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I am introducing a new section in the August issue of the Journal entitled ‘Great Drug Classes’ and it will be a full description of drug classes by internationally known writers

I have mentioned before in this column my belief that, especially with the modern multidisciplinary approach to treating urological cancer, we must listen carefully to what our colleagues in medical and radiation oncology have to say.

In this way we can resolve some of the misunderstandings which sometimes exist between our disciplines, and we can offer our patients the best treatment for their condition. To allow us to hear the views of these colleagues, and to see what new drugs are being used in oncology, we will be publishing several mini-reviews and original articles from leading authors which will outline new therapeutic strategies, and which will, I am certain, be of interest to the readers of the BJU International.

In keeping with this ideal, I am introducing a new section which will have its first appearance in the August issue of the Journal. The Pharmaceutical Review section, which appears towards the end of each issue of the Journal and written by Mike Wyllie, has received very positive reviews, and it allows readers to see what is happening to drug development in many areas of urological interest. The new section will be entitled ‘Great Drug Classes’ and will be a full description of drug classes by internationally known writers, which will appear roughly twice annually. This new concept will receive a formal introduction in this column next month by Mike Wyllie and myself, but I felt it was such an important innovation that I would announce it in an informal way this month.

This multidisciplinary approach is not confined to just urological oncology. We must listen also to the views of many allied specialists in virtually every other field of urological interest. This view is reflected in the types of papers published in the other sections of the Journal, and in the number of non-urologists on the Editorial Board of the Journal, and in the sections which have their own Editorial Boards, such as Sexual Medicine and Investigative Urology. It is only in this way that we can advance our knowledge in urology in general.

I hope you will let me know your views on the new section, as well as on the oncology papers, and indeed on any other aspect of the Journal which you feel strongly about. I also very much hope you enjoy the innovations which I try to introduce on a regular basis.

JOHN M. FITZPATRICK
Editor - in - Chief
INTRODUCTION

The recent paper by Smith [1] is timely and provocative. Dr Smith proposes that the minimally invasive surgical (MIS) approach to radical retropubic prostatectomy (RRP) via robotics does not deliver the distinct advantages of MIS. The advantages of reduced pain and early return to normal activity found in other surgery, e.g. laparoscopic nephrectomy and cholecystectomy, are clear. The assertion is made by Dr Smith that this is not so in open RRP compared with laparoscopic RRP, despite the technology of robotics delivering a three-dimensional view, ×10 magnification, 540° ‘wristing’, motion scaling, tremor elimination, seven degrees of freedom and ergonomic comfort.

In his institution and his personal series, Dr Smith achieved equivalence in the outcomes of blood loss, pain control and early discharge with open RRP compared with laparoscopic robotic RRP. His contends that a lower midline subumbilical incision is not painful, as the linea alba is split and there is no muscle cutting. However, it may be that the muscle retraction provided by the self-retaining instrument causes much postoperative pain not seen after a laparoscopic approach.

Most other centres, certainly those outside North America, are unable to provide such a comfortable journey after surgery for their patients treated with open RRP. I think that the patients’ expectation of early discharge in the USA, because of the very significant hospital-stay costs, contributes to a greater acceptance of discomfort at the time of hospital discharge. The hospital stay at my institution for open RRP is 5 days, compared with 2 days for robotic RRP.

If the advantage of this ‘high-tech, new-tech’ computer robotic approach is not in its MIS role, Dr Smith suggests it may be in the delivery of improved cancer control and the reduction of the twin major morbidities of RRP, i.e. erectile dysfunction and urinary incontinence.

Despite much better understanding of the anatomy of the neurovascular bundle (NVB) [2], sexual dysfunction remains the key malady associated with RRP. Robotics provides three significant advantages over open surgery which may translate into improved sexual outcomes after robotic RRP. Indeed, Dr Smith reports his impression in his series of an earlier return to erectile function after telerobotic radical prostatectomy.

The pneumoperitoneum provides haemostatic tamponade, allowing a better visualization of the NVB unobscured by bleeding. The ×10 magnification certainly improves visualization here. The robotic dissection of the NVB in an antegrade fashion from bladder neck to apex, with less traction on the NVB, which is clearly visually identifiable, provides a surgical advantage. Will this improved recognition and dissection provide robotics with a compelling modern surgical advance? I agree with this view and in my series, erectile function seems to improve earlier.

Urinary incontinence after RRP remains an issue in ≈8% of patients and 1–2% need an artificial sphincter implanted surgically. The absolute control of bleeding from a dorsal vein complex at robotics permits an astonishingly precise dissection of the sphincter musculature at the prostatic apex. This makes apical dissection much more refined (a site commonly associated with positive margins) and facilitates preservation of the sphincter. It is here also that the cavernosal nerves can be damaged if they are not clearly visible. Recent studies from Japan [3] show that there are several variations of the anatomical relationship of the cavernosal nerve to the urethra at this point. In a dry surgical field with the magnification available via robotics, there is conceptually and practically less chance of nerve damage at this location. The same authors also describe the branch from the NVB to the urethral sphincter at this point.

Ahlering et al. [4] recently described a robotic technique to reduce the pT2 positive-margin rate at the apex. Robotic dissection and visualization of the NVB and apical tissue allowed a reduction in the positive apical rate from 36% to 16.7% in their series.

In conclusion, according to Smith, the advantages in robotic RRP lie not in its MIS character but in its improved precision, visualization and haemostatic tamponade. These technical enhancements may in turn lead to robotics providing better outcomes in cancer control, erectile function and return to complete urinary continence. Certainly, marketing of robotic services has concentrated more on the former advantages than the latter. We await reports from other robotic centres for verification of Dr Smith’s postulate. I would contend that another advantage not mentioned by Smith is the easy transfer of MIS laparoscopic skills to the untrained laparoscopic surgeon via robotics.

Finally, cost issues also need to be considered. The high capital and disposable cost of the Da Vinci robotic system creates a ‘have and have not’ environment. As with computers, television sets and mobile telephones, robotics will be cheaper over time. Maybe the time has come to acknowledge that putting a
INTRODUCTION

The management of advanced prostate cancer has changed relatively little in the 60 years since the therapeutic role of castration was identified by Huggins and Hodges [1]. Androgen suppression, currently using an LHRH agonist (medical castration), remains the mainstay of treatment. Castration-resistant disease progression is inevitable and while further hormonal manipulations, using androgen receptor antagonists, oestrogens or corticosteroids, can be of some benefit, none of these agents has been shown to prolong overall survival. Some 9000 men die from prostate cancer every year in the UK, which is the equivalent of one death every hour.

Prostate cancer was once considered to be a relatively chemo-resistant disease. A review of 17 prostate cancer chemotherapy trials before 1985 found a disappointing overall response rate of <5% [2]. A clinical role for prostate cancer chemotherapy was first confirmed by the landmark trial reported in 1996 by Tannock et al. [3]; 161 men with progressive, symptomatic castration-resistant metastatic prostate cancer were randomized to receive mitoxantrone (12 mg/m²) at 3-week intervals. There was a significant advantage for the use of chemotherapy in terms of the pain relief response (29% vs 12%, P = 0.01). This was the first of two randomized trials which showed that adding mitoxantrone chemotherapy could improve symptom palliation in castration-resistant prostate cancer [3,4]. However, the use of chemotherapy in these trials had no apparent effect on overall survival.

The role of prostate cancer chemotherapy is now set to change significantly, with the recent publication of two large randomized studies, which for the first time show that chemotherapy can improve overall survival. In the TAX 327 trial, 1006 patients with castration-resistant disease received prednisolone 5 mg twice daily and were entered into a three-way randomization between weekly docetaxel, 3-weekly docetaxel, and standard chemotherapy with mitoxantrone [5]. There was a significant overall survival advantage for docetaxel chemotherapy (median survival 18.2 vs 16.4 months, hazard ratio 0.83, 95% CI 0.70–0.99; P = 0.03), with a trend favouring treatment at 3-weekly intervals rather than weekly. Importantly in this study, treatment with docetaxel was also associated with a significant advantage in both pain response and quality of life, while the toxicity of docetaxel chemotherapy was comparable to that of mitoxantrone. In the second trial, by the South-west Oncology Group in >700 patients, the combination of docetaxel plus estramustine was compared with mitoxantrone plus prednisolone [6]. There was a significant survival advantage for the docetaxel/estramustine combination (median 18 vs 16 months, hazard ratio 0.80, 95% CI 0.67–0.97; P = 0.01) but at the cost of greater toxicity. Taken together, these two trials show that docetaxel is more effective than mitoxantrone, and suggest that adding estramustine increases the toxicity, but not the efficacy, of docetaxel-based chemotherapy.

What are the implications of these new findings? Certainly, docetaxel given at 3-weekly intervals combined with a low-dose steroid should be the new standard of care for chemotherapy in patients with progressive, symptomatic castration-resistant prostate cancer. However, in the past many UK clinicians took the view that the benefits of treatment with mitoxantrone were not large enough to justify its widespread use. The key question now is whether the proven survival advantage, in addition to a further improvement in quality of life, alters the balance of harm and benefit to a point where it will become the norm for men with castration-resistant prostate cancer to receive chemotherapy. Some may still argue that a ‘2-month survival benefit’ is a poor return for the effort and expense associated with up to 10 cycles of chemotherapy. However, the modest difference in median survival does not adequately describe the benefit that patients stand to gain from docetaxel. First, in comparison with mitoxantrone, 3-weekly docetaxel was associated with a 24% reduction in the hazard of death. Second, the benefits in terms of pain control and quality of life are at least as important as any survival benefit, if not more so.

Prostate cancer should no longer be considered a chemo-resistant disease. There are ≈ 10 000 new cases of castration-resistant prostate cancer in the UK every year. If half of them were to receive chemotherapy for an average of 4 months each, this would...
translate into 33 patients on prostate cancer chemotherapy at any one time in each of 50 cancer centres. This would represent a major additional pressure on already stretched resources.

Docetaxel is the first drug that has been shown to improve survival for men with castration-resistant prostate cancer. By analogy with other solid cancers, the survival benefit of chemotherapy may be greater if used earlier in the natural history of the disease. The Medical Research Council STAMPEDE trial is important in this regard, and aims to recruit >3000 patients starting long-term androgen suppression for locally advanced or metastatic disease, to test the effect of adding docetaxel chemotherapy, either with or without zoledronate and celecoxib, on overall survival.

The increased use of chemotherapy for castration-resistant disease, and its potential role earlier in the course of the disease, will have a significant impact on the way that patients with prostate cancer are managed in the UK. At one time prostate cancer was managed largely by urologists, with clinical oncologists providing palliative radiotherapy when required [7]. Prompted by the National Health Service Cancer Plan, there has been recent progress towards multidisciplinary care. The new opportunities provided by docetaxel chemotherapy, together with developments in radiation therapy, and the plethora of molecular-targeted agents under evaluation, mean that all patients with prostate cancer should have access to specialist uro-oncological services throughout their cancer journey. This will require both a significant cultural shift and a major increase in the National Health Service resources devoted to uro-oncology.

CONFLICT OF INTEREST

Chris Parker and Mark Emberton have acted as medical advisors to Sanofi-Aventis.

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α-ACYLMETHYL CO-ENZYME A RACEMASE: A TUMOUR MARKER FOR THE 21ST CENTURY? DEBASHIS DAS, PROKAR DASGUPTA and ASISH CHANDRA* – Departments of Urology and *Histopathology, Guy’s and St. Thomas’ Hospitals and GKT School of Medicine, London, UK

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INTRODUCTION

The end of the last century saw an exponential growth in the screening of early carcinoma of the prostate, using serum PSA levels. TRUS and needle-biopsy can then allow pathologists to make a definite diagnosis, but sadly up to 24% of specimens are ambiguous and as a result, thousands of men have to have expensive and time-consuming repeat biopsies [1]. The recent article by Stamey et al. [2] further fuelled the controversy by indicating that in the last 5 years, the use of serum PSA testing has mainly been related to BPH, stating that the ‘PSA era’ for prostate cancer is over in the USA, and presenting the need for new serum markers.

α-Acylmethyl co-enzyme A racemase (AAMCR) is a normally occurring prostatic enzyme that has been widely shown to be significantly up-regulated in prostate cancer [3]. Initial reports showed that staining for AAMCR could diagnose cancer in histological specimens with up to 97% sensitivity and 100% specificity, and further studies highlighted its suitability in verifying the minute foci of prostate cancer often found in the samples taken from needle-biopsies [4].

Previously available immunohistochemical stains for high molecular weight cytokeratins (HMWCKs) and p63 highlight the absence of the basal-cell layer in the neoplastic prostate, and hence serve only as an indirect indicator of malignancy. False-positives can occur through artefact during specimen processing, or in certain benign conditions like adenosis, which mimics invasive carcinoma in its architecture, cytology and occasional lack of demonstrable basal cells.

However, AAMCR indicates the presence of neoplastic epithelial cells and thus, for the first time, provides direct evidence of neoplastic transformation. Moreover, it may also be used with, or even on the same section as, stains for HMWCK or p63. Such an ‘antibody cocktail’ would not only show the absence of the basal cell but also highlight the presence of neoplastic epithelial cells, simultaneously.
However, AAMCR is not fool-proof, and most series show <100% accuracy [5]. Despite this, interest in the enzyme continues, and a more specialized role is rapidly emerging, i.e. to reach a definitive diagnosis of cancer when traditional histopathology and immunohistochemistry have yielded only an ‘atypical’ diagnosis [6,7]. This alone could avoid both re-biopsy and a delay to any ‘atypical’ diagnosis [6,7]. This alone could become one of its most important discoveries.

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THE ‘FLARE’ PHENOMENON: SHOULD WE BE CONCERNED?
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INTRODUCTION
The ‘flare’ phenomenon was initially described over 20 years ago in patients with advanced breast cancer who were given hormonal treatment. The term ‘tamoxifen flare’ was designated for women who had a transient but severe period of increased bone pain and worsening clinical status after the initiation of tamoxifen therapy for advanced breast cancer [1]. A similar phenomenon was seen when LHRH analogues were used for androgen ablation therapy (ABT) in patients with advanced and metastatic prostate cancer [2]. Steroidal and nonsteroidal antiandrogen drugs, when used in combination with LHRH...
analuges, are extremely potent in obliterating the effects of the flare phenomenon [3]. Therefore, a combined androgen blockade (CAB) approach in the form of an antiandrogen drug and an LHRH analogue is used frequently to treat patients with prostate cancer requiring hormone deprivation. However, without antiandrogens, will all patients on LHRH analogues initially suffer the flare phenomenon, and is this effect always clinically deleterious?

**LHRH ANALOGUE**

Treatments for advanced and metastatic prostate cancer require suppression of testosterone in the form of surgical or chemical castration. Historically, orchidectomy was used to reduce testosterone levels but because of the profound psychological impact, patients increasingly preferred chemical methods, e.g. diethylstilbestrol, antiandrogens and LHRH analogues, in the treatment of their prostate cancer [4]. As diethylstilbestrol increased the risk of cardiovascular side-effects in patients, LHRH analogues have become the major medical option for castration [5].

The sustained administration of LHRH analogues creates a blockade of the pituitary and gonadal axis through the process of ‘down-regulation’ of LHRH receptors and ‘desensitization’ of pituitary gonadotrophins, by reducing the synthesis and release of LH and FSH [6]. Through the deprivation of LH and in turn testosterone, the growth and proliferation of prostate cancer is halted. However, LH and testosterone levels may transiently increase up to 10-fold and twice or more, respectively, from 2–3 days and lasting up to 10–20 days after the initial injection of LHRH analogue [6,7]. This is known as a ‘biochemical flare’ and is also characterized by an increase in PSA levels [3,8]. It is now well accepted that a rising PSA level is secondary to tumour growth and reflects the proliferation of prostate cancer. Therefore, in some patients, the first 2–3 weeks of LHRH analogue treatment may be associated with worsening clinical status in the form of increasing bone pain, cord compression, BOO and cardiovascular problems related to increased hypercoagulability. This is described as a ‘clinical flare’. In addition, a scintigraphic flare has also been identified on bone scans of patients started on monotherapy with LHRH agonists [9].

Patients at increased risk of the flare phenomenon, particularly clinical flare, are men with metastatic prostate cancer (D1 and D2 disease). Studies show that 4–63% of this group of patients with prostate cancer have the flare phenomenon [3,10]. The wide variation in incidence may be the result of the poor distinction between clinical and biochemical flare, and the subjective and objective aggravation of cancer-related symptoms. However, for patients with advanced but not metastatic prostate cancer (D0 disease) commencing LHRH analogue therapy, the flare phenomenon is extremely rare. This assessment is important, particularly as the use of LHRH analogues for treating patients with prostate cancer has become more prevalent. Over the last decade, there has been an increasing incidence of prostate cancer, with the advent of PSA testing, and the increasing elderly population and public awareness. Furthermore, with early hormone deprivation in patients with advanced or asymptomatic metastatic prostate cancer being shown to delay disease progression, LHRH analogues are now being administered in more men with prostate cancer [11]. This has resulted in LHRH analogues being mainly used to treat advanced rather than metastatic prostate cancer [8].

**ANTI-ANDROGENS FOR NON-METASTATIC DISEASE**

Conventionally, patients with locally advanced prostate cancer are started on antiandrogens for a few days up to 2 weeks before and up to 2 weeks after their first injection with LHRH analogue. However, there is no robust evidence showing the incidence of the flare phenomenon and the requirement for antiandrogens in these patients. Most patients requiring ABT for adjuvant and neoadjuvant purposes are unlikely to have prostatic bone metastases, and are therefore not at risk of bone pain or spinal cord compression secondary to the flare phenomenon after LHRH analogue treatment. Similarly, at the time of diagnosis, if these patients had not presented with LUTS related to an enlarged prostate, the initial testosterone surge and tumour growth as a result of LHRH analogue therapy is unlikely to be significant enough to cause urinary obstruction. The lack of evidence of flare symptoms in these patients in previous trials might be because of the routine use of antiandrogens with LHRH analogues [12]. The flare response has also yet to be identified after recommencing treatment in patients on trials of intermittent ABT. More significantly, it is still not clear if adding antiandrogens to LHRH analogue therapy improves survival outcomes in patients with non-metastatic prostate cancer. Although given for a short period to cover a flare response, antiandrogens are known to cause side-effects which include: liver function abnormalities, hepatic encephalopathy, diarrhoea and other gastrointestinal disturbances (flutamide); pulmonary toxicities, decreased light accommodation, alcohol intolerance and rash (nilutamide); and gynaecomastia, breast tenderness and cardiovascular complications (cyproterone acetate) [13]. Anti-androgen drugs add to the total cost of CAB treatment and require biochemical monitoring of liver function, which is significant, particularly as there is limited evidence about the appropriate time of starting and duration of antiandrogen administration before LHRH analogue therapy.

**CONCLUSION**

We are aware that antiandrogens are vital in preventing the detrimental effects of bone pain, cord compression and urinary obstruction secondary to the flare phenomenon in patients with metastatic prostate cancer. However, there is no robust evidence to support the almost routine use of initial antiandrogens to cover the flare phenomenon for patients with non-metastatic disease starting LHRH analogues. Moreover, this may add to the costs and the side-effects. Recently, LHRH antagonists have proven to be effective and more convenient in treating prostate cancer without causing the flare phenomenon [6]. Further studies are required to identify an ideal ABT for patients with prostate cancer.

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Molecular staging of bladder cancer

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THE DISEASE

Cancer of the urinary bladder is a major epidemiological problem that continues to grow each year. Bladder cancers encompass urothelial carcinomas (UCs, or TCCs), squamous cell carcinomas, adenocarcinomas and certain other infrequent tumour types. It is the fourth most common malignancy in males and the ninth most common malignancy in females in the USA. An average of 260,000 new cases of urinary bladder cancer are diagnosed worldwide every year, with an estimated 63,210 new cases in 2005 in the USA alone, with ~13,180 deaths [1].

THE PROBLEM

The current treatment for UC is based on the pathological staging of the tumour. The staging therefore is crucial for clinical decision-making and exploring the various treatment options, and the therapy thus chosen can result in significant morbidity and financial burden to the patient. The traditional TNM classification or the WHO classification system for UC [2] relies on pattern recognition and nomenclature for reporting biopsies, the interpretation of which can be highly subjective and can have a high frequency of inter- and intra-observer variability. Interpretations of biopsies can be confounded by sampling problems such as the absence of the muscular layer in the specimen, or the exclusion of the bladder wall in biopsies of large tumours growing exophytically that can affect the staging. Even among trained pathologists there are no uniformly accepted definitions for micro-invasion, which is an important criterion to determine the risk of metastasis. Most significantly, the basic tools available to determine tumour behaviour, malignant potential and chance for recurrence provided by the current pathological staging methods can be highly subjective. Thus, while current histopathological criteria can provide important morphological information about tumours in patient populations, they are unable to specify the risk for progression or response to treatment for an individual patient with UC. Esrig et al. [3] showed the wide difference in recurrence and survival rates between patients of the same pathological stage with differences in their tumour p53 status. In a cohort of 243 patients with UC treated by radical cystectomy, the recurrence rates for stage pT1, pT2a and pT2b tumours with negative p53 nuclear reactivity were 7%, 12% and 11%, respectively, in contrast to 62%, 56% and 80%, respectively, for tumours that had p53 immunoreactivity. That study indicated the need to incorporate objective staging methods using molecular markers specific to UC to complement the morphological approach, and devise a refined system of staging that focuses on the biological behaviour of the tumour and its predicted clinical outcome, thereby equipping the clinician with a better insight on the appropriate treatment regimen to be instituted.

THE PLAYERS

Recent studies in the molecular biology of UC have opened new avenues for investigative...
research to identify molecular markers for the disease. While loss of heterozygosity on chromosome 9 has been implicated in UC, recent studies have shown that allelic loss on chromosome 9 is found exclusively in early well-differentiated tumours, while late tumours showed other genetic lesions [4]. The tumour suppressor genes on chromosome 9 (characterized by allelic losses in 9p and 9q, and deletions between 9p12 and 9q34, which spans the p16INK4A locus) have been implicated in UC formation. These tumours have a good prognosis and low invasive and metastatic potential. This is in contrast to alterations on chromosome 17p, the site for the p53 gene, which is associated with a more aggressive phenotype [5].

p53

The gene for p53 is critical for regulating the cell cycle and apoptosis, and plays a key role in mediating growth arrest and DNA damage at the G1/S transition [6]. The p53 gene acts as a tumour-suppressor gene, and loss of heterozygosity of one allele followed by mutation of the remaining allele is an important mechanism for gene inactivation. This mutation results in a longer half-life for the protein, which is then localized to the nucleus and can be detected by immunohistochemistry.

Our studies have shown that nuclear accumulation of p53 is significantly associated with a greater risk of recurrence of UC and decreased overall survival (both \( P < 0.0001 \)) [3]. As noted previously, immunohistochemical analysis of organ-confined UC showed lower 5-year recurrence rates for stage pT1, pT2a and pT2b tumours with no detectable p53 nuclear reactivity than for the same tumours with positive p53 immunoreactivity. A multivariate analysis stratified according to grade, pathological stage and lymph node status showed that nuclear p53 accumulation was an independent predictor of recurrence-free and overall survival (\( P < 0.0001 \)). These results support the hypothesis that within organ-confined carcinoma, it is the presence or absence of nuclear p53 accumulation (suggestive of a p53 alteration/mutation) and not the depth of invasion that determines survival.

While many other studies also concluded that p53 can serve as an independent prognostic marker in UC, some have failed to show this relationship as independent from other prognostic factors. A meta-analysis by Schmitz-Drager et al. [6] of 138 publications reporting on 43 studies comprising 3764 patients with UC showed considerable differences in the clinical outcome, which the authors attributed to the p53 immunohistochemistry protocols used, patient selection and study design. They opined that the current need is for new prospective multicentre clinical trials examining p53 alterations in patients with UC.

While adjuvant chemotherapy, radiation and/or immunotherapy are effective for patients with locally advanced UC, such treatment is not traditionally used for patients with invasive organ-confined disease, as only a proportion of them are at risk of progression. However, there is an urgent need to re-categorize these patients by considering their p53 status, so as to better define those who are most likely to progress and those who would benefit from systemic adjuvant chemotherapy. With this in mind, an international randomized p53-targeted therapy trial is currently underway to study the effects of three cycles of adjuvant methotrexate, vinblastine, adriamycin and cisplatin chemotherapy after radical cystectomy for pathological T1-T2 tumours with negative lymph nodes and altered p53 expression. This is the first UC clinical trial targeting a molecular lesion, led by us at the University of Southern California, along with other collaborators from institutions across the USA and Europe; it is based on the data that tumours with altered p53 are at greater risk of progression and selectively respond to chemotherapy containing cisplatin. The trial thus aims to elucidate the prognostic value of p53 in organ-confined UC [7].

p21

The cyclin-dependent kinase inhibitor p21 \(^{WAF1/CIP1}\) (p21) is a downstream effector of p53 and thus a potential tumour-suppressor gene. Shariat et al. [8] showed that positive p21 expression was independently associated with UC recurrence and progression in carcinoma \( \text{in situ} \) with no muscle-invasive disease, possibly by p53-independent modulation of p21. Our studies have shown that patients with p53-altered, p21-negative tumours have a significantly greater recurrence rate and lower survival rate than those with maintained p21 expression levels, irrespective of tumour grade, pathological stage and lymph node status. The association between p21 status and tumour progression was particularly notable in patients with organ-confined (carcinoma \( \text{in situ} \), T1, T2a, T2b) and extravesical disease (T3, T4) with no evidence of lymph node metastases. Also, maintenance of p21 expression appeared to negate the deleterious effects of p53 alterations on UC progression [9].

**RETINOBLASTOMA (RB) GENE AND PROTEIN**

The RB gene forms a phosphorylated nucleoprotein (pRb) that interacts with many cell-cycle regulatory proteins involved at the G1/S transition [5]. Deletion of chromosome 13q is the most common cause of RB gene inactivation. Miyamoto et al. [10] showed that RB gene mutations are involved in low-grade and noninvasive UC, and in high-grade and invasive cancers. The loss of expression of pRb is important in the progression of UC. However, we have shown that a significant proportion of tumours expressing the highest levels of pRb have clinical outcomes similar to those with no detectable pRb with lower recurrence-free and overall survival [11]. We explained the biological basis for this by showing that hyperphosphorylation is associated with loss of p16 expression and/or cyclin D1 overexpression [12].

**OTHER MOLECULAR MARKERS**

Various other molecular markers are being explored for the determination of prognosis in UC. MDM2, involved in an autoregulatory feedback loop with p53, is amplified in UC. This amplification frequency increases with stage (Ta to T4) and grade (low-grade to high-grade) [13].

Overexpression of cyclin D1 has been described in a variety of tumour types [14]. As noted above, we also showed that overexpression of cyclin D1 along with a loss of p16 may cause hyperphosphorylation and functional inactivation of pRb [12]. Cyclin E overexpression may also be associated with aggressive tumour growth in UC [15]. Cellular transition through the G1 to S phase is regulated by cyclin-dependent kinases (CDKs). The frequency of amplification of the CDK4
gene increases with the stage and grade of UC [13]. In addition, there are frequent deletions and methylation of the INK4A gene in noninvasive UC, but only those that affect both p16(RSK) and p14AR, the major CDK inhibitors, correlate with the worst prognosis [16]. There is also evidence to suggest that low expression of p27(Kip1) correlates with decreased disease-free and overall survival in UC [17].

Alterations in the angiogenesis pathway are also important in UC. Our studies have shown that expression of thrombospondin-1, a potent angiogenesis inhibitor, is significantly associated with disease recurrence and overall survival in UC, and is significantly associated with altered p53 levels and microvessel density counts [18].

High expression of epidermal growth factor receptor on human UC cells corresponds to decreased responsiveness to standard modes of therapy [19]. Immunohistochemistry studies have correlated loss of TGF-β receptors (TβR) with tumour grade, pathological stage and lymph node status (TβR-I and II), and with tumour progression and decreased survival [TβR-I] [20]. Loss of E-cadherin expression correlates with an aggressive phenotype of primary UC [21]. Increased expressions of proteinases degrading the extra-cellular matrix, e.g. matrix metalloproteinase-9 [22] and urokinase plasminogen activator [23], also correlate with an unfavourable prognosis. Increased cytoplasmic expression levels of tenascin-C correlate with better survival rates [24].

THE TRANSLATION

From this discussion it is apparent that there is a wide array of molecular markers available for determining the prognosis in a patient with UC. Unfortunately, no single marker can be solely relied upon to provide a complete prognostic picture.

THE LOGIC USED

The absence of a single ‘gold standard’ predictive molecular marker in the case of UC, and indeed in the case of any cancer, although seemingly disappointing, is not a surprise. It has been acknowledged that UC is a multistep genetic process [6] wherein individual alterations in single molecular determinants may offer important predictive and prognostic information, but because the disease is multifactorial, their individual roles become restricted.

Several studies have tried to show the interplay between individual markers as a combined tool to determine outcome. This is partly based on the grouping of major genes or proteins according to their functional role in the cell cycle, apoptosis, angiogenesis and other independent cascades in the oncogenesis control machinery, that might reveal a cooperative or synergistic effect on clinical outcome.

Data from our laboratory show that alterations in both p53 and pRb may act cooperatively or synergistically to promote tumour progression [11]. Examination of 185 cases of UC showed that patients with altered p53 and pRb had significantly greater rates of recurrence and lower survival (both P < 0.001) than those with no alterations in either p53 or pRb. Stein et al. [9] showed that the combination of p53 and p21 status provides a better indicator of prognosis than any one indicator analysed alone. In that study, examination of UC specimens from 242 patients who underwent cystectomy showed that patients with p53-altered/p21-negative tumours had a higher rate of recurrence and worse survival than those with p53-altered/ p21-positive tumours (P < 0.001).

Recently, we published results examining the combined effects of p53, p21 and pRb expression in the progression of UC [25]. While altered expressions of these markers are independent determinants of prognosis, we showed that they acted co-operatively or synergistically to promote tumour progression. The patients were classified into four groups: group I (no alteration in any marker, 47), group II (any one marker altered, 51), group III (any two markers altered, 42) and group IV (all three markers altered, 24). The 5-year recurrence rates in these groups were 23%, 31%, 60% and 93%, respectively (log-rank P < 0.001), and the 5-year survival rates were 68%, 56%, 28% and 8%, respectively (log-rank P < 0.001). These findings point strongly towards the use of multiple markers to better stage tumours, and to better determine the prognosis and predict the therapeutic response of individual patients to specific treatment. Our group is currently working on developing a panel of markers, using >70 genes controlling various aspects of the cell cycle, apoptosis, angiogenesis, transcription, signal transduction, cell growth, and invasion, that will be studied in combination to develop a new molecular paradigm to refine the pathological staging of UC, to enhance its correlation with prognosis, therapeutic response and final clinical outcome.

THE TECHNOLOGY USED

The compilation, analysis and application of results from a comprehensive molecular marker panel as described above, to better stage an individual patient with UC, requires the use of sophisticated, high-throughput technology that can produce accurate and reproducible results reflective of the molecular grade of the tumour (Fig. 1). We briefly discuss below certain applications that can be used in the expression profiling of UC [26].

Analysis and validation of tumour subclassification by expression microarray analysis is being widely used at present. These arrays offer overall views of gene expression to present comprehensive pictures of cell function. Incorporating high degrees of sensitivity, specificity and reproducibility, these arrays can sort through the activities of thousands of genes and recognize the major players. Monitoring many genes in parallel allows the identification of reliable classifiers or ‘signatures’ of UC. Dyrskjot et al. [27] identified clinically relevant subclasses of UC (Ta, T1 and T2-4, with Ta tumours being further subclassified) by expression microarray analysis of 40 well-characterized bladder tumours. Using bioinformatics, such data generated from microarray analysis can also be used to design genetic algorithms to classify subsets of expression profiles into different categories, depending on the clinical outcome.

Apostolakis et al. [28] and Crawford et al. [29] developed a modified quantitative method of standardized competitive RT-PCR (StaRT-PCR) that allows simultaneous measurements of many genes, using nanogram amounts of cDNA. The transcript levels are expressed as numerical values per million molecules of β-actin, thus affording intra- and intersample comparisons. We have used this technique to obtain transcript profiles of >70 genes crucial in various cellular pathways, as noted above.
Our preliminary analysis shows that medium- to high-throughput transcript profiling can identify important gene subsets that can be used in molecular class prediction, identification of high-risk patients who can benefit from adjuvant therapy, and help in determining the prognosis.

We are also experimenting with the use of genetic programming to obtain an outcome analysis of patients [30]. This is a machine-learning approach based on the concepts of natural selection and population dynamics to ‘evolve’ diagnostics in-silico. It is an iterative analytical process wherein baseline patient and tumour information obtained from a retrospective resource database is fed into the system, with the corresponding clinical staging and patient outcome. From these inputs a set of rules, in the form of computer programs, are produced that will accurately predict staging or patient outcome endpoints. Validation sets of similar information are then used to check the generality of the rules developed. Such programs can then be used to formulate generic criteria for patient staging, using multiple molecular determinants to predict the clinical outcome and tailor the therapy accordingly. Our analysis shows that using a machine-learning approach to analyse expression profiles for molecular staging may prove to be more accurate than traditional histopathology (manuscript in preparation).

LOOKING INTO THE FUTURE

The clues to unravel the propensity of a tumour to metastasize are hidden within the complexity of tumour cells and objective criteria are needed to classify patients appropriately, such that the staging is reproducible with minimum subjectivity and representative of the final clinical outcome. A staging based on a panel of molecular markers can complement the current clinicopathological staging to accurately indicate the risk of disease progression and identification of specific molecular targets that may be more amenable to specific therapies. Such a staging can then be used for administering targeted therapy that can be...
individually tailored to meet a patient's molecular profile and projected response, thus decreasing morbidity.

From a research standpoint, the application of a revised molecular staging of UC can be used to design better clinical trials (such as p53-targeted therapy trial in bladder cancer) to identify new molecular targets that are responsive to chemotherapy. The follow-up of patients can be in the least obtrusive and most cost-effective way possible, by analysing the changes in the marker panel and thereby identifying the high-risk subpopulation where intervention can be instituted earlier. We will need to incorporate demographic variables and physiological differences in these studies, and intercalate them with the molecular findings into computer programs derived from genetic programming, to obtain high-throughput techniques that can subclassify patient types for the purposes of screening and diagnosis.

The ultimate goals of molecular staging will be to assign patients to more logical tiers of classification, based on their predicted response to therapy and projected clinical outcome. This will provide patients with a better quality of life, and clinicians with a clearer idea of how to tailor the treatment for each patient so as to obtain an optimal therapeutic response.

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CONFLICT OF INTEREST

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Abbreviations: UC, urothelial carcinoma; RB, retinoblastoma (gene); CDK, cyclin-dependent kinase; StaRT-PCR, standardized competitive reverse transcriptase polymerase chain reaction.
The role of hand-assisted laparoscopy in urology: a critical appraisal

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laparoscopy, hand assistance, nephrectomy

INTRODUCTION

Laparoscopic techniques are now part of the standard armoury for extirpative and reconstructive urological procedures. Hand-assisted laparoscopy (HAL) is a variant of laparoscopy, a pneumoperitoneum is created, a laparoscope inserted and laparoscopic instruments used for the surgery, with the only difference between standard laparoscopy and HAL being that the surgeon is able to introduce a hand into the operative field.

The objective of this review is to examine the advantages and disadvantages of the selective use of hand-assistance in laparoscopic urology, and the evidence comparing its efficacy with standard laparoscopic techniques.

HISTORY OF HAL IN UROLOGY

HAL surgery is being widely used in general surgery and gynaecology for colon resections, splenectomy, distal pancreatectomy, partial heptatectomy and hysterectomy [1–4]. In urology, HAL was first introduced in 1996 when Bannenberg et al. [5] performed the first HAL nephrectomy in a pig. They reported that HAL nephrectomy was quick and easy, and compared with conventional laparoscopic nephrectomy, the surgery was quicker (30–45 vs 90–120 min). In 1997, Nakada et al. [6] performed the first HAL nephrectomy in a human for a chronically infected kidney from stone disease. Since 1997 many investigators have reported their experience with HAL for complex laparoscopic urological procedures, including radical nephrectomy (RN), nephroureterectomy (NU), donor nephrectomy (DN), partial nephrectomy (PN) and cystectomy.

HAND-ASSISTANCE DEVICES

Several devices are commercially manufactured which allow the hand to be introduced into an insufflated abdomen while maintaining the pneumoperitoneum. The Pneumosieve® (Dexterity Inc., Atlanta, USA) was the first device, introduced in 1997. The Intromit® (Applied Medical, Rancho Santa Margarita, USA) and the HandPort® (Smith and Nephew, Huntingdon, UK) followed shortly thereafter, but all have been discontinued in favour of the three superior ‘second-generation’ products currently available.

The GelPort® (Applied Medical), based on coaptative gel, is snapped onto an abdominal ring. The LapDisc® (Hakko Ltd, Tokyo, Japan, marketed by Ethicon Endo-Surgery, Bracknell, UK) is based on an the principle of an iris valve creating an airtight seal for the surgeon’s hand. Finally, the Omniport® (ASC Limited, Wicklow, Ireland, marketed by TYCO, Gosport, UK) is inflated with air to fix it into place and maintain pneumoperitoneum. All of these devices are effective (Fig. 1), and selection depends on surgeon preference, the patient’s habitus and history of previous abdominal surgery.

TECHNIQUE

The patient is placed supine or in a partial (not complete) flank position, and secured to the table by several cloth tapes; the table is then rotated laterally allowing the viscera to fall away inferiorly. One common port configuration for left-sided renal surgery is shown in Fig. 2, and is similar to that used by both the present authors. An assisting port is sometimes placed caudal and well lateral to the camera port. The figure assumes the configuration for left-sided renal surgery is chosen, general principles dictate that the hand should have easy access to the renal hilum while maintaining full flexion/extension at the wrist, and avoid clashing with the laparoscope and/or laparoscopic instruments.

DISCUSSION

Since the first reports of HAL nephrectomy by Nakada et al., there have been numerous publications explaining the efficacy and efficiency of the technique. Several comparative studies have been reported, where HAL has been compared with open surgery, standard laparoscopy and retroperitoneoscopy for RN, radical NU and DN.

SIMPLE AND RADICAL NEPHRECTOMY

Nakada et al. [8] compared a group of 18 patients who underwent HAL RN with a contemporary cohort who had an open surgical RN. Patients were matched for age, body mass index and American Society of Anesthesiology score. In the HAL group, the mean operating–room time was 220.5 min, the length of stay 3.9 days, the time to return to normal activity 15.8 days, and the time taken to return to work 26.8 days. The median time taken to return to completely normal was 28.0 days. In the open group, the corresponding times were 117.8 min, 5.1 days, 23.5 days, 52.2 days and 150 days; three patients never recovered normal activity. The authors concluded that HAL nephrectomy offers considerable benefits for patient
recovery, at the expense of longer surgery.

Comparing 22 HAL and 16 standard laparoscopic RNs, Nelson and Wolf [9] noted significantly faster surgery with the HAL approach, at 4.5 vs 3.4 h. There were no significant differences in analgesic use, time to oral intake, duration of hospital stay or time to full recovery. Three other studies [10–12] comparing HAL and standard transperitoneal laparoscopic RN found no significant improvement in operative time with HAL; however, the comparisons were confounded by issues with case order and previous experience.

Rehman et al. [13] reported a series of three patients who had simultaneous HAL bilateral nephrectomy for end-stage renal disease and symptoms resulting from autosomal dominant polycystic kidney disease. The mean operative duration was 5.5 h and mean estimated blood loss 200 mL. Patients resumed oral intake on the first day after surgery, had a mean hospital stay of 4.3 days and returned to normal activity after a mean of 2 weeks. Similar results were reported by Troxel et al. [14] for bilateral nephrectomy before renal transplantation.

There is only one reported comparison of the HAL vs the retroperitoneoscopic route for RN, wherein data by Batler et al. [15] showed that the HAL approach did not result in a longer time to oral intake or longer hospital stay; in addition, there was no significant difference in narcotic usage or time to normal activity in both groups. The same group published an elegant small study suggesting that HAL RN may be safe when used by urologists with minimal laparoscopic experience [16].

NU

Stifelman et al. [17] compared their results of HAL NU in 11 patients with a matched group of contemporary open NUs. The surgery was slower with the HAL approach (mean 291 min vs 232 min for the open procedure), but the mean blood loss was 144 vs 311 mL, oral narcotic requirement 5.8 vs 16 tablets, and length of stay 4.6 vs 6.1 days for the HAL NU and open groups, respectively.

Seifman et al. [18] reported similar results comparing 16 patients who underwent HAL NU with 11 contemporary patients undergoing open surgery. The surgery was slower with the laparoscopic approach (320 vs 199 min) but the hospital stay was 3.9 vs 5.2 days, time taken to resume driving 17.1 vs 37.7 days, and time to achieve normal light activity 18.2 vs 38.1 days, in the HAL NU and open groups, respectively. Minor complications occurred in 19% of laparoscopic and 27% of open surgical procedures. Cancer control was similar in both groups.

In a comparison of 11 standard and 16 HAL NUs performed at the same institution, slower with the laparoscopic approach (320 vs 199 min) but the hospital stay was 3.9 vs 5.2 days, time taken to resume driving 17.1 vs 37.7 days, and time to achieve normal light activity 18.2 vs 38.1 days, in the HAL NU and open groups, respectively. Minor complications occurred in 19% of laparoscopic and 27% of open surgical procedures. Cancer control was similar in both groups.

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Landman et al. [19] found the latter speeded the surgery by 72 min. Convalescence measures were similar in the two groups, except that the hospital stay was longer after HAL NU (3.3 vs 4.5 days).

DN

Wolf et al. [20] performed a randomized controlled trial between HAL DN and open DN, with 50 patients randomly assigned to undergo each (live DN). This trial showed that in the HAL group there was 47% less analgesic use, 35% decrease in inpatient hospital stay, 33% faster return to light activity and 73% less pain at 6 weeks after surgery than in the open group. The HAL DN patients had complete recovery sooner and had fewer long-term residual effects. There were no significant differences in graft function.

Stifelman et al. [21] compared 60 patients who had undergone HAL DN with 31 who had had open surgery. The time to patient recovery, blood loss, analgesic use and hospital stay were all less in the HAL DN group, while operative times and complication rates were similar. Again, there were no significant differences in graft function.

Ruiz-Deya et al. [22] compared patients who had undergone open surgery, laparoscopic surgery and HAL DN, noting that HAL DN was faster than a conventional laparoscopic
approach and offered significantly shorter warm-ischaemia times. There were no differences in long-term graft function.

Table 1 summarizes six published comparisons of HAL and standard laparoscopic DN [22–27]. HAL is faster, associated with a shorter warm-ischaemia time, less frequently required conversion to open surgery, had fewer complications, and is followed by a shorter hospital stay. However, in the studies that assessed narcotic use, there tended to be somewhat more postoperative narcotic use or longer duration of convalescence after HAL.

### TABLE 1 Published series comparing standard transperitoneal laparoscopic with HALS donor nephrectomy

<table>
<thead>
<tr>
<th>Ref</th>
<th>N patients, route</th>
<th>Operative duration, min</th>
<th>Warm ischaemia, min</th>
<th>Complications</th>
<th>Conversion</th>
<th>Hospital stay (days)</th>
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<tbody>
<tr>
<td>[22]</td>
<td>11 standard</td>
<td>215</td>
<td>3.9</td>
<td>1/1</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>[23]</td>
<td>23 HAL</td>
<td>165</td>
<td>1.6</td>
<td>2/0</td>
<td>1</td>
<td>2.0</td>
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<tr>
<td>[24]</td>
<td>40 standard</td>
<td>255</td>
<td>5.0</td>
<td>3/0</td>
<td>0</td>
<td>6.5</td>
</tr>
<tr>
<td>[25]</td>
<td>15 standard</td>
<td>276</td>
<td>3.8</td>
<td>1/0</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>[26]</td>
<td>29 standard</td>
<td>205</td>
<td>2.4</td>
<td>2/0</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>[27]</td>
<td>18 HAL</td>
<td>269</td>
<td>3.4</td>
<td>2/1</td>
<td>1</td>
<td>4.1</td>
</tr>
<tr>
<td>Total</td>
<td>134 standard</td>
<td>278*</td>
<td>3.7*</td>
<td>14.2%†</td>
<td>5.2%†</td>
<td>3.1*</td>
</tr>
<tr>
<td>[28]</td>
<td>15 HAL</td>
<td>260</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Total</td>
<td>158 HAL</td>
<td>232*</td>
<td>2.5*</td>
<td>8.1%†</td>
<td>2.5%†</td>
<td>2.8*</td>
</tr>
</tbody>
</table>

*weighted mean; †% occurrence of summed totals.

