td>17/56</td>
<td>BPH</td>
<td>4.9</td>
<td>PIN PIN PCa</td>
<td>6</td>
<td>80</td>
</tr>
<tr>
<td>18/65</td>
<td>BPH</td>
<td>8.6</td>
<td>PIN BPH n/a</td>
<td>n/a</td>
<td>68</td>
</tr>
<tr>
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<td>PIN PIN BPH</td>
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<tr>
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<td>PIN BPH BPH</td>
<td>n/a</td>
<td>80</td>
</tr>
<tr>
<td>21/65</td>
<td>indurated</td>
<td>6.0</td>
<td>PIN PIN BPH</td>
<td>n/a</td>
<td>108</td>
</tr>
</tbody>
</table>

*This patient also had atypical small acinar proliferation on initial biopsy; n/a, not applicable.*
disease would result in a better outcome, rather than using the history, DRE, PSA levels and the rate of PSA change to direct when to take a repeat prostate biopsy, with subsequent therapies for any detected cancer administered then.

It is also unknown if the outcome of prostate cancer would be better if men were treated for a finding of isolated PIN rather than the conservative approach described. Radical prostatectomy in men with PIN alone shows that significant proportions have cancer present [1]. There is no data to suggest that radical prostatectomy and pelvic lymphadenectomy for isolated PIN resulted in identifying understaged locally advanced or metastatic prostate cancer. Androgen deprivation or 5α-reductase inhibitors may also be therapies for PIN, but because the results are conflicting, it is unknown if these therapies will abolish PIN [14,17–22]. It is also unknown if radiation therapy can abolish PIN, although the prevalence and extent of PIN appears to be decreased [23–25]. Because of the long latency period between PIN and cancer, any therapies may be delivered before they are necessary. Furthermore, all of these therapies can result in significant side-effects that can adversely affect quality of life, and therefore using therapy for isolated PIN is not advocated [1]. One proactive management option may involve chemoprevention strategies for PIN, which are under study and hold much promise if carcinogenesis can be selectively inhibited in these patients at apparently higher risk of developing cancer.

The present study was retrospective and with relatively few patients, and therefore selection bias will be an inherent limitation. Also, the number and location of biopsy cores obtained introduced sampling bias. All patients did not have prostate biopsies taken at regular intervals or at the end of the study to obtain more accurate results in terms of the development of histologically confirmed prostate cancer. However, based on the length of follow-up, the patients’ advancing age and comorbidities developed, routine prostate biopsies were not deemed to be necessary in all patients who were unlikely to develop clinically significant cancer.

The current practice of following patients with PIN conservatively if subsequent prostate biopsies are negative appears to be a reasonable management option. Whether PIN is a premalignant lesion, or merely a lesion associated with prostate cancer, needs to be addressed with multicentre studies with a follow-up of >10 years.

CONFLICT OF INTEREST

None declared.

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Abbreviations: PIN, prostatic intraepithelial neoplasia.
Increasing the number of biopsy cores improves the concordance of biopsy Gleason score to prostatectomy Gleason score

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OBJECTIVE
To evaluate taking more biopsy cores for predicting the radical prostatectomy (RP) Gleason score compared with the biopsy Gleason score, as although random sextant biopsies are the standard for a tissue diagnosis of prostate cancer, and taking more biopsies increases the detection rate, it is uncertain whether taking more cores improves the prediction of the RP Gleason score.

PATIENTS AND METHODS
We analysed retrospectively 404 patients from three centres (Seattle 162, Washington 107 and Chicago 135) who had RP for prostate cancer. Six, eight or 10 biopsies were taken based on the physician’s preference and the patient’s characteristics.

RESULTS
Before RP, 158 (39%) patients had six, 65 (16%) had eight and 181 (45%) had 10 biopsy cores taken. The accuracy of the Gleason sum of the three groups was 65/158 (41%), 26/65 (40%) and 104/181 (57.5%), respectively (P < 0.004, 10-core vs six-core). However, when comparing the Gleason score separately (i.e. 4 + 3 is not equal to 3 + 4), the accuracy of the three groups was 48/158 (30%), 20/65 (31%), and 95/181 (52.5%), respectively (P < 0.001, 10-core vs six core).

CONCLUSIONS
Taking more biopsy cores improves the accuracy of the biopsy Gleason score in predicting the final Gleason score at RP; the predictive accuracy of the final Gleason score may be increased from 41% to 58% by increasing the number of biopsies from six to 10.

KEYWORDS
prostate cancer, Gleason sum, Gleason score, biopsy

INTRODUCTION
Prostate cancer is the second commonest malignancy in men, with an estimated incidence in the USA of 230 110 in 2004 [1]; with a positive biopsy rate of 25–30% [2,3], this translates into >600 000 prostate biopsies being taken annually in the USA. The standard of prostate cancer diagnosis has been the sextant biopsy technique described by Hodge et al. [4]. Recently, urologists have been taking more biopsy cores to enhance the detection of prostate cancer [5–8], but it is debatable whether taking more biopsies improves not only the detection rate, but also the accuracy of predicting the final radical prostatectomy (RP) specimen Gleason score.

There are many options for treating localized prostate cancer, including RP, brachytherapy, external beam radiation therapy, watchful waiting and androgen-deprivation therapy. In choosing among these options the patient and urologist must carefully evaluate all appropriate clinical features, including PSA level, age, clinical stage, comorbidities and the histological features on needle biopsy. Unfortunately, the accuracy of predicting the final RP specimen Gleason score from the biopsy Gleason score is currently reported to be ~43% [9]; biopsies under-graded the prostate cancer in 39% and over-graded it in 16% of biopsies [9]. Because of the strong prognostic significance of the Gleason score [10,11], increasing the predictive value of prostate biopsies would better enable patients and urologists to make a more informed decision about the treatment options and the disease.

In this study we examined whether taking more prostate biopsies would increase the predictive value of the biopsy Gleason score when compared with the RP specimen Gleason score. An increase in the predictive value of the prostate biopsies would benefit patients by helping to guide urologists through the decision algorithm for each patient’s management of their prostate cancer.

PATIENTS AND METHODS
We retrospectively reviewed 404 patients who had RP and had either six, eight or 10 prostate needle biopsies taken at the time of diagnosis. All surgery was performed between 1996 and 2002 at the authors’ institutions (Seattle, 162 patients; Washington, 107, and Chicago, 135). The number of needle biopsies taken was determined at the time of biopsy, based on the patient’s clinical characteristics and physician’s preference. Initially, TRUS was used to estimate prostate volume, followed by prostate biopsy; six biopsies were taken using the standard sextant technique [4]. Patients who had eight cores taken had one biopsy at the apex, two (both laterally and medially) in the mid-section of the prostate, and one at the lateral aspect of the base. This was then repeated on the opposite side of the prostate.
Patients who had 10 biopsies taken had two at the base and mid-section of the prostate (both laterally and medially), and one at the apex, again repeated on the opposite side of the prostate. Eight patients had transitional zone biopsies and were excluded from the study.

The patients’ characteristics before and after RP, including age, PSA level, clinical stage, race, biopsy Gleason score, RP Gleason score, number of biopsies and margin status, were recorded. Patients who had fewer than six biopsies or more than 10 were excluded from the study. We then compared the Gleason scores before and after RP for each patient and determined the concordance rate of the Gleason score and Gleason sum. The results were analysed statistically using the chi-square and Mantel-Haenszel chi-square tests.

RESULTS

All 404 patients either had six, eight or 10 prostate needle biopsies taken, based on clinical characteristics and physician preference. The patients’ median (range) age at surgery was 63 (42–79) years, the mean follow-up 2.3 (0.17–6.4) years and median preoperative PSA level 6.5 (0.28–113.9) ng/mL, which was similar in the three biopsy groups (P > 0.05).

The clinical preoperative stage and RP pathological stage are summarized in Table 1. Those who had 10 cores taken had a slightly higher pathological stage, probably because of the higher clinical stage before RP.

However, there was no statistically significant difference among the three groups in age, clinical stage, pathological stage, mean biopsy Gleason score and mean RP specimen Gleason score (Table 1).

The accuracy of the Gleason score of the three groups compared with the RP Gleason sum is also shown in Table 1; the difference between the 10-core and the other two groups was statistically significant (P < 0.001 and <0.004, respectively), with again no difference between the six- and eight-core groups (P = 0.954). Thus, the predictive accuracy of the Gleason sum and individual Gleason score was better in patients who had 10 biopsy cores taken.

The discrepancy in grading is also shown in Table 1; the improvement in under-grading by taking more biopses was not statistically significant (P > 0.05), but that in over-grading was between the 10-core and the other two groups (P < 0.03 and <0.02, respectively).

DISCUSSION

Because of the differences in success rates and morbidities of the various procedures for treating prostate cancer, counselling before therapy about the aggressiveness and extent of disease is paramount for patients. Given the prognostic significance of the Gleason score [6,7], increasing the accuracy of biopsies will better enable patients to make a more informed decision about their treatment algorithm. While many authors have tried to increase the detection rate of prostate cancer by taking more prostate biopsies [5–8], we propose that it will also increase the accuracy of predicting the Gleason score and Gleason sum of the final RP specimen.

The present results show that taking more cores (10 rather than six) improved the accuracy of predicting the Gleason sum, from 41% to 57.5%, and the Gleason score, from 30% to 52.5%. Both of these improvements were statistically significant when comparing the 10-core with the other two groups. There was no large difference in the other variables among the three groups, in age, clinical stage, pathological stage, mean biopsy Gleason score and mean RP Gleason score. The mean PSA level was lower in the 10-core group (6.7 ng/mL) than in the other two (10.0 and 11.1 ng/mL, respectively). We attribute this difference to a few outliers with extremely high PSA levels in the six- and eight-core groups. For clinical and pathological stage, those who had 10 cores taken had a higher stage on final analysis, but this was not statistically significant (P > 0.05).

Grossklaus et al. [12] and Egevad et al. [5] also reviewed the value of taking more biopsies to
better predict the pathological Gleason score, finding that this prediction was only marginally improved by doing so. However, their studies only assessed a total of 135 and 121 patients. As in the study by Egevad et al., under-grading still appears to be a major problem, occurring in 35% of all the present patients, with over-grading in 16% (Table 1). Because there is such a large proportion of under-grading of prostate cancer, many patients and urologists may be underestimating the severity of the patients’ disease when discussing their treatment options, based on the pathological Gleason score and sum of the prostate biopsy.

While the Gleason score still has strong prognostic significance, other authors have evaluated other clinical predictors for prostate cancer. Sebo et al. [13] recently reported that the percentage of needle–biopsy cores and surface area positive for cancer are the strongest predictors of pathological stage and tumour volume on multivariate analysis. Grossklaus et al. [12] also concluded that the percentage of positive cores is the best predictor of both pathological stage and tumour volume. We did not analyse the percentage of positive cores for its predictive value of pathological stage and tumour volume, but are currently updating our database.

A recent study agreed with the present conclusions; San Francisco et al. [14] reviewed 466 men who had retropubic RP, dividing the patients into two groups based on the number of needle biopsies. One group comprised 126 men who had extended needle biopsies (≥10 cores) and the second 340 patients diagnosed with prostate cancer by restricted needle biopsies (≤9 cores); the concordance rate was 76% and 63%, respectively. The present study is unique in that we evaluated and compared patients who had exactly six, eight or 10 biopsy cores at the time of diagnosis. The study by San Francisco et al. had only 34/126 (27%) patients who had exactly 10 biopsy cores, while 75/126 (59.5%) in the extended group had 12–14 cores taken. These patients were combined and compared with those who had nine or fewer cores. This may be why they had a high concordance rate of 76%, vs 57.5% in the present study.

Not only does taking more cores improve the concordance rate, but it also may indirectly help to predict recurrence after RP. San Francisco et al. [15] reported that the percentage of cores positive for prostate cancer was a better predictor of cancer recurrence than PSA level. The percentage was calculated by the number of cores positive for cancer divided by the total number of cores, multiplied by 100. Patients with >28% positive cores were at significantly greater risk of prostate cancer recurrence. Clearly this finding, with a greater concordance rate with 10 biopsy cores, will add to the value of prostate cancer biopsies.

Furthermore, nomograms for predicting final pathological stage and failure are available for use by urologists and patients [16–19]. It is clear that biopsy Gleason score and PSA are important preoperative predictors for these nomograms. Increasing the concordance rate of prostate biopsies by taking more cores will also increase the accuracy of the preoperative nomograms and physicians will be better able to counsel patients on their disease.

One of the limitations of the present study is that it was a retrospective review of the charts of patients who had had RP for prostate cancer. As such, there was no standard protocol devised for obtaining prostate biopsies. There were also many pathologists from different institutions reviewing both the prostate biopsies and the prostate specimen after surgery.

The optimum number of prostate biopsies to be taken remains unknown. Because of recent studies delineating an increase in detection rate by taking more biopsy cores, the patients who were treated more recently had more biopsies (10 cores) than those treated earlier in the study (six cores). Arguably, 12 or 14 biopsies could increase the accuracy of predicting the final Gleason sum and Gleason score even further. In the present patients there was no identifiable increase in morbidity when taking six rather than 10 biopsies. Berger et al. [20] reported that, except for haematospermia, there was no increase in morbidity when taking six rather than 15 cores. We are currently expanding our database to further evaluate the accuracy of taking 12 biopsy cores.

In conclusion, taking more biopsy cores improved the accuracy of predicting the final RP specimen Gleason sum, from 41% to 57.5% for six and 10 cores (P < 0.004), but when comparing the Gleason score separately, the accuracy improved from 30% to 52.5%. This improvement in accuracy will be of benefit to patients when choosing among their treatment options for prostate cancer.

CONFLICT OF INTEREST

None declared.

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Abbreviations: RP, radical prostatectomy.
Serum thyroid-stimulating hormone is elevated in men with Gleason 8 prostate cancer

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INTRODUCTION

Men with prostate cancer of Gleason score ≥8 have a high risk of skeletal metastases and should be considered for bone scintigraphy at diagnosis [1]. However, the mechanism for these metastases is uncertain and various causes have been suggested [2]. The skeleton is a dynamic organ whose structural integrity depends on constant remodelling, controlled by many local and systemic factors, including TSH, an important regulator of this process.

PATIENTS AND METHODS

We evaluated 51 men referred for treatment of localized prostate cancer and 10 with biopsy-confirmed benign prostatic hypertrophy. Serum TSH was determined with a chemoluminescent immunoassay and a commercially available instrument (Immulite, Diagnostic Products Corporation, Los Angeles).

RESULTS

There was significant variation in TSH levels with Gleason score (P = 0.004); men with Gleason 8 tumours had the highest serum TSH levels. Because serum TSH levels increase with age, we used a multivariate analysis of variance with both age and Gleason score as covariates. The effect of Gleason score on TSH level was significant (P = 0.036) and independent of the effect of age (P = 0.392).

CONCLUSION

We propose that the high serum TSH levels in men with Gleason 8 prostate cancer is a result of the elaboration of TSH by cancer cells. Bone mineral density in the face of normal levels of thyroid hormone depends on an intact response to TSH, which ordinarily suppresses both osteoblast and osteoclast differentiation, thereby exerting control over bone remodelling. However, with abnormally high TSH levels this process may become deranged, promoting the development of bone metastases. If TSH production by prostate cancer cells could be suppressed, the incidence of bone metastases might be reduced.

KEYWORDS

prostate, cancer, thyroid-stimulating hormone, bone metastasis

DISCUSSION

Bone metastases in prostate cancer are predominantly osteoblastic, with more irregular bone trabeculae; markers of bone resorption also increase, although there are similar numbers of osteoclasts [2]. Prostate cancer cells release PSA, a kallikrein serine
protease that can cleave parathyroid hormone-related peptide, released by tumour cells [6], at the N-terminal. This cleavage may block tumour-induced bone resorption. In patients with prostate cancer high PSA levels are associated with bone metastases, but levels of bone resorption markers are also high in patients with bone metastases and reflect the extent of metastases more accurately than PSA level [2].

We suggest that the elevated serum TSH levels in men with Gleason 8 prostate cancer result from the elaboration of TSH by the cancer cells within the bone itself. The very high local level of TSH in the bone is reflected in the elevated serum TSH levels of patients with Gleason 8 tumours.

Bone mineral density in the face of normal levels of thyroid hormone depends on an intact response to TSH [4]. TSH ordinarily suppresses both osteoblast and osteoclast differentiation, thereby exerting control over bone remodelling. However, in the presence of abnormally high TSH concentrations, this process may become deranged, promoting the development of bone metastases. If TSH production by prostate cancer cells could be suppressed, the incidence of bone metastases might be reduced.

CONFLICT OF INTEREST

None declared.

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Inguinal hernia repair with polypropylene mesh during radical retropubic prostatectomy: an easy and practical approach

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OBJECTIVE
To report the results of the simultaneous inguinal hernia repair during radical retropubic prostatectomy (RRP) with the preperitoneal tension-free Stoppa technique, using a polypropylene mesh.

PATIENTS AND METHODS
During 855 consecutive RRPs, 40 (5%) patients (median age 66.9 years, range 52–81) with 49 inguinal hernias had a simultaneous inguinal hernioplasty. The RRP was performed according to the Walsh modified technique. After the prostate and seminal vesicles were removed and the urethrovesical anastomosis completed, a polypropylene mesh of maximum size 15 x 7.5 cm and a small slit on its medial side was then created and placed in the preperitoneal space, embracing the spermatic cord and covering the myopectinal orifice. Preoperative risk factors, e.g. constipation, pulmonary disease or urinary obstructive symptoms, were collected retrospectively from the files. Complications after surgery, including wound infection, pelvic collections, urinary fistula and recurrence of the hernia, were assessed.

RESULTS
Preoperative risk factors for hernia development were identified in 23 (58%) patients; three had recurrent hernias. With a median 23.1 months of follow-up period two (4%) hernias recurred. There were no complications after surgery.

CONCLUSION
Preperitoneal hernia repair with polypropylene mesh is safe, effective and practical. The procedure simultaneous with RRP gave a 96% success rate and with no significant increase in operating time or additional complications.

KEYWORDS
prostatectomy, inguinal hernia, surgical mesh, polypropylene

INTRODUCTION
With the increased use of PSA testing, cases of localized prostate cancer have become more frequent and currently nearly 70% of these tumours are diagnosed at stage T1c [1]. The most frequent treatment for localized prostate cancer is radical retropubic prostatectomy (RRP), with half of patients so treated [2]. It is estimated that inguinal hernias are present in 5–12% of patients who are candidates for radical surgical treatment for localized prostate cancer [3–5].

Data from the National Center for Health Statistics show that there were ~800 000 groin hernia repairs in the USA in 2003, and it is the most common operation by general surgeons in the country [6]. If these hernias are left untreated they can potentially result in serious complications, e.g. bowel strangulation or ischaemia, and a 14% mortality rate for emergency operations is reported in these cases. Also, any abdominal incision tends to weaken the abdominal wall and predispose the patient to developing hernias [3,4,7].

The simultaneous correction of inguinal hernia during prostate surgery was first described in 1949 by McDonald and Huggins [8], who used two separate incisions. However, after the description of a simultaneous preperitoneal approach with the Nyhus technique to correct the hernia defect during prostate surgery [3], this procedure became more popular. Subsequently, the simultaneous correction of hernia defects during prostate surgery, using the tension-free preperitoneal technique with a prosthetic repair as described by Stoppa et al. [9], was reported [4,5,10].

Despite the clear advantages of the simultaneous approach in avoiding future complications from the hernia defect and potential emergency surgery, to date there is no published consensus about the best method to treat inguinal hernias during prostate surgery. The reported concerns that have also contributed to stimulate urologists to choose different methods, and even to avoid simultaneous procedures, are the risk of infection, the risk of recurrence of the hernia defects, a longer operation and anaesthetic time, unfamiliarity with the techniques and concerns over performing procedures usually done by general surgical specialists [3,11]. Therefore, different methods have been used by many authors [3–5,11,12].

It was recently proposed that a modified Pfannenstiel incision with no use of the preperitoneal approach is an ideal method for inguinal hernia repair concurrent with RRP [13]. In the present study we report the results of inguinal hernia repair during RRP with the preperitoneal approach, using a polypropylene mesh.

PATIENTS AND METHODS
From September 1999 to August 2004, 855 RRPs were performed at the authors'
TABLE 1

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<thead>
<tr>
<th>Type</th>
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<tbody>
<tr>
<td>I</td>
<td>Infantile indirect inguinal hernias with no distension of the deep inguinal ring</td>
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<tr>
<td>II</td>
<td>Indirect inguinal hernias with distension of the deep inguinal ring</td>
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<td>III A</td>
<td>Direct inguinal hernias</td>
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<td>III B</td>
<td>Large indirect inguinal hernias</td>
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<td>III C</td>
<td>Femoral hernias</td>
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<td>IV</td>
<td>Recurrent hernias</td>
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The Nyhus classification [14]

The mesh is placed in the preperitoneal space to cover the myopectinal orifice.

FIG. 1. The polypropylene mesh, with a small slit on its medial side to accommodate the cord structures.

FIG. 2. The mesh is placed in the preperitoneal space to cover the myopectinal orifice.

FIG. 2. The polypropylene mesh, with a small slit made on its medial side to accommodate the cord structures.

INGUINAL HERNIA REPAIR WITH MESH DURING RADICAL PROSTATECTOMY

All the patients routinely received i.v. prophylactic antibiotics (first-generation cephalosporins), beginning 2 h before surgery and continued until the indwelling catheter was removed. For the procedure the patients were placed supine with hyperextension of the hips. After bladder catheterization, a median longitudinal incision was made and bilateral pelvic lymph nodes dissected. RRP was done according to the Walsh modified technique [15]. After the prostate and seminal vesicles were removed and urethrovescical anastomosis completed, the spermatic cord was dissected, the hernia sac identified, and treated according to the position and local characteristics. If an indirect hernia sac was present it was dissected off and the peritoneum closed at the neck, while if there was a direct hernia, the sac was teased from its envelope of transversalis fascia in the abdominal wall. The spermatic cord elements were separated from the peritoneum through the parietalization manoeuvre. If there was a lipoma of the chord it was also dissected off.

The hernias were repaired according to the technique of Stoppa et al. [9]. A polypropylene mesh (maximum 15 × 7.5 cm) was used, with a small slit made on its medial side to accommodate the cord structures (Fig. 1) and placed in the preperitoneal space to cover the myopectinal orifice (Fig. 2). Care was taken to avoid contact with the anastomosis and any excess mesh discarded by cutting with scissors. No stay sutures were used to immobilize the mesh. For bilateral repair the procedure was repeated on the opposite side. No antiseptic lavage of the surgical field was used after placing the mesh. A Penrose drain was inserted in the prevesical space, externalized through the lower extremity of the incision and removed 4 days after surgery; care was taken to avoid drain contact with the mesh. The median (range) follow-up was 23.1 (1–60) months; complications, including wound infection, pelvic collections, urinary fistula and recurrence of the hernia, were assessed.

RESULTS

There were 49 inguinal hernias diagnosed and repaired in the 40 patients (median age 66.9 years, range 52–81). Preoperative risk factors for hernia were identified in 23 (58%) patients; all reported LUTS, two reported intestinal constipation and one reported chronic pulmonary disease through tobacco abuse. Sixteen hernias were on the right, 15 on the left and nine were bilateral. The diagnosis was before surgery in 36 (90%) patients; nine had had a previous inguinal hernia repair, four each on the right and left, and one bilateral. Three patients had recurrent hernias. The hernia repair added a mean of 10 min to the operative duration.

With a median 23.1-month follow-up, two (4%) patients had evidence of recurrence; both complained of LUTS before surgery, had unilateral right inguinal hernias and were younger than the median age of the whole group (66 years). The first had had a previous hernia repair at the same side and this was the second recurrence. The recurrences were at 18 and 24 months. Both patients had a repeat herniorrhaphy through an inguinal approach.

There were no complications after surgery, e.g. wound infection, pelvic collections and urinary leakage, despite using the mesh. Three patients reported mild and transient testicular pain at the side of the hernia repair, but required no treatment.

DISCUSSION

To date there is no consensus on the ideal method for correcting inguinal...
hernias during RRP. In the present study, with a 23-month follow-up, the recurrence rate was 4%, which is comparable to that of other methods with the same follow-up, and with no complications during this period.

A groin hernia begins within one weak area, termed the myopectinal orifice; this area is limited superiorly by the internal oblique and transverse abdominal muscles, laterally by the ilio-psoas muscle, medially by the rectus muscle and inferiorly by the pecten of the pubis. The femoral vessels and the spermatic cord cross this area, which is divided in half by the inguinal ligament and sealed on its inner surface by the transversal fascia. Any failure of the transversalis fascia or in the shutter mechanism (a movement produced by the transverse aponeurotic arch when the transverse abdominal and internal oblique muscles are tense) to contain the peritoneum and its contents will produce a groin hernia [4].

McDonald and Huggins [8] were the first to report an inguinal hernia repair concomitant with prostatectomy, but this was done with two separate incisions, with the hernia defect repaired through the inguinal approach. A Pfannenstiel incision was subsequently proposed by others for the herniorrhaphy during prostatectomy, but also with and inguinal approach to correct the hernia defect [16].

The preperitoneal hernia repair was first reported by Annandale and subsequently modified by Nyhus [cited in [4]]. According to the Nyhus technique, the transverses arch is approximated to the ilipubic tract with interrupted polypropylene sutures and then these initial sutures are placed down to Cooper's ligament to close the femoral canal, preventing potential recurrences in this area [17].

However, one important limitation of the Nyhus procedure is the resultant high tension on the repair, which is often unacceptable for a large defect when the supporting tissues are extremely weakened. This tension may also be worsened after closing the abdominal incision. A study with 1186 patients (aged 18–96 years) treated with this technique showed a recurrence rate of 6.6% with a mean follow-up of 3.5 years [18].

Some authors indicate the use of the Nyhus technique to repair inguinal hernias during RRP. Schlegel and Walsh [3] performed 41 preperitoneal herniorrhaphies in 32 patients simultaneously with 343 RRP and 26 radical cystoprostatectomies, using the preperitoneal Nyhus technique. They had no evidence of recurrence and no complications related to the hernia repair. However, their median follow-up was only 11.4 months, and as we show in the present report, the hernia can recur after this period, as in two of the present patients, at 18 and 24 months.

The repair introduced by Stoppa et al. [9] remains one of the most reliable methods of hernia repair [19]; they proposed that the prosthetic mesh could replace the weakened transversalis fascia by eliminating any potential protrusion of the abdominal contents. This procedure has been the preferred method to correct hernial defects during prostate surgery for many authors. Filiadis et al. [10] evaluated the results of hernia correction with a simultaneous Stoppa procedure and open surgical prostatectomy for benign disease in 22 patients; with a median follow-up of 20.4 months there was one case of wound infection with urinary fistula that required no treatment and one recurrence that was clinically insignificant; also treated conservatively. Patients with recurrent hernias were excluded from that study.

The Stoppa procedure was also described simultaneously with RRP by Choi et al. [4], who retrospectively reviewed the results of 70 hernioplasties with the preperitoneal approach (35 with and 35 without mesh) in 48 patients. In the prosthetic group they used a 12–cm mesh that was tapered to avoid approximation with the anastomosis, and fixed with one or two stay sutures to stabilize the mesh. The group with no mesh had the preperitoneal Nyhus technique, as described by Schlegel and Walsh [3]. The mean (range) age of the patients was 60.9 (43–73) years.

With a mean follow-up of 24 (6–66) months there was no recurrence in the mesh group and five (14%) in the no-mesh group. All recurrent hernias were detected within a year of the initial operation. There were no complications. More recently, Drachenberg and Bell [5] reported their experience with the preperitoneal mesh-plug herniorrhaphy in 15 patients undergoing RRP. With a median follow-up of 18 months there were no recurrences or orchalgia. The laparoscopic repair of inguinal hernias, which is almost always based on the Stoppa operation, was also described simultaneously with transperitoneal laparoscopic RP [20].

Recently, Manoharan et al. [13] described a modified Pfannenstiel incision to allow inguinal hernia repair during RRP. The authors repaired hernias using the tension-free technique described by Lichtenstein et al. [21], and reported their initial experience with 15 patients, who had no complications or recurrences with a mean follow-up of 5.5 months. However, the technique seems to be more laborious, as it requires further dissection of the subcutaneous plane along the inferior aspect of the Pfannenstiel incision to expose the external oblique aponeurosis, and an additional incision of this aponeurosis before assessing the spermatic cord and exploring the hernia sac. Also, the results of this technique need to be re-evaluated with more patients and longer follow-up.

To date there are no prospective controlled trials comparing different techniques of herniorrhaphy during prostate surgery. The study by Choi et al. [4] was retrospective and did not mention the types of inguinal hernia according to the Nyhus classification, creating a possible bias in the results. Perhaps it is reasonable to avoid the use of prosthetic mesh techniques only in patients with a high risk of wound infection, as in cases of preoperatively documented UTIs or rectal perforations during the procedure.

In the present series there were two recurrences after the first year from surgery; these failures may have resulted from an inadequate size of prosthesis, insufficient to cover the entire myopectoral orifice, or from migration of the mesh, as we did not use stay sutures. According to the Stoppa principle, the prosthesis is held in place mainly by intra-abdominal pressure, and no stay sutures are necessary, but when simultaneous with RRP much more dead space is left after dissecting
the prostate, seminal vesicles and lymph nodes, and this might influence recurrence. The use of immobilising sutures could prevent mesh migration and reduce the present 4% recurrence rate.

In conclusion, preperitoneal hernia repair with polypropylene mesh is safe, effective and practical; the simultaneous approach gave excellent results (success rate of 96%) with no significant increase in operative duration or additional complications. We therefore advocate this method in patients undergoing RRP who have concomitant inguinal hernias, to avoid future surgery and potential complications from these unrepaired defects.

CONFLICT OF INTEREST

None declared.

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Abbreviations: RRP, radical retropubic prostatectomy.
An office-based immunodiagnostic assay for detecting urinary nuclear matrix protein 52 in patients with bladder cancer

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OBJECTIVE
To report the rapid (5 min) and simple detection of a nuclear matrix protein (NMP) in the urine of patients with bladder cancer, using a newly developed office-based dot-enzyme-linked immunosorbent assay (ELISA).

PATIENTS AND METHODS
Western blot and specific immunoglobulin-G antibody were used to identify the urinary NMP marker. Urine samples from 149 patients with bladder cancer and 72 controls were evaluated using the developed dot-ELISA. The initial responses of 43 patients treated by irradiation were followed using the assay.

RESULTS
The NMP marker was identified in the urine of patients with bladder cancer at 52 kDa (NMP-52) by Western blot. The dot-ELISA detected the urinary NMP-52 marker in 92% of patients with squamous cell carcinoma, 98% with transitional cell carcinoma, and all six of those with adenocarcinoma of the bladder, with a specificity of 94%. The positive and negative predictive values (97% and 94%, respectively) and efficiency (96%) of the dot-ELISA were high. In addition, the NMP-52 tumour marker was not detected in the urine of patients who showed a response after radiotherapy.

CONCLUSION
Detecting the urinary NMP-52 marker using dot-ELISA would be helpful in the rapid diagnosis and follow-up of patients with bladder cancer.

KEYWORDS
bladder, tumour, marker, NMP, 52-kDa, urine, diagnosis, follow-up, dot-ELISA

INTRODUCTION
The highest incidence rates of bladder cancer are generally found in industrially developed countries, particularly North America and Western Europe, and areas associated with endemic schistosomiasis, including parts of Africa and the Middle East [1]. The appropriate treatment of patients with bladder cancer mandates early detection and regular follow-up for recurrences [2]. Currently, cystoscopy is the standard method for diagnosing and monitoring bladder cancer recurrence, but it is an invasive and relatively costly technique, and may sometimes be inconclusive, particularly in cases of cystitis [3,4]. Also, routine urinary cytology commonly used in conjunction with cystoscopy is costly, requires an experienced cytopathologist and offers poor sensitivity for low-grade tumours [5]. These characteristics have prompted the search for more reliable noninvasive markers of bladder cancer.

During the last few years, several urinary markers of bladder cancer have been introduced and evaluated for the noninvasive detection of bladder cancer [6,7]. However, most of the currently available urinary markers have lower specificity than urinary cytology, particularly when the markers are used singly [8]. The nuclear matrix is central in regulating important cellular processes such as DNA replication and transcription [9]. The protein composition of the nuclear matrix is tissue-specific and can serve as a ‘fingerprint’ of each cell and/or tissue type [10,11]. Differences in nuclear matrix protein (NMP) composition are found in several human tumours, including prostate [12], renal [13], breast [14], colon [15], cervical [16], and head and neck [17]. Thus, identifying nuclear matrix biomarkers has potential in the diagnosis and prognosis of cancer [18]. In the present study we identified a urinary NMP marker and evaluated a newly developed, reliable and more convenient office-based dot-ELISA for the rapid diagnosis and monitoring of patients with bladder cancer after radiotherapy.

PATIENTS AND METHODS
Urine samples were collected from 149 patients with histopathologically diagnosed bladder cancer (126 men and 23 women, mean age 62.6 years, range 40–88) of different types and grades. The patients were subdivided according to the types of tumour into 25 with squamous cell carcinoma (SCC) type, 118 with TCC and six with adenocarcinoma. They were also subdivided according to the stages of tumour into two with Ta, three with T1, two with T2, 29 with T3a, 59 with T3b, 33 with T4a and 21 with T4b. Urine samples from 57 apparently healthy volunteers (47 men and 10 women, aged 25–65 years) and 15 patients with malignancy other than bladder cancer (five men and 10 women, aged 30–65 years) were...
used as controls. The collected urine samples were centrifuged at 2100 × g to remove any cellular debris and tested fresh or stored at −20 °C with no additions or treatment until tested. The Ethical Committee of the Mansoura University Hospitals approved the study. Informed consent was obtained from all participants and they were fully informed about the diagnostic procedures involved and nature of the disease.

In a follow-up study, 43 patients with histopathologically diagnosed bladder cancer (38 men and five women, mean age 63.5 years, range 40–82; 27 stage T3 and 16 stage T4) received radiotherapy at a dose of 65 Gy, using a linear accelerator, in two phases. In Phase I the patient received a total treatment dosage of 45 Gy over 5 weeks (five daily fractions a week, 1.8 Gy/day), and in Phase II, after 2 weeks interruption, the patients received 20 Gy over 2 weeks (five daily fractions a week, 2 Gy/day). The status of the tumour size, lymph nodes metastasis and distant metastasis were followed using CT to evaluate the initial response to radiotherapy. Urine samples were collected from all treated patients before and at the end of each phase (i.e. at 5 and 9 weeks) of radiotherapy and then 4 weeks after stopping radiotherapy.

PRODUCTION OF ANTI-NMP-52 IgG ANTIBODIES

Specific IgG antibodies were produced in four New Zealand white rabbits immunized subcutaneously in three different inoculation sites with the 52-kDa purified marker (see below). In brief, equal volumes (500 µL) of the antigen (500 µg/mL) and complete Freund’s adjuvant (CFA) or incomplete Freund’s adjuvant (IFA) were homogenized together using two Luer-lock syringes connected to a three-way stainless-steel valve. Each rabbit was immunized subcutaneously three times, once with antigen in CFA (on day 0) and twice with antigen in IFA (on days 15 and 28) before being killed on day 32. Blood samples were collected from all rabbits at 0, 28 and 32 days of immunization. The sera were separated, purified and stored at −20 °C until used. The reactivity of the collected rabbit sera was tested against urine samples of histopathologically diagnosed patients with bladder cancer, the purified 52-kDa antigen, and the NMP-22 antigen (Matritech, Newton, MA, USA). The specificity of the rabbit sera was tested against urine samples of 16 healthy individuals and patients with malignancy other than bladder cancer including colorectal cancer (three), hepatocellular carcinoma (four), breast cancer (four) and prostate cancer (four) using the dot-ELISA.

SDS-PAGE AND GEL ELECTRO-ELUTION

Urine samples were subjected to analytical SDS-PAGE, at 100 µg/lane, using vertical slabs of 16% polyacrylamide [19]. Molecular-weight standards (Sigma Chem Co., St Louis, MO) were run in parallel. In preparative slab gel electrophoresis, the running condition was adapted to reduce smear of proteins and to enable a long migration distance between bands in the 52-kDa region, according to the pre-stained molecular weight marker. In each run, 250-µL of urine per preparative gel was electrophoresed, and a lane from electrophoresed preparative gel stained with Coomassie blue and immunoblotted to identify the 52-kDa band. In the unstained preparative gel, the adjacent band was then cut and the 52-kDa antigen electro-eluted from polyacrylamide gel at 200 V for 3 h in a dialysis bag (Sigma). Forty runs were completed to obtain 1 mg of the 52-kDa antigen. After dialysis, the electro-eluted antigen was concentrated using polyethylene glycol and 40% trichloroacetic acid (TCA), then centrifuged at 6500 × g for 15 min. The precipitate was washed twice using diethyl ether to remove excess TCA. The excess diethyl ether was removed by gentle drying and the pellet reconstituted in PBS (pH 7.2). The protein content of a sample of electro-eluted antibody was determined before the remainder was stored at −20 °C.

WESTERN BLOT

Urine samples separated on SDS-PAGE (as above) were electrotransferred onto nitrocellulose membrane (0.45 µm pore size, Sigma) in a protein transfer unit [20]. The nitrocellulose filter was blocked using 5% (w/v) nonfat dry milk dissolved in 0.05 mol/L Tris-buffered saline (TBS) containing 200 mmol/L NaCl (pH 7.4), rinsed in TBS and incubated with the anti-NMP-52 antibody diluted in blocking buffer with constant shaking. The blots were washed three times (30 min each) in TBS, followed by incubation for 2 h with goat antirabbit IgG alkaline phosphatase conjugate (Sigma) diluted 1:500 in TBS. After washing three more times with TBS (15 min each), the blots were soaked in substrate (premixed BCIP and NBT in 0.1 mol/L Tris buffer, pH 9.6; ABC Diagnostics, New Damietta, Egypt). The colour reaction was observed within 15 min, and dipping the blots in distilled water then stopped the reaction.

The purity of the purified antigen was assessed using analytical SDS-PAGE [19] and capillary zone electrophoresis (CZE) in a modification of the method described by Gordon et al. [21], an autosampler (model 1-LIFT; Prince Technologies, Emmen, the Netherlands), a 65-cm fused silica capillary (75 µm inner diameter) coated with polyimide film (Prince Technologies), a variable ultraviolet-visible detector (Lambda 1010; Metrhom, Herisau, Switzerland) and WinPrinc software (Version 5; Prince Technologies). For the CZE run, 10 µL of a dilution of the purified antigen (50 µg/mL distilled water) was injected through the capillary at high voltage (30 kV) and low pressure (2.5 kPa) for 10 s before the sample was eluted with borate buffer (pH 8.3) at high voltage (30 kV) for 15 min while the internal capillary temperature was kept at 20 °C. Eluents were detected by their ultraviolet absorption at 200 nm and signals analysed using Dax software (Version 5; Prince Technologies).

To create a simple and rapid assay, a previously described dot-ELISA [22] was adapted to detect the target urinary NMP-52 marker. All the steps of the dot-ELISA were carried out on the surface of a nitrocellulose membrane fixed in a plastic cartridge, and each reagent completely absorbed into the nitrocellulose membrane within 30 s (incubation time). After optimizing the reaction conditions, 200 µL of urine sample were added per dot. Different concentrations (50, 100 and 250 µg) of the NMP antigen and an irrelevant protein (e.g. BSA) were used as positive and negative controls, respectively. The nonspecific binding sites on the nitrocellulose membrane were blocked with 5% (w/v) BSA in PBS, pH 7.2. After washing three times using 100 µL/wash of PBS, 200 µL of the specific anti-NMP-52 antibody diluted 1:100 in PBS was added. After washing, 200 µL of the diluted antirabbit IgG alkaline phosphatase conjugate was added; after more washing, 200 µL of premixed NBT/BCIP alkaline phosphatase substrate in 0.1 mol/L Tris buffer (ABC Diagnostics) was added; 2 min later the reaction stopped by distilled water and the development of colour observed. The colour of the tested urine
RESULTS

The anti-NMP-52 antibody identified two reactive epitopes at 52 and 40 kDa in the urine of 32 selected patients with different types of bladder cancer (Fig. 1). The 52-kDa antigen was identified in all 32 urine samples, while the 40-kDa antigen was identified only in 19. To ensure that the 52-kDa marker was a feature of bladder carcinoma, 16 selected urine samples from normal volunteers and 15 from patients with malignancy other than bladder carcinoma were assessed using Western blot. Neither the target 52-kDa nor the 40-kDa degradation product was reactive with the anti-NMP-52 antibody. The 40-kDa antigen was identified only in the urine of patients with bladder cancer. Molecular weight markers (Mr) were not shown but indicated by arrows.

The purity of the eluted 52-kDa antigen was confirmed using CZE; there was a single polypeptide band at 52 kDa. In the native PAGE, a single polypeptide band corresponding in mobility to the 52-kDa marker was identified in all blots of the 31 control urine samples. The high molecular weight reactive epitope purified from urine was analysed by SDS-PAGE. A single polypeptide band was stained with Coomassie blue, at 52-kDa (Fig. 2A). The reactivity of the isolated antigen was confirmed using Western blot (Fig. 2B). The purity of the eluted 52-kDa antigen was also confirmed using CZE; there was a single peak at 6.3 min (Fig. 2C). Specific antibodies developed in rabbits to human p53 (53-kDa) or IgG fraction (= 50 kDa) showed no reactivity to the target NMP using indirect ELISA (data not shown). Partial biochemical characterization of the reactive epitope confirmed its protein moiety. The reactivity of the anti-NMP-52 antibody was lost (i.e. a negative result using dot-ELISA) towards the 52-kDa antigen, and the 40-kDa fraction showed reactivity of the NMP antibody. Lane 1, urine of a patient with bladder cancer; and lane 2, the purified 52-kDa fraction.
of the antigen with pepsin enzyme and it was completely lost at 20 min.

We developed a simple noninvasive dot-ELISA format based on the anti-NMP-52 IgG antibody for rapid detection (within 5 min) of the target marker in urine samples of patients with bladder cancer. The dot-ELISA allows a semiquantitative reading of the resulting coloured dot if the marker is detected (i.e. a positive test). A colourless dot was produced if no marker was detected (i.e. a negative test; Fig. 3). To assess the reproducibility of these endpoints, the dot-blot assay was performed on five separate occasions for a selected group of urine samples of patients with bladder cancer, showing low (+), moderate (+++) and high (++++) colour endpoints (four samples each). The urine sample was tested in triplicate on each occasion. The percentage intra-assay and interassay coefficients of variation were <10%, ascertaining the reproducibility of these colour endpoints. In addition, the reactivity of the target NMP-52 marker in urine samples with low dot-ELISA colour endpoints was maintained with no change for at least as year after being stored frozen at −20°C. In urine samples with high and moderate dot-ELISA colour endpoints and stored frozen at −20°C, the reactivity of the target marker was maintained with no changes for long periods (>18 months).

FIG. 3. Rapid and office-based detection of NMP-52 marker in urine samples of patients with bladder cancer using the dot-ELISA. A and B represent urine samples from healthy individuals showing no detection of the target NMP-52 (negative), C represents a urine sample from a patient with bladder cancer, showing a low level (+) of the target NMP-52 marker, and D another urine sample from a patient with bladder cancer showing a high level (+++) of the target NMP-52 marker.

To evaluate the diagnostic performance of the developed test for clinical use, urine samples from 149 patients with histopathologically diagnosed bladder cancer and 72 controls were evaluated. The results were negative in four patients, low in 57, moderate in 48 and high in 40. The dot-ELISA assay showed only one false-positive result among 15 patients with malignancy other than bladder cancer, and three false-positive results among 57 apparently healthy controls, giving an overall specificity of 94%. The sensitivity, specificity, efficiency and predictive values of a positive and a negative result for the dot-ELISA compared with standard histopathology are shown in Table 1. The dot-ELISA assay was able to detect the urinary NMP-52 marker with high sensitivity in the different types and grades of bladder tumour (Table 1), in the early stages of bladder tumour (pTa, pT1 and pT2), and late stages (T3 and T4; Table 1).

Urine samples collected from all 43 patients before, during and after radiotherapy were tested for the target urinary marker using dot-ELISA (Table 2). Before radiotherapy the dot-ELISA result of these patients were low in 18, moderate in 17 and high in 8. During radiotherapy, reduced tumour size, no regional lymph node metastasis and no evidence of distant metastasis were characteristics for all 22 patients showing a
response to treatment. The intensities of the dot blots were significantly ($P < 0.05$) decreased in the urine of all those responding and were unchanged ($P > 0.05$) in 21 treated patients in whom the disease progressed. Four weeks after radiotherapy the urinary NMP was not detected using dot-ELISA in all those responding but was detected in all those not responding to radiotherapy.

**DISCUSSION**

Bladder tumour markers are potentially useful in screening for cancer, monitoring the course of the disease, and detecting relapse or recurrence after treatment [7]. Studies aim to develop easily applicable, noninvasive, inexpensive and reliable tools with high specificity and sensitivity to a certain tumour. However, no tumour marker of high specificity and sensitivity has become a routine diagnostic or screening tool for bladder carcinoma [23]. Several NMPs were identified and evaluated as markers of bladder cancer [24,25]; the one most widely evaluated, NMP-22, is a nuclear mitotic apparatus that is involved in the distribution of chromatin to daughter cells during cellular replication, and its concentration is at least 25 times greater in bladder cancer than the mean levels isolated from normal bladder [7]. A urine-based test for NMPs has been used for detecting bladder cancer, with variable results. The sensitivity of the NMP-22 test is 68.5–88.5% and the specificity 65.2–91.3%, depending on the thresholds used. However, this assay is usually not a point-of-care test and needs a laboratory with trained technicians [25]. In the present study we identified highly reactive epitopes at 52 and 40 kDa in the urine of patients with different types of bladder cancer, using specific anti-NMP IgG antibody. Based on the presence of proteolytic enzymes in urine, we speculated that the 40-kDa antigen might be a stable urinary degradation product of the 52-kDa antigen. In addition, the 40-kDa protein was not detected alone in urine samples from patients with bladder cancer tested using western immunoblotting. However, further characterization studies are required to confirm our assumption that it might represent a degradation product. An intense and diffuse nuclear and cytoplasmic immunoperoxidase reaction was shown in formalin-fixed paraffin-embedded sections of different types of bladder carcinoma (data not shown). The target NMP-52 is probably released from the nuclei of the tumour cells during apoptosis. Partial biochemical characterization experiments indicated the characteristic polypeptide nature of the reactive epitope isolated from urine. Interestingly, this NMP-52 has not been described previously in bladder cancer, but further molecular studies are required to confirm its identity. Attallah et al. [22] used a dot-ELISA based on a specific monoclonal antibody to detect a target cytoskeratin in urine samples from patients with bladder cancer, with high sensitivity, specificity and efficiency (90%). The dot-ELISA format does not require sophisticated equipment nor highly trained technical staff, and can be completed in ~30 min. In the present study, we developed a more convenient, reliable, inexpensive and easily applicable dot-ELISA format for the rapid detection (5 min) of the target NMP marker in the urine of patients with bladder cancer. The newly developed office-based format is suitable for the clinic and field use. We evaluated the performance characteristics of the developed assay for the rapid diagnosis and monitoring of bladder cancer after radiotherapy. The dot-ELISA detected the target NMP-52 marker in all types of bladder cancer with a sensitivity of 92–100% and a specificity of 94%. In addition, the assay had a high predictive values, positive (98%) and negative (93%), with an efficiency of 97%. An accurate rapid test for detecting bladder cancer in the follow-up must have high accuracy in patients with tumour stage pTa or pT1. The assay identified the target marker in patients with stage pTa, pT1 and pT2 disease. However, more patients with early stages of bladder tumour should be assessed before drawing final conclusions. In patients with no previous diagnosis of bladder cancer, the NMP-22 test had a greater sensitivity (80.9% vs 40%) but a lower specificity (64.3% vs 100%) than voided urine cytology [26]. However, these values for sensitivity and specificity are still significantly lower than those obtained using the NMP-52 test, further emphasising the bladder cancer-specific nature of NMP-52. It would be wiser to compare NMP-52 results with established urinary markers of bladder cancer. However, this approach is beyond the scope and limited financial resources of the present work.