Several centres have reported that HAL PN is safe and reproducible. Stifelman et al. [28] performed HAL PN in 11 patients, nine of whom had suspicious lesions and two of whom had duplex systems with nonfunctioning upper moieties. The harmonic scalpel (Ethicon, Cincinnati, Ohio, USA) was used to excise tissue; haemostasis was aided with gel-foam and the argon beam coagulator. The mean operative duration was 273 min and the estimated blood loss 319 mL. Patients resumed oral intake at a mean of 1.7 days and were discharged home in 3.3 days. There was one conversion; no major complications were reported. The average tumour diameter was 1.9 cm and there were no positive surgical margins.

In another study, Wolf et al. [29] compared 10 laparoscopic PNs (eight with hand assistance) to a contemporary cohort of 10 who had open PN. Most tumours were peripheral, exophytic and of a similar size (mean 2.4 cm in both groups) Data on patient satisfaction and recovery were obtained via self-administered questionnaires. The mean operative time was 24% longer in the laparoscopic group. However, in the HAL group, there was 62% reduction in parenteral narcotic use, 43% reduction in hospital stay, 64% more rapid return to normal light activity, and improved pain and physical health scores taken at 2 and 6 weeks.

### CONTRAINDICATIONS

HAL does not seem to have a niche for any reconstructive procedure. e.g. pyeloplasty or cyst decortication, which can be performed safely and effectively with standard laparoscopic techniques. In young children, during deep pelvic surgery and during retroperitoneoscopy, the hand in the operative field takes up too much working space, making visualization and exposure difficult. Adrenal surgery, and small/!

### CONCLUSION

HAL surgery offers clear advantages over traditional open surgery, including decreased blood loss, pain medication requirement, hospital stay and convalescence. It appears to be at least as effective as conventional laparoscopic techniques, and offers the benefits of proprioception and three-dimensional spatial orientation. In summary, HAL surgery appears to be a safe, reproducible and minimally invasive technique to perform extirpative renal surgery.

### CONFLICT OF INTEREST

None declared.

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Abbreviations: HAL, hand-assisted laparoscopy; NU, nephroureterectomy; RN, DN, PN, radical, donor, partial nephrectomy.
The role of photodynamic diagnosis in the contemporary management of superficial bladder cancer

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bladder cancer, photodynamic diagnosis, TURBT, 5-ALA

INTRODUCTION
Photodynamic diagnosis (PDD) for bladder tumours was reported as long as 40 years ago [1], but the modern era was heralded with the first clinical report of the use of 5-aminolaevulinic acid (5-ALA) as a photosensitizing agent [2]. Numerous studies have followed, most promoting the increased sensitivity that PDD offers in detecting bladder cancer. However it is still not in widespread use, perhaps because clinicians are unsure of exactly which patient groups are best served by this technique. This review will attempt to clarify, using the evidence available, where exactly PDD fits into the options available to contemporary urologists.

MECHANISM OF 5-ALA-INDUCED BLADDER TUMOUR FLUORESCENCE
5-ALA is the starting point of the haem biosynthesis pathway (Fig. 1), haem being a vital element of the cytochromes involved in the respiratory chain. The substance immediately before haem in this pathway is protoporphyrin IX (PPIX) and it is this intermediate that is fluorescent, appearing red when viewed under blue-violet light. Normally the accumulation of PPIX is prevented in the presence of adequate haem molecules by negative feedback on the synthesis of 5-ALA, but exogenous administration of 5-ALA overcomes this.

PDD relies on the selective accumulation of PPIX in neoplastic cells, up to 10 times more in tumour than normal tissue [3]. Although the precise reason for selective PPIX accumulation is unknown, there are several plausible explanations. The penetration of 5-ALA into tumour cells may be eased by their relative permeability; there is also some evidence that neoplastic cells actively accumulate 5-ALA. Normally PPIX is metabolized to haem by the enzyme ferrochelatase and this enzyme’s activity appears to be reduced in tumour cells, perhaps because of limited iron availability.

5-ALA is generally administered intravesically 2 h before cystoscopy through a urethral catheter. The procedure requires special telescopes and a specific light source (D-Light, Karl Storz, Germany). Using a foot pedal or a push-button on the camera it is possible to switch between white or blue light during cystoscopy and resection. Papillary tumours appear intensely red when viewed under blue light and red mucosal patches may represent carcinoma in situ (CIS) (Fig. 2).

5-ALA ESTER
The speed of onset and degree of PPIX accumulation are related to the amount of 5-ALA that penetrates the urothelium, and in its standard form 5-ALA is highly charged and passes across the lipid cell membrane relatively poorly. A standard pharmacological solution to this problem is esterification of the molecule, which can then easily cross the cell membrane. Non-specific esterases within the cell then release the active compound. After extensive preclinical work the optimum formulation for esterification of 5-ALA was found to be hexylester ALA (h-ALA) [4]. A clinical study showed that when h-ALA was used for PDD there was twice the fluorescence, with a decreased dwell time and a 20-times lower concentration [5]. Subsequently this has been confirmed in bladder biopsies, which show greater PPIX accumulation within cells when h-ALA is used [6]. h-ALA is likely to become the standard agent for PDD but at present it is not widely available and has not been investigated extensively. Unless stated otherwise, the studies reported below used standard 5-ALA.

THE ROLE OF PDD AT INITIAL TRANSURETHRAL RESECTION OF BLADDER TUMOUR (TURBT)
The high recurrence rate of superficial bladder cancer, up to 70% at 5 years, is responsible for a huge workload for urologists, and much inconvenience for patients. The recurrence rate at the first check cystoscopy (3 months) varies enormously, even when known risk factors are allowed for, suggesting that incomplete resection or failure to detect small additional tumours may be a risk factor [7]. Many studies show that taking additional biopsies using PDD yields a higher rate of tumour detection at initial TURBT. In three recent studies, patients were randomized when undergoing the first TURBT to either white-light cystoscopy (WLC) or PDD and then the area re-resected 2–6 weeks later (Table 1) [8–10]. All reports showed that those patients having resection under PDD had significantly less residual tumour, suggesting that this technique improves the technical quality of TURBT. Ultimately it is the reduction in long-term recurrence that is important. This was assessed by Filbeck et al. [10] in a group of 191 patients with superficial bladder cancer. These authors showed that the recurrence rate at 2 years was 34% in the WLC group and only 10% in the PDD group (P = 0.004). That study provides strong support for the routine use of PDD at first TURBT to reduce subsequent recurrence rates.

Another method of reducing the recurrence rate of superficial bladder cancer is the administration after TURBT of intravesical chemotherapy, as confirmed by a recent meta-analysis [11]. None of the above studies used this, despite evidence that it is the ‘standard’, and hence it is not possible to say if PDD has any additional effect. Definite acceptance of PDD will require a further
randomized controlled trial that includes adjuvant chemotherapy.

DETECTION OF CIS; IMPLICATIONS FOR PROGRESSION

CIS is notoriously difficult to recognize on standard cystoscopy, as it may appear macroscopically identical to normal urothelium. The progression rate of untreated CIS to invasive disease is about 50% and ‘missed’ cases may be responsible for progression in otherwise ‘low-risk’ patients. Two studies have produced meaningful data on the detection of CIS by PDD.

In the first, a large study of 1012 fluorescence endoscopies, CIS was found on 88 occasions [12]; 50 of these cases (57%) were not detected on a preliminary standard WLC. A more recent study specifically set out to consider the diagnosis of CIS using PDD. h-ALA was used as the photosensitizer [13]. Patients were selected to be particularly at risk for CIS and indeed the detection rate was high, at 39% (83/211). A standard WLC was carried out together with a single random biopsy, and this was followed by PDD cystoscopy. Of the 83 patients, 62 were detected both on WLC and PDD, 18 on PDD alone and three on WLC alone.

Because of its high potential for progression, random biopsies have been taken to try to detect CIS. Neither of the above studies compared PDD with random biopsies. Also, although it would be hoped that any improved method for detecting CIS could potentially contribute to a reduction in the development of invasive disease, it was shown that random biopsies do not influence this [14]. Hence a randomized trial would be required to clarify this issue for PDD.

DETECTION OF HIGH-GRADE DYSPLASIA

As well as CIS there have been reports that PDD can be used to detect bladder dysplasia with much greater sensitivity than WLC [15,16]. The significance of this is uncertain, as there is controversy as to the natural history of dysplasia. However, given that some have suggested it is associated with progression in superficial disease, it will be interesting to see if increased detection allows clarification of this issue.

ROLE OF PDD AT CHECK CYSTOSCOPY

In the current situation, where PDD is not standard practice, the use of PDD at check cystoscopy may have value even in low-risk patients. This would be related to the detection of previously invisible tumours, especially CIS. In a group of patients undergoing a second TURBT using PDD 6 weeks after conventional TURBT, 14% (seven of 50) had tumours in previously unresected areas of the bladder [17]. Assuming that all patients have received PDD at the first resection, the question arises as to which ones require it at subsequent cystoscopy. There are some specific groups to consider.

AFTER INTRAVESICAL THERAPY (IVT, E.G. BCG OR CHEMOTHERAPY)

PDD can give false-positive results in conditions such as inflammation, and therefore the effect of previous IVT, particularly BCG, is of interest. The largest study examining this found that patients who had undergone recent IVT (within 6 months) had significantly more false-positive biopsies using PDD (65/164, 40%) than those who were >6 months after IVT or had never had it (205/753, 27%) [18]. Despite the high false-positive rate, additional cases of residual tumour or CIS are still found using PDD in this patient group, and so it may still be of value.

RE-RESECTION

When G3pT1 bladder tumours are diagnosed most urologists will be suspicious of the possibility that invasive disease is being missed and will perform early re-resection at 6 weeks. This involves a thorough resection of all scar tissue. In this situation PDD is unlikely to be of benefit because it detects only superficial disease. There have been no formal studies of PDD in re-resection of G3pT1 disease, but a small study that included a variety of histological types highlighted...
another problem, frequent false-positive fluorescence of the scar [17].

THE ROLE OF PDD IN A HAEMATURIA CLINIC

Flexible cystoscopy using PDD has recently become possible with the development of a more powerful light source (D-Light C). The potential role in the haematuria clinic is not clear. Detecting additional tumours is unlikely to be of benefit if patients are undergoing subsequent TURBT using PDD. There would no doubt be a few occasions where solitary tumours that might have been missed completely on standard flexible cystoscopy will be detected, but whether this is cost-effective will need to be determined.

OTHER POTENTIAL USES OF PDD

UPPER TRACT STUDIES

The patient with persistently positive urinary cytology but a normal cystoscopy and upper tract imaging is a clinical dilemma. Flexible ureteroscopy is used to directly visualize the ureters and renal collecting system, but may miss flat lesions. With the new more powerful light source, PDD of the upper tracts might be possible and is being investigated.

CYTOLOGY

In the follow-up of patients with low-grade TCC, noninvasive methods are continually being sought. Urine cytology has been used but lacks sensitivity for low-grade lesions. A recent report described fluorescence cytology as a possible method of overcoming this [19]. However, the sensitivity was only increased from 79% to 86% and clearly all patients have to be catheterized, meaning this technique is unlikely to become popular in its present form.

PROBLEMS WITH PDD

Current formulations of 5-ALA are unstable and require preparation immediately before administration, usually by a pharmacist. This may cause logistical problems in getting patients ready for an early-morning operating list. Newer preparations are being developed that can be made up by nursing staff on the ward, and ultimately it is hoped that a stable formulation can be created. PPIX is degraded when exposed to light (blue or white) and its fluorescence reduced. In initial demonstrations of PDD this was a problem, but it has not been a practical issue in its routine use.

While training is required in the use of PDD it is a relatively straightforward procedure. One source of error for trainees is the fluorescent appearance of tangentially viewed mucosa because of the nonspecific accumulation of PPIX in normal urothelium. This can be clarified by varying the observation angle.

Up to a third of fluorescent areas on PDD may be histologically benign. These can occur in previous resection sites and be a result of cystitis, squamous metaplasia or previous IVT. It will be impossible to completely eliminate false-positive results but one approach to reducing them has been in vivo quantification of PPIX fluorescence [20]. This relies on digital calculation of the ratio of fluorescence to background and has been shown to reduce false-positive results by 30% without compromising sensitivity.

PHOTODYNAMIC THERAPY

Photodynamic therapy uses the selective uptake of photosensitizers by bladder cancer cells as a method of treatment. Essentially, when these cells are exposed to light in the
The presence of oxygen, local hyperthermia leads to cell death. This is an attractive idea for the therapy of widespread CIS or multifocal papillary disease. Unfortunately, initial attempts were associated with significant rates of bladder contracture because of the nonspecific accumulation of photosensitizers in the detrusor muscle. It was suggested that this is much less of a problem with 5-ALA, which does not penetrate the urothelium to any significant extent. There are relatively few clinical reports to date, but they are encouraging in terms of safety and tolerability [21–23].

**HYPERICIN**

Hypericin is a naturally occurring substance derived from St John’s Wort (*Hypericum perforatum*) and has been used clinically as an antidepressant. It was shown to be a potent photosensitizer in TCC. Initial clinical reports claim a higher specificity than 5-ALA-based PDD, although there were false-positive results in patients who had received intravesical BCG [24]. While offering some advantages over 5-ALA, such as relative ease of preparation, there is not enough data available on hypericin at present to be able to evaluate its clinical role.

**CONCLUSIONS**

In the decade since its first clinical use it has been shown that PDD using 5-ALA is a safe adjunct to cystoscopy and which allows the detection of otherwise invisible tumour. One randomized study showed that its use can reduce the recurrence of superficial TCC. It is a relatively easy technique to learn and because it allows an accurate assessment of the adequacy of resection at TURBT, it is likely to be a useful aid for training junior urologists in the future. With the development of h-ALA and more user-friendly formulations, it will be used increasingly and should become the standard method of primary TURBT. However, evidence for its use in other situations is at an earlier stage and indications will be better defined over the next 10 years.

**CONFLICT OF INTEREST**

None declared.

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Abbreviations: PDD, photodynamic diagnosis; PPIX, protoporphyrin IX; 5-ALA, h-ALA, 5-, hexylester-, aminolaevulinic acid; IVT, intravesical therapy; CIS, carcinoma in situ; TURBT, transurethral resection of bladder tumour; WLC, white-light cystoscopy.
INTRODUCTION

Hypospadias is a common congenital disorder affecting 1 in 300 live male births. Classically it is a triad consisting of a malpositioning of the urethral meatus, a ventral curvature of the penis and an abnormal distribution of foreskin, giving a ‘hooded’ appearance [1]. The resulting defect varies, with 80% having a distal malpositioning of the meatus, requiring a single-stage repair, and 20% having a more severe, proximal malpositioning, which may require two or three operations to repair. The more severe the initial problem the higher the complication rate, and the more operations the patient is likely to require.

There are many reports describing the surgical management of hypospadias. The results may be measured easily in terms of complications. The appearance and the acceptability to the patient are just starting to be considered [2,3]. The difficulty is that the patients are children and it may not be their opinions that are sought, but only those of their parents [2]. Although the many operations described suggest that none is perfect, the results now are sufficiently good that nonsurgical factors, and particularly the psychological aspects, should be considered in assessing the outcomes [4]. However, there is little published that describes what psychological problems need addressing (if any), what interventions have proved useful or how often they are offered.

Adult patients who are now available for outcome analysis had their surgery in the 1970s; much of the evidence cited in the present review comes from surgery that is even older. Techniques have changed and surgical results have improved. However, against this must be set the increase in patient expectations that has come with greater education. In this review, publications on the nonsurgical aspects of hypospadias repair are reviewed and compared with the practices of surgeons in the UK, as assessed by a questionnaire.

THE TIMING OF SURGERY

Early practice guidelines from the American Academy of Pediatrics recommended that children with hypospadias should be operated on after 4 years of age [5]. It was felt that the parental separation from the child would be detrimental to the child’s development, and surgery should be delayed until after the phase of separation anxiety, thought to end at 4 years old. This recommendation has changed for two reasons; first, surgical and anaesthetic techniques for infants have improved [6], and second, there was a large move within paediatrics as a whole to encourage parents to stay with their child. This allowed the admission of young children with no increase in anxiety as a result of separation [7]. More recently, hypospadias has been repaired as a day-case procedure, avoiding the concerns of separation anxiety.

Four papers focused on the timing of elective surgery in hypospadias and agreed that an early operation minimizes the potential psychological damage caused by genital surgery [6,8–10]. Surgery when aged 6–15 months avoids five particularly sensitive phases of psychosocial development, the disruption of which is thought to predispose to psychological problems in later life (Table 1) [11]. This period was derived on the basis of theory rather than research, a point noted by the authors. Indeed, little research, least of all prospective or controlled, has been done to verify these theories [12]. Mondaini et al. [13] studied 40 hypospadias patients and compared them to over 10 000 unaffected controls; the age at which surgery took place was not associated with abnormal psychological adjustment later in life.

Usefully, the timing of the development of body image also corresponds to the Freudian ‘phallic stage’ of development and the Oedipal Complex, where the child is supposed to experience rivalry with the parent of the same sex and develop an attachment with the parent of the opposite sex. The guidelines from the American Academy of Pediatrics now recommend operations for hypospadias before 30 months old, to minimize the psychological impact on body image and gender identity [14].

BODY IMAGE

BEFORE SURGERY

Impending surgery for hypospadias induces anxieties in both parents and children that are not seen in families with other surgical conditions of similar severity. Genital awareness is thought to develop at 3–5 years old, earlier if there are older male siblings [8]. This is when the child begins to widen his social circle in nursery and play-groups, and has the opportunity to compare genitals [9].

In severe hypospadias the genitalia may be ambiguous [15]. If the parents are anxious about the ‘maleness’ of the child, there may be an adverse affect on body image [9]. A preoperative assessment of children aged 2–6 years with hypospadias found that parents were indeed concerned about the ‘maleness’ of their child, which mirrored findings in the children themselves, who showed predominantly genital-centred anxieties, as measured on the Robertson Auditory Projective Test [16]. The assessment by Robertson and Walker also compared the anxieties of the parents of children with cleft palate with those who had hypospadias. They found that the parents of the hypospadias group had anxieties based on the future potency of the child, whereas the children themselves, and both the parents and children in the cleft palate group, had anxieties based more on the present operation. The authors felt that the main difference between the groups was the presence of a hidden ‘guilty secret’ which prevented the parents from discussing the operation within the family.

Ironically, there are many men who have a hypospadiac meatus for which they have
the surgeon’s view has little correlation with cosmetic result, although it was reported that there is little research into what makes a good outcome, being less important [21]. Overall satisfaction, with micturition and satisfactory sexual function, were correlated with the cosmetic appearance, together with other factors that are important for future psycho-sexual functioning. Importantly, one study found that the better the genital body image, the better the psychological function [22]. Therefore, in children who have had an operation for hypospadias a good cosmetic result is most important. Surgically correctable:

- meatal position
- glans shape
- scars
- scrotum
- general appearance.

Uncorrectable:

- volume of the glans
- penile size
- penile thickness.

Much disagreement among surgeons and patients centres on the uncorrectable features. Studies of the long-term effects of hypospadias surgery on body image have found that patients were more embarrassed than controls about their penis, and had more sexual inhibitions as a result [22]. Genital body image in hypospadias patients was negatively correlated with the initial severity of the hypospadias [13,19], and positively with the terminal position of the meatus [20]. Size may also be a cause of dissatisfaction. The hypospadiac penis is often said to be short. In part this may be because of the circumcised appearance, especially in societies where infant circumcision is unusual. However, where a formal measurement has been made, a fifth of hypospadiac penises were below the 10th centile. The finding was most marked in adolescents, with four of seven being below the 10th centile (Table 2) [3].

Penile size is a source of considerable anxiety in many adolescents. Limited research is available on the relationship of penile size to sexual satisfaction. Men with micropenis and with epispidias report intercourse that is less pleasurable than that of a circumcised one [24].

Early operations concentrated on functional correction, believing that ‘minor’ cosmetic abnormalities, such as a coronal hypospadias, could be ignored. Surgeons felt that the cosmetic appearance was only of concern to the parents, not the patients [18]. Nowadays it is felt that poor cosmetic results are not accepted by patients [1] and have an effect on the patient and was reflected in their level of satisfaction [3,20]. This finding was replicated by another study which found that the cosmetic appearance, together with other factors that are important for future psycho-sexual functioning, were correlated with overal satisfaction, with micturition being less important [21].

There is little research into what makes a good cosmetic result, although it was reported that the surgeon’s view has little correlation with that of the patient [3]. In that study, the surgeon based a good cosmetic outcome on the variables that were surgically corrected, such as meatal position. Patients tended to place emphasis on other factors that are not currently operable. Mureau et al. [3] identified eight features of importance in judging the outcome of hypospadias surgery:

Surgically correctable:

- meatal position
- glans shape
- scars
- scrotum
- general appearance.

Uncorrectable:

- volume of the glans
- penile size
- penile thickness.

Much disagreement among surgeons and patients centres on the uncorrectable features. Studies of the long-term effects of hypospadias surgery on body image have found that patients were more embarrassed than controls about their penis, and had more

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**TABLE 1** The ages of main psychosexual milestones in infancy and early childhood

<table>
<thead>
<tr>
<th>Age</th>
<th>Psychological event</th>
<th>Other high risk periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infancy</td>
<td>Maternal bonding</td>
<td>18–24 months</td>
</tr>
<tr>
<td>18 months</td>
<td>Rapprochement</td>
<td>4–6 years</td>
</tr>
<tr>
<td>3–4 years</td>
<td>Development of genital body image</td>
<td></td>
</tr>
<tr>
<td>2–7 years</td>
<td>Cognitive development</td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>Oedipal/phallic awareness</td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>Castration anxiety</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2** The number of patients in three age groups with a stretched penile length in different centiles; adapted from [3]

<table>
<thead>
<tr>
<th>Age group, years (n)</th>
<th>No. of patients</th>
<th>&lt;10th centile</th>
<th>Average</th>
<th>&gt;90th centile</th>
</tr>
</thead>
<tbody>
<tr>
<td>9–12 (16)</td>
<td>1</td>
<td>12</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>13–15 (10)</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16–18 (7)</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

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emotional or behavioural differences between patients and controls [22,28], although Sandberg et al. [28] noted that higher rates of hospital admissions were associated with an increase in emotional problems.

LATE EFFECTS

The studies assessing late effects of hypospadias surgery divide their findings into three groups: psychological, psychosocial and psychosexual. Although there have been several studies evaluating the psychological sequelae of hypospadias, most comprise few patients and have poor controls, often comparing the surgical patient with a community control. Where papers assessed behavioural changes after surgery the sample patients were frequently those who had been operated on when aged 3–6 years, and it could be argued that the resulting behaviour was a consequence of operating during a psychologically vulnerable age. Up to 20% of patients with severe hypospadias and genital ambiguity felt that their psychological well-being had been impaired, and 10% had evidence of mild depression [29].

However, with those less severely affected there is some variation in findings. One study, using open interviews, found that patients who had been operated on for hypospadias showed more neurotic symptoms, e.g. depression and anxiety, used immature defence mechanisms and had poor relationships as a result [27]. In the same patient group, a second study, using Rorschach’s test, found that patients who had been operated on for hypospadias had more neurotic symptoms, less self-esteem and less capacity for relations, which was felt to prove the existence of the castration complex [30]. The converse was found in another study, again using an unblinded interview, which found no psychological problems in adulthood [31].

Research into psychosocial factors is similarly divided. Those studies which have found psychosocial deficits showed that some patients who have been operated on for hypospadias have less capacity to form relationships or felt that their relationships were affected by the disorder [20,27]. One study found that the marriage rate was 20% lower in a community population [32]. In addition, one study suggested that adults who had had a hypospadias repair were employed in less competitive and less responsible jobs than surgical controls [27].

However, importantly in the study of Bracka [20], those men who were satisfied with their penile appearance had a near-normal age of sexual debut (15.6 years), the delay being mainly in those who were dissatisfied (debut at 19.0 years). In other reports, up to 19% of hypospadias patients had more sexual difficulties and inhibitions, and anticipated more ridicule, than surgical controls. They were older at the first sexual intercourse and they had fewer partners as a result [20,34].

Some studies have focused on the development of general and gender-role behaviour. An early study reported that compared with matched controls, patients with hypospadias were less secure in their maleness and had a tendency to take a more feminine sex role, although had similar sexual orientation to controls [35]. Other research groups reported more behavioural problems and lower social competency than in nonclinical controls, but found better adjustment than a psychiatric control group. Boys with hypospadias showed more cross-gender behaviour than the psychiatric controls [26], although this was associated with hospitalization rather than severity of the condition. In a larger sample the same research team subsequently reported fewer men exhibiting masculine behaviour, although once again the number of hospitalizations for surgery were correlated with gender-atypical behaviour rather than severity of the hypospadias [36]. The significance of the data in that study is very dependent on the selection of the subjects. Apparently the reasoning for the gender-atypical behaviour was the hypo-androgenization associated with hypospadias rather than the surgery itself. In most patients with hypospadias the pituitary and testicular axis is normal, and so the presence of hypo-androgenization must imply some selection bias [20].
Several risk factors for a poor psychological outcome can be conjectured:

- Surgery outside the optimum age bracket.
- Severity of the hypospadias.
- Number of operations.
- Child’s/parent’s unfavourable view of hypospadias.

Most have not been rigorously tested and rely on psychodynamic theories. Probably the most important are the severity of the hypospadias and the number of operations needed for correction. These two factors are understandably difficult to differentiate, as the one will frequently lead to the other. Poor surgical results (Figs 2 and 3) are associated with a delayed age of sexual debut.

TREATMENTS

Most authors cited have agreed that psychological support would be beneficial for this group of patients. This rarely occurs and one study found that two-thirds of patients had received no guidance, 60% stating that they had never even heard of the term ‘hypospadias’! [20]. Studies, especially of severely affected patients, emphasize ‘the importance of follow-up in adolescence and adult life with adequate counselling when necessary’ [29]. However, to date there are no studies that have assessed what therapies are useful, for how long they should run, or which patients are suitable; all are questions worth investigating.

One case study reported a prepubescent boy who was described as having subjectively experienced the appropriate hypospadias surgery as repeated abuse, and had subsequently become a sexual offender [37]. A cognitive behavioural model was used to break a pattern of offending. The authors argue that a psychodynamic model would have been more appropriate if the boy had engaged with the treatment consistently. Treatment for his offending failed, although earlier treatment (implying during surgery and before offending) may have altered the outcome. The difficulty with this view is that the huge majority of men born with hypospadias are not criminals and therapy aimed at preventing such behaviour in all patients would not be practical or useful.

Work has been carried out in clients with eating disorders or body dysmorphic disorder, and shows some success in challenging and changing distorted views on body image using a cognitive-behavioural model [38,39]. This is an area of potential development for the psychological treatment of hypospadias. However, again it would not be practical to provide all hypospadias patients with individual cognitive therapy. It would be useful to establish the risk factors for a poor psychological outcome and so be able to target psychological treatments effectively.

It is also reasonable to question whether psychological treatment for all patients would be beneficial. Bracka [20] suggested that all children should have regular follow-up to identify surgical and psychological problems at an early stage. Possibly a regular visit to hospital for an inspection of the genitalia (which the child may think are normal) might generate a psychological problem that previously had not existed. Work in our unit with adolescents born with exstrophy has suggested that patients wish to be considered normal, and that facilities provided by adults to help in fact serve only to emphasize the abnormalities from which they wish to escape [40].

SURVEY

The psychological factors in hypospadias might be considered sufficiently well documented that surgeons would be aware of them and adjust their practices accordingly. After all, the operations are, in many cases, cosmetic in intent, so that the psychological outcome ought to be very important [4];
sadly, at least in the UK, this appears not to be the case.

In a mailing to 193 urology centres (71% response rate) we identified 34 surgeons in 30 hospitals who regularly carried out hypospadias surgery. Ten were paediatric urologists, 20 were general urologists and four were plastic surgeons. The mean age at which the hypospadias was repaired was strongly related to the specialty and experience of the surgeon, at 21, 36 and 40 months (range 12–120) for the three groups of surgeons, respectively; and 21, 30 and 51 months for those with a case load of more than seven, two to four, or ‘rarely’ each year. Paediatric surgeons and those with a greater case load usually operated within the optimum age range. Most surgeons apparently were aware of the relevant psychological factors, but those who operated late misinterpreted the data which were cited as a reason to justify their practice. Some surgeons who operated late were under the illusion that the penis grows significantly between 18 and 36 months.

Of the surgeons, 60% had no concerns about psychological problems in the children under their care. Many of those who did have such concerns thought that the immediate per-operative period was the most important, often more for the parents than the child. The support used was mainly from nurses and play therapists; only 18% of current support was from a psychologist (or similar psychosocial professional). When it was realized that psychological help was needed, 80% had an idea about how to refer, with 56% referring to a paediatric psychiatrist or psychologist.

The opinions expressed did not always seem to be based on the medical evidence, and were sometimes self-contradictory. Some surgeons felt that having the child in nappies was helpful, while others thought it to be a disadvantage. Penile size and anaesthetic risk were used to support surgery at all ages. The contradicitions were even more marked when surgeons were citing the psychological reasons for their choice. It would appear that surgeons use an often spurious psychological reason to support their personal prejudice.

There appeared to be little support for the notion that all children had psychological problems as a result of hypospadias and its surgery. Of more concern, the views did not always accord with the available evidence. Where surgeons did have concerns, most made referrals only when a specific problem occurred.

CONCLUSIONS

Which nonsurgical factors are important in successful hypospadias treatment? There is good evidence that a better surgical result does produce a happier adult. However, there is also evidence that the patients and surgeons do not agree on that which constitutes a good result. For the surgeon, successful relocation of the meatus and correcting chordee may constitute success. For the patient with hypospadias, the goal is a normal penis. This is a significant challenge when some of the features of hypospadias are not surgically correctable, with a particular difficulty in the increasing number of societies which do not routinely circumcise.

Although psychological evidence is limited, several consistent findings suggest that in the long term some patients show a greater tendency towards low mood and low self-esteem. Whilst patients are later in initiating intercourse and feel that their relationships have been affected (often expecting more ridicule from their partners) most ultimately experience a satisfying sexual relationship. However, importantly, most of these studies were focused on patients who would have been operated at the standard age (i.e. 3–6 years) which is different from current accepted practice.

Several risk factors for a poorer psychological outcome can be conjectured. These include the severity of hypospadias, the timing of the operation and individual ways of dealing with hypospadias, e.g. negative cognitive schema associated with physical appearance. In our survey, practising surgeons identified the main psychological issues as parental anxiety, anticipatory anxiety and some anxieties about future sexual function. However, so far there is little rigorous evidence that these really are risk factors for postoperative psychological distress.

Given these hypotheses, it should be possible to consider carefully that which surgeons could be doing to facilitate improved psychosocial outcomes in both the short- and long-term. It is not clear at what point or what type of psychological support would be most helpful or to whom it should be offered. This is an area that needs further research.

Although we still lack proof that surgery is less traumatic, in the broadest sense, in infants aged < 30 months, the theoretical basis for early surgery is sound. Those surgeons, particularly those with no specialist practice, should pay attention to the established psychological aspects and operate within the recommended age limits.

CONFLICT OF INTEREST

None declared.

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In a multi-institutional study authors from the USA and Austria attempt to determine if there are differences in several indices between African-American and white men undergoing radical prostatectomy. They did not find race to be an independent risk factor for PSA recurrence, but found that other variables commonly associated with PSA recurrence are also important in African-Americans.

Using data extracted from the Hospital Episodes database, authors from England describe national trends in radical nephrectomy between 1995 and 2002. They found a considerable increase in the annual number of radical nephrectomies, with an expected increase in the number of laparoscopic procedures. They also found a decrease in emergency admissions and length of hospital stay.

Racial differences in serum prostate-specific antigen (PSA) doubling time, histopathological variables and long-term PSA recurrence between African-American and white American men undergoing radical prostatectomy for clinically localized prostate cancer

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OBJECTIVE
To determine if there are significant differences in biochemical characteristics, biopsy variables, histopathological data, and rates of prostate-specific antigen (PSA) recurrence between African-American (AA) and white American (WA) men undergoing radical prostatectomy (RP), as AA men are twice as likely to die from prostate cancer than their white counterparts.

PATIENTS AND METHODS
We established a cohort of 1058 patients (402 AA, 646 WA) who had RP and were followed for PSA recurrence. Age, race, serum PSA, biopsy Gleason score, clinical stage, pathological stage, and PSA recurrence data were available for the cohort. The chi-square test of proportions and t-tests were used to assess basic associations with race, and log-rank tests and Cox regression models for time to PSA recurrence. Forward stepwise variable selection was used to assess the effect on the risk of PSA recurrence for race, adjusted by the other variables added one at a time.

RESULTS
The AA men had higher baseline PSA levels, more high-grade prostatic intraepithelial neoplasia (HGPIN) in the biopsy, and more HGPIN in the pathology specimen than WA men. The AA men also had a shorter mean (SD) PSA doubling time before RP, at 4.2 (4.7) vs 5.2 (5.9) years. However, race was not an independent predictor of PSA recurrence.
(P = 0.225). Important predictors for PSA recurrence in a multivariable model were biopsy HGPIN (P < 0.014), unilateral vs bilateral cancer (P < 0.006), pathology Gleason score and positive margin status (both P < 0.001).

CONCLUSIONS
This study indicates that while there are racial differences in baseline serum PSA and incidence of HGPIN, race is not an independent risk factor for PSA recurrence. Rather, other variables such as pathology Gleason score, bilateral cancers, HGPIN and margin positivity are independently associated with PSA recurrence. The PSA doubling time after recurrence may also be important, leading to the increased mortality of AA men with prostate cancer.

KEYWORDS
prostate cancer, African-American, PSA doubling time, pathological stage, radical prostatectomy, PSA recurrence

INTRODUCTION
The number of new cases of prostate cancer was estimated at 513 000 worldwide and 173 000 in the USA, accounting for 15.3% of all cancers in men in developed countries in 2000 [1]. Within the next 15 years, prostate cancer is predicted to be the most common cancer in men [1]. The incidence of prostate cancer varies widely among ethnic populations, and the rate of this disease can differ by as much as 90 times among various populations. Specifically, African-American (AA) men in the USA have the highest incidence of prostate cancer (137 per 100 000 per year) [2].

According to the 2000 USA Census, AAs comprise the second largest racial group in the USA; that AA men have a 2.5 times greater mortality from prostate cancer than white Americans (WA) has become a significant health concern in the USA [2]. This strikingly higher mortality for AA men raises several questions. Is the difference in mortality a result of diagnosis at later, more advanced disease stages? Or is it because prostate cancer is more biologically aggressive in AA men? [3]. It may also be possible that AA men are receiving different treatments for their prostate cancer than other populations [4]. In addition, is there a role for competing causes of death such as comorbidity and age? Finally, do poverty, socio-economic status and education have a role? [4].

Several authors [4–10] have examined an important surrogate, PSA recurrence, as a measure of prostate cancer-specific mortality. These studies evaluated clinical and pathological variables before and after surgery, socio-economic factors, and delivery of healthcare; the results are conflicting [4–10]. Recently there has been interest in the PSA doubling time as a surrogate for the ultimate outcome of prostate cancer [11]. Differences in PSA doubling times before and after treatment among races have been studied in a few series [8], but there is no comprehensive report of this subject in AAs in an equal-access setting. To address this issue, we evaluated differences in PSA doubling times, histopathological variables, and the incidence of PSA recurrence between AA and WA men with clinically localized prostate cancer. Accordingly, the analysis was limited to patients undergoing radical retropubic prostatectomy (RP) to ensure the availability of histopathological data from the excised surgical specimen.

PATIENTS AND METHODS
This retrospective cohort study was aimed at comparing pathological stage and PSA recurrence in AA and WA men with prostate cancer and who were treated with RP at a large healthcare system in the mid-West USA, the Henry Ford Health System (HFHS), a vertically integrated healthcare system incorporating the nation’s 10th largest health maintenance organization. The population served by HFHS is large and racially diverse, with =30% of the patients being AA. The HFHS has a computerized medical information system and medical record database. Comprehensive data are also available from computerized health-claims databases. This study was part of an Institutional Review Board-approved project for evaluating the effect of various demographic, clinical and histopathological variables on prostate cancer recurrence.

HFHS maintains a computerized tumour registry database accredited by the American College of Surgeons. Registry staff use a thorough case-finding system, including a review of all pathology and cytology reports, and radiation and oncology consultations. The American Joint Commission on Cancer system is used to determine the stage of disease by evaluating tumour size, extent of invasion, microscopic involvement of lymph nodes and presence of metastases. HFHS Registry staff link this data with the Detroit area Surveillance, Epidemiology and End Results Program records, and conduct an annual follow-up for vital status and recurrence; the annual follow-up is estimated at 94%. Thus the tumour registry of the HFHS was searched for all patients with an ICD-9 code of 185 (prostate cancer), who were treated by RP and followed during the period 1 January 1990 to 31 December 2000; only men with localized cancer (i.e. a negative bone scan) were included in the study.

Patients were excluded who were not AA or Caucasian, as were those who had incomplete follow-up information. Patients who developed bone metastasis within a year of diagnosis were also excluded, as we felt that these men most likely had pre-existing metastatic disease. Patients were also excluded if they received preoperative hormonal, radiation, cryotherapy, or received immediate adjuvant hormonal or radiation therapy for extracapsular disease, including seminal vesicle involvement.

The diagnosis of cancer was established by histological examination of prostate biopsy specimens by HFHS pathologists; tumour grade was reported as Gleason score 2–10, and the disease was staged using the 1992 TNM classification.

Relevant baseline variables were recorded, e.g. race, age, clinical stage, serum PSA, biopsy Gleason score (minor and major), side(s) of positive cores, perineural infiltration (PNI), high-grade prostatic intraepithelial neoplasia (HGPIN), and prostatic inflammation.
Pathological variables after RP were also recorded, e.g. specimen weight, pathological stage, PNI, HGPIN, prostatic inflammation, percentage tumour, margin status, seminal vesicle involvement, and lymph node spread. The baseline (preoperative) PSA doubling time was calculated by a linear regression model using at least four PSA values.

The primary endpoint of the analysis was the difference in PSA recurrence and doubling time. PSA recurrence was defined as two or more consecutive samples with a PSA level of >0.2 ng/mL. The PSA doubling time after recurrence required at least three PSA values during the follow-up [11]. The secondary endpoints were differences in pathological variables, e.g. percentage cancer, HGPIN, PNI, inflammation, margin status, seminal vesicle invasion and lymph node spread.

A univariate analysis was used to compare the pathological variables at baseline and after RP between the racial groups. Comparisons of PSA recurrence were based on survival analysis. The Cox proportional-hazards model was used for multivariate survival analysis, which allowed an estimate of the PSA recurrence time, controlling for differences in follow-up time and risk factors that may affect survival, including confounding variables and effect modifiers. All relative risks were derived from the multivariate Cox models. Adjusted survival curves were generated using the empirical cumulative hazard estimate of the survivor function. Differences in the duration of survival were calculated by measuring differences in the adjusted survival curves at median survival. All P values were two-sided.

RESULTS

The baseline characteristics of the cohort of 1058 patients are summarized in Table 1. An important and surprising finding of the study was that at baseline, AA men had a shorter PSA doubling time than WA men (Table 1).

Table 1 also summarizes the study endpoints between the cohorts; even though PSA recurrence was no different between the racial groups, after PSA recurrence was diagnosed the PSA level increased at a faster rate in AA men than WA men. This was not statistically significant because the doubling time was calculable only in a few patients who eventually had PSA recurrence. However,
these data showed that there was >3 years difference in PSA doubling time between the racial groups. None of the other study endpoints, i.e. margin positivity, seminal vesicle infiltration and lymph node spread, were statistically different between the cohorts.

As shown in Table 2, AA origin was not an independent predictor of PSA recurrence in the univariate model. Other known variables, e.g. clinical stage, Gleason score, percentage cancer and pathological stage, were significantly associated with PSA recurrence. Importantly, HGPIN and specimen weights were also important predictors of recurrence. HGPIN had a hazard ratio (HR) of 1.8, and greater than margin positivity (1.76) and Gleason score (1.46; Table 2).

In the Cox proportional-hazard model race was not an independent predictor of PSA recurrence (Table 2, HR 1.21), but other variables, e.g. biopsy HGPIN, unilateral cancers in the biopsy, pathology Gleason score and positive margin status, were independent predictors of PSA recurrence. Interestingly there was a counter intuitive significant association between clinical stages T1 and T2 cancers, showing that T2 cancers had a lower HR for recurrence; we do not know the significance of this finding.

### DISCUSSION

Overall mortality from prostate cancer is greater in AA than WA men; historically, this has been attributed to a more advanced tumour stage in AA men at diagnosis. More recently, increased awareness and the widespread use of PSA screening has dramatically increased the detection of earlier stage cancers. This has been associated with a clear improvement in mortality and survival rates, especially in AA men, where there has been an improvement of 21% in organ-confined disease. Some have argued that the treatment outcome has been better in WA than AA men for localized prostate cancer, but others have argued that there is no significant racial difference in treatment outcome [4–7,9,10,12].

The present study indicates that there are racial differences in baseline serum PSA, PSA doubling times, clinical and pathological stages, and the incidence of HGPIN. However, race alone did not appear to be an independent risk factor for PSA recurrence. Rather, other variables, e.g. pathology Gleason score, bilateral cancers, HGPIN and margin positivity, were independently associated with PSA recurrence. The present study is unique in that it includes a significant proportion of AA patients (38%) and has many additional variables that could affect PSA recurrence. Further, we also showed that while PSA recurrence rates are no different between AA and WA men, PSA levels double much faster in the former than the latter. The biological significance of a rapid PSA doubling time is currently unknown, but given the association of a rapid PSA doubling with earlier development of metastasis [11,13], it can be postulated that after PSA recurrence, AA men may have a more aggressive course of disease. This finding is in contrast with results from a detailed study by Banerjee et al. [5], who reported that the mean average relative PSA velocity for AA and WA men having disease recurrence was 0.25 and 0.11 ng/mL per month, respectively (P = 0.21). The rate of PSA increase in patients who developed disease recurrence after RP was 18.9% per month for AA men and 16.3% per month for WAs (P = 0.73). The present results may differ because the follow-up was longer (78 months) than that assessed by Banerjee et al., where the median follow-up was 39 months. The brevity of the PSA doubling time was also noted before RP in the present series, but the significance of this finding is unknown. Several studies reported that a rapid PSA doubling time may be associated with more aggressive disease [4–7,9,10,12].

We postulate that even though PSA recurrence rates are comparable, AA men who develop PSA recurrence may have a more accelerated course of disease. This hypothesis is currently being tested at our centre in patients for whom long-term survival data are available.