The integration of urinary markers in follow-up protocols is another potential field of use. The good sensitivity and negative predictive values can reduce the need for follow-up cystoscopy. However, an apparent limitation of the available urine-based markers in the follow-up of bladder cancer appears to be the high rate of false-positive results [27].

<table>
<thead>
<tr>
<th>Sample time (week)</th>
<th>N</th>
<th>dot-ELISA</th>
<th>% negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before radiotherapy (0)</td>
<td>43</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>During radiotherapy:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>end of phase I (5)</td>
<td>43</td>
<td>37</td>
<td>6</td>
</tr>
<tr>
<td>end of phase II (9)</td>
<td>43</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>After radiotherapy (13)</td>
<td>43</td>
<td>7</td>
<td>90</td>
</tr>
</tbody>
</table>

In conclusion, the sensitive and specific detection of urinary NMP-52 using the simplified dot-ELISA would be helpful in the rapid diagnosis and follow-up of patients with bladder cancer. Further studies are needed to confirm the efficacy of NMP-52 for monitoring the responses to surgery and detecting recurrence.
IMMUNODIAGNOSTIC ASSAY FOR URINARY NMP-52 IN BLADDER CANCER

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CONFLICT OF INTEREST
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Abbreviations: NMP, nuclear matrix protein; SCC, squamous cell carcinoma; CFA, IFA, complete, incomplete Freund’s adjuvant; TCA, trichloroacetic acid; TBS, Tris-buffered saline; CZE, capillary zone electrophoresis.
Detrusor myectomy was introduced as an alternative to enterocystoplasty for refractory detrusor overactivity. The early results of this procedure were described by the authors from Bristol as encouraging, and they now present their long-term follow-up with a median of 79 months. They found that the results were sustained in a significant group of these patients.

The value of frequency-volume urinary diaries is undoubted in patients of either sex who have LUTS, particularly in those with symptoms suggestive of overactive bladder. Authors from Chicago compare such diaries between asymptomatic controls and women with symptoms of overactive bladder. A whole range of diary variables was compared, and the interesting findings used as a potential method to define important outcome goals in therapeutic trials.

**OBJECTIVES**

To assess the long-term results of detrusor myectomy, which has obvious theoretical advantages over enterocystoplasty for refractory detrusor overactivity (DO), and for which the early results have been encouraging.

**PATIENTS AND METHODS**

The medical records were reviewed of 30 consecutive patients (median age 33 years, range 10–62) who had a detrusor myectomy between November 1992 and April 2002 in our unit. Twenty-four patients (80%) had idiopathic DO (six males and 18 females) and six (20%) had neurogenic DO (four males and two females). The median (range) follow-up was 79 (28–142) months. All patients were confirmed to have DO on urodynamics before surgery and 26 (87%) had urodynamics afterward.

**RESULTS**

Nineteen (79%) of those with idiopathic DO and two with neurogenic DO showed a continued overall improvement. The cystometric capacity improved in 80% of patients after surgery, whilst the detrusor pressure at maximum flow and the bladder contractility index decreased in 60% and 78% of the patients, respectively. Ten patients (45%) had to start clean intermittent self-catheterization afterward.

**CONCLUSIONS**

Detrusor myectomy is successful in ≈80% of patients with idiopathic DO, although detrusor contractility is affected in most and almost half of the patients required clean intermittent self-catheterization afterward. This procedure should be offered as an alternative to enterocystoplasty as it is less morbid and does not preclude subsequent surgery if required. However, further evaluation of this technique is required in neuropathic patients.

**KEYWORDS**

detrusor myectomy, detrusor overactivity, overactive bladder

**INTRODUCTION**

In patients with refractory detrusor overactivity (DO), enterocystoplasty has the highest overall rate of success, but with a much higher likelihood of early and delayed complications [1,2]. Detrusor myectomy (DM) is a surgical technique that attempts to bridge the gap between the medical and the surgical treatment alternatives for refractory DO. Since its original description by Cartwright and Snow in 1989 [3], there have been few reports of this procedure.

One-year follow-up data were published from the authors’ institution in 1998 [4]; the
The present report is a longer-term follow-up of that data. Published results have been variable, with some reporting good outcomes in patients with neurogenic DO (NDO) whilst others have been unable to duplicate these results [5,6]. There have been few published reports of children who have had DM, although the results appear poor [7,8]. Laparoscopic bladder autocaustion has also been reported, with reasonable short-term results [9,10]. Thus the aim of the present study was to assess the long-term results of DM.

PATIENTS AND METHODS

Thirty patients with troublesome overactive bladder symptoms had DM between November 1992 and April 2002; the underlying cause was idiopathic DO (IDO) in 24 (80%) and NDO in six (20%). All patients were refractory to conventional anticholinergic treatment and were keen to explore surgical treatment options. DM was offered to them as an alternative to enterocystoplasty. Patients were counselled about the relative novelty of the procedure and that long-term data as to the effectiveness of the technique were unavailable. The possible need for clean intermittent self-cathereterization (CISC) and the likelihood of further surgery was discussed. As early results showed that a high proportion of patients would need to use CISC, all later patients were taught to use the technique before surgery, to ensure compliance after surgery if CISC proved necessary. All patients had undergone urodynamics before surgical intervention.

For urodynamics, initial free urinary flow rates and residuals were measured in all patients. After counselling them as the patient, bladder and rectal lines were inserted and the initial residual urine usually drained and recorded. All patients had their urethral pressure profiles measured and bladder filling commenced at 50 mL/min. Quality control was checked throughout the test by asking the patient to cough, to ensure that both pressure lines were working properly. If the patients had severe DO the filling rate was slowed to ≈20 mL/min. Urodynamics were usually conducted with the males standing and the females sitting. The filling line was removed when the patient's bladder was full or when the patient reported a severe urge to void. The patient was then asked to void and the voiding pressures were measured. Video X-ray screening was used routinely in all patients.

For the DM, an extraperitoneal approach was used via a Pfannenstiel incision. The bladder was distended with 250 mL of saline mixed with methylene blue. The peritoneum was dissected off the dome and the supra-posterior aspect of the bladder and a disk of detrusor muscle of 8–12 cm was excised. The mucosa was left intact to develop into a broad-based superiorly situated diverticulum. After excising the detrusor muscle, the peritoneum was opened and the omentum brought down and sutured anteriorly and laterally to the detrusor edge. When the mucosa was torn the defects were repaired immediately with six '0' absorbable polyglactin sutures. In later cases a small piece of the excised detrusor muscle was used as a buttress over which six '0' sutures were tied. This appeared to improve the closure of the defects, which were usually tears of <0.5 cm in maximum diameter. If there was no leak at the end of the procedure when the bladder was distended carefully, then bladder cycling was started on the evening of the operation. Bladder distension was started 2 days after surgery if there was leakage after the mucosal tears were repaired.

The urethral catheter was removed after 24 or 48 h depending on the patients' mobility and according to whether or not the mucosa had been intact during surgery. If the mucosa had been torn then a drain was left in situ and gentle bladder cycling continued until a cystogram was taken 7 days after DM. Patients were then followed up at 3-, 6- and 12-monthly intervals. Urodynamics were performed after DM in 26 (87%) of the patients (Fig. 1). The main urodynamic variables assessed before and after DM were the cystometric capacity, maximum detrusor pressure at maximum flow (pdetQmax), and the bladder contractility index (BCI; pdetQmax + 5Qmax) [11].

RESULTS

The median (range) follow-up was 79 (28–142) months; the mucosa was breached during DM in 15 patients (50%) and this was sutured immediately. There was no correlation between mucosal breach and the final outcome (Table 1). There was one major complication, a bowel perforation that probably occurred during mobilization of the omentum. This patient required a laparotomy and closure of the perforation, with a significant stay in intensive care. She did not have a good result from the DM and ultimately went on to have an ileal conduit.

TABLE 1 The correlation between mucosal tears and final outcomes

<table>
<thead>
<tr>
<th>Mucosal tears</th>
<th>Improvement</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>10 (33)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>No</td>
<td>11 (37)</td>
<td>4 (13)</td>
</tr>
</tbody>
</table>

Twenty-one patients (70%) had a continued improvement, of whom 19 had IDO and two NDO. One of the latter patients had an artificial urinary sphincter (AUS) inserted because of stress incontinence on urodynamics (Table 2). Amongst the patients
with an improvement, only 14 (67%) had no evidence of DO on cystometry after DM. The values assessed before and after DM are shown in Table 3. Overall, the cystometric capacity increased in 80% of the patients by a mean of 165 mL (P < 0.001). The change in pdetQmax was not significant, even though it decreased in 60% of the patients, whilst the BCI decreased in 78% (P < 0.06) after DM. Ten patients (45%) had to start CISC after surgery. The mean (range) volume obtained on CISC was 325 (200–500) mL. The main indications for CISC were UTI in seven patients, persisting NDO in 14, and were likely to have had associated detrusor sphincter dyssynergia (DSD). The one neurogenic patient in the present series who had a spinal cord injury had associated urethral sphincter weakness, subsequently necessitating the insertion of an AUS.

**DISCUSSION**

DM or bladder autoaugmentation is an alternative surgical option described by Cartwright and Snow for refractory DO [3]. This procedure has produced mixed results in different groups of patients, and therefore has not replaced enterocystoplasty as a standard treatment for refractory DO. In addition, several variations of DM have been described which aim to achieve better results. Animal models have shown that bladder capacity could be maintained if a graft (e.g. omentum, synthetic membrane or (myoplicated dura) is inserted at the time of intervention, either in place of the removed detrusor muscle disk or between the muscle edges where the muscle has been split, allowing the exposed mucosa to bulge through. In either case the aim was to prevent adhesion of the muscle edges and/or the mucosa either to the surrounding tissues or, in the case of the incised (rather than removed) detrusor, to prevent the edges from re-uniting [12]. Bladder autoaugmentation with rectus muscle backing was also described, where both recti are dissected from the anterior and posterior sheaths and sutured to the detrusor edges [13]. All patients in the rectus muscle study showed better compliance and increased cystometric capacity, but there were few patients and the follow-up was short.

Laparoscopic bladder augmentation was reported in children and in patients with spinal cord injury [9,10]. The long-term results of this procedure in children with NDO have been poor [7,8]. The fate of the mucosal diverticulum is unknown, but it was speculated that ultimately the diverticulum will undergo fibrosis, or that muscular re-growth may occur [14]. However, we showed that in patients with IDO the symptomatic improvement was maintained even in the long term; 45% of the patients had to use CISC after DM, often because of recurrent UTIs.

The use of sterile intermittent catheterization in the management of spinal cord injury was introduced by Guttman and Frankel [15] in 1947, and was slow to gain acceptance. Lapides et al. [16] went on to develop the concept of clean rather than sterile intermittent catheterization. Although the improvement in continence rates was >90% after CISC in all groups of patients [17,18], 41–48% were infection-free after starting CISC [17,18] and >95% were satisfied with CISC [18].

Patients with NDO had a high failure rate in the present series. Initial published reports were encouraging in patients with NDO [5] and these results were further supplemented by a longer-term follow-up by Stohrer et al. [6]. The present initial results did not support this and suggested that the outcome of this procedure was better in patients with IDO [4]. However, most patients in the study by Stohrer et al. had neurogenic voiding dysfunction secondary to spinal cord injuries, and were likely to have had associated detrusor sphincter dyssynergia (DSD). The one neurogenic patient in the present series who had a spinal cord injury had associated urethral sphincter weakness, subsequently necessitating the insertion of an AUS.

**Autoaugmentation with detrusor myotomy** rather than myectomy has also been reported, with encouraging early results in patients with myelomeningocele [19]. The long-term results in one published series of 21 patients with a mean follow-up of 6 years showed an effective reduction in intravesical pressure, with a significant increase in bladder capacity [20]. However, the results have been poor, and in one series the authors conclude that this procedure cannot be recommended for managing congenital neuropathic bladder [21]. Autoaugmentation was also reported with variable results using demucosalized gastric flaps, peritoneal flaps and seromuscular colonic flaps to cover the

**TABLE 2 Secondary procedures**

<table>
<thead>
<tr>
<th>Diagnosis before DM</th>
<th>Urodynamics after DM</th>
<th>Secondary procedures</th>
<th>Time after DM, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spina bifida</td>
<td>USI/No NDO</td>
<td>Insertion of AUS</td>
<td>29</td>
</tr>
<tr>
<td>Spina bifida</td>
<td>Persisting NDO</td>
<td>Ileocystoplasty</td>
<td>14</td>
</tr>
<tr>
<td>Spina bifida</td>
<td>Persisting NDO</td>
<td>Sling + ileocystoplasty</td>
<td>26</td>
</tr>
<tr>
<td>Spina bifida</td>
<td>Persisting NDO</td>
<td>Colocystoplasty</td>
<td>11</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>Persisting NDO</td>
<td>Ileocystoplasty + AUS</td>
<td>13</td>
</tr>
<tr>
<td>IDO</td>
<td>USI/No IDO</td>
<td>Colposuspension</td>
<td>3</td>
</tr>
<tr>
<td>IDO</td>
<td>Persisting IDO</td>
<td>Ileal conduit</td>
<td>62</td>
</tr>
<tr>
<td>IDO</td>
<td>Persisting IDO</td>
<td>Ileal conduit</td>
<td>50</td>
</tr>
</tbody>
</table>

**USI, urodynamic stress incontinence.**

**TABLE 3 Urodynamic variables before and after surgery**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before</th>
<th>After</th>
<th>% showing change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (range):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystometric capacity, mL</td>
<td>228 (41–555)</td>
<td>405 (120–1040)</td>
<td>+80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pdetQmax</td>
<td>38 (15–110)</td>
<td>26 (0–49)</td>
<td>−60</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>BCI</td>
<td>122 (50–241)</td>
<td>89 (0–185)</td>
<td>−78</td>
<td>&lt;0.06</td>
</tr>
</tbody>
</table>
REFERENCES

None declared.

CONFLICT OF INTEREST

None declared.

REFERENCES


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Abbreviations: DM, detrusor myectomy; (N)(I)DO, (neurogenic) (idiopathic) detrusor overactivity; BCI, bladder contractility index; AUS, artificial urinary sphincter; CISC, clean intermittent self-catheterisation; DSD, detrusor sphincter dyssynergia.
Are conventional pressure–flow measurements dependent upon filled volume?

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OBJECTIVE
To determine, in a prospective study, whether detrusor pressure (p\text{\textsubscript{\text{det.Q\text{\textsubscript{max}}}}}) and maximum urinary flow rate (Q\text{\textsubscript{max}}) measurements obtained after filling to maximum cystometric capacity (MCC) differ from those obtained with filling restricted to average voided volume (V\text{\textsubscript{void}}), as standard protocols for pressure flow studies (PFS) mandate bladder filling until the subject has a strong desire to void, which aids standardization but further divorces the test from real-life experience.

PATIENTS AND METHODS
After calculating the appropriate sample size, 84 patients attending for PFS with an adequately completed 3-day frequency–volume chart were recruited. Each underwent two consecutive PFS with filling to MCC and average V\text{\textsubscript{void}} in a random order, and measurements of p\text{\textsubscript{\text{det.Q\text{\textsubscript{max}}}}} and Q\text{\textsubscript{max}} were compared. For men, the agreement for a diagnosis of obstruction between the tests was also assessed.

RESULTS
Complete data were obtained from 76 (90%) of the patients, with a mean (range) age of 64 (20–94) years. The mean (SD) difference between MCC and average V\text{\textsubscript{void}} was 134 (113) mL (P < 0.01). There were no significant differences between estimates of Q\text{\textsubscript{max}} at −0.1 (3) mL/s (P = 0.75), and of p\text{\textsubscript{\text{det.Q\text{\textsubscript{max}}}}} at −1 (13) cmH\textsubscript{2}O (P = 0.91), obtained within each patient. For men there was 91% agreement (32 of 38) in the classification of obstruction.

CONCLUSIONS
Restriction of filling to the average V\text{\textsubscript{void}} during PFS allows a closer approximation to normal voiding and results in no clinically relevant change to the value of standard pressure–flow measurements or alters individual classification of obstruction.

KEYWORDS
urodynamics, bladder outlet obstruction, pressure–flow study, frequency–volume chart, voided volume

INTRODUCTION
Voiding dysfunction in patients with bothersome LUTS is currently diagnosed by plotting on a nomogram simultaneous readings of maximum urinary flow rate (Q\text{\textsubscript{max}}) and detrusor pressure at Q\text{\textsubscript{max}} (p\text{\textsubscript{\text{det.Q\text{\textsubscript{max}}}}}) obtained from a conventional pressure–flow study (PFS), allowing urodynamic categorization into obstructed, equivocal or unobstructed groups [1]. The reliability of such categorization depends on several factors, which may include the filled volume of the bladder [2]. Based on recommendations of the ICS, standard practice for PFS is to continue bladder filling until the subject experiences a strong desire to void, this being defined as maximum cystometric capacity (MCC) [3]. The main reasons for this practice are the increased likelihood of detecting detrusor overactivity (DO) during an extended filling phase, and to encourage the ability to void in an atypical setting. However, a possible disadvantage of filling beyond the functional capacity is over-stretching the detrusor, leading to reduced force of contraction [4] and hence reduced Q\text{\textsubscript{max}} [5].

As the role of PFS is to provide diagnostic measurements in the context of symptoms experienced by an individual, it would seem reasonable to recreate real-life conditions as far as possible within the clinical environment of the urodynamic laboratory. As part of this aim, restricting filling to an individually comfortable bladder volume might be advantageous, provided the diagnostic reliability was not compromised. We therefore carried out a prospective study to test the following hypotheses: (i) There is no difference in simultaneous measurements of Q\text{\textsubscript{max}} and p\text{\textsubscript{\text{det.Q\text{\textsubscript{max}}}}} made during voids initiated at MCC and average voided volume (V\text{\textsubscript{void}}); (ii) the classification of obstruction is not changed by restricting bladder filling to the average V\text{\textsubscript{void}}.

PATIENTS AND METHODS
A preliminary calculation of sample size showed that the recruitment of ≥75 subjects was required to detect clinically relevant differences of 15 cmH\textsubscript{2}O or 2 mL/s in p\text{\textsubscript{\text{det.Q\text{\textsubscript{max}}}}} and Q\text{\textsubscript{max}}, respectively, at 5% significance [6]. All adult patients with no neurological disease who attended our urodynamic laboratory for conventional PFS and with an adequately completed 3-day frequency–volume chart (FVC) were invited to participate in the study. After a careful explanation of the aims and methods of the study, and obtaining of informed consent, we recruited 84 patients over a 5-month period.

For each patient the average V\text{\textsubscript{void}} was calculated by summing the volumes of all voids recorded during three consecutive 24-h periods and dividing the total by the number of voids. The patients then had two consecutive PFS with filling to both MCC, indicated by a strong desire to void, and to average V\text{\textsubscript{void}}, the sequence being determined by previous randomization. A standard technique for PFS was used which conformed to ICS good practice [2]. Briefly, after a private void and under aseptic conditions, the bladder
was catheterized with a 10 F filling line and a 4 F bladder pressure line, whilst a 6 F catheter covered with a vented finger cot was inserted into the rectum for abdominal pressure recording. The water-filled lines were connected to pressure transducers placed at the level of the pubic symphysis and zeroed to atmospheric pressure. The initial fill was with the patient supine before changing to standing or seated for voiding, whilst the second fill was with the patient standing (men) or seated on a commode (women). Both voids were therefore in the same position for each patient. Non-physiological filling at a rate of 100 mL/min was used throughout.

Intravesical pressure (p ves), abdominal pressure (p abdom), subtracted detrusor pressure (p det), flow rate (Q void) together with filled and voided volume (V max) were continuously recorded at a sampling frequency of 10 Hz. Residual urine was estimated at the end of the second void by abdominal ultrasound.

Values for filled volume, Q max and p det,max for each PFS were recorded and expressed as the mean (sd). Values for residual volume obtained at the end of the second void were noted but not added to the measured filled volumes during PFS. The Abrams-Griffiths (AG) number (p det,max − 2Q max) was calculated for each void and men were categorized as being obstructed, equivocally obstructed or unobstructed by plotting the values of Q max and p det,max from each void on the provisional ICS nomogram [1]. Cystometry traces recorded for the two fills were examined by two experienced urologists for the presence of DO, defined according to current ICS criteria [3]. Differences in paired values of Q max and p det,max within each patient were assessed using Student’s t-test and the Bland-Altman analysis to estimate bias and measurement error [7]. The 95% CI for the mean was calculated when appropriate and statistical significance assessed at the 5% level.

RESULTS

In all, 84 patients were recruited (63 men and 21 women, mean age 65 years, range 20–94); of these, 76 (90%; 59 men and 17 women) completed the study, whilst eight were excluded because of expulsion of the bladder pressure line during voiding (two), failure to void into the uroflowmeter (two), or failure to void (four). Two of those who failed to void did so on both the fills, and another two failed after filling to the average V void alone. Of those who completed the study, 38 were randomized to filling to MCC first, whilst 38 were initially filled to average V void.

The mean (sd) infused volume required for MCC, at 327 (135) mL, was 134 (114) mL (70%) higher than that of average V void at 193 (78) mL, calculated from the FVC (P < 0.001). Overall, measurements of Q max and p det,max obtained after filling to MCC and average V void showed no statistical or clinically relevant differences (Table 1, Fig. 1a,b). In all, 30 (39%) patients had a difference in Q max of >2 mL/s and 16 (21%) had a >15 cmH2O difference in p det,max. There were systematic differences between voiding variables recorded from the first and second fills, irrespective of filled volume, with both Q max and p det,max being significantly greater on the first fill (Table 1). The residual urine volume after the second void was >150 mL for 12 (16%) patients, with a mean (range) of 290 (170–500) mL. Analysis of the data from this group showed no significant differences from those with more complete bladder emptying (Table 1).

For men the diagnostic category for obstruction was changed for four (5%), with three moving from obstructed to equivocal and one from equivocal to obstructed (Table 2). The reproducibility of the diagnosis of BOO using filling to average V void in the subgroup of 59 men was 91%. All those with no BOO were correctly identified by filling to average V void. There were phasic pressure rises indicative of DO in 24 (32%) patients (Table 3). Statistical analysis of the detection rates for DO according to filled volume and order of filling, assuming all cases of DO were identified, showed significantly more cases during the second fill, irrespective of the filled volume used (P < 0.001, Fisher’s exact test), whilst the increased detection rate after filling to MCC compared to average V void was of only marginal significance (P < 0.041, Fisher’s exact test).

<table>
<thead>
<tr>
<th>Random (76 patients):</th>
<th>Mean (sd) [95% CI] difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value at MCC – value at average V void for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q max, mL</td>
<td>-0.1 (3.2) [-0.9 to 0.6]</td>
<td>0.75</td>
</tr>
<tr>
<td>p det,max cmH2O</td>
<td>-0.2 (12.6) [-3.0 to 2.7]</td>
<td>0.91</td>
</tr>
<tr>
<td>AG number</td>
<td>0.08 (11.8) [-2.6 to 2.8]</td>
<td>0.95</td>
</tr>
<tr>
<td>V void, mL</td>
<td>134 (114) [108–160]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sequential, value at 1 – value at 2 (76 patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q max, mL</td>
<td>1.3 (3.0) [0.6–2.0]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>p det,max cmH2O</td>
<td>5.3 (11.4) [2.7–7.9]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AG number</td>
<td>2.7 (11.5) [0.1–5.3]</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>V void, mL</td>
<td>13 (179) [-27 to 54]</td>
<td>0.5</td>
</tr>
<tr>
<td>Residual volume &gt; 150 mL (12 patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Value at MCC – value at average V void for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q max, mL</td>
<td>-1.4 (2.6) [-3.0 to 0.2]</td>
<td>0.08</td>
</tr>
<tr>
<td>p det,max cmH2O</td>
<td>-5.7 (12.7) [-13 to 2.4]</td>
<td>0.15</td>
</tr>
<tr>
<td>AG number</td>
<td>-2.8 (11.8) [-10 to 4.7]</td>
<td>0.42</td>
</tr>
<tr>
<td>V void, mL</td>
<td>188 (153) [91–286]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>at MCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V void – V void, mL</td>
<td>135 [220] [-5.0 to 274]</td>
<td>0.06</td>
</tr>
<tr>
<td>at average V void</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V void – V void, mL</td>
<td>-27 [100] [-90 to 37]</td>
<td>0.37</td>
</tr>
</tbody>
</table>

TABLE 1

Comparison of Q max, p det,max and V void measured during PFS after filling to MCC and V det,max, with randomized order of filling, with sequential filling, and as a comparison between the two fills for patients whose residual urine was >150 mL.

<table>
<thead>
<tr>
<th>Fill at</th>
<th>BOO</th>
<th>Equivocal</th>
<th>Unobstructed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC</td>
<td>35</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Average V void</td>
<td>33</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>P</td>
<td>0.85</td>
<td>0.84</td>
<td>0.57</td>
</tr>
<tr>
<td>First</td>
<td>36</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Second</td>
<td>32</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>P</td>
<td>0.57</td>
<td>0.55</td>
<td>0.55</td>
</tr>
</tbody>
</table>

TABLE 2

Comparison of diagnostic categorization of men, using the provisional ICS nomogram, according to V void and fill sequence.

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FIG. 1. Bland-Altman analyses of: a, Qmax (MCC) and Qmax (average Vvoid); and b, pdet.Qmax (MCC) and pdet.Qmax (average Vvoid).

TABLE 3 Comparison of the rate of diagnosis of phasic DO during filling cystometry according to filled volume and sequence of fill for 24 patients who had DO on at least one fill

<table>
<thead>
<tr>
<th>Fill at</th>
<th>DO</th>
<th>No DO</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Average Vvoid</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>P</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Second</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The present results suggest that for the purposes of a conventional PFS used to investigate the urodynamic cause of voiding dysfunction, restriction of bladder filling to average Vvoid allows a valid estimation of Qmax and pdet.Qmax and does not alter the classification of BOO for >90% of patients.

The effect of filled volume on measurements obtained during conventional PFS has not been systematically characterized to date. Studies examining the volume-dependency of free urinary flow rate showed an increase with Vvoid, particularly at 0–200 mL [8]. Theoretical modelling and experimental studies suggest that over-stretching can impair detrusor contraction strength and hence reduce Qmax [4,5]. Further information was provided by studies comparing ambulatory natural-fill urodynamics with conventional PFS, which showed that the 40–50% reduction in Vvoid after natural-fill was associated with increases in both Qmax and pdet.Qmax compared to sequential conventional PFS [9,10]. A subsequent comparative study where nonphysiological filling during conventional PFS was restricted to ‘normal desire to void’ found that the increase in Qmax recorded after ambulatory filling was less pronounced and voiding pressure was similar, suggesting a dependency on filled volume rather than fill rate [11]. In an attempt to characterize more precisely the effect of filled volume with a constant fill rate, Groen et al. [12] compared values of Qmax and pdet.Qmax obtained by conventional PFS after filling to both MCC and modal functional capacity in young female volunteers. They found that flow rate was lower and voiding pressure unchanged during voids initiated at functional capacity. These findings were an advance on existing knowledge, but the quality of their data was compromised by a high exclusion rate, use of unpaired data and lack of randomization of voiding sequence. The effect of void sequence is illustrated by findings from most studies investigating the test/re-test reliability of conventional PFS, which have shown sequence bias with values of both Qmax and pdet.Qmax being higher on the first fill [6,13], changing the urodynamic classification of obstruction for ~30% of cases [13,14], as also in the present study. These changes are thought to reflect variation in contraction strength and outlet status rather than measurement error [13].

The present data showed no overall bias in measurement of Qmax and pdet.Qmax when filling was restricted to average Vvoid, but there was a degree of random variation. Analysis of this variation using pre-set limits (Qmax < 2 mL/s, pdet.Qmax < 15 cmH2O) showed clinically significant changes for flow rate in 39% of patients and for pressure measurement in 21%, compared to values of 20% and 15%, respectively, in a recent reliability study [13]. In contrast, Bland–Altman analysis, where random variation is expressed by the SD of differences, showed similar findings to...
previous studies [6,12]. On balance, it therefore appears reasonable to conclude that our methods caused no substantial increase in random variation of standard pressure-flow measurements.

The FVC is now well established as a useful tool for evaluating patients with LUTS but for the purpose of informing the conduct of PFS, a representative standard \( V_{\text{void}} \) for an individual must be defined [16]. Previous authors suggested the maximum \( V_{\text{void}} \) (previously defined as functional capacity), modal \( V_{\text{void}} \) and average \( V_{\text{void}} \) over 24 h or during daytime alone. A review of relevant reports suggests that the average \( V_{\text{void}} \) over three or four consecutive 24-h periods is best, a measure that has consistently been shown to be \( \approx 60\% \) of MCC [15,17–19]. The precise volume within the bladder just before voiding will be the sum of filled volume, urine production during the test and residual volume. We decided to measure filled volume only, as this represents the major component of bladder capacity during conventional PFS, can be accurately measured and can be related directly to data from the FVC. It is possible that high residual urine volumes may affect the reliability of pressure-flow measurements, but in the few present patients with residuals of >150 mL the results were unchanged.

Given the lack of bias found in measurements of \( Q_{\text{max}} \) and \( p_{\text{det}, \text{Qmax}} \) it is not surprising that there were no clinically significant changes in classification of obstruction using either the AG number or position on the provisional ICS nomogram. Although such classification has only been validated for men with LUTS, we felt it appropriate to include women in the overall study, to give a wide range of pressure and flow readings and hence widen the applicability of the findings.

The present results, together with previous published work, suggest that pressure-flow measurements are consistent irrespective of whether the void was initiated at functional or maximum capacity, indicating that reduced contraction strength caused by overfilling is not of practical importance during conventional PFS. Part of the rationale for filling to MCC is to detect DO, although filled volume is only one of many factors that influence this diagnosis [20]. The present study was not designed to answer this question, but there were marked variations in detecting DO, with fill sequence appearing the more important factor rather than filled volume. This may have been influenced by performing the second fill standing or sitting, a known provocative factor for DO [20]. Another reason for filling to MCC is to encourage the subject to void in the laboratory environment. This has not been tested before, but in the present study only 2% of patients failed to void only when filling was restricted to functional capacity. The patient experience during cystometry has not been well documented. A recent study suggested that men in particular find the procedure uncomfortable, whilst women experience more shame and embarrassment [21]. Whether this discomfort was lessened by restricting filling to a functional capacity was not assessed in the current study, but it may benefit those who experience urge at higher volumes.

In conclusion, in men with LUTS and no significant urge component who solely require diagnosis of possible BOO, a PFS with filling restricted to average \( V_{\text{void}} \) calculated from the FVC allows valid pressure-flow measurements. Those patients with mixed symptoms, particularly involving urgency or urge incontinence, require further provocation by extended filling, change to upright position or a second fill, to reliably document DO. It may be possible therefore to better tailor invasive urodynamic evaluation to individual requirements provided the rationale and conduct of the examination is fully documented in the report.

CONFLICTS OF INTERESTS

None declared.

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Abbreviations: Qmax, maximum urinary flow rate; pdet.Qmax, detrusor pressure at Q max; PFS, pressure-flow study; MCC, maximum cystometric capacity; DO, detrusor overactivity; Vvoid, fiv, voided or filling volume; PVC, frequency-volume chart; AG, Abrams-Griffiths (number).
Validation of a patient-administered questionnaire to measure the severity and bothersomeness of lower urinary tract symptoms in uncomplicated urinary tract infection (UTI): the UTI Symptom Assessment questionnaire

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Accepted for publication 21 February 2005

OBJECTIVE

To develop and validate a self-administered questionnaire to assess the ‘severity’ and ‘bothersomeness’ of the most frequently reported signs and symptoms of uncomplicated urinary tract infection (uUTI).

SUBJECTS AND METHODS

The UTI Symptom Assessment questionnaire (UTISA) is a 14-item instrument asking about the severity and bothersomeness of seven key uUTI symptoms. It was developed after comprehensive literature and data review and administration in draft form to a sample of 30 women with uUTI. The final questionnaire was completed by 276 women with uUTI who participated in a noncomparative clinical trial of ciprofloxacin. The women completed the questionnaire in electronic format at baseline (before the first dose of ciprofloxacin once-daily), at 3-h and 8-h intervals until all UTI symptoms were resolved, and at the test-of-cure visit. Baseline scores on the King’s Health Questionnaire (KHQ) were used to assess convergent and divergent validity; responses to the Global Rating of Change (GRC) were used to assess both responsiveness and the ‘minimally important difference’. Discriminant validity and responsiveness were assessed by comparing UTISA scores with a clinical evaluation of UTI symptoms performed by the investigator at baseline and at the test-of-cure visit.

RESULTS

The UTISA was found to comprise three four-item domains named ‘urination regularity’, ‘problems with urination’, and ‘pain associated with UTI’. Two questions asking about haematuria loaded on a fourth factor. The three domains were homogeneous (with high inter-item correlations) and internally consistent. Convergent validity was shown by high correlations between similar UTISA and KHQ domains (all \( r_s > 0.40 \)), and divergent validity by small correlations between unlike domains (all \( r_s < 0.15 \)). In general, the UTISA domains showed excellent discriminant validity, with scores on selected domains discriminating between women with different clinical evaluations. The responsiveness of the UTISA was also excellent, with high correlations between changes in domain scores and the clinical evaluation and GRC items. Symptom improvement was highest in the first 3 h, leading to greater responsiveness and minimally important difference during this period. However, the UTISA could detect even small subsequent changes.

CONCLUSION

The three-domain UTISA has excellent psychometric properties and it is likely to prove an excellent tool for assessing uUTI outcome from a patient’s perspective, both in research and clinical settings.

KEYWORDS

urinary tract infection, urinary symptoms, questionnaire, validity, reliability.

INTRODUCTION

UTIs are considered to be the most common bacterial infection, with an estimated seven million physician visits and one million emergency department visits each year in the USA alone. Half of all women have at least one UTI in their lifetime [1,2]. However, the incidence of UTI is difficult to assess accurately, as UTI is not well reported. Moreover, although diagnosis ideally involves confirmation of the presence of symptoms and a positive urine culture, in practice, a diagnosis is frequently made without the benefit of culture results.

An acute uncomplicated UTI (uUTI; also referred to as cystitis, acute cystitis or dysuria–frequency syndrome) has been defined in several ways. In women, it includes a clinical syndrome characterized by various combinations of dysuria (painful urination), frequency, urgency, gross haematuria, lower back and/or abdominal/suprapubic discomfort with pyuria and bacteriuria [3]. Usually there is no identified underlying renal or urological dysfunction or obstruction, and up to half of patients with uUTI do not have bacteriuria according to established criteria [4,5]. Frequent sexual intercourse, diaphram use, the use of a spermicide, not voiding after intercourse and a history of recurrent infection are risk factors for UTIs in women [6,7].

While there has been some research on the impact of uUTI on everyday activities, with one study reporting that each episode of UTI results in an average of 6.1 symptomatic days and 2.4 restricted-activity days, as well as time lost from work [8], there has been little research on symptom duration, bothersomeness, or the impact of symptoms on patients’ quality of life (QoL) [9], although an association is recognized [10]. Generalized symptoms of feeling ‘out of sorts’, especially...
feeling unwell, weak and tired, or irritable and restless, are common [11], while women in one study described the dominant feeling as a burning and scorching pain: ‘It feels like peeing barbed wire.’ [12].

One recent study examined the impact of uUTI on QoL [13] using the Medical Outcome Study Short-Form 36 Health Survey (SF-36) [14] to assess QoL, and found that women with uUTI reported significantly poorer QoL in all SF-36 domains, both emotional and physical. However, no validated instrument exists to measure symptoms in uUTI.

The present study was designed to develop and validate a questionnaire, the UTI Symptom Assessment questionnaire (UTISA), to assess the ‘severity’ and ‘bothersomeness’ of the most frequently reported symptoms and signs of uUTI. The questionnaire was developed on the basis of the results of a series of comprehensive reviews to identify the key symptoms associated with uUTI. The data for the validation study came from a noncomparative clinical trial of the treatment of women with uUTI. The trial was specifically designed to validate the new instrument, and incorporated validated measures as well as clinical and patient evaluations of symptoms for the purposes of psychometric validation. The psychometric properties of the questionnaire were assessed primarily by the pattern of associations between UTISA scores and scores on the King's Health Questionnaire (KHQ) and Global Rating of Change (GRC) scores. The assessed properties included internal consistency, convergent and divergent reliability, discriminant validity, and responsiveness. The Minimally Important Difference (MID) of the UTISA was also assessed.

SUBJECTS AND METHODS

The UTISA was developed as a means of assessing symptoms in uUTI and its development has been reported in full elsewhere [7]. In brief, key clinical signs/symptoms of uUTI were identified from comprehensive literature and data reviews. Seven key symptoms were identified: frequency of urination, urgency of urination, pain and burning during urination (dysuria), inability to empty the bladder completely, pain or discomfort in the lower abdomen, low back pain, and urine leakage. A questionnaire to assess these seven symptoms was then developed. For each symptom, the patient rated bothersomeness on a 10-point Likert-type scale and indicated the time to symptom resolution. Focus on ‘bothersomeness’ arose from research in BPH, which suggested that the perceived bother from urinary symptoms is a stronger predictor of healthcare-seeking behaviour for urinary dysfunction than is the absolute frequency of symptom occurrence.

The questionnaire was administered to 33 women aged 18–55 years who had been diagnosed with uUTI. The women were drawn from two ethnically and socio-economically diverse USA communities, and 30 of the 33 women completed the questionnaire. The responses of these 30 women were reviewed to finalise the UTISA questionnaire. The questionnaire was modified to make it self-administered and to capture symptom severity as well as bothersomeness. The Likert scales for each item were changed from 10-point to 4-point scales, and the symptom of urine leakage was replaced with haematuria (blood in urine/dark urine), primarily for clinical diagnostic value rather than for symptomatic importance. The questionnaire (baseline and follow-up versions) is shown in Figs 1 and 2. For permission to use it, please contact the Bayer Pharmaceuticals Corp., Global Health Economics and Outcomes Research Department, West Haven, CT.

The assessment of reliability and validity was conducted in the context of a prospective, open-label, noncomparative, multicentre clinical trial of ciprofloxacin once-daily 500 mg for 3 days. Women with uUTI were recruited for entry to this trial between 18 June 2003 and 14 January 2004. At their first visit, just before their first dose of ciprofloxacin once-daily, the women gave written informed consent, and gave a urine sample for dipstick biochemical analysis for nitrates or leukocyte esterase, and a clean-catch midstream urine specimen for culture and sensitivity. The women also provided demographic and medical history details (age, ethnicity, years of education, employment status, previous history of uUTI, number of days since onset of uUTI before seeing physician), and completed the patient-reported questionnaires. The clinical evaluation was performed and the ciprofloxacin once-daily dispensed. During treatment (days 1–3), the women completed the UTISA, the KHQ and the GRC. If the patient discontinued the treatment prematurely during this time (days 1–3) a further clean-catch midstream urine specimen was taken and was clinically evaluated. Otherwise, these assessments were taken at the second test-of-cure visit 5–9 days after treatment, at which point the patient-reported measures were also completed.

Clinical evaluation involved the investigator assessing five UTI symptoms (dysuria, frequency, urgency, suprapubic pain, gross haematuria) rated either as ‘none’, ‘mild’, ‘moderate’, ‘severe’, and scored as 0–3. The clinical evaluations took place at the first visit, at any premature discontinuation (day 1–3), and at the test-of-cure visit. Bacteriological assessments were also made, but the data were not used for validation as it was considered that the validity of the instrument would be related primarily to symptom severity and bothersomeness. Of the 273 women with culture data, those with (189, 69%) and those without positive cultures (defined as > 10³ colony-forming units/mL at first visit) did not differ significantly in terms of any baseline demographic or clinical data.

Patient-reported outcomes were recorded electronically (using ‘palm pilot’ computers with information downloaded to a host computer) at the first visit (just before the first dose of ciprofloxacin once-daily), and at 3-h and then 8-h intervals until the symptoms had resolved, or at the test-of-cure visit, whichever came first. The palm pilots prompted the patients to complete the questionnaires at the correct times.

The UTISA is a self-administered, 14-item questionnaire that assesses, for each of the seven most frequently reported symptoms and signs of UTI (frequency, urgency, pain/burning on urination, incomplete voiding, pain in pelvic area, low back pain, blood in urine), levels of ‘severity’ and ‘bothersomeness’. Each item has a Likert-type response scale, the ‘severity’ item response options being ‘did not have’, ‘mild’, ‘moderate’, ‘severe’, scored 0–3; and the bothersomeness item response options being ‘not at all’, ‘a little’, ‘moderately’, ‘a lot’, scored 0–3. The UTISA was administered at the first visit, every 3 h for the first 24 h while awake, then every 8 h until all UTI symptoms were resolved (for at least 24 h or three data capture points, whichever was longer), and finally at the test-of-cure visit.

The KHQ is a self-administered questionnaire designed to assess the impact of urinary incontinence on QoL. The measure was
Assess symptom improvement. As formulated

The GRC is a three-item measure used to

cure visit.

whichever was longer), and at the test-of-

24 h, or over three data capture points,

symptoms had resolved (no symptoms for

The KHQ was administered at visit 1, on day 3,

these problems affect the woman at present.

urgency, bladder pain, etc.), and how much

questions on bladder problems (frequency,

as overactive bladder [16,17]; it contains

list of 10 individual bladder problems plus an

impairment. Part III of the questionnaire is a

better'). The GRC was administered at the first

great deal better', 'a good deal better',

improvement ('a very great deal better', 'a

('about the same', 'better', 'worse'), and item 3

severity of their UTI symptoms ('no symptoms

whether there have been any changes in their

UTI since last completing the questionnaire

('about the same', 'better', 'worse'), and item 3

asks women to rate the level of any symptom

('no symptoms at all', 'mild', 'moderate', 'severe'), item 2 asks

questions (frequency, urgency, bladder pain, etc.), and how much

these problems affect the woman at present.

The KHQ was administered at visit 1, on day 3,

when the patient indicated that the UTI

symptoms had resolved (no symptoms for

24 h, or over three data capture points,

whichever was longer), and at the test-of-

cure visit.

The GRC is a three-item measure used to

assess symptom improvement. As formulated

for this study, item 1 asks women to rate the

severity of their UTI symptoms ('no symptoms

at all', 'mild', 'moderate', 'severe'), item 2 asks whether there have been any changes in their

UTI since last completing the questionnaire

('about the same', 'better', 'worse'), and item 3

asks women to rate the level of any symptom

improvement ('a very great deal better', 'a

great deal better', 'a good deal better',

'moderately better', 'somewhat better', 'a little

better'). The GRC was administered at the first

visit (item 1 only) and at all subsequent

administrations of the UTISA.

Data were analysed by ANOVA with post hoc

Tukey tests (or nonparametric Kruskal–Wallis

test with Bonferroni-corrected post hoc

Mann–Whitney tests to compare group

scores. Associations between continuous

variables (absolute or change scores) were

assessed using Pearson’s $r$ or Spearman’s $r_s$

correlation coefficients. Throughout the

analysis, missing values were dealt with by

excluding cases pairwise, and $P < 0.05$ was

considered to indicate statistical significance.

Exploratory factor analysis using principle-

components extraction and varimax rotation

was used on the 14 UTISA items. Factors were

extracted if their eigenvalue was $> 1$. Domain

scores of any resulting factors were calculated as a sum of the component item

scores, where the higher the score the greater

the symptoms. The normality of the

distribution of the resulting domain scores

was assessed using Kolmogorov-Smirnov

statistics. Where data were not normally

distributed, nonparametric tests were used.

A correlation matrix was calculated for the 14

UTISA items to assess domain homogeneity,

and Cronbach’s $\alpha$ statistics calculated to

assess the internal consistency reliability.

Convergent validity was assessed in terms of

the strength of the associations between

UTISA domain scores and similar individual

symptom and domain scores of the KHQ. For

example, the UTISA ‘urination regularity’

domain was expected to be strongly

associated with the KHQ ‘frequency’ and

‘urgency’ items; the UTISA ‘pain associated

with UTI domain’ with the KHQ ‘bladder pain’

item; and the UTISA ‘problems with urination’

domain with the KHQ ‘difficulty urinating’

item. As short-term UTI is not expected to

have a major impact on personal

relationships, divergent validity was assessed.

---

About Your Symptoms and Their Impact on Your Life (for use at visit 1)

<table>
<thead>
<tr>
<th>SYMPTOMS</th>
<th>Did not have</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of urination (going to the toilet very often)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Urgency of urination (a strong and uncontrollable urge to pass urine)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Pain or burning when passing urine</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Not being able to empty your bladder completely/passing only small</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>amounts of urine</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Pain or uncomfortable pressure in the lower abdomen/pelvic area caused</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>by your urinary tract infection</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Low back pain caused by your urinary tract infection</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Blood in your urine</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

8. Please give an overall rating of the severity of your urinary tract infection symptoms as they are at this moment (Please circle the number of your answer)

   0  No symptoms at all          1  Mild          2  Moderate        3  Severe
by calculating the degree of association between the UTISA domains and the KHQ 'personal relationships' domain; only small correlations were expected. The discriminant validity of the UTISA was assessed by comparing UTISA domain scores (at first administration) between the initial clinical ratings for dysuria, frequency, urgency, suprapubic pain and gross haematuria. It was hypothesized that the UTISA 'urination regularity' domain would discriminate between different clinical evaluations of 'frequency' and 'urgency'; that the 'problems with urination' domain would discriminate between the evaluations of 'dysuria'; that the 'pain associated with UTI' domain would discriminate between the different clinical evaluations of 'suprapubic pain'; and the 'blood in urine' domain between evaluations of 'gross haematuria'. In addition,
the responses to the GRC item 1 were examined in relation to the UTISA scores. Those reporting more severe symptoms on the GRC were expected to have higher UTISA scores.

Responsiveness was assessed by comparing the improvement in UTISA domain scores (from the first visit to when symptoms were no longer present) to the improvement in the clinical evaluation of UTI; the greater the improvement in clinical severity, the greater the expected improvement in domain scores. To explore the responsiveness of the UTISA domains to patient-reported changes, changes in domain scores were compared among the three categories of responses to GRC item 2 (on the categories recorded as worse = −1, about the same = 0, better = 1). The analyses were repeated at the second, third fourth and fifth assessments (i.e. in the first 1–2 days of the study after the initial administration of ciprofloxacin once-daily). In addition, the responsiveness of the UTISA domains to the level of patient-reported improvement was assessed by correlating changes in domain scores at the second, third, fourth and fifth assessments with changes in GRC item 3. A responsiveness index was calculated for the UTISA domains by dividing the mean change in score for patients who report feeling ‘a little better’ (GRC item 3) by the SD for those patients who feel ‘about the same’ (GRC item 2). Index of responsiveness scores of 0.2 are regarded as small, 0.5 as moderate and 0.8 as large [18]. To assess whether the responsiveness of the UTISA is consistent across time, these analyses were conducted for assessments 1–5 of the UTISA and GRC questionnaires.

The MID is the difference in the measure associated with the smallest detectable symptom improvement. The MID was calculated by selecting patients who reported that they felt ‘better’ on GRC item 2 and ‘a little better’ on GRC item 3. The change scores for the UTISA were then calculated (e.g. time 1 minus time 2). This analysis was repeated at assessments 2–5.

RESULTS

In all, 276 women were recruited to the ciprofloxacin once-daily trial and thus to the validation study; 267 women (96.7%) were followed up to the test-of-cure visit. The mean (SD, range) age of the women was 33.0 (11.46, 18–78) years. Although the sample was ethnically diverse, 12% (34) were Black, 4% (12) American Indian, 2% (five) Hispanic, and most of the women (70%, 193) were White. Overall, 175 women (64.9%) were working full time, 65 (23.8%) were working part time, 107 (39.2%) women reported that they were looking after the house and/or children full time, 53 (19.4%) were studying at university either full or part time, and 46 (6.8%) reported that they were engaged in some other role.

Most of the women reported that their present overall health was either very good (110, 40.3%) or good (136, 49.8%), and that their bladder problem affected their life either a little (108, 39.6%) or moderately (83, 30.4%). Most of the women (171, 62.0%) reported no previous episode of uUTI, although 26.4% (73) reported one previous episode, 11.2% (31) two previous episodes and 0.4% (one) three previous episodes. In terms of the duration of the current episode, 14.1% [39] reported that it had been present for 1 day, 38.4% [106] for 2 days, 44.2% [122] for 3 days, and 3.3% (nine) for 4 days. There was no association between number of previous episodes and duration of the current episode.

In terms of the investigator-reported clinical evaluations at baseline, most of the women were considered to have moderate or severe dysuria (208, 75.4%), frequency (238, 86.2%) and urgency (237, 85.9%). Suprapubic pain was less common, with most women (179, 64.9%) rated as having either mild or moderate suprapubic pain. Haematuria was reported as absent in 151 women (54.7%). Colony counts (colony-forming units/mL) for the 273 women with culture data were as follows: ≤10³, 84 (30.8%); >10³–10⁶, nine (3.3%); >10⁶–10⁸, 28 (10.3%); >10⁸, 152 (55.7%).

On exploratory-factor analysis of the baseline UTISA scores, four components were extracted which explained 72.6% of the variance (Table 1). For each symptom, the severity and bothersomeness items loaded onto the same factor. Four items [items relating to incontinence to completely empty bladder and pain/discomfort in the lower abdomen] loaded on to two components. The items were allocated to the component on which the loading was highest. The four components were termed ‘urination regularity’, ‘problems with urination’, ‘pain associated with UTI’, and ‘blood in urine’. The last component contained only two items and it was retained in the UTISA for clinical diagnostic value rather than symptomatic importance.

The domains were internally homogeneous with average intra-item correlations being notably higher than average inter-item correlations: urination regularity (0.644 vs 0.284); problems with urination (0.610 vs 0.284); pain associated with UTI (0.641 vs 0.284); and blood in urine (0.657 vs 0.284).

<table>
<thead>
<tr>
<th>TABLE 1 Factor loadings (loadings &gt;0.4) on the four extracted components showing the structure of the UTISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
</tr>
<tr>
<td>1. Frequency – severity</td>
</tr>
<tr>
<td>2. Frequency – bothersomeness</td>
</tr>
<tr>
<td>3. Urgency – severity</td>
</tr>
<tr>
<td>4. Urgency – bothersomeness</td>
</tr>
<tr>
<td>5. Pain – severity</td>
</tr>
<tr>
<td>6. Pain – bothersomeness</td>
</tr>
<tr>
<td>7. Empty – severity</td>
</tr>
<tr>
<td>8. Empty – bothersomeness</td>
</tr>
<tr>
<td>9. Discomfort – severity</td>
</tr>
<tr>
<td>10. Discomfort – bothersomeness</td>
</tr>
<tr>
<td>11. Low back pain – severity</td>
</tr>
<tr>
<td>12. Low back pain – bothersomeness</td>
</tr>
<tr>
<td>13. Blood in urine – severity</td>
</tr>
<tr>
<td>14. Blood in urine – bothersomeness</td>
</tr>
<tr>
<td>% of variance explained</td>
</tr>
</tbody>
</table>
The Cronbach’s α coefficients for the three four-item domains were >0.8 (0.87, 0.85, and 0.83 for urination regularity, problems with urination, and pain associated with UTI, respectively), showing high internal consistency. The coefficient for the two-item domain was 0.72.

Mean (SD, median) scores for the four domains were: urination regularity, 9.01 (2.63, 9); problems with urination, 7.98 (2.97, 8); pain associated with urination, 5.69 (3.41, 6); and blood in urine, 1.52 (1.87, 1). Respondents used the full range of scores for each domain (0–12 for the four-item domains and 0–6 for the two-item domain).

The only statistically significant correlation between domain scores and age was between the ‘problems with urination’ score and age ($r_i = -0.13, P < 0.05$), indicating that younger women reported more of these problems (pain/burning when urinating and a feeling of incomplete emptying). In terms of association with ethnicity, ‘pain associated with UTI’ scores differed significantly between the ethnic groups ($\chi^2 = 9.397, d.f. 3, P < 0.05$), with White women reporting significantly less pain than Black women, at a mean (SD) of 5.34 (3.30) vs 7.03 (3.26); $z = -2.53, P < 0.05$.

Scores for each item were consistent with Black women scoring higher than White women.

While there was no association between UTISA scores and number of previous episodes, the ‘pain associated with UTI’ and ‘blood in urine’ scores differed significantly between those with different duration (Kruskal–Wallis $\chi^2 = 12.14, P = 0.007$, and 11.31, $P = 0.010$, respectively). Pain associated with UTI scores tended to increase with increasing duration of the episode ($r_i = 0.185, P = 0.002$) and ‘blood in urine’ scores to decrease ($r_i = -0.177, P = 0.003$).

The Spearman’s correlation coefficients between the UTISA and KHQ domains and selected KHQ items are shown in Table 2. The correlation coefficients for the assessment of convergent validity were all positive (i.e. the higher the UTISA score the higher the KHQ score) and statistically significant. In particular, the correlation coefficients between the UTISA domain scores and the corresponding KHQ items were all $> 0.4$. In terms of divergent validity, although there was a statistically significant association between the KHQ ‘personal relationships’ domain and the UTISA ‘pain associated with UTI’ domain, the correlation coefficient was small, and neither of the other domains showed a significant association.

The mean UTISA domain scores by clinical evaluation of UTI (both assessed at the test-of-cure visit) are shown in Table 4. The UTISA domains of urination regularity, problems with urination, and pain associated with UTI were responsive to changes in the clinical evaluation of ‘dysuria’, ‘frequency’, and ‘urgency’, with all correlation coefficients being at least moderate and statistically significant. In addition, the domain pain associated with UTI was responsive to changes in the clinical evaluation of ‘suprapubic pain’, and the domain blood in urine to changes in ‘gross haematuria’. All other correlations, even if significant, were small ($<0.20$). Mean change scores in the UTISA domains at the first five assessments by patient-reported level of change are shown in Table 5, with the Spearman’s correlation coefficients between the UTISA change scores and the GRC items 2 and 3. At assessments two and three, change in each UTISA domain (from the previous assessment) was highly responsive to patient-reported change with mean scores differing at $P < 0.001$ between the patient-reported change categories, and Spearman’s correlation coefficients being moderate and also significant at $P < 0.001$. Patients who reported that they felt worse generally reported deterioration in UTISA domain score, while those who reported that they felt better reported an improvement. The associations were weaker at assessments four and five, and the blood in urine domain scores were not significantly associated with patient-reported change. However, in terms of the degree of improvement (GRC item 3), only at the second assessment (3 h) were any of the UTISA domains responsive. The responsiveness indices of the UTISA domains during the first 1–2 days of treatment in relation to the GRC responses are shown in Table 6. The largest changes in domain scores were during the first two assessments. For example, the mean (SD) urination regularity scores at baseline, and the second, third, fourth and fifth assessments were 9.01 (2.63), 6.43 (3.09), 4.86 (3.03), and 3.81 (2.86), respectively (Table 7). The responsiveness of the UTISA domains was also higher at these first two assessments than at the subsequent
assessments, although most indices indicated moderate (>0.50) or high (>0.80) responsiveness [18]. In other words, the change in UTISA scores associated with a response of ‘a little better’ to the GRC was greater at the early stages than at the later stages of treatment. Thus, the UTISA is responsive to this difference in pattern of improvement.

As the degree of improvement in UTISA scores was higher at the first two assessments after treatment than at subsequent assessments, the estimated MID for each domain tends to be larger in the early stages of treatment than in later stages (Table 8). This means that the smaller levels of improvement in scores in the later stages of treatment are indeed detectable by the patients. However, these analyses are based on relatively few patients, so to obtain an overall MID for each scale, a mean was taken of the estimated MIDs at each assessment. The mean scores were rounded for ease of use, giving MIDs of 1.75, 1.25, and 0.50 for the urination regularity, problems with urination, pain associated with UTI, and blood in urine domains, respectively.

### DISCUSSION

This study reports the development and validation of a new instrument, the UTISA questionnaire, to measure the severity and bothersomeness of the most frequently reported symptoms and signs of uncomplicated uUTI. Strengths of the study include comprehensive reviews of published material in the questionnaire-development stage, combined with administration of a draft measure. The validity of the revised questionnaire was tested in a large sample of 276 ethnically and socio-economically diverse women with uUTI in the context of a noncomparative clinical trial.

One interesting feature of the trial was its use of electronic recording devices. Palm pilot devices are inexpensive, small, light, and well suited for collecting patient-reported outcomes. Alarms can be set to trigger data collection at specific intervals and, as the patient responses are time-stamped, the time at which the data were gathered is more accurate than with paper diaries. The devices worked well in the present study, which involved repeated collection of data for several measures over frequent and varying periods. Electronic methods of data collection have been used successfully in other studies [19–21] showing that such methods are reliable and valid [21], and can yield a more complete and accurate profile of signs and symptoms than paper questionnaires [19,20].

The UTISA is a 14-item questionnaire that asks about the ‘severity’ and ‘bothersomeness’ of seven key symptoms and signs of uUTI, and includes three principal domains of urination regularity (frequency and urgency), problems with urination (pain/burning on urination and a feeling of incomplete emptying), and pain associated with UTI (pain/pressure in lower
abdomen and low back pain, both caused by UTI). The domains were internally homogeneous and had good internal consistency, with a Cronbach α in the optimum range of 0.8–0.9. A Cronbach α of >0.9 is considered to indicate that the scale items are too similar [22,23]. The blood in urine domain had two items and weaker psychometric properties, and was retained for clinical diagnostic value only.

The mean domain scores indicated that items related to urination regularity are the most troublesome symptoms in acute uUTI. Interestingly, Black women reported more problems with pain than White women, having significantly higher scores on the UTISA pain associated with UTI domain (P < 0.05). This is consistent with other reports of different experiences of pain across racial and ethnic groups, with African-Americans reporting higher levels of pain than White Americans in a variety of different conditions [24].

All three UTISA domains had excellent psychometric properties. Convergent validity was indicated by high and significant correlations between the UTISA domains and related KHQ items and domains. Divergent validity was indicated by the small and generally non-significant correlations between the UTISA domains and the KHQ personal relationships domain. The small but statistically significant correlation between the UTISA pain associated with UTI domain and the KHQ personal relationships domain (r = 0.13, P < 0.05) might be explained in that one of the KHQ items within this

<table>
<thead>
<tr>
<th>TABLE 5</th>
<th>Mean (SD) UTISA domain scores by change in UTI symptoms (GRC item 2) and Spearman’s correlation coefficients between UTISA change scores and GRC item 2 (scored as worse, same, better) and item 3 during the first five assessments for evaluation of responsiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change score</td>
<td>UTISA domain</td>
</tr>
<tr>
<td>Time 1–2</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>1.75 (2.55)</td>
</tr>
<tr>
<td>PAU</td>
<td>0.89 (2.11)</td>
</tr>
<tr>
<td>BIU</td>
<td>0.41 (1.30)</td>
</tr>
<tr>
<td>Time 2–3</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>0.52 (1.93)</td>
</tr>
<tr>
<td>PAU</td>
<td>0.46 (1.70)</td>
</tr>
<tr>
<td>BIU</td>
<td>0.12 (0.73)</td>
</tr>
<tr>
<td>Time 3–4</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>0.63 (1.58)</td>
</tr>
<tr>
<td>PAU</td>
<td>−0.11 (3.55)</td>
</tr>
<tr>
<td>BIU</td>
<td>0.17 (0.68)</td>
</tr>
<tr>
<td>Time 4–5</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>0.39 (1.83)</td>
</tr>
<tr>
<td>PAU</td>
<td>0.68 (3.21)</td>
</tr>
<tr>
<td>BIU</td>
<td>0.14 (0.58)</td>
</tr>
</tbody>
</table>

UR, urination regularity; PWU, problems with urination; PAU: pain associated with UTI; BIU, blood in urine; K-W, Kruskal–Wallis; *P < 0.05, †P < 0.01, ‡P < 0.001.

<table>
<thead>
<tr>
<th>TABLE 6</th>
<th>Responsiveness indices for each UTISA domain at each assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change score</td>
<td>UTISA domain</td>
</tr>
<tr>
<td>Time 1–2</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>2.40</td>
</tr>
<tr>
<td>PAU</td>
<td>1.65</td>
</tr>
<tr>
<td>BIU</td>
<td>0.70</td>
</tr>
<tr>
<td>Time 2–3</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>0.96</td>
</tr>
<tr>
<td>PAU</td>
<td>1.59</td>
</tr>
<tr>
<td>BIU</td>
<td>0.67</td>
</tr>
<tr>
<td>Time 3–4</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>1.11</td>
</tr>
<tr>
<td>PAU</td>
<td>0.44</td>
</tr>
<tr>
<td>BIU</td>
<td>0.11</td>
</tr>
<tr>
<td>Time 4–5</td>
<td>UR</td>
</tr>
<tr>
<td>PWU</td>
<td>1.17</td>
</tr>
<tr>
<td>PAU</td>
<td>1.22</td>
</tr>
<tr>
<td>BIU</td>
<td>0.39</td>
</tr>
</tbody>
</table>

UR, urination regularity; PWU, problems with urination; PAU: pain associated with UTI; BIU, blood in urine.
domain is ‘Does your bladder problem affect your sex life?’ It is possible that a woman’s sex life might be affected more by pain associated with UTI than by any other symptom.

The three UTISA domains generally had excellent discriminant validity in relation to the clinical evaluation. They also had very high levels of responsiveness, with strong associations between changes in domain scores and changes in clinical evaluation. Responsiveness was also shown by the strong associations between UTISA domain change scores and GRC items, particularly GRC item 2, which asks whether the patient felt their condition had worsened, stayed about the same, or improved since the last assessment.

In terms of the calculated responsiveness indices, these were generally at least moderate and mirrored the reported changes in UTISA domain scores, which were greatest in the two assessments immediately after treatment. Thus the UTISA is responsive to this difference in pattern of improvement, with the responsiveness indices at the first two assessments generally being greater than those at the subsequent assessments.

The estimated MID values also reflected the greater improvement in symptoms immediately after treatment, being greater initially than after a few hours of treatment. This probably reflects that, as the improvement in symptoms is initially rapid, the symptom improvement (as measured by improvement in the UTISA domain scores) associated with patient reports that they felt ‘a little better’ is likely to be greater than the associated improvement at a time when symptom resolution is less rapid. To take account of this, the estimated MID was taken as the rounded mean value of all MIDs calculated over the four assessments 2–5.

In conclusion, the UTISA questionnaire measures the severity and bothersomeness of key signs and symptoms associated with uUTI. The questionnaire comprises three four-item domains (urination regularity, problems with urination, and pain associated with UTI), with two additional items measuring haematuria. The three domains have excellent psychometric properties. The questionnaire was designed for use in a clinical setting, but is also likely to be suitable for use in an epidemiological context.

<table>
<thead>
<tr>
<th>UTISA Domains</th>
<th>Change score</th>
<th>Time 1–2</th>
<th>Time 2–3</th>
<th>Time 3–4</th>
<th>Time 4–5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urination regularity</td>
<td>2.58 (2.87)</td>
<td>2.38 (2.75)</td>
<td>1.26 (2.57)</td>
<td>0.70 (2.15)</td>
<td></td>
</tr>
<tr>
<td>Problems with urination</td>
<td>1.58 (2.43)</td>
<td>1.31 (2.32)</td>
<td>0.99 (2.05)</td>
<td>0.31 (0.99)</td>
<td></td>
</tr>
<tr>
<td>Pain associated with UTI</td>
<td>1.07 (2.01)</td>
<td>1.20 (2.12)</td>
<td>0.28 (3.10)</td>
<td>0.25 (0.84)</td>
<td></td>
</tr>
<tr>
<td>Blood in urine</td>
<td>0.80 (1.85)</td>
<td>0.72 (1.85)</td>
<td>1.13 (2.80)</td>
<td>0.17 (0.68)</td>
<td></td>
</tr>
</tbody>
</table>

**CONFLICT OF INTEREST**

None declared. Source of funding: Bayer Healthcare Pharmaceuticals.

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Abbreviations: uUTI, uncomplicated urinary tract infection; UTISA, UTI Symptoms Assessment questionnaire; QoL, quality of life; SF-36, Medical Outcome Study Short-Form 36 Health Survey; KHQ, King’s Health questionnaire; GRC, Global Rating of Change; MID, Minimally-Important Difference.
A novel midstream urine-collection device reduces contamination rates in urine cultures amongst women

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OBJECTIVE
To evaluate a novel urine-collection device (UCD) that automatically collects a midstream urine (MSU) sample, and compare contamination rates to those of the conventional MSU sampling method, as the contamination of urine samples for microbiological analysis in women leads to diagnostic ambiguity and unnecessary costs, and may result in part from an incorrect collection procedure.

PATIENTS AND METHODS
In all, 2823 women from four centres, most from antenatal clinics, were randomized to two urine-collection methods: conventional MSU collection and collection with a novel MSU UCD (the Whiz®; JBOL Ltd, Oxford, UK). Semi-quantitative growth and user acceptability were compared between the collection methods.

RESULTS
MSU samples collected with the UCD had significantly fewer mixed growth samples (9% vs 14%, P = 0.001; 36% relative reduction), significantly fewer heavy mixed growth samples (1.2% vs 3.0%, P = 0.004; 60% relative reduction) and required significantly fewer re-tests (11% vs 16%, P = 0.002; 31% relative reduction). There were more samples with clinically insignificant growth than the conventional MSU group (86% vs 82%, P = 0.005). Those using the UCD preferred it to the conventional method (67.5%) and experienced significantly less spillage during sample collection (27% vs 46%, P = 0.001; relative reduction 41%).

CONCLUSION
The UCD reduced contamination rates in urine samples and improved the predictive value of the urine culture in a manner acceptable to patients and staff.

KEYWORDS
clean-catch, mid-stream, preterm labour, urine contamination, urine collection

INTRODUCTION
UTIs and symptoms mimicking UTI are common in women. The diagnosis of UTI is based on urine sampling and testing with reagent sticks and/or laboratory culture, both of which require a high-quality specimen free of perineal, fecal or vaginal contaminating organisms and inflammatory cells [1]. The interpretation of the urine culture uses semiquantitative methods, including the number of colony-forming units per unit volume, the number of species of organism cultured and the identification of species as likely pathogenic organisms. Urine can be sampled by suprapubic puncture, catheter insertion or midstream urine (MSU) collection. Inherent in catheter insertion and MSU sampling is possible contamination by perineal, vaginal, fecal or skin flora. While suprapubic aspirate is free of contaminating organisms, the procedure is invasive and limited to a few specific clinical situations. MSU sampling is the commonest method, but has a high inherent contamination rate, defined by mixed-growth cultures and growth of non-pathogenic commensals [2]. Contamination rates as high as 30% have been reported [2]. Such contamination obscures interpretation of the urine culture and may mask underlying bacteriuria. The importance of reducing contamination levels in MSU samples is not limited to possible cost savings to health services. The threshold for treating bacteriuria is lower in some clinical settings, including pregnancy, dialysis and those who are immunocompromised, where bacteriuria may lead to complications, e.g. premature labour, or may indicate subclinical infections with slow-growing organisms [3].

To standardize the method of collecting a MSU sample, to remove dependence on patients to produce an adequate specimen, to simplify the compliance required from patients and the need for time-consuming instruction by clinical staff, a novel urine collection device (UCD) was developed for urine sampling in women. The device automatically (i.e. independent of user intervention) collects a MSU sample by excluding the initial low-flow portion of the urinary stream and with no interruption of urine flow, consistent with British Standard Operating Procedure for urine testing [4]. We conducted a clinical trial to investigate whether the novel UCD reduced contamination rates when compared with conventional MSU sampling methods, and to establish patient preference.

PATIENTS AND METHODS
The MSU collection devices (Whiz® UCD, JBOL Ltd, Oxford, UK) were donated by the supplier. Conventional MSU samples were collected using methods and supplies usually available in the participating centres.

Women attending outpatient clinics in four different centres, and who were required to provide a urine sample for microbiological analysis, were asked to participate in the trial. Most women were recruited from antenatal clinics (85%), with a minority from general practice (15%). The indication for urine testing was recorded. Women at each centre...
were randomized into two equal test groups using serially numberd sealed envelopes. Individual samples and the group sample method were further identified by unique serial numbers, and thus laboratory staff were unaware of the collection method for each sample when reading the culture plates.

The practice of collecting conventional MSU samples (group 1) was according the usual procedure of instruction in each particular setting, and this varied among the centres. Not all centres advised patients on perimeatal cleansing or labial separation for conventional MSU sample collection.

In group 2 (UCD), patients were given the pack including the device, and asked to read the instructions provided on the side of the box (Fig. 1) and give a sample. No cleansing procedures or labial separation were advised.

To determine the patient response to the UCD and gauge opinions on the conventional methods, patients were asked to complete a questionnaire after giving a sample. Questionnaires provided details of age, posture during urine sampling, reason for the urine test, spillage during sample collection and preference for MSU collection technique. In addition, ease of use of the device and conventional method was scored using a numeric scale (1 to 10). The UCD user questionnaire contained an extra question, ‘Have you ever given a urine sample before?’ to enable a comparative evaluation amongst UCD users only between their use of the UCD and their previous conventional methods of collection. Patients using the UCD were asked to indicate whether they had given a conventional urine sample in the past. At the end of the trial the acceptability of the UCD to clinical staff was assessed by an interview and questionnaire to the clinical staff involved with the trial. In all, 2823 participants were recruited from four different centres and randomized to either conventional MSU collection (1420) or collection with the UCD (1403).

The results were assessed statistically using Pearson chi-squared tests to compare the percentages, and Mann–Whitney/Wilcoxon tests where outcomes were in ordered categories. ANOVA or logistic regression methods were used to investigate potential confounding factors.

RESULTS

The age distribution of the subjects was: <20 years, 4.3%; 20–35, 72.1%; 36–49, 21.2%; 50–64, 1.7%; ≥65, 0.69%; the mean (SD) age was 31 (8.1) years, using midpoints for the age categories, with most patients in both trial groups aged 20–35 years (72.1%). There was no difference in the age groupings of the different arms of the trial (data not shown). Of the 2823 samples collected for culture, the results of 641 (315 conventional and 326 UCD samples) were lost to the study through labelling errors and sample processing errors. This loss of laboratory data did not introduce a bias towards one or other method (chi-square 0.446, one degree of freedom, P = 0.504).

Microbiological culture results were obtained on 2182 urine specimens; Table 1 shows how the laboratory results were interpreted clinically, and Table 2 the results of semiquantitative culture, the relative reduction or increase between the arms of the trial and statistical values. In the study as a whole, 13.3% of samples required a re-test (see Table 1 for the criteria). However, samples collected with the UCD were significantly less likely to require re-testing than the conventional MSU samples (UCD 11%, conventional 16%, P = 0.002; relative reduction 31%). The reason for the test, posture during sampling and the centre at which the sample was taken had no effect on the significance of the results shown in Table 2 (Pearson chi-square P = 0.004, 0.364 and 0.916, respectively).

User acceptability data showed that half the respondents experienced little difficulty in collecting the urine sample with either method (score 1 or 2 from 10 on the numerical scale). There was no significant difference in ease-of-use scores between the methods of collection (Wilcoxon/Mann–
Acceptance of the device by clinical staff was assessed through interview, and all 12 interviewed indicated a preference for the UCD, citing time-saving and improved hygiene as the main reasons for their preference.

The indication for MSU testing in all patients was 'routine antenatal screening'. Indications for specimens collected in general practice (15% of patients) were 'UTI' in 1% of all patients, 'other' in 8%, and 'routine' in the remaining 6%.

Both groups in all centres, except the GP surgeries, use dipstick testing, and did this by pouring a small portion of the urine sample collected onto the dipstick, rather than dipping in the sample, and the remaining urine in the collection bottle was sent to the laboratory.

The practice of collecting conventional MSU samples grouped varied among centres; instructions to patients on collection varied. At United Hospital Trust (Mid Ulster Hospital) patients were instructed to catch the middle part of the urine flow but they were not instructed on cleaning or separating the labia. At the John Radcliffe Women's Centre all patients in group 1 were given a sterile pack containing the Whiz UCD, which contained the peri-meatal area and a small cup for urine collection, and users were instructed by staff on how to collect the MSU sample, which included separating the labia and to void into the toilet, then to stop, then to collect a sample and finish voiding into the toilet. The sample collected was then transferred to a universal sample bottle. At Royal Hampshire County Hospital group-1 patients were asked to give an MSU; some of the GP surgeries instructed on separating the labia and the need to give an MSU, but others did not. No further instructions were given.

All centres for group 2 were given the packet containing the Whiz UCD, which contained the instructions and device, and no further instructions were given. At Stoke Mandeville women were instructed to read the instructions and make sure the device was held against the perineum, i.e. not used as a funnel.

**DISCUSSION**

The ability of clinicians to accurately diagnose a UTI is impaired by high rates of contamination, particularly in samples obtained from women [2], children [5], the elderly [6] and in general practice [7]. In addition, clinical situations in which low levels of bacteriuria may be clinically significant, e.g.
in antenatal and urogynaecological patients, are complicated by bacterial contamination of samples. This may lead to a delay in the diagnosis of a urinary infection, with consequential adverse outcomes for the patient (e.g. pre-term labour) and consequent morbidity, mortality and healthcare costs.

The reasons for high levels of contamination in women include anatomical factors (proximity of urethral meatus to vulva and covering by labia [8]) and compliance factors in collecting the sample. Most samples in clinical practice are collected unsupervised, and while careful instruction to patients on technique may be beneficial in reducing contamination, this may be difficult to achieve in busy clinical environments and may equally have an undesired negative effect on contamination rates [9]. Physical constraints (e.g. old age, pregnancy) and urinary pathology (e.g. stress incontinence) may further limit compliance and good technique.

The collection of a MSU sample requires understanding and implementation by the patient, with little opportunity for intervention by clinical staff. It has been found that conventional MSU sampling with additional cleansing procedures does not significantly alter culture outcome or levels of contamination [2,10]. This is attributed at least in part to the lack of supervision in sample collection. For this reason, we hypothesized that removing the need for patient intervention, and standardization of MSU sample collection, might reduce contamination levels. The UCD used is not simply a funnel, but incorporates a flow-sensitive sampling channel and diverter that, using urodynamic principles, excludes the initial low-flow portion of the urinary stream, thus discarding the contaminated early stream volume, and automatically collects the midstream volume without interrupting the stream. The British Standard Operating Procedure for MSU testing states that ‘the first part of voided urine is discarded and without interrupting the flow, approximately 10 mL is collected into a sterile container’ [4]. The new device is also used with no need for cleansing or separating the labia.

The present data show that urine samples collected with the UCD had significantly lower contamination levels than conventional MSU samples. Use of the UCD resulted in a reduction in mixed-growth samples and consequently more samples with no significant growth. The lower levels of contamination resulted in fewer re-tests required for these patients and improved the predictive value of the urine culture, i.e. more true-negative and fewer false-positive results. Taken together, the quality of urine sampling was significantly improved.

A urine culture is a frequently requested investigation; the present data suggest that up to 15% of urine cultures collected by conventional MSU sampling cannot be interpreted clinically. This finding is similar to that of Valenstein and Meier [11], who found that a median 18% of outpatient urine cultures were contaminated. Furthermore, as sample collection is a fraction of the cost of the administrative, staffing and laboratory costs of the urine culture, we assert that a significant portion of the microbiology budget for urine culture is consumed by samples with little clinical worth. A greater accuracy in diagnosis of urinary symptoms, including those that require exclusion of a urinary infection, will positively affect patient care and is likely to improve outcomes for patients, and reduce wastage of laboratory and clinical resources. The trend towards detecting many UTIs using the UCD, while not statistically significant in the present study of asymptomatic patients, suggests further study on different patient groups, e.g. those who have symptoms suggestive of UTI.

The present data indicate that the UCD is both easy to use and more acceptable to the patients providing the samples. Reasons for this may include the reduction of spillage during collection, removal of the need to ‘aim’ or control the urine stream, and improved hygiene of sample collection. Clinical staff supervising the use of the UCD preferred it to conventional MSU sampling because they thought it reduced the time taken to collect and process samples. They also considered that the UCD improved hygiene for staff, because it removed the need to transfer the sample from a collection pot to a sample container.

This study was limited to women predominantly aged 20–35 years. Further studies are required to establish the possible benefit of the UCD in other populations, particularly men, children and the elderly, or where there are high rates of sample contamination and possibly lower rates of proper compliance with the MSU technique.

The trial samples were identified to the laboratory database by a unique trial identification number, to ensure that laboratory staff were unaware of the sample method. Several samples were excluded because of an administrative error while attaching labels to some of the specimens. This mislabelling affected both groups equally and was not a source of bias to the primary outcomes of the study.

In conclusion, we show that the rate of contamination of urine specimens can be reduced by using an automatic midstream UCD with no need for perimeatal cleansing or labial separation, and that such a device is more acceptable to patients and staff than conventional MSU sampling. Use of this UCD should be considered for collecting all MSU samples in women, where the specimen is to be used for bacterial culture. This may be particularly important during pregnancy, where UTI may result in serious complications, including premature delivery and its associated morbidity [12]. Further studies are needed in other clinical settings in which urine culture poses a diagnostic difficulty or leads to additional complications.

ACKNOWLEDGEMENTS
We thank the clinical staff of the centres involved for their enthusiastic and competent participation in the trial and thank all those who read and commented on the manuscript.

CONFLICT OF INTEREST
None declared. Source of funding: R. Weatherall was employed by the NHS at the start of the research so was paid by JBOL.

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Abbreviations: MSU, midstream urine; UCD, urine-collection device.
How do urinary diaries of women with an overactive bladder differ from those of asymptomatic controls?

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Accepted for publication 30 March 2005

OBJECTIVE
To quantify clinically important differences in common diary variables between asymptomatic controls and women with symptoms of overactive bladder (OAB), controlling for the effects of age and race.

PATIENTS, SUBJECTS AND METHODS
The 24-h urinary diaries of 49 women with symptoms of OAB were compared to those of age- and race-matched asymptomatic controls. Control subjects did not have a physical examination.

INTRODUCTION
Treatments for overactive bladder (OAB) symptoms of urgency, frequency and/or urge incontinence should restore normal urinary tract function. As few studies have documented the urinary habits of asymptomatic women, there is little published information comparing normal voiding habits to those of women with LUTS [1,2], and therefore there are almost no data available on the likely magnitude of the treatment effect needed to restore normality. This lack of information can impede clinical decision-making and the design of research trials.

In a previous study of the urinary diaries of 300 asymptomatic women [3] we found that urinary diary variables, e.g. 24-h frequency and mean voided volume, depended on the subject’s age and race. The aim of the present study was to quantify clinically important differences in diary variables between asymptomatic controls and women with symptoms of OAB, controlling for the effects of age and race.

RESULTS
The 49 patients with OAB symptoms had a median (range) age of 51 (20–85) years, a body mass index of 25 (17–46) kg/m² and a parity of 2 (0–5). The median number of voids was significantly greater in women with OAB than asymptomatic controls (P<0.001). The median value for mean voided volume was significantly lower in women with OAB than asymptomatic controls (P=0.014). There was no difference in the maximum voided volume, total voided volume, daytime or night-time diuresis rates, voids per litre intake, or total fluid intake.

CONCLUSIONS
This preliminary study suggests that a median reduction of three voids/24 h and an increase of 70 mL in the mean voided volume might be clinically important goals in therapeutic trials for treating OAB symptoms. This remains to be confirmed by further studies linking improvements in quality of life and the overall impression of bladder health with these quantitative diary variable changes.

KEYWORDS
urinary frequency, lower urinary tract, overactive bladder syndrome.

PATIENTS, SUBJECTS AND METHODS
After Institutional Review Board approval, 24-h urinary diaries were collected from consecutive women presenting to the Female Pelvic Medicine Center at Loyola University Medical Center as new patients with OAB symptoms (urinary urgency, frequency and/or urge incontinence). These diaries were compared with the diaries of 300 asymptomatic women with OAB symptoms (45%). The racial composition of the sample was 82% Caucasian, 12% African-American and 6% Hispanic.

Twenty-four women (49%) were premenopausal, 32 (65%) had concurrent symptoms of stress urinary incontinence, and 14 (33%) had concurrent symptoms of pelvic organ prolapse. The physical examination determined that there were pelvic organ support defects to the hymen or beyond in 19 patients with OAB symptoms (45%).

Patients with OAB and controls had similar parity and body mass index. Results of the comparison of diary data are detailed in Table 2. As expected, the median number of voids was significantly greater in women with...
OAB than asymptomatic controls (P < 0.001), and the median value for mean voided volume was significantly lower in women with OAB than in controls (P = 0.045). There was no difference in maximum voided volume, total voided volume, daytime or night-time diuresis rates, voids per litre intake, or total fluid intake.

DISCUSSION

The only published studies we are aware of that directly compare the diaries of symptomatic subjects with asymptomatic controls are those of Larsson et al. [4], who compared the diaries of 62 women with urge incontinence to those of 151 healthy controls, and found that women with urge incontinence had greater urinary frequency, smaller mean voided volumes and smaller maximum voided volumes than controls, as detailed in Table 3; the racial composition of the study participants was not stated, but is presumed to be predominantly Caucasian.

There are some striking similarities between the present results and those of Larsson et al. [4] of women with detrusor instability; the values for mean and maximum voided volumes are essentially identical in the two studies, and the greater urinary frequency recorded in the present study is partly explicable by the greater total voided volumes recorded by the present subjects. It is likely that the fluid intake was higher in the present sample, but this factor was not recorded in the report by Larsson et al. [4].

Published studies of the urinary habits of ‘normal’ adults [1,5–7] suggest that ‘normal’ habits might depend on race, culture and/or geography. In most areas of medicine it is appropriate to establish normative values before interpreting abnormal values. It seems appropriate to do this when interpreting urinary diary variables, to allow researchers to recognise clinically important changes in urinary function. Although quantifying change is irresistible for researchers, the change for the patient in terms of her life impact, treatment goals, treatment satisfaction and overall quality of life cannot be replaced with arbitrary numerical thresholds.

The urinary diary is an excellent clinical and research tool in women with OAB. Although certain diary variables are known to be different in patients with OAB, the magnitude of this difference is understudied. The present preliminary study suggests that a median reduction of three voids/24 h and an increase of 70 mL in mean voided volume might be clinically important goals, apparently distinguishing normal voiding patterns from those clinically diagnosed with OAB. Further research is needed to study the relationship between these quantitative diary improvements and the quality-of-life improvement and overall impression of bladder health. Clinicians are acutely aware that lower urinary tract attributes such as bladder volume and urinary frequency, while recorded well in a urinary diary, reflect only part of the clinical picture, e.g. urinary urgency might be far more troublesome to patients with OAB than urinary frequency per se, but it is not reflected in simple urinary diaries.

The present study is limited because we used a simple 1-day diary rather than a longer record of urinary habits. However, we have found such 1-day records to be clinically useful, and a study by van Melick et al. [8] suggested little difference in the value of voiding diary variables when a 1-day diary

### TABLE 1 Diary formulae

<table>
<thead>
<tr>
<th>Variable</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime urine output</td>
<td>Sum of total urine volume after the first morning void, through to the last void before retiring.</td>
</tr>
<tr>
<td>Daytime diuresis rate</td>
<td>Daytime urine volume divided by the total number of minutes between the first void of the day, and the last void before retiring.</td>
</tr>
<tr>
<td>Night-time urine output</td>
<td>Sum of total urine volume voided during the night (after retiring with the intention of sleeping) plus the first void of the next morning.</td>
</tr>
<tr>
<td>Night-time diuresis rate</td>
<td>Night-time urine volume divided by the number of minutes between the last void before retiring and the first void of the following morning.</td>
</tr>
</tbody>
</table>

### TABLE 2 Diary variable values in age- and race-matched women (49 in each group) with and without symptoms of OAB. Values are the median (range), with the sign test result (P)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with OAB</th>
<th>Asymptomatic controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of micturition/24 h, n</td>
<td>11 (9–16)</td>
<td>7 (4.5–9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of daytime voids, n</td>
<td>10 (8–13)</td>
<td>6.5 (4.5–8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Number of night-time voids, n</td>
<td>1 (0–3)</td>
<td>0.5 (0–1)</td>
<td>0.045</td>
</tr>
<tr>
<td>Total voided volume, mL</td>
<td>1950 (1365–2760)</td>
<td>1218 (827–2499)</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean voided volume, mL</td>
<td>171 (145–205)</td>
<td>200 (171–266)</td>
<td>0.014</td>
</tr>
<tr>
<td>Maximum voided volume, mL</td>
<td>360 (300–480)</td>
<td>390 (357–560)</td>
<td>0.2</td>
</tr>
<tr>
<td>Total fluid intake/24 h, mL</td>
<td>2040 (1470–2610)</td>
<td>1725 (979–3147)</td>
<td>0.7</td>
</tr>
<tr>
<td>Daytime diuresis rate, mL/min</td>
<td>1.4 (1–2.1)</td>
<td>0.8 (0.4–1.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Night-time diuresis rate, mL/min</td>
<td>1 (0.7–1.4)</td>
<td>0.4 (0.3–1.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>Voids per litre intake, n</td>
<td>5.6 (4.4–7.8)</td>
<td>4 (3.2–4.6)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

### TABLE 3 Comparison of results from present study and those by Larsson et al. [4] comparing urinary habits of asymptomatic women to those with detrusor instability. Values represent medians

<table>
<thead>
<tr>
<th>Variable</th>
<th>Present study: women with OAB</th>
<th>[4]: women with detrusor instability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total voided volume, mL</td>
<td>1890</td>
<td>1490</td>
</tr>
<tr>
<td>Total voids/24 h, n</td>
<td>9.5</td>
<td>11</td>
</tr>
<tr>
<td>Mean voided volume, mL</td>
<td>172</td>
<td>170</td>
</tr>
<tr>
<td>Maximum voided volume, mL</td>
<td>330</td>
<td>360</td>
</tr>
</tbody>
</table>
was compared to diaries of 2 or 3 days' duration in patients with motor urge incontinence. We were also limited by the relatively few matched pairs, but a strength of the present study is that we were able to control for race and age using this paired analysis.

CONFLICT OF INTEREST

None declared.

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Abbreviations: OAB, overactive bladder.
A comparison of the effect of 1.5% glycine and 5% glucose irrigants on plasma serum physiology and the incidence of transurethral resection syndrome during prostate resection

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OBJECTIVE

To examine changes in the pathophysiology and frequency of the transurethral resection (TUR) syndrome with two irrigation fluids, as variable amounts of irrigation fluid are absorbed during TUR of the prostate (TURP), and although polar solutes are required to prevent an effect on diathermy, the solutes may have effects when absorbed.

PATIENTS AND METHODS

Between December 2001 and March 2003, 250 patients were included in a prospective randomized trial comparing glycine 1.5% with 5% glucose irrigation fluids. We measured blood loss, fluid absorption, temperature change, biochemistry including a glycine assay, and peri-operative symptoms. Blood samples were taken immediately before and immediately, 5 and 24 h after TURP. Irrigating fluid absorption during TURP was measured with 1% ethanol as a marker and breath ethanol measurements. Operative details were recorded, including the type of anaesthesia (with or without sedation), resection time and weight of resected tissue. Peri-operative symptoms were documented prospectively. TUR syndrome was defined as a serum sodium level of ≤125 mmol/L with two or more associated symptoms or signs of TUR syndrome.

RESULTS

Five (2%) patients had TUR syndrome; all five were irrigated with glycine, although this difference was not statistically significant (P = 0.06). Of the five men, three had hypotension, four were tired, one was nauseous, two had parasthesia, two had ‘uneasiness’, one had blurred vision and two were confused; none had chest pain. There was a large variation between the groups in levels of normal. In other studies, glycine was reportedly toxic, and that the levels recorded were many times the upper limit of normal may have both immediate and long-term effects.

CONCLUSIONS

An increase in serum glycine was associated with TUR syndrome; there were large variations in the amounts of glycine absorbed, reaching levels many times the upper limit of normal. In other studies, glycine was reportedly toxic, and that the levels recorded were many times the upper limit of normal may have both immediate and long-term effects.

KEYWORDS

glycine toxicity, osmolar concentration, TURP, irrigation fluid, TUR syndrome, adverse events

INTRODUCTION

Since the introduction of TURP by McCarthy in 1926, the problem of which irrigation fluid to use during the procedure has caused wide-ranging debate, up to and including the present. For standard TURP the criteria for an ideal irrigant are: it must irrigate the surgical field; not be an electrical conductor and not affect diathermy; have good visual acuity and be ‘user-friendly’; have similar osmolarity to serum; minimal side-effects when absorbed; and can be detectable by the surgeon when excess volume is absorbed.

Glycine solution is the most commonly used irrigant and has been used in TURP for >50 years. In 1948, Nesbitt and Glickman used glycine at 1.1% and 2.1% to prevent the haemolysis that occurred when sterile water was used as an irrigant. TURP has several recognized complications; one of the more serious and potentially fatal is the TUR syndrome. Estimates of the incidence of TUR syndrome range from 0% [2], 1% [3], 2% [4], 7% [5] to 10% [6–8], but it is currently poorly defined and many mild cases can be falsely attributed to old age, anaesthetic complications and excessive blood loss. The symptoms arising might also differ depending on the choice of irrigating fluid [9].

For over 20 years, urologists in one district general hospital used only 5% glucose as their irrigant of choice, and their clinical experience lead them to think that 5% glucose solution is not toxic and is entirely satisfactory as an irrigating fluid for use during endoscopic surgery [10]. These urologists have apparently not been aware of problems with stickiness of the instruments or caramelization of the cutting loop diathermy during surgery, and consider 5% glucose to appear optically identical to 1.5% glycine.

PATIENTS AND METHODS

Between December 2001 and March 2003, 250 patients undergoing TURP in two hospitals (Southmead and Torbay) were recruited to a prospective randomized trial,
levels on an alcohol meter, using a nomogram absorbed was measured by breath ethanol into unmarked bags. The amount of irrigant required then lethargy was not included as a symptom of TUR syndrome. The irrigant included in the analysis and their operative results compared with values before TURP.

Only patients with spinal anaesthesia were assessed with immediate preoperative blood analysis and electrocardiograms [11].

TABLE 1 TUR syndrome by treatment group and the glycine level after TUR

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TUR syndrome?</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td>113</td>
<td>5</td>
<td>118</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>228</td>
<td>5</td>
<td>233</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine after TUR: µmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;400</td>
<td></td>
<td>138</td>
<td>0</td>
<td>138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥400</td>
<td></td>
<td>88</td>
<td>5</td>
<td>93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>226</td>
<td>5</td>
<td>231</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and randomly allocated to either irrigation during TURP with glycine 1.5% or 5% glucose. All patients gave fully informed consent and were assessed with immediate preoperative blood analysis and electrocardiograms [11].

Only patients with spinal anaesthesia were included in the analysis and their operative details recorded. The protocol requested that sedation was not used, but if sedation was required then lethargy was not included as a symptom of TUR syndrome. The irrigant selected was unknown to both the surgeon and anaesthetist, as the irrigants were put into unmarked bags. The amount of irrigant absorbed was measured by breath ethanol levels on an alcohol meter, using a nomogram [12]; the total irrigation fluid absorbed by each patient was recorded. The duration of TURP, weight of resected tissue, volume of irrigant used and evidence of prostatic capsule perforation were recorded. Blood transfusions were recorded and a standard protocol was for two 8-hourly bags of normal saline to be prescribed; no patient received i.v. dextrose or dextrose saline after undergoing TURP.

After TURP, in recovery, blood samples were taken to measure haemoglobin, haematocrit, sodium, potassium, urea, creatinine, glucose, osmolality, calcium and glycine. Glycine was analysed using anion-exchange chromatography with ninhydrin detection on an amino-acid analyser. Blood samples were rechecked at 5 and 24 h after TURP, and all results compared with values before TURP.