Whether race is truly predictive of PSA recurrence after RP has been controversial. Earlier reports stated that race is an independent predictor of outcome, while recently many authors reported that there is

### TABLE 2 Univariate and multivariate modelling assessing the effect of the recorded variables on PSA recurrence

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1.18 (0.93–1.48)</td>
<td>0.169</td>
</tr>
<tr>
<td>Age, decades</td>
<td>0.86 (0.72–1.03)</td>
<td>0.093</td>
</tr>
<tr>
<td>PSA</td>
<td>1.00 (0.99–1.00)</td>
<td>0.613</td>
</tr>
<tr>
<td>Log PSA</td>
<td>1.01 (0.90–1.12)</td>
<td>0.924</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>0.71 (0.53–0.94)</td>
<td>0.018</td>
</tr>
<tr>
<td>Gleason (biopsy)</td>
<td>1.43 (1.29–1.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Biopsy inflammation</td>
<td>1.15 (0.43–3.08)</td>
<td>0.788</td>
</tr>
<tr>
<td>Biopsy PNI</td>
<td>1.17 (0.78–1.77)</td>
<td>0.449</td>
</tr>
<tr>
<td>Biopsy HGPIN</td>
<td>1.80 (1.22–2.64)</td>
<td>0.003</td>
</tr>
<tr>
<td>Biopsy % cancer</td>
<td>1.02 (1.01–1.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uni- or bilateral +ve cores</td>
<td>0.84 (0.67–1.07)</td>
<td>0.157</td>
</tr>
<tr>
<td><strong>RP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen weight</td>
<td>0.99 (0.98–0.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percent cancer</td>
<td>1.01 (1.00–1.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Primary Gleason grade</td>
<td>1.38 (1.29–1.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gleason score</td>
<td>1.46 (1.33–1.60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N stage</td>
<td>1.39 (0.86–2.25)</td>
<td>0.174</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>1.58 (1.18–2.12)</td>
<td>0.002</td>
</tr>
<tr>
<td>Margin positive</td>
<td>1.76 (1.40–2.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pathological stage</td>
<td>1.49 (1.19–1.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PNI</td>
<td>1.08 (0.81–1.43)</td>
<td>0.611</td>
</tr>
<tr>
<td>HGPIN</td>
<td>0.80 (0.59–1.09)</td>
<td>0.162</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.65 (0.24–1.76)</td>
<td>0.401</td>
</tr>
</tbody>
</table>
no racial disparity in progression-free survival among men with clinically localized prostate cancer. In an elegant study by Powell et al. [3,5,10,14–18] a subgroup of younger AA patients (aged <65 years) were evaluated, and they had a greater risk of recurrence. Using multivariate modelling, we re-examined this issue in the present cohort, but there was no statistically significant racial difference in younger AA patients. This discrepancy could be attributed to an inherent difference in the study populations; in contrast to the series of Powell et al., the present had <1% clinical stage T3 cancer.

The present findings are robust because the analysis involved patients from an equal-access system, which minimizes potential access-related issues in healthcare. Other studies [9] showed that lack of healthcare insurance and barriers to access could delay diagnosis and affect outcome. Therefore, the present study is more suitable for determining whether race is an independent factor for PSA recurrence; we conclude that race is not independently associated with PSA recurrence but must acknowledge that AA men have a shorter PSA doubling time, which may translate into more aggressive disease. Our centre is currently studying long-term data from these patients.

Another strength of the present study is that it includes several additional histopathological variables which could affect PSA recurrence. Specifically, we assessed differences in PNI, inflammation and percentage cancer in the biopsy. None of these variables was more prevalent in AA patients, but the incidence of HGPIN was significantly greater in AA men and associated with a greater incidence of PSA recurrence. An important limitation of the study is its retrospective design, but the large sample size and inclusion of several confounding variables potentially offset this limitation.

In conclusion, AA race is not an independent risk factor for PSA recurrence in patients undergoing RP, and AA men have no greater incidence of HGPIN, higher baseline PSA level, or rapid PSA doubling before RP or after recurrence. Early PSA testing and aggressive evaluation of abnormal PSA, and of HGPIN, could result in the diagnosis of early prostate cancer and ultimately prolong survival.

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CONFLICT OF INTEREST
None declared.

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Abbreviations: AA, African-American; WA, white American; RP, radical prostatectomy; HFHS, Henry Ford Health System; HGPIN, high-grade prostatic intraepithelial neoplasia; PNI, perineural invasion; HR, hazard ratio.
Installation of telerobotic surgery and initial experience with telerobotic radical prostatectomy

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OBJECTIVE
To assess the ability of untrained laparoscopic surgeons to learn and implement laparoscopic telerobotic radical prostatectomy (TRP) using the daVinci Surgical System (Intuitive Surgical, CA), and assess the education, safety and efficacy issues when instituting this system.

PATIENTS AND METHODS
Between December 2003 and October 2004, 122 consecutive TRPs were performed by two surgeons for clinically localized prostate cancer. The individual robotic surgeon was assisted at the bedside by another surgeon. The TRP was performed robotically by the surgeon at the remote console unit. Perioperative data and pathological results were recorded. The two surgeons spent 1 week in a skills laboratory using a porcine model of laparoscopic TRP, and then cadaveric robotic prostatectomy. The first six cases were mentored by an experienced telerobotic surgeon.

RESULTS
The TRP was conducted by two surgeons with no previous laparoscopic experience. There were no conversions to open surgery. Assessing the complications, postoperative continence, operating time and transfusion rates showed equivalent efficacy and safety to open and pure laparoscopic methods.

CONCLUSION
TRP represents a novel computer-based surgical approach to prostate cancer, which offers the benefits of minimally invasive surgery without the extensive experience associated with the laparoscopic method. It remains to be seen whether the robotic approach can deliver better outcomes in continence and potency over time.

KEYWORDS
telerobotic, radical prostatectomy, Da Vinci system, outcome

INTRODUCTION
Telerobotic surgery allows a closed laparoscopic abdominal approach, placing a computer between the patient and surgeon. The surgeon’s hand movements are digitized to improve dexterity. The system has the added benefit of three-dimensional visualization compared to the conventional laparoscopic approach. Pure laparoscopy is counter-intuitive compared with telerobotic radical prostatectomy (TRP), which is intuitive for the surgeon.

Robotic surgery is a beguiling surgical innovation and some of the enthusiasm during installation of robotic systems relates to maintenance or increasing surgical market share. The novelty of the technology means that it is at present unproven, with high capital cost.

The DaVinci Surgical Robotic System (Intuitive Surgical, CA, USA) is a master-slave telemanipulation system (Fig. 1). The master-slave system consists of a remote console where the operating surgeon [master] directs the robotic surgical arms [slave] via a telerobotic videoscopic link.

The DaVinci system represents an important technological breakthrough. It has transformed conventional laparoscopic surgery from a two-dimensional counter-intuitive procedure to a fully intuitive natural surgical procedure using excellent visualization. Previous laparoscopic surgery has some advantages over open approaches for RP. These relate to reduced pain, early discharge and early return to normal activity. Laparoscopic TRP has the potential to improve patient outcomes compared with open RP.

Tangible benefits relate to improve visualization via pneumoperitoneum, which also provides tamponade reducing the intraoperative bleeding. The absence of an abdominal incision means less postoperative pain, improved cosmesis and early discharge. At present there is no evidence to suggest that there is any improvement in the rates of return to urinary continence, and it is too early to determine whether improved surgical dexterity and visualization will actually improve postoperative potency. This is a key area where technology may improve the results [1].

The need for transfusion is much reduced in our TRP series compared to our open series. Three patients in the first 100 required a blood transfusion. Historically in our open RP series, 60% of patients usually had an autologous transfusion. There are reports worldwide with much lower open transfusion rates than ours [1]. However, in our hands one of the remarkable advances of laparoscopic prostatectomy relates to haemostasis and reduced blood loss.

To establish a functioning telerobotic surgical service, ideally it should be multidisciplinary. Significant training requirements were necessary before establishing the service for operating room nurses and technicians, and engineering staff responsible for maintaining the equipment. The operating room had to be reconfigured. Cardiovascular surgeons have...
also embraced the technology, mainly for the repair of mitral valves and atrial septal defects.

Surgeons who are skilled open surgeons can transfer their skills very easily to a telerobotic laparoscopic approach. There appears to be no requirement for previous general laparoscopic skills [2]. Certainly a single-team approach with two surgeons and consistent table-side assistance, and trained operating room nursing staff, has made the institution of this programme much easier [3].

**ELEMENTS OF THE DA VINCI SURGICAL SYSTEM**

The surgical console provides the computer interface between surgeon and surgical robotic arms. The surgeon controls the robotic arms through the use of master handles, which are located in virtual three-dimensional space below the visual display. The surgeon's hand movements are digitized and transmitted to the robotic arms, which perform in identical movements in the operative field. Foot controls are used to activate electrocautery, for repositioning the master handles and for focusing. The surgeon views the surgical field through the binocular display in the hood of the console. The robotic arms are deactivated when the surgeon’s eyes are removed from the display. The surgeon's console and the robotic-arm cart are connected via a data cable. In the USA, Food and Drug Administration approval for this technology mandates that the operating surgeon is in the same room as the patient. However tele-surgery in which the patient and surgeon are remote is possible, and has been reported [4].

**MASTER HANDLES**

In addition to providing direction to the robotic arms, the master handles are also used to control other aspects of the video display system and robotic arms, such as endoscope selection and motion-scaling ratio. The master handles filter tremor in the surgeon's hands and arms (Fig. 2). The majority of tactile feedback is provided indirectly by the video monitor, that is visually, and the tensile feedback through the robotic arms.

The robotic-arm cart is placed beside the patient on the operating table. It holds three, or more recently four, robotic arms on a central tower. One arm holds the videoscope and the others are used to attach instrument adapters which are connected to robotic instrumentation through reusable trocars. Stereoscopic vision is supplied by a 30° or 0° specialized three-dimensional endoscope, which provides the surgeon at the console with binocular vision in the operative field.

The robotic surgical instruments have both an elbow joint and wrist, enabling seven degrees of freedom and two degrees of axial rotation, mimicking the natural motions of open surgery. This is in contrast to conventional laparoscopic surgery, where the surgeon’s hand movements are counter-intuitive and in two dimensions. There is a range of different instruments available which can be used up to 10 times, after which the robotic system deactivates them and prevents further use.

**PATIENTS AND METHODS**

In all, 122 men (mean age 61.2 years, range 48–72) underwent TRP by two surgeons.
between December 2003 and December 2004. Information on continence after TRP was collected by questionnaires sent to all patients, with a return stamped, self-addressed envelope included.

SURGICAL TECHNIQUE

The technique of TRP was adapted from that described by surgeons using a purely laparoscopic approach [5,6]. A protocol of surgical steps was used in all the present patients, and all were transperitoneal. The technique we adapted was originally described as the Montsouris technique, modified at the University of California Irvine. The same surgical steps are used in all cases: (i) Establishing pneumoperitoneum via Hassan cannula [used in preference to Veress needle after the first 30 cases]; (ii) placing the trocars; (iii) docking the robot; (iv) taking down the urachus and defining the space of Retzius for dividing the superficial dorsal veins of the penis; (v) incision of the lateral pelvic fascia; (vi) dividing the puboprostatic ligaments; (vii) staple ligation of the dorsal venous complex; (viii) dividing the junction of the bladder neck and prostate; (ix) dividing the fascial layer above the seminal vesicles, with dissection of the vasa deferentia and control of the blood vessels supplying these structures; (x) dividing the anterior layer of Denonvilliers' fascia, exposing the anterior wall of the rectum; (xi) dividing the prostate pedicles; (xii) dissecting bilaterally the neurovascular bundles; (xiii) dividing the prostate and urethra at the apex; (xiv) removing the prostate in an endoscopic bag.

This technique has been used in all but one patient at our centre; in the one case, the dissection had to be retrograde, from apex of the prostate to bladder neck, because of difficulty with rectal dissection.

The modifications to the technique in the present series relate to the use of a suprapubic needle to the lasso in the Foley catheter after dividing the bladder neck. The needle is passed through the eye of the catheter with a one Nylon suture, which is then brought out suprapublically to add traction to the prostate anteriorly. Two other surgical ports are placed, one in the left iliac fossa and one below the left costal margin. These ports are used by the bedside surgeon for instrumentation being suction, irrigation and surgical retraction.

RESULTS

No patient required conversion to open surgery. The mean (range) preoperative PSA level was 8.4 (1.2–25) ng/mL, the prostatic volume 44.7 (20–106) mL and the body mass index 27.2 (20.2–38.1) kg/m². The clinical and pathological T stage is shown in Table 1. The mean (range) stay after TRP was 2 (1–9) days, and the indwelling catheter time 8.4 (5–33) days (median 7). The margin status is shown in Table 2; the overall positive margin rate (tumour at the inked margin) was 16.3%, including six patients who had positive seminal vesicle involvement.

Data were available on urinary continence in 93 patients at 3 months (Table 3); four patients were incontinent before TRP and wore pads, thus they were excluded from the analysis of continence after TRP. Only one patient declined to complete the continence questionnaire. At 3 months, 65 patients (73%) reported they were pad-free or wearing one ‘security’ pad; by 6 months 82% of patients were continent.

Preliminary data were available for erectile function but were too premature for a meaningful assessment of long-term erectile dysfunction after TRP. It may take up to 2 years for the return of erectile function after nerve-sparing robotic RP. Four patients (3%) received blood transfusions; other complications are listed in Table 4.

DISCUSSION

TRP was popularized and championed at the Vattikuti Urology Institute by Menon and Tewari [7] and Tewari et al. [8]. The present report shows the replacement of an open operation with TRP. Laparoscopic RP has an equivalent oncological outcome to reported open series [9]. Weider and Soloway [10] reported overall positive margin rates of 28%; those for laparoscopic RP are reportedly 19–23% [11,12].

The morbidity (safety) of this new procedure were at least equivalent to the experience in major centres with open surgery [11]. The rate of return to continence at 6 months was 82%, with patients using no or one pad per day, which would appear to be acceptable. Further follow-up beyond a year for both continence and erectile function is necessary and underway.

<p>| TABLE 1 Clinical and pathological staging of the 122 men |
|-----------------------------------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Stage</th>
<th>n (%)</th>
<th>Positive margin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td>(of T stage)</td>
</tr>
<tr>
<td>T1a</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>T1b</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>T1c</td>
<td>87 (73)</td>
<td>15 (17)</td>
</tr>
<tr>
<td>T2a</td>
<td>14 (12)</td>
<td>0</td>
</tr>
<tr>
<td>T2b</td>
<td>16 (13)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>T3a</td>
<td>1 (1)</td>
<td>1</td>
</tr>
</tbody>
</table>

Pathological
pT2a 11 (9)
pT2b 22 (18)
pT2c 63 (53)
pT3a 19 (16)
pT3b 5 (4)

*tumour at inked margin.

<table>
<thead>
<tr>
<th>TABLE 2 Margin status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margins, n (%)</td>
</tr>
<tr>
<td>Capsule</td>
</tr>
<tr>
<td>Seminal vesicle</td>
</tr>
</tbody>
</table>

*three patients, two margins +ve; two patients, no data.

| TABLE 3 The frequency of incontinence during the follow-up, and pad use/24 h |
|-----------------------------------------------|------------------|
| n (%) | 3 months (89 men) | 6 months (49 men) |

Incontinent
Never 14 (16) 8 (16)
Almost never 17 (19) 16 (33)
Sometimes 43 (48) 21 (43)
Always 15 (18) 4 (8)
N pads/24 h
0 27 (30) 24 (49)
1 38 (43) 16 (33)
2 2 (3) 4 (8)
≥3 14 (16) 4 (8)

The true benefits of this procedure over open RP clearly relate to reduced blood loss, absence of abdominal incision, early discharge and early return to normal activity. Nerve sparing was attempted in almost all...
patients (step xii). The vision system allows excellent visualization of the neurovascular bundles.

Robotic technology has long been present in industry but only recently has it been an option for surgeons [13]. A cholecystectomy was conducted between New York and Paris by telerobotic means [4]. The Zeus System has been trialled for several years, as a voice-activated surgical robot. We think that the introduction of telerobotic laparoscopic surgery is a watershed in surgical innovation. If robotics are expected to be embraced by surgeons, a need for institutional development and application of this technology is likely [14] and application of this system is limited only by the surgeon's imagination.

The ability to view the surgical field in three dimensions using natural hand and arm movements, and the use of filters for hand and arm tremor, is significant. The addition of motion scaling, such that large movements are reduced to fine movements, is an advantage. The robotic system removes surgical tremor, which is compensated on the end motion by computer filters. A further advantage to the surgeon is improved visualization via the three-dimensional camera system, which has ×10 magnification in a more appropriate comfortable ergonomic environment. The three-dimensional magnified view is a dramatic improvement over conventional two-dimensional laparoscopic visualization.

Pelvic open surgery for retropubic RP requires the surgeon and assistant to adopt sometimes anatomically difficult positions, stressing the cervical and lumbar spines. Benefits are conferred to the operating surgeon as a result of the ergonomic set-up of the surgeon console.

Retropubic RP is a difficult open surgical operation and lends itself to the telerobotic laparoscopic approach, improved dexterity and visualization in an anatomically confined area of subpubic access; it is macrosurgery performed in a more appropriate comfortable ergonomic environment. The three-dimensional camera system, which has ×10 magnification in a more appropriate comfortable ergonomic environment.

These advantages perhaps outweigh the clear problems of the shift to robotics. The high capital costs, lack of compatible instrumentation, large physical size of the robot and eventual obsolescence are obviously concerns to be addressed over time. If robotics are expected to be embraced widely, exciting additional advances could ensue. The overlay of MRI and CT images for surgical guidance, and the addition of haptic feedback, are potentially feasible. The application of telerobotics across all surgical disciplines is likely [14] and application of this system is limited only by the surgeon's imagination.

In urology TRP now seems to have an enduring position. Further applications in urology will relate to partial nephrectomy, cystoprostatectomy [15], pyeloplasty and ureterolysis.

CONFLICT OF INTEREST

None declared.

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Abbreviations: (T)RP, (telerobotic) radical prostatectomy.
Robot-assisted vs pure laparoscopic radical prostatectomy: are there any differences?

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OBJECTIVE
To compare our experience of pure laparoscopic radical prostatectomy (LRP) with robot-assisted radical prostatectomy (RAP).

PATIENTS AND METHODS
The two techniques were compared retrospectively in 100 patients with localized prostate cancer who had LRP or RAP (50 each). Both groups were similar in age, serum prostate-specific antigen level, Gleason score and clinical stage. Their charts were reviewed, collating intraoperative data and early functional outcome.

RESULTS
The mean surgical time for LRP and RAP was 235 and 202 min (P > 0.05) and mean (95% confidence interval) blood loss 299 (40) and 206 (63) mL (P = 0.014), with no transfusions in either group. The positive margin rate did not differ significantly (14% LRP and 12% RAP) and there was no biochemical recurrence in either group. Early functional outcomes were similar.

CONCLUSIONS
Both LRP and RAP are technically demanding, but feasible, with the patient clearly benefiting. There were no major surgical differences between the techniques, but RAP is more costly.

KEYWORDS
continence, erectile function, laparoscopy, outcomes, prostatectomy, prostate cancer, robotics

INTRODUCTION
Laparoscopic surgery for localized prostate cancer is becoming standard in many institutions. Recently, laparoscopic technology [e.g. robots] and technique have advanced, resulting in significant progress in the development of minimally invasive surgery (MIS) for prostate cancer. The combination of lower postoperative morbidity, improved cosmesis, shorter convalescence and comparable oncological outcome has driven the demand for pure laparoscopic radical prostatectomy (LRP) compared to the open retropubic approach [1,2]. Consequently, various centres now regularly use LRP routinely for prostate cancer.

At our institution we have found this procedure to be technically reproducible, performing >70 LRPCs. These skills were transferred to perform robotic-assisted prostatectomies (RAP), now used in the USA and Europe, with early results still being reported [1,3,4]. The initial benefits are similar to LRP, except that it is more easily learned by surgeons trained in open techniques. We assessed the operative, pathological and functional outcomes from our unique experience of LRP and RAP.

PATIENTS AND METHODS
We retrospectively assessed LRP and RAP in the last 50 patients undergoing LRP and RAP (total 78 and 200, respectively); the LRP series was completed before the RAP series. Perioperative data with early oncological and basic functional results were recorded by chart review. The indications for surgery were identical to those for open retropubic RP. Patients were counselled about all possible treatments for prostate cancer [5].

Some patients had previous surgery (bilateral hernia mesh repair in two, appendicectomy in two and previous laparotomy in one). Two of these men required conversion to a transperitoneal approach (bilateral hernia mesh repair), as the extraperitoneal space could not be opened sufficiently for surgery.

All patients were positioned similarly; after general anaesthesia was established, an orogastric tube was placed. The supine position was adopted, with Trendelenburg tilt (15–20° for RAP and 30–40° for LRP), and the legs abducted to allow access for the robot and/or to the perineum. The arms were tucked beside the body and thoracic ‘X’ straps placed across the chest. After aseptic preparation and draping of the surgical field, a 16 F urethral catheter was inserted.

A balloon dilator was then placed through a 2-cm para-umbilical incision, under visual control, which developed the extraperitoneal space. Five ports (two robotic arms, one robotic camera port, two assistant ports) were required for the RAP and four ports for the LRP (two working ports, one camera, one assistant). The endopelvic fascia was dissected from the base to the apex of the prostate. The dorsal vein complex (DVC) was suture-ligated and the bladder neck dissected from the base of the prostate. The vas deferens and seminal vesicles were then dissected to the level of posterior Denovilliers’ fascia. The vascular pedicles were bipolar cauterized, preserving the neurovascular bundles. The apical urethra and DVC were transected. Lymph nodes were sampled if the Gleason score was >7 and/or the PSA level >10 ng/mL, but in the 100 patients reviewed this was not required (Table 1). The vesico-urethral anastomosis was made using two continuous polyglactin sutures. The specimen was extracted through the para-umbilical incision in an entrapment bag, and a drain placed at the anastomosis.

After surgery all patients were mobilized within 4 h of surgery, and generally...
discharged home within 23–48 h. The urethral catheter was routinely removed at 7 days after surgery.

One team of genitourinary pathologists analysed the pathological specimen, noting pathological stage, Gleason grade and surgical margin. All perioperative data, including age, preoperative PSA, clinical stage, Gleason grade, prostate weight, pathological variables (pathological Gleason grade, stage, margin status), blood loss, nerve-sparing and operating room times (total time including anaesthesia time, pre-docking/after undocking times, robot operating times) were recorded.

Early functional outcomes were assessed during the follow-up, including erectile function (interview and the International Index of Erectile Function-5, IIEF-5, questionnaire). Spontaneous erections were documented at the consultations before surgery; the quality of the erections was not noted afterward, but IIEF-5 scores were recorded at 3 months. Continence was defined as being totally dry and using no pads, either for wetness or security. The time to total urinary continence was assessed by interview and examination (Valsalva manoeuvre and coughing with a full bladder). Data are expressed as the mean (95% CI) and compared using the paired \( t \)-test, with \( P < 0.05 \) taken to indicate statistical significance.

RESULTS

The demographics were similar in both groups (Table 1); the mean total operating-room time (including anaesthesia) and surgery time were similar (Table 1); the estimated blood loss differed but there were no blood transfusions in either group. Nerve sparing was less in LRP than RAP, but the mean specimen weights were similar.

There were four bladder neck contractures (one after LRP and three after RAP) treated by urethral dilatation, and two urinary leaks (one in each group) treated by prolonged catheterization (10 days). There were no other minor or major complications up to 30 days after surgery.

The pathological staging was comparable in the two groups (Table 1), with a mean Gleason grade of 6. The positive surgical margin rate was similar for both groups, at 14% (LRP) and 12% (RAP). There was no biochemical recurrence of prostate cancer in any of the 100 patients at a mean (range) of 5.3 (2–9) months after surgery.

Continence was verified by the absence of urinary leakage on Valsalva manoeuvre or coughing after catheter removal, at intervals (immediately, 4, 8, 12 and >12 weeks). At 3 months after catheter removal, 46 patients (92%) in the LRP group and 45 (90%) in the RAP group were totally continent; the other patients were still using an underwear liner for security only (Table 1).

The assessment of erectile function after surgery was inconclusive as the data are immature. When interviewed, the LRP group had 22% spontaneous erections, with 36% requiring drug aid (sildenafil or tadalafil), with a mean IIEF-5 score of 37 (15); when interviewed, the RAP group reported spontaneous erections in 40%, with a further 46% requiring drug aid, and an IIEF-5 score of 34 (11).

DISCUSSION

LRP aims to combine the advantages of open retropubic RP with those of minimally invasive surgery, to allow better intraoperative and functional outcomes. The LRP and RAP groups were comparable for patient and cancer demographics, and outcomes during and after surgery (Table 1).
ROBOT-ASSISTED VS PURE LAPAROSCOPIC RADICAL PROSTATECTOMY

Limitations of the present study are the single-centre experience with relatively few patients and use of chart review, all increasing observer and comparison bias. Both groups had a higher prevalence of clinical stage T1c tumours, probably a result of patient selection over time. An important consideration was that the LRP series preceded the RAP series, and thus experience was gained in the laparoscopic anatomy required for these operations. To avoid any comparison bias caused by this factor (robotic set-up, team training, laparoscopic anatomy), we compared the last 50 patients in each group, allowing a comparison after gaining the experience, thereby minimizing the bias. Ultimately the goal was to show not only the ease of conversion from LRP to RAP, but also to compare the clinical outcomes, which should be similar with either technique.

The mean surgical times were no different between the groups; this is interesting, as the suturing aspects of this form of surgery are easier with the robotic arms in unskilled hands [6], but if already skilled in pure laparoscopy there should be no anticipated difference. The blood loss was less in the RAP group, with no blood transfusions in either group. This difference was not expected and may primarily be a result of the magnified, three-dimensional imaging of the robot, which allows the surgeon to perform more deliberate and accurate haemostatic manoeuvres.

The anatomical bilateral nerve-sparing dissection appeared to be easier during RAP than LRP, with a 92% preservation rate (Table 1). The comparable LRP group had a 48% bilateral nerve-preservation rate. The obvious bias is that patient selection may have improved, as suggested by the slightly higher incidence of T3 prostate cancers in the LRP group. The surgeon may also have been more confident with the margins of surgery as experience increased and the optics improved, and thus the RAP group benefited from the LRP experience.

There are usually complications during surgery; the objective of any improvement in a surgical procedure is that the morbidity is reduced and outcomes improved. There were some urinary leaks or bladder neck contractures in the present patients. The leaks may have occurred as a result of loose suturing, as our technique involves a continuous suture. However, as our technique of prostatectomy used an extraperitoneal approach with its obvious advantages [7], the leaks were localized. The contractures are difficult to explain, as there was good mucoso-mucosal apposition at the time of suturing, because of the excellent visualization afforded by MIS techniques. We can only suggest that a combination of overzealous diathermy at the bladder neck with over-tight suturing may have caused the problem, although we have no direct evidence for this.

The pathological/oncological outcome is an important measure of the effectiveness of a modified technique compared with the standard. The present two groups had comparable clinical staging, with most being biopsy-confirmed localized prostate cancer (T1c). The mean weights of the prostate specimens were similar, at 51–53 g, but this includes glands of 23–105 g. The positive surgical margins were similar in both groups, and lower than other reported in initial series [4,8].

Functional outcome is also an important benchmark for a newer procedure; the present results are early, but the outcomes are encouraging compared with open surgery. By 3 months, both groups had reached >90% total continence (Table 1); this high rate (with no use of pads) is promising. The mechanism for achieving this is probably a combination of preserving the length of the distal urethra and bladder neck urethra, and the neurovascular bundles and pubo-prostatic ligaments [9,10]. Also, accurate bipolar cautery and controlled dissection below the endopelvic fascia avoids nerve and muscle damage, which may be important in preserving the continence mechanism [11].

Assessing early erectile function for comparison with other series of RP is difficult, as the recovery of the neurovascular supply to the erectile tissue occurs 12–18 months after surgery [12]. Interestingly, in the RAP group nearly half the patients reported spontaneous erections, but the IIEF scores were not equally high; these data require further maturity before any meaningful comments can be made.

The present study highlights the feasibility of successful RP by either technique. The importance of this to the health-policy makers and providers cannot be over-emphasized. The use of MIS for the prostate allows patients to leave hospital within 1–2 days, and to return to their initial routine within a few weeks. Although this is difficult to measure from the present retrospective series, it is a reasonable assumption; we are planning long-term studies in this area. In contrast, open surgery, even in the best hands, may have a similar outcome to MIS in terms of hospital stay but the patients’ return to preoperative activity usually takes several weeks, in our experience. The important message from this is that as the incisions and overall trauma of surgery decrease, return to preoperative activity should logically take less time [13].

The overall cost affects two areas, i.e. the hospital and society. The latter is not easily measurable, but the cost of losing days from work/life affects everyone. In the present study we compared two minimally invasive procedures, which both provide a good recovery after surgery. If cost is measured as the clinical outcome alone, either technique is good, but financially RAP is a costly procedure.

Laparoscopically experienced surgeons with assistants generally untrained in laparoscopy undertook all the present surgery. The changes in the team added time and effort to the operations but this depiction of reality is important if these technologies are to be embraced. The laparoscopic skills allowed a smoother transition to RAP. Importantly, after our experience we suggest that surgeons should train appropriately and not simply take a 1–2 day course before attempting this demanding procedure. Indeed, the level of laparoscopic skills required to perform RAP has been underestimated [6], although there has been some attempt to address this [14], by groups who have laparoscopic experience available to them. If a surgeon wishes to use RAP, he or she should have appropriate laparoscopic experience available, or be a trained laparoscopist. The current disadvantage is the enormous cost of the robot-assisted approach compared with pure laparoscopy.

CONFLICT OF INTEREST
None declared.

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Abbreviations: L(RP), laparoscopic (radical prostatectomy); RAP, robot-assisted prostatectomy; MSI, minimally invasive surgery; DVC, dorsal vein complex; IIEF-5, International Index of Erectile Function-5.
Radical prostatectomy versus high-dose rate brachytherapy for prostate cancer: effects on health-related quality of life

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OBJECTIVE

To examine the effects of different treatments on the health-related quality of life (HRQoL) of men with localized prostate cancer.

PATIENTS AND METHODS

Between October 1997 and August 2002, 182 men diagnosed with prostate cancer (T1c to T3bN0M0) had radical prostatectomy (RP, 89) or 192iridium high-dose rate brachytherapy (HDR-BT, 93) with external beam radiotherapy, and were followed for ≥6 months. A postal survey was sent in which HRQoL was assessed using the 36-item Short-Form Health Survey (SF-36) QoL questionnaire, and disease-specific QoL using the University of California Los Angeles Prostate Cancer Index (UCLA-PCI).

RESULTS

Questionnaire responses were obtained from 151 of 182 patients; there was no significant difference in SF-36 scale scores between men treated with RP or HDR-BT. In the UCLA-PCI, the HDR-BT group had better urinary function (P < 0.001) and sexual function scores (P = 0.043). Men treated with RP had better bowel bother scores (P = 0.027). In patients with ≥2 years of follow-up, urinary function (P < 0.001) and sexual bother (P = 0.029) were better for men treated with HDR-BT than for men treated with RP. Men treated with HDR-BT had significantly better urinary function (P = 0.009) and sexual bother (P = 0.013) even than 30 men treated with unilateral nerve-sparing RP.

CONCLUSIONS

In terms of HRQoL, RP and HDR-BT did not differ, but HDR-BT resulted in better urinary and sexual function than RP. When planning treatment, QoL concerns, including mental health issues associated with prostate cancer, need to be addressed with the patients, as do the potential side-effects.

KEYWORDS
prostate cancer, quality of life, high-dose-rate brachytherapy, prostatectomy

INTRODUCTION

The prognosis associated with localized prostate cancer is excellent, with a 5-year relative survival rate approaching 100% [1]. Quality of life (QoL) issues therefore are key to deciding among treatment options which can impinge on everyday activities to differing extents. Several therapeutic options for localized prostate cancer, including radical prostatectomy (RP), external beam radiotherapy (EBRT), and brachytherapy (BT), affect sexual, urinary, and bowel function deleteriously for many men. In general, men who undergo RP report more urinary dysfunction (greater incontinence and greater need to use absorptive pads) and more sexual dysfunction (reduced erectile capacity and decreased sexual desire) than men treated with EBRT [2–7]. Bowel dysfunction (urgency and diarrhoea) and irritative urinary dysfunction are reported more often by men treated with EBRT and BT than by men who undergo RP [4,6].

Several tools have been developed for evaluating QoL. The concept of health-related QoL (HRQoL) is multidimensional and includes physical, psychosocial and emotional status, as well as patient autonomy, and is applicable to various medical conditions. A generic measuring instrument, the 36-item Short-Form Health Survey (SF-36), is used extensively throughout the world [8,9]. This survey is considered to be valid and comprehensive, without being time-consuming, and is readily applicable to assessing individual patients. In addition, numerous international cross-cultural adaptations of the original instrument, as well as validation data for normal subjects and patients with various chronic conditions, are available [10]. The University of California Los Angeles Prostate Cancer Index (UCLA-PCI) was the first, and is the most often used, measure of disease-specific QoL available for evaluating treatments for early-stage prostate cancer [11].

The primary objective of the present study was to examine the effects of different treatments on the HRQoL of men with localized prostate cancer. We measured HRQoL (using the SF-36) and disease-specific QoL (using the UCLA-PCI) in men treated with 192Ir high-dose-rate brachytherapy (HDR-BT), and in men treated with RP.

PATIENTS AND METHODS

Between October 1997 and August 2002, 182 men diagnosed with prostate cancer (T1c to T3bN0M0) were treated with RP (89) or HDR-BT (93) with 36.8 Gy EBRT, and were followed for 6–64 months. A postal survey was sent to the patients, including HRQoL assessment using the SF-36, and a urinary and bowel symptom assessment using the UCLA-PCI. We obtained questionnaire responses from 151 of 182 patients (83%), including 70 treated with RP and 81 treated with HDR-BT. Survey
participation rates of RP and HDR-BT patients were 78.7% and 87.1%, respectively. The patients’ characteristics at presentation are summarized in Table 1. Clinical classification (HDR-BT) and pathological classification (RP) were determined in accordance with the 1997 unified TNM system. Neoadjuvant hormone therapy was administered to 12 of the 70 RP patients (17%) and 34 of the 81 HDR-BT patients (42%). Unilateral nerve-sparing surgery (NSS) was used in 30 of the 70 RP patients (43%).

HDR-BT, using $^{192}$Ir followed by EBRT, consisted of external irradiation (four-port) of the prostate at 2.3 Gy x 16 times (36.8 Gy) and HDR-BT using a microSelectron (Nucletron) at 6 Gy x 4 times (24.0 Gy) within 30 h.

We used the Japanese version of the SF-36 (version 1.2); this contains 36 questions to assess eight aspects of HRQoL: physical functioning; role-physical functioning; bodily pain; general health; vitality; social functioning; role-emotional functioning; and mental health. Each question was given a score from 0 to 100, and a mean score was obtained for each, with higher scores indicative of a better outcome. We also used the Japanese version of the UCLA-PCI (version 1.2), a disease-specific instrument focusing on health concerns of men treated for prostate cancer. The questions assess levels of bowel, urinary, bladder and sexual functioning, and the degree to which such symptoms were burdensome: urinary function; urinary bother; bowel function; bowel bother; sexual function; and sexual bother. All scores in each section were given equal weight, being linearly transferred from a scale of 0–100, with higher scores representing a better level of functioning and less burden.

All descriptive data are reported as the mean (SD) with differences in mean values between RP and HDR-BT patients analysed by two-tailed, unpaired t-tests or the Mann–Whitney test, or by ANOVA as appropriate. Significance was defined at the 5% level.

**RESULTS**

Data from the SF-36 are shown in Fig. 1a,b. There was no apparent significant difference for any scale score between RP and HDR-BT, and no significant difference in patients with ≥2 years and <2 years of follow-up. When patients were divided according to age, those aged ≥65 years (HDR-BT, 66; RP 56) showed no significant difference in any SF-36 scale score. Men treated with RP including unilateral NSS (30) had significantly higher physical functioning ($P = 0.007$) and role-physical functioning ($P = 0.031$) scores than men treated with HDR-BT (Fig. 1b). There were no patients with bilateral preservation of neurovascular bundles in the present study.
HRQoL AFTER DIFFERENT TREATMENTS FOR PROSTATE CANCER

Figure 1c,d present data from the UCLA-PCI; men in the HDR-BT group reported better urinary function ($P < 0.001$) and sexual function ($P = 0.043$) than men in the RP group, whereas men in the RP group had better bowel bother scores ($P = 0.027$). There were no significant treatment-related differences for urinary bother, bowel function, and sexual bother. Among patients with $\geq 2$ years of follow-up (HDR-BT, 51; RP, 46) men treated with HDR-BT had significantly better urinary function ($P < 0.001$) and sexual bother scores ($P = 0.029$). Among patients with $<2$ years of follow-up, men treated with HDR-BT (30) had significantly higher scores for urinary function ($P < 0.001$) and sexual function ($P = 0.029$) than men treated with RP (24). Men aged $\geq 65$ years treated with HDR-BT had significantly higher scores for urinary function ($P < 0.001$) and sexual function ($P = 0.019$) than men of similar age treated with RP. Men in the RP group, including unilateral NSS, had significantly higher scores for bowel bother (men in the HDR-BT group. Men in the HDR-BT group had significantly better scores for urinary function and sexual bother than men treated with RP including unilateral NSS. There were no significant treatment-related differences for urinary bother, bowel function and sexual function between RP with unilateral NSS and HDR-BT (Fig. 1d).

The SF-36 and UCLA-PCI results showed no significant differences among HDR-BT or RP patients between subgroups with neoadjuvant hormone therapy or not. There were no significant differences comparing patients with the same clinical stage, with the same duration of follow-up.

DISCUSSION

The primary objective of the present study was to examine the effects of different treatments on the HRQoL of men with localized prostate cancer, comparing disease-specific and general HRQoL outcomes between RP and HDR-BT. The general HRQoL (from the SF-36) showed no significant difference between RP and HDR-BT in overall outcome. Men treated with RP including unilateral NSS had significantly higher physical functioning and role-physical functioning scores than men treated with HDR-BT, but no HRQoL data were obtained before treatment.

As for disease-specific QoL (UCLA-PCI), HDR-BT was associated with better urinary and sexual function than RP. Men treated with HDR-BT had significantly better scores for sexual bother than men treated with RP and unilateral NSS. There was no significant difference in sexual function between RP with unilateral NSS and HDR-BT. The present results show that HDR-BT has a better outcome for sexual life than RP with unilateral NSS.

Brandeis et al. [12] compared men treated with BT (low-dose rate BT, both with and with no pretreatment EBRT) with men who had RP; the RP group reported greater urinary leakage than the BT group, but the BT group reported more obstructive and irritative urinary symptoms (increasing frequency and urgency, nocturia, and weak urine stream). Other authors also concluded that BT has significant effects on the urinary tract. Arterbery et al. [13] evaluated short-term complications of low-dose rate BT, reporting nocturia,
frequency, dysuria and hesitancy lasting 12–24 weeks. Kaye et al. [14] reported that half of patients treated with low-dose rate BT had irritative and/or obstructive urinary symptoms (acute urinary retention, 4%; some degree of incontinence, 13%; urethral stricture, 3%; significant perineal pain, 18%). Thus, while patients treated with either RP or BT had urinary tract symptoms, specific symptoms differed. By contrast, HDR-BT showed an advantage for sexual function compared with RP. Desai et al. [15] reported that after 125I-interstitial implantation of the prostate gland, IPSS and acute urinary side-effects peaked at 1 month and gradually returned to baseline at 24 months.

When comparing the present HDR-BT data with the results from other reports of radical EBRT (Table 2; [16–19]) there was no difference in SF-36 scores. The present UCLA-PCI data showed that HDR-BT had better results for sexual bother, but Japanese patients might be less concerned about sexual function before treatment.

In the present study, patients treated with HDR-BT received subsequent EBRT (36.8 Gy). In the future, treatment with HDR-BT omitting EBRT in low-risk patients with low PSA levels, low-grade tumour histology and tumours of < T3 could result in much better HRQoL. Better results would be expected for urinary and sexual function, and bowel function might not differ from that after RP.

There are a few limitations of the present study; no information was obtained on pretreatment function, so no firm conclusions can be drawn about treatment-related changes. Future studies will need to be prospective, longitudinal and long-term. Assessing patients at baseline before treatment and following them over time will provide important insights into treatment-related differences in QoL.

No significant differences were evident between RP and HDR-BT for general HRQoL. By contrast, patients treated with HDR-BT showed better urinary and sexual function than those treated with RP when disease-specific QoL was assessed. In planning treatment, QoL concerns, including mental health issues associated with prostate cancer, need to be addressed with patients, as do the potential side-effects.

CONFLICT OF INTEREST

None declared.

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Abbreviations: HR(QoL), health-related (quality of life); RP, radical prostatectomy; BT, brachytherapy; HDR, high-dose rate; EBRT, external beam radiotherapy; 36-item, short-form health survey; UCLA-PCI, The University of California Los Angeles Prostate Cancer Index; NSS, nerve-sparing surgery.
Does neoadjuvant hormone therapy for early prostate cancer affect cognition? Results from a pilot study

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OBJECTIVE
To examine, in a prospective study, the influence that temporary reversible medical castration for localized prostate cancer has on cognition, by assessing whether temporary 3–5 month treatment with a luteinizing-hormone releasing hormone (LHRH) agonist before radical radiotherapy had a short- or long-term affect on cognitive function.

PATIENTS, SUBJECTS AND METHODS
Thirty-two patients with localized prostate cancer had cognitive assessments at baseline (T1) before the start of drug treatment, at 3 months (T2) or on completing drug treatment but before radiotherapy, and 9 months later (T3). Eighteen men with no prostate cancer (controls subjects) completed the cognitive tests at the same times. In addition, psychological functioning and quality of life were assessed at the same times, together with serum free and bound testosterone, β-oestradiol and sex hormone-binding globulin levels.

RESULTS
There was a significant cognitive decline (on at least one cognitive task) at T2 in 15 (47%) patients vs three (17%) of controls (odds ratio 4.412, \( P = 0.033 \)). Most patients (nine of 15) who had a change in performance declined on tasks of spatial memory and ability. At T3 there was significant cognitive decline in 11 (34%) patients and five (28%) control subjects (odds ratio 1.37, \( P = 0.631 \)).

CONCLUSION
This pilot study suggests that short-term LHRH therapy for early-stage prostate cancer has modest short-term consequences on men’s cognitive functioning; a larger prospective study is warranted.

KEYWORDS
prostate cancer, LHRH therapy, cognition

INTRODUCTION
Over the last 30 years there have been significant advances in the detection, prognosis and management of prostate cancer [1]. Prostate cancer is highly responsive to hormone therapy. Huggins and Hodges [2] first described the effects of castration on advanced metastatic prostate cancer in 1941, yet the indications and timing of hormone therapy are only recently being clarified. In localized prostate cancer a short course of LHRH therapy before radical radiotherapy reduces the planning target volume, improves the therapeutic ratio [3] and has benefits in some groups of patients [4]. The guidelines from the National Institute of Clinical Excellence [5] suggest that hormone therapy for early prostate cancer (in terms of medical castration) and potential side-effects associated with this treatment should be discussed fully with patients. Some of the side-effects are well recognized by the medical profession, e.g. erectile dysfunction, hot flushes and decreased quality of life (QoL) [6,7], but an area that has come under recent scrutiny is whether these drugs have a detrimental affect on cognition.

The neuropsychological literature suggests that testosterone is related positively to cognitive functioning [8,9] and therefore the impact of hormone therapy on prostate cancer, which reduces the amount of bioavailable testosterone, may be negative. Three recent cognitive studies in Australia, America and Finland involving men with prostate cancer produced variable results [10–12].

The Australian study (6-month data reported in 2002 [10,13]) involved 77 men with extraprostatic prostate cancer, randomized to receive leuprorelin, goserelin (both LHRH analogues), cyproterone acetate (an antiandrogen) or close monitoring (no treatment). Memory and attention after randomization to treatments were assessed at baseline (77 men), 6 (65 men) and 12 months (62 men). Patients randomized to the drug treatments had a significant decline in performance for several tasks that required complex information processing, relative to a group of 15 healthy controls. However, as the authors noted, the control group had a higher than average intelligence and education level than the patients, which makes cognitive comparisons problematic.

Conversely, in the American study [11] there were no differences in performance on cognitive tasks between a group of patients who received 9 months of androgen suppression (flutamide or bicalutamide for 2 weeks followed by monthly injections of leuprolide for nine doses) and a healthy control group. In that study the participants performed cognitive assessments at baseline, 9 and 12 months. However, when the patients’ performance was examined using a calculation of reliable change, almost half the group had a clinically significant decline for seven of eight tasks. Unfortunately the authors did not report the extent of significant decline that also would have occurred in some members of the control group.

In contrast to the results from both studies, the Finnish study [12] reported improvements in semantic memory and object recall in a group of men receiving androgen deprivation therapy. Cognitive function, QoL and mood were assessed in 25 newly diagnosed patients...
treated with combined androgen therapy (flutamide and an unspecified LHRH analogue) at baseline, 6 and 12 months. Healthy controls (52) were also recruited and assessed on the same measures, although they were only tested at baseline. Cognitive deficits in sustained attention, verbal and visual motor performance were apparent in the patients at baseline (before treatment) compared with controls. The researchers reported cognitive improvements during androgen deprivation at 6 and 12 months in episodic and semantic memory, with no cognitive impairment on any other measure or any decline in any of the QoL measures. The authors concluded that cognitive function is maintained in patients with prostate cancer when treated with combined androgen therapy. However, with no similar testing protocol in the healthy controls the interpretation of the results is limited and the impact of any practice effects cannot be adequately assessed or statistically analysed.