The TUR syndrome was defined as a sodium level after TURP of ≥125 mmol/L [4], with two or more symptoms or signs of TUR syndrome. Symptoms or signs attributed to TUR syndrome were nausea, vomiting, bradycardia, hypotension, hypertension, chest pain, mental confusion, anxiety, paraesthesia, and visual disturbances. Any symptoms or signs of TUR syndrome noted during TURP were also recorded.

Analysis of covariance was used to test for differences between the blood values the day after TURP for the two treatment groups, adjusting for baseline (before TURP) values. Interactions between treatment group and baseline values were examined and retained if significant at the 5% level. If the baseline values did not influence the next-day values, either a two-sample t-test or a Wilcoxon test was used to assess the next-day values as appropriate. Model assumptions were also assessed graphically.

RESULTS

In all, 124 patients were randomized to receive 5% glucose and 126 to receive 1.5% glycine; the mean (SD, range) age of the patients was 74.3 (8.9, 48–96) years. There was no significant difference between the groups in sodium levels (Fig. 1) or for the changes in potassium, urea, creatinine, osmolality, calcium, haemoglobin or haematocrit.

Five of the 233 patients for whom the TUR syndrome status could be determined had TUR syndrome (2.1%, 95% CI 0.7–5.0; Table 1). Of the five patients who had TUR syndrome, one had bradycardia, three had hypotension, four were drowsy, one was nauseous, two had pricking, two experienced uneasiness, one had blurred vision and two were confused; none had chest pain. Although all five patients with TUR syndrome were in the glycine group and none of the patients in the glucose group developed TUR syndrome, this difference did not reach statistical significance (Fisher’s exact test, P = 0.06, n = 233).

All five patients with TUR syndrome (Table 1) had glycine levels above the normal range (150–399 µmol/L), at a mean (range) of 28 915 (16 686–36 800) µmol/L, a fluid absorption of 3.6 (2.6–4.1) L, and a resection time of 47.6 (35–58) min; four had prostate capsule perforation noted during TURP. In the glycine group overall, there was significant variation in the glycine assay results, with some values many times the upper limit of normal.

There was evidence of an association between TUR syndrome and raised glycine levels at the end of TURP (Fisher’s exact test, P = 0.01, 231 men). Patients with TUR syndrome were more likely to have a glycine level outside the normal range than those who did not have TUR syndrome; the median level of

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glycine in the glucose, glycine, TUR syndrome group and non-TUR syndrome group groups was (μmol/L) 241, 791, 29 665 and 317, respectively.

In the glycine group there was no evidence to suggest an association between glycine levels and osmolality at the end of TURP (Spearman’s rank correlation coefficient 0.05, 119 men), but the sodium levels tended to decrease with increasing glycine levels (Spearman’s rank correlation coefficient –0.57).

There were 124 patients in the glucose group, of whom 13 were diabetic; they were managed peri-operatively with a standard protocol for diabetic patients, receiving i.v. insulin infusion. Immediately after TURP in these 13 patients the median (interquartile range, range) glucose level was 8.1 (5.6–10.3, 5.6–23.9) mmol/L, but by the second blood sample at 5 h after TURP all patients were within the normal range.

None of the patients in the glucose group developed TUR syndrome, although one had a serum sodium value of <125 mmol/L after TURP (Fig. 2) but did not fulfil the criteria for TUR syndrome, as he had no symptoms or signs of TUR syndrome. This patient, according to the breath ethanol nomogram [7], had absorbed just under 3 L of 5% glucose immediately after TURP were 40 and 119 mmol/L, respectively; 5 h later the respective levels had returned to normal, at 5.9 and 135 mmol/L; Table 2 gives values for all patients in the glucose group.

DISCUSSION

Endoscopic surgery of the genitourinary tract requires the use of an irrigating fluid. The absorption of some irrigant occurs during almost every TURP [12]. Volumes of irrigation fluid absorbed can be difficult to predict, although the volume tends to be greater in extended and bloody operations [13]. In the 1950s, several studies were undertaken to determine the amount of fluid absorbed during TURP. Hagstrom (1955) weighed patients before and after TURP, and calculated that ~20 mL/min of fluid was absorbed by the patient. However, there appeared to be a wide variation among patients; Oester and Madsen, using a double-isotope technique, showed in 1969 that the mean was ~1 L, and that a third of the fluid was absorbed intravenously, when the venous sinuses were opened, meaning that most of the fluid was in the periprostatic area. Currently surgeons are more aware of the dangers of irrigant absorption and most would attempt to limit the duration of TURP; however TUR syndrome still occurs.

Several irrigant solutions are available, including sorbitol-mannitol and glycine; the former is used in Europe but glycine is most common in the UK and North America. There is now increasing evidence highlighting the toxicity of 1.5% glycine solution when absorbed during TURP [14]. Glycine is an amino acid present in humans at <400 μmol/L; at higher concentrations research has shown it has direct and indirect cardiotoxic effects in animal studies [15,16], and pathophysiological action in stimulating, amongst other things, the release of atrial natriuretic peptide, thereby enhancing sodium loss and contributing to hyponatraemia, which is part of the TUR syndrome [17]. The amount of glycine absorbed seems to have an independent contribution to cerebral effects in volunteers [18] and to mortality in mice [19].

The metabolism of glycine gives rise to glycolic acid and ammonia, and high levels of blood ammonia have also been suggested as a possible cause of TUR syndrome [12]. Previous studies showed a correlation between symptoms and hyperammonaemia after infusion of glycine 2.2% [18] and TURP [20].

Nausea, vomiting and confusion occur six to nine times more often when 1–2 L of glycine solution is absorbed than when no absorption is detected [21]. Most patients with a transient deterioration in mental status after TURP have absorbed irrigant [22]. Consciousness appears to be lowered when even more glycine is absorbed [22,23], and this has also been associated with hyperammonaemia [12].

Alternatively, a direct toxic effect of glycine may be part of the mechanism of the cerebral side-effects of glycine absorption [24]. Glycine is an inhibitory neurotransmitter, and the visual disturbances in TUR syndrome are not the same as those expected in cortical oedema. The condition can proceed to transient blindness and is sometimes the only sign of fluid absorption [25]. Light perception is usually lost in cortical oedema, but not in TUR syndrome [26]. Fundoscopy is normal [25] and measurement of intraocular pressure is unchanged in TUR syndrome, which would indicate that visual changes are not due to cerebral oedema secondary to hyponatraemia [27]. However, there is little doubt that the hyponatraemia resulting from a dilutional effect of all irrigating fluids eventually causes neurological symptoms related to cerebral oedema. Istre et al. [28] detected cerebral oedema by CT that correlated with nausea after the absorption of as little as 1 L of glycine 1.5% in females undergoing transcervical resection of endometrium. Restlessness and epileptic seizures are signs of massive absorption; they are most likely caused by hyponatraemia, as these symptoms have been associated with various irrigants, e.g. glycine [6], sorbitol 3% [29], sorbitol-mannitol [30] and sterile water [31].

<table>
<thead>
<tr>
<th>Sodium at end of operation, mmol/L</th>
<th>Glycine at end of operation, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>120</td>
<td>120</td>
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<tr>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>140</td>
<td>140</td>
</tr>
</tbody>
</table>

FIG. 2. A plot of the sodium and glycine levels at the end of TURP for 231 patients; values from those men with TUR syndrome are shown as crosses.

TABLE 2 Serum glucose values after TURP in the glucose group (124 patients)

<table>
<thead>
<tr>
<th>Glucose, mmol/L</th>
<th>Median (interquartile range, range) [number]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately after TURP</td>
<td>6.1 (5.2–9.9, 4.2–40.1) [121]</td>
</tr>
<tr>
<td>5 h after TURP</td>
<td>6.5 (5.7–7.9, 3.8–15.8) [113]</td>
</tr>
</tbody>
</table>
However, the hyponatraemia associated with TUR syndrome is not simple dilutional hyponatraemia; there is a loss of sodium during the osmotic diuresis associated with irrigant absorption, therefore the urinary excretion of sodium represents an absolute loss, as the irrigant contains no electrolytes [9]. Also, large amounts of glycine have been shown to increase the release of atrial natriuretic peptide in excess of that expected from the volume absorbed, which will further promote natriuresis [17]. This supports our findings that an increase in glycine level is not associated with a change in osmolality caused by a simple dilutional effect, but still results in a lower sodium level.

Serum osmolality usually remains normal or decreases by ≤10 mosmol/kg when fluid is absorbed. However, the change in osmolality correctly indicates tissue oedema only when mannitol is absorbed. As glycine and glucose enter the cells they will be accompanied by water through osmosis, even when serum osmolality is normal. Tissue oedema will therefore be greater than indicated by the serum osmolality. Thus, the terminology ‘isotonic hyponatraemia’ is not useful when glycine or glucose is used. It is particularly irrelevant when ethanol is used to indicate fluid absorption, as this agent increases the osmolality provided by 5% glucose solution which is 190 mosmol/kg. This higher osmolality of 5% glucose is 285 mosmol/kg, therefore the urinary irrigant absorption, therefore the urinary

A solution of 5% glucose is a standard crystalloid; because glucose is metabolized throughout the body it requires 13 L to be given/absorbed intravenously to expand the intravascular compartment by 1 L. Normal serum osmolality is ~290 mosmol/kg. The osmolality of 5% glucose is 285 mosmol/kg, as opposed to the osmolality of 1.5% glycine, which is 190 mosmol/kg. This higher osmolality provided by 5% glucose solution may be beneficial in reducing the possible side-effects of cerebral oedema, which can occur after inadvertent absorption of irrigating fluids.

In the present study, for glucose control, there seemed to be no adverse effect of absorbing large quantities of glucose. The largest values were immediately after TURP (range 4.2–40 mmol/L), even in the patient whose glucose level was 40 mmol/L after absorbing 2.8 L of irrigant, who had a normal level of 5.9 at 5 h and 6.3 mmol/L at 24 h after TURP.

Another potentially safer alternative to glycine irrigation is normal saline with bipolar diathermy [32–35]. This relatively new development allows TURP using a familiar technique. Like 5% glucose, normal saline is a more physiologically solution that can be given intravenously and with minimal known side-effects. However, there are as yet no large scale randomized studies comparing the clinical effectiveness and cost-effectiveness of this technique compared to standard TURP; such studies are urgently needed.

In conclusion, hyponatraemia and the toxicity of glycine and/or its metabolites explain the clinical symptoms of TUR syndrome. Although endoscopic procedures on the genitourinary system are currently limited to using irrigation fluids, the choice of irrigant is not limited. We recommend that surgeons should consider the use of alternative irrigants to glycine or alternative surgical techniques that allow crystalloids such as normal saline to be used as an irrigant.

**CONFLICT OF INTEREST**

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The management of penile fracture based on clinical and magnetic resonance imaging findings

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OBJECTIVE
To present our experience with repairing penile fracture, based on clinical and magnetic resonance imaging (MRI) findings.

PATIENTS AND METHODS
Between December 2002 and October 2004, 14 men (19–64 years old) presented to our centre with a penile fracture. Two patients had urethral bleeding. MRI was used before surgery in all patients, and the repair comprised a localized longitudinal penile incision in 13 men. This incision was designed according to the tunical tear site and size already depicted by MRI. One case was managed conservatively, as MRI confirmed an intercavernosal haematoma with no tunical tear. The follow-up was 4–21 months.

RESULTS
The tear involved one corpus cavernosum in 11 patients; two were associated with urethral injury. The course after repair was uneventful in all men; the follow-up showed no erectile dysfunction in any. The patients reported neither pain nor penile curvature during erection.

CONCLUSION
MRI is a simple and informative investigation for evaluating and documenting a penile fracture, and it improves the management plan.

KEYWORDS
penis, fracture, MRI, tunica, tear

INTRODUCTION
Penile fracture is a urological emergency, being the rupture of the tunica albuginea of the corpus cavernosum caused by trauma to the erect penis [1]. The most common cases reported in the Western world occur during sexual intercourse [2]. In Middle Eastern countries many of these injuries are self-inflicted during masturbation, as the patient bends the erect penis abruptly in an attempt to achieve detumescence after prolonged erection [3]. Other causes of fracture are variations of striking the erect penis, e.g. falling on the erected penis or even rolling over on the erect penis in bed [4]. It is a rare event, which is why the best management plan is not well defined [5].

Surgical intervention is generally recommended to preserve the tunical integrity that is essential for erection. Beforehand an accurate assessment of the location and size of the tunical tear, and whether there is associated urethral injury, is important. For this purpose many authors have tried cavernosography [4,6] and...
urethrography [7]. However, these investigations have their problems. Others used ultrasonography [8–11] but it was unhelpful [12]. MRI was used recently to evaluate penile fracture, and was found to be effective [12–15]. In the present study we report our experience in managing penile fracture, using MRI as a noninvasive documentary tool, in addition to its efficacy in depicting the anatomical details of the condition, which not only facilitates surgical intervention but also minimizes its morbidity.

**PATIENTS AND METHODS**

Between December 2002 and October 2004, 14 patients with penile fracture were treated in our centre. The delay in presentation was 1–17 h from injury; all patients reported hearing a cracking sound, followed by rapid detumescence associated with pain and penile swelling. Two patients had urethral bleeding. On physical examination all patients had various degrees of penile swelling and ecchymosis. Because of penile tenderness and the associated swelling, the definite site of tunical tear could not be adequately palpated in 10 patients. All patients had emergency MRI, using a surface coil. With the patient supine the penis was taped against the abdominal wall and the surface coil placed on the penis. Sagittal T2-weighted spin-echo images (repetition time/echo time, 3750/100 ms) were used as a ‘scout’ for the subsequent axial and coronal T2-weighted fast-spin echo images, with the same parameters. In some cases additional axial and sagittal T1-weighted images (repetition time/echo time 400/10 ms) were obtained to confirm the penile haematoma. The field of view of all sequences was 215–320 mm. All patients required surgical intervention except one in whom MRI showed intercavernosal haematoma with no tunical tear. This patient was managed conservatively using analgesics, ice-packs and a pressure bandage. Surgical exploration was by a longitudinal incision placed at the site of the tunical tear as determined by MRI. The haematoma was evacuated and the tunical tear repaired using interrupted 2–0 polyglactin sutures. The urethral injury was repaired on a urethral catheter using interrupted 4–0 polyglactin sutures and the catheter was left for 1 week. Patients were discharged from hospital 24 h after surgery; all were instructed to avoid sexual intercourse for 1 month. The patients' demographic data, cause of injury and MRI findings are shown in Table 1.

**RESULTS**

The most common mechanism of penile fracture in the patients was bending the erect penis (nine of 14). MRI detected that the tunical tear involved the right corpus in eight men and the left in five. Other MRI data on tear size, site and associated injuries are shown in Table 1 and Figs 1–5. In no case were the MRI findings refuted at operation. There were no significant complications during or soon after surgery. The patients were followed for 4–21 months and all reported subjectively good erectile function, with neither penile curvature nor pain during erection.

**DISCUSSION**

Penile fracture is a rare condition that is easy to diagnose clinically; immediate surgical repair is recommended to avoid the complications of conservative management, i.e. penile curvature and deformity, fibrosis and sexual dysfunction. Moreover conservative treatment requires a prolonged hospital stay [16–18].

Locating the tunical tear site and associated urethral injury before surgery is important to make a correct incision for the repair. Although the tear can be palpated manually, it may be obscured by the swelling and haematoma even if the examination is under anaesthesia.

Some authors advocated the use of cavernosography to locate the tear site [4,6], but this requires manipulation of the tender and swollen penis. Also, some patients with normal findings on cavernosography have a rare related injury requiring open exploration, i.e. deep dorsal vein rupture [19]. Cavernosography also cannot be used in patients allergic to contrast material. Moreover, it can give false-negative results [16,20].

Penile fracture is associated with urethral injury in up to 38% of patients [1].

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**TABLE 1** Patient demographic data, cause of injury and MRI findings

<table>
<thead>
<tr>
<th>Patient/age, years</th>
<th>Cause</th>
<th>Site, cm from coronal sulcus</th>
<th>Tear size, mm</th>
<th>Laterality</th>
<th>Associated injuries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/23</td>
<td>Bending erect penis</td>
<td>5</td>
<td>13</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>2/39</td>
<td>Bending erect penis</td>
<td>6</td>
<td>9</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>3/21</td>
<td>Bending erect penis</td>
<td>4.5</td>
<td>12</td>
<td>L</td>
<td>Urethral injury</td>
</tr>
<tr>
<td>4/25</td>
<td>Bending erect penis</td>
<td>6.5</td>
<td>10</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>5/19</td>
<td>Sexual intercourse</td>
<td>5.5</td>
<td>6</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>6/37</td>
<td>Bending erect penis</td>
<td>6</td>
<td>11</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>7/26</td>
<td>Bending erect penis</td>
<td>7</td>
<td>14</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>8/28</td>
<td>Sexual intercourse</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Intracavernosal haematoma</td>
</tr>
<tr>
<td>9/33</td>
<td>Falling on the erect penis</td>
<td>6</td>
<td>12</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>10/29</td>
<td>Bending erect penis</td>
<td>6.5</td>
<td>11</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>11/35</td>
<td>Bending erect penis</td>
<td>5</td>
<td>14</td>
<td>R</td>
<td>Urethral injury</td>
</tr>
<tr>
<td>12/64</td>
<td>Sexual intercourse</td>
<td>4</td>
<td>13</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>13/33</td>
<td>Trauma by axe while farming</td>
<td>5</td>
<td>11</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>14/49</td>
<td>Bending erect penis</td>
<td>5.5</td>
<td>8</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>
Urethrography was used by some authors to confirm associated urethral injury [7]. Moreover, a Consensus Group [21] recommended retrograde urethrography as a routine in suspected cases of penile fracture, as the presence of blood at the external meatus is neither sensitive nor specific for urethral injury. Mydlo [22] reported that some patients with blood at the meatus had no evidence of urethral injury on exploration, while others had urethral injuries with no blood at the meatus. Nevertheless, retrograde urethrography is an invasive examination, needs contrast material, and carries the risk of extravasation and introduction of infection.

Recently MRI was used to evaluate penile fractures, where it was highly accurate in identifying the tunical tear and associated urethral injury [12–15]. Thus we used MRI as a non-contact investigation for such a tender and painful condition, to determine the site and size of the tear and associated urethral injury. According to the MRI findings we used a localized longitudinal incision centred over the tear. We think that this approach is minimally invasive and less morbid than the most commonly used exploratory subcoronal penile degloving incision. In the present series the tear site was 4–7 cm from the penile coronal sulcus. Accordingly, using penile degloving leads to unnecessary dissection of 4–7 cm of the penis to get access to the tear site. Moreover, penile degloving was reported to carry a high complication rate (14–25%) including infection, abscess formation and skin necrosis [23,24]. None of these complications occurred in the present patients. Our localized approach was easy and provided a 'skin window' directly over the tear, allowing an adequate repair of the tunica and the urethral injury. It decreased the hospital stay, as all of the patients were discharged 24 h after surgery, sooner than reported (1–4 days) by many authors using penile degloving [7,25,26].

Penile fracture carries a risk of erectile dysfunction; in our locality it is not infrequent that the patient (after surgical repair) claims that they have de novo erectile dysfunction as a result of faulty surgery. Thus before surgery MRI is an effective measure providing objective documentation of the condition for such medicolegal purposes. Moreover, MR images can be used for teaching purposes and for comparing results from different centres.

Notably, MRI saved one of the present patients from surgical intervention; in this patient, although presenting with typical findings of a penile fracture during sexual intercourse, MRI proved that there was an intercavernosal haematoma with no tunical tear (Figs 4 and 5).

In conclusion, MRI is an easy and informative investigation for evaluating and documenting penile fracture; it also improves the management plan. However, MRI is expensive and needs available apparatus and an expert operator.

**ACKNOWLEDGEMENTS**

The authors acknowledge Salah El-Din Shaker, MD (Urology Department, Assiut University

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**FIG. 1.** MRI findings of patient no. 1: Tear of the tunica albuginea. Coronal T2-weighted MR image shows a discontinuity of the low-signal-intensity tunica albuginea (arrow) and surrounding haematoma (*) in the ventral aspect of the right corpus cavernosum. The tear was 5 cm from the coronal sulcus of the penis, confirmed by the scale on the images.

**FIG. 2.** MRI findings in patient no. 3: A tunical tear with urethral rupture. Coronal T2-weighted MR image shows a tunical tear in the ventral aspect of the left corpus cavernosum 4.5 cm distal to the penile coronal sulcus (arrowhead). A large haematoma adjacent to the tear (H) and disruption of the corpus spongiosum and urethra (arrow) are also clear.
CONFLICT OF INTEREST

None declared. Source of funding: Assiut University Hospital, Egypt.

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Outcome from percutaneous nephrolithotomy in patients with spinal cord injury, using a single-stage dilator for access

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Accepted for publication 30 March 2005

OBJECTIVE
To present our experience of percutaneous nephrolithotomy (PCNL) for treating urolithiasis in patients with spinal cord injury (SCI) using a single-stage dilator for percutaneous access.

PATIENTS AND METHODS
A prospective database of patients with SCI having PCNL using the single-stage dilator was assessed, analysing patient data, stone-free rates, morbidity and the follow-up outcome.

RESULTS
In all, 26 patients had 54 PCNLs on 32 kidneys; 20 had unilateral and six bilateral stone disease; there were many staghorn calculi (24/54). Major complications occurred in three of 54 PCNLs (6%). The complete stone-clearance rate was 87% for PCNL alone, rising to 29 of 32 kidneys (91%) or 24 of 26 patients (92%) with adjuvant procedures. A further three kidneys required no further treatment and were monitored, having residual fragments of ≤2 mm.

CONCLUSIONS
PCNL has a high success rate and acceptable complication rate compared to extracorporeal shock-wave lithotripsy, and remains a valid first-line treatment option for kidney stones in patients with SCI.

KEYWORDS
spinal cord injuries, percutaneous nephrostomy, lithotripsy, kidney calculi, surgical instruments

INTRODUCTION
Patients with spinal cord injury (SCI) have impairment of sensory, motor or autonomic function. When their primary neurological lesion is coupled with the associated problems of immobilization, metabolic alteration, and neurogenic bladder and bowel dysfunction, they are predisposed to a variety of secondary complications [1]. Historically neurogenic bladder dysfunction was often poorly managed, predisposing patients to extremely high detrusor pressures, VUR, poor bladder emptying, detrusor muscle deterioration, and chronic infection, which contributed to urolithiasis [2–4]. Urolithiasis...
often involved large calculi, because of the use of long-term catheter drainage predisposing them to chronic bacteriuria (Fig. 1) [5,6]. The stones that form are primarily struvite (magnesium ammonium phosphate), and often contributed to by infections with urea-splitting organisms such as Proteus, Ureaplasma or Klebsiella [3].

Contemporary urodynamic evaluation and management of detrusor and sphincter function, combined with clean intermittent catheterization, has resulted in most patients with SCI having low-pressure urinary drainage systems and reduced rates of urinary infection [3]. Despite this, patients with SCI still have a significant incidence of urolithiasis, and a recent longitudinal study found almost no change in the past 25 years [1]. Furthermore, 34% of patients with SCI developing one stone will have a second stone episode within 5 years [7]. Effective stone treatment is very important, as the presence of stones is associated with decreasing renal function [8].

Percutaneous nephrolithotomy (PCNL) revolutionized stone surgery over 20 years ago, replacing open surgery as the treatment of choice for large calculi [2,9,10]. However, in the past decade ESWL, with its low morbidity and improved stone-clearance rates, has been considered a valid option for treating calculi in patients with SCI [5]. This trend has been supported by some series that have found high morbidity when managing struvite stones, particularly in patients with SCI [2,11]. We present a series of PCNLs for treating urolithiasis in patients with SCI from a specialized spinal urology unit, using a single-stage dilator for access, and compare it to published series of PCNL with complete data, and those primarily using ESWL to treat similar stones.

**PATIENTS AND METHODS**

A prospective database of patients with SCI who had PCNL in the past 8 years was examined, as were medical records where any data were inconclusive or incomplete. This yielded 26 consecutive patients who had 54 PCNLs (Table 1). The greatest length and width of solitary stones were measured by CT, and the values multiplied together to give a measure of stone area.

The surgical procedure of PCNL was consistent throughout, with no patients excluded, and CT was undertaken because of the complex nature of the stones. Urine cultures were also taken before surgery, and appropriate antibiotic cover began at least 24 h before surgery and ceased 48 h after the procedure. Patients all received general anaesthesia and underwent cystoscopy with a ureteric catheter placed under fluoroscopy whilst in the lithotomy position (except for a patient with an ileal conduit). The patients were then placed prone on the operating table, with appropriate use of support to obtain the best position for percutaneous access to the affected renal system. The most appropriate calyx, based on preoperative imaging and patient positioning (i.e. the calyx giving the best access to the stone mass, or a targeted calyx containing an isolated stone, e.g. parallel lie), was punctured under bi-planar C-arm fluoroscopic control and a ‘slippery’ guidewire inserted (0.97 mm, Cook

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**TABLE 1 Patient characteristics, stone locations and results of PCNL**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>26</td>
</tr>
<tr>
<td>Bacteriuria</td>
<td>22</td>
</tr>
<tr>
<td>Indwelling catheterization</td>
<td>11</td>
</tr>
<tr>
<td>Condom urinary drainage</td>
<td>7</td>
</tr>
<tr>
<td>Condom/CISC</td>
<td>4</td>
</tr>
<tr>
<td>CISC alone</td>
<td>2</td>
</tr>
<tr>
<td>Ileal conduit</td>
<td>2</td>
</tr>
<tr>
<td><strong>Level of injury</strong></td>
<td></td>
</tr>
<tr>
<td>Quadriplegics</td>
<td>12</td>
</tr>
<tr>
<td>Paraplegics</td>
<td>14</td>
</tr>
<tr>
<td><strong>Stones</strong></td>
<td></td>
</tr>
<tr>
<td>Kidneys with a solitary calculus</td>
<td>39</td>
</tr>
<tr>
<td>Staghorn calculi</td>
<td>24</td>
</tr>
<tr>
<td>Kidneys with multiple calculi</td>
<td>15</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
</tr>
<tr>
<td>Renal pelvis</td>
<td>29</td>
</tr>
<tr>
<td>Upper ureter</td>
<td>7</td>
</tr>
<tr>
<td>Upper pole calyx</td>
<td>8</td>
</tr>
<tr>
<td>Lower pole calyx</td>
<td>10</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td></td>
</tr>
<tr>
<td>Operated kidneys</td>
<td>32</td>
</tr>
<tr>
<td>Previously operated kidneys</td>
<td>3</td>
</tr>
<tr>
<td>PCNLs</td>
<td>54</td>
</tr>
<tr>
<td>PCNLs per patient</td>
<td>2.12</td>
</tr>
<tr>
<td>PCNLs per kidney</td>
<td>1.67</td>
</tr>
<tr>
<td>Mean duration of operation, min</td>
<td>103</td>
</tr>
<tr>
<td>Patients stone-free, n (%)</td>
<td></td>
</tr>
<tr>
<td>After one PCNL</td>
<td>37/44 (84)</td>
</tr>
<tr>
<td>After two PCNLs</td>
<td>47/54 (87)</td>
</tr>
</tbody>
</table>

*CISC, clean intermittent self-catheterization.*
Urological Inc., Spencer, IN, USA). A single-stage renal and fascial dilator ('Webb' dilator, William A. Cook Australia Pty Ltd, Brisbane, Australia) of 28 F (Fig. 2) was used to create the track for the nephroscope sheath. This is a purpose-designed target dilator that dilates to 28 F with a single passage over a guidewire. A second universal or ‘safety’ guidewire was then placed once access was obtained. The nephroscope was then introduced (Fig. 3). A lithoclast or ultrasonic lithotripter (both Karl Storz GmbH & Co., Tuttlingen, Germany) was used to fracture and fragment the stones, with forceps used to extract larger stone fragments that were not aspirated or flushed out. At the end of the procedure, a nephrostomy tube (10 F Cope type, Cook Urological) was placed and only removed when a nephrostogram and ureterogram showed clear and free drainage of the operated system 2 days after surgery. Data were analysed using the chi-square test for categorical variables.

RESULTS

In all, 26 patients with SCI had 54 PCNLs on 32 kidneys in during the past 8 years at our institution (Table 1), including 23 male and three female patients (12 quadriplegics, 14 paraplegics) with a median age of 50 years. Six patients had bilateral stone disease and 20 had unilateral stone disease. Of those with a single calculus (39/54), the mean (range) stone area was 480 (70–3500) mm$^2$ and 24/54 PCNLs involved staghorn calculi (stone occupying all the calyces or 80% of the collecting system space). The stone was struvite in 50 of the 54 PCNLs, and calcium oxalate in the other four. The mean (range) operation time was 103 (30–220) min.

Multiple calyceal punctures were required in 13 of the 54 PCNLs, and 23 punctures were supracostal.

The overall success rate for complete stone clearance per kidney after one PCNL was 84% (37/44 PCNLs), increasing to 87% (47/54 PCNLs) with two such procedures. Of the kidneys with residual stone fragments, two had 2-mm stone fragments after treatment that were monitored with no further management. A further two kidneys had ESWL (one with 2-mm fragments remaining), one had open pyelolithotomy, and two had ureteroscopic stone removal. In summary, the stones were completely cleared in 29 of 32 kidneys (91%) with three kidneys being monitored as they had fragments £2 mm, because of the high risk of larger stones recurring.

Major complications occurred in three of 26 patients (12%) or three of 54 PCNLs (6%) (Table 2); two were pneumothorax requiring

<table>
<thead>
<tr>
<th>Complication</th>
<th>Patients, n (%)</th>
<th>Procedures, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>2 (8)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Urosepsis</td>
<td>1 (4)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (12)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Minor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever (&gt;38.5°C)</td>
<td>15 (58)</td>
<td>26 (48)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>3 (12)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Calyceal perforation*</td>
<td>2 (8)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (77)</td>
<td>31 (57)</td>
</tr>
</tbody>
</table>

*calyceal perforation, entrance into and then out of the collecting system as visible by extravasation of contrast medium on the image intensifier during surgery.
for patients with SCI, as they are still at risk of urolithiasis will continue to be a problem influenced by the method of urinary drainage than in the general population and is not patients with SCI (34% at 5 years) is higher Furthermore the stone-recurrence rate in management of patients with SCI [1,7].

Conservative management of upper tract calculi was abandoned 20 years ago, following advances in endourological techniques and lithotripter technology, as serious infection or significant deterioration in renal infection often developed with no extension of routine perioperative prophylactic antibiotic use. The blood transfusion rate was 6% for 54 PCNLs.

DISCUSSION

Conservative management of upper tract calculi was abandoned 20 years ago, following advances in endourological techniques and lithotripter technology, as serious infection or significant deterioration in renal infection often developed with no intervention [12]. Recent reviews concluded that there has been a relatively constant incidence of initial kidney stones in patients with SCI over this period, and the recurrence rate of kidney stones has not been significantly reduced despite advances in the management of patients with SCI [1,7]. Furthermore the stone-recurrence rate in patients with SCI (34% at 5 years) is higher than in the general population and is not influenced by the method of urinary drainage [7]. Urolithiasis will continue to be a problem for patients with SCI, as they are still at risk of UTI, are immobile and often have poorly draining urinary systems. The optimum contemporary management in this group should still be determined by the basic principles of stone evaluation, including stone size and site, drainage of the system, and the presence of infection.

A review of series reporting stone treatment with PCNL, ESWL or combined treatments in patients with SCI identifies overall success rates of 38–89% (Table 3) [2,5,13–18]. In the present series of 26 patients, the number of PCNLs was 1.69 per kidney treated, remarkably similar to other series of PCNL (1.44 and 1.67) [2,13]. The major complication rate of 12% (three of 26) was less than in other series of PCNL (17% and 20%), but not significantly less (P > 0.06 and 0.39, respectively) [2,13]. Success rates with PCNL alone, including the present series, were all similar (81–89%).

ESWL has been suggested both as a primary and adjuvant treatment to PCNL for patients with SCI [3,5,7]. Struvite stones in patients with SCI are often soft and are easily visualized with fluoroscopy or conventional radiography, making them suitable targets for ESWL. However, difficulty in locating renal calculi for ESWL must be anticipated in patients with SCI, who can have significant spinal curvature and extremity contractures. Inadequate positioning for ESWL reduces success rates [4]. Furthermore, because of positioning problems related to the potential kyphoscoliosis and pelvic tilt, and consequent difficulties in the accurate location of shock-waves on renal calculi for lithotripsy, these patients might be at greater risk of developing renal parenchymal and vascular damage after ESWL [19]. The potential long-term effects on renal parenchyma dictate strict control of its repeated use [18]. Other common issues that arise with ESWL are the elimination of stone fragments that can cause obstruction, and incomplete stone clearance permitting ongoing infection and nephrolithiasis [18].

Despite these limitations, acceptable results (53–73%) have been obtained using ESWL in patients with SCI (Table 3). In the largest series, 32 patients had ESWL on 41 kidneys, with a mean stone burden of 2.9 cm per kidney [5]. All stones were treated without previous debulking on a HM-3 lithotripter (Dornier Medizintechnik GMBH, Germering, Germany). A stone-free rate at 3 months of 73% (26/41) was reported. ESWL has some advantages in that it can be undertaken in selected patients with SCI using either no or local anaesthetic, without autonomic

<table>
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<th>Variable</th>
<th>Present [13]</th>
<th>[2]</th>
<th>[5]</th>
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<th>[15]</th>
<th>[16]</th>
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<td>35</td>
<td>23</td>
<td>32</td>
<td>15</td>
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<td>5</td>
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<tr>
<td>Procedures, n</td>
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<td>47</td>
<td>46</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>10</td>
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<tr>
<td>Kidneys treated, n</td>
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<td>28</td>
<td>41</td>
<td>24</td>
<td>10</td>
<td>13</td>
<td>8</td>
</tr>
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<td>Quadraplegic, n</td>
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<td>27</td>
<td>18</td>
<td>27</td>
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<td>8</td>
<td>4</td>
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<td>8</td>
<td>5</td>
<td>57</td>
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<td>0</td>
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<td>0</td>
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<td>PCNL</td>
<td>PCNL</td>
<td>ESWL</td>
<td>ESWL</td>
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<tr>
<td>Mean age, years</td>
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<td>43</td>
<td>52</td>
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<td>Procedure per kidney, n</td>
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<td>1.44</td>
<td>1.67</td>
<td>1.44</td>
<td>1.5</td>
<td>1.8</td>
<td>1.5</td>
<td>1.25</td>
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<tr>
<td>% Stone-free after one procedure</td>
<td>84</td>
<td>54</td>
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<td>NA</td>
<td>44</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>% or n/N</td>
<td>Overall stone-free rate per kidney</td>
<td>87</td>
<td>89</td>
<td>83</td>
<td>76</td>
<td>67</td>
<td>5/10</td>
<td>5/13</td>
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<td>11</td>
<td>11</td>
<td>38</td>
<td>NA</td>
<td>25</td>
<td>10</td>
<td>88</td>
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<tr>
<td>Postoperative fever</td>
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<td>74</td>
<td>44</td>
<td>13</td>
<td>20</td>
<td>2/8</td>
<td>2/10</td>
<td>NA</td>
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<tr>
<td>Blood transfusion</td>
<td>6</td>
<td>49</td>
<td>19</td>
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<td>Major complications†</td>
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<td>20</td>
<td>17</td>
<td>0</td>
<td>3</td>
<td>1/8</td>
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<td>1/8</td>
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<tr>
<td>Death</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NA, not available; *Includes ESWL after PCNL, open pyelolithotomy, ureteroscopic stone removal, open drainage of perirenal abscesses, nephrostomy, open ureterolithotomy and PCNL after ESWL; †Perirenal abscess, respiratory arrest, aspiration pneumonia, fistula, hydathorax, ura sepsis requiring nephrostomy.
dysreflexia in some cases [14,18]. It also has lower morbidity, with no deaths and fewer blood transfusions.

Culkin et al. [13] found that major morbidity after PCNL was significantly higher in patients with SCI (20% per patient operated) than in ambulatory patients (1.4%). Minor morbidity such as blood transfusions per patient operated was also higher in the SCI group (49% vs 20%). However, these results must be interpreted with caution, as the patients were not matched for stone size, age or other morbidities. The SCI group had 31% of patients with staghorn or partial staghorn calculi, compared to only 9% in the ambulatory group.

There are many reasons for patients with SCI to be at higher risk of postoperative complications than ambulatory patients; they often have poor respiratory reserves, leading to airway problems such as pulmonary atelectasis and pneumonia, their surgical wounds take longer to heal, and they are at higher risk of infection than the general population. Despite this perceived additional morbidity, stone-free rates of >80% are attainable [4].

Surgical technique is important, and the benefits of using a single-stage dilator such as the ‘Webb’ [20], can be applied to patients with SCI. Single-stage dilatation offers more accuracy, as repeated passage of multiple targeted sheaths is not required. Also, because of the large stones in patients with SCI, there is little room in the collecting system in which to dilate and place a wire. Repeated movements in and out using multiple dilators can dislodge a wire, compromising the procedure and leading to further unnecessary punctures or resulting in haemorrhage [20]. A single-stage dilator with a marker indicating where the sheath meets the tapering of the trocar (Fig. 2) prevents unnecessary advancement of the sheath without the trocar, minimizing parenchymal damage and reducing the reliance on an image intensifier to gauge the position of the sheath. Finally, chronically infected kidneys also can bleed with repeated trauma, and single dilatation might minimize this risk, and this might explain the lower haemorrhage rate in the present series than in other reported series that used different methods of access (Table 3). Single-stage dilators might also be better than balloon dilators because extruding the balloon from the collecting system, or advancing a sheath over an incompletely dilated balloon, might result in haemorrhage [20].

We agree with Chen et al. [7] that, when considering kidney stones in patients with SCI, choice of treatment should be determined by stone size, location, patient’s medical condition and the experience of the urologist, rather than by a preconceived bias that one procedure is better than another. However, we would also consider specifically the presence of UTI and drainage of the affected system, with the principle that PCNL is more appropriate than ESWL for larger stones, in poorly draining systems or in the presence of UTI, where complete stone clearance is paramount. Furthermore, from available evidence, PCNL has high success rates and acceptable complication rates compared to ESWL, and remains a valid treatment option for patients with SCI. Consideration should be given to using a single-stage dilating system, which was a reliable technique in the present series. Finally, we stress the importance of preventing calculi and detecting them when they are small, which will improve treatment outcomes for upper tract calculi in all patients with SCI.

CONFLICT OF INTEREST

None declared.

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Abbreviations: SCI, spinal cord injury; PCNL, percutaneous nephrolithotomy.
Multimodal management of urolithiasis in renal transplantation

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Accepted for publication 8 March 2005

OBJECTIVE
To report the largest single series of renal transplant patients (adults and children) with urolithiasis, assess the risk factors associated with urolithiasis in renal transplant recipients, and report the outcome of the multimodal management by endourological and open procedures.

PATIENTS AND METHODS
The records of all patients undergoing renal transplantation between 1977 and 2003 were reviewed. In all, 2085 patients had a renal transplant at our centre and 21 (17 adults and four children) developed urinary tract calculi. Their mode of presentation, investigations, treatments, complications and outcomes were recorded. Investigations included one or more of the following; ultrasonography (US), plain abdominal X-ray, intravenous urography, nephrostogram and computed tomography. Management of these calculi involved extracorporeal shock wave lithotripsy (ESWL), flexible ureteroscopy and in situ lithotripsy, percutaneous nephrolithotomy (PCNL), open pyelolithotomy and open cystolitholapaxy.

RESULTS
Thirteen patients had renal calculi, seven had ureteric calculi and one had bladder calculi. The incidence of urolithiasis was 21/2085 (1.01%) in the series. Urolithiasis was incidentally discovered on routine US in six patients, six presented with oliguria or anuria, including one with acute renal failure, four with a painful graft, three with haematuria, one with sepsis secondary to obstruction and infection and in one, urolithiasis was found after failure to remove a stent. Ten patients (63%) had an identifiable metabolic cause for urolithiasis, two by obstruction, two stent-related, one secondary to infection and in six no cause was identifiable. Thirteen required more than one treatment method; 13 (69%) were treated by ESWL, eight of whom required multiple sessions; eight required ureteric stent insertion before a second procedure and four required a nephrostomy tube to relieve obstruction. Two patients had flexible ureteroscopy and stone extraction, three had a PCNL and one had open cystolitholapaxy. PCNL failed in one patient who subsequently had successful open pyelolithotomy. All patients were rendered stone-free when different treatments were combined.

CONCLUSIONS
The incidence of urolithiasis in renal transplant patients is low. There is a high incidence of metabolic causes and therefore renal transplant patients with urolithiasis should undergo comprehensive metabolic screening. Management of these patients requires a multidisciplinary approach by renal physicians, transplant surgeons and urologists.

KEYWORDS
nephrolithiasis, renal transplantation, treatment, outcome

INTRODUCTION
Since the first calculus in a renal transplant [1] was described in 1975, urolithiasis has been recognized as a complication, although uncommon [2], of renal transplantation. It is currently thought that urolithiasis complicates 0.4–1% [3] of all renal transplants.

With advances in immunosuppression, donor card distribution and national transplant programmes, including laparoscopic and hand-assisted laparoscopic surgery, renal transplantation is becoming increasingly common. The resulting improvement in graft and patient survival has led to frequent presentation of the less common and longer-term complications of transplantation. It is conceivable that increasing transplantation is likely to have increased the number of renal grafts placed with calculi already in situ; the so-called ‘donor-gifted allograft lithiasis’ [4]. Several general and transplant-specific conditions may predispose a patient to developing urolithiasis, e.g. more concentrated and alkaline urine [5]. Although the presentation of urinary stones is often atypical and with no pain in most of these patients, heightened awareness of this condition has enabled clinicians to diagnose transplant urolithiasis at an earlier stage.

The steady development of minimally invasive methods has revolutionized the management of urolithiasis in renal transplant patients. Simultaneous progress in interventional radiological techniques and expertise has made the emergency management of acute obstruction easier. However, such facilities and expertise are not widely available and hence such patients are best managed in centres that are well equipped and have expertise to offer the appropriate treatment/intervention. We reviewed our renal transplant patients (adults and children) with urolithiasis, assessed the risk factors associated with the condition, and report the outcome of management by endourological and open procedures.

PATIENTS AND METHODS
All cases of renal transplantation at our institution from 1977 to 2003 were reviewed, comprising a series of 2085 consecutive renal
transplants. All procedures were either performed or assisted by a transplant consultant using a standard technique. Information on the incidence and management of urolithiasis was accumulated by retrospective case-note analysis.

For the overwhelming majority of cases a Lich-Gregoir extravesical ureteroneocystostomy with an extravesical seromuscular tunnel was performed, using the upper transplant ureter. A 7F, 16-cm JJ stent is routinely placed before completing the anastomosis, and removed after 6 weeks by flexible cystoscopy (Fig. 1). Although stenting is controversial and some centres use it selectively, in our unit it is used routinely. A recent meta-analysis [6] comparing stented to unstented extravesical ureteroneocystostomy showed that stented anastomoses have lower complication rates (3.2% vs 4.8% in case series analysis). Although stenting generally predisposes to urinary calculi, it reduces other complications and our stone incidence rate of ≈1% is similar to that in other centres not routinely stenting. No patient in this series developed stent encrustation.

A Foley catheter drained the bladder for at least 4 days. Immunosuppression included prednisolone, azathioprine and cyclosporin until 1999, when mycophenolate mofetil replaced the azathioprine.

Urolithiasis presenting with graft dysfunction, haematuria, unexplained fever, pain, or anuria was investigated by immediate ultrasonography (US) and early liaison with the urological surgeon was implemented for cases of obstruction.

RESULTS

The mean (range) lead time to presentation after transplantation was 3.6 (0.5–18) years. Twenty-one patients presented with renal allograft urolithiasis (eight men and 13 women; mean age 41 years, range 15–64), although there were six patients aged >60 years and five aged ≤18 years (Table 1). There were 18 cadaveric and three live-donor kidney recipients in the series. The predisposing renal diseases included reflux nephropathy in six patients, hypertension in four, glomerulonephritis in three and two had adult polycystic kidney disease. Over half of the patients (11) were treated with a triple immunosuppressive regimen of prednisolone, cyclosporin and azathioprine. The remainder have now been switched to a combination of mycophenolate mofetil, tacrolimus and prednisolone.

Patients presented with urolithiasis at a mean (range) of 3.7 (0.17–18) years after transplantation; eight presented within a year of their transplant. Presentation was with loin pain over the graft in four patients, haematuria in three, oliguria or anuria in six, sepsis in one, acute renal failure detected by rising serum creatinine levels in one or on routine US in six. All but six of the patients had a recognized predisposing condition towards renal tract lithiasis. Most commonly this was recurrent confirmed UTI (six patients) although four had hyperparathyroidism and four had uric acid calculi associated with hyperuricaemia. Other predisposing factors included ileal conduit urinary diversion, Mitrofanoff diversion, and heavy stent encrustation at the time of removal (Table 2).

Only two patients were able to spontaneously pass their stones with no intervention; both calculi were in the upper pole and were 4 and 4.5 mm in diameter. Three required percutaneous nephrostomy drainage and subsequent ESWL. In all, 13 patients had ESWL, with eight requiring multiple sessions to clear their stones. ESWL was delivered with the patient prone, with the stones located using US and X-ray on a Modulith (Storz, Germany). Five patients needed a ureteric JJ stent, two a percutaneous nephrolithotomy (PCNL) and two flexible ureteroscopic stone removal. Two patients required open procedures for stone clearance; an open pyelolithotomy for a large staghorn calculus in the allograft after an unsuccessful PCNL, and an open cystolithotomy for multiple large bladder calculi. After treatment all patients were entered into a surveillance programme with regular US of their renal transplant. No grafts failed as a result of urolithiasis and at present all patients are stone-free, although one is continuing to form regular stones but which are amenable to ESWL. One patient was left with significant residual fragments after ESWL, which subsequently passed spontaneously.

DISCUSSION

The incidence of urolithiasis in renal transplants at our institution is ≈1% (21/2085), with women outnumbering men, which is similar to previously published series [7]. Predisposing risk factors for stone formation were present in most renal allograft recipients. Some of these were documented before transplantation, e.g. gout and ileal conduit formation, while others were only discovered after biochemical investigation following calculus formation, e.g. hyperparathyroidism and recurrent UTIs. Attempts have previously been made to identify the importance of risk factors in this situation; Harper et al. [5] investigated risk factors for stone formation in five patients and compared them to 41 transplant patients with no stones. They reported that although
TABLE 1 The demographics of patients with renal transplant urolithiasis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at presentation</th>
<th>Original kidney disease</th>
<th>Donor, year</th>
<th>Immunosuppression</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>Glomerulonephritis</td>
<td>Cadaveric 1991</td>
<td>Cy, Az, Pr</td>
<td>1998</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>APKD</td>
<td>Cadaveric 1988</td>
<td>Cy, Az, Pr</td>
<td>2000</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>Chronic pyelonephritis, reflux</td>
<td>Cadaveric 1993</td>
<td>Cy, Az, Pr</td>
<td>1995</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>Bilateral VUR</td>
<td>Live 1984</td>
<td>Cy, Pr</td>
<td>1989</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>Henoch Schonlein purpura</td>
<td>Cadaveric 1991</td>
<td>Cy, Az, Pr</td>
<td>1997</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>Nephrotic syndrome</td>
<td>Cadaveric 1990</td>
<td>Cy, Az, Pr</td>
<td>1993</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>Reflux nephropathy</td>
<td>Cadaveric 1997</td>
<td>Cy, MMF</td>
<td>2000</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>Analgesic nephropathy</td>
<td>Cadaveric 1997</td>
<td>Az, Pr</td>
<td>1995</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>Glomerulonephritis</td>
<td>Cadaveric 1996</td>
<td>Cy, Az, Pr</td>
<td>1996</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>Hydronephrosis, hypertension</td>
<td>Live 1990</td>
<td>Cy, Az, Pr</td>
<td>1990</td>
</tr>
<tr>
<td>11</td>
<td>63</td>
<td>APKD, hypertension</td>
<td>Cadaveric 1994</td>
<td>Cy, Az, Pr</td>
<td>1994</td>
</tr>
<tr>
<td>12</td>
<td>61</td>
<td>NIDDM, hypertension</td>
<td>Cadaveric 1994</td>
<td>Cy, Az, Pr</td>
<td>1994</td>
</tr>
<tr>
<td>13</td>
<td>61</td>
<td>Genitourinary TB, adenocarcinoma</td>
<td>Cadaveric 1990</td>
<td>Cy, Az, Pr</td>
<td>1994</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>Reflux nephropathy</td>
<td>Cadaveric 1999</td>
<td>MMF, Cy, Pr</td>
<td>1999</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>Reflux nephropathy</td>
<td>Cadaveric 1999</td>
<td>Cy, MMF, Pr</td>
<td>1999</td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td>Reflux nephropathy</td>
<td>Cadaveric 1999</td>
<td>MMF, Cy, Pr</td>
<td>1999</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>Reflux nephropathy</td>
<td>Cadaveric 1999</td>
<td>MMF, Cy, Pr</td>
<td>1999</td>
</tr>
<tr>
<td>18</td>
<td>63</td>
<td>Single kidney, hypertension</td>
<td>Cadaveric 1999</td>
<td>MMF, Cy, Pr</td>
<td>1999</td>
</tr>
<tr>
<td>19</td>
<td>48</td>
<td>Glomerulonephritis</td>
<td>Cadaveric 1999</td>
<td>MMF, Cy, Pr</td>
<td>1999</td>
</tr>
<tr>
<td>20</td>
<td>52</td>
<td>Glomerulonephritis</td>
<td>Cadaveric 1999</td>
<td>MMF, Cy, Pr</td>
<td>1999</td>
</tr>
<tr>
<td>21</td>
<td>39</td>
<td>Reflux nephropathy</td>
<td>Cadaveric 1999</td>
<td>MMF, Cy, Pr</td>
<td>1999</td>
</tr>
</tbody>
</table>

Cy, cyclosporin; Az, azathioprine; Pr, prednisolone; N, Neoral; MMF, mycophenolate mofetil; Tac, tacrolimus; APKD, adult polycystic kidney disease; TB, tuberculosis; NIDDM, non-insulin dependent diabetes mellitus.

TABLE 2 The predisposing conditions, treatments and outcome of patients with renal transplant urolithiasis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Predisposing conditions</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None identified</td>
<td>Passed stone</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>Gout</td>
<td>Nephrostomy, ESWL × 1</td>
<td>Clear</td>
</tr>
<tr>
<td>3</td>
<td>Ileal conduit, spina bifida</td>
<td>ESWL × 2</td>
<td>Clear</td>
</tr>
<tr>
<td>4</td>
<td>Recurrent UTIs</td>
<td>ESWL × 1</td>
<td>Clear</td>
</tr>
<tr>
<td>5</td>
<td>Hyperparathyroidism</td>
<td>PCNL + Open pyelolithotomy</td>
<td>Clear</td>
</tr>
<tr>
<td>6</td>
<td>Hyperparathyroidism</td>
<td>PCNL + stent, 3 × ESWL</td>
<td>Clear</td>
</tr>
<tr>
<td>7</td>
<td>Gout</td>
<td>Stent + ESWL</td>
<td>Clear</td>
</tr>
<tr>
<td>8</td>
<td>None identified</td>
<td>Stent + ESWL</td>
<td>Clear, recurrent × 3</td>
</tr>
<tr>
<td>9</td>
<td>Stent encrustation at removal</td>
<td>ESWL + spontaneous passage</td>
<td>Clear</td>
</tr>
<tr>
<td>10</td>
<td>None identified</td>
<td>Nephrostomy, ESWL × 4</td>
<td>Clear</td>
</tr>
<tr>
<td>11</td>
<td>Gout</td>
<td>ESWL × 3</td>
<td>Clear</td>
</tr>
<tr>
<td>12</td>
<td>Nephrostomy in intensive unit</td>
<td>Stent, ESWL × 2</td>
<td>Fragments &lt;2 mm</td>
</tr>
<tr>
<td>13</td>
<td>Hyperparathyroidism, gout, UTIs</td>
<td>FURS via cutaneous ureterostomy × 3</td>
<td>Clear</td>
</tr>
<tr>
<td>14</td>
<td>Stent encrustation</td>
<td>Stent + ESWL × 6</td>
<td>Clear</td>
</tr>
<tr>
<td>15</td>
<td>Hyperparathyroidism, UTIs</td>
<td>Stent + ESWL × 4</td>
<td>Clear</td>
</tr>
<tr>
<td>16</td>
<td>Recurrent UTIs, Mitrofanoff</td>
<td>Open cystolithotomy</td>
<td>Clear</td>
</tr>
<tr>
<td>17</td>
<td>Recurrent UTIs</td>
<td>Stent + FURS</td>
<td>Clear</td>
</tr>
<tr>
<td>18</td>
<td>None identified</td>
<td>Nephrostomy + JJ stenting + ESWL</td>
<td>Clear</td>
</tr>
<tr>
<td>19</td>
<td>Recurrent UTIs</td>
<td>Passed stone</td>
<td>Clear</td>
</tr>
<tr>
<td>20</td>
<td>None identified</td>
<td>Nephrostomy + passed stone</td>
<td>Clear</td>
</tr>
<tr>
<td>21</td>
<td>None identified</td>
<td>PCNL</td>
<td>Clear</td>
</tr>
</tbody>
</table>

FURS, flexible ureteroscopic removal of stone; Success was complete clearance (clear)/fragments <2 mm. Mean (range) stone size 8.1 (4–17) mm.
patients with calculi in transplants passed significantly more concentrated and alkaline urine, there were other factors contributing to stone formation. This is reflected in the present series, where seven different risk factors were identified (Table 2).

The question of whether grafts may have been transplanted with calculi already in situ has been investigated by several centres. Donor-graft lithiasis was described by Van-Gansbeek et al. [8] in 1985 and has led to calls for preoperative screening for renal lithiasis in some transplant centres. Transplanted kidneys with pre-existing stones possibly require conservative and expectant treatment to preserve renal function, as stones are likely to recur. In contrast, Lu et al. [9] reported that these patients can be successfully treated with percutaneous procedures soon after transplantation, and suggest that pre-transplant US should be implemented to reduce the risk of ‘donor-gifted calculi’. Most centres would delay PCNL in the initial period after transplantation because of the higher levels of immunosuppression and potential risks of sepsis and poor wound healing. Torrecilla et al. [10] suggested that the detection of renal calculi in cadaveric renal donors is not a reason to refuse the graft for further transplantation, as long as appropriate and careful endourological expertise is available. Recently the group from Michigan [11] reported the ex-vivo ureteroscopic clearance of renal calculi in 10 kidneys before transplantation, finding it to be a feasible means of rendering a stone-bearing kidney stone-free without compromising ureteric integrity or renal allograft function.

Treatment protocols for calculi in the transplanted kidney should mimic those for single kidneys in general. Because of the superficial position of the transplanted kidney, nephrostomy drainage and subsequent PCNL is relatively straightforward, although we recommend that this be carried out on larger calculi (>1.5 cm) in specialist centres with a large PCNL experience, because of the greater importance of the single kidney. Percutaneous removal of calculi from transplanted kidneys was first described in 1985 by Hulbert et al. [12], and is now often the treatment of choice when there is a significant stone burden. Encrusted stents and obstructing clots can also be removed with this technique [13], and it has the advantage of potentially removing all stone fragments at one procedure. Although reported as the primary procedure of choice by some centres [14], we only used three PCNLs in the present 21 patients, probably because most calculi were <1.5 cm in diameter and there is an on-site ESWL machine. In general, for patients with obstructing calculi, we opted for swift resolution of the obstruction via nephrostomy (in four patients) or insertion of a ureteric JJ stent (in eight), followed by one or more sessions of ESWL. For unobstructive smaller stones of <1.5 cm, treatment with ESWL is usually sufficient. This has led to successful stone clearance in the 13 cases managed in this way. Fears that the position of the transplanted kidney would impair stone clearance with ESWL do not appear to have been a feature of the present series. There are potential difficulties in locating transplant calculi because of the overlying bony pelvis [4], but most patients can be treated while prone. Flexible ureteroscopy was necessary in two patients after the failure of ESWL; this method and disintegration of stones with electrohydraulic lithotripsy or holmium laser is a challenging but effective means for treating stones in transplant kidneys. Access to these kidneys may be difficult because of their position in the pelvis and the location of the neo-ureteric orifice. This is usually achieved by introducing the ureteroscope over a guidewire. Instruments with ‘active’ secondary deflection are particularly useful in reaching calculi in transplanted kidneys. The position of calculi within the renal collecting system tends to vary considerably from day to day because these kidneys are relatively horizontal, making it difficult to compare clearance rates between the upper and lower poles. All patients presenting with obstructed kidneys clearly had calculi at the PUJ or within the upper ureter.

In conclusion, despite significant improvements in immunosuppression, surgical advances and diagnostic imaging, the incidence of transplant urolithiasis is essentially unchanged over the last 20 years [15]. Transplant urolithiasis requires renal physicians and urologists to maintain vigilance and a high index of suspicion. We recommend that such patients be managed in specialist centres that have all readily available endourological methods. There should be access to an on-site lithotripsy machine, flexible ureterorenoscopes with holmium laser, and urologists with significant experience of PCNL. Most patients with calculi of <1.5 cm can be rendered stone-free with ESWL. If this failed, flexible ureteroscopy and holmium laser fragmentation, although technically difficult in these kidneys, can be attempted. For larger stones, PCNL gives the best chance of complete stone clearance.

CONFLICT OF INTEREST

None declared.

REFERENCES


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Abbreviations: US, ultrasonography; PCNL, percutaneous nephrolithotomy.
Asymptomatic bacteriuria in men with orthotopic ileal neobladders: possible relationship to nocturnal enuresis

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OBJECTIVE
To assess prospectively the incidence with time of asymptomatic bacteriuria in patients with orthotopic ileal neobladders, and the possible effect on neobladder function.

PATIENTS AND METHODS
In all, 47 patients (mean age 52.7 years, SD 8.7, range 31–68) with uncomplicated orthotopic ileal neobladders were prospectively evaluated. With no antibiotic manipulation, consecutive urine cultures were assessed monthly. Continence was assessed by direct information from the patients at each follow-up visit.

RESULTS
Overall, 797 samples were cultured from the 47 patients (mean 17.6, SD 7.1). There was a steady decrease in the incidence of positive cultures, from 74.5%, to 35.6% and 6.7% at 1, 6 and 18 months, respectively. While there was persistently sterile urine in only eight patients (17%), 32 had occasional and seven had persistent bacteriuria. Escherichia coli was the commonest organism (76.6%) followed by Klebsiella pneumonia (15.7%); 54% of E. coli and 38% of K. pneumonia infections were sensitive to nitrofurantoin. Diurnal continence was achieved in 98% of the patients at 6 months after surgery. There was a gradual decrease in the frequency of nocturnal enuresis (NE) with time, from 87%, to 42%, 28% and 27% at 1, 6, 12 and 18 months, respectively. There was a significant correlation between the presence of bacteriuria and NE during the first 6 months, but it was not sustained after that. The age of the patients was also related significantly to the incidence of NE; at 6 months, only one of 18 men aged ≤50 years had NE, while 19 of 29 aged >50 years had (P < 0.001). At 1 year all patients aged ≤50 years were nocturnally continent, while half of those aged >50 years had NE (P = 0.001).

CONCLUSIONS
Ileal neobladders are associated with a high incidence of asymptomatic bacteriuria during the first year after surgery. There was spontaneous clearance of bacteriuria with time, with no antimicrobial manipulation. Soon after surgery there was a significant association between bacteriuria and NE. The effect of antimicrobials on patients with NE should be evaluated.

KEYWORDS
neobladders, bacteriuria, nocturnal enuresis

INTRODUCTION
The orthotopic ileal neobladder has been considered by many investigators as the standard method of urinary diversion after
radical cystectomy for bladder cancer [1–3]. Bacteriuria is frequent but asymptomatic in most patients with an orthotopic neobladder; such neobladders have lower rates of positive urine cultures (3–34%) [4–6] than cutaneous reservoirs, which are almost always colonized with bacteria [7]. The importance of such bacteriuria remains controversial. The harmful effects of bacteriuria on refluxing kidneys, confirmed experimentally in dogs, cannot be directly related to humans [8]. One of the main complications of the orthotopic ileal neobladder is nocturnal enuresis (NE). Large series show that 15–40% of patients require pads or other protection during sleep, compared with <10% during the daytime [1–3,9]. Studies investigating the incidence of such bacteriuria with time and its impact on nocturnal urinary control are sparse.

In the present study we evaluated the incidence of asymptomatic bacteriuria and its change with time after surgery, and determined the most common pathogenic organisms. The possible relationship between such bacteriuria and NE was also assessed.

PATIENTS AND METHODS

Of 173 patients with orthotopic neobladders (147 men and 26 women) constructed between January 2002 and January 2004, 47 men were included in the present study (mean age 52.7 years, SD 8.7, range 31–68). Women were excluded as most of them had a large residual urine volume after voiding, that predisposed to bacteriuria. All patients had an orthotopic bladder substitution after radical cystectomy for bladder cancer. The neobladders were constructed using detubularized terminal ileal segments with fashioning of a W-shaped pouch. A 5-cm long intact 'chimney' was preserved for direct uretero-intestinal anastomosis on one side, while the other ureter was implanted using a serous-lined extramural tunnel, as previously described [1,10]. Nerve-sparing cystectomy was attempted only in a few patients (five) and was therefore unsuitable for statistical analysis. Our policy was to start broad-spectrum parenteral antibiotics on the morning of the operation and continued for 5 days afterward, then change to an oral antimicrobial (trimethoprim-sulphamethoxazole) as long as the urethral catheter was indwelling (20 days) and for 2 weeks after its removal. None of the present patients was treated with antimicrobial drugs thereafter. Inclusion criteria were normal kidneys before surgery, no upper tract obstruction afterward, and no stones, chronic retention, local recurrences or distant metastases. All selected patients had a residual urine of <50 mL after voiding, confirmed by catheter insertion after voiding once, then verified by monthly pelvic ultrasonography. All patients had no history of cardiac diseases, hypertension, diabetes mellitus or administration of systemic chemotherapy or radiation therapy, or other comorbidities that could affect the outcome.

Clean-catch midstream urine samples were obtained monthly for cultures and antimicrobial susceptibility testing. The well-mixed urine was sampled with a 1 μL calibrated microbiological loop and plated onto the surface of the following culture media: cysteine lactose electrolyte-deficient, blood agar, Schaedler anaerobic agar and Sabouraud dextrose agar. Cultures were incubated (respectively for each type), aerobically at 35–37 °C for 18–24 h, anaerobically in the Oxoid Anaerobic System, and in ambient air for 1–4 weeks for fungal isolation. All isolated bacteria were identified by using MicroScan WalkAway 40 (Dade Behring Inc., USA) dried identification panels for Gram-negative bacteria type 2 and antimicrobial susceptibility tested using MIC determination panels (Negative Urine MIC panel type 10) in the same machine. A urine culture containing >10^5 colony-forming units/mL was considered as positive.

Patients were followed up regularly every month after surgery (mean 17.6 months, SD 7.1, range 7–30) by a history, physical examination and urine culture. The radiological evaluation consisted of renal and pelvic ultrasonography monthly. CT and a bone scan were used when clinically indicated. Continence and a voiding diary were assessed by direct information from the patients at each follow-up visit. Patients were considered continent if they were completely dry during the day and night, with no need for protection by pads or condom catheters.

The results were evaluated statistically with Fisher’s exact test, using two-tailed P values, with P < 0.05 considered to indicate significant differences.

RESULTS

Overall, 797 samples were cultured from the 47 patients (mean 17.6, SD 7.1); there was significant bacterial growth in 239 cultures (29.6%). The incidence of positive cultures during the first follow-up visit was 74.5%; there was then a steady decline in the incidence of positive cultures to 35.6%, 33% and 6.7% at 6, 12 and 18 months, respectively. None of urine cultures were positive at 24 months (22 patients).

Escherichia coli was the commonest organism, constituting 76.7% of positive cultures, followed by Klebsiella pneumonia (15.7%) (Fig. 1). Proteus and Pseudomonas infections were detected in 5.5% of positive
cultures. Thirty-two patients (68%) had occasional bacteriuria, 20 with the same organisms and 12 with different organisms. Eight patients (17%) had persistently sterile urine cultures on all sampling occasions. Only seven patients (15%) had persistent bacteriuria; three with the same organism and four with different organisms (Table 1).

Table 2 shows the antimicrobial susceptibility for different organisms. The most effective antimicrobial agents against *E. coli* were imipenem, amikacin and piperacillin-tazobactam. The susceptibility of *E. coli* to the commonly used antimicrobial nitrofurantoin was 54%. The most effective antimicrobial agents against *K. pneumonia* were imipenem and amikacin; none of cultures with *K. pneumonia* were sensitive to trimethoprim-sulphamethoxazole, while 38% were sensitive to nitrofurantoin. Daytime continence was assessed in all patients at 1 month after surgery; 18 were totally continent, 15 were incontinent requiring condom-catheter drainage and 14 had stress urinary incontinence. At 6 months after surgery diurnal continence was complete in 46 patients (98%).

For nocturnal continence, during the first month 87% of patients were enuretic; there was a gradual decrease in the frequency of enuretic patients with time, the frequency of NE being 42%, 28% and 27% at 6, 12 and 18 months, respectively (Table 3). Four of 22 patients (18%) had NE 2 years after surgery; three of them had occasional enuresis and one used a condom catheter. The age of the patients was significantly related to the incidence of NE; at 6 months only one of 18 men aged ≤50 years had NE, while 19 of 29 aged >50 years had NE (P < 0.001). At 12 months all patients aged ≤50 years were nocturnally continent, while half of those aged >50 years had NE (P = 0.001).

There was a significant correlation between the presence of positive cultures and nocturnal continence during the first 6 months (Table 3, Fig. 2). During the first follow-up visit, 94% of patients with bacteriuria had NE, compared to 67% of those with sterile urine. After 6 months, 69% of patients with bacteriuria had NE compared to 28% of those with sterile urine. These significant correlations were not sustained thereafter.

### DISCUSSION

The clinical significance of bacteriuria in patients with an orthotopic ileal neobladder is controversial. Advocates of using an antireflux system propose that the high incidence of bacteriuria may lead to renal deterioration. However, the cited findings were generally based on occasional urine sampling, and studies meticulously investigating the rate of bacteriuria over time and its impact on reservoir function are lacking. By repeated monthly urine culture we found substantial bacterial colonization of the urinary tract. The high incidence of

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### Table 1

**Pattern of asymptomatic bacteriuria among 47 patients with orthotopic ileal neobladders**

<table>
<thead>
<tr>
<th>Pattern of urine culture</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistently sterile</td>
<td>8 (17)</td>
</tr>
<tr>
<td>Occasional bacteriuria:</td>
<td></td>
</tr>
<tr>
<td>same organism</td>
<td>20 (43)</td>
</tr>
<tr>
<td>different organisms</td>
<td>12 (26)</td>
</tr>
<tr>
<td>Persistent bacteriuria:</td>
<td></td>
</tr>
<tr>
<td>same organism</td>
<td>3 (6)</td>
</tr>
<tr>
<td>different organisms</td>
<td>4 (9)</td>
</tr>
</tbody>
</table>

### Table 2

**Antimicrobial susceptibility to cultured organisms**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Susceptibility, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>54</td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole</td>
<td>8.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18.6</td>
</tr>
<tr>
<td>Norfloxacir</td>
<td>25</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>91.9</td>
</tr>
<tr>
<td>Amoxicillin-calvulinic acid</td>
<td>57.3</td>
</tr>
<tr>
<td>Imipenem</td>
<td>100</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>50</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>40</td>
</tr>
<tr>
<td>Amikacin</td>
<td>98</td>
</tr>
</tbody>
</table>

### Table 3

**Correlation between bacteriuria and NE**

<table>
<thead>
<tr>
<th>Follow-up, months</th>
<th>Cultures</th>
<th>Bacteriuria, n (%)</th>
<th>Nocturnal continence, n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sterile 4</td>
<td>8</td>
<td>12 (25)</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 2</td>
<td>33</td>
<td>35 (75)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>sterile 8</td>
<td>12</td>
<td>20 (43)</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 3</td>
<td>24</td>
<td>27 (57)</td>
<td>0.033</td>
</tr>
<tr>
<td>3</td>
<td>sterile 12</td>
<td>9</td>
<td>21 (45)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 6</td>
<td>20</td>
<td>26 (55)</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>sterile 17</td>
<td>6</td>
<td>23 (49)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 5</td>
<td>19</td>
<td>24 (51)</td>
<td>0.001</td>
</tr>
<tr>
<td>5</td>
<td>sterile 20</td>
<td>8</td>
<td>28 (60)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 6</td>
<td>13</td>
<td>19 (40)</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>sterile 21</td>
<td>8</td>
<td>29 (64)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 5</td>
<td>11</td>
<td>16 (36)</td>
<td>0.001</td>
</tr>
<tr>
<td>12</td>
<td>sterile 18</td>
<td>6</td>
<td>24 (67)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 8</td>
<td>4</td>
<td>12 (33)</td>
<td>0.7</td>
</tr>
<tr>
<td>18</td>
<td>sterile 20</td>
<td>8</td>
<td>28 (93)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>bacteriuria 2</td>
<td>–</td>
<td>2 (7)</td>
<td>0.7</td>
</tr>
<tr>
<td>24</td>
<td>sterile 18</td>
<td>4</td>
<td>22 (100)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>bacteriuria –</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
asymptomatic bacteriuria during the first year after surgery is in agreement with the results of Iwakiri et al. [6]. E. coli was the most common organism isolated in the present cultures, which coincides with the results of Keegan et al. [11], who found that the E. coli strains isolated from the reservoirs were less likely to carry determinants of virulence, e.g. P and 5 fimbriae and toxins, compared to community-acquired E. coli strains.

Repeated cultures showed not only a spontaneous decrease in the incidence of bacteriuria, but also a spontaneous change in the organisms in 41% of patients with bacteriuria, with no antimicrobial manipulation. Reaching maturity both in the urodynamic characteristics of the reservoir, and the patients’ understanding of their new voiding pattern, might explain the steady decline in asymptomatic bacteriuria with time.

The reasons for the greater incidence of bacteriuria in ileal neobladders than in normal bladders and in those after radical prostatectomy remain a matter of controversy [6]. It has been hypothesized that the intestine is incapable of inhibiting bacterial proliferation, in contrast to urothelium. Thus intestine that normally exists in symbiosis with bacteria, with no inflammatory reaction, may render the urine less bacteriostatic, promoting bacterial growth and may thereby serve as a source of infection [12]. In contrast, Mansson et al. [13] showed that the chemical differences in reservoir urine did not promote bacterial growth compared with urine from an intact bladder. There was a spontaneously decreasing incidence of bacteriuria with time. The mechanism of this acquired defence with time is unknown. Studies investigating host-response variables in patients with a neobladder and bacteriuria showed no significant host reaction, indicating a ‘silent’ colonization rather than an active infection [14]. A high level of immunoglobulin in urine from patients with intestine incorporated into the urinary tract was reported [6] but the significance of this finding remains obscure. Terai et al. [15] reported high levels of secretory IgA in the urine of intestinal reservoirs, and concluded that long-term secretory IgA secretion in such reservoirs is an important host defence system.

Urinary continence after orthotopic substitution appears to improve with time, with most studies showing that patients reach the new baseline at 6–12 months after surgery [3]. Although daytime continence is generally satisfactory, NE remains a concern; it develops as a consequence of absent sensation that permits excessive nocturnal urine volumes to overcome the impaired continence mechanisms of the urethra. This situation is mainly caused by loss of the physiological storage reflexes after cystoprostatectomy [16]. Jakobsen et al. [17] reported a decrease of ≥20% in the urethral closure pressure during the deep stage of rapid-eye movement sleep. The definition of nocturnal continence used in the present study was complete dryness with no pad use or other methods of protection. We recommend that all patients should use regularly timed voiding soon after surgery. The present incidence of NE at 1 month after surgery was 87% and decreased consistently with time. Daytime continence was achieved by 98% of patients at 6 months and remained stable during the follow-up; these results are similar to those published previously [1–3].

The mean age of the present patients was 10 years less than that of a ‘standard’ cohort with a neobladder [2,3], possibly explaining the rapid regaining of continence in these younger patients, who had early development of bladder cancer associated with schistosomiasis in Egypt [18]. The effect of attempted nerve-sparing cystectomy on continence is confirmed by many investigators, as the preserved autonomic innervation probably contributes to pressure generation by the sphincter mechanism at rest [19,20]. The incidence of local pelvic recurrence after radical cystectomy and an orthotopic Kock pouch was ≈21% in a series of 353 men from our centre [21]. This high rate restricted the indication of nerve-sparing radical cystectomy to a highly selected group of patients in this series.

El-Bahnasawy et al. [22] reported that the incidence of bacteriuria was significantly higher among enuretics, but they did not assess the influence of treating such infection on the level of continence. Urodynamic assessment of patients with asymptomatic bacteriuria showed no correlation with any of the cystometric characteristics [23]. In the present study there was a significant correlation between bacteriuria and NE during the first 6 months after surgery. The dramatic decline in bacteriuria, from 75% at 1 month to 36% at 6 months, paralleling pouch maturation, could explain the lack of a significant correlation of NE with bacteriuria after 6 months. The decline in the number of patients from 47 to 22 over the 2-year study period might also contribute to the lack of a significant correlation after 6 months. However, these correlations could be coincidental and not a cause-and-effect relationship.

The relationship between bacteriuria and incontinence can be detected from the reciprocal effect of bacterial flora and intestinal motility. It is well known that decreased intestinal motility leads to bacterial overgrowth and subsequent bacterial translocation. However, bacterial overgrowth either suppresses or exaggerates motility, depending on the type of organism and/or its endotoxins [24]. Experimental data showed that applying enterotoxins of E. coli and some other species leads to an exaggerated migrating motor complex and acceleration of spontaneous motility [24]. Not only this effect, but these enterotoxins may lead to secretory fluid production. Both effects, if

![FIG. 2. Association between bacteriuria (red) and NE (green) during the 2 years after surgery. The number of patients assessed at 6, 12, 18 and 24 months was 45, 36, 30 and 22, respectively.](image-url)
occurring within intestinal reservoirs, will result in NE.

Moreover, the associated inflammatory effect of bacteria and/or its enterotoxins may also induce contractile changes even after the disappearance of the bacteria. This was documented by some, in that an inflammatory process involving the intestinal wall, whether clinically as in Crohn’s disease and pouchitis of the ileal reservoir after total colectomy, or experimentally, is known to be associated with changes in contractile response of enteric smooth muscles [25].

Whether treating asymptomatic bacteriuria is beneficial remains controversial. While some authors found that prophylactic antibiotics did not seem to reduce the bacterial burden [5–11], some gastroenterologists have reported that oral bacteriotherapy not only modified intestinal microflora but also clinically improved intestinal motility in irritable bowel syndrome [26].

Despite there being no data showing the benefit of prophylactic antibiotic therapy in patients with a reconstructed lower urinary tract, some authors advocate this regimen. Further studies are needed to elucidate the long-term effects of antimicrobial treatment on microbial ecology, and the clinical benefit of such treatment in patients with NE. Long-term studies on renal function in patients with an orthotopic neobladder should also be conducted to assess the possible effects of such asymptomatic bacteriuria in uncomplicated cases.

In conclusion, ileal neobladders are associated with a high incidence of asymptomatic bacteriuria during the first year after surgery. There was spontaneous clearance of bacteriuria with time, with no antimicrobial manipulation. Soon after surgery there was a significant association between bacteriuria and NE. The effect of antimicrobials in patients with orthotopic bladder substitution and NE should be assessed further.

CONFLICT OF INTEREST

None declared.

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Abbreviations: NE, nocturnal enuresis.
Vesicostomy revisited: the best treatment for the hostile bladder in myelodysplastic children?

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Accepted for publication 18 March 2005

OBJECTIVE
To evaluate the effects of vesicostomy on the urinary tract of myelodysplastic children in whom conservative bladder management with clean intermittent catheterization (CIC) has failed to preserve upper and lower urinary tract function.

PATIENTS AND METHODS
Sixteen children with myelodysplasia underwent vesicostomy. Indications included worsening hydronephrosis, vesico-ureteric reflux (VUR), recurrent urinary tract infections (UTIs), and increasing renal insufficiency despite CIC and/or difficulty with CIC. The mean (range) age at vesicostomy was 36.5 (9–82) months and the follow-up 7.4 (2–16) years.

RESULTS
Hydronephrosis resolved or improved in 12 of 14 children, the incidence of UTI decreased to one or fewer per year in 10, VUR resolved or improved in nine, and renal function improved or stabilized in six of seven patients. One patient initially presented with renal insufficiency and subsequently required dialysis despite vesicostomy. Complications occurred in three of 15 children, and included stomal stenosis and bladder calculi. The vesicostomy was closed in six patients after a mean of 4.4 (1.5–9) years. Four of these patients required concomitant bladder augmentation.

CONCLUSIONS
Vesicostomy in myelodysplastic children is effective in preventing and/or resolving the deleterious consequences of a ‘hostile’ bladder. The procedure is uncomplicated, well tolerated, reversible and should be considered in managing children in whom conservative management by CIC has failed.

KEYWORDS
vesicostomy, myelodysplasia, treatment outcome

INTRODUCTION
The goals of urological care in children with myelodysplasia are to prevent urinary tract deterioration and achieve continence at an appropriate age. With >90% of these children having neurological involvement of the bladder [1,2] early detection and proactive treatment can significantly decrease incapacitating bladder dysfunction and the future need for surgical intervention [3–5]. The primary bladder management in spina bifida is clean intermittent catheterization (CIC). The initiation and use of CIC has been shown to decrease or eliminate hydronephrosis and upper tract damage in...
>80% of patients with myelodysplasia [6]. McGuire et al. [7] reported that high detrusor storage or voiding pressures and low bladder compliance may put the upper tracts at risk in myelodysplastic children. Hence, the aim of long-term bladder management in these patients is to maintain low storage pressures and efficient bladder emptying. Older children refractory to this treatment may additionally require augmentation cystoplasty to protect the urinary tract. Younger patients in whom conservative bladder management fails may undergo vesicostomy to maintain low-pressure drainage. It is often temporary and reversed later in life, when the child can be managed with anticholinergics and CIC with or without bladder augmentation. We retrospectively reviewed a cohort of patients with spina bifida who had a vesicostomy after failed conservative management.

PATIENTS AND METHODS

The institutional review board approved this retrospective study. A review of 380 patients with myelodysplasia seen from 1988 to 2002 identified 16 children (10 girls and six boys) treated with vesicostomy. Indications included worsening hydronephrosis, recurrent UTI, VUR, impaired renal function, noncompliance with CIC, and urethral false passage (Table 1). Impaired renal function was defined as a serum creatinine level of >125 μmol/L or unilateral renal function of <35% on a nuclear scan. All patients had more than one indication for vesicostomy. The procedure was carried out according to the Blocksom technique, which emphasizes creating the vesicostomy from the bladder dome to minimize the risk of prolapse [8]. Conservative bladder management with CIC, anticholinergics and prophylactic antibiotics had failed in all patients. Prophylactic antibiotics were given to all patients with VUR and/or recurrent UTI. After vesicostomy, antibiotics were discontinued when imaging studies showed resolution of VUR, and in patients with recurrent UTI after an infection-free period of ≥6 months.

The upper tracts were followed with periodic renal ultrasonography and serum creatinine measurements. Renal nuclear scans were obtained in children with high-grade VUR, evidence of renal scarring or renal size discrepancy on ultrasonography. Yearly fluoro-urodynamics were used to determine vesico-urethral function and the degree, if any, of VUR. When required, vesicostomies were occluded using a Foley catheter balloon to facilitate urodynamic studies. Urodynamics were performed with an infusion rate of 8–20 mL/min. An improvement in the incidence of UTI was subjectively defined as a decrease from four or more to one or fewer per year.

RESULTS

Ten girls and six boys (mean age at vesicostomy 36.5 months, range 9–82) were identified. The operative time was <1 h for each case; the mean (range) follow-up was 7.4 (2–18) years. Vesicostomy provided overall resolution, improvement or stabilization of the preoperative indications in 14 of 15 patients. One patient with recurrent UTIs and hydronephrosis died from unrelated causes 2 years after a successful vesicostomy. Although the patient’s indications for vesicostomy resolved, he was excluded from the analysis of outcome because of the limited follow-up.

Of the 14 patients with hydronephrosis, eight achieved complete resolution, four improved and two stabilized. Ten of 14 patients with recurrent UTIs improved after vesicostomy to one or fewer UTI per year. Four patients are currently maintained on suppressive antibiotics for recurrent infections. Of the 11 patients with VUR, five resolved after vesicostomy while four had a significant improvement. Two of the four patients with minimal residual VUR are maintained on suppressive antibiotics. Despite resolution of upper tract dilation, high-grade reflux continued in two patients, necessitating ureteric reimplantation at the time of vesicostomy reversal.

Six of the seven patients with impaired renal function before surgery had improved or stabilized serum creatinine levels after vesicostomy. One patient with long-standing hydronephrosis and renal insufficiency progressed to end-stage renal failure after vesicostomy.

Of the four patients with an indication for vesicostomy being either noncompliance or difficulty with CIC, one has had the vesicostomy closed and learned self-catheterization.

The incidence of complications was low and included peristomal dermatitis, bladder calculi and stomal stenosis, easily managed with dilatation (Table 1). Bladder prolapse did not occur in any patient. No child developed upper tract calculi.

Six patients had the vesicostomy closed at a mean (range) of 4.4 (1.5–9) years after diversion. Concomitant procedures at the time of closure included ureteric reimplantation in two patients for continued high-grade reflux, augmentation cystoplasty in four for small bladder capacity and cystolitholapaxy in one. Two children who did not require augmentation were found to have a bladder capacity of >300 mL and normal compliance during urodynamic studies. With a mean of 9.5 years after vesicostomy closure no patient has had recurrent VUR, worsening hydronephrosis or frequent UTIs.

To date, nine patients have not had the vesicostomy closed; the mean time since placing the vesicostomy was 7.2 (2–16) years. Four patients did not have the social support necessary for adequate bladder management. Three patients have been lost to follow-up and two are due to have the vesicostomy closed.

DISCUSSION

Early proactive treatment of the high-pressure bladder with CIC and anticholinergics significantly decreases the incidence of poor bladder compliance, upper tract deterioration and the subsequent need for surgery [3–5]. In the subset of patients with myelomeningocele refractory to medical
management, surgery may be the only option to prevent continued renal deterioration. Since its introduction in children by Duckett [9] and Michie et al. [10] vesicostomy has been shown to reverse upper tract dilatation associated with neurogenic bladder dysfunction [11–16]. Mandell et al. [15] used vesicostomy in 10 infants with neurogenic bladder dysfunction, with resulting improvements of the upper urinary tract in all patients; however, the median follow-up was short, at only 24 months. Bruce et al. [16] reported on vesicostomy in 24 children with hostile bladders. After a follow-up of <2 years, the results were successful in 23 patients, while only one went on to require suprapical diversion.

In the present series, the long-term results in 15 high-risk children with spina bifida show that vesicostomy is effective. There was an improvement in the upper urinary tract in 13 patients, and the complications after vesicostomy were minor, with four of 15 patients developing peristomal dermatitis, each easily and successfully managed with topical therapy. One boy formed bladder calculi and had litholapaxy at the time the vesicostomy was taken down and the bladder augmented. Also, one patient developed mild stomal stenosis that was treated with intermittent dilatation. With a mean follow-up of >7 years, no patient had a stomal prolapse. The present complication rate is similar or better than in other published studies. With a mean follow-up of 22 months, Bruce and Gonzalez [16] reported a 17% complication rate, mainly consisting of stomal stenosis. The authors did not comment on peristomal skin excoriation. Noe et al. [11] reported complications in 15 of 35 (43%) patients after a Blocksom vesicostomy, including recurrent UTI in six and significant bladder prolapse in two. In addition, although not considered a complication by the authors, nearly a third of patients were treated for peristomal reactions and dermatitis.

To date, only one other study has examined the long-term outcomes of vesicostomy in patients with myelomeningocele; Hutcheson et al. [17] published the 13-year results of 18 patients treated with vesicostomy at a mean of 2.6 years old. Similar to the present findings, indications for vesicostomy were corrected or improved in 89% of patients. Complications included stomal stenosis, temporary ureteric obstruction and peristomal skin excoriation. Furthermore, eight patients (44%) formed upper or lower urinary tract calculi and two (11%) reported recurrent pyelonephritis. In the present study, one child had a bladder stone while no patients developed upper tract calculi or recurrent pyelonephritis. It is possible that the prophylactic use of antibiotics in selected patients in the present cohort may have diminished the incidence of infection and stone formation.

Four patients had concomitant procedures at the time of vesicostomy closure. One patient with a diminished bladder capacity had bladder augmentation. Augmentation cystoplasty and bilateral ureteric reimplantation was used in two children with small-capacity bladders and persistent VUR. One boy with a bladder calculus had cystolitholapaxy and augmentation at the time of closure. There were no complications from any additional procedure. No patients had recurrent VUR, hydrenephrosis or UTIs after vesicostomy closure.

Nine patients, with a mean duration of >7 years since placing the vesicostomy, have not had it closed. Complications in this group were minimal and included peristomal dermatitis in three and stomal stenosis in one. The reasons for prolonged vesicostomy are varied; most include lack of social support to allow for adequate bladder management after reversal.

In conclusion, the long-term outcomes of vesicostomy in myelodysplastic patients are effective in reversing the deleterious consequences of a hostile bladder when conservative treatments fail. Vesicostomy obviates the need for more invasive procedures and effectively postpones definitive therapy in patients who are not yet suitable for lower urinary tract reconstruction. The procedure is uncomplicated, well tolerated, easily reversible and should be strongly considered in myelodysplastic patients in whom conservative therapy fails.

CONFLICT OF INTEREST
None declared.

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Abbreviations: CIC, clean intermittent catheterization.
Inguinal hernia in female infants: a cue to check the sex chromosomes?

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Accepted for publication 30 March 2005

INTRODUCTION
Androgen insensitivity syndrome (AIS) is one cause of XY sex reversal; it arises from target tissue resistance to the actions of androgens due to molecular abnormalities in the androgen receptor. Patients with CAIS are born with normal external female genitalia, and although inguinal hernias are uncommon in female infants, they are a well-known presentation of CAIS. Such patients were identified from the Cambridge Intersex Database and details of presentation, presence and laterality of inguinal hernia and contents, and family history of CAIS, were recorded. A questionnaire detailing different indications for considering CAIS in female infants with a hernia was distributed to members of the British Association of Paediatric Surgeons and the British Society for Paediatric Endocrinology and Diabetes.

RESULTS
More than half of patients with CAIS presented with inguinal hernia, of which half were bilateral and a third contained gonads. Completed questionnaires were returned by 87 surgeons and 64 endocrinologists, and most of the surgeons and endocrinologists would consider CAIS in all female infants with a hernia. Bilateral hernias, hernias containing gonads and a family history of CAIS would prompt clinicians to consider the diagnosis.

CONCLUSION
Most clinicians agreed that CAIS should be considered in all female infants with inguinal hernia, as this is the commonest mode of presentation in childhood. Macroscopic inspection of the internal genital structures coupled, perhaps, with gonadal biopsy is recommended. Fluorescence in situ hybridization offers a rapid and reliable method to check the sex chromosomes. Liaison between the paediatric surgeon and endocrinologist is essential in management of infants with CAIS.

KEYWORDS
inguinal hernia, androgen insensitivity syndrome, female
RESULTS

Analysis of the Cambridge Intersex Database identified 120 cases of CAIS (Fig. 1). The median (range) age at presentation was 11 (0.4–64) years; 57% presented with inguinal hernia, half had bilateral hernias, and equal proportions were right or left-sided. Gonads were palpable in the hernial sacs in a third of cases. There was a family history of CAIS in 85% of cases. In 3% of cases the diagnosis was established antenatally because of the need to perform a karyotype for unrelated reasons.

Completed questionnaires were returned by 87 of 102 surgeons approached (85%) and by 64 of 89 (72%) endocrinologists, from 28 endocrine centres throughout the UK. Most of the surgeons (62%) and endocrinologists (80%) would consider the diagnosis of CAIS in any female infant presenting with inguinal hernia. Bilateral hernias were considered an indication to consider the diagnosis of CAIS by nine surgeons and eight endocrinologists; 12 surgeons and four endocrinologists chose the ‘palpable gonads’ option, and 16 surgeons and eight endocrinologists were encouraged to consider the diagnosis in the presence of a family history of CAIS. No surgeon or endocrinologist chose the option of a family history of inguinal hernia in a sibling or a cousin (Table 1).

Two surgeons were opposed to considering CAIS in girls presenting with inguinal hernia because of the low incidence of the association with inguinal hernia, and four surgeons commented that inspecting the gonads and the internal genitalia was a very useful guide to further management.

DISCUSSION

The possibility that female infants presenting with inguinal hernia might have CAIS has been the subject of several studies. Of 17 prepubertal girls admitted for inguinal herniotomy, two had an XY karyotype [11]. The inguinal masses were biopsied and this confirmed the presence of testicular tissue. In a larger series of 124 infants and children with inguinal hernias, three were found to have CAIS [12]. A similar incidence was reported in other comparable series of patients [13,14]. In a prospective study of 32 girls admitted for hernia repair, all had a 46XX karyotype [15].

In the present study, most surgeons reported that they consider CAIS in all girls presenting with inguinal hernia. This view was strengthened if the hernia was bilateral, contained gonads or there was a family history of CAIS. The Cambridge Intersex Database contained an equal incidence of unilateral and bilateral hernias and only a third of CAIS patients had hernias containing gonads. There was also a positive family history in this group of patients, which would certainly influence the decision to investigate. However, several instances were recorded where an older relative had presented with inguinal hernia and the diagnosis not made until this was established in the index case. Examining the contents of the hernial sac might not readily distinguish a testis from an ovary in infancy and biopsy has been recommended before hernia repair proceeds [16].

Burge et al. [10] surveyed 32 surgeons in the UK and Ireland to determine if they excluded CAIS in girls with inguinal hernia. In contrast to the present study, 41% did not investigate for CAIS because of a low incidence of an association with inguinal hernia. Nevertheless, the authors believed that this practice should change and there appears to be a consensus from the present study that a diagnosis of CAIS and related conditions should be considered in female infants presenting with inguinal hernia.

The approach will depend on available facilities and expertise. In some centres, obtaining a preliminary karyotype result by fluorescence in situ hybridisation is possible within 24 h of sample collection, thus minimizing parental anxiety whilst awaiting a full karyotype result. An experienced ultrasonographer can provide precise details on the location and morphological nature of inguinal masses in infants. Inspection of the gonads at surgery should determine their nature. The Fallopian tube and ovary are found in 15–20% of sliding hernias in girls [13]. This might be sufficient to definitively exclude the presence of a testis but many surgeons will biopsy the gonad and perhaps

<table>
<thead>
<tr>
<th>TABLE 1 Response to the questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAIS should be considered in: options</td>
</tr>
<tr>
<td>All female infants presenting with an inguinal hernia</td>
</tr>
<tr>
<td>Only in female infants with bilateral inguinal hernias</td>
</tr>
<tr>
<td>Only for inguinal hernias containing gonads</td>
</tr>
<tr>
<td>In female infants with a family history of inguinal hernias (female sibling/cousin)</td>
</tr>
<tr>
<td>In infants with a family history of CAIS (female sibling/cousin)</td>
</tr>
<tr>
<td>Only in female infants with bilateral inguinal hernias</td>
</tr>
<tr>
<td>Only for inguinal hernias containing gonads</td>
</tr>
<tr>
<td>Other options chosen by surgeons:</td>
</tr>
<tr>
<td>Inspection of gonads and internal genitalia</td>
</tr>
<tr>
<td>CAIS diagnosis not worth considering as association with hernia presentation is low</td>
</tr>
</tbody>
</table>

FIG. 1. The mode of clinical presentation of CAIS. IH, inguinal hernia; FH, positive family history of CAIS; PA, primary amenorrhoea; AD, antenatal diagnosis. Percentages of total number in each category are shown on the bars.
explore the contralateral side. It is essential that gonads should not be removed until full discussion has taken place with the family and appropriate investigations to establish a diagnosis have been completed.

An inguinal hernia is the commonest presentation of CAIS in childhood and there is now a firm opinion, amongst paediatric surgeons and endocrinologists, that the diagnosis of CAIS and other causes of complete XY sex reversal should be considered in all female infants with inguinal swellings. Accordingly, investigating such infants to exclude CAIS is probably justifiable, although the approach chosen should be decided by the clinician, based on individual circumstances and available expertise.

ACKNOWLEDGEMENTS

The participation of members of the British Association of Paediatric Surgeons and the British Society of Paediatric Endocrinology and Diabetes is gratefully acknowledged.

CONFLICT OF INTEREST

None declared.

REFERENCES


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Abbreviations: (C)AIS, (complete) androgen insensitivity syndrome.
Nocturnal enuresis at 7.5 years old: prevalence and analysis of clinical signs

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OBJECTIVE
To determine the prevalence of nocturnal enuresis (NE) in a large cohort of children at 7.5 years old, and to examine the frequency of variables such as gender, severity, associated elimination problems, and clinical signs within the identified group.