In the present study we examined the effects of temporary and reversible medical castration with an LHRH agonist before radical radiotherapy for localized prostate cancer. This gave an opportunity to study the effects of cognition and QoL before, during and on recovery from testosterone suppression, and to assess any correlation with serum testosterone levels.

PATIENTS AND METHODS

Patients with localized prostate cancer whose management would routinely (in local practice) include neoadjuvant LHRH therapy were identified by the clinician (D.B.) in the clinic and recruited to the study by the research psychologists. Participants consented to cognitive, QoL and blood assessments at three times, i.e. at baseline (T1) before starting drug treatment, at 3 months (T2) or on completing drug treatment but before radiotherapy, and 9 months later (T3). In addition, psychological functioning and quality of life were measured at the same times, together with serum free and bound testosterone, $\beta$-oestradiol and sex hormone-binding globulin (SHBG) levels. Cognitive assessments were conducted at the participants’ homes or in a quiet room in the unit at each sample time. All participants in the study gave fully informed written consent.

In all, 36 of 41 (88%) patients consented to the study and 32 were analysed (two protocol violation, two withdrew with no reason given). All were treated with 3 weeks of cyproterone acetate (antiandrogen) to prevent tumour flare, followed by monthly injections of goserelin (LHRH analogue) for 3–5 months before a course of radical radiotherapy to the prostate alone.

The control group comprised 25 healthy men and intelligence. All of the tests are fully standardized and validated, and were taken from published test batteries with population norms.

Intelligence was estimated using the National Adult Reading Test [14]. This task requires participants to read aloud 50 irregular words that cannot be determined phonetically, e.g. ‘deny’, ‘quadraped’, ‘capon’. The number of errors made is calculated and used to predict the Full Scale Intelligence Quotient.

Verbal ability was assessed using a phonemic verbal fluency task. Participants are given 60 s to generate as many exemplars as they can, beginning with a given letter, e.g. (F, A, S), avoiding proper nouns and additional verb derivatives. Alternate letter forms are used at different sample times. The total number of words generated for the three letters is recorded.

Verbal memory was assessed using the Rey Auditory-Verbal Learning Test [15], which consists of five presentations with recall of a 15-word list, one presentation of a second 15-word list and a sixth recall trial, which altogether takes 10–15 min. Retention of the list is also tested after a 30-min delay. Alternate word lists are used at each sample time to avoid practice effects. The scores reported from this test are the ‘supraspan’ (number of words recalled from the first presentation of the list), and delayed recall score (total words recalled after a 0.5 h delay).

Visual memory was assessed using the Complex Figure Task, with two alternate forms used at different sample times [16,17]. This task requires participants to copy a complex geometric figure and then reproduce the figure from memory in an immediate recall and after a 30-min delay. The figures are scored according to the correct reproduction of different aspects of the figure; the highest possible score is 36.

Visual-spatial memory was assessed using a computerized mental rotation task [18]. Participants are asked to judge whether pairs of two-dimensional figures are mirror images or the same images when presented at selected angles of rotation. Reaction times (milliseconds) and the number of errors (%) are recorded for each trial, to provide a measure of speed and accuracy.

Working memory capacity reflects the ability to maintain substantial quantities of information whilst sometimes performing manipulations of or calculations involving the data being stored. It is assessed using two Wechsler Memory Scale III tasks, the digit-span task and the spatial-span task [19]. In the first the participant is read an increasingly long string of digits which they must repeat in the same order (condition a) and in reverse order (condition b). Performance scores are
and repeated measures performance at T1, T2 and T3 using one-way ANOVA, and chi-squared tests as appropriate. Pearson’s correlations were used to examine the relationship between serum testosterone levels, QoL and cognitive test scores. The reliable change index (RCI), with a correction for observed practice effects on each measure, was used to assess individual performance across sample times [23]. In neuropsychological testing, group comparisons can sometimes mask significant impairment in a subgroup of the population, which can lead to the under- or overestimation of cognitive impairment.

Using the method proposed by Jacobson and Truax [24], an RCI was calculated for each cognitive measure using the baseline and follow-up data of the control subjects. The RCI was calculated as follows. The test-retest reliability coefficient ($r_{xx}$) was computed for each measure. The SE of measurement ($SE_m$) was calculated as $SD_l \sqrt{1 - r_{xx}}$, where $SD_l$ is the SD of the baseline score. The SE of the difference ($SE_{diff}$) was calculated as $\sqrt{2(SE_m)^2}$. The $SE_{diff}$ describes the spread of distribution of change scores that would be expected if no change occurred.

To establish a 90% CI for the RCI the $SE_{diff}$ was multiplied by $±1.64$ SD [25]. These thresholds were corrected for practice effects [23], which for each variable is the mean difference between the follow-up and baseline scores. Thus, for each variable a 90% CI was calculated as $SE_{diff} \times (±1.64) +$ practice effect. For each participant, a difference score was calculated representing the performance difference on each measure (T2 − T1). If this score fell outside the RCI, a statistically significant change in performance was considered to have occurred.

### RESULTS

There was a significant reduction in free and total testosterone and $\beta$-oestradiol levels for the patients after LHRH treatment ($P < 0.001$ in all three analyses) and at 12 months testosterone levels remained significantly lower than at baseline ($P = 0.035$ and 0.026, respectively). $\beta$-oestradiol levels returned to baseline and SHBG levels did not change significantly with treatment (Fig. 1).

For the cognitive test performance, repeated-measures ANOVA showed no difference between the groups on any task (Table 2). Table 3 shows the changes within each group using the reliable-change analysis. There was a decline (on at least one task) in 15 (47%) patients and three (17%) of controls (odds ratio 4.412, $P = 0.033$, two-sided) at T2, but it was not significant at T3, when 11 (34%) patients and five (28%) controls declined on at least one task (odds ratio 1.37, $P = 0.631$). In contrast seven patients and six controls had a reliable improvement at T2, and at T3, five and three, respectively.

Broadly grouping the tasks into verbal, spatial and processing speed categories provides an indication of which aspect of cognition was most affected (Table 4). At both T2 and T3 many patients had reliably declined on visual spatial tasks compared with the control group.

At T1, seven of 32 (22%) patients scored above the threshold on the GHQ12 ($≥$4 denotes probable psychological morbidity). At T2 this had decreased to five (16%) and by T3 only two (6%) still had raised levels. In contrast, only one control had scores above the threshold at one sample (T2). A recent health study stated that 20% of the population will score above the threshold on the GHQ12 [26]. There was no relationship between levels of anxiety above the threshold and the decline in cognitive task performance.
The overall score on the FACT-P showed a trend towards a significant decrease in QoL from T1 to T2 (mean 127.9 to 123.1, \( P = 0.051 \)) followed by a recovery to baseline levels at T3 (127.7). The prostate-specific symptom scale showed a clear decline at T2 from T1 (38.3 to 35.8, \( P = 0.034 \)), which also recovered by T3 (36.7), as did the TOI, with values at T1–3 of 85.5, 81.0 and 84.1, \( P = 0.024 \) and 0.121). Seventeen (53%) men had a clinically reliable decline in QoL after LHRH therapy at T2 and T3, and 11 (34%) still had a clinical decline compared with baseline. These scores indicate that intense combined therapy for early-stage prostate cancer affects a patient’s QoL, but it recovers in over half the patients by 12 months.

Neither QoL, level of anxiety nor decline in performance on the cognitive tasks correlated with a decline in free and total testosterone levels. All men had a significant reduction in serum testosterone after LHRH therapy but not all had a significant decline in QoL.

In the semistructured interviews, at T3 eight (25%) patients considered that their memory had become worse during the treatment, and five of them had reliably declined on at least one cognitive task.

### DISCUSSION

Despite the lack of an overall group effect of the treatment, the significant change, as measured by the RCI method, clearly shows that LHRH therapy affects cognitive functioning for some men. The pattern of deficit was more noticeable for tasks measuring spatial ability and spatial memory. There is some evidence to suggest a beneficial relationship between higher testosterone levels and spatial ability in older men [27], but others investigating cognition and treatments for prostate cancer do not support this finding. In the present pilot study there was no clear correlation between a decrease in bioavailable testosterone and performance.

### TABLE 2 The test scores for the patient and control group at each time

<table>
<thead>
<tr>
<th></th>
<th>Mean (sd) score</th>
<th>T1 Patient</th>
<th>T1 Control</th>
<th>T2 Patient</th>
<th>T2 Control</th>
<th>T3 Patient</th>
<th>T3 Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS total</td>
<td></td>
<td>45.0 (13.96)</td>
<td>42.2 (8.11)</td>
<td>44.1 (13.66)</td>
<td>44.1 (12.79)</td>
<td>46.7 (14.36)</td>
<td>47.2 (11.93)</td>
</tr>
<tr>
<td>AVLT supraspan</td>
<td></td>
<td>10.4 (1.9)</td>
<td>10.2 (2.4)</td>
<td>10.5 (2.6)</td>
<td>10.7 (2.4)</td>
<td>11.0 (2.6)</td>
<td>11.5 (2.3)</td>
</tr>
<tr>
<td>AVLT delayed score</td>
<td></td>
<td>7.16 (3.4)</td>
<td>7.5 (2.8)</td>
<td>7.5 (3.4)</td>
<td>8.0 (3.07)</td>
<td>8.5 (3.5)</td>
<td>8.33 (2.9)</td>
</tr>
<tr>
<td>Digit span total</td>
<td></td>
<td>17.0 (4.28)</td>
<td>17.6 (3)</td>
<td>17.1 (4.89)</td>
<td>18.5 (4.14)</td>
<td>17.2 (4.62)</td>
<td>18.5 (3.73)</td>
</tr>
<tr>
<td>Spatial span total</td>
<td></td>
<td>14.7 (2.56)</td>
<td>14.2 (2.77)</td>
<td>15.2 (2.68)</td>
<td>15.1 (2.59)</td>
<td>14.4 (2.64)</td>
<td>15.1 (3.42)</td>
</tr>
</tbody>
</table>

### TABLE 3 The number of participants who either significantly declined, significantly improved, both improved and declined (mixture) on one or more cognitive task, or showed no changes in performance after RCI analyses

<table>
<thead>
<tr>
<th>Reliable change</th>
<th>Significant decline</th>
<th>Significant improvement</th>
<th>Mixture</th>
<th>No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/T2 changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>15</td>
<td>7</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Controls</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>T2/T3 changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>11</td>
<td>5</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

### TABLE 4 Participants who showed a reliable decline on specific areas of cognition

<table>
<thead>
<tr>
<th>Area</th>
<th>T2 Patients</th>
<th>T2 Controls</th>
<th>T3 Patients</th>
<th>T3 Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal (FAS, AVLT)</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Visual-spatial (Mental rotation, complex figure)</td>
<td>13</td>
<td>4</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Working memory</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Digit span, spatial span</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

FAS, phonemic verbal fluency task; AVLT, Auditory-Verbal Learning Test; KCDT, Kendrick Assessment of Cognitive Ageing.
on cognitive tasks, probably because there was insufficient power in this exploratory study.

While Cherrier et al. [11] reported a decline in spatial ability in their group of patients, others reported either a decline across a range of tasks associated with complex information-giving [10], or an improvement in semantic and spatial memory [12]. The lack of consensus is probably caused by differences in the methods used in these studies and the interpretation of the data.

One confounding variable when trying to identify a common theme in previous reports is that a wide variety of neuropsychological tasks was used, purporting to measure spatial and verbal ability. Some studies did not use adequate tests to measure visual-spatial functioning, which traditionally is thought to be most susceptible to fluctuations in testosterone levels. Other studies used many different tests to assess the same general areas of cognitive function, yet the tasks involved may not be assessing exactly the same construct, e.g. whilst a city-map task and a block-design task can both be considered measures of spatial or visual-spatial ability, the measures themselves have different intrinsic qualities. Block design can be classified as a relatively pure measure of visuo-spatial organizational ability where the subject has to construct a three-dimensional object from a picture. In contrast, a city-map task requires the subject to memorize a route marked in a city map for 2 min and later to draw the learned route on an unmarked map. Therefore studies using one task may find a relationship between visuo-spatial memory and testosterone levels, whereas no such relationship is found using the other task. This leads to the disparity of findings and confusion surrounding the role of testosterone in mediating cognitive function.

In addition to the types of tasks used, age and intelligence are factors known to influence cognitive test performance. As individuals age, performance on some tasks has a tendency to decline and therefore the older patient may have reduced performance from the outset. The same argument applies to those who have below-average intelligence. In this pilot study, age and intelligence quotient were not perfectly matched between groups, which is one possible confounding factor, together with the small sample size, that needs to be addressed in future work.

Although a consensus is difficult to determine in such a new area, it is clear that testosterone has some role in mediating cognitive ability, and therefore is of potentially great relevance when considering the possible side-effects of hormonal treatment for prostate cancer, particularly if it is prescribed long-term.

We acknowledge that there were too few participants and the differences detected are subtle, but pooled with results from newly emerging studies, we suggest that a larger prospective study is warranted.

There is a good evidence-base for much of the current use of hormone therapy in prostate cancer, but there seems to be increasing use of LHRH therapy in early disease with uncertain indications [28] and anecdotal impression of the extensive early use of hormones for asymptomatic PSA failure of radical treatments, which is understandable given the significance of the PSA result to patients [29]. However, it is therefore very important that both clinicians and patients are made aware of any potentially harmful side-effects, to be balanced against benefit, before determining treatment route.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

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analogue and cyproterone acetate: a randomized controlled trial. BJU Int 2002; 90: 427–32


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Abbreviations: QoL, quality of life; SHBG, sex hormone-binding globulin; GHQ12, General Health Questionnaire; FACT-P, Functional Assessment of Cancer Therapy – Prostate; TOI, Trial Outcome Index; RCI, reliable change index.
Surgical treatment of stage pT3b renal cell carcinoma in solitary kidneys: a case series

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OBJECTIVE

To describe the surgical management of patients with renal cell carcinoma (RCC) in a solitary kidney (managed preferentially by nephron-sparing surgery, NSS, to avoid dialysis) and extending into the renal vein or inferior vena cava (T3b).

PATIENTS AND METHODS

We identified 13 patients treated surgically between 1977 and 2002 for stage T3b RCC in a solitary kidney; their charts were reviewed to ascertain details of management, pathology and outcomes.

RESULTS

NSS was successful in seven patients (four in situ and three extracorporeally). Five patients had radical nephrectomy (RN), four after failed NSS. The mean (SEM) operative duration was longer for NSS, at 5.8 (0.7) h, than RN, at 3.3 (0.6) h. There was one death during surgery before nephrectomy, and eight other complications in six patients. At a median (range) follow-up of 24 (0–204) months, eight patients had died, four from RCC (all having had NSS) at a median interval of 9.5 (7–16) months. Of the five patients alive at a median follow-up of 25 months, four had no identifiable disease, whilst one had systemic recurrence.

CONCLUSIONS

NSS combined with venous tumour thrombectomy for treating T3b RCC involving a solitary kidney is feasible, albeit complicated. There was oncological success in a third of the patients. The treatment of these patients needs to be individualized, as alternatives to NSS (RN or observation) have obvious disadvantages.

KEYWORDS
carcinoma, renal cell, nephrectomy, vena cava, inferior, surgery
transplantation had a JJ stent placed, which was subsequently removed after recovery.

RESULTS

Five patients had had a previous nephrectomy for contralateral RCC, a median of 7 (2–17) years previously. The contralateral kidney was congenitally absent in four patients and had been removed or rendered atrophic secondary to benign disease in the other four, each in the distant past. Nine patients presented with haematuria, two with pain, one with a pathological fracture related to metastasis, and one with constitutional symptoms.

The mean operative time was 4.78 (0.61) h but was significantly longer in patients who had NSS, at 5.84 (0.74) h, than in those who did not, at 3.30 (0.62) h, reflecting the additional time required for reconstruction or auto-transplantation. The median recorded blood loss was 1200 (650–5400) mL, and all patients required some blood transfusion. Four patients were managed in the intensive care unit (ICU) after surgery, while another required admission to the ICU 3 days after surgery for respiratory difficulties. The median hospital stay was 13 (8–27) days.

There were nine early complications in seven patients (Table 1); one patient died during surgery secondary to pulmonary embolus. Five secondary procedures were required in three patients, to repair a calyceal fistula, to de-bride and close a wound disruption, to explore and wash out a haemoperitoneum, and to undertake arterial thrombectomy from an auto-transplant. One perinephric abscess was treated successfully by percutaneous drainage; there was no associated urine leak or haematoma. Two patients had respiratory complications; one perinephric fat abscess was successfully treated by percutaneous drainage; there was no associated urine leak or haematoma. Two patients had respiratory complications; one of whom required endotracheal intubation and admission to the ICU.

Initially after surgery the serum creatinine level increased from the baseline, at 135.7 (13.3) μmol/L in all patients, more so in those patients rendered anephric, to 1312 (149.8) μmol/L, than in those who were not, at 442 (115.3) μmol/L. Two patients had acute tubular necrosis after NSS, requiring transient haemodialysis, while all five patients who were anephric started long-term haemodialysis. Before discharge the serum creatinine level improved, to 266 (106) μmol/L. All patients rendered anephric started long-term haemodialysis. Before discharge the serum creatinine level improved, to 266 (106) μmol/L. No patients required dialysis.

All tumours were of the clear-cell subtype, with a mean size of 8.34 (0.81) cm. All tumours were classified as T3b and were grade 1, 2, 3 and 4 in two, five and one patient, respectively. Perinephric fat invasion, positive resection margins, involved regional lymph nodes and distant metastasis were found in one patient each, all in the NSS group. The patients were followed for a median (mean, range) of 24 (55.2, 0–204) months, during which four died from RCC, at a median interval of 9.5 (10.5, 7–16) months. Four other patients died from unrelated causes, including the one death during surgery. Five patients were alive at the last follow-up, at a median of 25 (73.4, 8–204) months, one with systemic recurrence, and four free of disease, and in this group the serum creatinine was 266 (106) μmol/L. All four deaths from cancer and the systemic recurrence were in patients who had had NSS.

DISCUSSION

This study of the surgical treatment of stage T3b RCC in a solitary kidney shows the
feasibility of NSS in conjunction with venous tumour thrombectomy, thus preserving renal function and avoiding dialysis. Bench surgery with auto-transplantation was used in five of the earlier patients, as in situ cooling and reno-protection seemed unreliable at that time [9,10]. Currently, improved preoperative imaging and extensive experience with NSS mean that many more patients should be amenable to partial nephrectomy in situ [5,6]. However, given that many of T3b RCCs are not ideally suited to NSS, there was a significant failure rate in the present series, with a third of patients requiring RN. In a previous report of nine RCCs with venous involvement treated by NSS [8], no information was provided on whether some patients had undergone RN as a result of failed NSS.

Those patients who had RN had quicker surgery and fewer complications, but a similar length of hospital stay, than those who had NSS. The difficulty of combined NSS and venous tumour thrombectomy is shown by the fact that, compared with published data [6,11–13], the present patients fared worse in terms of blood loss (250–350 mL), operative times (2–3 h), hospitalization (median 5 days) and complication rates (15–30%). NSS in a solitary kidney is associated with higher complication rates than NSS that is not imperative [11]. Also, series of venous tumour thrombectomy [1,2], which include patients with more extensive vena caval thrombus than the present, reported similar operative times and blood loss to the present NSS group, perhaps indicating similar operative complexity. However, complications are fewer and, perhaps partly as a consequence of that, hospital stays are shorter for routine venous tumour thrombectomy [1,2] than those carried out with NSS in the present series.

The oncological outcome of this series was that four of the patients died from disease recurrence and one had systemic recurrence, all within 18 months; all were patients who had undergone NSS. However, there were also adverse findings, e.g. nodal and distant metastasis, invasion of the perinephric fat and involvement of the resection margin, in these patients, but not in those who had RN. Empirically, none of these adverse features except the one positive margin would have been more effectively treated by RN. Four patients died from unrelated causes but there was prolonged disease-free survival (median 72 months, mean 76.8, range 8–204) in seven, including both NSS and RN. Encouragingly, despite the adverse pathology represented, four patients were alive with no evidence of disease at a median of 81.5 months (mean 93.8, range 8–204). These results are comparable to those reported by Angermeier et al. [8], where four of nine patients died from RCC at a mean of 35.5 months after surgery, with the remaining five alive with no evidence of disease at a mean follow-up of 33.2 months.

The patient and physician faced with venous tumour thrombus in the context of a solitary kidney or bilateral disease has a difficult decision to make. We hope that our results will help with that decision, where it is important to consider the advantages and disadvantages of the alternative treatment strategies. NSS allows these patients to avoid dialysis, but is associated with prolonged and complex surgery. In well-selected RCCs, NSS can provide an equivalent oncological outcome to RN [14], but in the present series all deaths from cancer were after NSS. Thus, in the face of adverse pathological features such as nodal or distant metastases, positive surgical margins or perinephric fat invasion, NSS may be oncologically inadequate and should probably be avoided.

However, RN renders these patients anephric and exposes them to the significant morbidity [15,16] and mortality [17,18] of chronic dialysis. Given the few patients in the present series, it remains unclear whether RN prolongs survival in patients with T3b RCC in a solitary kidney, compared to NSS. Although the site of relapse was not identified in this study, for the subset of patients who relapse at distant sites as a result of micrometastatic disease, outcomes after RN and NSS are likely to be similar. Thus, the limited life-expectancy of such patients would be compromised after RN by having to undergo dialysis.

The third management option in such patients is observation, which would lead inexorably to the subsequent development of local complications and morbidity, which can be troublesome and difficult to palliate effectively [19,20]. Leaving the tumour untreated may also accelerate the development of systemic disease and death. Unfortunately, given the retrospective nature of this study, we cannot comment on how often this management strategy was chosen, and what the outcome was. No single protocol can be recommended for all patients with T3b RCC in a solitary kidney. Ultimately, treatment needs to be individualized to the patient, and may be dictated largely by patients’ preferences.

CONFLICT OF INTEREST

None declared.

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Abbreviations: ICU, intensive care unit; NSS, nephron-sparing surgery; RN, radical nephrectomy.

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OBJECTIVES

To describe national trends in the practice of radical nephrectomy (RN) in England between 1995 and 2002.

METHODS

Data were extracted from the Hospital Episode Statistics database of the Department of Health in England between 1995/1996 and 2001/2002. Patients were included in the study if an International Classification of Diseases diagnosis code (ICD-10) for malignant neoplasm of the kidney, renal pelvis or ureter, and an operative procedure code (OPCS-4) describing total or partial excision of the kidney by either a laparoscopic or open approach, were present in any of the diagnosis or operative procedure fields. Overall, 17 308 patients were included.

RESULTS

Patient age and the proportion who were men did not change over the study period. The proportion of patients admitted as an emergency decreased from 14.0% to 7.5% over this period (P < 0.001). The mean waiting duration increased by almost 6 days (P < 0.001) and length of stay by 1 day, from 11.7 days in 1995 to 10.8 days in 2001 (P < 0.001). In-hospital mortality decreased from 2.0% to 1.5% (P = 0.134). In-hospital mortality and length of stay were higher in older patients and in those admitted as an emergency. Women had a longer stay than men (11.5 vs 11.1 days), but in-hospital mortality was higher in men (2.3% vs 1.6%). The national number of RNs per year increased by ≈20%, from 2254 in 1995 to 2671 in 2001. Over the same period the mean annual hospital volume of RN increased by ≈40%, from 17 in 1995 to 24 in 2001. The annual number of laparoscopic RNs nationally increased from seven in 1995 to 84 in 2002.

CONCLUSIONS

The annual number of RNs in England increased by almost a fifth and this was accompanied by an increase in annual hospital volume of about two-fifths. There was a large proportional increase in the number of laparoscopic RNs. Emergency admission rates and length of stay decreased but this was not accompanied by a significant change in in-hospital mortality rate.

KEYWORDS

radical nephrectomy, outcomes, England

INTRODUCTION

Renal malignancies account for ≈2% of newly diagnosed malignancies per year in the UK [1]. Over the last 20 years the annual number of renal malignancies diagnosed has increased worldwide by ≈70%, with most recent values indicating that ≈5700 people are affected each year in the UK [2]. Each year an estimated 95 000 deaths worldwide are from renal malignancy, with >3000 such deaths annually within the UK [2]. In the USA, 31 800 new cases of carcinoma of the kidney and of the renal pelvis were diagnosed in 2002, with 11 600 deaths attributed to these diseases [3]. Urological surgical intervention in the form of radical nephrectomy (RN) or nephroureterectomy is considered the primary curative treatment [4,5].

Large-scale national audits to investigate surgical activity and the quality of surgical care are expensive and labour-intensive. An alternative approach is to conduct studies using national administrative databases. This approach has been used increasingly in the USA, in particular using the Medicare database [6–10]. The Hospital Episode Statistics (HES) database of the Department of Health in England could be considered as the English equivalent of the Medicare database; it records medical, demographic and administrative data relating to all patients admitted to National Health Service (NHS) hospitals in England [11]. Over 12 million records are collected each year, which include private patient admissions to NHS hospitals but do not include patients treated within independent-sector hospitals. HES data are being increasingly used to illustrate variations in health status and delivery of care. They are also used in medical research, assessing performance and in policy development [12,13].

The objective of the present study was to describe changes in surgical activity, and patient characteristics and outcomes for RNs in England between 1995 and 2002, using the HES database.

METHODS

Data were extracted from the HES database for 1995/1996 to 2001/2002 for all patients recorded as having undergone a RN. Patients were included in the study if first, there was an International Classification of Diseases code (ICD-10) for malignant neoplasm of the kidney, renal pelvis or ureter in any of the diagnosis, second, there was a Hospital Episode Statistics (HES) database code (OPCS-4) indicating RN and excision of perirenal tissue (M021), nephroureterectomy not elsewhere classified (M022), bilateral nephrectomy (M023),
The national number of RNs annually decreased from 198 to 162. The annual number of hospitals recorded as using RN decreased from 176 to 162. The mean waiting duration increased by almost 6 days over the study period. The mean waiting duration increased by almost 2.5 times higher in those admitted as emergency than those admitted electively.

In-hospital mortality rates increased with age (Table 2), e.g. in those aged ≤55 years it was 0.6% but 4.7% in those aged >80 years. In-hospital mortality was also slightly higher in men than women. There appeared to be no statistically significant association between waiting duration and in-hospital mortality. Furthermore, in-hospital mortality was almost 2.5 times higher in those admitted as an emergency than those admitted electively. Patients who died had a longer stay in hospital before death than those who were discharged (15.8 and 11.1 days, respectively).

The national number of RNs nationally increased by ≥20%, from 2254 in 1995 to 2671 in 2001 (Table 1). Over the same period the mean annual hospital volume of RN increased by >40%, from 17 in 1995 to 24 in 2000. However, in this period the total number of hospitals recorded as using RN decreased from 198 to 162. The annual number of laparoscopic RNs (LRNs) nationally increased from seven in 1995 to 84 in 2001, and the number of hospitals recorded as...
using LRN increased from seven in 1995 to 24 in 2001. The annual number of partial nephrectomies (PNs) also increased from 49 to 108, and these were in 35 hospitals in 1995 and 52 in 2001 (Table 1).

**DISCUSSION**

In summary, the annual number of RNs in England increased by almost a fifth over the study period, and this was accompanied by an increase in annual hospital volume of about two-fifths. There was also a large proportional increase in the number of LRNs and PNs. Length of stay was longer in older patients, in women and in those admitted as an emergency. In-hospital mortality was higher for older patients, men, and in those admitted as an emergency.

There are several limitations associated with the use of administrative data sources such as HES. First, although the 20% increase in the annual number of RNs over the study period may partly be explained by the 2% annual increase in the incidence of renal malignancies also reported over this period, it may also be explained by lower or varying thresholds for surgical intervention, or by improvements in the accuracy and coding of clinical data. It is not possible to determine the relative contributions of these effects from the present data.

Second, in-hospital mortality rates decreased from 2.0% to 1.5% over the period; this could reflect improvements in the quality of surgical care, but also could be related to the simultaneous decrease in the length of hospital stay over the period. This explanation cannot be excluded, as shorter hospital stays increase the chances of observing higher mortality rates in the period after discharge [16].

The finding that the proportional increase in the annual hospital volume of RN was about twice that of the national number of RNs was surprising. Several explanations could account for this. First, this could suggest a degree of centralization of cancer services for RN in England. However, national guidance recommending that local hospitals should not perform RN in cases where the procedure was likely to be complex (e.g. for tumours invading major blood vessels, or for patients with von Hippel-Lindau disease) was issued only in 2002, and therefore should not affect the results of the present study [17]. Second, the number of hospital trusts in England submitting data to the HES database decreased over this period, partly through hospital mergers, and this may also explain the observed increase in hospital volume [18].

The demographic characteristics of the patients included within this study are similar to those included within several previous studies of RN [3,19]. The present mean hospital stay was 11.2 days, which compares with a French study of 656 patients undergoing RN between 1986 and 1997, that reported a stay of 11 days [19], but contrasts with a nationwide USA study of the Medicare database (only including patients aged >65 years), which reported a stay of 7.5 days [20]. The reasons for this difference are likely to be multifactorial, but will include physician, hospital, cultural, social and financial factors.

The surgical approach clearly also influences the length of stay after RN. In a single-centre USA study, the length of stay for open RN was 3.6 days, vs 1.7 days for LRN [21]. In the present study, the mean stay for LRN was 8.4 days, compared to 11.3 days for open RN. A national UK audit of LRN for several indications between 2001 and 2002 reported a median stay after surgery of 4 days in 263 patients [22], of which 113 (43%) were for cancer. Although the study periods do not overlap exactly, in the final year of the present study we identified 84 RNs that were coded as having been laparoscopic. Importantly, the present study included patients only from England, whereas the nationwide audit included patients from elsewhere in the UK. Without formal case-note validation it is not possible to determine how many of the patients included in these two studies overlap. In comparison to the USA, in the UK the proportion of RNs that were laparoscopic is smaller, but this is increasing rapidly [23].

The present in-hospital mortality rate of 2.1% is lower than a national USA study of 58 990 Medicare patients undergoing RN between 1994 and 1999, where it was 3% [7]. However, for only those patients aged >65 years in the present study the in-hospital mortality rate was 2.9%, very similar to the national USA
study. In a large single-centre French study of 656 patients, the mortality rate was only 0.6%, considerably lower than either the present or the USA study [19]. Another single-centre study between 1995 and 2002 of 1049 patients in the USA undergoing RN reported a perioperative mortality rate of 0.2% [24]. This illustrates the low mortality rates that can be attained in some centres, but it is not clear how generally applicable these single-centre results are to a national level, given the possible role of confounding factors such as differences in disease severity and degree of comorbidity.

This study used the HES database because it is the only source of national information on all NHS admissions in England. These data will provide useful indicators for comparative local audit and clinical governance. In the future, studies using the BAUS Complex Operations database should allow similar assessments that address some of the limitations associated with the HES database, including accuracy of clinical coding and paucity of information on disease severity and stage. These studies may also permit some element of validation of the HES data, and report on the effect of recent national targets which aim to improve access to cancer services, on waiting times and clinical outcomes [25].

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CONFLICT OF INTEREST

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Abbreviations: R(L)P(N), radical (laparoscopic) (partial) nephrectomy; NHS, National Health Service; HES, Hospital Episode Statistics.
The relationship between angiogenesis and cyclooxygenase-2 expression in prostate cancer

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OBJECTIVE
To test the hypothesis that angiogenesis in prostate cancer is associated with tumour invasion and metastasis, and that this is mediated through increased cyclooxygenase-2 (COX-2) expression.

PATIENTS AND METHODS
Angiogenesis was assessed in 105 patients with either prostate cancer (79) or benign prostatic hyperplasia (BPH, 26) and these data correlated with levels of COX-2 expression in the same dataset. The mean microvessel density (MVD) was analysed as a marker of angiogenesis, using the endothelial antigen CD34 stained by immunohistochemistry.

RESULTS
There was no difference in MVD in progressive tumour stages compared with BPH. There was a negative correlation between MVD and COX-2 expression, but the effect of increased COX-2 expression on MVD was not marked.

CONCLUSION
These data suggest that COX-2 drives tumour spread in prostate cancer by means other than the promotion of angiogenesis.

KEYWORDS
prostate cancer, angiogenesis, COX-2

INTRODUCTION
Angiogenesis, the formation of new blood vessels, is vital in the growth, progression and metastasis of many cancers [1]. Solid tumours induce the formation of new capillaries to avoid oxygen starvation and obtain the required nutrients to grow beyond 2–3 mm in diameter [1]. The regulation of angiogenesis depends on a complex interplay between pro-angiogenic factors, e.g. vascular endothelial growth factors (VEGFs) and inhibitory factors, known as the 'angiogenic switch' [1,2]. Immunohistochemical (IHC) studies for endothelial antigens such as von Willebrand’s factor (Factor VIII), CD31 and CD34 are frequently used to quantify neovascularization in tumours. Such studies have suggested that estimating the mean microvessel density (MVD) predicts local spread and recurrence in several cancers [1,3,4]. In addition, studies suggest that angiogenesis contributes to the metastatic potential of prostate cancer [1,5]. The administration of angiogenesis inhibitors suppresses the primary and metastatic growth of prostate tumours in vivo [1]. Several retrospective studies showed that mean MVD correlates with increasing Gleason score and disease progression (from extraprostatic extension to metastasis) in prostate cancer [4,5]. However, the clinical value of measuring angiogenesis remains controversial; estimates of mean MVD have not always been shown to predict local recurrence, nor relate to Gleason score or stage [6]. In addition, some studies were unable to confirm that MVD can act as an independent prognostic variable for prostate cancer when subjected to multivariate analysis [1,7].

Cyclooxygenase-2 (COX-2) is the inducible form of COX which is involved in the formation of prostaglandins (PGs) such as PGE2 from arachidonic acid, which in turn regulates VEGF production, thereby promoting angiogenesis [8]. aberrant or increased expression of COX-2 has been implicated in the pathogenesis of several human cancers, including colorectal [9] and breast [10,11]. A recent report revealed a correlation between COX-2 expression and tumour MVD as measured by CD31 in breast cancer [11]. This provides the rationale for the use of selective COX-2 inhibitors in treating selected cancers, to reduce neovascularization and therefore cell growth, which are currently under investigation [1].

Increased expression of COX-2 has already been shown in the prostate cancer cell lines LNCaP (androgen-sensitive) and PC-3 (androgen-insensitive) [10,12]. Studies using IHC, including ours, have confirmed high levels of COX-2 expression in human prostate cancer tissue and high-grade prostatic intraepithelial neoplasia [13–17]. A correlation between COX-2 staining intensity, Gleason score and poor prognosis in prostate cancer was reported in some studies [16–18], but not others [13]. We recently showed higher levels of COX-2 expression in locally advanced prostate cancer [19]. Studies in hormone-resistant prostate cancer cell lines show a link between COX-2, PGE2 production and the hypoxic up-regulation of VEGF, which may be reversed by adding a selective COX-2 inhibitor [20]. Similarly, studies of prostate cancer in mice show that treatment with selective COX-2 inhibitors prevents the up-regulation of VEGF, decreasing tumour MVD and tumour growth [21].

We tested the hypothesis that angiogenesis in prostate cancer is associated with tumour invasion and metastasis, and mediated through increased COX-2 expression. The confirmation of angiogenesis and COX-2 as consistent prognostic markers in prostate cancer would support further clinical assessment of angiogenesis and COX-2 inhibitors, which have already shown promise in various trials [1,22].

PATIENT AND METHODS
A database of 105 tumour biopsies was established with archival tissue specimens (formalin-fixed, paraffin-embedded). Detailed data on stage, presence of metastasis,
Gleason grade and survival data, if available, were documented by reviewing case notes. To develop this patient cohort, we obtained multiple research ethical committee approval and the support of the Scottish Urological Oncology Group, and recruited patients throughout Scotland. Tissue sections were then divided into two groups by stage (T1/2 and T3/4), with BPH specimens as a control. Two further subgroups with nonmetastatic and metastatic disease at presentation were also identified. COX-2 expression data were also available for each tumour in this cohort [19].

Tumour angiogenesis was assessed by IHC using a monoclonal antibody to the endothelial cell-surface marker CD34 (mouse IgG1, QBEnd/10, Novocastra, UK). Tissue sections (5 μm) were dewaxed in xylene and rehydrated through graded alcohols. Antigens were retrieved by incubating sections in 0.1% trypsin in 0.1% calcium chloride (w/v, pH 4) for 25 min at 37 °C. Sections were then incubated with primary antibody at 1:50 dilution for 30 min at room temperature. Negative control sections were incubated with an isotype-matched control antibody. Bound antibody was visualized using a biotinylated secondary antibody, streptavidin-horseradish peroxidase complex (DAKO, UK) and 3,3′-diaminobenzidine as chromogen (Vector Laboratories, Burlinghame, CA). Tissue sections were counterstained with haematoxylin and dehydrated through graded alcohols and xylene. Subjective analysis of the tissue sections was used to identify the four most vascular regions or ‘hot spots’ (areas of maximum endothelial cell staining of microvessels) at low magnification (×200) (Fig. 1). The vessels in each hot spot were then counted at higher magnification (×400) in four fields of vision; the mean of these counts was taken as the mean MVD. Every tenth slide was double-scored by an independent observer.

COX-2 expression was previously determined using a monoclonal antibody (mouse IgG1, Cat. No. 160112, Cayman Chemical Co., USA) at 1:80 concentration. This was quantified by the same two observers, while unaware of sample origin, using a weighted histoscore method, calculated from the sum of (1 x percentage weak staining) + (2 x percentage moderate staining) + (3 x percentage strong staining), providing a semiquantitative classification of staining intensity. The interclass correlation coefficients between each observer for each protein were >0.7, which is classed as excellent.

MVD scores are shown as the mean (±0.5), and results were analysed statistically using Student's t-test to compare differences in scores between BPH and individual tumour stages. Spearman rank correlation coefficient was used to determine any correlation between COX-2 expression, angiogenesis (as determined by the mean MVD), and Gleason score. Kaplan-Meier survival plots were used to correlate MVD scores with survival.

RESULTS

In all, 105 patients were retrospectively recruited into the study; 79 had prostate cancer (46 with stage T1/2 and 31 with stage T3/4, and two stage unknown) and 26 had BPH (Table 1). Tumour groups were also subdivided into metastatic (11 T1/2, seven T3/4, two unknown) and nonmetastatic (35 T1/2, 24 T3/4) at presentation. The median age, Gleason sum and mean survival for all patients in the database are shown in Table 1. There was no significant difference in angiogenesis, as measured by MVD, between tumour groups (all groups combined) compared with BPH, nor with T3/4 compared with T1/2 (Table 1) when assessed using a t-test. Nor were there significant differences between patients with or without metastasis at diagnosis, either in total or when further subdivided by individual tumour stage (Table 2). In patients with a high MVD (above the mean) there was no significantly different survival time from those with a low MVD (below the mean; P = 0.15).

There was a negative correlation between COX-2 expression and angiogenesis, as measured by MVD, between tumour groups (all groups combined) compared with BPH, nor with T3/4 compared with T1/2 (Table 1) when assessed using a t-test. Nor were there significant differences between patients with or without metastasis at diagnosis, either in total or when further subdivided by individual tumour stage (Table 2). In patients with a high MVD (above the mean) there was no significantly different survival time from those with a low MVD (below the mean; P = 0.15).

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DISCUSSION

We previously reported an association between high COX-2 expression and increased tumour stage (T3/4), and increased COX-2 expression in prostate cancer compared with BPH [19]. This confirmed previous studies in which COX-2 expression was associated with aggressive disease in prostate cancer [16–18], and led to the hypothesis that COX-2 drives increased neovascularization. In support of this, a recent study of breast cancer reported a positive relationship between COX-2 expression (as measured by a weighted histoscore) and angiogenesis as measured by the mean MVD using the CD31 antigen [11]. Interestingly, in the present study there was a significant
negative correlation between angiogenesis, as measured by CD34 expression, and COX-2 expression. This would contradict the hypothesis that COX-2 acts as a pro-angiogenic stimulant, at least in prostate cancer, and could suggest that COX-2 inhibits new blood vessel formation. However, the decrease in MVD associated with an increase in COX-2 expression was lower than the observed variation in MVD at individual COX-2 expression levels (Fig. 2). For example, an increase in COX-2 expression from 100 to 300 histoscore units would result in a theoretical reduction in MVD from 15.88 to 10.48 microvessels/field (a decrease of 5.4 units). However, the actual mean (± SD) MVD between COX-2 histoscores of, e.g., 140–160, is 17.76 (9.9) microvessels/field. At this point the variation in MVD scores is almost twice the maximum change predicted (as 90% of samples have a COX-2 histoscore of 100–300). This suggests that the variation in estimates of MVD would preclude its use as a prognostic or predictive factor. Furthermore, the large variation in MVD scores for individual COX-2 scores would undermine the biological significance of the observed negative correlation between MVD and COX-2 expression. Perhaps a sub-analysis comparing the mean MVD in focal areas of high or low COX-2 expression alone may have produced less variation, but in practice this would not be technically feasible because of the diffuse and heterogeneous nature of COX-2 staining within individual tissue sections. There are also inherent difficulties in accurately comparing protein expression within specific areas between different tissue sections, in the absence of a dual-staining technique. The weighted histoscore technique for measuring COX-2 expression is therefore well established and was used previously in comparison with mean MVD scores in breast tissue [11,16–19].

Whilst the present results therefore may reflect the methodological problems associated with measuring MVD it may also imply that COX-2 drives tumour progression independently of neovascularization. For example, COX-2 has well documented roles in promoting the inflammatory response, inhibiting apoptosis via the Akt/bcl-2 pathway, and is involved in the control of cellular proliferation via the interleukin--6 pathway [23]. Studies in breast cancer show that cellular proliferation, as measured by the mitotic activity index, have no association with angiogenesis as measured by MVD [3].

The importance of angiogenesis in tumour metastasis has been well established for over 30 years [1]. In theory, inhibiting angiogenesis might provide a further therapeutic option by targeting cancer growth and spread. However, in the present study angiogenesis, as measured by mean MVD in prostate cancer, did not increase with increasing tumour stage or metastases, in line with other studies [6,7]. However, others reported a relationship between mean MVD and advancing disease in prostate cancer [4,5,24,25]. There are similar inconsistencies with the use of MVD as a prognostic indicator in colorectal cancer, where MVD was lower in metastatic than primary tumours [26], and to a lesser extent in breast cancer [27]. These differences may be related to the use of different antibodies, as the present MVD scores were lower than those previously published using Factor VIII and CD31 as an endothelial antigen [5,6,25]. This was also reported in breast cancer when CD31 was compared to Factor VIII [6]. However, disparities between MVD scores were also reported between studies using the same antibody [4,6]. Furthermore, a recent report associating MVD with the outcome after radical prostatectomy cited scores of a similar magnitude to the present when using CD34 as an antigen [28]. Similar scores were also reported when the present protocol for measuring MVD using CD34 was incorporated into a pilot study of breast cancer specimens within our laboratory (unpublished data). There are other well documented controversies in determining angiogenesis, e.g. the presence of tumour heterogeneity [1,7]. Other aspects of the methods, e.g. the actual region selected for vessel counting, may be as important [1,6]. If measuring

<table>
<thead>
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<th>Variable</th>
<th>Pathology</th>
<th>pT1/pT2</th>
<th>pT3/pT4</th>
<th>Non-metastatic</th>
<th>Metastatic</th>
<th>All tumours</th>
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<tr>
<td>Median (IQR) age, years</td>
<td>BPH</td>
<td>75 (71–75)</td>
<td>64 (60–63)</td>
<td>73 (63–76)</td>
<td>64 (61–74)</td>
<td>74 (68–80)</td>
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<td>Mean (± SD)</td>
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<td>Gleason score</td>
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<td>7 (2)</td>
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<td>7 (1)</td>
<td>–</td>
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<td>58 (24)</td>
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<td>40 (33)</td>
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<tr>
<td>time to death, months</td>
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<td>38 (7)</td>
<td>44 (15)</td>
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<tr>
<td>Mean (± SD) MVD</td>
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<td>12.9 (6)</td>
<td>13.4 (9)</td>
<td>13.0 (7)</td>
<td>14.2 (9)</td>
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</table>

IQR, interquartile range; N/A, not available; % death, % of patients dying from prostate cancer during the follow-up.
Angiogenesis alone is to be exploited for clinical use, current methods involving MVD analysis need to be simplified and standardized [1]. Further prospective studies are needed to explore its potential as a prognostic marker in prostate cancer. However, despite the possible inverse correlation between the mean MVD and COX-2 expression in the present study, it does not preclude the targeting of angiogenesis as a treatment method. Angiogenesis is regulated by a complex series of molecular pathways which, whilst they include the modulation of VEGF via PGF2 produced by COX-2, are subject to many other modulatory factors. We therefore conclude that it is unrealistic to correlate COX-2 with a distant endpoint such as angiogenesis. A more appropriate relationship may be found if VEGF expression was determined directly.