SUBJECTS AND METHODS
Of an original cohort of 13 971 infants alive at 12 months, 11 251 who were still active in the Avon Longitudinal Study of Parents and Children (ALSPAC) survey, were followed at 91 months. The mother or main carer was given a questionnaire which asked, amongst other items, about the presence and frequency of bedwetting, other elimination problems, and signs related to the wetting behaviour; 8269 (73.5%) questionnaires were returned and 8151 contained information on the frequency of bedwetting.

RESULTS
In all, 1260 children (15.5%) at 7.5 years wet the bed, but most wet once or less a week, and only 215 (2.6%) met the Diagnostic and Statistical Manual of Mental Disorders (fourth edition) criteria of NE (wetting at least twice a week). A higher prevalence was reported in boys than girls and 266 children (3.3%) had both daytime wetting and bedwetting, with 189 (2.3%) having both daytime soiling and bedwetting. Daytime urgency increased with severity of bedwetting and occurred in 28.9% of children with NE.

CONCLUSION
At 7.5 years old the incidence of bedwetting is high, but only 2.6% of this large population-based sample wet at a frequency meeting the definition of NE. Although a small percentage of children had both daytime wetting and bedwetting, the evidence suggests that these are discrete problems. Amongst children with NE, indicators of bladder overactivity were present, supporting the view of heterogeneity and the importance of individual assessment in deciding on appropriate treatment.

KEYWORDS
nocturnal enuresis, prevalence, children
common than monosymptomatic NE. The categorization between NE that is monosymptomatic or not has recently emerged as important, based on the absence or presence of bladder dysfunction [21,22]. Monosymptomatic enuresis refers to children who report no bladder or voiding problems associated with their wetting, whereas non-monosymptomatic enuresis refers to bedwetting associated with bladder overactivity and voiding problems. This classification is important in considering the most appropriate treatment, and forms a fundamental tenet of the ‘three-systems’ model advanced by Butler and Holland [22], which proposes that NE arises from: (i) bladder overactivity and/or (ii) a lack of nocturnal arginine vasopressin release, leading to polyuria; and (iii) an inability to wake from sleep to bladder sensations. Although using different terminology, Neveus [23] also proposed that three causative factors underpin NE.

It was suggested that each system can be identified through the presence of clinical signs [22]. Bladder overactivity can be identified by frequent daytime voiding (>7 voids/day); a sense of urgency; low or variable functional bladder capacity; small voided volumes; variability in the size of the wet patch; and waking during or immediately after wetting [24–26]. Wetting soon after going to sleep and large wet patches might indicate a lack of vasopressin release [25,27].

The aim of the present study was to determine the prevalence of NE in a large cohort of children at 7.5 years old, and to examine the frequency of variables such as gender, severity, associated elimination problems and clinical signs within the identified group.

### SUBJECTS AND METHODS

The Avon Longitudinal Study of Parents and Children (ALSPAC) [28] is an ongoing population-based study investigating a wide range of environmental and other influences on the health and development of children. Pregnant women resident in the former Avon Health Authority in South-west England, having an estimated date of delivery between 1 April 1991 and 31 December 1992, were invited to take part, resulting in a cohort of 14 541 pregnancies, of which 13 971 children were alive at 12 months old. The primary source of data collection was by self-completed questionnaires administered during pregnancy (8, 18 and 32 weeks of gestation) and at various ages of the child. More detailed information on the ALSPAC study is available at http://www.alspac.bris.ac.uk.

Of the original cohort, 11 251 (80%) remained active in the study at 91 months old. When the child reached this age, all mothers or main carers were sent a questionnaire and invited to complete several items, amongst which were questions about the child’s night-time wetting, daytime wetting and soiling. The carer was asked to indicate how often the child: (i) soiled his/her pants during the day; (ii) soiled his/her pants at night; (iii) wet his/ herself during the day; (iv) wet the bed at night. The following options were given: (a) never; (b) occasional accidents – less than once a week; (c) about once a week; (d) 2–5 times a week; (e) nearly every day; (f) more than once a day. The mothers were further asked to identify whether their child: (i) woke soon after wetting; (ii) seemed upset when going to sleep; (iii) seemed upset when the bed was wet, with the frequency options of (a) never; (b) sometimes; (c) often; or (d) always.

The representative nature of the ALSPAC sample has been investigated by comparison with the 1991 National Census data of mothers with infants aged <1 year who were resident in the county of Avon. The ALSPAC sample had a slightly greater proportion of mothers who were married or cohabiting, who were owner-occupiers and who had a car in the household. The study had a smaller proportion of ethnic minority mothers. Ethical
approval for the study was obtained from the ALSPAC Ethics Committee and the Local Ethics Committees of United Bristol and North Bristol Trusts (formerly Southmead and Frenchay Health Care Trusts).

RESULTS

In all, 8269 questionnaires were returned at 91 months (response rate 73.5%), of which 8151 responded to the question on the frequency of bedwetting. Of those responding, there was a shortfall in the more socially disadvantaged groups (maternal education level; housing tenure; maternal social class and maternal age; chi-square P < 0.001) than in those not responding. However, there were no significant differences evident in these factors and the bedwetting variables (multiple wetting; NE; infrequent bedwetting; no bedwetting). Despite the sample attrition, there appeared to be no relation between socio-economic status and bedwetting, and so the sample estimates are considered as representative of the general population.

At 7.5 years old, 1260 children (15.5%) wet the bed, most of whom (978; 12%) wet 'less than once a week' and 67 (0.8%) wet 'once a week', thus 1045 (82.9%) of bedwetting children wet 'at most once a week'. The remaining 215 children (2.6%) wet at a frequency meeting the DSM IV definition of NE (at least twice a week); 112 (1.4%) wet 2–5 times a week, 84 (1.0%) nearly every night, and 19 (0.2%) 'more than once a night'.

Table 2 shows a higher rate of bedwetting in boys (20.2%) than in girls (10.5%), and when frequency of bedwetting 'at least twice/week' is considered, the relative difference between boys and girls is even greater, at 150 boys (3.6%) vs 65 girls (1.6%).

For further analysis, bedwetting was categorized according to clinical usefulness as follows: (i) multiple bedwetting (at least once a night); (ii) frequent bedwetting (in line with the definition of NE; at least twice a week); (iii) infrequent bedwetting (no more than once a week); (iv) not bedwetting (never wet the bed)

As shown in Table 2, 990 children (12.1%) had isolated bedwetting (bedwetting with no daytime wetting), whilst 358 (4.4%) had isolated daytime wetting. There was some daytime wetting in 624 children (7.7%), mostly 'infrequent' (no more than once a week) but 79 (0.9%) had 'severe' daytime wetting. Only 266 (3.3%) children had both daytime wetting and bedwetting, of whom only a very few (0.2%) had both 'frequent/multiple' bedwetting and 'frequent/multiple' daytime wetting.

Daytime soiling was reported in 553 children (6.8%), mostly 'infrequent' (no more than once a week), with 66 (0.8%) having severe daytime soiling. Only 189 children (2.3%) had both daytime soiling and bedwetting, while 364 (4.5%) of the children had daytime soiling and no bedwetting (Table 2). Night-time soiling was rare; only 66 children (0.8%) had night-time soiling, and this was mostly 'infrequent' (no more than once a week).

Nearly 80% of non-bed wetting children did not wake to void (Table 2). Just over 40% of 'frequent' and 'infrequent' bedwetting children woke to void during the night, some more than once. During the day, 60 children (10.6%) at 7 years of age still required a reminder to toilet (Table 3) and children with NE were far more likely to need a reminder than children who were dry at night (P < 0.001).

The need to hurry to the toilet to pass urine in the day (urgency) increased with the severity of bedwetting (Table 3; P < 0.001); 28.9% of children with NE showed urgency. In terms of daytime urinary frequency, children with no NE very rarely voided as often as 10 times/day (Table 3), whereas the proportion of children

### Table 2 Severity of bedwetting related to gender, daytime wetting, soiling and arousability to void

<table>
<thead>
<tr>
<th>Bedwetting severity</th>
<th>1 (multiple)</th>
<th>2 (frequent)</th>
<th>3 (infrequent)</th>
<th>4 (never)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bedwetting, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boy</td>
<td>13 (0.3)</td>
<td>173 (7.0)</td>
<td>491 (19.7)</td>
<td>1315 (95.0)</td>
<td>1515</td>
</tr>
<tr>
<td>Girl</td>
<td>6 (0.2)</td>
<td>59 (1.5)</td>
<td>351 (9.3)</td>
<td>1327 (39.5)</td>
<td>1520</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19 (0.2)</td>
<td>232 (2.4)</td>
<td>842 (9.5)</td>
<td>1642 (84.5)</td>
<td>2007</td>
</tr>
<tr>
<td><strong>Daytime wetting, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple (&gt;1/day)</td>
<td>4 (21.1)</td>
<td>5 (2.6)</td>
<td>0</td>
<td>2 (0)</td>
<td>11</td>
</tr>
<tr>
<td>Frequent (≥2/week)</td>
<td>3 (15.8)</td>
<td>11 (5.7)</td>
<td>23 (2.2)</td>
<td>31 (0.4)</td>
<td>68</td>
</tr>
<tr>
<td>Infrequent (≤1/week)</td>
<td>5 (26.3)</td>
<td>36 (18.4)</td>
<td>179 (17.2)</td>
<td>325 (4.7)</td>
<td>545</td>
</tr>
<tr>
<td>No daytime wetting</td>
<td>7 (36.8)</td>
<td>144 (73.5)</td>
<td>839 (80.6)</td>
<td>6522 (94.8)</td>
<td>7512</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19 (0.2)</td>
<td>196 (2.4)</td>
<td>1045 (12.8)</td>
<td>6885 (84.5)</td>
<td>8145</td>
</tr>
<tr>
<td><strong>Soiling, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple (&gt;1/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>1 (5.3)</td>
<td>2 (1)</td>
<td>0</td>
<td>2 (0)</td>
<td>7</td>
</tr>
<tr>
<td>Night</td>
<td>1 (5.3)</td>
<td>2 (1)</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Frequent (≥2/week)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>1 (5.3)</td>
<td>9 (4.6)</td>
<td>13 (1.3)</td>
<td>36 (0.5)</td>
<td>59</td>
</tr>
<tr>
<td>Night</td>
<td>0</td>
<td>0</td>
<td>2 (0.2)</td>
<td>7 (0.1)</td>
<td>9</td>
</tr>
<tr>
<td>Infrequent (≤1/week)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>6 (31.6)</td>
<td>19 (9.7)</td>
<td>136 (13.1)</td>
<td>326 (4.8)</td>
<td>487</td>
</tr>
<tr>
<td>Night</td>
<td>2 (10.6)</td>
<td>6 (3.1)</td>
<td>27 (2.6)</td>
<td>19 (0.3)</td>
<td>54</td>
</tr>
<tr>
<td>No soiling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>11 (57.9)</td>
<td>165 (84.6)</td>
<td>891 (85.5)</td>
<td>6521 (94.7)</td>
<td>7588</td>
</tr>
<tr>
<td>Night</td>
<td>16 (84.2)</td>
<td>185 (95.9)</td>
<td>1012 (97.2)</td>
<td>6855 (99.6)</td>
<td>8068</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19 (0.2)</td>
<td>195 (1.0)</td>
<td>1042 (1.3)</td>
<td>6885 (1.1)</td>
<td>8141</td>
</tr>
<tr>
<td><strong>Arousability to void, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never wakes</td>
<td>11 (73.3)</td>
<td>103 (53.6)</td>
<td>586 (56.9)</td>
<td>5433 (79.7)</td>
<td>6133</td>
</tr>
<tr>
<td>Wakes once/night</td>
<td>0</td>
<td>57 (29.7)</td>
<td>384 (37.3)</td>
<td>1190 (17.5)</td>
<td>1631</td>
</tr>
<tr>
<td>Wakes twice/night</td>
<td>2 (13.3)</td>
<td>16 (8.3)</td>
<td>33 (3.2)</td>
<td>54 (0.8)</td>
<td>105</td>
</tr>
<tr>
<td>Wakes ≥3 times/night</td>
<td>1 (6.7)</td>
<td>6 (3.1)</td>
<td>2 (0.2)</td>
<td>12 (0.2)</td>
<td>21</td>
</tr>
<tr>
<td>Don’t know</td>
<td>1 (6.7)</td>
<td>10 (5.2)</td>
<td>25 (2.4)</td>
<td>127 (1.9)</td>
<td>163</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15 (0.2)</td>
<td>192 (10.3)</td>
<td>1030 (10.3)</td>
<td>6816 (68.5)</td>
<td>8053</td>
</tr>
</tbody>
</table>
with >10 voids/day increased with wetting severity (P < 0.001).

Children with multiple night-time wetting rarely woke after wetting, but 21.4% of children with NE and 58.8% of children with infrequent bedwetting tended to wake ‘often’ or ‘always’ after wetting (Table 4; P < 0.001). Rates for children wetting ‘soon after sleep’ are shown in Table 4. Of children with NE, 57.9% ‘sometimes’, ‘often’ or ‘always’ wet soon after sleep, unlike children with infrequent bedwetting, who tended not to wet soon after sleep.

DISCUSSION

Epidemiological surveys of NE have been undertaken in various parts of the world. Many of these have adopted a cross-sectional methodology and use varying definitions of what constitutes NE, and for any given age, they typically include <1000 individuals. The present study, part of a longitudinal survey of all children born over a 21-month period in the County of Avon, UK, in 1991–92, selecting 7.5 years as a clinically appropriate age, provided >8000 responses. This is the largest epidemiological sample of bedwetting reported since the national cohort of 1958.

Three studies have reported prevalence rates based on two criteria for NE, with predictably disparate results. Verhulst et al. [4] compared the (WHO International Classification of Diseases) ICD-10 definition of bed wetting ‘at least once a month’ [29] with wetting ‘more than twice a month’; Hellstrom et al. [8] examined rates at ‘more than once in 3 months’ and ‘more than once a week’; whilst Chiozza et al. [15] compared DSM III (wetting ‘at least once a month’) with DSM IV (wetting ‘at least twice a week’). The present study examined the prevalence in relation to three clinically appropriate divisions: multiple

### TABLE 3

Severity of bedwetting related to the frequency the child voids during the day without a reminder, daytime urgency and daytime voiding frequency

<table>
<thead>
<tr>
<th>Bedwetting severity</th>
<th>1 (multiple)</th>
<th>2 (frequent)</th>
<th>3 (infrequent)</th>
<th>4 (never)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime voids without a reminder, n (%)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never goes without a reminder</td>
<td>1 (5.3)</td>
<td>16 (8.2)</td>
<td>76 (7.3)</td>
<td>767 (11.2)</td>
<td>860</td>
</tr>
<tr>
<td>Sometimes goes without a reminder</td>
<td>7 (36.8)</td>
<td>32 (16.4)</td>
<td>62 (6.0)</td>
<td>232 (3.4)</td>
<td>333</td>
</tr>
<tr>
<td>Often goes without a reminder</td>
<td>5 (26.3)</td>
<td>49 (25.1)</td>
<td>275 (26.4)</td>
<td>842 (12.3)</td>
<td>1171</td>
</tr>
<tr>
<td>Always goes without a reminder</td>
<td>6 (31.6)</td>
<td>98 (50.3)</td>
<td>629 (60.4)</td>
<td>5007 (73.1)</td>
<td>5740</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>195</td>
<td>1042</td>
<td>6848</td>
<td>8104</td>
</tr>
<tr>
<td>Daytime urgency, n (%)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dash to toilet</td>
<td>10 (55.6)</td>
<td>56 (28.9)</td>
<td>177 (17.1)</td>
<td>474 (6.9)</td>
<td>717</td>
</tr>
<tr>
<td>Can hold for &lt;5 min</td>
<td>2 (11.1)</td>
<td>65 (33.5)</td>
<td>309 (29.8)</td>
<td>1407 (20.6)</td>
<td>1783</td>
</tr>
<tr>
<td>Can hold for &gt;5 min</td>
<td>6 (33.3)</td>
<td>73 (37.7)</td>
<td>550 (53.1)</td>
<td>4954 (72.5)</td>
<td>5583</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>194</td>
<td>1036</td>
<td>6835</td>
<td>8083</td>
</tr>
<tr>
<td>Daytime frequency, n (%)‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voids &lt;5/day</td>
<td>2 (11.1)</td>
<td>48 (24.6)</td>
<td>306 (29.7)</td>
<td>2716 (39.9)</td>
<td>3072</td>
</tr>
<tr>
<td>Voids 5–9/day</td>
<td>10 (55.6)</td>
<td>101 (51.8)</td>
<td>488 (47.4)</td>
<td>2641 (38.8)</td>
<td>3240</td>
</tr>
<tr>
<td>Voids ≥10/day</td>
<td>3 (16.7)</td>
<td>11 (5.6)</td>
<td>23 (2.2)</td>
<td>72 (1.1)</td>
<td>109</td>
</tr>
<tr>
<td>Don’t know</td>
<td>3 (16.7)</td>
<td>35 (17.9)</td>
<td>213 (20.7)</td>
<td>1378 (20.2)</td>
<td>1629</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>194</td>
<td>1030</td>
<td>6807</td>
<td>8050</td>
</tr>
</tbody>
</table>

Chi-square tests (value, degrees of freedom, two-sided asymptotic significance): *Pearson (332.260, 9, <0.001); likelihood ratio (251.664, 9, <0.001); linear-by-linear association (24.892, 1, <0.001); †Pearson (367.817, 6, <0.001); likelihood ratio (301.447, 6, <0.001); linear-by-linear association (344.504, 1, <0.001); ‡Pearson (130.224, 9, <0.001); likelihood ratio (99.149, 9, <0.001); linear-by-linear association (1.333, 1, 0.248).

### TABLE 4

Severity of bedwetting related to waking after wetting and wetting soon after sleep

<table>
<thead>
<tr>
<th>Bedwetting severity</th>
<th>1 (multiple)</th>
<th>2 (frequent)</th>
<th>3 (infrequent)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wakes after wetting, n (%)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>14 (82.4)</td>
<td>100 (52.1)</td>
<td>164 (16.4)</td>
<td>278</td>
</tr>
<tr>
<td>Sometimes</td>
<td>2 (11.8)</td>
<td>51 (26.6)</td>
<td>250 (24.9)</td>
<td>303</td>
</tr>
<tr>
<td>Often</td>
<td>0</td>
<td>19 (9.9)</td>
<td>98 (9.8)</td>
<td>117</td>
</tr>
<tr>
<td>Always</td>
<td>1 (5.9)</td>
<td>22 (11.5)</td>
<td>491 (49.0)</td>
<td>514</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>192</td>
<td>1003</td>
<td>1212</td>
</tr>
<tr>
<td>Wets soon after sleep, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>4 (26.7)</td>
<td>73 (42.2)</td>
<td>766 (85.6)</td>
<td>843</td>
</tr>
<tr>
<td>Sometimes</td>
<td>5 (33.3)</td>
<td>84 (48.6)</td>
<td>116 (13.0)</td>
<td>205</td>
</tr>
<tr>
<td>Often</td>
<td>2 (13.3)</td>
<td>15 (8.7)</td>
<td>6 (0.7)</td>
<td>23</td>
</tr>
<tr>
<td>Always</td>
<td>4 (26.7)</td>
<td>1 (0.6)</td>
<td>7 (0.8)</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>173</td>
<td>895</td>
<td>1083</td>
</tr>
</tbody>
</table>

Chi-square tests (value, degrees of freedom, two-sided asymptotic significance): *Pearson (178.135, 6, <0.001); likelihood ratio (173.148, 6, <0.001); linear-by-linear association (150.539, 1, <0.001).

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wetting (‘more than once a night’); NE defined in terms of DSM IV criteria (wetting ‘at least twice a week’); and infrequent bedwetting (‘at most once a week’).

In the Avon study, at 7 years old the prevalence of bedwetting (at any frequency) was 15.5%. Whilst caution is required in comparison with other surveys, because of different methods, this rate is in accord with other European countries. Verhulst et al. [4] found a prevalence rate of 15.3% for boys in Holland; Devlin [9] reported 13–17% in the Republic of Ireland, and Serel et al. [14] found a prevalence of 15.1% in Turkey. However, such rates are noticeably higher than those reported in northern Europe, with Jarvelin et al. [7] reporting a prevalence of 6.4% in Finland, and Hellström et al. [8] reporting a prevalence of 11.9% for boys and 7.7% for girls in Sweden.

The prevalence of NE (using the DSM IV definition) in the Avon study was much lower, the rate in this survey being 2.6%, corresponding in general with Chiozza et al. [15], who found wetting ‘twice a week’ in 3% of boys and 1.5% of girls. Although bedwetting is reported to have a fairly high prevalence rate, the proportion of children matching DSM IV criteria is substantially smaller. In Europe, 2–3% of children at 7 years old might be expected to be bedwetting at a severity to be defined as NE. However, severity is not the sole determining factor in a clinical context; both DSM IV [1] and Butler [2] advocate the presence of accompanying distress, concern and potential psychological impact as important in determining both definition and clinical appropriateness.

Only a minority of children wet every night or nearly every night; including those who wet ‘more than once a night’, only 1.2% of the total population (8.2% of the bedwetting population) wet every night. This is many fewer than in the USA, where 15% of children are reported to wet every night [20], or in Malaysia, where 20% of the enuretic population wet every night [18]. Most children (12.8% of the total population; 82.9% of those who wet the bed) wet infrequently (‘no more than once a week’). This compares with Bower et al. [12] in an Australian survey of children aged 5–12 years, who found 13.8% of the total population wet infrequently (‘no more than once in 2 weeks’), and an Italian survey that found the most frequent category of bedwetting was ‘less than once a week’ [15].

Nearly twice as many boys as girls were bedwetting at 7 years of age, the same ratio as Verhulst et al. [4] reported in Holland. For NE, the rate of 3.6% of boys and 1.6% of girls is almost identical to the Italian epidemiological work, where 3.0% of boys and 1.5% of girls were found to be bedwetting ‘at least twice a week’ [15].

Most bedwetting children had no daytime wetting or soiling, and might be regarded as having isolated bedwetting. Similarly, a large percentage of children with daytime wetting showed no NE (isolated daytime wetting), suggesting the presence of discrete problems with differing causes. A few children (3.3%) had both daytime wetting and bedwetting, a rate in accord with the Australian epidemiological survey where, in a sample of children aged 5–12 years, 4% had both daytime wetting and bedwetting [12]. Most children with daytime soiling showed no bedwetting, suggesting that, as with daytime wetting, the two problems have different causes. The few children (2.3%) with both daytime soiling and bedwetting suggests a significant clinical challenge. Night-time soiling was rare (0.8%), and shows that although bowel control at night might be expected to be achieved at 36 months by virtually all children [30], a small but clinically significant group lack nocturnal bowel control at 7 years.

Nearly 80% of children who never wet the bed also never wake to void, suggesting that they have low nocturnal urine production and adequate functional bladder capacity [22]. That ~20% who do wake to void (nocturia) suggests that their arousability to full bladder signals enables them to remain dry at night. Of the frequent and infrequent bedwetting children, 40% also wake at least once a night to void, suggesting that they have either severe NE (wet at night despite also waking to void), or have inconsistent arousability (able to wake to void on some nights and not on others).

The heterogeneity of NE is now well accepted, with current thinking distinguishing children with monosymptomatic NE (lack of daytime signs of bladder overactivity) from children with non-monosymptomatic NE (bedwetting with associated daytime signs of bladder overactivity). It has been argued both theoretically and empirically [22,27] that this categorization enhances clinical effectiveness by identifying appropriate treatments. Butler and Holland [22] highlighted various clinical signs to assist identification. Signs of bladder overactivity include daytime urgency, frequency and waking during or immediately after wetting [24–27]. The current epidemiological survey suggests urgency is rare in children not wetting the bed, yet occurs in 28.9% of those with NE. This is entirely in accord with Watanabe et al. [10], who found bladder overactivity in 28% of Japanese bedwetting children, and Kosar et al. [31] in 38% of Turkish children. Daytime toileting frequency (≥10 voids/day) was rare in non-bedwetting children, in line with Swedish studies which suggest children who are dry tend to void 2–8 times/day, with no differences between boys and girls [8,32]. Frequency was increased with severity of bedwetting in the present study, although as the upper limit a criterion was set at >10 voids/day, there were few children with such a degree of frequency overall. Waking after wetting was reported in many children with NE, particularly in those with infrequent bedwetting, where 49% always woke after wetting, suggesting that these children have a high degree of bladder overactivity. The presence of the three signs of bladder overactivity endorses the notion of heterogeneity amongst bedwetting children and highlights the importance of identifying children with non-monosymptomatic enuresis. The observed rates of the three signs of bladder overactivity are not equally spread through the population of children with NE, emphasizing the importance of individual assessment and intervention in clinical practice.

Wetting soon after sleep has been identified as a sign of low vasopressin release [24]. It is, in practice, often difficult to determine the point at which a child wets at night, unless there is a parental check a few hours after the child goes to sleep or if the wetting happens just before waking in the morning. Nevertheless, respondents to the present survey were able to judge the degree to which their child wet soon after wetting. By its nature, multiple wetting would be associated with wetting soon after sleep. As many children with NE wet soon after sleep, it suggests that a high proportion have low vasopressin release, as suggested by Norgaard et al. [33] and Rittig et al. [34]. This contrasts with children with infrequent wetting.
wetting, most of whom did not wet soon after sleep.

ACKNOWLEDGEMENTS

We are extremely grateful to all the mothers and other carers who have taken part in this study for several years and continue to do so, and to the midwives for their cooperation and help in recruiting the mothers during pregnancy. The whole ALSPAC study team comprising of interviewers, computer technicians, clerical workers, research scientists, volunteers, and managers, continue to make the study possible. The ALSPAC study could not have been undertaken without the financial support of the Wellcome Trust, the Medical Research Council, National Institutes of Health, and various UK Government Departments and Charitable Trusts. The ALSPAC study is part of the WHO initiated European Longitudinal Study of Pregnancy and Childhood. We are also grateful to: the Enuresis Information and Resource Centre in Bristol, East Leeds Primary Care Trust, Leeds Mental Health Trust Library, and Sarah Gasson Psychological Assistant, for their support and assistance during the course of this study.

CONFLICT OF INTEREST

None declared.

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Abbreviations: DSM, Diagnostic and Statistical Manual of Mental Disorders; ALSPAC, The Avon Longitudinal Study of Parents and Children; NE, nocturnal enuresis.
Efficacy of tolterodine as a first-line treatment for non-neurogenic voiding dysfunction in children

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OBJECTIVE

To assess the effect of antimuscarinic treatment with tolterodine combined with behavioural modification as a first-line treatment, before invasive investigation, in children with non-neurogenic voiding dysfunction but no obvious anatomical or neurogenic cause.

PATIENTS AND METHODS

The study comprised 44 children presenting with voiding dysfunction (30 girls and 14 boys, mean age 7 years, range 5–14); all had a noninvasive evaluation consisting of a history, urine analysis, renal and bladder ultrasonography and physical examination, with specific emphasis on the voiding pattern. Anticholinergic treatment with tolterodine (1 mg twice daily) was started in all patients; they were also informed about conservative management, including timed voiding, double voiding and relaxation of the pelvic floor during voiding. At the start and after 3 months, the dysfunctional voiding symptom score (DVSS) was completed twice by all patients.

RESULTS

For all patients the mean (sd) DVSS was 14.0 (2.67) and 6.68 (3.67) before and after treatment, respectively; the difference was statistically significant (P<0.001). The mean scores for girls and boys, respectively, were 13.8 (2.79) and 14.5 (2.44) before and 6.43 (3.79) and 7.50 (3.34) after treatment.

CONCLUSION

Tolterodine combined with behavioural modification for dysfunctional voiding in children with no neurological or anatomical abnormality can be recommended as a first-line treatment before invasive evaluation. Additionally, the DVSS appears to provide accurate and objective data for monitoring the effect of treatment in such children.

KEYWORDS

voiding dysfunction, tolterodine, dysfunctional voiding symptom score

INTRODUCTION

Dysfunctional voiding is one of the commonest clinical entities in paediatric urology, with ~40% of such patients presenting to paediatric urologists [1,2]. Symptoms include daytime and night-time wetness, urgency, frequency or infrequency, constipation or fecal incontinence. UTI is very common in these patients [3–5]. Anticholinergic medication and strict behavioural modification are the cornerstones of treating functional voiding disorders and incontinence in children [1,6]. As a widely used agent for treating incontinence and overactive bladder in adults, tolterodine is effective and safe, with reportedly fewer adverse effects than oxybutynin in children in recent studies [7–11].

There is no consensus on the diagnostic features or detection and monitoring of dysfunctional voiding. A history, physical examination, voiding diaries and uroflowmetry curves, with detection of postvoid residual urine, are methods for diagnosis [12]. There is no agreement about the value of urodynamics and radiological or cystoscopic evaluation [13–16]. The results of many recent studies support the view that the routine use of urodynamics, radiological evaluation and cystoscopy do not change therapy or influence final outcome in most children with voiding dysfunction, and these methods may not be necessary, provided that a thorough history is taken and the child examined physically [15,16]. The lack of a universally accepted objective means for detecting and monitoring the effect of therapy is another negative factor that hampering the reporting of standardized outcomes for voiding dysfunction studies in children.

The aim of the present study was to assess the effect of antimuscarinic treatment with tolterodine combined with behavioural modification as a first-line treatment before invasive investigation in children with no obvious anatomical or neurogenic cause. To quantitatively standardize dysfunctional voiding symptoms in children, the dysfunctional voiding symptom score (DVSS) was used [12].

PATIENTS AND METHODS

Voiding dysfunction was defined as incontinence, frequency, urgency or obstructive symptoms with or without recurrent afebrile UTI, with no obvious anatomical or neurogenic cause. The study included 44 children presenting with these features (30 girls and 14 boys, mean age 7 years, range 5–14). All patients had a noninvasive evaluation consisting of a history, urine analysis, renal and bladder ultrasonography and a physical examination, with specific emphasis on voiding pattern. Exclusion criteria were detection of an abnormality with renal ultrasonography, a large post-void residual urine volume, a history of febrile UTI, a history of failed previous therapy, and a positive neurological examination, including back lesions and an abnormal voiding pattern. Informed consent was obtained from the parents. Anticholinergic treatment with tolterodine (1 mg twice daily) was started in all patients [17] and maintained for 3 months. Patients were also informed about conservative management, including timed voiding, double
voiding, and relaxation of the pelvic floor during voiding. Patients with dysfunctional bowel elimination (15, eight girls and seven boys) were treated with a high-fibre diet and laxatives if necessary. At the start and after 3 months, the DVSS (Appendix) was completed twice by all patients. Parents were asked to record the side-effects during drug treatment. The paired t-test was used to compare the means, with P < 0.05 considered to indicate statistical significance.

RESULTS

A summary of the presenting symptoms and associated complaints is shown in Table 1. Compliance, including the use of medication as scheduled and adhering to behavioural modification, was good for all patients. For all patients, the mean (±) DVSS was 14.0 (2.67) and 6.88 (3.67) before and after treatment, respectively; the difference was statistically significant (P < 0.001). The respective mean (±, range) DVSS of girls and boys before treatment was 13.8 (2.79, 8–20) and 14.5 (2.44, 3–20), and after treatment was 6.43 (3.79) and 7.50 (3.34). The mean scores for a subgroup of DVSS questions (numbers 1, 2, 6 and 7) related to detrusor hyperactivity were also significantly different before and after treatment, at 7.63 (1.97) and 2.59 (1.04) (P < 0.001).

No patient discontinued therapy because of side-effects of tolterodine; 14 (31%) reported dry mouth and two (4%) reported headache after the first few days of treatment, but the effects were not severe. All but four patients (three girls and one boy) improved with tolterodine treatment; three of these had the highest DVSS (20, and two of 17) before treatment. All four had a urodynamic study and voiding cysto-urethrography (VCUG). An α-blocking agent was initiated in one girl because she had a ‘spinning-top’ deformity detected on VCUG and detrusor-sphincter dyssynergia on urodynamics. Detrusor instability was detected in two girls and one boy on urodynamics, and grade 1 unilateral reflux with VCUG in one girl. They remain under medical treatment and behavioural modification.

DISCUSSION

There is no universally accepted approach for evaluating and treating voiding dysfunction in children. Although behavioural modification is the most important, pharmacological and biofeedback methods have also been used [14,18–20]. Children with voiding dysfunction often undergo radiological, urodynamic or even cystoscopic evaluation to identify an anatomical or neurogenic cause [15]. However, recently the role of these methods in evaluating, managing and in the outcome has been intensively criticised [12–16]. Although video-urodynamics have increasingly gained favour for evaluating dysfunctional voiding, and non-neurogenic voiding dysfunction has also been classified according to urodynamic findings, its role in management and final outcome compared with the cost-effectiveness has been debated [4,15,21]. Soygür et al. [16] did not recommend video-urodynamics in children with non-neurogenic voiding dysfunction, as it does not generally change the management of these children. They concluded that a detailed voiding history and physical examination is sufficient for a correct diagnosis.

Parekh et al. [15] retrospectively evaluated the records of 1153 children with dysfunctional voiding disorder and concluded that the incidence of upper tract changes and positive anatomical findings in these children was too low to justify routine radiological evaluation and cystoscopy. They used video-urodynamics in only 40 of the patients who remained refractory to standard treatment for voiding dysfunction, and in those with positive radiological or cystoscopic findings. They concluded that, in these patients, a urodynamic study may possibly have affected treatment, e.g. changing the dose of anticholinergics or considering the addition of a second-line agent, but it was unlikely that urodynamics would have affected the ultimate outcome. The coexistence of voiding dysfunction with VUR is well known, with a reported incidence of up to 50% by some groups [22–25]. However, in the study by Parekh et al. [15], again the incidence of high-grade VUR in the patients with voiding dysfunction presenting with afebrile UTI was negligible (0.4%), which differs from the rate in previous studies. The incidence of other positive anatomical findings, in the form of trabeculations, a dilated posterior urethra, etc., was also very low in their series, at an 10%.

Although the precise pathophysiology of dysfunctional voiding is unclear, uninhibited bladder contractions, pelvic floor overactivity and poorly learned voiding are the main preceding factors. Among the drugs used for treating the hyperactive bladder, antimuscarinic agents are still regarded as first-line therapy. To date there are few reports on the use of tolterodine in children. Goessl et al. [11] published the first study of tolterodine in children with detrusor hyper-reflexia, and reported that the drug improved the compliance of the bladder in these children. Munding et al. [7] reported that tolterodine may be beneficial for reducing wetting episodes in children with voiding dysfunction, with no severe adverse effects. Hjalmas [8] published pharmacokinetic data and reported that the overactive bladder in children may be a potential future indication for tolterodine. Raes et al. [10] evaluated the efficacy and tolerability of tolterodine in unselected children with detrusor hyperactivity, and concluded that tolterodine is well tolerated in children and offers an effective treatment which is better than unselective antimuscarinic drugs for adverse effects.

We used the DVSS to evaluate the effect of tolterodine for dysfunctional voiding in the present children; the DVSS includes 10 quantitative and qualitative urological variables assessed by age-appropriate questions for children, and has been used as an objective instrument to grade voiding dysfunction in children [12]. In that study, there was a significant difference in the scores of 104 patients referred for dysfunctional voiding and 54 age-matched controls with a normal voiding history. Subsequently the score was reported to reliably quantify the improvement in voiding symptoms in 48 of 104 patients who had behavioural modification and completed a mailed questionnaire [26]. They also reported the beneficial use of the DVSS in predicting

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diurnal enuresis</td>
<td>29</td>
</tr>
<tr>
<td>Frequency/urgency</td>
<td>17</td>
</tr>
<tr>
<td>Constipation and/or encopresis</td>
<td>16</td>
</tr>
<tr>
<td>Nocturnal enuresis</td>
<td>14</td>
</tr>
<tr>
<td>History of afebrile UTI</td>
<td>10</td>
</tr>
<tr>
<td>Giggle incontinence</td>
<td>1</td>
</tr>
</tbody>
</table>

TABLE 1 The presenting symptoms and associated complaints in 44 children
resolution of VUR in children with voiding dysfunction [27]. In the present study, although the mean score of the girls after treatment was slightly higher, the scores recorded were generally consistent with the optimum thresholds determined (6.03 for boys and 9.02 for girls) in the original study [12].

The limitation of the present study is that there was no comparative group treated by only behavioural modification. The response to the drug might not be as good for those with initially higher scores, possibly because the natural history of the disorder is that a proportion of the children will improve spontaneously with no therapy, or the improvement obtained with the combination of tolterodine and behavioural modification in all patients was mainly a result of behavioural modification.

In conclusion, we recommend the use of tolterodine combined with behavioural modification as a first-line treatment for dysfunctional voiding in children with no neurological or anatomical abnormality, before invasive evaluation; tolterodine is well tolerated in children. The DVSS appears to provide accurate and objective data in monitoring the effect of treatment in children with voiding dysfunction.

CONFLICT OF INTEREST

None declared.

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Abbreviations: DVSS, dysfunctional voiding symptom score; VCUG, voiding cystourethrography.
APPENDIX: DYSFUNCTIONAL VOIDING SCORING SYSTEM

Patient Name:  
Hospital Number:  
Reason for Referral:  
Date:  

<table>
<thead>
<tr>
<th>Over the last month</th>
<th>Almost never</th>
<th>Less than half the time</th>
<th>About half the time</th>
<th>Almost every time</th>
<th>Not available</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I have had wet clothes or wet underwear during the day.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>2. When I wet myself, my underwear is soaked.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>3. I miss having a bowel movement every day.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>4. I have to push for my bowel movements to come out.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>5. I only go to the bathroom one or two times each day.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>6. I can hold onto my pee by crossing my legs, squatting or doing the &quot;pee dance&quot;.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>7. When I have to pee, I cannot wait.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>8. I have to push to pee.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>9. When I pee it hurts.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>10. Parents to answer. Has your child experienced something stressful like the example below?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>NA</td>
</tr>
</tbody>
</table>

TOTAL NO (0) YES (3)
The src-family kinase inhibitor PP2 suppresses the in vitro invasive phenotype of bladder carcinoma cells via modulation of Akt

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OBJECTIVE

To evaluate PP2 as a modulator of the cadherin/catenin complex in late-stage bladder carcinoma cells, and to assess its potential invasion-suppressor activity in this model.

MATERIALS AND METHODS

A panel of five human bladder carcinoma cells, characterizing late-stage disease, was used to determine the concentration for 50% inhibition of PP2 in cell-proliferation assays. Modulation of cadherin/catenin expression by PP2 was determined in Western blot analysis, with an assessment of the activation status of mitogen-activated protein kinase and Akt signalling pathways. Altered invasive capacity linked to these variables was determined in standard in vitro invasion assays.

RESULTS

PP2 elicited concentration-dependent growth inhibition in all bladder cell lines within the panel, with growth suppression recorded at 10–35 μmol/L PP2. Distinct morphological changes were recorded in cell lines exposed to PP2, accompanied by up-regulation of plakoglobin expression in a subset of lines. Exposure of cells to PP2 resulted in inactivation of Akt in all cells and a concomitant reduction in in vitro invasive capacity.

CONCLUSIONS

These results show that PP2 inhibits bladder carcinoma cell growth and can modulate plakoglobin expression in a subset of cell lines. In addition, PP2 can suppress the in vitro invasive capacity of bladder carcinoma cells by modulating the activation status of Akt.

KEYWORDS

bladder, carcinoma, PP2, invasion, Akt signalling

INTRODUCTION

Identifying the molecular events underlying bladder tumorigenesis defines different pathways to bladder cancer, facilitating the design of targeted therapeutics [1] to complement established therapies. Recent examples of such investigational therapeutic strategies used in bladder cancer trials include farnesyl transferase inhibitors designed to inhibit cell signalling in ras-transformed cells and the use of growth factor inhibitors, targeted at the tyrosine kinase family of membrane receptors [2]. Presently, grade and stage best define the risk of progression in bladder cancer, where the 5-year survival rate after surgery for muscle infiltrative disease (T2–T4), remains poor, as low as 10% for stage T4 disease [3–5]. Novel agents with invasive-suppressor activity would be desirable additions to the current options for chemotherapeutic agents in bladder cancer.

To adopt a targeted therapeutic approach to invasive bladder cancer it is necessary to understand the events that underlie this very complex process. The frequency of p53 mutations in bladder tumours is higher in high-grade and high-stage tumours than in low-grade lesions [6,7]. Along with loss of expression of the retinoblastoma gene product in late-stage bladder disease [8,9], alterations associated with these two loci are indicative of a poor outcome in patients with bladder cancer [10–12]. Alternative molecular changes implicated in the prognosis in bladder cancer include FGFR-3, c-myc, H-ras, c-erbB-1 and c-erbB-2 (for review see [13]), where changes associated with each loci may define a subpopulation of bladder tumours with different clinical behaviours. However, there is little direct evidence that changes associated with these gene loci are primarily involved in the acquisition of the invasive phenotype in bladder tumours. In contrast, the cadherin family of adhesion molecules is directly involved in invasion [14,15], where loss of E-cadherin in bladder cancer progression has been recorded as a frequent event in late-stage disease and is indicative of poor survival [16]. Restoration of E-cadherin expression to carcinoma cells has been shown to suppress invasion, both in vitro and in vivo models. In bladder cancer, novel expression of N-cadherin has also been identified in tumour progression [17] linked to an invasion-promotion role in carcinoma cells [18]. In addition, loss or altered expression of different catenin members in bladder tumours has been linked to poor prognosis [19,20]. Within this adhesion complex there are several different players, altered in bladder tumorigenesis, with potential roles in invasion.

To target the modulation of the cadherin complex, with the goal of suppressing invasion, it is important to know the mechanism of inactivation, or activation, of the cadherin/catenin complex, with the goal of suppressing invasion, it is important to know the mechanism of inactivation, or activation, of...
different components throughout tumour progression. For instance, loss of expression of the invasive-suppressor gene E-cadherin in bladder cancer is reportedly regulated by methylation events associated with the promoter region of the gene [21,22]. Loss or reduced expression of plakoglobin in late-stage bladder cancer occurs, at least partly, as a result of acetylation events [23,24]. Hence, demethylating and/or histone deacetylase inhibitors, are suitable agents for testing the potential to restore cadherin function.

Another class of agents representing src kinase-family inhibitors have been shown to modulate cadherin/catenin expression and have antimitastatic activity in vivo [25,26]. To date these have been tested in models where elevated levels of src activity accompany progression [27,28], an event that does not occur in urothelial neoplastic progression [29]. In the present study we investigated the potential invasive-suppressor activity of the src family kinase inhibitor PP2 within a panel of bladder cell lines, evaluating the expression status of different cadherin complex components and changes in signalling events triggered by exposure to PP2.

**MATERIALS AND METHODS**

The human bladder carcinoma cell lines J82, T24, UM-UC-3, EJ, KK47 and HU456 were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 7.5% fetal bovine serum and penicillin/streptomycin.

A stock solution of PP2 [3.3 mmol/L; Sigma, St. Louis, MO], dissolved in dimethyl sulphoxide (DMSO), was aliquoted and stored at −20 °C. The effect of PP2 or DMSO on the growth and viability of bladder carcinoma cells was determined by cell counting using a haemocytometer. Cells were seeded at a density of 5 × 10⁵ cells per 60 mm dish in DMEM supplemented with 7.5% serum, and allowed to attach overnight. After 24 h the medium was replaced daily with fresh medium containing the appropriate concentration of PP2 or DMSO. Cells were counted at various intervals after the start of treatment. At each time point cell viability was assessed by trypan blue exclusion. All experiments were performed in triplicate.

For Western blot analysis, subconfluent dishes of cells were washed in PBS followed by lysis in hot sample buffer (2 x ESB-0.08 mol/L Tris, pH 6.8; 0.07 mol/L SDS, 10% glycerol, 0.001% bromophenol blue) and sheared through a 26 G needle. Lysates were then assayed for protein concentration using the BSA method (Pierce, Rockford, IL). After determining the protein content, β-mercaptoethanol (1%) was added to each sample. Samples were boiled for 5 min and protein was loaded in each lane of a 7.5% or 12.5% polyacrylamide gel. Proteins were transferred overnight onto nitrocellulose. Membranes were blocked in 10% milk in Tris buffered saline with 0.05% Tween (TBST) and placed on primary antibody overnight at 4 °C. After incubating with the primary antibody, blots were washed in TBST three times for 15 min each, and secondary antibody linked to horseradish peroxidase was incubated with the blots for 1 h at room temperature. Blots were then washed as described above and developed with an ECL kit (Amersham, Arlington Heights, IL).

For immunoprecipitation and Src kinase assay, subconfluent dishes of cell lines were lysed in PBS-TDS buffer (PBS pH 7.4, 1% Triton X-100, 0.5% sodium deoxycholate, 2 mmol/L phenylmethylsulphonyl fluoride, 100 units aprotinin). Lysis was carried out on ice for 20 min followed by clarification in a microfuge at 4 °C. Supernatants were removed and a 10-μL aliquot taken for protein estimation using the BSA protein assay system. For immunoprecipitation, the protein concentration was standardized between samples, using 400 μg protein for each preparation. Lysates were incubated overnight at 4 °C with Mab327 (OncoGene Science, Manhasset, NY), followed by adding protein A-Sepharose beads and a further 90 min incubation. Immune complexes were washed three times in PBS-TDS and three times in 0.1% PBS. Immunocomplexes were incubated with [32P]-ATP in kinase buffer (50 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid, pH 7.4, 5 mmol/L MnCl₂, 5 mmol/L MgCl₂) for 20 min at room temperature. The antibody bead complex was washed three times in PBS and run in 12.5% polyacrylamide gels. Gels were dried and exposed to X-ray film.

For the transfection assay, J82 cells were plated at 3 × 10⁵ cells per 60 mm dish 24 h before lipofection. Either the puromycin gene alone or the wild-type PTEN gene (generous gift from Dr KM Yamada, Bethesda, MD) plus the puromycin-resistance gene were mixed with lipofectin reagent (Gibco BRL, Gaithersburg, MD, USA) and incubated with cells at 37 °C overnight in the absence of serum. After incubation, cells were washed and maintained in standard medium with serum. At 48 h after transfection, cells were split into puromycin-containing medium (2 μg/mL) to select for successfully transfected colonies. Individual colonies selected in puromycin were ring-cloned 2 weeks later.

The following antibodies were used: N-cadherin clone 13A9 (1 : 200; kindly provided by Dr M Wheelock, University of Nebraska, Omaha, NE); α-catenin (1 : 200), β-catenin, γ-catenin and p120 (Transduction Laboratories, Lexington KY); mitogen-activated protein kinase (MAPK), phosphorylated MAPK, Akt and phosphorylated Akt (Cell Signalling Technology, Beverly, MA); E-cadherin (1 : 500, Zymed, San Francisco, CA), PTEN (Chemicon Int., Temecula, CA), β-actin (Sigma). All antibodies were used at 1 : 1000 dilution unless otherwise indicated. All biochemical inhibitors (PD98059, 40 μmol/L, LY294002, 20 μmol/L, PP2 15 μmol/L, Akt inhibitor; 1 L-6-hydroxymethyl-chiro-inositol 2-(R)-2-0-methyl-3–0-octadecylcarbonate 20 μmol/L) were purchased from Calbiochem (San Diego, CA).