In summary, tumour angiogenesis, as measured by the MVD using an antibody to CD34, does not increase with tumour stage, and appears to have a weak negative relationship with the expression of the pro-angiogenic factor COX-2. The present study raises the possibility that COX-2 may influence tumour progression in prostate cancer through mechanisms other than the promotion of angiogenesis. The use of selective COX-2 and angiogenesis inhibitors may still have a role in the targeted treatment of prostate cancer in the future, and indeed this study suggests that these agents could be used in combination.

ACKNOWLEDGEMENTS

The authors thank Dr W J Angerson for his statistical advice. The work was supported by grants from Prostate Research Campaign UK and Glasgow Royal Infirmary Research Endowment Fund.

CONFLICT OF INTEREST

None declared.

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Abbreviations: VEGF, vascular endothelial growth factor; IHC, immunohistochemistry; MVD, microvessel density; COX-2, cyclooxygenase-2; PG, prostaglandin.
Testicular–sparing microsurgery for suspected testicular masses

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OBJECTIVE
To describe a microsurgical technique for removing suspected testicular masses with sparing of the testicular parenchyma, and to describe case studies.

PATIENTS AND METHODS
Six men were referred with testicular lesions (3–6 mm) detected on ultrasonography (US); in one, the lesion was palpable. US showed hypoechoic lesions and in two cases were mixed hypoechoic and anechoic. In these men, the testicular lesion was identified by US before surgery, giving three-dimensional coordinates to facilitate intraoperative recognition. A traditional inguinal incision was used and the funiculus clamped subinguinally without opening the canal. The testicle was isolated after sectioning the gubernaculum testis. In a separate operative field, an equatorial incision of the albuginea was made in a plane orthogonal to the major axis of the testicle, sparing the subcuticular vasa. The parenchymal lobuli were dislodged and the seminiferous tubules dissociated, the nodule identified and completely removed, together with 1 mm of surrounding healthy tissue. This technique can also be used for microsurgical testicular sperm extraction (MicroTESE), to retrieve sperm in infertile men.

RESULTS
In two infertile men MicroTESE was also performed. Histology revealed one case each of seminoma, Leydig-cell tumour, Leydig cell hyperplasia, atrophy, normality in the testicular lesions, particularly in infertile men, calls for a surgical approach that must be as conservative as possible for the testicular parenchyma. We think that microsurgery should be the first-line technique in small suspected testicular lesions in infertile men.

CONCLUSIONS
The increasingly frequent detection of benign testicular lesions, particularly in infertile men, calls for a surgical approach that must be as conservative as possible for the testicular parenchyma. We think that microsurgery should be the first-line technique in small suspected testicular lesions in infertile men.

KEYWORDS
infertility, testicular neoplasm, conservative surgery, Leydig cell tumour

INTRODUCTION
The increasing use of testicular ultrasonography (US) to evaluate infertile men or to monitor scrotal pathology has led to the detection of more tumours. Furthermore, the greater attention paid by men to their genitals, in the form of self-examination or visits to their GP, makes early identification of small palpable testicular lesions possible. As a significant percentage of these lesions are benign, especially in infertile men [1,2], the surgical approach should be as conservative as possible of the testicular parenchyma.

Schlegel [3] developed a microsurgical approach specifically for detecting seminiferous tubules containing residues of spermatogenesis in men with unobstructive azoospermia (microsurgical testicular sperm extraction, MicroTESE); this approach, which is now used by others [4], allows optimal sparing of the albuginea vasa and of the intraparenchymal branches of the testicular artery, as well as a greater precision in removing testicular parenchyma for clinical evaluation [4]. The microsurgical technique can also be used advantageously for MicroTESE in men with unobstructive infertility [5].

The objective of the present study was to describe a microsurgical technique for removing suspected testicular masses, with sparing of the testicular parenchyma, and to report our case studies.

PATIENTS AND METHODS
The microsurgical technique was that described by Goldstein [5], with a few variations. The testicular lesion is identified by US before surgery, giving three-dimensional coordinates to facilitate intraoperative recognition. A traditional inguinal incision is made and the funiculus clamped subinguinally without opening the canal. The testicle is isolated, the gubernaculum testis sectioned, and the tunica vaginalis opened. The lesion site is checked during surgery with a 15 MHz ultrasound probe. Using a ×15–20 operating microscope, in a separate operative field, an equatorial incision of the tunica albuginea is made in a plane orthogonal to the major axis of the testicle, from one quarter to about three-quarters of its circumference, depending on the depth of the lesion, sparing the subcuticular vasa. Haemostasis of the vessels that cannot be spared is ensured by using microsurgical bipolar forceps, while 1–2 min compression with gauze soaked in saline solution and gentamicin is sufficient to stem microbleeding of the parenchyma (ordinarily haemostasis is carried out when the spermatic cord is unclamped). The parenchymal lobuli are dislodged and the seminiferous tubules dissociated; the nodule is identified and completely removed, together with 1 mm of surrounding healthy tissue.
tissue. The tissue is then sent for extemporaneous histological examination. If the man also has unobstructive azoospermia, surgery is always by a circumferential incision for three-quarters of the tunica albuginea, and completed by taking ≈30 microsamples of seminiferous tubules from the two sides of the open parenchyma, as described by Schlegel [3]. In the presence of histological evidence of malignancy, the inguinal canal is opened while the funiculus is clamped, and the inguinal portion of the spermatic cord recovered. In the case of histologically benign nodules, the tunica albuginea is sutured with 5/0–6/0 slow-absorption monofilament using an atraumatic needle. The tunica vaginalis is closed with continuous nonabsorbable suture with an intracavitory cortisone instillation before completion.

Between April 2001 and February 2004, six men were referred to us with testicular lesions detected by US; in five the lesion was an incidental finding during a scrotal scan for infertility, and in one the lesion was palpable with a hypotrophic contralateral testicle. In all men the preliminary tumour markers were negative. Table 1 shows the ages of the men and the US dimensions of the lesions; the mean age of the men was 39.8 years.

### RESULTS

Six men had microsurgery using the method described; microTESE was also used in two with unobstructive azoospermia, and in one of these a varicocele was also corrected. The frozen-section examination yielded a diagnosis of germ cell tumour in one case and benign findings in the remaining five (Table 1). Definitive histology revealed one each of classic seminoma, Leydig cell tumour, Leydig cell hyperplasia, atrophy, normality in the incidental forms, and some complicated cysts of the albuginea in the man with a palpable lesion. Orchifuniculectomy with inguinal canal opening was used in the man with seminoma, identified as a germ cell tumour in the extemporaneous histological examination; CT and chest X-ray was negative, and he had precautionary radiotherapy, as advised by the oncologists. In the follow-up for infertility reasons, no scarring was observable on the tunica albuginea in the men who had conservative therapy.

### DISCUSSION

An increase in the incidence of testicular lesions was reported recently [6,7]; these lesions are found fairly often in infertile men [6], hence the need to spare as much testicular parenchyma as possible, and many of these lesions are benign. From the present data, microsurgery appears to be the least invasive treatment, allowing most or all of the subcutaneous vessels to be spared, and all the branches of the testicular artery, thus greatly reducing possible areas of ischaemia. Microsurgery also allows the lesion to be better identified, enabling as little peri-lesional tissue as possible to be removed in the case of benign pathologies. Furthermore, in cases in which a TESE must be used, it can be done microscopically, selecting the most appropriate tissue; the amount of tissue extracted is thus limited, increasing the percentage of men with positive recovery of spermatozoa, as already described [3,4]. The choice of subinguinal clamping of the spermatic cord is a compromise between the traditional inguinal approach and the scrotal approach. Vascular clamping takes place before any manipulation of the testicle and in case of malignancy clamping is maintained, the inguinal canal is opened, and the remaining part of the funiculus removed. The lesions discussed are relatively small, and infiltration of the funiculus in this type of lesion does not appear very likely. The advantages of microTESE have already been described [4].

The pathologist is important in determining the choice of treatment. Diagnosis from inspection of frozen sections has achieved a high degree of certainty, so the surgeon should rarely be faced with a difficult decision [8,9]. However, the decision might be difficult in the case of stromal tumours, i.e. in Sertoli cell tumours, because they are difficult to diagnose, and in Leydig cell tumours because it is difficult to differentiating between benign and malignant forms.

The increase in incidental US findings of testicular lesions, particularly in infertile men, calls for an approach that must be more conservative of the testicular parenchyma; this can be obtained by microsurgery. This technique is effective and minimally invasive, and is useful if a concurrent TESE is required. We think it should be the first-line approach in the case of small suspected testicular lesions in infertile men.

### CONFLICT OF INTEREST

None declared.

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Abbreviations: MicroTESE, microsurgical testicular sperm extraction; US, ultrasonography.
HIFU FOR THE PROSTATE

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OBJECTIVE
To investigate the efficacy and safety of extracorporeal prostatic tissue ablation using high-intensity focused ultrasound (HIFU) in vivo in animals, and in a clinical feasibility study in men, as this is an investigational minimally invasive treatment alternative for locally confined prostatic carcinoma, but may have significant side-effects.

PATIENTS, MATERIALS AND METHODS
Ultrasound (1.04 MHz excitation frequency) was generated by an extracorporeal cylindrical piezo-ceramic element and focused by a paraboloidal reflector to a focal size of 32 × 4 mm. The focal distance and aperture diameter were both 100 mm. HIFU was applied extracorporeally at different intensities and pulse duration (up to 6 s) to 11 dog prostates in vivo (median intensity 1192 W/cm²) and eight patients (median intensity 3278 W/cm², range 2384–3576) under general anaesthesia. The lesions were assessed macroscopically and histologically after HIFU and any side-effects evaluated.

RESULTS
Thermoablation was feasible in vivo and in all patients. Macroscopic analysis and histology showed sharply demarcated coagulative necrosis. Side-effects, including skin and rectal burns, occurred only after transvesical application in the in vivo study. There were no side-effects in patients after perineal application.

CONCLUSION
Extracorporeal HIFU is technically feasible and induces sharply demarcated tissue damage in the prostate. From the early results of this phase 1 study, the perineal approach seems to be safe.

KEYWORDS
high-intensity focused ultrasound, tissue ablation, prostate

INTRODUCTION
Over the last few years there has been a rapid development of minimally invasive therapies for treating infravesical obstruction caused by BPH or locally confined prostatic carcinoma. New energy sources using different temperatures to destroy prostatic tissue have been promulgated as alternatives to TURP, the reference standard of treatment, or to radical prostatectomy. For treating BPH these...
prostatic tissue, and the objective of the designed for the extracorporeal ablation of We have developed a HIFU application system partly caused because the ultrasound waves [11,13]. These significant side-effects are 10% [12] and impotence in 0.5–5% [11,12], urethral strictures in up to burns in 0.7–15% [11], recto-urethral fistula transrectal treatment include rectal mucosal [et al.]. promising [2,4–10]. According to Thüroff carcinoma [1,2], with the probe used to apply the energy inserted transrectally. Contrary to the invasive approach of cryoablation, the energy source used for HIFU need not be inserted into the prostatic tissue to be treated. Ultrasound waves are applied outside the organ and focused on a target area within the tissue. The power density of the converging ultrasound waves is highest when the waves reach the focus. This allows high energy densities to be reached even in deeper tissue layers. Absorption of the acoustic energy by the tissue and its conversion into thermal energy induces thermonecrosis [3]. Although the reported follow-up periods are short, the oncological results obtained to date after treating locally confined prostatic carcinoma using transrectal HIFU are promising [2,4–10]. According to Thüroff et al. [11], side-effects encountered after transrectal treatment include rectal mucosal burns in 0.7–15% [11], recto-urethral fistula in 0.5–5% [11,12], urethral strictures in up to 10% [12] and impotence in ~70% of patients [11,13]. These significant side-effects are partly caused because the ultrasound waves are applied transrectally. We have developed a HIFU application system designed for the extracorporeal ablation of prostatic tissue, and the objective of the present study was to investigate the efficacy and safety of extracorporeal prostatic ablation using HIFU in vivo in dogs and in a clinical feasibility study in men.

PATIENTS, MATERIALS AND METHODS

Ultrasound waves were generated by a cylindrical piezo-ceramic element and focused by a paraboloidal reflector (Fig. 1). The excitation frequency was 1.04 MHz, and aperture diameter and focus distance both 100 mm. The average size of the rotational ellipsoid was 32 × 4 mm (focal size). The geometric dimensions of the applicator and focus were identical for in vivo and clinical application.

During the animal tests, ultrasound waves were coupled to the bodies of the dogs inside a water basin filled with degassed water (37 °C). The main feature of the generator used for clinical application is that it allows ultrasound waves to be directly coupled to the body surface using a cushion filled with cooled (16 °C) degassed water. The amount of water inside the cushion can be controlled electronically to allow the penetration depth to be adjusted to each patient and the specific distance between the skin surface and prostate (Fig. 2).

For both the dog and human application, the tissue areas to be treated and the focal position inside the tissue were located and monitored using of a diagnostic 3.5 MHz ultrasound transducer (B-mode imaging) positioned in the centre of the cylindrical piezo-ceramic element. The transducer was moved and positioned with a mechanical positioning device which can be manually moved in all directions. A dedicated computer program is used to identify the focal position in the diagnostic ultrasonogram.

In each prostate, different discrete areas in the centre of the prostate parenchyma were selected by the integrated diagnostic ultrasound transducer. The treatment areas were 25 mm apart and were subjected to one ultrasound pulse with an interval of 30 s between each application.

The in vivo model comprised 11 male beagle dogs under intubation anaesthesia and fixed in a basin filled with degassed water (animal test permission: ref. no. 37–9185.81/80/93, Land Government Office, Karlsruhe). The abdominal skin was shaved and degreased in the coupling area. Focused HIFU was applied to the prostate transvesically through the filled urinary bladder. Because of the anatomy of the animal, it was not possible to find a coupling window for perineal application. Focused HIFU was applied at an intensity of 1192 W/cm² and with a pulse duration of 1–6 s; the median (range) focal depth was 46 (38–59) mm. Identical treatments were repeated at least five times each. During autopsy, within 1 h of completing HIFU, the prostatic lesions and possible thermonecrosis

FIG. 1. Schematic diagram of the generator for applying HIFU.

FIG. 2. Applicator with a water coupling cushion for applying clinical HIFU.
in the skin and ultrasound path distal from the focus were evaluated. The prostates were cut into 3 mm tissue slices for macroscopic tissue preparation, and the maximum lesions per area measured using a sliding calliper. The tissue was then fixed in a 10% formalin solution for histological assessment (haematoxylin and eosin staining; 4 μm sections).

For the clinical study, eight patients originally scheduled for open prostatic adenoma enucleation (five) or TURP (three) were recruited for HIFU. After the patients had been informed about the treatment and risks involved, and given their consent (ref. no. 166/98, Ethics Commission of the Baden–Württemberg Medical Association), they were prepared for surgery in accordance with the clinic’s internal standards. To allow HIFU treatment patients were placed in the lithotomy position, had their perineum shaved and the skin degreased. Before HIFU application the source shown in Fig. 2 was perineally coupled to the patient’s body using ultrasound gel, and the water cushion filled with water according to the required penetration depth (Fig. 3). The integrated ultrasound transducer was used to locate an optimum sonic window for coupling the water cushion and to make sure that no bone structures (pubic bone) or air (rectum) could interfere with the propagation of the ultrasound waves by absorption or reflection.

All patients were treated under intubation anaesthesia. For each prostate, focused HIFU was applied at different intensities (median 3278 W/cm², range 2384–3576) and total number of pulses (median 10.5, range 3–50). In all, 131 pulses were applied to eight prostates, with a pulse duration in all cases of 4 s (Table 1). The skin in the perineal region was examined after completing the treatment to identify thermal skin lesions. The patients were then positioned for open prostatic adenoma enucleation or TURP, which followed the standard procedure. In the case of adenoma enucleation, the prostatic tissue was examined macroscopically immediately after surgical removal, then fixed in a 10% formalin solution for histological tissue preparation (haematoxylin and eosin, 4 μm) to allow the induced lesions to be examined. After discharge, the patients were followed up by the office urologist.

RESULTS

In the dog model, it was possible to induce thermal lesions in all 11 prostates; 5–31 areas were treated in each prostate. The macroscopic lesions showed a sharply circumscribed long, oval white/yellow lesion with a haemorrhagic seam (Fig. 4). With


FIG. 4. Macroscopic findings of sharply demarcated coagulation necroses after applying HIFU to prostatic tissue in vivo.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Penetration depth, mm</th>
<th>Number of pulses</th>
<th>Intensity, W/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>17</td>
<td>3576</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
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<td>2682</td>
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<td>3</td>
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<td>12</td>
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</tr>
<tr>
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<td>80</td>
<td>5</td>
<td>2980</td>
</tr>
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<td>69</td>
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<td>7</td>
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<td>50</td>
<td>3576</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>7</td>
<td>2980</td>
</tr>
</tbody>
</table>
**TABLE 2 The maximum prostatic lesion size after HIFU (intensity 4769 W/cm²)**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Pulse duration, s</th>
<th>Maximum lesion size, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>6 x 3</td>
</tr>
<tr>
<td>2</td>
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</tr>
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<td>3</td>
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<td>20 x 4</td>
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</tr>
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<td>9</td>
<td>5</td>
<td>20 x 4</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>22 x 10</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>8 x 5</td>
</tr>
</tbody>
</table>

**FIG. 5.** Microscopic findings of a coagulation necrosis after applying HIFU to prostatic tissue in vivo (×25).

Identical treatment parameters (pulse duration and penetration depth) the lesion size was reproducible but there was a difference in size in different animals. The mean diameters of the macroscopic lesions are shown in Table 2; the median diameter was 8 x 4 mm, the largest 22 x 10 mm and the smallest 6 x 3 mm. There was no correlation between lesion size and pulse duration.

Histological analysis (Fig. 5) showed that the central coagulation necrosis was characterized by complete and homogeneous denaturation of all tissue structures, with immediate interruption of the blood circulation caused by vascular coagulation. Incomplete tissue destruction was identified in the boundary zone, along with ruptured blood vessels.

After HIFU application to the prostate at an intensity of 1192 W/cm² and a pulse duration of 6 s (21 areas), one animal had muscular haemorrhage of 40 x 35 mm in the penetrated healthy tissue, caused by ruptured blood vessels. Histology showed no thermonecrosis in this area. One animal had some minor diffuse bleeding in the pre-prostatic fat tissue. Ulceration of the rectal mucosa was identified in all cases, with a median (range) diameter of 9 x 4.5 (35 x 20 to 3 x 2) mm. A whitish serosal necrosis appeared in four animals, and first-degree skin burns in two.

Perineal extracorporeal HIFU application was possible in all eight patients; all had surgery immediately after HIFU. Macroscopic examination of the prepared prostatic tissue sections after adenoma enucleation in five showed sharply circumscribed lesions with tissue destruction. Because of the surgical procedure it was not possible to measure lesion size exactly. The lesions corresponded to the anatomical areas that had been exposed to HIFU under ultrasonographic guidance. The histological analysis of the tissue sections showed that most lesions were mechanical, with haemorrhage and minor tissue necrosis. Histology showed sharply demarcated lesions.

As assessed immediately after HIFU and during the clinical follow-up, there was no thermal or mechanical injury of the urethra, bladder and rectal mucosa, or fistula. None of the patients had thermal skin lesions; in the five treated by open prostatectomy there were no lesions in the tissue traversed by the ultrasound waves in the abdominal wall incision or in the bladder. No other HIFU-specific side-effects and complications after surgery were identified.

Because of the multidirectional flexibility of the water cushion, it was possible to couple the transducer and to place the focal point in the prostate in different anatomical positions in the eight patients. Using the central 3.5 MHz diagnostic inline ultrasonography, good visualization of all areas of the prostate was possible and the target tissue could be precisely positioned in the focal point of the system.

During HIFU it was not possible to observe and monitor the time-dependent development of the lesion. This was caused by the back-scattering of the HIFU waves in the tissue, which over-modulated the diagnostic ultrasonography and created a totally white image in the B-mode scan. After the power pulse, a hyper-echogenic area at the expected focal region was sometimes apparent in the undisturbed B-mode scan. Therefore, online monitoring and reliable visualization of the thermonecrosis by diagnostic ultrasonography was not possible.

**DISCUSSION**

HIFU has been used for ≈50 years to treat patients with various indications; organs treated to date include the brain (Parkinson’s disease [14]), eyes (glaucoma [15,16]), liver [17], uterus [18] and urinary bladder [19]. Apart from extracorporeal application to these organs, HIFU has also been applied transrectally to treat prostatic tissue. Initial results of an in vivo study published in the 1990s documented the feasibility of the contact-less induction of thermonecrosis [20]. In 1993, Foster et al. [21] reported on the first 15 patients who had received transrectal prostatic treatment by HIFU. Since then, various groups have treated BPH by HIFU, and these studies provided histological evidence of coagulation necrosis within the prostate, followed by a reduction of obstructive urinary...
disorders on subsequent clinical examination [1,22]. However, because HIFU was not effective in the long-term, treatment of BPH by HIFU was eventually discarded [23].

In parallel with its use for prostatic adenoma tissue, HIFU was also used to treat prostatic carcinoma. Initial results showed the possibility of local tumour control with negative control biopsies in half the patients [24]. After assessing PSA values and biopsy results at different follow-up intervals, various groups provided evidence of promising remission rates [2,4–10]. As noted previously, there were various significant side-effects after transrectal treatment [11–13], with stress incontinence identified in 3.9–24% of patients [2,13], partly caused by applying HIFU transrectally.

In a histopathological study of nine prostatectomy tissue sections (radical prostatectomy; 7–12 days after transrectal HIFU treatment of a prostatic carcinoma), Beerlage et al. [25] showed that thermonecrosis was induced in the treated prostatic tissue, but that it was incomplete on the dorsal side in two samples. These authors concluded that incomplete thermonecrosis is caused by several factors specific to transrectal HIFU. The first was the safe distance (3–6 mm) between the rectal wall and dorsal prostatic capsule required for transrectal application, to avoid thermal rectal lesions, such that tissue directly at the prostate capsule is not exposed. Second, the ellipsoidal focal configuration causes the maximum energy concentration to be reached in the centre of the ellipse, whereas the energy dose in the treated tissue at the ends of the ellipse may be insufficient to induce thermonecrosis. Third, the ultrasound waves were coupled to the prostate using a balloon filled with cooled water and placed in the rectum. Because of this cooling effect the temperature required to induce thermonecrosis on the dorsal side of the organ may fall below the threshold temperature of 60 °C.

The transrectal applicators used have a penetration depth of up to 45 mm, and consequently, complete tissue treatment may be anatomically impossible for larger prostates (>40 mL). Thus Chaussy et al. [7] suggested TURP before HIFU to ensure effective treatment even of peripheral areas, after reducing the tissue mass. Thus in view of these technical drawbacks and side-effects of transrectal treatment, in the present study we assessed the suitability of a generator for extracorporeal HIFU treatment of prostatic tissue.

Using the integrated diagnostic ultrasound transducer, good visualization of all areas of the prostate was possible and the target tissue could be positioned precisely in the focal point of the system. However, online visualization of the thermonecrosis was not possible. Several groups [26,27] providing comparable findings described the limitations of diagnostic ultrasonography in terms of visualizing detectable tissue changes during or after HIFU. Possible solutions for improving online monitoring of complete tissue ablation might be provided by direct computer-aided evaluation of ultrasound signals, Doppler ultrasonography or MRI [28]. These new technologies are currently in the development stage.

Macroscopic analysis of the present prostates showed sharply demarcated lesions similar to those identified after transrectal application [1], and histology showed that mechanical lesions, in the form of ruptured tissue and haemorrhages, prevailed over thermal lesions. Susani et al. [29] described similar histological findings immediately after transrectal HIFU: epithelial cells had dark-staining pycnotic nuclei, with the surrounding cytoplasm being narrow and irregularly vacuolated. The epithelium was detached from the basal membrane and single cells were dissociated. Electron microscopy of the fresh lesions (2–3 h after HIFU) showed severe changes at the subcellular level. After 7 days, the target area appeared to be a classic haemorrhagic necrosis; within 10 weeks, the coagulative necrosis was resorbed by tissue rich in macrophages and capillary sprouts, and a scar was formed. The border between HIFU treated and untreated tissue was extremely sharp, comprising 5–7 cell layers.

In the present in vivo dog study, the lesions were of variable size and the dimensions not reproducible. The side-effects on the rectal mucosa were basically caused by the anatomical proximity of the prostate and rectum in dogs (3–5 mm apart [30]). It was not the aim of this study to ablate large tissue volumes and thus only small tissue areas were treated. Because the technique is new, we did not know the exact pulse duration and number to be delivered in human prostates. Only experience will enable us to progress and treat larger areas with more pulses; further clinical studies are necessary.

In conclusion, extracorporeal HIFU therapy of prostatic tissue, using a cylindrical piezoceramic element and a paraboloidal reflector to focus the ultrasound waves, induces sharply demarcated tissue necrosis. The application is flexible and technically feasible. With monitoring by diagnostic inline ultrasonography, HIFU tissue ablation was safe and reliable in a pilot study of eight patients. This method is an alternative to transrectal HIFU, and larger-scale follow-up studies are required to confirm that the perineal is better than the transrectal approach.

CONFLICT OF INTEREST

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Abbreviations: HIFU, high-intensity focused ultrasound.
Association of cigarette smoking, alcohol consumption and physical activity with lower urinary tract symptoms in older American men: findings from the third National Health And Nutrition Examination Survey

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OBJECTIVES
To examine the association of cigarette smoking, alcohol consumption and physical activity with lower urinary tract symptoms (LUTS) in older men.

SUBJECTS AND METHODS
The study included 2797 men participating in the Third National Health and Nutrition Examination Survey (NHANES III), who were aged ≥ 60 years. During an interview, LUTS, smoking history, alcohol consumption and physical activity were assessed. Cases comprised men with at least three of the symptoms of nocturia, hesitancy, weak stream and incomplete emptying. Men who had had prostate surgery unrelated to cancer were not included as cases. Controls were men with no symptoms or surgery. We adjusted for age and race in logistic regression models and used sampling weights to account for selection probability.

RESULTS
Current cigarette smokers had no higher odds of LUTS than ‘never’ smokers, but former heavy smokers (≥ 50 pack-years) had a higher odds of LUTS than never smokers (odds ratio 2.01; 95% confidence interval 1.04–3.89). Men who drank alcohol daily had a lower chance of LUTS than non-drinkers (0.59; 0.37–0.95; P trend, 0.07). All levels of moderate and vigorous activity were statistically significantly inversely associated with LUTS (P trend, 0.06), whereas men who reported no leisure-time physical activity had a greater odds of LUTS (2.06; 1.26–3.39).

CONCLUSIONS
Moderate alcohol consumption and physical activity may be protective against LUTS. Current cigarette smoking was not consistently associated with the condition. The possible association in former smokers warrants further investigation.

KEYWORDS
NHANES III, LUTS, smoking, physical activity, alcohol consumption

INTRODUCTION
LUTS are a common bothersome condition in older men. Although BPH is thought to be one cause of these symptoms not all men with symptoms have an enlarged prostate [1]; changes in the tone of prostate and bladder smooth muscle may also contribute to these symptoms. Despite the high prevalence of LUTS not much is known about their causes. Age is the only well-established risk factor, but it has been hypothesized that common lifestyle factors such as smoking, consumption of alcohol, or physical inactivity might contribute to the symptoms.

While physical activity generally has been found to be inversely associated with the prevalence of LUTS [2,3] the associations of cigarette smoking and alcohol consumption with LUTS are more controversial. Most studies found either no [3–8] or a positive [9–11] association between cigarette smoking and LUTS. Fewer studies have evaluated the association of alcohol intake with LUTS; two studies reported an inverse association [4,11] whereas in others there were positive associations [3,7,9].

The Third National Health and Nutrition Examination Survey (NHANES III) is a large American cross-sectional study conducted between 1988 and 1994. Using the data collected in NHANES III, we evaluated the association of cigarette smoking, alcohol consumption and physical activity with LUTS in a multi-ethnic group of older men representative of the USA.

SUBJECTS AND METHODS
NHANES III is a nationally representative cross-sectional study of the non-institutionalized civilian USA population conducted between 1988 and 1994 [12]. A multistage probability sampling design was used with oversampling of non-Hispanic blacks, Mexican-Americans and older participants. Subjects participated in an interview conducted at home and had an extensive physical examination. In the present analysis we included 3117 men who were aged ≥ 60 years at participation. Of these, we excluded those men with a mobility impairment (103) or who were not self-respondents (133); we further excluded 84 men who reported during the interview of having had a diagnosis of prostate cancer at
some point before the interview. The remaining 2797 men were included in the analysis.

During the interview, all men who were aged ≥60 years were asked to report the following symptoms, which are part of the AUA Symptom Index [13]: (a) How many times per night do you usually get up to urinate (pass water)? (‘nocturia’); (b) when you urinate (pass water), do you usually feel like you have not completely emptied your bladder? (‘incomplete emptying’); (c) do you usually have trouble starting to urinate (pass water)? (‘hesitancy’); and (d) has the force of your urinary stream of water decreased over the years? (‘weak stream’). In the present analysis, men were considered as having LUTS if they reported at least three of the four symptoms. Nocturia was included as a symptom when men had to get up at least twice per night. Men were also asked if they had ever had surgery for their prostate not related to cancer. Those men who reported such surgery were excluded from the cases because removing the hyperplastic tissue may have reduced or eliminated symptoms. The controls were men who reported none of the four symptoms and had never had prostate surgery unrelated to cancer. Men with only one or two symptoms were excluded from the analysis to increase the specificity of the LUTS definition. In a cohort of similarly aged men unselected for urological problems and in which the AUA Symptom Index was administered [11], the Pearson correlation coefficient between the AUA symptom score and the index using only the four symptoms of the abbreviated score was 0.7 (P < 0.0001). The agreement between using as the threshold a score of 3 or 4 on the abbreviated index and using a score of 15+ on the full index as the indicator of high moderate/severe LUTS was 69%.

Smoking history was assessed during the interview and men were classified according to their smoking habit into current (1–34, or ≥35 cigarettes/day), former, or never smokers. We also calculated pack-years of smoking from smoking history, a pack-year being defined as 20 cigarettes/day for 1 year. The consumption frequency of alcoholic beverages (beer, wine, liquor) during the past month was assessed using a food-frequency questionnaire during the interview. This method captures long-term habits of alcohol consumption. We categorized men into those who consumed none of these three alcoholic beverages, those who drank up to once per week, more than once per week but less than once per day, and those who drank alcohol once a day or more. During the physical examination at the Mobile Examination Center a 24-h dietary recall was administered, which assessed the amount of alcohol consumed during the previous day. From these data, the daily intake of alcohol (in grams) was calculated. We grouped men as having an intake of 0, 1–15, 16–37 or ≥38 g/day. Furthermore the type and frequency of leisure-time physical activity in the past month were ascertained during the interview. Physical activities were coded and classified by rate of energy expenditure (i.e. by intensity) according to a standardized coding scheme developed by Ainsworth et al. [14]. Men were grouped by their weekly frequency of moderate and vigorous activity, defined as walking, jogging or running, biking, swimming, aerobic, dancing, calisthenics, gardening, lifting weights, and other physical activities, if the metabolic equivalent of the activity compared to at rest (METS) was ≥2.4 for men aged 60–64, >1.9 for men aged 65–79, or >1.25 for men aged ≥79 years. We further evaluated the frequency of vigorous activity only, which was defined as walking (for men aged >79), jogging or running (all men), biking (for men aged >64), swimming (all men), aerobics (all men), dancing (for men aged >64), calisthenics (for men aged >64), gardening (for men aged >64), lifting weights (for men aged >79), and other physical activity if METs were >5.9 for men aged 60–64, >4.7 for men aged 65–79, or >2.9 for men aged >79 [15]. The waist circumference of the participants was measured during the physical examination. Men were considered to have a history of hypertension if they currently used medication to treat hypertension or if they were told by their doctor on two occasions that they had hypertension/high blood pressure.

The results were analysed statistically using SAS v8.1 (SAS Institute, Cary, NC) and SUDAAN [16] software. We used sample weights that took into account several features of the NHANES III survey, i.e. the specific probabilities of selection for the individual domains that were over-sampled as well as non-response and differences between the sample and the total USA population [12]. Logistic regression was used to calculate the odds ratio (OR) and 95% CI of LUTS for cigarette smoking, alcohol consumption and physical activity. In the logistic regression models, we adjusted for age (5-year categories) and race (non-Hispanic black, non-Hispanic white, Mexican-American, other). We further included in the models the waist circumference (continuous variable) as a possible confounder, and mutually adjusted cigarette smoking, alcohol consumption and physical activity. Trends for alcohol consumption and physical activity were tested by assigning to each man the median value for the exposure category into which he fell and modelling this term as a continuous variable, the coefficient for which was evaluated by the Wald test.

RESULTS

Of the 2797 men in the analysis, 28.8% had no LUTS and had never had prostate surgery (controls), 46.7% reported one or two symptoms and 10.3% reported three or four symptoms (cases). Men with LUTS were older than men in the control group and had fewer years of education (Table 1). These men also drank less alcohol, but smoking patterns and weekly frequency of physical activity did not differ.

Men who currently smoked up to 35 cigarettes/day had no greater odds of LUTS, but there was insignificantly greater odds of LUTS in men who smoked ≥35 cigarettes/day (Table 2). However, this association was strongly attenuated after adjusting for waist circumference, the frequency of alcohol consumption, and the frequency of moderate and vigorous activity. Of these factors, waist circumference caused the strongest attenuation of the OR for heavy smoking. Former smokers had a slightly but not statistically significant greater odds of LUTS than never smokers. Men who had ever smoked ≥50 pack-years had a higher odds of LUTS than never smokers. This association was limited to former smokers; there was no association among current smokers who had smoked ≥50 pack-years. Further adjustment for waist circumference, but not for hypertension, attenuated the association of pack-years with LUTS in both ever and former smokers.

There was an inverse association between the frequency of alcohol consumption and LUTS in this group of older men (Table 2). Compared to non-drinkers, men who drank alcohol daily had a significantly lower odds of LUTS. This association was not substantially altered after
TABLE 1 Age-adjusted baseline characteristics of men aged ≥60 years in the NHANES III, 1988–94

<table>
<thead>
<tr>
<th>Factor</th>
<th>Controls</th>
<th>Cases</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unweighted sample size</td>
<td>715</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>% of total sample</td>
<td>28.8</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>67.6 (0.3)</td>
<td>71.0 (0.6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Current waist size, cm</td>
<td>100.4 (0.68)</td>
<td>101.2 (0.65)</td>
<td>0.46*</td>
</tr>
<tr>
<td>Years of education</td>
<td>11.3 (0.2)</td>
<td>10.4 (0.4)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Smoking habits, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>28.7</td>
<td>23.6</td>
<td>0.21†</td>
</tr>
<tr>
<td>Former</td>
<td>48.3</td>
<td>57.5</td>
<td></td>
</tr>
<tr>
<td>Current (1–34/day)</td>
<td>19.0</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Current (≥35/day)</td>
<td>4.1</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency†, n/month</td>
<td>15.6 (1.89)</td>
<td>9.5 (1.25)</td>
<td>0.02*</td>
</tr>
<tr>
<td>median</td>
<td>0.85</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Intake, g/day</td>
<td>11.1 (1.19)</td>
<td>7.0 (1.60)</td>
<td>0.02*</td>
</tr>
<tr>
<td>median</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>85.9</td>
<td>86.2</td>
<td>0.17†</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>6.9</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Mexican-American</td>
<td>1.9</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>5.5</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Physical activity¶, mean (SEM) times/week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate + vigorous</td>
<td>6.90 (0.35)</td>
<td>5.97 (0.63)</td>
<td>0.24*</td>
</tr>
<tr>
<td>median</td>
<td>5.23</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Vigorous</td>
<td>3.58 (0.26)</td>
<td>3.18 (0.35)</td>
<td>0.46*</td>
</tr>
<tr>
<td>median</td>
<td>0.42</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

All percentages and means are calculated using sampling weights; adjusted for age; † χ²-test; ‡ t-test; † χ²-test; tassessed by food frequency questionnaire during the household interview; ¶ assessed by 24-h recall during the physical examination (see text).

FIG. 1. Age- and race-adjusted OR of LUTS by walking (miles/week) in men aged ≥60 years in the NHANES III, 1988–94.

![Graph showing OR and 95% CI for walking in men aged ≥60 years in the NHANES III, 1988–94.]

Men who reported no leisure-time physical activity had a significantly higher odds of LUTS (OR = 2.09, 95% CI 1.14–12.2, P trend 0.08) than men who reported some physical activity, and adjusting for smoking, alcohol intake and waist circumference did not change this association. All levels of moderate or vigorous physical activity were also associated with a significantly lower odds of LUTS than men who reported no moderate or physical activity (Table 2). These results did not change after further adjusting for history of hypertension or for the presence of the metabolic syndrome (data not shown). However, vigorous physical activity alone was not consistently inversely associated with LUTS. The most frequently reported activity was walking. Fewer men with LUTS than men without reported walking (33.2% vs 50.8%, P = 0.003). Men who reported walking had a lower odds of LUTS than men who did not, although the OR did not decrease monotonically (Fig. 1). Adjusting for total frequency of moderate and vigorous physical activity as well as waist circumference, smoking and alcohol drinking did not change the association for walking.

DISCUSSION

In this group of older men in the USA, alcohol consumption and physical activity (moderate and vigorous) were both inversely associated with LUTS. Men who walked regularly were less likely to have LUTS than men who did not. There was no association between current cigarette smoking and LUTS, but we could not exclude that former heavy smokers were more likely to have LUTS.

Several studies have examined the association between cigarette smoking and LUTS, with inconsistent results. Most studies found no statistically significant association between cigarette smoking and LUTS (3–8), whereas three studies reported a statistically significantly positive association (9–11). In an analysis of the Health Professionals Follow-up Study (11), heavy smokers had a significantly higher risk of LUTS than never smokers, whereas moderate smokers did not. Similarly, in NHANES III, there was no association for current cigarette smoking, but a suggestion of a higher occurrence of LUTS in heavier current, lifetime and former smokers.

There may be several explanations for LUTS possibly being more common in long-term heavy smokers. Nicotine increases sympathetic nervous system activity (17) and might contribute to LUTS via an increase in the tone of the prostate and bladder smooth muscle. Furthermore, smoking is thought to be associated with higher concentrations of testosterone (18). A higher testosterone concentration might be associated with higher intraprostatic dihydrotestosterone levels, which is thought to be important in the development of BPH and LUTS (19).

There was a slightly greater chance of LUTS in former smokers and these men also had a higher occurrence of LUTS in heavier current, lifetime and former smokers. The reasons for a greater risk of LUTS in former smokers are not clear. The greater risk of LUTS in former smokers is thought to be associated with higher concentrations of testosterone (18). A higher testosterone concentration might be associated with higher intraprostatic dihydrotestosterone levels, which is thought to be important in the development of BPH and LUTS (19).

There was a slightly greater chance of LUTS in former smokers and these men also had a higher occurrence of LUTS in heavier current, lifetime and former smokers. The reasons for a greater risk of LUTS in former smokers are not clear. The greater risk of LUTS in former smokers is thought to be associated with higher concentrations of testosterone (18). A higher testosterone concentration might be associated with higher intraprostatic dihydrotestosterone levels, which is thought to be important in the development of BPH and LUTS (19).
symptoms might be more likely to stop smoking than men without symptoms. In addition, we cannot exclude chance as an explanation for this finding.

Men who frequently consumed alcohol were less likely to have LUTS than men who did not. There was also a lower odds of LUTS with increasing daily alcohol intake when using a second dietary assessment tool that captured intake the day before the interview. These results support the findings of two other studies reporting negative associations between alcohol consumption and LUTS [4,11], whereas the association was positive in two others [3,7]. Platz et al. [11] reported lower odds in moderate drinkers, but this protective effect was attenuated in men who consumed >50 g alcohol/day (= 3.5 or more drinks per day). This pattern was also apparent in another USA cohort study [9], in which African-American men with an intake of >72 g/day [five or more drinks per day] had a significantly higher odds of LUTS than non-drinkers, whereas there was no association in moderate consumers. Light to moderate alcohol consumption is associated with improved insulin sensitivity [21] and decreased testosterone concentration [22]. As an alternative explanation, we cannot exclude that the observed inverse association between the frequency of alcohol consumption and LUTS is caused by avoidance of fluids, especially of alcoholic beverages that have a diuretic effect, by men with LUTS, as there was an insignificantly lower odds of LUTS in men who drank caffeinated beverages at least four times a week (data not shown).

In NHANES III, men who were physically active in their leisure time were less likely to have LUTS. All levels of moderate and vigorous activity were inversely associated with LUTS, but the association for vigorous activity did not decrease consistently. Two previous studies reported inverse associations between the frequency of physical activity and LUTS [2,3].

Physical activity is associated with improved insulin sensitivity [23]. We previously reported statistically significant positive associations of glycosylated haemoglobin, a long-term marker of glucose and insulin metabolism, and the metabolic syndrome with LUTS in this group of men [24]. Alternatively, reductions in the odds of LUTS by physical activity might be caused by changes of sympathetic nervous system activity. Aerobic exercise training may elicit adaptations in the adrenergic system, because the sympathetic nervous system is activated through each bout of exercise, and repeated activation of this system could result in a reduction of the resting system activity [25]. In contrast to Platz et al. [2] there was no consistently inverse association between vigorous physical activity and LUTS in the

**TABLE 2 The OR of LUTS by current smoking status, pack-years of smoking, alcohol consumption and physical exercise**

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR (95% CI)*†</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoking status‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Former</td>
<td>1.46 (0.88–2.40)</td>
<td>1.37 (0.79–2.36)</td>
</tr>
<tr>
<td>Current, cigarettes/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–34</td>
<td>0.84 (0.46–1.54)</td>
<td>0.78 (0.39–1.56)</td>
</tr>
<tr>
<td>≥35</td>
<td>1.83 (0.74–4.53)</td>
<td>0.75 (0.31–1.82)</td>
</tr>
<tr>
<td>Pack-years of cigarette smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>&lt;21</td>
<td>1.27 (0.78–2.04)</td>
<td>1.22 (0.68–2.19)</td>
</tr>
<tr>
<td>21–49.9</td>
<td>1.10 (0.66–1.83)</td>
<td>1.22 (0.68–2.19)</td>
</tr>
<tr>
<td>≥50</td>
<td>1.72 (0.99–2.99)</td>
<td>1.43 (0.80–2.59)</td>
</tr>
<tr>
<td>Current smokers only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>&lt;21</td>
<td>0.52 (0.14–1.91)</td>
<td>0.64 (0.13–3.06)</td>
</tr>
<tr>
<td>21–49.9</td>
<td>1.08 (0.50–2.32)</td>
<td>0.78 (0.25–2.44)</td>
</tr>
<tr>
<td>≥50</td>
<td>1.19 (0.55–2.56)</td>
<td>1.08 (0.44–2.65)</td>
</tr>
<tr>
<td>Former smokers only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>&lt;21</td>
<td>1.34 (0.82–2.09)</td>
<td>1.21 (0.66–2.25)</td>
</tr>
<tr>
<td>21–49.9</td>
<td>1.07 (0.59–1.94)</td>
<td>1.22 (0.64–2.31)</td>
</tr>
<tr>
<td>≥50</td>
<td>2.16 (1.12–4.17)</td>
<td>1.91 (0.97–3.78)</td>
</tr>
<tr>
<td>Alcohol consumption, frequency§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>&lt;1/week</td>
<td>0.60 (0.33–1.09)</td>
<td>0.53 (0.24–1.18)</td>
</tr>
<tr>
<td>&gt;1/week but &lt;1/day</td>
<td>0.74 (0.37–1.45)</td>
<td>0.99 (0.47–2.08)</td>
</tr>
<tr>
<td>≥1/day</td>
<td>0.59 (0.36–0.97)</td>
<td>0.59 (0.34–1.03)</td>
</tr>
<tr>
<td>Frequency of physical activity, times/week¶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>0.1–3.0</td>
<td>0.48 (0.24–0.99)</td>
<td>0.32 (0.14–0.74)</td>
</tr>
<tr>
<td>3.1–6.0</td>
<td>0.41 (0.18–0.91)</td>
<td>0.23 (0.09–0.57)</td>
</tr>
<tr>
<td>&gt;6.0</td>
<td>0.49 (0.29–0.84)</td>
<td>0.35 (0.18–0.67)</td>
</tr>
<tr>
<td>P trend</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Vigorous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>0.1–2.0</td>
<td>0.52 (0.25–1.10)</td>
<td>0.36 (0.15–0.87)</td>
</tr>
<tr>
<td>2.1–4.0</td>
<td>0.85 (0.40–1.82)</td>
<td>0.78 (0.32–1.88)</td>
</tr>
<tr>
<td>≥4.0</td>
<td>0.80 (0.46–1.40)</td>
<td>0.77 (0.37–1.60)</td>
</tr>
<tr>
<td>P trend</td>
<td>0.88</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*All results were calculated using sampling weights; †adjusted for age and race; ¶second column: smoking status: adjusted for age, race, frequency of moderate and vigorous physical activity, frequency of alcohol consumption and current waist circumference (continuous); ‡second column: alcohol consumption: adjusted for age, race, frequency of moderate and vigorous physical activity, smoking status and current waist circumference (continuous); §second column: physical activity: adjusted for age, race, frequency of alcohol consumption, smoking status and current waist circumference (continuous).
present study. Only men who reported vigorous activity up to twice a week had a statistically significantly lower odds of LUTS, but the association was weaker in men who were more vigorously active. However, in this general population, few men reported participating in vigorous physical activity more than twice a week.

In addition to an inverse association between total moderate and vigorous activity, men who walked, the most often reported physical activity in this group of older men, were less likely to have LUTS. This association was reported previously in the Health Professionals Follow-up Study [2]. A small case-control study in Japan [26] reported that walking 10 000 steps or more per day for 12 weeks was inversely associated with sympathetic nervous activity and blood pressure in hypertensive men compared with sedentary men. Therefore, men who walk regularly might be less likely to have LUTS because of the lower tone of the prostate and bladder smooth muscle, and lower blood pressure, previously been shown to be positively associated with LUTS [9,20,24].

Several aspects of the study design merit further discussion. First, NHANES III is a cross-sectional study representative of the USA population of older men, thus aiding in the broad general applicability of these results. Also, the elderly were over-sampled, allowing for more stable estimates in the analysis of older men. Second, the questions on LUTS in NHANES III covered four of the seven questions of the AUA Symptom Index, which also includes frequency, intermittency and urgency, which together discriminated between men with and with no BPH in a clinical setting [13]. To increase the specificity of the present analysis, we included only men with three or four symptoms in the case group; we exclude men with only one or two symptoms in the control or case group because individually these symptoms are not specific for LUTS. Third, we cannot completely exclude that some men in the control group did not report LUTS because they were taking medications to treat their symptoms. However, this is unlikely because NHANES III was conducted between 1988 and 1994, and medication for treating BPH symptoms was not approved until 1992 (finasteride) [27] and 1993 (terazosin) [28]. Finally, smoking, alcohol consumption and physical activity were assessed concurrently with LUTS. Therefore, the results reflect associations and are not necessarily causal.

In conclusion, physical activity, even moderate activity like walking, may be beneficial for LUTS. Additionally, moderate alcohol consumption might be associated with a reduction in the occurrence of LUTS, whereas heavy cigarette smoking in the past may increase the occurrence of LUTS in older men. Intervention studies are needed to determine whether the frequency of LUTS can be modulated by changes in these lifestyle factors.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

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Abbreviations: NHANES III, Third National Health and Nutrition Examination Survey; MET, metabolic equivalent of the activity compared to at rest; OR, odds ratio.
Relevance and variability of the severity of incontinence, and increased daytime and night-time voiding frequency, associated with quality of life in men with lower urinary tract symptoms

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OBJECTIVES
To estimate the distribution of the severity of urinary incontinence (UI) and daytime and night-time voiding in patients with lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH); to estimate the proportion of ‘subjectively relevant’ symptoms within each severity category; to identify differences in quality of life (QoL) by degree of subjectively relevant daytime and night-time symptoms; and to identify differences in QoL in men with subjectively relevant UI or no UI.

PATIENTS AND METHODS
Data from a group of 480 men awaiting urological assessment for LUTS suggestive of BPH were collected by questionnaire shortly after referral from their general practitioner in 1997–2000. The International Continence Society – Benign Prostatic Hyperplasia Index, Sandvik’s Incontinence Severity Index, and the World Health Organization Quality of Life Survey – Abbreviated Version (WHOQoL-bref) were used to assess symptoms and QoL.

RESULTS
There was a large heterogeneity of self-reported symptom severity and related bother in the three symptoms of UI, increased daytime voiding frequency and night-time voiding in these referred patients. The WHOQoL-bref showed significant group differences of subjectively relevant symptoms.

CONCLUSION
The perception of increased night and daytime frequency, as measured by symptom severity and bother, varied greatly. The severity of UI and its effect on men waiting for a urological assessment of LUTS suggestive of BPH also varied widely. In general, the symptoms and their impact were slight to moderate. The WHOQoL-bref could be used to differentiate among groups of subjectively relevant symptoms, and in so doing supported information generated by the bother question.

KEYWORDS
LUTS, BPH, quality of life, storage, urinary incontinence, subjective relevance, bother

INTRODUCTION
Urinary incontinence (UI) and increased daytime and night-time voiding frequency are often regarded as the most bothersome LUTS [1–5]. Bothe reflects men’s overall distress with having LUTS [6]. Subjectively relevant symptoms must be distinguished from healthy functioning. For UI and increased daytime and night-time voiding frequency, this differentiation may be achieved by estimating the bother associated with leakage and the number of voids during day or night.

Generally, it seems that LUTS suggestive of BPH do not greatly impair quality of life (QoL) [7,8]; there are few reports on the association between the subjective relevance of storage symptoms and QoL, e.g. to date, the QoL questionnaire developed by the WHO (brief version, WHOQoL) has not been used to investigate differences in QoL in patients bothered by LUTS suggestive of BPH. However, two studies investigated the association between the ‘bothersomeness’ of LUTS and well-being/health status, concepts closely related to QoL; the correlations were high in these studies [9,10].

The purpose of the present study was to estimate the distribution of the severity of UI, and daytime and night-time voiding in patients with LUTS suggestive of BPH, and to estimate the proportion of subjectively relevant symptoms within each severity category. Further, we identified differences in QoL by the degree of subjectively relevant daytime and night-time symptoms, and differences in QoL in men with subjectively relevant UI and men with no UI.

PATIENTS AND METHODS
Men waiting for a urological evaluation at Trondheim University Hospital, Norway, in 1997–2000 were eligible for the study. Based on an overall assessment of his GP’s description of the patient in the referral letter, the urologist made the tentative diagnosis of BPH for 612 referred patients, who were subsequently enrolled in the study. The eligible patients were sent information about the waiting list situation, a request to participate in the study and a questionnaire. Patients agreed to participate by returning the questionnaire and a signed consent form in a pre-stamped envelope; one reminder was sent. Ethics approval was obtained from the regional ethics review board.

The ICS has defined UI as the complaint of any involuntary leakage of urine [1]. Increased daytime frequency is defined as the complaint by the patient that he/she voids too often by day [1]; night-time frequency is defined as voids that occur from the time the individual wakes with the intention to sleep, to the time the individual wakes with the intention of rising [1,11,12].
Symptoms were defined as 'subjectively relevant' when the patient regarded the symptom as at least 'quite a problem' for daytime and nighttime voiding. This group was divided into two categories, i.e. 'some bother' and 'much/major bother'. When the patient associated the UI with at least 'some bother', it was defined as subjectively relevant. This group was divided into two further categories, i.e. 'a bit of a problem' and 'quite a problem/a serious problem'. Men reporting no UI, or reporting the impact of their UI as 'no problem/a small nuisance', were defined as having no subjectively relevant symptom.

The following indices were chosen as outcome variables: Sandvik's Incontinence Severity Index, the ICS–BPH and a five-category impact scale. The Sandvik index was used to characterize the degree of UI [13] (Table 1). Men who stated the presence of UI or who gave answers about frequency, amount and type of leakage were considered to have incontinence.

This index was validated against a 48-h pad-weighting test in women, according to which slight, moderate, severe and very severe incontinence represent mean (95% CI) leakages (g/24 h) of 6 (2–9), 23 (15–30), 52 (38–65) and 122 (84–159), respectively [13]. Hanley et al. [14] reported good test-retest reliability and ability to detect change after treatment using the index. Cronbach’s $\alpha$ was not reported in the validation studies; the present study had a Cronbach’s $\alpha$ of 0.83. The impact of UI was measured by a five-category scale, i.e. no problem, a small nuisance, some bother, much bother and a major problem. Missing responses were not replaced.

The ICS-BPH is a 34-item questionnaire examining symptoms relating to lifestyle and sexual function, including five questions on incontinence incidents, flow rate, and two open-ended questions. Twenty-six (score range 0–4) of the 34 items are immediately followed by a corresponding bother issue (score range 0–3) [15,16]. Two items addressing increased daytime and nighttime frequency and their corresponding bother issue were assessed in this study, i.e. the time interval between voiding (item 1) and nighttime frequency (item 2).

Validation studies showed differentiation between community and clinical samples, but a poor relation between questions assessing strength of stream and uroflowmetry [15]. Excellent test-retest reliability was reported and Cronbach’s $\alpha$ is 0.69–0.85 for the symptom and bother subscales [15]. Lifestyle items are ‘fairly’ related to the Short-Form Health Survey (SF-36) and have a Cronbach’s $\alpha$ of 0.59 [16]. In the present study the Cronbach’s $\alpha$ was 0.41 for this subscale. Missing responses were not replaced. The validity and reliability of the Norwegian version of ICS–BPH have not been published.

The 26-item WHOQoL-bref is based on a definition of QoL as the individuals’ perception of their position in life in the context of the cultural and value systems in which they live.
examined separately, i.e. overall perception of QoL and overall perception of health.

Where >20% of data were missing from an individual assessment, that assessment was excluded from the analysis. Where the response to an item was missing, the mean value of the other items in the same domain was substituted. Each domain had a range of 4–20, with the higher scores indicating better QoL. The scores for the two single items were 1–5, with the higher scores indicating a better QoL or better health [17]. The psychometric properties of the Norwegian version of WHOQoL-bref were tested recently [18].

Frequency analyses and simple distributions were obtained to estimate the symptom severity distribution and the distribution of subjectively relevant symptoms within each severity category. ANOVA was used to assess differences of mean QoL scores in the groups of subjectively relevant symptoms.

RESULTS

Of the 612 questionnaires, 480 (78%) were returned and analysed. The mean (SD, median, range) age of the patients was 67.0 (10.6, 69, 39–91) years. Involuntary leakage was reported by 176 patients (37%); most with UI had moderate or slight leakage. The proportion of men with subjectively relevant UI increased markedly with increasing severity. When the men with no subjectively relevant symptoms were excluded, 20% of men reporting one night-time void reported that this was a serious problem or ‘quite a problem’. This proportion increased to >80% for patients reporting four or more voids per night (Fig. 1c).

Between the categories of subjectively relevant UI and no UI there were significantly different mean scores of QoL among all three groups in the physical dimension (Table 1). For the other domains there were differences in mean QoL between several of the domains, but not in overall health.

For daytime voiding frequency, the men with subjectively relevant symptoms differed in mean QoL from those who regarded their number of daytime voids as no problem, or ‘a bit of a problem’. In the social relationships domain, there was no difference in overall QoL, whereas for the physical domain there were differences among all three categories of the condition (Table 1). There was a similar tendency in the groups of subjectively relevant night-time frequency (Table 1).

DISCUSSION

This study shows the distribution of self-reported symptom severity and related bother in UI, increased daytime voiding frequency, and night-time voiding in a sample of patients with LUTS suggestive of BPH. All patients had been referred by their GP to a urologist for evaluation. Despite the GPs having first evaluated and then referred the patients, the self-reported symptom severity and degree of bother varied widely.

Peters et al. [19] stated that symptom occurrence alone does not necessarily reflect the degree of bother caused by LUTS, so it is also important to consider the bother caused by the symptoms. Berges et al. [20] suggested that the bother level may discriminate between individuals with moderate symptoms who may be followed by ‘watchful waiting’ and those in need of active therapy. Perry et al. [21] referred to the group with significant incontinence as potential patients, and Boyle et al. [22] found that bother associated with UI was a more important
determinant of visiting the doctor than the level of UI symptoms. In the present study, most patients referred by their GP for a urological evaluation did not have severe UI, or increased daytime or night-time voiding frequency. Furthermore, they were not particularly bothered by their symptoms, even though the three symptoms measured are regarded as the most bothersome. More studies are needed to investigate which men with subjectively relevant symptoms are potential patients in need of active treatment.

When assessing night-time voids, only 3% of patients reported them as a serious problem, and 28% as ‘quite a problem’. The small proportion of men bothered by night-time voids might reflect that we not only registered the number of times the patient woke to void, but also the number of voids before sleep and after waking. Nevertheless, we hesitate to conclude that to get up twice or more per night is extremely bothersome to patients as other studies have and thus must be considered symptomatic [23,24]. The ICS committee concluded that the number of voids per night is not important for definition purposes, as long as the patient is awake before voiding and they return to sleep afterwards [12]. The ICS committee defines nocturia as waking at night to void. It is problematic that this definition does not indicate when nocturia becomes bothersome [24].

For the three symptoms a few men found each a serious or major problem. The mean QoL decreased with increasing bother but the differences between the groups were very small, so the clinical relevance of the conclusion that this definition does not indicate when nocturia becomes bothersome [24].

In conclusion, what is regarded as increased night- and daytime frequency among patients referred for urological evaluation of LUTS suggestive of BPH seems to vary greatly, as measured by self-reported QoL, symptom severity and bother. Further, the severity of UI and its impact among patients referred for a urological evaluation varies widely, with only a few patients considering it a major problem. The WHOQoL-bref could be used to differentiate between groups of subjectively relevant storage symptoms, and in doing so supported the information generated by the bother question.

CONFLICT OF INTEREST

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Abbreviations: UI, urinary incontinence; QoL, quality of life.
The prevalence and correlates of urinary tract symptoms in Norwegian men: The HUNT Study

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OBJECTIVES
To estimate the prevalence of lower urinary tract symptoms (LUTS) by severity (using the International Prostate Symptom Score, IPSS) in a population-based study of men aged ≥20 years, and to assess the association between putative risk factors and the presence of moderate to severe LUTS.

SUBJECTS AND METHODS
Between 1995 and 1997, LUTS data were collected from 21,694 male residents aged ≥20 years in Nord Trøndelag County in Norway, using the IPSS; from the IPSS (score 0–35) LUTS was defined as a score of ≥8, indicating moderate to severe symptoms. We estimated the prevalence of LUTS and used logistic regression analysis to study lifestyle and anthropometric factors, and comorbidity related to LUTS.

RESULTS
The overall prevalence of moderate to severe LUTS was 15.8% (13.2% moderate and 2.6% severe). The prevalence of LUTS increased strongly with age, from ~5% among men aged <40 years to >30% when aged ≥70 years. Factors positively associated with an increased risk of moderate and severe LUTS were anthropometric (body mass index and waist hip ratio) and lifestyle factors (alcohol consumption and smoking), as well as comorbid conditions, including diabetes, history of stroke, muscle complaints and osteoarthritis.

CONCLUSION
The findings from this population-based study suggest that the prevalence of LUTS among men aged ≥20 years may be lower than previously estimated. Although LUTS may be viewed as an inevitable consequence of ageing, it appears to be exacerbated by lifestyle factors and comorbid conditions.

KEYWORDS
LUTS, prevalence, men, Norway, comorbidity, lifestyle

INTRODUCTION
LUTS can cause personal suffering and reduced quality of life for many men [1–5]; in the USA, ~380,000 TURPs are carried out annually to alleviate LUTS, and the procedure is also common in other western societies. However, the reported prevalence of LUTS varies considerably among studies. This can partly be explained by the lack of consensus on a common definition of LUTS; e.g. some authors [6,7] restrict the diagnosis of LUTS to patients with severe symptoms, whereas others have used a broader definition.

Recently, the definition of LUTS was standardized and the IPSS has now been widely adopted [8,9]. Nonetheless, studies that have used the IPSS have also shown substantial variation in the prevalence of LUTS. In one study comparing the community prevalence of LUTS among four countries, the proportion of men who reported moderate to severe symptoms was 14%, 18%, 38% and 56% in France, Scotland, Olmsted County (USA) and Japan, respectively [10]. A recent study showed less variation in prevalence (16–25%) in the Netherlands, France, UK and Korea [11], but other community studies reported different prevalence estimates among populations [12,13].

Despite the evidence that LUTS represent a burden, especially among elderly men, little is known about their causes [14]. Apart from the positive association with age, high levels of circulating androgens appear to increase the risk of LUTS, possibly via its association with benign prostate enlargement [9,14–16]. LUTS also appear to be negatively associated with socio-economic status and positively associated with obesity [14,17]. Other lifestyle factors, e.g. cigarette smoking, alcohol consumption and consumption of coffee or tea, may also be positively associated with LUTS [14,17,18], whereas physical activity may be inversely related to the symptoms [19,20]. However, the results of studies are conflicting [14,17–21]. LUTS also tend to occur in conjunction with other age-related conditions. Thus, studies have shown that LUTS may be present together with cardiovascular disease, diabetes and metabolic syndrome, neurological disorders, and rheumatic diseases [14,19,22].

The purpose of the present study was to estimate the prevalence of LUTS by severity (using the IPSS) in a population-based study of men aged ≥20 years, and to assess the association between putative risk factors and the presence of moderate to severe LUTS.

SUBJECTS AND METHODS
Data were derived from the second Nord-Trøndelag Health Study (HUNT-2), a population–based study of residents in Nord-Trøndelag County in Norway who were aged ≥20 years between 1995 and 1997. The HUNT study was conducted as a collaboration between the National Health Screening Service, the National Institute of Public Health, and the Norwegian University of Science and Technology, and has been described in detail elsewhere [23,24]. Briefly, participants were asked to complete a baseline questionnaire, which was mailed together with the invitation to attend a physical examination. The examination...
information on age, height, weight, waist and
recommendations made by the developers of severe symptoms (20–35), according to (IPSS 0), mild (1–7), moderate (8–19) or according to total score as no symptoms of 0–35. In the analysis, we categorized LUTS the sum of the seven items, with a total score (items). Each item has response categories of responded to the seven symptom questions of the University Medical Center.

All participants signed a consent form that included information about the study objectives. The study was approved by the regional committee for ethics in medical research, by the Norwegian Data Inspectorate, and by the Institutional Review Board of Duke University Medical Center.

The IPSS was calculated for all men who responded to the seven symptom questions (items). Each item has response categories of 0 (not at all) to 5 (almost always); the IPSS is the sum of the seven items, with a total score of 0–35. In the analysis, we categorized LUTS according to total score as no symptoms (IPSS 0), mild (1–7), moderate (8–19) or severe symptoms (20–35), according to recommendations made by the developers of the IPSS [8].

From the general health survey we also used information on age, height, weight, waist and hip circumference, cigarette smoking, consumption of alcohol, coffee or tea, and information on comorbidity, including diabetes, muscular complaints, osteoarthritis, and history of stroke or coronary heart disease.

The overall prevalence of LUTS was calculated by clinical severity in 10-year age groups, and related to factors previously associated with LUTS. We used logistic regression analysis to estimate the association between the independent variables and the presence of LUTS, by comparing men who reported moderate to severe LUTS with men who reported mild or no LUTS. The associations are presented as odds ratios (OR, corrected for age) with 95% CIs.

### RESULTS

Among the 21 694 men, 15.8% reported moderate to severe LUTS (13.2% moderate and 2.6% severe; Table 1); the prevalence of LUTS increased rapidly with age, such that >95% of men aged <40 years reported no LUTS, whereas of men aged 60–69 years, 26.3% had LUTS. The prevalence increased gradually from hardly any LUTS in the younger groups to more than a third with moderate to severe LUTS. Among men aged ≥70 years, the prevalence of LUTS was very rare, but one in five men aged ≥70 years reported moderate to severe LUTS. Among men aged ≥70 years reported moderate to severe LUTS was 16%, but it increased rapidly with age. Before the age of 40 years LUTS was very rare, but one in five men aged ≥40 years reported moderate to severe LUTS. Among men aged ≥70 years reported a slightly higher prevalence of LUTS among those with a higher WHR, but with no clear trend across categories (quintiles) of WHR.

Several lifestyle factors were also associated with the risk of LUTS. Men who reported having 6–10 drinks (of beer, wine or liquor) per week were more likely (OR 1.41, 95% CI 1.19–1.66) to report LUTS than men who abstained from alcohol (Table 2). Comparing current and former smokers to never-smokers, cigarette smoking was associated with a higher prevalence of LUTS. Among former smokers the results indicated a dose-response relationship between the number of cigarettes and prevalence of LUTS. Men who had smoked >20 cigarettes/day were ~50% more likely to have LUTS than men who had never smoked. Among current smokers the pattern was somewhat different; smoking >16 cigarettes/day was positively associated with LUTS. Although not significantly, smoking <16 cigarettes/day seemed to be associated with a lower prevalence of LUTS. Consumption of coffee or tea also showed weak positive associations with LUTS.

Men with comorbid conditions were also more likely to report LUTS (Table 2); men with diabetes were more likely to have LUTS than men without. There was a similar association between muscle complaints and LUTS, and between osteoarthritis and LUTS. Men with a history of stroke also had higher prevalence of LUTS.

### DISCUSSION

In this population-based study of nearly 22 000 Norwegian men aged ≥20 years, where the IPSS was used to assess the presence of LUTS, the total prevalence of moderate to severe LUTS was 16%, but it increased rapidly with age. Before the age of 40 years LUTS was very rare, but one in five men aged ≥40 years reported moderate to severe LUTS. Among men aged ≥70 years reported a slightly higher prevalence of LUTS among those with a higher WHR, but with no clear trend across categories (quintiles) of WHR.

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### TABLE 1

<table>
<thead>
<tr>
<th>Age group (n)</th>
<th>% within each IPSS category</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPSS</td>
<td>N</td>
<td>0</td>
<td>1–7</td>
<td>8–19</td>
<td>20–35</td>
</tr>
<tr>
<td>20–29 (2414)</td>
<td>6406</td>
<td>11</td>
<td>855</td>
<td>2865</td>
<td>568</td>
</tr>
<tr>
<td>30–39 (3515)</td>
<td>43.6</td>
<td>50.4</td>
<td>5.4</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>40–49 (5067)</td>
<td>36.7</td>
<td>53.0</td>
<td>8.9</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>50–59 (4192)</td>
<td>24.0</td>
<td>58.5</td>
<td>14.6</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>60–69 (3260)</td>
<td>15.7</td>
<td>58.0</td>
<td>21.6</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>70–79 (2570)</td>
<td>12.7</td>
<td>57.8</td>
<td>23.6</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>80–89 (640)</td>
<td>10.0</td>
<td>52.0</td>
<td>30.8</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>≥90 (36)</td>
<td>11.1</td>
<td>41.7</td>
<td>33.3</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Total (21694)</td>
<td>29.5</td>
<td>54.6</td>
<td>13.2</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>

The proportion of LUTS as measured by the IPSS in different age groups.
the present study [11]. These differences suggest that the prevalence of LUTS may have geographical or racial variations, but the results also suggest that men in different cultures may have different thresholds for discomfort before reporting the presence of urinary symptoms.

Previously, some investigators reported a negative association between cigarette smoking and LUTS [19,20,28,29], but others found a positive association [7,14,17,30]. In the present study there was a positive association between cigarette smoking and LUTS among former smokers. A possible negative association between light or moderate smoking and LUTS among current smokers has been shown by others [31]. Previous studies also reported discrepant findings for the consumption of coffee or tea and related LUTS [18,32]. Because coffee and tea are ubiquitous exposures, the potential for public health intervention can be sizeable if the association is causal. However, there was no clear association with tea or coffee consumption in the present men.

Men with comorbid conditions were also more likely to report LUTS. Possibly, these conditions may have a general impact on lower urinary tract function, or the medical treatment of the conditions may influence lower urinary tract function or urine excretion. Thus, men with diabetes were more likely to report LUTS than men without diabetes, suggesting that the pathogenesis may either be vascular or neurological. Patients whose diabetes is not optimally treated will tend to have glucosuria, and thereby be more likely to report urinary symptoms. Similarly, men who have muscle complaints may experience pain that could possibly influence smooth muscle function in the urinary system through neurological mechanisms. However, pain-relieving medication per se has been suggested to increase the risk of LUTS [22]. A positive association between osteoarthritis and LUTS was also reported by others [22].

In conclusion, ≈20% of men aged ≥40 years reported moderate to severe LUTS; in men aged ≥70 years about a third reported these symptoms. Although LUTS may be viewed as an inevitable consequence of ageing, our results also suggest that LUTS are associated with lifestyle factors, and may be exacerbated by comorbid conditions.

### TABLE 2

| Variable                          | No. of men with IPSS | OR (95% CI) |* |
|-----------------------------------|----------------------|-------------|
| **Age**                           |                      |             |
| 20–29                             | 2,316                | 1.0 (Reference) |
| 30–39                             | 3,306                | 1.49 (1.16–1.90) |
| 40–49                             | 4,547                | 2.69 (2.16–3.38) |
| 50–59                             | 3,459                | 4.99 (4.01–6.19) |
| 60–69                             | 2,404                | 8.38 (6.75–10.4) |
| 70–79                             | 1,813                | 9.83 (7.89–12.2) |
| 80–89                             | 397                  | 14.4 (11.1–18.6) |
| ≥90                               | 19                   | 21.1 (10.6–41.8) |
| **BMI, kg/m**^2                   |                      |             |
| <25                               | 6,526                | 1.0 (Reference) |
| 25–29                             | 9,156                | 1.13 (1.04–1.23) |
| 30–34                             | 2,206                | 1.20 (1.06–1.35) |
| 35–39                             | 286                  | 1.39 (1.04–1.85) |
| ≥40                               | 45                   | 1.79 (0.90–3.56) |
| **WHR (quintiles)**               |                      |             |
| <0.851                            | 3,940                | 1.0 (Reference) |
| 0.851–0.881                       | 3,683                | 1.20 (1.05–1.38) |
| 0.882–0.906                       | 3,663                | 1.22 (1.07–1.39) |
| 0.907–0.942                       | 3,642                | 1.11 (0.97–1.26) |
| ≥0.943                            | 3,273                | 1.32 (1.15–1.50) |
| **Height, cm (quintiles)**        |                      |             |
| <173                              | 4,116                | 1.0 (Reference) |
| 173–176                           | 3,973                | 1.10 (0.99–1.23) |
| 177–179                           | 3,070                | 1.15 (1.02–1.29) |
| 180–183                           | 3,641                | 1.11 (0.99–1.26) |
| ≥184                              | 3,419                | 1.04 (0.90–1.18) |
| **Lifestyle**                     |                      |             |
| **Alcohol, units/week**           |                      |             |
| 0                                 | 3,007                | 1.0 (Reference) |
| 1–5                               | 10,616               | 1.12 (1.01–1.25) |
| 6–10                              | 1,515                | 1.41 (1.19–1.66) |
| ≥11                               | 323                  | 1.23 (0.88–1.72) |
| **Smoking, n cigarettes/day**     |                      |             |
| Never                             | 7,147                | 1.0 (Reference) |
| Former 1–5                        | 592                  | 1.08 (0.89–1.32) |
| 6–10                              | 1,920                | 1.10 (0.97–1.25) |
| 11–15                             | 909                  | 1.25 (1.06–1.49) |
| 16–20                             | 839                  | 1.34 (1.13–1.59) |
| ≥20                               | 470                  | 1.52 (1.24–1.87) |
| Current                           | 1–5                  | 558                 | 0.99 (0.79–1.24) |
| 6–10                              | 1,728                | 0.94 (0.81–1.09) |
| 11–15                             | 1,442                | 0.89 (0.75–1.06) |
| 16–20                             | 731                  | 1.38 (1.14–1.68) |
| ≥20                               | 195                  | 1.72 (1.23–2.39) |
| **Coffee/tea consumption, cups/day**|                  |             |
| 0–5                               | 9,976                | 1.0 (Reference) |
| ≥6                                | 8,071                | 1.09 (1.01–1.17) |
| **Disease status**                |                      |             |
| **Diabetes**                      |                      |             |
| No                                | 17,753               | 1.0 (Reference) |
| Yes                               | 474                  | 1.25 (1.04–1.49) |
| **Stroke**                        |                      |             |
| No                                | 17,966               | 1.0 (Reference) |
| Yes                               | 251                  | 1.61 (1.30–2.00) |
| **Muscle complaints**             |                      |             |
| No                                | 16,031               | 1.0 (Reference) |
| Yes                               | 947                  | 1.68 (1.46–1.93) |
| **Osteoarthritis**                |                      |             |
| No                                | 16,307               | 1.0 (Reference) |
| Yes                               | 1,054                | 1.69 (1.50–1.91) |

*Age-adjusted.
ACKNOWLEDGEMENTS
The authors thank Jaspreet Chowdhary, MPH, for technical help with the manuscript. The Norwegian Medical Research Council and Merck Co supported the study financially.

CONFLICT OF INTEREST
None declared.

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Abbreviations: BMI, body mass index; WHR, waist/hip ratio; OR, odds ratio.
A prospective randomized trial comparing transurethral prostatic resection and clean intermittent self-catheterization in men with chronic urinary retention

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Accepted for publication 9 February 2005

OBJECTIVE
To determine whether a preliminary period of clean intermittent self-catheterization (CISC) before transurethral resection of the prostate (TURP) improves bladder contractility and surgical outcome in men with chronic urinary retention (CUR), and whether pressure-flow studies (PFS) before TURP predict the outcome.

PATIENTS AND METHODS
The study was a two-centre, pragmatic and randomized trial. Included were 41 men scheduled for TURP with lower urinary tract symptoms (LUTS), an International Prostate Symptom Score (IPSS) of >7, benign prostatic enlargement and a persistent postvoid residual urine volume (PVR) of >300 mL. They had conventional PFS using unphysiological filling. The patients then gave consent and were randomized into two treatment groups; the first had TURP after stabilizing renal function by indwelling catheterization if indicated, and the second was taught CISC. Men in both groups were reviewed at 3 and 6 months after surgery or the start of CISC, by the IPSS, urine culture and assay of plasma creatinine, and upper tract imaging and repeat PFS at 6 months. The primary outcome variables were IPSS, maximum urinary flow rate, voiding and end-filling pressures, and mean PVR; secondary variables included treatment failure, complications and other urodynamic measures.

RESULTS
Of the 41 patients, 17 (mean age 67 years, range 52–84) were randomized to immediate TURP and 24 (mean age 69 years, range 55–88) to CISC. There was a significant improvement in IPSS and quality of life at 6 months in both groups (P<0.001). In the CISC group there was a significant improvement in voiding and end-filling pressures, indicating recovery of bladder function (P<0.001 for each). Of the 41 men, nine (22%) with voiding pressures of ≤45 cmH₂O had no significant improvement in symptoms or urodynamic variables. Detrusor overactivity was found in 17 (41%) patients, of whom six had upper tract dilatation which resolved after treatment.

CONCLUSION
The present results emphasize the usefulness of CISC in ensuring the recovery of bladder function in men with CUR. Measuring the voiding pressure before TURP can predict the surgical outcome. Both CISC and immediate TURP are effective for relieving LUTS and result in a better quality of life. A preliminary period of CISC before TURP for men with CUR and low voiding pressure may be valuable. The presence of upper tract dilatation is associated with high end-void and end-fill bladder pressures, and such men have a good outcome from surgery.

KEYWORDS
prostate, urinary retention, prostatic hyperplasia.

INTRODUCTION
Chronic urinary retention (CUR) is defined as the consistent presence of a significant residue after voiding. The magnitude of the postvoid residual volume (PVR) taken to define CUR in previous studies has been arbitrarily set at 300 mL [1]. Common clinical manifestations include nocturnal incontinence, a palpable but painless bladder, dilatation of the upper urinary tracts and impaired renal function, whilst causative factors include detrusor hypocontractility, chronic BOO and neurological bladder dysfunction. At present most men with CUR are treated by catheter drainage and fluid replacement until the creatinine level is stabilized, followed by surgery to relieve the likely BOO, but with no prior urodynamic assessment. Previous studies that used pressure-flow studies (PFS) to obtain a urodynamic diagnosis lend some support for this empirical line of management, but also reveal some possible disadvantages [1,2]. Clean intermittent self-catheterization (CISC) is now a well-established method of ensuring complete bladder emptying for those with incomplete voiding, particularly patients with neurological bladder dysfunction [3].

The relief of BOO, which will have the effect of decreasing opening pressure, may not be sufficient to allow bladder emptying for all men with CUR, particularly those with detrusor hypocontractility. Contractility has been shown to improve after starting CISC [4], whilst other management options, such as muscarinic agonists and prokinetic agents, have not been successful.

In the present study we investigated first whether re-establishing the normal filling and emptying cycle by a period of CISC can improve both bladder contractility and results of bladder outlet surgery for men with CUR, and second, whether a symptomatic and urodynamic assessment after a period of CISC will aid selection for bladder outlet surgery in such cases. We also tried to identify if preoperative PFS can predict a poor outcome of TURP and hence identify a
subgroup of men who will not benefit from surgery.

PATIENTS AND METHODS

The study was a two-centre pragmatic randomized controlled trial, stratified by centre. All analysis was by intent-to-treat, following CONSORT guidelines [5,6]. The study was approved by the local ethics committees at each participating centre and all patients provided written informed consent before study entry.

The study included men with LUTS and an IPSS of >7 [7], together with CUR, defined as a PVR of >300 mL measured by ultrasonography on two occasions [1], with patients and physicians agreeing that the findings justified intervention. Patients were excluded from study if there was clinical evidence of prostate cancer, previous prostatic surgery, uncontrolled renal impairment, a life-expectancy of <6 months, proven neurological bladder dysfunction, or inability to practise CISC. The urine of all patients was sterile at the time of the study.

Those men who elected to participate in the trial had conventional unphysiological filling cystometry and PFS with no drainage of residual urine, according to ICS approved methods [8]. Measurements taken from the resultant recordings included cystometric capacity, end-filling pressure, voiding pressure (P\text{det.Q\text{max}}) and maximum flow rate (Q\text{max}). Prophylactic oral antibiotics were administered before and for 3 days after the investigation.

The patients gave consent and were randomized into two treatment groups (Fig. 1); the first was managed conventionally by TURP, =4 weeks after stabilization of the creatinine level by indwelling catheterization, and the second was also managed by an initial period of indwelling urethral catheterization but were then taught CISC, using a 12 or 14 F catheter every 6 h.

Both groups were reviewed at 3 and 6 months after TURP or the start of CISC. At the 3- and 6-month review the IPSS, urine culture and serum creatinine assay were repeated, with additional renal ultrasonography and PFS at 6 months. Men in the CISC group with urodynamic evidence of BOO were advised to have TURP at the end of the study, and men with no urodynamic evidence of obstruction were offered the choice of TURP, continued CISC or an indwelling catheter.

The symptomatic outcome was measured by the change in the IPSS and associated quality-of-life score; the urodynamic outcome was determined by the change in the end-filling pressure, P\text{det.Q\text{max}} P\text{det.max}, and PVR. Differences between means of the outcome variables were analysed using a paired Student’s t-test.

RESULTS

In all, 65 patients were considered for inclusion, but 11 were excluded before randomization because of prostate cancer (one), PVR < 300 mL (eight) and inability to use CISC (two). Three men with uncontrolled severe renal impairment or significantly dilated upper tracts on ultrasonography, characteristic of high-pressure CUR, were excluded and treated as an emergency, because it was difficult to stabilize their renal function during the trial. After randomization three patients in the CISC group withdrew from the study, being sufficiently happy with their management, and seven (five TURP, two CISC) failed to attend for follow-up visits (Fig. 2). This left 41 men who completed the study, of whom 17 (42%); mean age 67 years, range 52–84) were randomized to TURP and 24 (58%; 69 years, range 55–85) were randomized to CISC.
FIG. 2. The chronic retention trial profile.

The baseline primary outcome variables were similar in both groups (Table 1); the end-filling pressure was significantly higher in men randomized to TURP ($P = 0.004$). In all, eight (20%) men (four in each group) had an elevated creatinine level which was associated with hydroureteronephrosis in all but one. These men had significantly higher end-filling pressure ($P < 0.001$) and voiding pressure ($P = 0.016$) than those with no upper tract dilatation. The mean (range) resting end-void subtracted bladder pressure (baseline filling-phase pressure), i.e. the intrinsic pressure within the bladder at the end of micturition when the bladder is supposedly empty, was significantly higher in these patients, at $25.75\, (12–42)\, \text{cmH}_2\text{O} \quad (P < 0.001)$. They all had a 4–6-week period of indwelling catheterization to stabilize renal function before starting the allocated management. There was phasic detrusor overactivity (DO) during filling in 17 (42%) patients, of whom six had upper tract dilatation. Nine (22%) patients (seven CISC, two TURP) found to have a voiding pressure of $\leq 40\, \text{cmH}_2\text{O}$ (two CISC) had an acontractile bladder.

There was a significant improvement in the IPSS and quality-of-life score at 3 months in both groups ($P < 0.001$). Four patients from the CISC and two from the TURP group developed complications during the follow-up, i.e. symptomatic infection (two), bleeding (two) or both (two). Renal function remained stable for all men of both groups.

At 6 months, both treatment groups had a significant improvement in IPSS and quality-of-life score at 6 months ($P < 0.001$). In the CISC group, there was a significant increase in end-filling, end-void pressures and $P_{\text{detQmax}}$ ($P < 0.001, 0.001$ and 0.015, respectively); conversely, in the TURP group, there was a significant decrease in all three variables ($P = 0.01, 0.004$ and $<0.001$, respectively). The PVR decreased significantly from baseline to the 6-month follow-up ($P < 0.001$ for both groups).

There was a good outcome overall, with complete bladder emptying, in 32 (78%) patients, the remainder having persistent symptoms and poor bladder emptying, although most were happy continuing CISC. Nine (22%) patients (seven CISC and two TURP) with a voiding pressure of $\leq 45\, \text{cmH}_2\text{O}$ had no significant improvement, whilst two in the CISC group with voiding pressures of 45 and 40 cmH$_2$O, respectively, had a minimal

### TABLE 1 Baseline comparability of the two treatment groups, and the changes in primary variables from baseline to the 6-month follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>CISC</th>
<th>TURP</th>
<th>P (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N patients</td>
<td>24</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mean (SD):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>69 (7.3)</td>
<td>67 (8.0)</td>
<td>0.46</td>
</tr>
<tr>
<td>IPSS</td>
<td>23.2 (6.1)</td>
<td>25.8 (4.2)</td>
<td>0.13</td>
</tr>
<tr>
<td>IPSS quality of life</td>
<td>4.2 (1.1)</td>
<td>4.4 (0.9)</td>
<td>0.42</td>
</tr>
<tr>
<td>Q$_{\text{max}}$, mL/s</td>
<td>5.5 (4.2)</td>
<td>5.2 (3.4)</td>
<td>0.78</td>
</tr>
<tr>
<td>PVR, mL</td>
<td>963 (503)</td>
<td>954 (531)</td>
<td>0.96</td>
</tr>
<tr>
<td>Pressures, cmH$_2$O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>voiding</td>
<td>85 (57.1)</td>
<td>102 (44.7)</td>
<td>0.305</td>
</tr>
<tr>
<td>End-filling</td>
<td>11 (7.5)</td>
<td>22 (15.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>End void</td>
<td>10.3 (8.1)</td>
<td>13.9 (11.4)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

### Change, baseline to 6-month follow-up

<table>
<thead>
<tr>
<th>Mean (95% CI)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IPSS quality of life</td>
<td>$-2.54 [-3.11, -1.97]$</td>
<td>$-3.00 [-3.75, -2.25]$</td>
<td></td>
</tr>
<tr>
<td>PVR, mL</td>
<td>$-600.5 [-826.6, -374.3]$</td>
<td>$-854.4 [-1078.1, -630.7]$</td>
<td></td>
</tr>
<tr>
<td>Pressures, cmH$_2$O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>voiding</td>
<td>8.96 (1.94–15.97)</td>
<td>$-47.7 [-67.12, -28.17]$</td>
<td></td>
</tr>
<tr>
<td>end-filling</td>
<td>3.54 (1.84–5.24)</td>
<td>$-7.12 [-12.32, -1.92]$</td>
<td></td>
</tr>
<tr>
<td>end void</td>
<td>2.33 (1.12–3.54)</td>
<td>$-7.41 [-12.17, -2.65]$</td>
<td></td>
</tr>
</tbody>
</table>
improvement and required continued CISC to empty their bladder. In summary, 19 (79%) patients from the CISC and 15 (88%) from the TURP group had a satisfactory symptomatic and urodynamic outcome after surgery. For patients with a successful outcome, 29% from the CISC and delayed TURP group, and 33% from the immediate TURP group, required CISC after TURP for 6–8 weeks. All the patients with DO and upper tract dilatation had a good surgical outcome, with persistent dilatation in only one. Eight (33%) patients from the CISC and two (12%) from the TURP group developed complications during the follow-up, i.e. symptomatic infection (six), bleeding (two) or both (two).

**DISCUSSION**

Previous randomized controlled trials have tended to exclude men with CUR from analysis because it was expected that they would be generally less healthy at the onset than those with uncomplicated LUTS, and would be more likely to have a poor outcome and complications [9,10]. Despite this exclusion, men with CUR are a clinically important group, comprising up to a quarter of men undergoing TURP in the UK [11,12].

The present study confirmed the findings of others, that most patients with high-pressure CUR have a good outcome after bladder outlet surgery. The renal function was usually improved or remained stable and bladder empting was satisfactory in most cases [13,14]. Patients with high-pressure CUR often present with late-onset enuresis, a tense, palpable bladder, hypertension, and progressive impairment associated with bilateral hydronephrosis and hydrourerter, commonly leading to uraemia and death, whilst voiding urological symptoms are typically absent in uncomplicated cases. The diagnosis was confirmed by finding an abnormally high end-void and bladder pressure during cystometry before draining residual urine [13].

Similar to other series, the present study confirmed that patients with CUR are commonly elderly and present, not necessarily to a urologist, with late-onset enuresis or symptoms of cardiac decompensation [13]. After appropriate management most can be expected to make a satisfactory recovery.

A few patients refused to enter the trial because of a fear of CISC; while many reports acknowledge that CISC improved the patients’ quality of life, very few go on to identify and discuss daily-life activities that are affected by having to use CISC [15]. The main reason for the failure of CUR is that patients do not comply, and with a better understanding of the problems carers would be able to give practical help and support [15]. The present study shows that CISC and TURP are effective for relieving LUTS, and improving quality of life. There was a significant increase in end-filling and voiding pressure on conventional urodynamic assessment after a 6-month period of CISC. A previous report also emphasized the usefulness of this technique in ensuring recovery of bladder function after the acute detrusor failure sometimes subsequent to other than urological surgery [4]. The voiding and end-filling pressures decreased significantly after relieving the obstruction in the TURP group, but the end-filling pressure was higher in the TURP than in the CISC group; the reason for this finding is uncertain.