Cells used in growth factor assays were maintained until subconfluent and then washed with PBS, followed by an overnight incubation in serum-free DMEM. Assays designed to assess the signalling pathways activated by the action of growth factors (epidermal growth factor, EGF, Sigma; heregulin-β, EGF domain, US Biological, Swampscott MA; human basic fibroblast growth factor (bFGF), Peprotech, Rock Hill NJ; human insulin, Lilly, Indianapolis, IN) involved exposure of cells to growth factor (20 ng/mL) for 1 h in the continued absence of serum. Cells were then lysed and analysed by Western blot. The same protocol was followed for invasion assays where the presence of the growth factor was maintained throughout the course of the assay.

In vitro invasion was assayed using modified Boyden chambers consisting of Transwell (Corning Costar Corp., Cambridge, MA) membrane filter inserts in 24–well tissue culture plates. For invasion assays the upper surfaces of the membranes were coated with Matrigel (Collaborative Biomedical Products, Bedford, MA) and placed into 24–well tissue culture plates containing 600 μL of NIH/3T3 conditioned media (experimental) or serum-
free DMEM (control); 1 × 10² cells were added to each Transwell chamber and allowed to invade toward the underside of the membrane for 8 h (J82), 16 h (UM-UC-3) or 24 h (T24, EJ, KK47) at 37 °C. Cells that passed through the membrane were fixed in methanol, stained with crystal violet and counted under light microscopy. In additional assays, cells were pre-incubated for 1 h (PD98059, 40 μmol/L; SB203580, 20 μmol/L; LY294002, 20 μmol/L; PP2, 15 μmol/L, Akt inhibitor, 20 μmol/L) before adding to the Matrigel-coated wells and maintained over the course of the assay in the presence of inhibitor. All assays were performed in triplicate and repeated three times.

Immunocytochemical staining was performed on an automated immunocytochemical processor (Ventana ES, Medical Systems, Tucson, AZ). Plakoglobin and acetylated histone 4 antibody were used at dilutions of 1 : 500 and 1 : 50, respectively. Cells were plated at subconfluent levels on sterile glass slides and allowed to attach and spread over a 24-h period. PP2 or DMSO vehicle was added to the medium and cells maintained for a 24-h period. PP2 or DMSO vehicle was added to each Transwell chamber and allowed to invade toward the underside of the membrane for 8 h (J82), 16 h (UM-UC-3) or 24 h (T24, EJ, KK47) at 37 °C. Cells that passed through the membrane were fixed in methanol, stained with crystal violet and counted under light microscopy. In additional assays, cells were pre-incubated for 1 h (PD98059, 40 μmol/L; SB203580, 20 μmol/L; LY294002, 20 μmol/L; PP2, 15 μmol/L, Akt inhibitor, 20 μmol/L) before adding to the Matrigel-coated wells and maintained over the course of the assay in the presence of inhibitor. All assays were performed in triplicate and repeated three times.

**RESULTS**

Figure 1A shows that PP2 elicited dose-dependent growth inhibition in all the bladder carcinoma cell lines in the study panel. The concentration required to inhibit growth by 50% (IC₅₀) was 10–35 μmol/L of PP2. Trypan blue exclusion, assayed on floating cells and those remaining attached to dishes, revealed >99% loss of viability in the former, with only 60% of attached cells retaining the ability to exclude the dye. Cell viability from these experiments was further confirmed in colony-forming assays (data not shown). Figure 1B shows the time-dependent growth inhibition of bladder carcinoma cells after continuous exposure to one concentration of PP2 representing the IC₅₀. Half the cells were killed by 48 h in cell lines exposed to PP2, causing a progressive decline in cell number sustained during the experimental period.

**MODULATION OF CADHERIN/CATENIN EXPRESSION**

All bladder carcinoma cell lines within the panel lack E-cadherin expression and four of the five cell lines showed a significant reduction in plakoglobin expression. Each of the cell lines is derived from a late-stage tumour and harbour molecular changes indicative of invasive bladder cancer [30]. Cell morphological changes associated with exposure to PP2 were recorded in several bladder carcinoma cell lines, consistent with the observations by Nam et al. [25] in colon carcinoma cells, where alterations were accompanied by up-regulation of different elements of the cadherin/catenin complex. Figure 2 is a representative blot showing a concentration-dependent increase in plakoglobin expression in bladder cell lines after a 48-h exposure to PP2. In cell lines expressing low levels of plakoglobin, increased expression of this catenin member, in response to PP2 exposure, was recorded in cell lines EJ, UM-UC-3 and T24, but not J82. Bladder cell line KK47 constitutively expresses high levels of plakoglobin comparable to that recorded in E-cadherin-expressing bladder carcinoma cells (Hu456). Re-probing blots with antibodies to E-cadherin, N-cadherin, α-, β- and p120 catenins revealed no discernible change in expression of these cadherin complex components within the same experiment (data not shown).

Immunocytochemical staining of morphologically responsive cell lines revealed increased membrane-associated plakoglobin after exposure to PP2 (Fig. 3).

**ALTERED IN VITRO INVASIVE POTENTIAL IN THE PRESENCE OF PP2**

The effect of exposure to PP2 on the invasive potential of N-cadherin expressing bladder carcinoma cell lines was analysed using standard in vitro Matrigel invasion assays. As J82 and UM-UC-3 were highly invasive in this assay, the effect of exposure to PP2 was assayed over 8 and 16 h, respectively. The same invasion assay involving T24, EJ and KK47 were performed over 24-h. Figure 4 shows decreased invasion associated with all bladder carcinoma cell lines exposed to PP2.

**MECHANISM OF ACTION OF PP2 INHIBITION OF INVASION**

As PP2 is a src-family kinase inhibitor we initially assayed pp60⁵⁰ kinase activity in the
INHIBITION OF BLADDER CARCINOMA CELL INVASION BY PP2

Panel of bladder cell lines exposed to PP2. In contrast to many carcinoma cell lines derived from different organs, the endogenous level of src kinase activity is low in bladder cell lines derived from late-stage tumours [29]. After exposure to PP2 no discernible change in pp60src activity was detected (data not shown). To identify alternative signalling proteins altered by the action of PP2, possibly linked to the invasive phenotype, we investigated the activation status of MAPK (Erk1 and Erk2) and Akt proteins, both of which have been implicated in many cellular pathways linked to tumorigenesis and invasion. Figure 5 is a Western blot, showing no change in the expression or phosphorylation status of MAPKs in bladder cells exposed to PP2. In contrast, the same cell lines showed a reduction in phosphorylated Akt in the presence of PP2, indicative of deactivation of this signalling molecule. As these two different signalling proteins were analysed on the same cell lysates in the same electrophoretic run, it is likely that inactivation of Akt is a targeted PP2 event.

MODULATION OF AKT ACTIVITY AND EFFECT ON INVASION

To further establish a role for Akt in in vitro bladder cell invasion we used different experimental approaches to modulate the Akt activation status in selected bladder carcinoma cell lines. The J82 bladder

FIG. 2. Levels of plakoglobin expression detected in Western blot analysis on total cell lysates of bladder carcinoma cells exposed to escalating concentrations of PP2. HU456 control represents plakoglobin levels in E-cadherin expressing bladder carcinoma cells. Lanes were equalised for protein concentration within each experiment represented within one panel.

Fig. 3. Immunocytochemical staining for plakoglobin in bladder carcinoma cell line T24 treated with DMSO (A) and PP2 (B) for 18 h. Note the morphological change in the PP2-treated cells, characterized by a transition from a fusiform shape (A) to a flattened epithelial shape (B), accompanied by accumulation of plakoglobin at the intercellular membrane (compare A and B).

FIG. 4. In vitro invasion assay involving five bladder carcinoma cell lines conducted in the presence of DMSO (control, green) or PP2 (15 μmol/L, red). There was a significant reduction in invasive potential in all cell lines in the presence of PP2.

FIG. 5. Western blot analysis of paired total cell lysates from the bladder carcinoma cell panel from cells exposed for 18 h to DMSO (control) or PP2 (15 μmol/L). Blots were probed using antibodies to the MAPK, phosphorylated MAPK, Akt and phosphorylated Akt. Each protein-standardized lysate was from the same experiment and analysed in the same electrophoretic run. There was no change in the phosphorylation status of MAPK within each treated and untreated pair, but significant dephosphorylation of Akt in the same lysate from PP2-treated cells.
carcinoma cell line showed activated Akt and is highly invasive in in vitro invasion assays. Akt activation in this cell line probably resulted from the loss of PTEN expression by gene deletion [31]. Restoring PTEN expression in the J82 cell line (Fig. 6A) significantly reduced the phosphorylation status of Akt in transfected cells (Fig. 6B) and their invasive potential in in vitro invasion assays (Fig. 7).

In reciprocal experiments we sought to activate Akt in bladder carcinoma cells to assess the effects on invasive potential. Figure 8 shows the baseline activation status of Akt within the panel of bladder carcinoma cells, where phosphorylated Akt was assayed after maintaining cells in serum-free medium for 24 h. Within this cell panel constitutive levels of phosphorylated Akt closely correlated with invasive potential, where EJ and KK47 had the lowest level of activated Akt and were the least invasive. In initial experiments we determined the effect of EGF, bFGF, heregulin and insulin on the activation status of MAPK and Akt. Adding EGF to serum-starved EJ cells activated both the MAP and Akt signalling pathways, and resulted in the most significant increase in in vitro invasion potential amongst the growth factors tested. Repeating this experiment in the presence of biochemical inhibitors of different signalling pathways showed significant inhibition of invasion associated with all inhibitors, with PP2 reducing invasion close to baseline levels (Fig. 9).

**DISCUSSION**

The src family kinase inhibitor PP2 can act to inhibit in vitro cell invasion in a panel of bladder carcinoma cell lines representative of late-stage tumours. In addition, PP2 caused concentration-dependent cell death in all bladder cell lines and up-regulation of plakoglobin in a subset of lines. Suppression of the invasive phenotype by PP2 was mediated through the Akt signalling pathway, where inactivation of Akt resulted in a reduction in in vitro invasive capacity in bladder carcinoma cells.

Different src family members are over-expressed and/or highly activated in several human cancers, including colon, ovary, lung, oesophagus, skin and gastric carcinomas [27,28]. In such cases the possibility of using tyrosine kinase inhibitors in treating these cancers is under investigation and remains a promising strategy for targeted therapeutics. In bladder cancer, no increased src family tyrosine kinase activity has been recorded as associated with neoplastic progression [29].
but src activity has been reported to modulate cadherin/catenin expression and function, contributing to the metastatic phenotype in alternative models [32]. The bladder cell carcinoma panel used in the present study is representative of late-stage disease, where loss of E-cadherin, accompanied by down-regulation of plakoglobin expression, was shown by all but the KK47 cell line. E-cadherin and plakoglobin have invasion [14,15] or tumour-suppressor activity [33,34], respectively, and are characteristically silenced in bladder cancer progression. These silenced adhesion components represent attractive targets for reactivation in a targeted therapeutic approach to bladder cancer. No restoration of E-cadherin expression was detected in bladder carcinoma cells after exposure to PP2, but up-regulation of plakoglobin was recorded in a subset of the cell panel. There was no modulation of alternative catenin molecules after exposure to PP2.

The src family of tyrosine kinases represent non-receptor protein kinases that are central in cell growth and differentiation. Indeed, src tyrosine kinases are becoming recognized as pivotal agents in diverse cell signalling pathways [26–28]. In contrast to previous studies in alternative carcinoma models, there was no discernible change in the pp60src kinase activity in the presence of PP2 in the present study. This may reflect the sensitivity of the assay, as the baseline level of src kinase activity is low; or that kinase-mediated changes in protein phosphorylation occurred via a different member(s) of the src family; or that alternative cellular kinases were affected by the action of PP2. Although PP2 is reported to inhibit src family kinase activity at nanomolar concentrations in in vitro kinase assays, micromolar concentrations are necessary to invoke the same inhibition in intact cells, probably because of the permeability of the compound. At higher concentrations, PP2 has been shown to inhibit alternative cellular kinase molecules [35], complicating the interpretation of the pathway of PP2 action in the bladder model.

Interestingly, a reduction in the phosphorylation status of Akt, representing deactivation of this signalling pathway, was consistently recorded in all bladder carcinoma cell lines exposed to PP2. Previous work from our laboratory [18], and a recent publication [36], identifies the PI3/Akt signalling pathway as important in regulating bladder cell invasion. Consistent with this hypothesis, all bladder carcinoma cell lines in the present study showed a reduction in phosphorylated Akt after exposure to PP2, and a concomitant reduction in invasive potential in in vitro assays. Further confirmation of a role for Akt in bladder cell invasion was shown by inactivation of Akt after restoring PTEN expression in bladder cells lacking the gene. Restoring PTEN expression in J82 cells resulted in inactivation of Akt and reduced invasive potential of PTEN-transfected cells compared to puromycin control transfectants. Interestingly, in a previous study we identified PTEN alterations within this bladder cell panel, revealing loss of the PTEN gene in two bladder cell lines and point-mutation events associated with an additional four cell lines [31]. Although we did not show the functional significance of these different PTEN point mutations, notably all bladder carcinoma cell lines reported as harbouring point mutational changes had an activated Akt status.

To further show the involvement of Akt activation in bladder cell invasion we identified the EJ cell line as having a low baseline level of activated Akt, a low in vitro invasive potential and Akt activation in response to the action of different growth factors. Exposure of the EJ cell line to EGF resulted in marked activation of Akt and a significant increase in invasive capacity. However, EGF also activated MAPK in EJ cells, hence, we used biochemical inhibitors to separate the effects of the MAP and Akt signalling pathways. Not surprisingly, different inhibitors of the PI3/Akt signalling pathway reduced the invasive potential of EJ in the presence of EGF, but the results were similar with the MAPK inhibitor. In a previous study we showed that novel expression of N-cadherin in bladder carcinoma cells elicits the invasive phenotype, with activation of Akt. In this model the MAPK inhibitor did not suppress the invasive phenotype. Although these results appear contradictory they probably indicate that several pathways can contribute to the invasive phenotype, and these may be activated via different routes. Should the presence or absence of specific growth factors play a determining role in the activation of different signalling pathways involved in invasion, consideration of in vivo autocrine and paracrine loops may be important in assessing the pathways to target.

In summary, we present data that PP2 can suppress the in vitro invasive potential of bladder carcinoma cell lines by modulating the activation status of Akt. In contrast to alternative carcinoma models there is no compelling evidence that this event is mediated through the src signalling pathway in the bladder model. However, consistent with published reports [25], PP2 exposure can act to modulate expression of the cadherin complex proteins. In the bladder model, PP2 exposure resulted in up-regulation of plakoglobin in a subset of bladder carcinoma cell lines with accumulation of this catenin family member at cell-cell borders. Plakoglobin has been reported to have a tumour-suppressor function in some cancer models [33,34], and one recent report of the action of PP2 in vivo showed restoration of the cadherin/catenin adhesion system and a reduction in in vivo metastases in a colon cancer model [25]. The results in the present study suggest that PP2, and other members of this class of molecule, may have potential as invasion-suppressor agents in bladder cancer.

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CONFLICT OF INTEREST

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Abbreviations: DMEM, Dulbecco’s Modified Eagle’s Medium; DMSO, dimethyl sulphoxide; TBST, Tris buffered saline with 0.05% Tween; MAPK, mitogen-activated protein kinase; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor.
Sildenafil inhibits the formation of superoxide and the expression of gp47phox NAD[P]H oxidase induced by the thromboxane A2 mimetic, U46619, in corpus cavernosal smooth muscle cells

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OBJECTIVE
To assess the effect of sildenafil on superoxide formation and gp47phox (the active subunit of NADPH oxidase) expression in cultured corpus cavernosal smooth muscle cells (CVSMCs).

MATERIALS AND METHODS
CVSMCs derived from rabbit penis were incubated with U46619 (thromboxane A2 analogue) with or without sildenafil for 1 or 16 h at 37°C. Superoxide dismutase-inhibitable superoxide formation was assessed using the reduction of ferricytochrome c measured spectrophotometrically, and gp47phox assessed using Western blot analysis. The role of NAD[P]H oxidase and cGMP was further studied by using specific inhibitors of each.

RESULTS
Superoxide formation was significantly greater in cells incubated with U46619 after 1 and 16 h incubation than in controls, an effect blocked by NADP(H) oxidase inhibitors. These effects of U46619 were inhibited by sildenafil (1 and 10 nmol/L), which in turn were negated by the guanylyl cyclase inhibitor, ODQ; 10 nmol/L sildenafil inhibited p47phox expression induced by U46619.

CONCLUSIONS
Sildenafil is a potent inhibitor of superoxide formation in CVSMCs. This effect is mediated through the inhibition of PDE-5 which in turn augments the inhibitory action of the NO-cGMP axis on NAD[P]H oxidase expression and activity. This mechanism constitutes a new pharmacological action of sildenafil, consolidates the potential role of superoxide in ED, and indicates that thromboxane A2 may be an important mediator of intrapenile oxidative stress.

KEYWORDS
erectile dysfunction, oxidative stress, sildenafil, thromboxane A2

INTRODUCTION
Penile erection is initiated by relaxation of the corpus cavernosum, which is mediated by an increase in nitric oxide (NO) formation released by both nonadrenergic, noncholinergic nerve fibres [1] and the intracavernosal endothelium [2]. NO relaxes cavernosal smooth muscle by activating guanylyl cyclase that generates cGMP [2,3]. In turn, cGMP activates protein kinase G, which suppresses calcium mobilization, resulting in an erectile response [2,3]. Therefore, any process that reduces NO formation impairs normal erectile function.

Major risk factors for vasculogenic erectile dysfunction (ED) are diabetes mellitus, smoking and dyslipidaemia [4]. In turn, these risk factors are associated with an increase in superoxide (O$_2^-$) formation in the vasculature [5,6]. O$_2^-$ reacts with NO to form peroxynitrite (ONOO$^-$) and other reactive nitrogen species [7]. As a result, the bioavailability of NO is decreased, thereby impairing NO-dependent smooth muscle relaxation and therefore the normal erectile response. There is increasing evidence that the excess production of O$_2^-$ is caused by the over-expression of endogenous vascular NAD(P)H oxidase and that the up-regulation of this enzyme may play a role in promoting ED [5–8]. NAD(P)H oxidase is rapidly up-regulated by cytokines [9,10] and vasoconstrictors, including thromboxane A2 [11]. In turn, thromboxane A2 is a potent constrictor of the human corpus cavernosum [12] and penile arteries [13], and as such is implicated in the causes of ED [14–16]. It is also possible that the pathogenic effect of thromboxane A2 in ED involves an up-regulation of NAD(P)H oxidase expression.

Sildenafil, now routine for treating ED, is a type-5 phosphodiesterase (PDE-5) inhibitor that augments the action of NO by preventing the hydrolysis of cGMP [17,18]. In the context of the above, it was shown that NO is also a potent inhibitor of NAD(P)H oxidase expression [10], which in turn reduces O$_2^-$ formation in vascular smooth muscle cells (SMCs) from other vascular tissue, an effect mediated by cGMP [10]. It follows therefore that sildenafil (by enhancing cGMP levels) may also inhibit the activity and expression of NAD(P)H oxidase. By doing so, reduced O$_2^-$ formation would augment NO bioavailability thereby improving the erectile response in patients with ED.

To test these hypotheses, the effect of the thromboxane A2 analogue, U44619, on O$_2^-$ formation and the expression of gp47phox (an active component of NAD(P)H oxidase [19]) was assessed in isolated cavernosal cells derived from the rabbit corpus cavernosum. The effects of sildenafil on these systems were then studied both acutely (1 h) and for longer
The roles of NAD(P)H oxidase and cGMP were further studied by using specific inhibitors of each.

**MATERIALS AND METHODS**

All animals used were given humane care in compliance with the rules and regulations of Bristol University and the UK Home Office. New Zealand White rabbits were killed with a lethal overdose of barbiturates and the penises rapidly excised. The corpus cavernosum was dissected from the surrounding the tunica albuginea. Cavernosal tissues were placed in Dulbecco’s Minimum Essential Medium (DMEM) with 10% fetal calf serum (FCS; both GibcoBRL, Paisley, Scotland) and cut into 2 mm² pieces. Cavernosal vascular SMCs (CVSMCs) were prepared using previously described methods [10]. CVSMCs were maintained in DMEM (containing 10% FCS, 100 units/mL penicillin and 100 µg/mL streptomycin) at 37 °C in a 95% air-5% CO₂ incubator. For experimentation, subconfluent cultures of CVSMCs were growth-arrested by incubating in quiescing medium (serum-free DMEM supplemented with 0.5% lactalbumin hydrolysate, 100 µunits/mL penicillin and 100 µg/mL streptomycin) for 72 h [10–12]. The following experiments were then carried out.

To assess the effect of U46619 with or with no sildenafil on O$_2^-$ formation, CVSMCs were incubated with the thromboxane A$_2$ analogue, U46619 for 1 or 16 h at 37 °C in a 95% air-5% CO₂ incubator. Cells were then equilibrated in DMEM with no phenol red for 10 min at 37 °C in a 95% air-5% CO₂ incubator (Heraeus, Hera Cell, Kandro Laboratory Products, Germany); 20 µmol/L horseradish cytochrome c (Sigma Chemical Co., Poole, UK) with or without 500 µU/mL copper-zinc superoxide dismutase (SOD; Sigma) was added and incubated at 37 °C in a 95% air-5% CO₂ incubator for 1 h. The reaction medium was removed and reduction of cytochrome c determined at 550 nm in an Anthos Lucy 1 spectrometer (Laboratory-tech International, Ringmer, East Sussex, UK) and converted to µmol O$_2^-$, using a ΔE$_{550nm}$ of 21.1 mmol/L.cm$^{-1}$ as the extinction coefficient. The reduction of cytochrome c that was inhibitable with SOD reflected actual O$_2^-$ release [9–11]. CVSMCs were rinsed in PBS, lysed with 0.1% v/v Triton-X100 and total protein content measured using BCA-protein assay kit (Pierce, Rockford, Illinois, USA). The raw data were expressed as µmol of O$_2^-$/mg protein per hour.

To determine whether the source of O$_2^-$ was NAD(P)H oxidase, cells were incubated with the NAD(P)H oxidase inhibitors diphenyleneiodonium chloride (DPI) and apocynin when measuring ferricytochrome c reduction, as described above. Furthermore, to determine the role of cGMP in mediating effects, cells were also incubated over 16 h with the soluble guanyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[3,4-d]quinoxalin-1-one (ODQ) [10].

To assess the effects on NAD(P)H oxidase protein, the effect of U46619 and sildenafil on the expression of gp47 (an active catalytic subunit of NAD(P)H oxidase [19]), in both the membranes and cytosol of CVSMCs was investigated using Western analysis. After 16 h of incubation with U46619 (± sildenafil), as described above, CVSMCs were rinsed in PBS and lysed with ice-cold hypotonic buffer (20 mmol/L HEPES, pH 7.5, 10 mmol/L potassium acetate, 1.5 mmol/L magnesium acetate). Cells were incubated for 15 min on ice, removed and homogenized using a glass homogenizer. Intact cells and nuclei were sedimented by centrifugation at 3000 g for 5 min at 4 °C [19]. Supernatants were removed and centrifuged at 20 000 g for 2 h at 4 °C, the resultant supernatant being the cytosolic fraction and the pellet the membrane-enriched fraction. Cytoplasmic and membrane fractions were boiled in a 1 : 1 ratio with Tris (50 mmol/L; pH 6.8 containing 4% w/v SDS; 10% v/v glycerol; 4% v/v 2-mercaptoethanol; 2 mg/mL bromophenol blue). Samples of equal protein (100 µg) were loaded onto 12% Tris-glycine SDS gels and separated by electrophoresis. After transfer to nitrocellulose, the blots were primed with MoAb 48 (2.5 µg/mL final concentration), then incubated with goat antirabbit antibody conjugated to horseradish peroxidase (1 : 2000 dilution) and developed by enhanced chemiluminescence (Amersham International). Rainbow markers (14–220 kDa; Amersham) were used for molecular weight determination.

Data are expressed as the mean (SEM) with the number of rabbits used. Student’s unpaired t-test or a one-way factorial ANOVA was used to determine difference among treatments, with P < 0.05 considered to indicate statistical significance. Multiple group comparisons were made using one-way ANOVA.

**RESULTS**

The thromboxane A$_2$ analogue U46619 was a potent stimulator of superoxide formation by CVSMCs incubated for both 1 and 16 h (Fig. 1). This effect was negated by the presence of the NADPH oxidase inhibitors, apocynin and DPI (Fig. 1). Over a 1-h incubation and after a 16-h incubation with U46619 and sildenafil, superoxide release by CVSMCs was inhibited by sildenafil in a dose-dependent manner (Fig. 2a,b). The inhibitory effect of sildenafil on superoxide formation by CVSMCs after a 16-h incubation was reversed by ODQ (Fig. 3). Sildenafil (10 nmol/L) reduced

![FIG. 1. Effect of the thromboxane A₂ mimetic, U46619 (10 nmol/L), on superoxide formation by rabbit CVSMCs over 1 (green bars) and 16 h (red bars) of incubation, and the effect of the NAD(P)H oxidase inhibitors, 10 µmol/L apocynin and 10 µmol/L DPI. Each point is the mean (SEM) from six animals. #P < 0.05, significantly greater than control; *P < 0.01 significantly less than U46619 alone.](image-url)
FIG. 2. The effect of sildenafil on superoxide formation elicited by incubation with 10 nmol/L U46619 by cultured rabbit CVSMCs over a 1-h (a) and 16-h (b) incubation. Data are the mean (SEM) from six animals. *P < 0.01, significantly greater than U46619-treated than untreated controls; #P < 0.01, significantly less than U46619-treated cells.

FIG. 3. The effect of the guanylyl cyclase inhibitor, ODQ (1 μmol/L) on the sildenafil-mediated inhibition of superoxide release from by rabbit CVSMCs after 16 h of incubation with U46619 (10 nmol/L). Each point is the mean (SEM) from six animals. *P < 0.01, significantly less than U46619 alone; $P < 0.01, significantly greater than sildenafil alone.

The present study shows that U46619 is a potent stimulator of O_2^- formation in corpus cavernosal smooth muscle cells, both acutely (over 1 h) and after a longer (16 h) exposure.

**DISCUSSION**

The present study shows that U46619 is a potent stimulator of O_2^- formation in corpus cavernosal smooth muscle cells, both acutely (over 1 h) and after a longer (16 h) exposure.

It has long been established that thromboxane A_2 is a potent constrictor of both cavernosal and pudendal arterial smooth muscle [12,13], and as such its over-production has been implicated in the causes of ED [14–16]. Although thromboxane A_2 is formed by all vascular tissues, the pathological effect of thromboxane A_2 is thought to be negated by the concomitant formation of prostacyclin (PGI_2), which has potent diametrically opposite properties to thromboxane A_2 [14], which include the inhibition of NADPH oxidase expression [11]. However, in disease states associated with ED, including diabetes mellitus and smoking, PGI_2 is diminished and thromboxane A_2 increased [14]. Thromboxane A_2 is also released by activated/adherent platelets that are hyper-reactive in such disease states [5]. It was suggested that as NO is a potent inhibitor of platelet adhesion, then reduced NO would augment transient platelet adhesion and therefore enhance local release of thromboxane A_2 [5]. Thus, it is proposed that thromboxane A_2 may be an important pathogen in ED through different pathways, i.e. (i) direct vasoconstriction and (ii) increased NAD[P]H oxidase activity and expression, resulting in prolonged augmentation of O_2^- generation and negation of NO bioavailability.

Second, the present study shows that sildenafil, via its effect on PDE-5 and elevation of cGMP, potently inhibits O_2^- formation by CVSMCs in response to U46619, both acutely and in the longer term. Sildenafil also blocked the expression of U46619-stimulated gp47^phox protein expression over the longer term (16 h). These effects were blocked by ODQ, showing that the inhibitory effects of sildenafil on NAD[P]H oxidase expression and activity are mediated by guanylyl cyclase. This is expected, as sildenafil is PDE-5 inhibitor, the enzyme which hydrolyses cGMP to inactive GMP [3]. In a previous study we showed that U46619 also up-regulated gp91^phox and promoted an increase in O_2^- formation by pulmonary arteries [11]. The 50% inhibitory dose (IC_50) of sildenafil on O_2^- formation and gp47^phox expression after 16 h incubation in the present study was 1–10 nmol/L. As sildenafil inhibits PDE-5 activity at an IC_50 of 3.5 nmol/L [20,21], these data indicate that the effects of sildenafil on O_2^- formation and gp47^phox expression are indeed mediated by the inhibition of PDE-5. Therefore, the inhibition of NADPH oxidase and the concomitant reduction in O_2^- formation by sildenafil
would enhance NO formation, as $O_2^-$ negates NO through a reaction that forms ONOO$^-$. This ‘antioxidative’ effect of sildenafil may constitute an additional mechanism to that of augmentation of NO-mediated relaxation of VSMCs, that would explain the therapeutic effect of the drug (Fig. 5).

These findings may be of therapeutic relevance, as the concentrations at which sildenafil elicits this indirect inhibitory effect on $O_2^-$ formation in the present study are well within the concentrations required to promote erection [1,2,20,21]. The inhibitory effect of sildenafil on $O_2^-$ formation in the present study was also apparent within 1 h, again at therapeutic plasma concentrations. In turn, sildenafil is recommended to be taken only 1 h before any sexual activity [17,18,22]. As sildenafil elicits an inhibition of $O_2^-$ formation within 1 h it is not unreasonable to suggest that this ‘antioxidative’ effect may augment the therapeutic action of the drug. Additionally, novel PDE inhibitors have been developed that act over a longer term, in that the patient can take the oral tablet in the morning and the effects of the drug persist all day, provided that the appropriate psychogenic inputs (i.e., sexual arousal) are also present [17]. Thus, the longer-term effects of sildenafil on the inhibition of $O_2^-$ formation, by suppressing NAD[P]H oxidase expression, may also be an important attribute of the drug.

In conclusion, thromboxane $A_2$ increases the activity and expression of NAD[P]H oxidase and the formation of $O_2^-$ by CVSMCs. As $O_2^-$ negates NO availability, reduced NO is associated with ED and increased thromboxane $A_2$ with risk factors for ED, so that thromboxane $A_2$ may be important in the causes of ED through promoting intrapenile oxidative stress. Second, sildenafil has a potent inhibitory effect on the formation of $O_2^-$ through a reduction of both NAD[P]H oxidase activity and expression. These effects are mediated through the inhibition of PDE-5 activity and therefore an augmentation of cGMP formation. The resultant decrease in endogenous $O_2^-$ would in turn increase the bioavailability of intrapenile NO. Apart from augmenting CSM relaxation, enhanced NO availability would further inhibit NAD[P]H oxidase activity and expression. This would create a ‘self-augmenting’ therapeutic loop that would not only augment erection acutely but may have longer-term beneficial effects in improving erectile function, particularly in...
those patients where increased tissue NAD(P)H oxidase expression is a causal factor. Further studies are required to explore this novel therapeutic pathway of PDE inhibition, which in turn may lead to improved drug design and efficacy.

CONFLICT OF INTEREST

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Abbreviations: NO, nitric oxide; (C)VSMC, (cavernosal) vascular smooth muscle cell; ED, erectile dysfunction; PDE–5, type-5 phosphodiesterase; DMEM, Dulbecco’s Minimum Essential Medium; FCS, fetal calf serum; SOD, superoxide dismutase; DPI, diphenyleneiodonium chloride; ODQ, 1H-(1,2,4)oxadiazolo[3,4]quinoxalin-1-one.
Loss of ryanodine receptor calcium-release channel expression associated with overactive urinary bladder smooth muscle contractions in a detrusor instability model

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OBJECTIVE
To investigate the changes in spontaneous bladder smooth muscle contractions that occur during detrusor instability (DI), and to test the possibility that altered function or expression of ryanodine receptors (RyRs) could account for the increased bladder contractions.

MATERIALS AND METHODS
After 8 weeks of partial bladder outlet obstruction, DI was confirmed in female experimental rats by filling cystometry. Muscle strips were dissected from freshly isolated bladders, and isometric tension recorded in strips from DI and normal bladders. The contractions were recorded during electrical stimulation or exposure to various agents. Western blot analysis was used to determine RyR expression in DI and normal bladder muscle.

RESULTS
In DI bladder muscle, spontaneous contractile activity persisted in the presence of blockers for known neurotransmitter receptors in the bladder wall. The RyR blocker ryanodine significantly increased the spontaneous contractile frequency in normal bladder strips, but failed to affect spontaneous contractions in DI muscle. Caffeine inhibited spontaneous contractile activity in both the DI and normal strips. After administering the L-type Ca\(^{2+}\) channel antagonist nimodipine, the myogenic spontaneous contractile activity was abolished in normal strips; in contrast, in DI strips, the amplitude of contractions was reduced but the frequency of contractions was unchanged. Western blot analysis showed that RyR expression was lower in DI muscle than in normal bladder muscle.

CONCLUSION
These results provide the first characterization of a loss of regulation of spontaneous contractile activity by RyRs in DI muscle associated with a significant decrease in RyR expression. RyRs in normal detrusor muscle act as negative-feedback regulators of spontaneous contractile activity, presumably by releasing Ca\(^{2+}\) that activates Ca\(^{2+}\)-dependent K\(^+\) channels to decrease contractility. This mechanism might be weakened in DI muscle, resulting in spontaneous contractile overactivity.

KEYWORDS
detrusor instability, ryanodine receptor, spontaneous contraction, sarcoplasmic reticulum

INTRODUCTION
Some studies and reviews have described a myogenic basis for the increased excitability and contractile activity of bladder smooth muscle that is associated with detrusor instability (DI) in both animal models and humans [1]. One of the most common causes of DI is BOO [2]. Spontaneous contractions occur in strips of detrusor dissected from animals or humans in vitro [3], and these contractions are increased in detrusor strips with DI resulting from BOO [4,5]. The spontaneous contractions are distinct from contractions mediated by motor nerves because they are insensitive to nerve blockers, but they are sensitive to some ion-channel antagonists [6,7]. Furthermore, spontaneous action potentials appear to initiate excitation-contraction coupling in bladder muscle [8,9]. Ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) are important modulators of excitation-contraction coupling in bladder myocytes [10,11]; Ca\(^{2+}\) release from RyRs might be triggered by Ca\(^{2+}\) influx via voltage-dependent Ca\(^{2+}\) channels (Ca\(^{2+}\)-induced Ca\(^{2+}\) release [10,12]); Ca\(^{2+}\) release from RyRs in the form of Ca\(^{2+}\) activates Ca\(^{2+}\)-activated K\(^+\) channels \(K_{Ca}\) including BK\(_{Ca}\) [13]. The characteristics of spontaneous contracture of urinary bladder smooth muscle appear to depend on these interactions [14,15]. In the present study, we focused on the role of RyRs in normal and DI bladder muscle.

MATERIALS AND METHODS
The studies used female Wistar rats aged 3–5 months from the Laboratory Animal Center of the Third Military Medical University. Rats were anaesthetized with sodium pentobarbital (40 mg/kg). The lower abdomen was shaved and scrubbed using povidone-iodine, and a vertical midline incision made. The urethra was exposed and a 3 F plastic catheter inserted into the bladder. Care was taken to identify the ureters and bladder base, and a 4/0 silk suture was passed around the proximal urethra and tied over the catheter. All BOO was induced by one investigator (J.H.). All rats were given three intraperitoneal injections of gentamicin within 48 h after surgery.

After 8 weeks of partial BOO, filling cystometry was used to determine bladder instability in obstructed and control rats. The rats, anaesthetized as above, were placed supine and the abdominal wall cleaned.
Through the same incision, the urinary bladder was exposed and gently freed from adhering tissues, emptied and then cannulated, via an incision at the dome, with a plastic cannula (0.6 mm internal diameter), which was purse-string sutured with silk thread. The free tip of the bladder cannula was connected to a pressure transducer (Nidoc970 urodynamic testing machine, Chengdu, China) and to a peristaltic pump for continuous infusion of warm furacillin solution (37 °C) into the urinary bladder at 10 mL/h. During infusion, intravesical pressure and voided urine volume were recorded continuously using a computer interface; DI was confirmed when recorded continuously using a computer.

During infusion, intravesical pressure was connected to a pressure transducer which was purse-string sutured with silk and a plastic cannula (0.6 mm internal diameter), cannulated, via an incision at the dome, with the bladder. Through the same incision, the urinary bladder was opened and the lamina propria was separated from the remaining bladder, labelled and harvested. The rat detrusor muscle in DI and normal physiological saline with periodic changes of bathing fluid. Steady-going spontaneous rhythmic contractions were generally observed after 60–90 min of equilibration, and were maintained for the remainder of the experiment. After 90 min of equilibration, 60 mmol/L KCl was added to the bathing medium to elicit a regular contractile response. Only strips showing reproducible contractile responses to 2–3 applications of 60 mmol/L KCl were studied. To minimize the possible effect of the activity of nerves in the detrusor strips, all experiments included a cocktail that contained tetrodotoxin (a blocker of neuronal Na+ channels) and antagonists for neurotransmitter receptors known to be present in the bladder [14].

The cocktail was prepared in physiological saline, and contained (in μmol/L): 1 atropine (muscarinic antagonist), 1 phentolamine (α-adrenergic antagonist), 1 propranolol (β-adrenergic antagonist), 1 α,β-methylene ATP (purinergic depletor), and 1 tetrodotoxin (Hebei Aquaculture Institute, Hebei, China). To verify the effectiveness of the antagonists on neurally mediated contractions, the strips were subjected to electrical stimulation to trigger nerve activity in the presence and absence of the cocktail. Strips were stimulated in a modified organ chamber equipped with a stimulating electrode for 2 s, using a 0.3-ms pulse of 20 mA at 20 Hz (produced by State Key Laboratory of Trauma, Burns and Combined Injury, Chongqing, China). The contractions evoked by three successive stimulations were averaged for each strip, before and after exposure to the cocktail. Ryanodine was prepared in dimethyl sulfoxide, caffeine in distilled water and nimodipine in ethanol (all from Sigma, and as stock solutions in small aliquots at −20 °C).

Crude SR microsomes were obtained from rat detrusor muscle in DI and normal preparations by differential centrifugation, and stored at −80 °C. Partially thawed tissues were homogenized in 10 volumes of homogenisation buffer (10 mmol/L Hepes, pH 7.2, containing 0.25 mol/L sucrose, 0.5 mmol/L EDTA, 1 mmol/L dithiothreitol, 0.2 mmol/L PMSF, 1 mmol/L benzamidine, 10 μmol/L leupeptin, 1 μmol/L pepstatin A and 100 mmol/L aprotinin). Homogenates were centrifuged (25 min at 4000 g) and the supernatants re-centrifuged for a further 60 min at 100 000 g. Microsomal pellets were re-suspended in four volumes of homogenisation buffer, then rapidly frozen and stored at −80 °C until required [16]. All procedures were conducted at 4 °C.

SDS-PAGE was used with 5% (w/v) separating and 3% (w/v) stacking gels cast in a minigel system. Rat detrusor muscle microsomes (50 μg, total protein determined by a modification of the Lowry method) were denatured for 5 min at 95 °C in loading buffer (0.1 mol/L Tris/HCl, pH 6.8, containing 2% w/v SDS, 2% v/v 2-mercaptoethanol, 10% v/v glycerol and 50 μg/mL bromophenol blue). Electrophoresis was performed at a constant current (20 mA per gel) and gels either stained with Coomassie Brilliant Blue R250 or electrophotographically transferred on to Immobilon-P (polyvinylidene difluoride) membrane (Millipore, USA) at 15 °C for 1 h at 28 v, then for 18 h at 400 mA. After blocking for 3 h with 4% w/v non-fat dried milk proteins in PBS, the blot was incubated overnight at 4 °C with RyR antibody (Santa Cruz Biochemicals, CA, USA). The immunoblot was developed with peroxidase-coupled secondary antibody (Beijing Zhongshan Biotechnology Co., Beijing, China) and 3,3'-diaminobenzidine/H2O2.

Data are presented as the mean (SEM) and the number of preparations. In the smooth muscle physiology experiments, the agents were applied for 15 min, with the last 5 min taken as the analysis period. Control data were obtained in the 5 min before applying the treatment agents. Differences were assessed by t-test or one-way ANOVA, where appropriate, and the null hypothesis rejected for P < 0.05.

RESULTS

To standardize the initial recording conditions, detrusor strips were stretched to a resting load of 2 mN in length increments of 2–3 mm after loading strips while slack for 20-min incubation. Spontaneous contractions appeared steadily =60 min after stretch. The strips were then exposed to physiological saline containing tetrodotoxin and a cocktail of blockers for transmitter receptors known to be expressed in bladder wall. In the presence of the cocktail, spontaneous contractions persisted in DI strips (Fig. 1); the mean spontaneous contraction frequency decreased while the mean amplitude increased (Table 1). This persistence of contractile activity in the presence of neurotransmitter antagonists suggests that
the spontaneous contractions in DI strips have a myogenic basis.

To verify that nerve-mediated contractions would be eliminated by the cocktail, electrical stimuli were applied to DI strips (five; Fig. 2); this evoked contractions with a mean amplitude of 4.89 (1.05) mN and a mean duration of 3.5 (0.8) s. The stimulus-evoked contractions almost disappeared after applying the cocktail for 15 min, and partially reappeared after 15 min washout with normal physiological saline; after washout, stimulus-evoked contractions were 2.44 (0.88) mN in amplitude and 3.1 (0.3) s in duration. Accordingly, we applied the cocktail for the rest of the experiments to minimize the impact of neurogenic contractions.

RyRs release Ca\(^{2+}\) from the SR into the cytoplasm, and ryanodine inhibits transient Ca\(^{2+}\) peaks and the associated spontaneous transient outward currents in bladder muscle cells [13]. In normal detrusor strips, ryanodine (10 \(\mu\)mol/L) increased the mean frequency of spontaneous contractions (Fig. 3b; Table 1). The mean contraction amplitude was not significantly affected (Table 1). In contrast, in DI strips, ryanodine did not alter either the frequency (Fig. 3a) or the amplitude of spontaneous contractions. The baseline spontaneous contractile activity before applying ryanodine was significantly different between the DI and normal detrusor strips in frequency and amplitude (Table 1), but in the presence of ryanodine, the contraction frequency was not significantly different between the DI and normal detrusor strips. The doubling of contraction frequency induced by ryanodine in normal strips suggests that RyRs modulate spontaneous contractions in normal conditions. It seems possible that Ca\(^{2+}\) peaks released by RyRs might act to decrease the whole contractile force; interestingly, this modulation by RyRs is lost in DI strips.

Caffeine releases Ca\(^{2+}\) from intracellular stores by RyRs, and caffeine-induced Ca\(^{2+}\) release is independent of carbachol-evoked Ca\(^{2+}\) release [17]. After applying 10 mmol/L caffeine to DI strips, spontaneous contractions gradually attenuated (Fig. 4a), and this attenuation was reversed only slowly after the caffeine was washed out with a fresh cocktail of physiological saline. It took a long time (>30 min) for rehabilitation of the steady-going and regular contractions. In DI strips, the mean frequency of contractions and the amplitude were reduced by caffeine (Table 1). There was a similar response to caffeine in normal detrusor strips, where the frequency and the amplitude were reduced (Table 1). Thus 10 mmol/L caffeine inhibited spontaneous contractions in detrusor strips in vitro from both normal and DI bladders.

Ca\(^{2+}\) influx is necessary for maintaining spontaneous contractions in detrusor muscle [14,17]. To test the role of Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels, we applied the dihydropyridine inhibitor, nimodipine (0.1 mmol/L). Nimodipine abolished the rhythm of spontaneous contractions and attenuated the contractile amplitude in normal detrusor strips (five, Fig. 5b), but was less effective in DI strips (five, Fig. 5a); in these, the contraction amplitude decreased sharply but the frequency was maintained. These results
Results were similar in normal detrusor strips. In DI strips, contractions were attenuated.

When the actions of known neurotransmitters were blocked [14], in the present study, we observed changes in RyR, and observed changes in targetted the RyR, and observed changes in expression and regulation of spontaneous contractions in vitro. In these experimental conditions, spontaneous contractions in isolated detrusor occur that do not appear to be mediated by nerves (Fig. 1); it is unclear whether they represent normal physiological contractile activity or are an artificial phenomenon in the condition of isolated preparations, but the increased frequency of spontaneous contractions in detrusor strips from DI bladder might correspond to the uninhibited contractions identified in vivo by filling cystometry. These contractions might represent a background activity of muscle function that determines the overall resting tone of the detrusor smooth muscle [23].

DISCUSSION

In the present study we used a BOO-induced model of DI in rats, which shares similar pathogenesis with DI after obstruction in other animal models, and in humans in clinical practice [2,3,18]. The bladder responds to the increased afterload by hypertrophy to normalize wall tension, and with persistent afterload, myogenic disorders appear, including DI. After surgically creating partial BOO in the rat, the uninhibitable rises in detrusor pressure measured during filling cystometry confirmed DI, but the precise course of the pathological changes is still elusive and needs further investigation [19]. The bladder has localized micromotions in isolation, or generalized contractions when the bladder fills [20,21]. The detrusor strips contract spontaneously, and there is widespread activity that is independent of input from the CNS [22], and which persists when the actions of known neurotransmitters are blocked [14]. In the present study, we targeted the RyR, and observed changes in L-type Ca\(^{2+}\) channels are essential for initiating the rise of intracellular Ca\(^{2+}\) that is associated with spontaneous contractions [14,17]. The contraction pathway evoked by various neurotransmitters (e.g. ATP and acetylcholine) or Ca\(^{2+}\) influx, is inhibited by dihydropyridine derivatives such as nifedipine in a dose-dependent manner in the urinary bladder [24]. There is no doubt that an increase in intracellular Ca\(^{2+}\) concentration is key to activating contraction in detrusor muscle; this increase can be due to Ca\(^{2+}\) influx from the extracellular space and/or Ca\(^{2+}\) release from intracellular stores. L-type Ca\(^{2+}\) channels might play a pivotal role in maintaining Ca\(^{2+}\) entry and in refilling intracellular Ca\(^{2+}\) stores [17,24]. Nimodipine, an inhibitor of L-type Ca\(^{2+}\) channels, inhibited spontaneous contractions of detrusor muscle strips, but the underlying rhythm of contractions was maintained in most DI strips, although their contraction amplitudes were too small to be analysed. To identify the Ca\(^{2+}\) entry pathways required for spontaneous contractile activity, further investigations are required (such as T-type Ca\(^{2+}\) channels [25] in DI).