There was a good surgical outcome, with adequate bladder emptying in 78% of patients with CUR. In contrast, Abrams et al. [1] reported a good surgical outcome in 59% of patients, the remainder having persistent symptoms and poor bladder emptying. Similar results were reported by George et al. [16], with 53% having a satisfactory result from bladder neck surgery. In both these studies a poor outcome was associated with low end-filling and voiding pressures on conventional urodynamic assessment before surgery. Others [2,14] reported a good overall improvement in upper urinary tract function and urodynamic variables, but commented that the PVR remained high in a significant proportion of patients (22% and 32%, respectively) and tended to increase with a longer follow-up. The latter study also found, in contrast to previous reports, that preoperative urodynamic values could not be used to predict the operative outcome. There is also evidence that men with CUR treated by outlet surgery may still progress to renal failure requiring dialysis [17]. In the present series, CISC for 6–8 weeks after surgery decreased the PVR significantly (P < 0.001) in men who had poor emptying after TURP.

A poor outcome was associated with low end-filling and voiding pressures; those with detrusor failure from the beginning are not expected to have a good outcome, especially when the voiding pressure was ≤40–45 cmH2O. The PVR decreased significantly in all patients with a good symptomatic outcome, but five in each group still had a PVR of >250 mL and needed CISC for 6–8 weeks after surgery. Seven (29%) patients from the CISC and two (12%) from the TURP group required CISC in the longer term after TURP failed to improve bladder emptying.

There was DO in 17 patients (41%) during filling cystometry, six of whom also had upper tract dilatation, and all had a good outcome after surgery. Styles et al. [2,18] confirmed the finding that involuntary detrusor contraction during ambulatory, long-term bladder-pressure monitoring was associated with upper tract dilatation, and correlated with an impaired GFR. Conventional cystometry using unphysiological filling tends to mask DO and it was suggested that CUR should be investigated by natural-filling cystometry [19]. The fill rate of 100 mL/min used in the present study may have hampered our ability to detect DO in these patients.

Complications such as renal failure, acute retention and UTIs are uncommon in men with a large, chronic PVR [20]. Eight (33%) patients from the CISC and two from the TURP group developed complications during the follow-up, i.e. infection, bleeding or both. CISC reduces infection hazards and greatly improves the lives of many patients with voiding disorders [21]. With a better understanding of the problems, carers would be able to give practical help and support [15].

In conclusion, urodynamics before surgery can be used to predict the outcome. CISC may be useful in ensuring recovery of bladder function after CUR and before a delayed TURP. DO in association with good voiding and end-filling pressures is a positive prognostic sign. Upper tract dilatation was significantly associated with high-pressure bladder filling, with a better response to TURP. The poor response of patients with low-pressure filling was a result of the high incidence of inadequate detrusor contraction, leading to a persistent PVR. Using CISC for a period before TURP may be worthwhile for patients with a low-voiding pressure but >40–45 cmH2O.
CONFLICT OF INTEREST

None declared.

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e-mail: ibrahim@ghalayini.com

Abbreviations: CUR, chronic urinary retention; PVR, postvoid residual volume; PFS, pressure-flow studies; CISC, clean intermittent self-catheterization; PdetQmax, voiding pressure at Qmax; Qmax, maximum urinary flow rate; DO, detrusor overactivity.
Preoperative administration of chlormadinone acetate reduces blood loss associated with transurethral resection of the prostate: a prospective randomized study

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Accepted for publication 8 February 2005

OBJECTIVES
To assess the effects of giving chlormadinone acetate (CMA) before surgery on blood loss associated with transurethral resection of the prostate (TURP), in a prospective randomized controlled study.

PATIENTS AND METHODS
Candidates for TURP among patients with benign prostatic hyperplasia were randomized to either treatment with CMA (CMA+) or not (CMA–). In principle, CMA was started at least 28 days before TURP and continued until just before surgery.

INTRODUCTION
Despite the development of various minimally invasive therapies for BPH, TURP is therapeutically beneficial and still remains the standard treatment for BPH. However, TURP is occasionally associated with considerable bleeding during and after surgery, sometimes leading to serious adverse events. Therefore, it is important for both the surgeon and patient that the blood loss associated with TURP is controlled. Recent reports show that taking the 5α-reductase inhibitor finasteride before surgery is effective in controlling the blood loss associated with TURP [1–3]. The exact mechanism by which intraoperative blood loss is reduced by finasteride is unknown, but seems to involve a decrease in prostate blood flow and microvessel density (MVD) within the prostate.

Since 1981, chlormadinone acetate (CMA), a steroidal antiandrogen shown to reduce the prostate blood flow in a rat model [4], has been widely used in treating BPH and prostatic cancer in Japan. CMA at 50 mg/day for treating BPH is sufficient to inhibit androgen uptake in the prostate, and competitively antagonises androgen receptors and androgen binding. Inhibition of androgen-receptor binding was reported to be 23 and 10 times greater for CMA than for hydroxyflutamide and bicalutamide, respectively [5]. CMA can induce apoptosis in prostate tissue and is effective for prostatic atrophy [6–9], and apoptosis-inducing and prostate-reducing activity were reported to be more potent for CMA than for finasteride [9,10].

The present prospective randomized study was designed to compare men treated with CMA before TURP with those not taking CMA for blood loss during and after surgery, to assess the effect of CMA in reducing the loss, and for the MVD of the resected prostate tissue; the correlation between MVD and blood loss was also evaluated.

RESULTS
In all, 33 patients in the CMA+ (median duration of treatment 34.5 days) and 38 in the CMA– group were evaluable. The mean blood loss during TURP was less in the CMA+ (237.3 mL) than in the CMA– group (263.1 mL), but the difference was not significant. There was significantly less blood loss per gram of resected prostate tissue in the CMA+ (9.6 mL/g) than in the CMA– group (13.3 mL/g) (P < 0.05). Haematuria on the day of and the day after TURP was also significantly less severe in the CMA+ than in the CMA– group (P < 0.001 and P < 0.05, respectively). The mean microvessel density of resected prostate tissue was significantly less after CMA treatment (P < 0.001).

CONCLUSIONS
CMA given for 1 month before TURP could reduce blood loss to some extent during and after TURP, and this may be related to a decrease in microvessel density.

KEYWORDS
prostate, chlormadinone acetate, TURP, blood loss, microvessel density
standard value; and patients with serious cardiovascular disorders.

To determine the period of CMA treatment a preliminary study was conducted in 10 patients with BPH; CMA 50 mg/day was administered for 4 or 8 weeks before TURP, and changes in prostate blood flow determined using power Doppler ultrasonography (SSD-2000, Aloka, Tokyo, Japan) every 2 weeks to calculate the resistive index [11,12]. Blood flow signals within the prostate decreased after the first 2-week period of CMA before TURP, and the mean resistive index of prostate vessels decreased from 0.81 (10 men) to the nadir level of 0.60–0.70 at 4 weeks. From these findings and those reporting that finasteride administered for 2 weeks before TURP reduced the blood loss associated with surgery [1], the administration period required to reduce the prostate blood flow was set to ≥4 weeks before TURP.

The present study required that the urologists had at least 10 years of experience with TURP. Variables measured were: age, weight of resected prostate tissue, duration of surgery, volume of the prostate and transition zone (TZ) before treatment, haematology findings, urine analysis findings, macroscopic urine analysis/urinary sediments, volume of the irrigation fluid and volume of blood loss during TURP, and the severity of haematuria afterward.

After the irrigation fluid was thoroughly stirred, blood loss during TURP was calculated by either the red blood cell (RBC) method or the visual scale (VS) method, or both. The RBC method was calculated as the volume of the collected irrigation fluid × RBCs (mL of irrigation fluid) divided by the RBCs (mL of blood) before surgery. The VS was calculated as the percentage of blood volume in the collected irrigation fluid × volume of the collected irrigation fluid. For the VS method, a 7-grade scale was used, with known concentrations of blood volume (grade A, ≥0.1%, to G, ≤20%) to check colour tone and thus assess blood volume in the irrigation fluid.

In addition to examining the correlation between blood loss as calculated by these methods, the correlation between blood loss calculated by the haemoglobin (Hb) method [3] and the VS method, and between blood loss calculated by the RBC method and the Hb method, were also evaluated. The Hb method uses the following equation: concentration of Hb (g/mL of the collected irrigation fluid) × volume of the collected irrigation fluid, divided by the concentration of Hb before surgery.

The blood loss per gram of resected prostate tissue was calculated for each patient, and the mean blood loss per gram of resected prostate tissue then calculated by dividing the total individual blood loss by the number of evaluable patients. Similarly, the mean blood loss per duration of surgery was calculated. After urine was pooled and thoroughly stirred, haematuria after TURP was visually assessed on a 7-grade scale using the VS (A–G) and the results compared between the groups.

Of 71 evaluable patients, 48 had the MVD measured in the resected prostate tissue, to assess the effects of CMA on microvessels in the prostate. The MVD was calculated as described by Nakano et al. [13]. The excised prostate was fixed in formalin, embedded in paraffin wax and cut into sections, and the sections incubated in the rabbit antihuman factor VIII polyclonal antibody (Dako A0082, DakoCytomation A/S, Glostrup, Denmark). The primary antibody was detected using a biotinylated goat antirabbit IgG. Antigen binding was visualized by diaminobenzidine incubation of the sections, after lightly counterstaining with haematoxylin.

The mean MVD was calculated as the number of microvessels in a ×200 field (×20 objective and ×10 ocular, 0.754 mm²), and expressed per mm². Large vessels with thick muscular walls and large vessels of lumina more than eight blood cells in diameter were excluded from the counts. Three visual fields per patient were selected to calculate the mean MVD, the groups compared, and the correlation between mean MVD and blood loss during surgery assessed.

The results are expressed as the mean (SD) unless otherwise indicated. The two-sample t-test, Mann–Whitney U-test and regression analysis were used to compare data as appropriate for the scale and properties of the data, with \( P < 0.05 \) considered to indicate statistical significance.

### RESULTS

During a 16-month period, 44 patients in the CMA+ and 48 in the CMA− group were enrolled; any men who had protocol deviations were excluded, and consequently the final totals were 33 and 38, respectively. The median (range) duration of CMA administration was 34.5 (28–141) days. There was no statistically significant difference between the groups in patient age, weight of the prostate before surgery, and weight of the TZ (Table 1). The resected weight for all 71 patients was 22.5 (11.3) g and the mean duration of surgery 60.6 (20.8) min. The median (range) surgeons’ experience with TURP was 18 (10–24) years.

For 20 patients in whom both the RBC and the VS method were used to determine blood loss during surgery, there was a positive correlation between them \( r = 0.70, P < 0.001 \). Blood loss was also assessed by the Hb method in the irrigant and preoperative blood in 17 men; these estimates were also closely correlated with those obtained by the VS and RBC methods (VS method, \( r = 0.85, P < 0.001 \); RBC method, \( r = 0.96, P < 0.001 \)).

### Table 1: Patient characteristics and outcomes of surgery

<table>
<thead>
<tr>
<th>Variable</th>
<th>CMA+</th>
<th>CMA−</th>
<th>(\text{P} &lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>71.5 (5.9)</td>
<td>73.5 (4.8)</td>
<td></td>
</tr>
<tr>
<td>Prostate volume, mL</td>
<td>44.3 (16.6)</td>
<td>42.5 (15.8)</td>
<td></td>
</tr>
<tr>
<td>TZ volume, mL</td>
<td>24.3 (10.0)</td>
<td>24.8 (12.4)</td>
<td></td>
</tr>
<tr>
<td>Resected weight, g</td>
<td>22.1 (11.2)</td>
<td>22.9 (11.4)</td>
<td></td>
</tr>
<tr>
<td>Duration of TURP, min</td>
<td>60.4 (20.6)</td>
<td>60.8 (21.3)</td>
<td></td>
</tr>
<tr>
<td>Blood loss, mL</td>
<td>237.3 (138.6)</td>
<td>263.1 (141.1)</td>
<td></td>
</tr>
<tr>
<td>Blood loss /resected weight, mL/g</td>
<td>9.6 (6.2)</td>
<td>13.3 (8.0)*</td>
<td></td>
</tr>
<tr>
<td>Blood loss /duration of TURP, mL/min</td>
<td>3.9 (2.0)</td>
<td>4.4 (1.7)</td>
<td></td>
</tr>
</tbody>
</table>

\*\( P < 0.05 \).
As there was a significant correlation between the RBC and the VS method, blood loss measured by the latter was converted values of the RBC method to compare blood loss during surgery between the groups. Blood loss during surgery was less in the CMA+ than in the CMA− group, although the difference was not statistically significant (Table 1). However, blood loss by weight of prostate tissue was significantly less in the CMA+ than in the CMA− group (P < 0.05; Table 1).

Haematuria after surgery on the day of TURP and the day afterward was statistically significantly less severe in the CMA+ than in the CMA− group (P < 0.001 and < 0.05, respectively). There was no blood loss of grade E (5%) or greater in the CMA+ group (Table 2).

Twenty-two patients in the CMA+ and 26 in the CMA− group were evaluable for MVD in the prostate sections. Blood loss was significantly greater in patients with a high MVD (r = 0.35; y = 0.03x + 23.57; P < 0.05; Fig. 1); the MVD was significantly lower in CMA+ than the CMA− group, at 25.5 (7.5) and 36.8 (9.4) vessels/mm² (P < 0.001; Fig. 2).

None of patients in the CMA+ group had a blood loss that required medical treatment, nor were there any adverse events associated with CMA. However, in the CMA− group two patients had blood loss requiring medical treatment (blood transfusion in one and a haemostatic agent after TURP in another).

**DISCUSSION**

None of the many current methods for treating BPH is better than TURP in terms of long-term outcome. Thus TURP has maintained its position as the standard treatment for BPH since its introduction half a century ago. Although TURP is highly beneficial for treating BPH, considerable bleeding is an inherent risk. As the visual field of TURP is prone to occlusion as a result of bleeding, a clear field should be ensured by an appropriate method to stop the bleeding associated with TURP. However, enlarged prostate glands should be resected before achieving haemostasis at the beginning of surgery, and thus considerable bleeding is likely during the first half of TURP [14]. In addition, TURP is generally associated with a high incidence of blood transfusion; Uchida et al. [15] reported an incidence of 13.4%. Blood transfusion may correlate with the
development of infection, immune reaction and graft-vs-host disease. Because of this, some medical institutions use autologous blood transfusion for patients with extremely large prostates, although blood transfusion-related risks are also inherent in autologous transfusion. Additional blood transfusion is occasionally required if there is insufficient transfusion of pooled blood. Despite conducting such autologous pooled blood transfusions, risks cannot be completely avoided. Numerous reports describe blood loss after TURP, including one indicating that the incidence of a loss requiring coagulation was 2.4% [15].

As noted, the management of blood loss during and after TURP is a challenge and reducing blood loss is essential. The usefulness of finasteride before TURP was investigated in a randomized, placebo-controlled trial [1], where finasteride given for 2 weeks before TURP reduced the amount of Hb in irrigation fluid and the amount of Hb per gram of resected tissue; the blood concentration of Hb immediately after surgery tended to be higher in the group treated with finasteride. Hagerty et al. [2] reported that blood loss requiring medical treatment was less after giving finasteride for a mean of 2.7 months before surgery in patients with BPH, particularly those with large prostates. Sandfeldt et al. [3] assessed blood loss by the Hb method and showed that 3 months of finasteride before surgery reduced blood loss during surgery in patients requiring larger resections. Finasteride and cyproterone acetate are reportedly effective for treating haematuria secondary to BPH [16–18]. Based on these results, we administered CMA before TURP to assess its activity in reducing blood loss during and after surgery, the loss being evaluated by two irradiation-fluid methods capable of rigorously reflecting true blood loss, the RBC and the VS method. The precision of blood loss estimated by the VS method was comparable (in the same patients) with the RBC or Hb method, indicating that the VS method is effective, simple and adequate for estimating blood loss, although giving slightly lower values than those estimated by the Hb or RBC method. To ensure comparability we converted values from the VS method to those of the RBC method.

All seven surgeons who conducted TURP had sufficient experience with the procedure. CMA given for 1 month before surgery significantly reduced blood loss per gram of resected prostate tissue in the CMA+ group (P < 0.05), although the 10% lower blood loss in the CMA+ group during TURP was not significantly different from that in the CMA– group (Table 1). However, the surgeon who operated on the most patients was associated with a 33% decrease (263.4 mL in the CMA+ vs 394.2 mL in the CMA– group). Accordingly, the present study may provide information on the clinically beneficial effects of CMA, although the secondary influences of several surgeons should be considered. The mean weight of the resected prostate was 22.5 g and the mean duration of surgery 60.6 min, values in good agreement with the results (23.3 g and 68.3 min, respectively) from a study which enrolled up to 1931 surgical patients in Japan [15]. Furthermore, Masumori et al. [19] reported that, although the mean predicted volume of the prostate for men in their 70s is smaller in Japanese (21.0 mL) than in American men (39.0 mL), the proportion of individuals who have both a prostate volume of >20 mL and a maximum urinary flow rate of <10 mL/s is higher in Japanese (41.8%) than in American men (17.2%). Thus the present surgical results also appear to be typical in Japan, and indicate that TURP is clinically effective for treating BPH.

Haematuria on the day of surgery and next day after TURP was significantly less severe in the CMA+ than in the CMA– group, in agreement with results obtained by Donohue et al. [1]. There was no blood loss requiring medical treatment in any patient in the CMA+ group, supporting the results of Hagerty et al. [2], confirming that CMA before TURP is effective in reducing the associated blood loss.

Possibly decreases in prostate blood flow and MVD within the prostate may be involved in the mechanism by which blood loss is reduced by finasteride. Support for this mechanism is that short-term dosing with finasteride is unlikely to reduce the prostate, but various reports show that finasteride was effective in treating haematuria secondary to BPH [17,18]; the haematuria recurred when finasteride was discontinued [17]; the haematuria was better at reducing prostate size [10,24] but CMA, being a stronger anti-androgen, requires only 12 weeks to do so [25,26]. In a double-blind controlled study comparing finasteride with CMA, the latter was better at reducing prostate size [10]. Thus it could be assumed that the difference in anti-androgenic activity between CMA and finasteride results in different reductions in MVD. As there was a positive correlation between MVD within the prostate and blood loss during surgery, MVD could be a predictor for blood loss during surgery.

Thus 1 month of CMA 50 mg/day before TURP could be used to reduce blood loss during and after TURP; CMA also decreased the MVD within the BPH tissue, suggesting that the effect of CMA in reducing the blood loss associated with TURP may be related to the MVD.

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CONFLICT OF INTEREST

None declared.

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Abbreviations: MVD, microvesSEL density; CMA, chlormadinone acetate; RBC, red blood cell (method); VS, visual scale (method); Hb, haemoglobin (method); TZ, transition zone.
Porcine small intestinal submucosa as a percutaneous mid-urethral sling: 2-year results

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OBJECTIVE

To report the 2-year follow-up results on patients treated with a novel minimally invasive outpatient procedure for placing a mid-urethral sling, using porcine small intestinal submucosa (SIS).

PATIENTS AND METHODS

Thirty-four women with urodynamic evidence of stress urinary incontinence (SUI, 19) or of SUI with a positive cough test (15) were treated. A curved ligature carrier was used to create a tract between bilateral suprapubic stab incisions and a 2-cm mid-urethral vaginal incision. A suture secured to each end of the SIS sling was placed through the eyelet of the ligature carrier. Extraction was used to position the sling at the mid-urethra, providing a backboard of support that was remodelled with ingrowth of the patient’s autologous tissue.

RESULTS

SUI was reportedly cured in 27 of the 34 women (79%) at the 2-year follow-up; three (9%) of those with no complete resolution were pleased with their results, because the improvement allowed them to wear an average one or fewer pads per day. One patient developed de novo urge incontinence. Three patients (9%) developed suprapubic inflammation at 10, 21 and 45 days after surgery; all resolved, but one had a recurrence of SUI. No prolonged retention, erosion or other complications were noted.

CONCLUSIONS

Early results with the percutaneous mid-urethral placement of SIS are promising and potentially comparable with those after using synthetic minimally invasive slings.

KEYWORDS

sling, incontinence, nonsynthetic, small intestine submucosa, percutaneous dissection was carried laterally only widely enough to palpate the undersurface of the pubic arch. The catheter guide was used for traction of the bladder neck and urethra away from the side of interest. A curved Stamey ligature carrier pierced the skin just medial to the pubic tubercle, and was advanced through the subcutaneous incision lateral to the proximal or mid-urethra, guiding the needle through the urethra-pelvic complex into the vagina; this was repeated on the opposite side (Fig. 1).

With both ligature carriers in position, the catheter was removed and cystoscopy used to confirm that the bladder had not been entered; the urethra was then inspected while manipulating the ligature carriers to confirm their proper placement. The needle was replaced if it had entered the urinary tract. The sutures securing each end of the sling were inserted through the ligature carrier eyelet and the carrier extracted. The sutures were gently pulled to advance the sling through the retropubic space, abdominal wall and skin. A 3–0 absorbable stay suture

INTRODUCTION

The surgical correction of stress urinary incontinence (SUI) by placing a sling beneath the bladder neck and proximal urethra can give excellent results [1], but traditionally requires major surgery, hospitalization and several weeks of convalescence. Although direct comparative data are lacking, similar results are reported using all the various operative approaches and sling materials [2].

Placing a mid-urethral sling is effective using minimally invasive techniques [3–5], by placing polypropylene mesh either percutaneously (PVT) or transvaginally (TVT, Gynecare, Somerville, NJ). The main disadvantage of these procedures is the risk of infection or erosion of the permanent synthetic material left in the surgical site [6,7]. Such experience with previous artificial sling materials has made many surgeons hesitant to use these procedures [8].

To avoid these risks, we placed a commercially available nonsynthetic small intestinal submucosa (SIS) sling using a percutaneous route in selected patients with type II SUI and report the 2-year follow-up from the initial series of patients.

PATIENTS AND METHODS

Thirty-four patients were selected based on urodynamic evidence of SUI (19) or a clear history of SUI combined with a positive cough test (15); those assessed by urodynamics before surgery had a mean (range) leak-point pressure of 74 (47–99) cmH₂O.

After inducing regional or general anaesthesia, the patient was placed in the dorsal lithotomy position and prepared using rectus fascia and the retropubic space, along the dorsal surface of the pubis. The subordinate index finger was placed into the suburethral incision lateral to the proximal or mid-urethra, guiding the needle through the urethra-pelvic complex into the vagina; this was repeated on the opposite side (Fig. 1).

With both ligature carriers in position, the catheter was removed and cystoscopy used to confirm that the bladder had not been entered; the urethra was then inspected while manipulating the ligature carriers to confirm their proper placement. The needle was replaced if it had entered the urinary tract. The sutures securing each end of the sling were inserted through the ligature carrier eyelet and the carrier extracted. The sutures were gently pulled to advance the sling through the retropubic space, abdominal wall and skin. A 3–0 absorbable stay suture
between the sling edge and the submucosal extents of the vaginal incision prevented rolling of the material.

The sling was positioned with no tension, as described for PVT and TVT, and no ‘cough test’ or other assessment of tension was used. The vaginal incision was closed with absorbable suture. The sling was then cut below the skin level and an adhesive strip placed. No vaginal pack was used. Patients returned to the office 1–3 days later to remove the catheter and have a voiding trial, depending on whether the operation was midweek or on Friday (respectively). Catheter use was based on the surgeon’s preference because of concerns that the material might slip more easily than polypropylene. We currently think that catheterization is unnecessary, based on subsequent experience, and no longer place a catheter after surgery.

With institutional review board approval, the patients’ charts were reviewed to assess the outcome at various intervals and results analysed, based on notes taken at the 2-year visit or the first visit after the 2-year period. All but two patients remained under the care of the institution, and information was available for visits either to the surgeon (in 28), the primary-care physician (in two), or the referring gynaecologist (in two). Because the two patients whose follow-up was at outside institutions were both known to have failed, their data were included as such on the assumption that they remained incontinent at 2 years. Failure was defined as either the patient stating she had persistent SUI, or pad use.

RESULTS

The SUI was reportedly cured in 27 of the 34 (79%) women at the 2-year follow-up; three (9%) of those with no complete resolution of SUI were pleased with the results of their procedure because of the improvement, and all three either wore one pad (two) or no pads (one) daily. These women they felt the operation had yielded a substantial improvement from baseline, which they felt was good enough that they desired no further intervention. Each had previously used at least three pads daily. The total cured or improved rate was therefore 88%.

Four women (12%) had persistent urge UI, but only one required the use of pads. Two of these four had a component of urge UI shown on urodynamics before surgery. Another who had surgery with no urodynamics beforehand had a clear history of urge UI, which was a secondary complaint to SUI requiring four pads/day. Only one woman had de novo urge UI, but was pleased with her outcome, with complete resolution of SUI. One patient with mixed urge UI and SUI at presentation failed to improve and eventually had an open pubovaginal sling, followed by placing a neuromodulation device. She continues to have only a partial response to therapy a year after her most recent intervention, and she is the only patient of the four with urge UI who has problems regularly enough to require the use of pads.

There was no prolonged urinary retention, although six patients (18%) required additional catheter drainage after the initial voiding trial (mean 8.6 days, maximum 18). These were early in the series, and presumably were caused by over-tightening of the sling. This is a subjective observation, but we noted that retention occurred early in the series, when there was greater concern that the material would migrate more easily than polypropylene. After 20 patients we decided to place the slings with the same spacing used for TVT, so a 1–2 mm space was left between the sling and urethra thereafter. This did not appear to correlate with a change in success rate, but seemed to address concerns of temporary retention. None of the patients with persistent urge UI had retention after surgery.

Four patients, including the woman described above with mixed UI, failed to have a significant prolonged improvement in SUI. One elderly patient was completely dry until she fell 3 weeks after surgery, fracturing several ribs; her incontinence thereafter was judged as only ‘improved’. Another elderly patient who was ‘improved’ had surgery early in the series; she had required 18 days of catheterization after the voiding trial. Urethral dilatation while placing downward traction on a Van Buren sound was used in an attempt to mobilize the sling; this manoeuvre was thought to have displaced or ruptured the sling.

Additional procedures during the surgery were anterior and posterior repair in five, major pelvic floor reconstruction (i.e. vaginal vault suspension) in four and diagnostic laparoscopy in two patients.

Three patients (9%) developed suprapubic inflammation 10, 21 and 45 days after surgery; two were minimally inflamed and they had complete resolution with oral fluoroquinolones, although one developed recurrent SUI a few weeks later and subsequently had an open autologous-fascia pubovaginal sling placed. A biopsy of her SIS would...
sling taken at the time of her re-operation was assessed (Fig. 2). The other patient had incision and drainage, revealing sterile inflammatory change in the subcutaneous tissues, which subsequently resolved during a period of empirical oral antibiotic coverage; she remains dry 30 months after surgery.

DISCUSSION

PVT and TVT procedures have minimized morbidity and recovery, but rely on synthetic materials. These procedures offer promising results, but carry the risk of infection, urinary retention or erosion, and must be surgically incised or removed if these complications occur [3]. Nonsynthetic slings such as SIS offer an alternative to those concerns.

Porcine SIS is a membrane from the animal’s small intestine, with no theoretical risks of human viral transmission from cadaveric fascia, pericardium or dermis. The cellular components are mechanically removed, leaving a biological scaffold for tissue remodelling. Functional growth factors, primarily fibroblast growth factor-2 [9], are thought to be vital to the regenerative process. Within weeks, the body absorbs the material and replaces it with a remodelled sling of neo-autologous tissue [10] (Fig. 2).

Although it seems to be a perception among pelvic surgeons that slings do not fail, peer-reviewed reports show that success rates are 80–85% [1]. Ward and Hilton [11] recently reported that TVT and colposuspension are both associated with success rates well below such perceptions. The inventor of the TVT reported an 85% success rate [12]; in that series, another 11% were judged to be improved.

The disadvantage in using SIS could potentially be related to its advantage, in that the material is not permanent. However, SIS is remodelled into a neo-autologous sling that appears histologically similar to an autologous fascia sling, as shown in Fig. 2. SIS placed using an open approach appears to be as effective as both autologous slings and TVT at 4 years after surgery, as reported recently [13], where 93% of patients remained dry after a mean of 48 months.

Three patients in the present series developed subcutaneous inflammation; two were mild and resolved with antibiotic coverage. The other had negative cultures, suggesting that the reaction could be inflammatory and not infectious. The material used in the present series is a four-layer, lyophilized version, in contrast to the eight-layer, air-dried version more commonly in use. The inflammatory process is poorly understood, but we consider, based on communication with the manufacturer, that this thinner tissue may be more readily remodelled and less likely to be associated with inflammation (Jason Hodde, personal communication, 2004). Ho et al. [14] recently reported that the inflammation associated with the eight-ply version appears to be usually self-limiting and unrelated to success rates. This risk must be weighed against the apparently lower risk of permanent urinary retention (which to our knowledge has never been reported with SIS, although it is likely that it will be at some time) than with synthetic slings. In addition, as the material is completely replaced with host tissue within months, the risk of vaginal or urethral erosion requiring surgical removal appears to be effectively negated.

The present study has the limitations of a retrospective chart review; it is well-recognized that patients may report a good outcome, stemming from a desire to please their surgeons. Thus we chose to use the most objective variable available from the chart review, i.e. pad use, in addition to notes of each patient’s response to questions about success. In addition, the presence or absence of urge UI was noted and reported even if not requiring pads. It is possible that some women had SUI that was not severe enough to wear pads and inaccurately denied it to their surgeons on questioning during follow-up visits.

These pilot data are relatively immature and must be validated with a longer-term follow-up, which would ideally involve pad testing and validated questionnaires. Comparison with established treatment options is needed. However, at 2 years, porcine SIS placed percutaneously as described appears to have success rates and complication rates comparable with TVT and other sling options.

In conclusion, a percutaneous nonsynthetic sling of porcine SIS offers a minimally invasive option for treating female SUI. Advantages include easy placement and safety, especially as it avoids placing a permanent prosthetic material near the urinary tract and vagina. The 2-year results are promising and rival those of synthetic slings such as PVT and TVT.

CONFLICT OF INTEREST

J. Stephen Jones is a research consultant for Cook Urological.

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Abbreviations: (S)UI, (stress) urinary incontinence; (P)(T)VT, (percutaneous) (transvaginal) vaginal tape; SIS, small intestinal submucosa.
Hyperbaric oxygen therapy for radiation-induced haemorrhagic cystitis

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OBJECTIVE
To assess the efficacy of hyperbaric oxygen (HBO) for treating haemorrhagic cystitis.

RESULTS
The haematuria resolved completely in all seven patients shortly after treatment; one had an improvement but died from complications relating to cancer shortly after completing treatment, and two had recurrence of gross haematuria. They were retreated with HBO until the haematuria resolved.

CONCLUSIONS
Radiation-induced haemorrhagic cystitis can be treated successfully with HBO primarily or after failure of standard regimens. This method was well tolerated even in patients debilitated by advanced cancer and blood loss. Long-term remission is possible in most patients, and re-treatment effectively manages recurrent bleeding.

KEYWORDS
hyperbaric oxygen therapy, radiation therapy, hemorrhagic cystitis

INTRODUCTION
Haemorrhagic cystitis can occur from 2 months to ≥10 years after pelvic irradiation. Levenback et al. [1] reported on 1784 patients who received radiotherapy for stage Ib cervical cancer over 29 years; haemorrhagic cystitis developed in 6.5%. Other studies reported an incidence of moderate to severe haematuria of 3–5% after radiotherapy for prostate cancer. The primary treatment for haemorrhagic cystitis is bladder irrigation; we initially start bladder irrigation with continuous saline, and the next step is cystoscopy and fulguration to stop bleeding bladder mucosa. If this treatment fails we initiate alum silver nitrate bladder irrigation. If all these methods fail we refer the patients for hyperbaric oxygen (HBO) therapy.

Oral and intravenous agents, e.g. aminocaproic acid, oestrogens and sodium pentosan polysulphate, have been tried with limited success. Intravesical treatments with alum silver nitrate, prostaglandins or formalin are sometimes used if bleeding persists. Finally, selective embolization of the hypogastric arteries, urinary diversion and cystectomy may be ultimately necessary in the most severe cases.

The potential clinical benefits of HBO have been reported for several decades. Among confirmed hyper-oxygenation physiological mechanisms operating in HBO are the induction of capillary angiogenesis and increased fibroblast concentration. These have also been established as micro-anatomical effects of HBO in irradiated tissues. HBO also induces healing of tissue damage, and decreases oedema, necrosis and leukocyte infiltration [2]. Recently HBO has emerged as a potential primary option for managing this challenging condition; we review our experience treating refractory haemorrhagic cystitis with HBO.

PATIENTS AND METHODS
Four men and three women (mean age 63 years, range 21–80) received HBO therapy for radiation-induced haemorrhagic cystitis. Ionizing radiation was administered for prostate cancer in the men, and metastatic breast cancer in one and primitive neuro-ectodermal tumour (PNET) in the women. Radiation was given for local disease and the mean dosage delivered was 64 Gy.

Patients with haemoglobin levels of <80 g/L, cardiac debilitated patients with haemoglobin levels of <90 g/L and patients with a fast decline in haemoglobin levels received a blood transfusion. Stabilization was defined as three consecutive haemoglobin levels of >100 g/L in 24 h.

The interval from original radiation treatment to HBO therapy was 3–180 months; before therapy six patients had cystoscopy and biopsies to exclude malignancy. All random biopsies showed histological changes consistent with post-radiation cystitis. One patient had had a nephroureterectomy for upper tract TCC before the diagnosis of prostate cancer and radiotherapy. Cystoscopy, ureteroscopy, urine cytology and random biopsy were used to exclude an underlying disease.

HBO was administered at 0.2 MPa for 90 min daily in a walk-in multiphase hyperbaric
Oxygen dissolved in the blood plasma in which a very high dose of oxygen may be achieved. The hyperbaric chamber provides conditions undersea and hyperbaric medicine criteria, the effect of HBO treatment on angiogenesis and osteogenesis in irradiated tissue is graded as level 1 [9].

The therapeutic effects of HBO for the treating long-term radiation effects were initially described by Marx and Ames [10] for post-irradiated head and neck cancer. Marx [3] redefined the sequence of the pathogenesis of radionecrosis as 1 (radiation), 2 (hypoxic-hypocellular-hypovascular tissue), 3 (tissue breakdown) and 4 (chronic non-healing wound). Beneficial effects of HBO on radiation-damaged tissue are related to the hyperoxia-induced primary neovascularization and secondary growth of healthy granulation tissue [11,12]. Additional benefits include vasoconstriction, which may help in reducing oedema, and improvements in wound healing and immune function [12–14].

HBO therapy enhances healing in a variety of radiation-injured tissues [12]. In an animal model, breathing 100% oxygen at normal atmospheric pressure produced no effect on angiogenesis in irradiated tissues. However, HBO at 2.4 AA produced an 8–9-fold increase in vascular density in irradiated tissues over normobaric oxygen and air-breathing controls. This stimulus for angiogenesis appears to be mediated at least partly through tissue macrophages responding to the steep oxygen gradient achieved in the hyperbaric environment [15]. Follow-up for up to 4 years after HBO therapy showed that transcutaneous oxygen measurements remain near normal levels, implying that the angiogenesis is essentially permanent [16].

Case series of radiation-induced haemorrhagic cystitis treated with HBO have been reported [17–29]; despite differences in the number of HBO treatments administered and characteristics of hyperbaric exposure among the various reports, most authors concluded that HBO therapy is effective for intractable radiation-induced haemorrhagic cystitis. If the earlier case series reported are combined, 82% of patients treated with HBO had an improvement or resolution of haematuria. The response to HBO depended on the severity of the presenting haematuria.

Although various authors reported a positive response to HBO for treating radiation-induced haemorrhagic cystitis the duration of follow-up varied. Del Pizzo et al. [25] reported on 11 patients treated with 28–64 HBO treatments and followed for a mean of 5.1 years. At a mean follow-up of 2.5 years eight of 11 patients were asymptomatic, while at 5.1 years five of the remaining eight had recurrent haematuria requiring hospitalization, blood transfusion and ultimately supravesical urinary diversion. Of these five patients two eventually required embolization and cystectomy. Of the 11 patients, three had a complete and durable resolution of symptoms at a mean of 5 years. This study highlights the progressive nature of radiation injury. The possibility that repeat HBO treatments might provide additional benefit has not been explored in detail. Investigators at Duke University analysed all published series and found that 40 HBO treatments was the optimum for acute resolution of symptoms and a long-term durable result [30].

The potential side-effects of HBO therapy are usually well tolerated. Some diabetic patients may have an exaggerated hypoglycaemic response to hyperoxia, the mechanism of which is yet to be defined. To minimize these side-effects, the patients’ glucose level should be closely monitored. CNS or any other obstructive pulmonary disease should be closely monitored. Patients with emphysema, a history of spontaneous pneumothorax and any other obstructive pulmonary disease should be closely monitored. CNS or pulmonary oxygen toxicity is unlikely in HBO treatment, which does not expose the patient to those oxygen side-effects.
In conclusion, haemorrhagic cystitis is a debilitating complication of radiation therapy for pelvic malignancy. Standard therapeutic methods have limited success and may have significant side-effects. HBO therapy is safe and noninvasive for treating the underlying histological changes that occur with radiation injury, resulting in long-term complete resolution in a large proportion of patients in whom standard treatment regimens fail. Early institution of HBO results in rapid resolution of haematuria. HBO therapy should be added as a preferred option for treating persistent haemorrhagic cystitis.

CONFLICT OF INTEREST

None declared.

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Abbreviations: HBO, hyperbaric oxygen; AA, absolute atmospheric (pressure); PNET, primitive neuro-ectodermal tumour.
Magnetic resonance imaging as a sole method for the morphological and functional evaluation of live kidney donors

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OBJECTIVE

To evaluate gadolinium-enhanced dynamic magnetic resonance imaging (MRI) as the sole method for the anatomical and functional assessment of potential live-kidney donors.

SUBJECTS AND METHODS

The study included 50 consecutive kidney donors; in addition to routine donor evaluation, the kidney was imaged with Gd-enhanced dynamic MRI, which was also used for selectively determining the glomerular filtration rate (GFR) of each kidney. All donors had a \textsuperscript{99m}Tc-mercaptoacetyltriglycine (MAG3) renal scan as the reference standard to measure GFR. The anatomical results of MRI were compared with the findings at donor nephrectomy, and the GFR estimated from MRI compared with that from MAG3 scintigraphy.

RESULTS

MR angiography had 100% sensitivity, 94% specificity and 96% overall accuracy for detecting the number of renal arteries, and 100% sensitivity, 98% specificity and 98% overall accuracy for the number of renal veins. There was a close correlation ($r = 0.54$, $P < 0.01$) between the GFR of each kidney estimated by MRI or MAG3. For the right and left kidneys the mean isotope clearance was not significantly different from that of mean MRI clearance. MR urography allowed visualization of the urinary tract and the detection of any abnormality.

CONCLUSION

Gd-enhanced dynamic MRI can provide accurate information about the anatomy of the urinary tract and vasculature of the kidney, and can be used to accurately estimate the selective GFR of each kidney. Therefore, we recommend MRI as a single imaging diagnostic method for assessing potential live kidney donors.

KEYWORDS

kidney, transplantation, living donors, MRI, GFR

INTRODUCTION

Renal transplantation is an effective treatment for patients with end-stage renal failure. Although cadaveric transplants continue to outnumber live-donor transplants by three to one, the number of live-donor renal transplants has increased...
steadily over the past decade. Transplants from living-related donors have better short- and long-term survival rates than do transplants from cadavers.

Selectively assessing the anatomy and function of each kidney is a fundamental part of evaluating potential donors. It is important for the urologist to have detailed anatomical information about the vasculature and morphology of the kidney and ureter, and to ensure that the donor has two well-functioning kidneys, and that renal function is evenly divided. If there is unusual asymmetry of function in the donor then measuring individual renal function should prevent the donor being deprived of the better kidney [1].

For decades, the morphology and function of the donor’s kidneys were traditionally assessed by several separate procedures, most commonly by ultrasonography (US), catheter angiography, excretory urography and radioisotope renal scans. In a recent study, contrast-enhanced spiral CT was recommended a single method for the anatomical and functional assessment of potential live-kidney donors [1]. However, CT subjects the patient to the risk of a high radiation dose. Moreover, the use of radiocontrast materials may increase the risk of renal and systemic toxicity. However, MRI offers donors the advantages of avoiding both radiation exposure and injection of potentially nephrotoxic iodinated contrast materials.

A combined MR examination including Gd-enhanced MR angiography (MRA), MR nephrography and MR urography (MRU) offers several potential advantages over conventional studies for anatomical evaluation [1,2]. In the present study, we describe a new ‘all-in-one’ MRI technique that provides both anatomical and functional information for each kidney. The diagnostic accuracy of the new technique for identifying the number of renal arteries and veins, and the morphology of the collecting system, was assessed by comparing the results with the findings at donor nephrectomy; the correlation between the GFR measured using the new MRI technique was also compared with that determined from a conventional radioisotope renogram.

SUBJECTS AND METHODS

Between January and December 2003, 50 consecutive potential kidney donors (30 men and 20 women, mean age 35 years, range 22–55) were prospectively included in the study. Apart from the routine evaluation of the donors, including abdominal US (to exclude renal stones or other abnormalities), the kidneys were imaged with Gd-enhanced MRI that was also used for selectively measuring the GFR of each kidney. All donors had a 99mTc MAG3 renal scan as the reference standard for measuring GFR; the two estimates of GFR were then compared. The anatomical results of MRI were compared with the findings at donor nephrectomy. All donors were evaluated clinically and had essentially normal biochemical clearance.

All MRI was conducted on a 1.5 T scanner (Signa Horizon LX Echo speed, General Electric Medical Systems, Milwaukee, WI, USA) with the use of phased-array torso surface coil. Before the start of MRI, 10 mg of frusemide was administered intravenously. The procedure started by obtaining a coronal localizer (scout image) to identify the abdominal aorta and the origins of the renal arteries, followed by a coronal T2-weighted sequence for the whole of both kidneys, and six coronal fast-spoiled gradient (FSPGR) slices of the centre of the kidney. Gadodiamide (Omniscan 0.5 mmol/mL Gd-DTPA-BMA; Nycomed, Ireland) was injected via a wide-bore veno-catheter in the antecubital vein at 3–4 mL/s. The contrast medium in the abdominal aorta, at suprarenal level, was automatically detected using SmartPrep software (General Electric Medical Systems). MRA used a breath-hold, three-dimensional (3D)-FSPGR acquisition in the coronal plane. The total amount of contrast medium was 20–30 mL, according to body weight, with a mean dose of 0.3 mmol/kg. The acquisition time was 12 s for each of the arterial and venous phases, with a 10-s gap between.

After finishing the arterial and venous phases of MRA, the pre-contrast six-slice coronal FSPGR at the centre of the kidney was repeated 10 times every 30 s and then at 15 min from injection of contrast medium. Gd-enhanced excretory MRU was then generated from a coronal contrast material-enhanced 3D-FSPGR with imaging parameters identical to those of MRA. No donors had contraindications for MRI; all studies were completed with no major complications. Only four subjects had claustrophobia, overcome by assurance.

The imaging parameters for coronal T2 were 5 mm thickness, no interslice gap, repetition time 8000–1000 ms, time to echo 75–95 ms, field of view 40 ¥ 40 cm and matrix 256 ¥ 196; the respective values for coronal FSPGR were 4 mm, no interslice gap, 30–40 ms, 2–3 ms, flip angle 70°, 42 ¥ 42 cm and 256 ¥ 160, for MRA were 2.6 mm, no gap, 0.9, 40 ¥ 32 cm, 256 ¥ 128 and slab thickness 30–50 mm. Reformatted maximum intensity projection (MIP) was used in different planes, e.g. coronal, sagittal oblique, axial and axial oblique, in the arterial and venous phases, to detect the number of vessels and to define any vascular abnormalities. Coronal and sagittal MIP for MRU was also used to identify the pelvicalyceal system and ureteric anatomy, and to define any abnormalities. Coronal T2 images were reviewed for any parenchymal or contour abnormalities, and to calculate parenchymal volume. The volume of the each renal unit was then calculated by drawing a manual region of interest (ROI) around each kidney at each T2 scan. The calculated surface area of pixels in each scan was transformed into millimetres automatically by the software. The total volume of the kidneys was calculated by adding the surface areas for each kidney and then the total surface area was multiplied by the slice thickness.