Ca\(^{2+}\) sparks are caused by opening of RyRs in the SR and are associated with the opening of L-type Ca\(^{2+}\) channels (Ca\(^{2+}\)-induced Ca\(^{2+}\) release), and can be modulated by ryanodine, voltage, Ca\(^{2+}\), caffeine and the location of events [26]. RyRs contribute high 'local' Ca\(^{2+}\) concentrations, which are not in equilibrium with Ca\(^{2+}\) buffers and which could locally reach much higher levels than the 'overall' Ca\(^{2+}\) concentrations measured during depolarization in bladder smooth muscle. In addition, the appearance of 'hot spots' of Ca\(^{2+}\) in bladder smooth muscle cells [9] closely parallels the rise in \(K_{\text{Ca}}\) currents, reaching a peak within 20 ms of the start of depolarization, whereas the rise in 'overall' Ca\(^{2+}\) is much slower. The Ca\(^{2+}\) 'hot spots' might be attributed to the site of SR in the subplasmalemma that has been called the 'superficial SR'; unlike Ca\(^{2+}\)-induced Ca\(^{2+}\) release in cardiac muscle, that in smooth muscle is not tightly linked to the gating of L-type Ca\(^{2+}\) channels [10]. Meanwhile, large-conductance Ca\(^{2+}\)-dependent K\(^+\) (BK\(_{\text{Ca}}\)) channel currents are activated by Ca\(^{2+}\) sparks
from RyRs in the SR, and BKca, channels are critical in regulating bladder smooth muscle excitability and contractility [27]. RyRs decreased spontaneous contractions in normal detrusor strips but this negative-feedback regulation was lost in DI muscle (Fig. 3). It seems reasonable that Ca2+ sparks by RyRs would decrease excitability through activation of K+ channels, leading to inhibition of Ca2+ influx through voltage-dependent Ca2+ channels. Ca2+ release by RyRs limits contraction frequency in detrusor muscle, as blocking RyRs doubled the contraction frequency. However, in DI muscle, ryanodine had no significant effect on spontaneous contractions, thus we hypothesized that RyRs are dysfunctional in DI muscle. RyR dysfunction could result from decreased expression of RyR protein, and Western blotting analysis supported this hypothesis (Fig. 6); RyR expression was significantly decreased in DI smooth muscle. Thus it seems possible that the decrease of RyR expression in DI muscle results in increased contraction frequency; this could result from either less hyperpolarization, or from less inactivation of voltage-dependent Ca2+ channels during the resting phases between bursts of action potentials.

However, RyR Ca2+ release and the sequence of negative regulation are complex and under the control of several factors. In isolated rat uterus, ryanodine had almost no effect on the spontaneous cytosolic Ca2+ or force transients, and Ca2+-induced Ca2+ release plays little role in SR Ca2+ release [28]. The discrepancy might also be attributed to the different tissue orientation or the absence of the cocktail. Further evaluation is needed of differences in Ca2+ sparks effects between the DI and normal smooth muscle.

Caffeine, which mobilizes intracellular Ca2+ stores via RyRs, markedly enhances spontaneous transient outward currents caused by activating BKca channels [29]. In both normal and DI bladder strips, caffeine generally blocked spontaneous contractions (Fig. 4). Caffeine-induced intracellular Ca2+ transients are independent of muscle potential under physiological conditions [17]. Some intracellular Ca2+ stores function as a superficial buffer barrier for Ca2+ influx, limiting the degree of contractile protein activation by diverting some of the Ca2+ that enters the cell away from the cytosolic compartment [30]. The subplasmalemmal Ca2+ store releasing Ca2+ (‘hot spots’ Ca2+) activates K+ currents in the plasmalemma to facilitate action potential re-polarization or hyperpolarization and inhibit Ca2+ influx [9]. Moreover, caffeine increases intracellular levels of cAMP via its phosphodiesterase activity; cAMP causes muscle relaxation, and might directly influence the [Ca2+]i-tension relationship. If so, the latter would play a major role [31,32].

In conclusion, the present results indicate that RyR Ca2+ release plays a negative role in spontaneous contractile activity. The presumed mechanism is that RyR Ca2+ release decreases contractility by inducing the activation of Kca channels [14]. The present results indicate that the effect of this cross-talk between RyRs and ion channels might be weakened in DI muscle, resulting in spontaneous contraction overactivity.

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CONFLICT OF INTEREST
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Abbreviations: DI, detrusor instability; RyR(s), ryanodine receptor(s); SR, sarcoplasmic reticulum.
Unseen forces at AUA 2005?

This time last year I was accused, even by my impeccable standards, of being unduly cynical and negative in my assessment of the impact and visibility of the pharmaceutical industry at the AUA meeting in San Francisco, which led me to conclude that "Those participants at the recent AUA meeting looking for new and exciting disclosures from the pharmaceutical industry would have been sorely disappointed. The other 99% of attendees could have been entering a re-run of the previous year’s AUA in Chicago, but in a more convenient downtown setting and at a higher ambient temperature" [1]. So what has changed about this year’s AUA? As a minimum, the words ‘convenient’, ‘higher ambient temperature’ and ‘Chicago’ can be omitted from the above quote and once again it was what was not being reported by industry in public that was probably more interesting and important than what was.

As would be anticipated from previous AUAs (and even more so at the recent EAU), audience attendance at the pre-AUA industry-sponsored symposia was low in both head-count and energy level. Like many investors on the stock market, at most symposia attendees appeared to be looking for their exit strategy at the time of entry. In part this may be due to the almost universal policy of vetoing interesting questions from the audience. Or is this my paranoia? Somewhat ironically, by way of contrast, was any symposium related to the use of dapoxetine in premature ejaculation. Certainly, at least in terms of novelty of data, dapoxetine was the star of the show. In terms of quantity Prof. Roehrborn, with 28 presentations (most of them personal), was well positioned but the overall prize on both the quality and quantity was the BJU International’s own Georg Bartsch, with at least 39.

On the basis of the data presented at the AUA, the interest in dapoxetine is easy to understand. Its pharmacokinetic profile, with short T_{max} and half-life that rendered it ineffective as an antidepressant, makes it near ideal for ‘on demand’ use in the treatment of premature ejaculation [2]. As the NDA was submitted at the end of 2004 we (assuming approval of dapoxetine) may well be about to enter the second phase of the sexual health revolution, i.e. the arrival of effective therapy for premature ejaculation. It is almost certain that other companies will be ‘dusting off’ their own SSRIs but dapoxetine will be difficult to match. In particular, the drug is effective (on demand) after a single dose, whereas the ‘off label’ use generally requires some degree of chronic drug exposure.

Although dapoxetine is potentially a drug ‘fit for purpose’, i.e. the rapidity of action and fast plasma clearance gives patients what they want, it may well represent the start of the story, as there are several unanswered questions. The first is the identity and even the location of the 5-HT (serotonin) receptor subtype involved in producing the acute effect of dapoxetine; the elevated synaptic 5-HT levels produced by inhibition of the serotonin transporter must act via a receptor. Rest assured the pharmaceutical industry, using the type of animal models described by Francois Guiliano, are actively researching this area. There are certainly enough 5-HT receptor subtypes to choose from, with up to 15 having been described so far. The other major question is how dapoxetine will eventually be described, e.g. could we be on the cusp of a description as an ejaculo-selective serotonin transport inhibitor (ESSTI) [2]?

This time last year, with the arrival of duloxetine in the European marketplace and the entry of RO 115-1240, the α1A/L...
agonist [3], we would have expected the AUA literature to be burgeoning with information on these or mechanistically similar agents. Unfortunately the development of the Roche drug has been terminated due to unexpected toxicology and the sponsors (Lilly) have removed duloxetine (hopefully only temporarily) from the FDA approval process.

For the pharmaceutical industry duloxetine would always have been a difficult act to follow as, with the possible exception of Karl Thor, no-one is really sure how it works. This merely reflects the relative complexity of understanding overall neurophysiological control mechanisms. In the case of \( \alpha \)-agonists, as several companies (including Abbott, Pfizer and Roche) have failed to deliver efficacy without blood pressure elevation, this may represent the end of the story. An alternative approach involving local delivery of the \( \alpha \)-agonist is being evaluated in phase II studies by Plethora Solutions Ltd. GSK may well hold ‘pole position’ in the search for effective therapy for stress incontinence; their \( \beta_3 \)-adrenoceptor agonist (solabegron, GW 427353) being in the early stages of development. A major issue will be whether it is possible to separate bladder and sphincter effects from obtrusive tachycardia and elevation of core temperature.

Elsewhere within the AUA there is considerable evidence that the pharmaceutical industry have not lost interest in \( \alpha \)-blockers for BPH/LUTS with ‘new’ data on naftopidil and silodosin (KMD 3213) emerging. Data interpretation is in general complicated by the comparison only with a homeopathic dose of tamsulosin (0.2 mg). Otherwise, in the ‘my \( \alpha \)-blocker is better than yours’ stakes, companies are concentrating on product ‘evergreening’ (i.e. patent extension) by launching new formulations; doxazosin (Cardura XL), alfuzosin (Uroxatral) and the TOCAS formulation of tamsulosin.

As in the case of BPH/LUTS, there appears to be little happening in urinary urge incontinence (UII)/overactive bladder (OAB). We may well be at the end of the road with strategies designed to reduce dry mouth, with new generation M3-selective agents and patches appearing to have little impact on the overall clinical profile. Few companies, with the possible exception of Roche, remain interested in selective antimuscarinics. The only sign of major activity is from Dynogen, who are looking for alternative mechanistic strategies, have a combination product in clinical development and have identified potentially exploitable calcium currents in bladder sensory neurones.

In many other areas, including oncology, although here presumably much basic research is being undertaken, beyond presentations on microtubule inhibitors and endothelin antagonists, industry is saying little.

None of the above in any way detracts from the value of the AUA to the urologist, only that for a variety of reasons (largely regulatory and patent) it is not a forum for the pharmaceutical industry to present high science or early stage clinical data.

Next month I will focus on combination products and ‘one drug fits all’ for the patient and his or her co-morbidities.

1 Wyllie MG. It’s new and exciting- can it be AUA 2004? BJU Int 2004; 94: 437–8
2 Wyllie MG. The era of ESSTIs is slowly approaching? BJU Int 2005; 96: 181–2
3 Musselman DM, Ford DP, Gennevois DJ et al. A randomized crossover study to evaluate RO 115-1240, a selective \( \alpha_1A/L \) partial agonist, in women with stress incontinence. BJU Int 2004; 93: 78–83

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A simple modification to the Albarran deflector enhances endoscopic control

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Accepted for publication 27 January 2005

INDICATIONS
The Albarran deflector is one of the oldest and most frequently used endoscopic instruments employed by urologists [1]. However, it is often cumbersome to control instruments in all planes of view through the Albarran. A simple modification of converting the flat Albarran plate into a cylinder facilitates control of catheters, laser fibres or electrodes placed through the instrument.

METHOD
A circumferential cylinder or ring is placed on the deflecting portion of the Albarran deflector, and the Bugbee electrode, ureteric catheter or laser fibre is placed through this cylinder or ring.

COMPARISON WITH OTHER METHODS
The cylinder or ring is no more difficult to use than the standard Albarran deflector. The ureteric catheter, laser fibre or electrode is threaded through the ring before placing the instrument into the cystoscope sheath. Using a 30° lens, the tip of any of these devices always remain in the surgeon’s field of view and cannot migrate from the field of vision, as can happen with the standard Albarran deflector (Figs 1 and 2).

FIG. 1.

a, Placing the electrode through the ring, and b, the field of vision maintained as the electrode is extended.
ADVANTAGE AND DISADVANTAGES

The distinct advantage of the cylinder over the deflector is that it maintains the position of any instrument placed through it in the surgeon’s visual field. The cylinder is proximal enough that it does not block the surgeon’s view of the tip of the instrument.

DIFFICULTIES AND COMPLICATIONS

The cylindrical ring, which is 3 mm long, is made of stainless steel. It has been used over 100 times in an ex-vivo setting and does not fatigue or lose its shape in any way. There has been no damage to any catheter, electrode or laser fibre placed through it.

CONFLICT OF INTEREST

None declared.

REFERENCE


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Cystoscopic removal of a JJ stent using a suture 'lasso'

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Accepted for publication 31 January 2005

INDICATIONS

Cystoscopic removal of a JJ ureteric stent in the neonate and young infant (inserted, e.g. after a neonatal pyeloplasty) can present technical difficulties because of the small calibre of the urethra. While a Wolf 7.5 F rigid cystoscope can be passed with ease, its working channel (4 F) will not accommodate a grasping forceps with jaws large and robust enough to grasp the JJ stent (usually 3 F or 4.7 F) adequately to enable removal without slipping. Alternatives are open cystotomy or to wait for several months for the urethra to enlarge sufficiently (with the risk of infection, stone encrustation, migration, etc.). We present a simple technique to circumvent this problem.

METHOD

A strong, stiff, monofilament suture e.g., 0 polydioxanone or 0 polypropylene, is kinked into a sharp, narrow apex to fit it into the channel (Fig. 1). It is then passed through the working channel of a 7.5 F Wolf cystoscope and the ends both brought out of the cystoscope (Fig. 2). Once inside the bladder, because of the stiffness of the monofilament, advancing one limb opens up a loop inside the bladder; advancing both limbs will not open the loop. The stiffness and monofilament nature of the suture used will prevent buckling inside the working channel. Suitable positioning and gentle withdrawal of the suture will 'lasso' the J-loop onto the stent (Fig. 3). The loop and the stent are then pulled snug onto the tip of the cystoscope that is then withdrawn en masse.

We have used this technique successfully on several occasions and its use can be extended to the older patient when suitable grasping forceps are unavailable.

CONFLICT OF INTEREST

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ANALYSIS OF HER2 EXPRESSION IN PRIMARY URINARY BLADDER CARCINOMA AND CORRESPONDING METASTASES

Sir,

We were interested in this recent article by Gardmark et al. [1], in which they describe their analysis of 80 patients with metastatic TCC of the urinary bladder to evaluate “the suitability of HER2 as a target in preparation for planned systemic therapies of HER2-positive urinary bladder carcinoma”. The authors examined HER2 expression using two different immunohistochemical stains (the HerceptTest and modified HerceptTest, MH).

According to these authors, HER2 staining was positive in 79% of the primary tumours and 62% of the metastases when the MH staining and target-score criteria were applied. Moreover, in their consecutive section, they underlined that the primary goal of the study was to assess over-expressed receptors for possible treatment with HER2 targeting agents.

To that end, in our opinion, essential requisites are those of evaluating not only HER2 expression, but also gene amplification and chromosome 17 aneusomy. Many studies have evaluated HER2 protein expression and gene amplification rates; they report HER2 expression rates of 2–74% [2]. This variation is partly a result of differences in the methods used to assess HER2 and variability in the reporting of data. Both overexpression with no gene amplification and heterogeneity in HER2 expression are more common in bladder cancer than in breast cancer [3,4]. This is mainly explained by a high level of chromosome 17 polysomy in TCC. Latif et al. [5], in 75 TCC classified as G3pT2, showed polysomy 17 in 97%, increased HER2 copy number in 92% and HER2 gene amplification in 7% of cases examined. In our previous investigation, we reported that chromosome 17 aneusomy is present in 84% of examined superficial bladder cancer, but also in 47% and 21% of normal-appearing adjacent proximal and distal mucosa, respectively [6]. Moreover, in our recent article, we found, in 48 advanced bladder cancers, chromosome 17 polysomy in 42% and HER2 gene amplification in 15% of specimens. Our study strongly confirmed the importance of chromosome 17 polysomy in detecting HER2 amplification [7]. Evidence from breast cancer suggests that only tumours with HER2 gene amplification respond to the anti-HER2 therapy (Herceptin) [8]. If this were true for bladder cancer, only a low proportion of patients would be suitable for treatment.

In conclusion, Gardmark et al. should be more careful in stating that immunohistochemical staining is sufficient to select HER2-targeting patients. We suggest that it would be useful to re-evaluate these results using fluorescence in situ hybridisation, which permits the simultaneous assessment of the chromosome 17 aneusomy and HER2 gene status.

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expression of the receptor on the cell surface is not important. Perhaps there may be some confusion as to the role of trastuzumab. Our approach involves the antibody merely as a carrier delivering the nuclide at the cell surface, not primarily acting alone or combined with chemotherapy, as in the treatment of breast cancer. In fact, the most recent work at our laboratory has been with other antibodies targeting the receptor.

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C-REACTIVE PROTEIN IS SIGNIFICANTLY ASSOCIATED WITH PROSTATE-SPECIFIC ANTIGEN AND METASTATIC DISEASE IN PROSTATE CANCER

Sir,
I read with interest this paper [1] investigating the relationship between PSA, stage of prostate cancer and the important but non-specific inflammatory marker, C-reactive protein (CRP). The authors state in the final paragraph of their discussion: “The present finding of a significant correlation of CRP and PSA levels suggests that more study may further illuminate the causal role of inflammation in prostate cancer.” The authors have used the statistical technique of multiple regression, as opposed to correlation. These are closely related; however, using multiple regression may have been unnecessary in this context. The latter technique gives a precise relationship between two variables, PSA and CRP in this instance, which is expressed as a regression equation. This has the implied use for predicting the dependent variable, CRP, and that this would be clinically useful. We implied nothing of the sort in our article, nor did we say anything about clinical utility. Dr. Abedin is reading into our work something that simply is not there. We did feel that the r² value of 0.207, contrary to Dr. Abedin’s assertion, was useful for the reader to know because it shows that the PSA level explained 20.7% of CRP level; therefore, regression was the appropriate analysis to use. (Dr. Abedin incorrectly states that the r² value of 0.207 was from a multiple regression; in fact, it was from the simple regression shown in Figure 2.)

Prostate cancer and PSA levels are complicated biological phenomena, poorly understood, and obviously do not correlate perfectly with inflammation, CRP, or, probably, anything else.

STEVEN LEHRER, MD

LAPAROSCOPY FOR IMPALPABLE TESTES

Sir,
I read with interest this article by Patil et al [1] investigating the relationship between PSA, stage of prostate cancer and the important but non-specific inflammatory marker, C-reactive protein (CRP). The authors state in the final paragraph of their discussion: “The present finding of a significant correlation of CRP and PSA levels suggests that more study may further illuminate the causal role of inflammation in prostate cancer.” The authors have used the statistical technique of multiple regression, as opposed to correlation. These are closely related; however, using multiple regression may have been unnecessary in this context. The latter technique gives a precise relationship between two variables, PSA and CRP in this instance, which is expressed as a regression equation. This has the implied use for predicting the dependent variable, CRP, and that this would be clinically useful. We implied nothing of the sort in our article, nor did we say anything about clinical utility. Dr. Abedin is reading into our work something that simply is not there. We did feel that the r² value of 0.207, contrary to Dr. Abedin’s assertion, was useful for the reader to know because it shows that the PSA level explained 20.7% of CRP level; therefore, regression was the appropriate analysis to use. (Dr. Abedin incorrectly states that the r² value of 0.207 was from a multiple regression; in fact, it was from the simple regression shown in Figure 2.)

Prostate cancer and PSA levels are complicated biological phenomena, poorly understood, and obviously do not correlate perfectly with inflammation, CRP, or, probably, anything else.

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REPLY
Dr. Abedin writes that we implied that the multiple regression we used would allow the prediction of the dependent variable, CRP, and that this would be clinically useful. We implied nothing of the sort in our article, nor did we say anything about clinical utility. Dr. Abedin is reading into our work something that simply is not there. We did feel that the r² value of 0.207, contrary to Dr. Abedin’s assertion, was useful for the reader to know because it shows that the PSA level explained 20.7% of CRP level; therefore, regression was the appropriate analysis to use. (Dr. Abedin incorrectly states that the r² value of 0.207 was from a multiple regression; in fact, it was from the simple regression shown in Figure 2.)

Prostate cancer and PSA levels are complicated biological phenomena, poorly understood, and obviously do not correlate perfectly with inflammation, CRP, or, probably, anything else.

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1 Patil KK, Green JSA, Duffy PG. Laparoscopy for impalpable testes. BJUI 2005; 95: 704–8

THE ROLE OF URINARY URGENCY AND ITS MEASUREMENT IN THE OVERACTIVE BLADDER SYMPTOM SYNDROME: CURRENT CONCEPTS AND FUTURE PROSPECTS

Sir,
I read this article [1] with interest and congratulate the eminent authors for tackling this issue. We have in common our concern to improve the treatment for this symptom syndrome, but I may have additional insight to offer, as I suffer from it. I began research into lower urinary tract physiology before I had any urological problems, but over the years my bladder has become seriously overactive, something that appears to happen to many who contracted poliomyelitis as young adults. Although I can only speak for what applies to me, I find that the authors are wrong in some of their assessments, and offer the following observations.

The definition of urgency as ‘a sudden compelling desire to pass urine, which is difficult to defer’ is accurate, and I agree with this. However, over the issue of whether urge and urgency are a continuum, or urgency is pathological and not experienced by normal people, in my experience it is the former. I seem to remember from childhood friends...
queuing for a lavatory, hopping around from one foot to the other desperate to get in, were they not experiencing urgency? Certainly for me the sensation of normal urge and compelling urgency differ only in intensity. What does differ though is that urgency now can come on suddenly, whereas before, normal urge if unattended to would progress to urgency.

Another thing that I disagree with is the concept that it is not possible to defer the void after an urgency episode, or that there is a ‘warning time’ that is measurable. For me the most important thing is to defer voiding after an urgency episode, because my motor disability means that it takes me a considerable time to get to the toilet, get out of my wheelchair, sort out garments and prepare to void (normally into a jug). While I am experiencing urgency I cannot do any of these things. Although I have several physical and psychological strategies to suppress urgency (I learnt a new one from Richard Turner Warwick the other day), they do not always work, and occasionally I leak urine before I can get up, but usually I manage to suppress it. Then if I have any sense, I empty my bladder. Otherwise it may be anything between a few minutes to half an hour before I get another attack, and then I am likely to leak. Even if I do suppress the first incidence of urgency and get to the bathroom, I am very likely to get a second urgency episode while I am sorting out garments, and more often than not I leak some urine. This most effectively stops the urgency, and I can then void normally. Urgency is clearly triggered by psychological stimuli, and by increasing bladder volume. In my experience it is also related to the composition of my urine; urinating into a jug gives plenty of opportunity to measure volume and assess the colour, and urgency comes on with small volumes of concentrated urine, but larger volumes of dilute urine.

With reference to quality-of-life issues, the most negative feature is the leakage; urgency is a nuisance, but it is the knowledge that it is very often associated with leakage that is the problem. It is not easy for me to change my clothes or to deal with any damp seats. Currently the volumes I leak are small enough to deal with using pads (I supplement these with Sainsbury’s super-absorbent clothes, which are easy to rinse out and re-use later). Urgency does not yet trigger a proper void – I hope it never will.

I do not routinely use drugs to control my bladder, although I may have to in the future, but use antimuscarinics when I travel. They are extremely effective for me initially, suppressing urgency and meaning that I can avoid leaking and cope with travelling. However, they are less effective with time, although usually being reasonably effective for ~10 days. However, when I stop taking them I have several days with very bad overactivity before things settle down again. As a pharmacologist, this is exactly what I would expect. The body is set up to adjust the sensitivity of receptive organs to the amount of stimulation received by the receptors. I would far prefer to use a drug that did not work through receptors, and am looking forward to a bladder-selective K-channel-opening drug, if ever they develop one.

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THE INCIDENCE AND TREATMENT OF LYMPHOCELES AFTER RADICAL RETROPUBIC PROSTATECTOMY

Sir,
We read with interest this article by Pepper et al [1], on the incidence and treatment of lymphoceles after radical retropubic prostatectomy and bilateral pelvic lymph node dissection (PLND). They rightly highlighted the need for suspicion of a lymphocele in such patients presenting with vague abdominal pain and discomfort. We would like to highlight a few issues that may need clarification.

We suggest the title should relate to ‘symptomatic lymphoceles’, as it is well documented that the incidence of sub-clinical lymphoceles is ~40% [2]. Interestingly, eight of the nine patients who had an ultrasonographic diagnosis of lymphocele proceeded to a confirmatory CT. The ninth patient ‘presumed’ clinically to have a lymphocele after failure to image on ultrasonography, did not have a CT scan. We think that this patient should not have been included in the data without confirmatory imaging.

We were surprised that all 260 patients had had bilateral PLND, irrespective of the stage of the disease. We would differ with the authors’ approach and only undertake PLND in higher-risk patients, as in other centres worldwide [3,4]. Indeed, the senior author of the paper (A.V.K.) took part in a multicentre audit of radical prostatectomy involving 854 patients, 701 of whom had PLND, the positive local node metastatic rate being only 4.9% [5]. We think that subjecting all patients to PLND, irrespective of the risk factors, carries extra morbidity (other than lymphoceles), increases the operative time and not least the financial burden on the health service. With such significant morbidity, the authors made no attempt to justify undertaking PLND. It would have been helpful if they had commented on the histological status of the dissected lymph nodes and whether there was a higher incidence of positive nodes in the patients with lymphocele, as there appears to be a greater risk of lymphocele formation in patients with tumour-bearing lymph nodes [6].

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1 Pepper RJ, Pati J, Kaisary AV. The incidence and treatment of lymphoceles after radical retropubic prostatectomy. BJU Int 2005; 95: 335–40


INTRODUCTION

Since the early 1980s ileocaecal segments have been used in the Mainz pouch technique for orthotopic bladder substitution to the bladder neck or to the urethra [1–3]. The Mainz pouch provides a low-pressure reservoir with good capacity [4]. In orthotopic bladder substitution to the urethra, functional characteristics compare favourably with other types of ileal-pouch bladder substitutes [5].

However, the surgical technique was criticised for being tedious and time-consuming, and that the technique of antirefluxive submucosal tunnel ureteric implantation was not applicable to dilated, irradiated or short ureters.

In 2001 we introduced surgical modifications to simplify the construction of an orthotopic Mainz pouch, and to use the same standardized technique of ureter implantation for normal, dilated and even very short ureters, but still providing antireflux protection [6].

PLANNING AND PREPARATION

Indications. The main indication for orthotopic bladder substitution to the urethra is bladder cancer, when cystoprostatectomy (or cystectomy in females) allows preservation of the sphincteric urethra. This is generally possible in the absence of urothelial cancer in the prostate (bladder neck in females) and at the surgical margins (frozen-section analysis) of the transected urethra. Orthotopic bladder substitution to the trigone (with no ureteric reimplantation) or to the bladder neck is indicated in benign conditions which nevertheless require subtotal cystectomy, e.g. interstitial cystitis or severe detrusor hyper-reflexia. Whether or not ureteric reimplantation into the pouch is preferable depends on the presence or absence of vesico-ureteric obstruction and/or reflux.

Patient selection is critical for the functional success of the procedure; in those aged ≥70 years the results for urinary control become less favourable. In patients with a neurogenic bladder and females, capability and willingness to perform transurethral clean intermittent self-catheterization (CISC) must be checked before surgery. In handicapped and very obese patients catheterization of a continent abdominal stoma might be preferable to transurethral CISC.
SPECIFIC INSTRUMENTS AND MATERIALS

- Optical loupes (×2.5–3.5, 50 cm focal length);
- Headlight;
- Babcock clamps;
- 4/0 glyconate monofilament sutures, double-armed with two 5/8 needles (FR 26) for urethral anastomosis;
- 4/0 poly p-dioxanone monofilament sutures on a 1/2 needle (HR22) for ileo-ascendostomy, pouch suturing;
- 4/0 polydioxanone on a straight needle;
- 4/0 polyglytone monofilament sutures on a 3/8 needle (P-13) for fixation of stents, pouchostomy;
- 7/0 polyglycolic acid braided sutures on a 3/8 needle (DR10) for ureteric anastomosis;
- 6/0 glyconate monofilament sutures on a 1/2 needle (HR13) for ureteric anastomosis;
- 6 and 8 F polyurethane/polypropylene ureteric stents;
- 10 F pigtail pouchostomy catheter;
- Poly GIA-75 staplers (lactomer absorbable copolymer staples).

The day before surgery the bowel is cleansed by administering 3 L of polyethyleneglycol solution. During surgery antibiotics (ampicillin/clavulanic acid and metronidazole) are administered and continued afterward for 7–10 days.

The patient is placed supine on the table, allowing access to the external meatus of the urethra to place a transurethral catheter during surgery. For urethro-intestinal anastomosis, a slight Trendelenburg position is helpful.

INTRAOPERATIVE DECISION-MAKING: SUTURING OR STAPLING THE POUCH?

Whether suturing or stapling of the pouch is used depends primarily on bowel diameter and secondly on the surgeon’s preference. In children and patients with a small diameter of small bowel and/or caecum and ascending colon, the standard sutured technique is preferable. In all others, it depends on the surgeon’s preference. However, as the length of bowel segments to be resected differs slightly with either technique, this decision has to be made before resecting the bowel. In the following, both the sutured version (Figs 1–7) and the stapled version (Figs 8–16) are illustrated.

If ureters have to be cut short because of irradiation damage or urothelial tumours, again this has to be considered before resecting the bowel segments, to allow ileal ureteric substitution (Figs 19 and 20) by resecting a somewhat longer segment of the prevallval ileum. However, for all variations of pouch formation, techniques of ureteric implantation and urethral anastomosis (Figs 17 and 18) are identical.
Sutured version, normal length ureters. The caecum and ascending colon are mobilized up to the hepatic flexure. The terminal 30 cm of ileum and 10 cm of caecum and ascending colon are isolated. A Foley balloon catheter is inserted into the ileum and the excluded bowel segments are irrigated with 1–2 L of saline. Thereafter, the proximal 20 cm of ileum is separated from the terminal 10 cm of prevalvular ileum, with transection of the superficial arcade of the mesentery only. The appendix is resected.
Figure 2
The intestinal continuity is restored by a spatulated end-to-end ileo-ascendostomy. The single-row seromuscular anastomosis is established with two running 4/0 glyconate sutures, here shown for the posterior wall.
Figure 3

The anterior wall of the spatulated end-to-end ileo-ascendostomy has also been closed by a running suture. The separated loop of 20 cm ileum is folded into a U-shape and opened along its antimesenteric border with cautery.
The posterior walls of the opened and into a U-shape arranged ileal segments are joined to each other by side-to-side anastomosis using a single row of all-layer running 4/0 polydioxanone on a straight needle. Stay sutures and Allis clamps help to align the segments. Caecum and ascending colon are opened thereafter at its antimesenteric tenia.
Figure 5

The posterior wall of the pouch is completed by side-to-side anastomosis of colon with the opened ileum. Again, a single row of all-layer 4/0 polydioxanone running sutures on a straight needle are used and the bowel segments are aligned with stay sutures and Allis clamps.
Figure 6

In a door-wing fashion, the bowel segments are opposed to each other and the anterior wall of the pouch is established by side-to-side all-layer running 4/0 polydioxanone sutures on a straight needle of ileum and colon.
Figure 7

The lower and the upper aspects of the pouch are closed, again using a single row of all-layer 4/0 running glyconate sutures.
Figure 8

Stapled version, normal length ureters. The caecum and ascending colon are mobilized up to the hepatic flexure. The terminal 25 cm of ileum and 15 cm of caecum and ascending colon are isolated. A Foley balloon catheter is inserted into the ileum and the excluded bowel segments are irrigated with 1–2 L of saline. Thereafter, the proximal 15 cm of ileum are separated from the terminal 10 cm of prevalvular ileum with transection of the superficial arcade of the mesentery only. The appendix is resected.
Figure 9

After the intestinal continuity is restored (see Figs 2 and 3), the proximal ileal segments are translocated towards the cecum and ascending colon with stay sutures.
Both the separate ileum and the caecum and ascending colon must be opposed to each other at their antimesenteric borders and fixed in this position with stay sutures or Allis clamps, to allow the Poly GIA stapler to divide both bowel segments longitudinally along their antimesenteric borders.
Both branches of a Poly GIA 75 stapler are inserted separately into each bowel lumen and thereafter closed in exactly that position, which clamps the antimesenteric borders of the bowel segments to each other. Before the stapler is released by advancing the (blue) slide with the thumb, care must be taken that no other tissue, such as mesentery, is interposed between the bowel segments. With advancing the slide of the stapler, in a one-step procedure both bowel segments are stapled side-to-side to each other in an inverting manner by placing two double staggered rows of lactomer absorbable staples and simultaneously divided along their antimesenteric borders between the two double rows of staples.
Figure 12

As the maximum length of the active stapling device is 75 mm, only part of the anastomosis has been established by the first application of a stapler. At the end of the incision, both rows of staples from either side of the anastomosis are unified, so that the anastomosis remains closed at its end.
Before the second stapler can be applied, the pouch has to be everted using Babcock clamps along the rows of staples. The blind end of the incision is cut open exactly in between the staple rows with Metzenbaum scissors over a distance of $\approx 0.5$ cm.
The branches of the second stapler are inserted in the same manner into each bowel lumen exactly between the first staple rows at their cut-open blind end, closed and released in the same manner as described above.
Figure 15

Again, with the help of Babcock clamps along the staple rows, the pouch is further step-wise everted for the third identical stapler application. The gaps between the staple rows of the first and second stapler application from cutting open the dead end are shown here. They are closed by running 4/0 glyconate sutures (see insert).
The upper and lower aspects of the pouch are sutured by all-layer running 4/0 glyconate sutures. The insert shows that in the stapled version the pouch is constructed from caecum and one loop of ileum only, compared with the two ileal segments of the sutured version. A counter-clockwise 180° rotation around the insertion of the ileocolic artery brings the ileocaecal valve into a cranial position and the ascending colon deep into the true pelvis. A tension-free urethro-intestinal anastomosis has been possible in every case, even in very obese patients.
At the deepest aspect of the pouch, a 20 F excision of the seromuscularis is made for urethro-intestinal anastomosis. The incised mucosa is everted and fixed by 4/0 polyglytone sutures. The anastomosis is established with the eight pre-placed double-armed 4/0 glyconate sutures. The ureter is implanted into the prevalvular ileum using a Nesbit technique for the right ureter and a Wallace technique for the left ureter. Before that, the left ureter must be pulled through the mesentery below the duodenum and above the inferior mesenteric artery into the right retroperitoneum. Ureteric stents must be inserted before ureteric implantation through the pouch wall and pulled through the ileocaecal valve. Optical magnification is used for the ureteric anastomosis. For either ureter, three 7/0 polyglycolic sutures are used for the spatulation, and the remainder of the anastomosis is completed by using 6/0 glyconate sutures. The ureteric stents are secured with 4/0 polyglytone sutures. In addition, a 10 F pigtail pouchostomy catheter is inserted through the anterior pouch wall and secured with a 4/0 polyglytone suture.
Figure 18

The urethral anastomosis is completed over a 20 F silicon Foley catheter.
Short ureters. If ureters had to be cut short because of irradiation damage or urothelial cancer involvement, the terminal prevulvular ileum can be used as an ileal ureter substitute for both ureters. The length of the terminal prevulvular ileum, which is used as ureteric substitute, must be chosen according to the length of the ureter defects.
Figure 20

For ureteric substitution, prevalvular ileum can be used up to the renal pelvis. Again, the right ureter is implanted in a Nesbit technique and, for Wallace anastomosis of the left ureter, the proximal segment of the prevalvular ileum is pulled through the mesentery below the duodenum and above the inferior mesenteric artery into the left retroperitoneum.
POSTOPERATIVE CARE

Medication. Antibiotics (ampicillin/clavulanic acid and metronidazol) are started before surgery and continued until 7–10 days afterward. For postoperative drainage of the stomach, we prefer intraoperative insertion of a 12 F balloon gastrostomy catheter as compared to a nasogastric tube. Patients are mobilized as early as 1–2 days. Gravity drains in the small pelvis and at the ureteric implantation site are removed as soon as the amount of drainage is <50 mL/24 h. Ureteric stents are removed at 10 and 11 days after surgery. When a pouchogram at 12 days shows no extravasation the transurethral catheter is removed and voiding initiated. At the time of pouchogram and catheter removal, in ≥85% of patients, the ileocaecal valve is able to prevent poucho-ureteral reflux. When the pouch capacity increases with time and pressures become lower, the ileo-caecal valve regain its competence and prevents reflux in >90% of patients. However, the pouchostomy catheter remains in place and is used to check for residual urine. When the postvoid residual is <50 ml the pouchostomy catheter is removed. Pelvic floor exercises are started as soon as the transurethral catheter is removed, and continued until complete continence is achieved.

SURGEON TO SURGEON

In determining the appropriate indication for orthotopic substitution, the most difficult part is to predict whether and how fast urinary continence will be achieved. Patients with cerebrovascular sclerosis are better with a continent cutaneous diversion. Furthermore, females who are very obese or have difficulties in using CISC are better using intermittent catheterization of an abdominal stoma.

In very obese patients with a fat mesentery, colonic mobilization up to the right flexure is the key to success, allowing a tension-free anastomosis with the urethra after 180° counter-clockwise rotation of the pouch. However, care must be taken not to harm the right colonic vein when deep incision of the colonic mesentery is necessary. If this happens and the right colonic flexure turns dark blue, resection is advised and an ileo-transversostomy must be performed rather than the ileo-ascendostomy.

If previously undetected polyps are found when opening the caecum/ascending colon, usually they are benign and should be excised and submitted to frozen section analysis. This is not an indication to abandon the planned procedure.

If there is leakage from the pouch and associated symptoms after catheter removal, this might be from the opening of the pouchostomy tube. Usually the problem is solved by reinserting a transurethral Foley catheter for 10 days. The problem may be prevented by reinserting the transurethral Foley catheter before planned removal of the pouchostomy tube. Leakage from the site of the ureteric anastomosis usually requires percutaneous nephrostomy drainage. If antegrade stenting is not possible this will most likely result in stricture at the implantation site, requiring later surgical revision. Early loss of ureteric stents usually has no sequelae. When this occurs, the upper tract should be checked by ultrasonography and attempts to reinsert a stent and/or nephrostomy catheter should be used only if there is a clinical need.

However, early loss of the transurethral catheter mostly results in extravasation and anastomotic stricture. Thus reinserting a transurethral catheter is advisable. In the male, this can be difficult. Transrectal guidance of the catheter tip and radiographic control of the catheter position are advised. Alternatively, urethroscopy may be used for transurethral placement of a 8 F ureteric catheter over which, as a guide, coaxial placement of an open-end 20 F balloon catheter (Integral® nephrostomy silicone catheter, Uromed) can be done safely. Early loss of the pouchostomy catheter usually has no sequelae and no attempts should be made to reinsert or replace it.

REFERENCES

5. Leibner J, Stein R, Hohenfellner R et al. Radical cystoprostatectomy combined with Mainz pouch bladder substitution to the urethra: long-term results. BJU Int 1999; 83: 964–70

e-mail: thueroff@urologie.klinik.uni-mainz.de
In [1], a number of values were incorrectly submitted in Figure 1 on page 1304. The values in the main body of the text were correct.

The correct version of Figure 1 is displayed below.

REFERENCE

### Abbreviations

Authors may use the abbreviations in this list, without definition when within the main text, but defined when in the Summary. Other abbreviations must be defined on first mention, both in the Summary and in the main text. Abbreviations of units should be those defined by SI.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>AUA</td>
<td>American Urological Association</td>
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<tr>
<td>BAUS</td>
<td>British Association of Urological Surgeons</td>
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<tr>
<td>BCG</td>
<td>bacille Calmette-Guérin</td>
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<tr>
<td>BPH</td>
<td>benign prostatic hyperplasia</td>
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<td>BSA</td>
<td>bovine serum albumin</td>
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<td>BOO</td>
<td>bladder outlet obstruction</td>
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<td>CI</td>
<td>confidence interval</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CT</td>
<td>computed tomography</td>
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<td>DMSA</td>
<td>dimercapto-succinic acid</td>
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<tr>
<td>DRE</td>
<td>digital rectal examination</td>
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<tr>
<td>DTPA</td>
<td>diethylene-triamine-penta-acetic acid</td>
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<tr>
<td>EDTA</td>
<td>ethylenediamine tetra-acetic acid</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>ESWL</td>
<td>extracorporeal shock wave lithotripsy</td>
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<td>FSH</td>
<td>follicle-stimulating hormone</td>
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<td>GFR</td>
<td>glomerular filtration rate</td>
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<td>gonadotrophin-releasing hormone</td>
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<td>hCG</td>
<td>human chorionic gonadotrophin</td>
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<td>human immunodeficiency virus</td>
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<td>HPLC</td>
<td>high-pressure liquid chromatography</td>
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<td>ICS</td>
<td>International Continence Society</td>
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<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
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<td>IgXz</td>
<td>immunoglobulin (class X, subclass z)</td>
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<td>International Prostate Symptom Score</td>
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<td>luteinizing hormone-releasing hormone</td>
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<td>MAG</td>
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<td>major histocompatibility complex</td>
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<td>NSAIDs</td>
<td>nonsteroidal anti-inflammatory drugs</td>
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<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
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<td>PBS</td>
<td>phosphate buffered saline</td>
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<tr>
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<td>prostate-specific antigen</td>
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<td>polytetrafluoroethylene</td>
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<td>pelvi-ureteric junction</td>
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<td>TGF</td>
<td>transforming growth factor</td>
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<td>tumour necrosis factor</td>
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<td>transrectal ultrasonography</td>
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<td>transurethral resection of the prostate</td>
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<td>Urinary tract infection</td>
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<td>VUR</td>
<td>vesico-ureteric reflux</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
august 26 | september 1
35th Annual Meeting of the International Continence Society, Montreal, Canada.
T +1 847 605 0850
E vicky@icsoffice.org
W http://www.icsoffice.org

september 15 | 17
PSA: Past, Present and Future, Milan, Italy.
Contact: Emilia Viaggi
T +39 051 235 993
F +39 051 291 4455
E evcongressi@emiliaviaggi.it
W http://www.emiliaviaggi.it/site/evcm_congresso/pg-20/num-34

september 21 | 24
57th Kongress der Deutschen Gesellschaft für Urologie, Düsseldorf, Germany.
Contact: Kongress-Sekretariat, Monika Langer, Gabriele Schwarzmann, Kongress-Sekretariat DGU 2005, Urologische Klinik, Städt.Klinikum Karlsruhe gGmbH, Moiتكrestr. 90, D-76133 Karlsruhe, Germany
T +49 0721 974-4102
F +49 0721 974-4149
E dgu2005@web.de
W http://www.urologenportal.de/580.html

september 29 | october 1
Contact: SIU Congress Office, 1155 University Street, Suite 1155, Montreal, Quebec, Canada H3B 3A7
T +1 514 875 5665
F +1 514 875 0205
E bariloche@siu-urology.org
W www.siu-urology.org/bariloche

september 30 | october 1
4th Biennial World Congress on Men’s Health & Gender (WCMH), Vienna, Austria.
Organization: WCMH Health & Congressmanagement, Lazarettgasse 9/5, 1090 Vienna, Austria
E office@wcmh.info
W http://www.wcmh.info
Congress Office: PROCON Conference, Incentive & Event Management, Odoakergasse 34-36/3, 1160 Vienna, Austria
E office@proconference.at
F +43 1 486 40 40 46
W http://www.proconference.at

october 5 | 8
10th Biennial Meeting of the Asia Pacific Society for Sexual & Impotence Research (APSSIR), Cairns, Australia.
Contact: Promaco Conventions Pty Ltd., P.O. Box 890, Canning Bridge, Western Australia 6153
T +61 8 93 32 29 00
F +61 8 93 32 29 11
E promaco@promaco.com.au

october 27 | 30
5th Meeting of the International Society for the Study of Women’s Sexual Health (ISSWSH), Las Vegas, Nevada, USA.
Contact: ISSWSH, 1111 N. Plaza Drive, Suite 550, Schaumburg, IL 60173, USA
T +1847 517 7225
F +1847 517 7229
E isswsh@wjweiser.com
W http://www.isswsh.org

october 31 | november 4
Urology Specialist Registrars’ Spinal Injuries Course. Twice Annually. Sheffield/Wakefield, UK.
Contact: Carole Gregory (secretary to Mr P R Tophill, Consultant Urological Surgeon), Princess Royal Spinal Injuries Unit, Northern General Hospital, Herries Road, Sheffield, S5 7AU, UK
T 0114 271 5645
F 0114 271 5649
E carole.gregory@sth.nhs.uk

november 9 | 11
4th Annual Meeting of the Mediterranean Association of Andrology (AMA), Alexandria, Egypt.
Contact: Dr Ashraf Samir, P.O. Box 125, Ibrahimia, Alexandria, Egypt
T +20 3 35 95 043
F +20 3 35 95 044
E drashraf@aast.edu
**November 17 | 18**


Organizer and further information: Aesculap Akademie GmbH, Am Aesculap Platz, 78532 Tuttlingen, Germany.

T +49 7461 95 2001
E info@aesculap-akademie.de
W www.aesculap-akademie.com

**November 17 | 19**

First World Congress on Hypospadias and Intersex Disorders, Istanbul, organised by the ISHID International Society on Hypospadias and Intersex Disorders.

Convenor: Serif Etker, MD

W http://www.ishid.org
W http://www.hypospadias-intersex.org

**December 1 | 4**

Fall 2005 Joint SBUR-ESUR Meeting
Miami Beach, FL, USA.

T 847 517 7225
F 847 517 7229
E info@sbur.org
W http://sbur.org/meetings/program2005december.asp

**December 4 | 7**

8th Congress of the European Society for Sexual Medicine (ESSM), Copenhagen, Denmark.

Contact: CPO Hanser Service, Zum Ehrenhain 34, 22885 Barsbüttel, Germany

T +49 40 67 08 820
F +49 40 67 03 283
E essir@cpo-hanser.de
W http://www.essm.org/index.cfm

**January 25 | 27**

14th Copenhagen Symposium on Endoscopic Urological Surgery in Copenhagen Denmark.

T +45 4488 3644
E suslen01@herlevosp.kbhambt.dk
W www.seus2006.dk

**February 9 | 12**

The 5th World Congress on The Aging Male, Salzburg, Austria.

Contact: 17 Rue du Cendrier, P.O. Box 1726, Ch-1211, Geneva 1 Switzerland

T +41 22 908 0488
F +41 22 732 2850
E aging@kenes.com

**June 21 | 24**


Contact: PCO: Status Plus BV

T +31 343 443888
F +31 343 442043
W http://www.statusplus.nl

**November 12 | 16**

28th Congress of the Société Internationale d’Urologie, Cape Town International Convention Centre, Cape Town, South Africa.

Contact: SIU Congress Office, 1155 University, Suite 1155, Montreal, Quebec, H3B 3A7, Canada

T +1 514 875-5665
F +1 514 875-0205
E siu2006@siu-urology.org
W www.siu2006.com