For dynamic scans, we first started by visually interpreting the images, comparing the series before and after contrast medium, to determine the corticomedullary differentiation, degree of parenchymal enhancement and the excretory power of each renal unit. Renographic dynamic MRI was generated by drawing ROIs over the kidney, excluding the renal pelvis. Using the functional software tool (GE Medical System) that merges all series, a curve resembling that from isotope renography was obtained. The MR dynamic renographic curve plots the enhancement units vs time (Fig. 1), and from the curve the time to the peak, the relative maximum units of enhancement (total enhancement units for each kidney minus the total on the unenhanced scan) and the response to diuretic were obtained. Curves were then obtained for the cortex and medulla for each kidney by applying a manual ROI over each at the same scan level, and from these curves the time at which the medullary response exceeded the cortex was also calculated (Fig. 2). Other circular ROIs were obtained from the aorta to determine the peak relative enhancement of the aorta.
To obtain an approximate GFR for each kidney, the total volume of each renal unit was multiplied by its peak relative enhancement, then divided by the peak relative enhancement of the aorta (to minimize the effect of differences in dose of contrast media and body weight of each subject, and differences in the rate of injection). The mean (range) post-processing time was 60 (45–70) min.

The results for vascular anatomy were reviewed and compared with operative data, considered the reference standard for vascular anatomy, including the number of renal arteries and veins. Functional data were correlated with the results of renographic clearance and 24-h total creatinine clearance. MRU results were also correlated with operative findings.

The sensitivity, specificity and overall accuracy of MRI for detecting the number of arteries were calculated. The proportion of surgically confirmed single renal arteries or veins was defined as sensitivity and the proportion of surgically confirmed multiple renal arteries or veins was defined as specificity. The correlation between MR clearance and isotope clearance was assessed using simple linear regression analysis, and mean MR and isotope clearance compared using Student’s t-test.

RESULTS

MRA in the 50 donors enabled the correct identification of the arterial supply to 48 kidneys, which included 30 single, 16 double and two triple renal arteries (Fig. 3a,b). One case was diagnosed as a single artery but was double, and one was diagnosed as double but was quadruple. Six cases with early branches were diagnosed accurately. MRA had 100% sensitivity, 94% specificity and 96% overall accuracy in identifying the arterial supply (Table 1).

For renal veins depicted at the second pass of 3D-FSPGR, MRA accurately diagnosed three cases with a retro-aortic left renal vein, two with circumaortic left renal veins and one with a double inferior vena cava. For identifying the number of veins, 45 cases with a single vein and four with double veins were diagnosed accurately. Only one case with double veins was diagnosed as a single vein. The sensitivity, specificity and overall accuracy for identifying renal veins at MRA was 100%, 98% and 98%, respectively (Table 1).

The MR nephrogram in the axial and coronal planes provided estimates of renal size and contour (Fig. 4a,b) that correlated with information from other complementary imaging. In one donor, a small (<1 cm) solitary renal cyst was seen on the MR nephrogram.

MRU correctly depicted the renal collecting system and ureters; 48 donors had single, unduplicated systems and ureters (Fig. 5). One donor had a unilateral malrotated pelvicalyceal system and another had a unilateral duplicated collecting system. These data agreed completely with the findings at donor nephrectomy.

For the functional assessment, the isotope clearance for each kidney was 54–77 mL/min, while the MR values were 217–277 units;
correlating the MR value with isotope clearance in each renal unit, the mean isotope clearance was 25 (20–33)% of the MR renographic value, and thus the corrected GFR for each unit was calculated from the MR study by dividing the MR value by 4.

A comparison between the isotope GFR of each kidney with the corresponding MRI GFR showed a close correlation \( r = 0.54, P < 0.01 \) and there was also a strong correlation between total 24-h creatinine clearance, total isotope GFR \( r = 0.44, P < 0.01 \) and total MR clearance \( r = 0.45, P < 0.01 \), confirming the validity of the isotope and MR techniques for assessing GFR. The mean isotope clearance was not significantly different from that of mean MR clearance for the right and left kidneys (Table 2). There was no significant difference between mean (SD) 24-h creatinine clearance and mean total MR clearance, at 118 (16) and 111 (19) mL/min, respectively.

The peak of enhancement was cortical and within the first 3 min, with a mean (SD) range of 114.5 (40.28, 68–179) s, followed by a smoothly decreasing slope. The corticomedullary exchange also occurred in the first 3 min, at 101.61 (35.58, 60–182) s, and then both curves decreased, the medullary at a slightly higher level than the cortical. The contrast media excretion began to appear in the cortex after 3 min, and the calyces and ureter were completely defined at the 15 min scan; this was represented in the whole-kidney MR renographic curve.

**DISCUSSION**

Gd-based MRI contrast agents that are small molecules, similar to inulin, are distributed in the vascular space and then rapidly into the extracellular space. These agents can serve as extracellular fluid markers and are freely filtered in the glomeruli. As they are neither secreted nor absorbed, they are an excellent indicator of renal function [3]. Nuclear medicine renography provides functional information but with relatively poor spatial resolution. US has better spatial resolution than nuclear methods but provides no functional information, although new US contrast agents could be promising for the study of renal dynamics. CT offers excellent spatial resolution with the potential to assess renal dynamics, but many exposures and iodinated contrast agents are needed, subjecting the patients to the risk of high radiation dose and increasing the risk of renal and systemic toxicity [4].

As MRI continues to develop, renal MRA will probably expand to include extensive functional information about creatinine clearance, flow and response to pharmacological agents, as well as spectroscopy, diffusion, perfusion and other techniques [5]. The advantages of comprehensive MRI include its noninvasive nature, no use of iodinated contrast materials, absence of exposure to ionizing radiation, decreased morbidity and reduced cost compared with that of conventional angiography combined with excretory urography [6].

Several studies have used contrast-enhanced MRA for evaluating potential living donors, with excellent results [7–10]. The present results showed a very high sensitivity, specificity and overall accuracy, similar to the results in previous studies for detecting accessory renal arteries, early branching and vascular pathology, as compared with the reference standard. Less accurate results were reported by Rajab et al. [11], who stated that MR interpretations correctly identified the vascular anatomy of the donor kidneys in 173 of 189 (91.5%). However, they concluded that noninvasive MR evaluation of donor renovascular anatomy is an acceptable substitute for traditional angiography, because the misinterpretation of the MR angiography did not adversely affect the recipients’ outcome. Hussain et al. [12] stated that MRI provides valuable information comparable with that from other imaging methods, e.g. US, IVU and digital subtraction angiography. CT angiography is currently not used in living kidney donors at their hospital.

Other investigators [9,13] compared CT and MRA, reporting substantial agreement. Interobserver disagreement in the interpretation of CT and MR angiograms is
related to 1–2-mm diameter vessels [7]. CT angiography and MRA provide all the information required by the surgeon. Both methods may miss small accessory renal arteries. MRA does not use potentially toxic contrast material or radiation and is the preferred investigation, with CT reserved for patients unable to tolerate MRI [9].

MRU is obtained from the same Gd-enhanced 3D-FSPGR acquisition used for the MRA, after a delay of ~15 min from injecting frusemide before the contrast material, leading to rapid uniform Gd distribution inside a relatively distended collecting system. The present results of MRU for depicting the collecting system and ureters were comparable with the operative data. Like CT with delayed images, Gd-enhanced MRI has a high sensitivity and specificity for depicting the pelvicalyceal anatomy and pathology. The accumulation of bright contrast material in the renal pelvis and calyces is sufficient to provide a high resolution of renal anatomy and good image quality. The overall ureteric position and morphology are evaluated well by Gd-enhanced MRI, with no ureteric blurring from the peristaltic waves [14,15].

Coronal images of the kidney (six slices, 5 mm thick) are acquired before and after injection with contrast material, to obtain a MR renographic study similar to the radionuclide renogram. The present MR renograms were similar to those obtained by Katzberg et al. [4], as there was an initial peak, representing the vascular component of contrast medium in the glomerular tufts in the cortex [3,16], followed by the tubular response that reflects the passage of contrast agent through the glomeruli into the tubular system, manifested by accumulation of contrast agent in the medulla with increasing concentration. The third phase of the dynamic renogram is the ducal phase, manifested by the higher plateau of the signal intensity curve over time for the medulla vs the cortex. The fourth phase is the calyceal phase, in which contrast medium is excreted into the pelvicalyceal system. However, the present results were markedly different from those reported by Semelka et al. [16], who found that the medullary signal intensity was lower than cortical intensity for all kidneys over time in normal kidneys and those with hydronephrosis. In the present study, the time at which the medullary curves of normal kidneys crossed those of the cortex was <3 min and the medullary curves continued at a slightly higher level than the cortical plateau. The brightness of signals at the calyces was apparent after ~3 min, depending on the excretory power of the kidney, and at delayed 15-min scans the ureter could be visualized adequately.

Beside general contraindications there are some limitations of MRI. It does not show most renal calculi and therefore we recommend that US be used beforehand. US is also used as a screening method to detect other urinary or extra-urinary abnormalities that might preclude safe renal donation. Moreover, motion artefacts may degrade the image quality of MRI and can be reduced with the use of fast and ultrafast sequences. Also, the relatively long post-processing time can be overcome by improving the software.

On the basis of a decision- and cost-effectiveness analysis, Liem et al. [17] concluded that digital subtraction angiography is the most cost-effective strategy if it has a specificity of >99% for detecting renal disease; otherwise, MRA with CT angiography is the most cost-effective strategy. The cost-effectiveness is not only direct but also includes the advantage of lack of exposure to ionizing radiation from conventional angiography, CT and renography, and avoiding the potential adverse reaction to iodinated contrast material, that may include anaphylaxis and nephrotoxicity.

In the present study we tried to use a simple technique for easy quantification of parenchymal enhancement by multiplying the total volume of each renal unit by its peak enhancement, and then divided by the aortic enhancement. Using this technique there was a good correlation between GFR values obtained by MR and those by isotope renography. By multiplying the MR renographic value by 0.25 we estimated a corrected MR GFR equivalent to isotope GFR.

In conclusion, Gd-enhanced dynamic MRI has several advantages for assessing potential live-kidney donors. In the vascular phase, MRA can clearly visualize the number of renal arteries and veins, and detect any congenital or acquired vascular anomaly. Moreover, in the parenchymal phase, MRI is as accurate as radioisotope renography in determining the relative GFR of each kidney, allowing selection of the kidney for nephrectomy. In addition, the MR nephrogram provides estimates of renal size and contour, and can identify parenchymal defects. Finally, MRU allows the visualization of the pelvicalyceal system, ureter and bladder, with the detection of any abnormality. Therefore, we recommend Gd-enhanced dynamic MRI as a single imaging method for assessing potential live-kidney donors.

CONFLICT OF INTEREST

None declared.

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**Abbreviations:** US, ultrasonography; MRA(U), MR angiography (urography); (3D)–FSPGR, (three-dimensional)–fast-spoiled gradient (scan); MIP, maximum intensity projection; ROI, region of interest.
Vitamin E therapy prevents hyperoxaluria–induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status

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OBJECTIVE
To determine whether vitamin E prevents hyperoxaluria–induced stone formation, using a new animal model of calcium oxalate stone disease, as our previous in-vitro and in-vivo studies showed that oxalate and hyperoxaluria induce free-radical generation, which results in peroxidative injury to renal tubular cells.

MATERIALS AND METHODS
Ethylene glycol (EG) was administered at 150 mg/day by gavage for 3 weeks to rats fed on diets with adequate (group 1), excess (group 2) or deficient (group 3) vitamin E. Several indicators of peroxidation, free radicals and enzymatic activity were then assessed.

RESULTS
EG treatment in group 1 lead to increased lipid peroxidation, protein thiol, excretion of urinary enzymes, oxalate and decreases in urinary calcium, antioxidant enzymes and altered glutathione redox balance. Although renal function was not altered, there was increased water intake, urine volume and lowered urinary pH in these rats. These changes were more intense, with extensive calcium-oxalate crystal deposition, in rats in group 3, and prevented in rats in group 2, except for urinary oxalate levels, which remained high. Histopathological examination showed that there was no deposition of calcium oxalate crystals in rats in group 2.

CONCLUSION
This is the first study to demonstrate in-vivo evidence that hyperoxaluria–induced peroxidative injury induces individual calcium oxalate crystal attachment in the renal tubules. In addition, excess vitamin E completely prevented calcium oxalate deposition, by preventing peroxidative injury and restoring renal tissue antioxidants and glutathione redox balance. Therefore, vitamin E therapy might provide protection against the deposition of calcium oxalate stones in the kidney of humans.

KEYWORDS
l lipid peroxidation, urolithiasis, hyperoxaluria, vitamin E, antioxidants, ethylene glycol, Sprague-Dawley rats

INTRODUCTION
Hyperoxaluria is one of the major risk factors for calcium oxalate kidney stone formation in humans [1]. Oxalate is normally excreted by the kidneys, and 60–80% of renal calculi are composed of calcium oxalate [1–3]. Oxalate present in many foods is poorly absorbed from the intestine, with only 5–15% of dietary oxalate appearing in the urine; the remaining 85% of the oxalate is produced endogenously [4]. A recent report showed an endogenous contribution closer to 50%, and the remainder being of dietary origin [5].

The prevalence of urinary tract stone disease is estimated to be 2–20 per 10 000 [3]. The recurrence rate with no treatment for calcium oxalate renal stones is 10% at 1 year, 33% at 5 years and 50% at 10 years [6]. Most patients with calcium oxalate renal calculi excrete large amounts of calcium and/or oxalate in their urine. Hypercalciuria can result from several causes, including increased gut absorption, reduced tubular reabsorption, and resorptive hypercalciures characterized by increased bone demineralization [7]. Levels of urinary oxalate are increased in 15–50% of patients with idiopathic calcium oxalate urolithiasis [1,8]. The diagnosis and initial management of urolithiasis has developed considerably in recent years. Various therapies, including alkali citrate, thiazide, dietary modifications, reduction in animal protein, and foods rich in glycolate and glyoxylate, have been tried in an attempt to prevent stone recurrence [7,9]. Despite recent advances in endourological, uroscopic and ESWL, stone recurrence can be reduced by only half.

It is clear from our previous in-vitro and in-vivo studies that oxalate–induced peroxidative injury is involved in the nucleation, aggregation and development of calcium oxalate stone disease [10–13]. The cell is endowed with several antioxidant systems, including enzymatic (superoxide dismutase, SOD, catalase and glutathione peroxidase, GPx) and non-enzymatic, e.g. reduced glutathione (GSH), vitamins E, A and C, to limit the extent of lipid peroxidation. Up to a certain limit, the cells are able to control the damage with GPx, catalase, SOD or other antioxidative mechanisms. However, once a threshold of damage or rate of damage is exceeded the cellular defences are overwhelmed and a very small additional insult results in severe cellular injury. Thus, the oxidant-antioxidant balance is a critical determinant of cell sensitivity to free-radical injury. Several laboratories reported that oxalate causes renal tubular injury by increase generation of free radicals [14,15]. In the present study therefore we sought to determine whether vitamin E offers promise as a therapeutic agent for preventing kidney stone formation in an animal model of hyperoxaluria, and describe...
a new model of calcium oxalate stone disease.

MATERIALS AND METHODS

Male Sprague-Dawley rats (40–45 g) were used; they were kept in a temperature-controlled room with 12-h light and 12-h dark cycles, housed individually in stainless-steel cages and given free access to diet and deionized water. The experimental protocol was reviewed and approved by the institutional animal care and use committee.

The rats received one of three diets: the first group had a diet adequate in vitamin E (Purified diet; Harlan Teklad, Madison, WI; 100 U vitamin E/kg) containing 50.0 g/kg corn oil with mineral mix #170915 and 0.2 g/kg \( \alpha \)-tocopherol acetate (specific activity 500 U/g), with the following nutrients: vitamin-free casein, 200 mg/g; DL-methionine, 3.0 mg/g; dextrose monohydrate, 674.5 mg/g; cellulose fibre, 50 mg/g; calcium carbonate, 3.5 g/g; choline dihydrogen citrate, 3.5 mg/g; dry vitamin A palmitate, 0.04 mg/g (specific activity 500 000 U/g); dry vitamin D3, 0.0044 mg/g (specific activity 500 000 U/g); vitamin B12, 0.05 mg/g; biotin, 0.0004 mg/g; calcium pantothenate, 0.066 mg/g; folic acid, 0.002 mg/g; inositol, 0.11 mg/g; menadione, 0.05 mg/g; niacin, 0.1 mg/g; pyridoxine HCl, 0.022 mg/g; riboflavin, 0.022 mg/g; and thiamine HCl, 0.022 mg/g. The \( \alpha \)-tocopherol acetate content of the diet adequate in semisynthetic vitamin E (normal control) was calculated based on the composition of standard rodent diet Teklad #8640 (109.54 IU/kg) and Teklad #8604 (90.18 IU/kg). In the second group, rats were fed a diet with excess vitamin E (Purified diet; Harlan Teklad, 2000 U/kg) containing corn oil 50.0 g/kg with mineral mix #170915 and 4.0 g/kg of \( \alpha \)-tocopherol acetate, with the above nutrients. The third group were fed a diet deficient in vitamin E, containing 10% \( \alpha \)-tocopherol-stripped corn oil (Purified diet; Teklad Test Diets), with the above nutrients. All diets were stored refrigerated in tight polythene bags. Food was changed every day to minimize vitamin E oxidation. The levels of vitamin E in the diet did not influence the growth rate of the rats for the treatment period.

MODEL VALIDATION

A preliminary study showed that the total intake of water per day was constant for each rat and differed among rats, thus we monitored water intake for 30 days for individual rats.

To estimate the amount of oxalate excretion after exposure to ethylene glycol (EG), rats were selected for a preliminary experiment as described below. The rats were divided into four groups with six in each and fed vitamin E-adequate diet: (A) control, (B) 100 mg EG, (C) 150 mg EG, and (D) 200 mg EG. EG was given once to each rat by gavage. Urine was collected for the first and second 24-h periods after giving EG and assayed for oxalate.

Based on the preliminary data obtained, a separate set of animals from a similar group were given 0, 100, 150 or 200 mg EG each day by gavage for 7, 14 or 21 days. The gain in body weight was monitored every week. The rats were killed 24 h after the last dose at 7, 14 and 21 days. The kidney was removed and fixed in formaldehyde for histopathological evaluation.

For the main study, rats were divided into six experimental groups (eight per group). The rats were selected based on food intake (18.5 ± 3 g/day), water intake (24 ± 2 mL/day) and body weight (initial body weight 40 ± 5 g; body weight on the first day of EG administration 340 ± 10 g). Groups I and II received a diet adequate in vitamin E, III and IV excess vitamin E, and V and VI deficient in vitamin E, for 6 weeks, after which hyperoxaluria was induced in groups II, IV and VI by EG (by gavage) at 150 mg/rat per day for 3 weeks. Groups I, III, and V were considered as controls (no EG). Food and water intake was recorded every day, and body weights monitored weekly. Twenty-four hour urine samples were collected at 0, 7, 14 and 21 days in 50 mL centrifuge tubes kept on ice and attached to urine-collecting funnels. Water intake, urine volume, crystalluria and pH were recorded. For the enzyme determinations, urine samples were dialysed for 3 h at 4 °C against distilled water. The rats were killed 24 h after the last oral dose of EG; the animals were anaesthetized with pentobarbital (50 mg/kg body weight), and the kidneys were quickly excised and used for the following analyses. A portion of the kidney was homogenized and assayed for enzyme activities. For the histopathological analyses, kidney tissue was fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5–6 μm, and stained with haematoxylin and eosin for microscopic examination.

Markers of oxidative stress were malondialdehyde (MDA) content, representing lipid peroxidation, determined by the thiobarbituric acid reactive method [16]. Protein carbonyls were measured according to the method of Levine et al. [17]. Antioxidants comprised vitamin E, determined by the method of Arnaud et al. [18] using HPLC. SOD was measured as described by Misra and Fridovich [19] and catalase using the method of Sinha [20]. The variables in the glutathione redox system, were GSH content, analysed with a modification of the enzymatic recycling assay using 5-thio-2-nitrobenzoate to form a spectrophotometrically detectable product at 412 nm (ε = 1.36 × 10^3 mole^-1 cm^-1) by the method of Tietze [21]. GPx activity was measured by the spectrophotometric method of Paglia and Valentine [22], and glutathione reductase activity in total cell homogenates with a spectrophotometric assay [23]. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined according to the method of Deutsch [24].

Markers of glomerular and tubular damage were blood urea nitrogen (BUN), measured according to the method of Crocker [25]. Serum creatinine was measured by the method reported previously [26] using a spectrophotometer; 24-h urine samples were processed for the determination of the following enzymes as described in our earlier studies [27]. Lactate dehydrogenase was analysed spectrophotometrically using pyruvate as a substrate [28]. A tubular brush border marker enzyme, alanine aminopeptidase, was determined by the method of Jung and Scholz [29]. Alkaline phosphatase and γ-glutamyl transpeptidase (GGT) were assayed using a commercial kit (Trinity Biotech, MO).

Urinary oxalate and calcium were determined by an ion-exchange chromatography method with some modifications [27], with a Dionex gradient ion-chromatography system equipped with a 0.4 x 25 cm AS11 anion exchange analytical column with a AG11 guard column for oxalate or CS12A cation exchange analytical column with a CG12A guard column for calcium. Sample (25 μL) was injected using an auto-sampling injector and eluted with 40 mmol/L NaOH/deionized water at 1.0 mL/min with a linear gradient from 10% to 75% NaOH, 90% to 25%
VITAMIN E AND HYPEROXALURIA-INDUCED CALCIUM OXALATE CRYSTAL DEPOSITION

TABLE 1 Scoring system for estimating crystal abundance in the kidney section of rats (six per treatment) fed a vitamin E–adequate diet followed by EG

<table>
<thead>
<tr>
<th>EG, mg/day</th>
<th>Days of EG</th>
<th>7</th>
<th>14</th>
<th>21</th>
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<tr>
<td>100</td>
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<td>150</td>
<td>–</td>
<td>+</td>
<td>+++</td>
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<td>200</td>
<td>+</td>
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</table>

–, no crystals; +, few crystals [one or two per field]; ++, moderate number of crystals [10–20 per field]; ++++, frequent crystals [≥20 per field]; ++++, abundant crystals [>100 per field].

deionized water for oxalate or 40% of 30 mmol/L methane sulphonic acid and 60% deionized water (isocratic) for calcium, after helium degassing. The column elution was monitored using a conductivity cell and peak area measured with Peaknet chromatography automation software v6.20. Background conductivity was minimized by using an ASRS anion self-regenerating micromembrane suppressor for oxalate or CSRS cation self-regenerating micromembrane suppressor for calcium and with recycled eluent. Sample oxalate or calcium concentration was then calculated based on an oxalate or calcium standard curve.

All experiments were repeated eight separate times in duplicate, with the results given as the mean (SD). Data were analysed by three-way ANOVA with Tukey’s multiple comparison, with P < 0.05 considered to indicate significant differences. Multivariate regression analysis were used to assess the relationship between excretion of oxalate and tissue antioxidants, urinary enzymes and lipid peroxidation, and between peroxidation, tissue antioxidants and urinary enzymes.

RESULTS

Most animal models of calcium oxalate stone disease have required the generation of fairly severe hyperoxaluria. Various approaches have been used for this, including exposure to EG, oxalate infusion or feeding, and pyridoxine depletion [30]. However, the utility of these treatments is compromised by one or more limitations [31]. When we used the previously available conventional model of urolithiasis (0.75% EG through drinking water) for our experiments with rats on purified diets with adequate, excess and deficient vitamin E, we had difficulty interpreting the experiments. This widely used model for human stone disease resulted in massive crystal deposition is some rats and no or fewer crystal deposits in others. Normal Sprague-Dawley rats drink 10–35 mL of water/day. When we monitored individual rats the water intake of one rat was 10.5 (3.0) mL/day; the water intake was constant for this rat and did not differ significantly from day by day. The intake of another rat was 35.2 (4.3) mL/day, and for a third 25.4 (2.9) mL/day. Therefore, each rat has a unique, constant drinking water requirement. Rats drinking 10 mL/day of 0.75% EG had no crystal deposition in the kidney by 30 days but those drinking 25 mL/day of 0.75% EG developed calcium oxalate depositions by 15 days, and those drinking 35 mL/day of 0.75% EG did so by 7 days. Moreover, EG consumption causes polyuria or polydipsia, and therefore increased consumption of EG, from 35 to 45–50 mL/day on day 5, resulted in increased oxalate synthesis and massive blockages of renal tubules with calcium oxalate crystals in all the groups. Therefore, we developed a new calcium oxalate stone model to induce controlled endogenous oxalate synthesis.

To estimate the amount of oxalate excretion after giving EG, male Sprague-Dawley rats were divided into four groups with different amounts of EG (0, 100, 150, 200 mg) given once to each rat by gavage. Oxalate was significantly increased in urine on the first day (0–24 h) after EG in the four groups (A-D, 0–200 mg EG), at 3.52 (0.42), 32.09 (5.23), 83.45 (9.85) and 125.46 (12.6) μmol/24 h. The following day’s collection (24–48 h after EG) showed that oxalate was completely excreted within 24 h for groups B and C, whereas the oxalate was still higher in group D, with respective values of 3.31 (0.26), 2.8 (0.32), 4.50 (0.52) and 6.36 (0.45) μmol/24 h. This shows that increased excretion of oxalate was directly proportional to the amount of EG administered.

The next set of animals, treated with 0, 100, 150 or 200 mg EG each day by gavage for 7, 14 or 21 days, showed that the EG-treated rats gained substantially less body weight than the control group (data not shown). In the histopathological evaluation, each kidney was scored for crystal deposition using the scoring system shown in Table 1. From these results we estimated that EG at 150 mg/day through gavage induces a controlled amount of oxalate synthesis to produce hyperoxaluria and may prove a useful in–vivo model to study stone disease. In addition, this model may offer an appropriate option for evaluating therapeutic approaches and advantages over the uncontrolled oxalate synthesis in 0.75% EG given to rats in drinking water. Although there were calcium oxalate crystals in the papillary region, a comparison between Randall’s plaque and the present observations is hampered by several difficulties. First, there is the obvious difference in species (human vs rat). Second, the approach (deductive vs inductive); and last, there is a difference between the electrolytes involved (calcium oxalate vs various calcium phosphates and carbonates) [32,33]. Khan [34] noted many similarities between the effects in experimental nephrolithiasis (including the EG protocol) induced in rats, and human kidney stone formation. It was also reported that the rat model of calcium oxalate nephrolithiasis can be used to investigate the mechanisms involved in human kidney stone formation [34].

As peroxidation was considered an important mechanism involved in many pathological conditions, we sought to determine whether vitamin E supplementation prevents peroxidation in rats treated with EG. Kidney tissue lipid peroxidation was estimated as MDA level (Table 2), and protein carbonyls were assessed as an indicator of protein peroxidation products (Table 2). Rats in group II had significant greater MDA levels and protein carbonyls than in group I. Hyperoxaluria-induced generation of MDA and protein carbonyls was significantly prevented in group IV. There was no significant change in MDA or protein carbonyl content in rats in groups I, III and V. However, rats in group VI had a dramatic increase in MDA and protein carbonyl contents. Supplementation with vitamin E therefore has a protective role against hyperoxaluria-induced oxidative injury.

To provide evidence that supplementation with vitamin E protects against hyperoxaluria-induced renal peroxidative damage, the kidney tissue vitamin E levels were also measured; as shown in Table 2, hyperoxaluria was induced in rats in group II and resulted in a significant decrease in the
levels of tissue vitamin E concentration; this may indicate the consumption of vitamin E by hyperoxaluria-induced free radical generation. The concentration of vitamin E in the kidney was significantly higher in the rats in group III and IV; exposure to EG was also associated with the consumption of vitamin E, as indicated by the decrease in the tissue vitamin E concentration, indicating that vitamin E positively combats hyperoxaluria-induced free radical generation. However, the tissue levels of vitamin E remained higher in group IV than in group V. Vitamin E deficiency in rats in groups I and V was validated by measuring the tissue vitamin E content. After vitamin E deprivation, there was a marked (93%) decrease in the levels of vitamin E in the kidneys. The tissue vitamin E content was significantly less in rats in group VI.

To examine the effect of vitamin E on hyperoxaluria-induced changes in kidney antioxidant and glutathione redox status, the enzymatic and non-enzymatic antioxidant levels were also assessed (Table 2). Rats in group II at 21 days had significant less SOD, catalase, GPx, glutathione reductase and G6PD activities, and GSH levels than in group I. In group IV the excess vitamin E significantly restored these enzyme activities and GSH levels towards the control levels. As expected, hyperoxaluria induced in rats in group VI produced a significant lower antioxidant enzyme and GSH concentration than in group II. There were no significant changes in the antioxidant or glutathione redox levels in rats on the three control diets. These data strongly indicate that dietary vitamin E strengthened the tissue antioxidative defence system. Vitamin E was accordingly found to reduce the hyperoxaluria-induced accumulation of reactive oxygen species and to significantly improve the tissue antioxidant status.

The indices of renal function are also summarized in Table 2. In groups II and IV, EG caused no significant increase in BUN or serum creatinine levels, whereas in group VI rats had significantly lower renal function, as indicated by the increased BUN and serum creatinine levels. As changes in renal tubular enzymes in 24-h urine are a sensitive index of renal tubular damage we studied the effect of vitamin E on EG-induced changes in the excretion of renal tubular enzymes. Rats in group II had a significant change in the activity of renal enzymes at all sample times. The cytosolic enzyme lactose dehydrogenase (LDH) had significantly greater activity in the urine of all EG-treated rats at 7, 14 and 21 days than in the control groups (Fig. 1a). Urine from rats in group IV had a significantly lower LDH activity than in group II. There were no significant differences among the control rats in any group, but the urinary excretion of LDH was significantly higher in group VI than in group II at 7 and 14 days. Brush border marker enzymes, GGT (Fig. 1b), alkaline phosphatase (Fig. 1c), and alanine amino peptidase (Fig. 1d), had significant changes in their excretion pattern in groups II, IV and VI (EG). The activity of brush border marker enzymes was significantly greater in urine at 7, 14 and 21 days in rats in group II. These enzyme excretions were further increased in group VI for up to 14 days and drastically decreased at 21 days. Rats in group IV had a significant restoration of these enzyme activities towards control levels. There were no significant changes in these urinary enzyme activities in the control rats in any of the vitamin E regimens.

Rats in groups II, IV and VI (EG-treated) had significantly greater urinary oxalate levels (Fig. 2) and lower calcium excretion (Table 2) than controls at all sample times. Rats in

<table>
<thead>
<tr>
<th>Mean (±SD) variable</th>
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<td>II</td>
<td>III</td>
<td>IV</td>
<td>V</td>
<td>VI</td>
</tr>
<tr>
<td>MDA, nmol/mg protein</td>
<td>1.23 (0.22)</td>
<td>3.26 (0.18)*</td>
<td>1.11 (0.16)*</td>
<td>1.36 (0.24)*</td>
<td>1.63 (0.21)*</td>
<td>5.77 (0.48)*</td>
</tr>
<tr>
<td>Protein carbonyls, nmol/mg protein</td>
<td>3.34 (0.42)</td>
<td>5.94 (0.39)*</td>
<td>2.88 (0.27)*</td>
<td>3.32 (0.35)*</td>
<td>3.72 (0.41)*</td>
<td>7.78 (0.37)*</td>
</tr>
<tr>
<td>α-tocopherol, µg/g</td>
<td>37.2 (3.2)</td>
<td>22.4 (2.5)*</td>
<td>70.6 (4.6)*</td>
<td>60.5 (4.2)*</td>
<td>2.5 (0.3)*</td>
<td>1.5 (0.2)*</td>
</tr>
<tr>
<td>Antioxidant enzymes and glutathione-redox system components</td>
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<tr>
<td>SOD, µmol/mg protein</td>
<td>30.2 (2.8)</td>
<td>16.8 (1.1)*</td>
<td>34.0 (1.9)*</td>
<td>26.2 (2.2)*</td>
<td>27.9 (3.4)*</td>
<td>11.2 (1.3)*</td>
</tr>
<tr>
<td>Catalase, µmol H2O2 consumed/min/mg protein</td>
<td>35.1 (2.4)</td>
<td>20.7 (1.8)*</td>
<td>33.6 (2.7)*</td>
<td>28.5 (2.5)*</td>
<td>31.9 (1.7)*</td>
<td>11.0 (1.3)*</td>
</tr>
<tr>
<td>GPx, µmol/mg protein</td>
<td>160.0 (14.3)</td>
<td>82.6 (6.5)*</td>
<td>166.2 (7.3)*</td>
<td>131.5 (10.8)*</td>
<td>155.2 (10.5)*</td>
<td>48.7 (3.4)*</td>
</tr>
<tr>
<td>Glutathione reductase, nmol NADPH oxidized/min/mg protein</td>
<td>120.5 (5.8)</td>
<td>55.2 (4.2)*</td>
<td>125.2 (7.3)*</td>
<td>113.4 (6.8)*</td>
<td>112.0 (6.5)*</td>
<td>32.6 (3.7)*</td>
</tr>
<tr>
<td>G6PD, µmol/mg protein</td>
<td>17.7 (1.3)</td>
<td>9.6 (0.8)*</td>
<td>18.5 (1.7)*</td>
<td>14.6 (1.1)*</td>
<td>16.4 (1.4)*</td>
<td>5.8 (0.8)*</td>
</tr>
<tr>
<td>GSH, nmol/mg protein</td>
<td>19.4 (1.5)</td>
<td>11.6 (0.9)*</td>
<td>22.6 (1.1)*</td>
<td>17.2 (1.2)*</td>
<td>17.8 (1.5)*</td>
<td>6.2 (0.7)*</td>
</tr>
<tr>
<td>Blood values</td>
<td></td>
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<td></td>
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<tr>
<td>BUN, mg/L</td>
<td>220 (22)</td>
<td>252 (18)</td>
<td>230 (21)</td>
<td>242 (21)</td>
<td>250 (21)</td>
<td>496 (34)*</td>
</tr>
<tr>
<td>Creatinine, mg/L</td>
<td>6.7 (0.5)</td>
<td>7.1 (0.5)</td>
<td>6.3 (0.6)</td>
<td>7.0 (0.8)</td>
<td>7.0 (0.3)</td>
<td>8.9 (0.4)*</td>
</tr>
<tr>
<td>Urinary variables</td>
<td></td>
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<tr>
<td>Water intake, ml/24 h</td>
<td>22.6 (2.2)</td>
<td>44.2 (3.6)*</td>
<td>23.0 (2.4)*</td>
<td>31.2 (2.0)*</td>
<td>22.9 (2.8)*</td>
<td>55.2 (3.2)*</td>
</tr>
<tr>
<td>Urine volume, ml/24 h</td>
<td>10.2 (1.3)</td>
<td>12.2 (1.2)*</td>
<td>18.6 (1.1)*</td>
<td>13.1 (1.7)*</td>
<td>42.2 (3.6)*</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.04 (0.04)</td>
<td>5.71 (0.05)*</td>
<td>6.10 (0.04)*</td>
<td>5.81 (0.05)*</td>
<td>6.05 (0.06)*</td>
<td>5.70 (0.05)*</td>
</tr>
<tr>
<td>Calcium, mg/24 h</td>
<td>1.72 (0.32)</td>
<td>0.64 (0.08)*</td>
<td>1.96 (0.22)*</td>
<td>0.78 (0.10)*</td>
<td>1.83 (0.17)*</td>
<td>0.51 (0.09)*</td>
</tr>
</tbody>
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*P < 0.05, eight rats. Comparisons: a, significant vs I; b, significant vs II; c, significant vs III; d, significant vs IV; e, significant vs V.
group VI had significantly greater urinary oxalate levels at 7 and 14 days, but significantly lower levels at 21 days. There were statistically significant increases in water intake and urine output, and a lower urine pH, in the rats in group II at 21 days (Table 2). These changes were drastically greater in group VI. Rats in group IV had water intake and urine output, and a lower urinary oxalate and urine pH, in the rats in group II at 21 days but precipitation of calcium oxalate crystals (+++)) showed focal involvement of the renal parenchyma, with some areas remaining free of crystal deposition. Disruption of crystal-containing tubules was also evident. Numerous crystals were present in the cortex, medulla and in the papilla. The kidney was enlarged and it was heavier than in group I (Fig. 5B). The kidney sections of rats in group IV after 21 days showed no calcium oxalate crystal deposition in any part of the nephron segment. Five of the eight rats had a normal kidney size and weight; three rats had mild enlargement of the kidney and the kidney was heavier (Fig. 5C). The pathological evaluation of kidney sections of rats in group VI showed diffuse and markedly extensive (+++) calcium oxalate deposits in the tubules of the cortex, medulla and collecting tubules (Fig. 5D). The crystal rosettes often completely occluded the tubular lumens. Intraluminal cellular debris and lymphocytic infiltration were sometimes found in association with crystal deposits. The decrease in urinary enzyme activities and reduced renal function, with the increase in urine volume in rats in group VI after 21 days may be caused by extensive calcium oxalate crystal deposition and significant blockage of renal tubules, which might
interfere with the filtration processes. The kidney was significantly larger than in the controls.

**DISCUSSION**

The present study provides direct evidence that vitamin E supplementation completely prevented calcium oxalate crystal deposition in the kidney, by preventing free radical-induced renal injury and by restoring antioxidant levels. Hyperoxaluria reduced vitamin E levels and increased MDA content and protein carbonyl in rats in group II, indicating increased demand for vitamin E during hyperoxaluria. This is direct evidence of increased use of vitamin E when free-radical generation is induced by hyperoxaluria, and therefore outlines the protective role of vitamin E in mitigating hyperoxaluria-induced free-radical generation. Earlier studies showed that increased lipid peroxidation reduces tocopherol levels in microsomal membranes [35].

We previously reported that oxalate-induced lipid peroxidation in renal tubular epithelial cells in culture was associated with a greater production of superoxide and hydroxyl free radicals. The production of these free radicals is greater when the cells are exposed to oxalate and calcium oxalate monohydrate crystals. This reveals that oxalate itself is injurious to cells and that calcium oxalate crystals potentiate the toxicity [11]. The significant increase in lipid peroxidation and protein carbonyl content in rats in group VI with hyperoxaluria is in agreement with the previous reports that vitamin E and selenium deficiency enhance free radical generation in rats [36]. Therefore, dramatically increased lipid peroxidation and protein carbonyls in the rats in group VI indicates that vitamin E deficiency potentiates the oxalate-induced free-radical production in the kidney. Grases et al. [37] showed that free radical-damaged cells produce a favourable environment for crystal development, and that phytic acid prevents calcium oxalate crystalization by its antioxidant properties. Recent studies show increased urinary excretion of MDA in human calcium-oxalate kidney stone formers [38]. In addition, another recent study provided more evidence indicating that oxidative stress plays a role in human calcium oxalate kidney stone formation [39]. The authors also reported increased MDA, decreased vitamin E, decreased GSH, and decreased GPx in human kidney stone formers [39], which is similar to the levels observed in our present studies with the rat model. The present results clearly show that excess dietary vitamin E significantly decreased oxalate-induced kidney lipid peroxidation, and strongly support the suggestion of our previous report that...
NADPH, which is a cosubstrate and is required by glutathione reductase and G6PD after antioxidant imbalance. The decrease in GSH level. However, the greatest depletion of hyperoxaluria significantly decreased renal damage.

The results indicate that EG-induced affect by hyperoxaluria. These results from peroxidative injury. In the absence of a optimum level by protecting renal tubules from peroxidative damage to the tissue caused by increased oxalate excretion, while supplementation with vitamin E contributed to maintaining the antioxidant enzymes at an optimum level by protecting renal tubules from peroxidative injury. In the absence of a sufficient concentration of vitamin E in the diet, the kidney antioxidants are adversely affected by hyperoxaluria. These results confirm that vitamin E acts as an excellent antioxidant for the kidney, which is greatly susceptible to oxalate-induced free radical damage.

The results indicate that EG-induced hyperoxaluria significantly decreased renal GSH level. However, the greatest depletion of renal GSH, in group VI, suggests tissue antioxidant imbalance. The decrease in glutathione reductase and G6PD after hyperoxaluria appears to indicate impaired reduction of oxidized glutathione (GSSG) to GSH by depletion of reducing equivalents of NADPH, which is a cosubstrate and is required for glutathione reductase activity [41].

In the current study the treatment of rats in group II resulted in hyperoxaluria, calcium oxalate crystalluria and enzymeuria. The excretion of tubular marker enzymes was further increased, indicating renal tubular damage, and appeared to correlate with the retention and deposition of crystals in the kidneys. However, renal function was unaltered. The excretion of oxalate by rats in group IV was increased and similar to that of in group II, indicating that neither oxalate synthesis nor calcium oxalate crystalluria was prevented by excess vitamin E. However, the extra vitamin E significantly reduced the levels of these enzymes in the urine of hyperoxaluria-induced rats, indicating that hyperoxaluria and formation of calcium oxalate crystals were eliminated without causing renal damage. Thus, vitamin E has a protective effect against hyperoxaluria-induced lipid peroxidative injury to the renal tubules. Earlier studies reported that increased excretion of urinary enzymes by rats occurs as a result of chronic hyperoxaluria induced by various hyperoxaluric challenges, including EG, hydroxyl-l-proline or ammonium oxalate [42]. An increase in urinary enzymes was also reported in patients with renal stones [43].

We are the first to demonstrate direct evidence in vivo that hyperoxaluria-induced peroxidation of renal tubular membrane binds individual calcium oxalate crystals and initiates kidney stone formation. The crystals are first formed in the renal proximal tubules, as calcium in calcium oxalate crystals is derived from the glomerular filtrate [44], and oxalate is readily filterable at the glomerulus and secreted by the proximal tubules [45,46]. In humans, various changes in urine chemistry, including hyperoxaluria, hypercalciumia and hypocitraturia, can lead to the development of abundant crystals within the renal tubules. Using calculations based on the concentration of ions in the renal tubules, Finlayson and Reid [47] reported that crystals are not usually retained and could not reach a size large enough to occlude the tubular lumen within the urinary transit time. In normal kidneys, it takes 3 min for urine to pass from the glomerulus to the renal pelvis; it would take several hours for crystals to become large enough to obstruct a collecting duct [47], suggesting that unless calcium oxalate crystals bind to the tubular membrane surface, stone development would not be possible. In agreement with Finlayson and Reid, we showed that hyperoxaluria-induced renal tubular peroxidative damage associated with antioxidant imbalance resulted in crystal attachment, subsequent aggregation and growth of calcium oxalate kidney stones.

Oxalate-generated free radicals disrupt the structural integrity of the membranes in renal epithelial cells [10,14]. Wiessner et al. showed [48] that coating crystals with urinary macromolecules enhanced the attachment of the crystals to injured renal cells at a pH of <6.0. Surface exposure and redistribution of phosphatidylserine was reported to mediate stone crystal attachment to the renal tubular cell epithelium [49–51]. Studies show that crystal formation results in cell damage and cell detachment from the basement membrane, and the released degradation products can promote heterogeneous nucleation of calcium salts such as calcium oxalate and calcium phosphate. The exposed region of the tubular basement membrane could serve as a site for crystal nucleation and aggregation [52]. Smith [53] showed that dilute acid-induced damage to the rat urinary tract mucus lining promoted calcium oxalate crystal adherence and treatment with glycosaminoglycan decreased this adherence. In addition, lipid asymmetry also increased the affinity of lipid for calcium oxalate monohydrate crystal attachment [54]. Lipid peroxidation reportedly correlates with changes in membrane phospholipid asymmetry [55]. The present histopathological studies also showed a relationship between hyperoxaluria-induced
crystal deposition and peroxidative damage to the renal tubular epithelium. Even though there was EG-induced hyperoxaluria and calcium oxalate crystaluria in rats in group IV for up to 22 days, histopathological findings showed complete prevention of calcium oxalate crystal deposition.

Vitamin E is the most effective chain-breaking lipophilic antioxidant found within biological membranes and that can prevent biological damage [56]. Trochophorics lack sufficient water solubility to be excreted directly in urine and the major route of elimination of its hydrophilic properties is through urine [57]. CEHC increased when the plasma level of RRR-α-tocopherol was exceeded by an excess α-tocopherol supply. Recently, it was reported that α-CEHC has antioxidant properties similar to those of troloxy, a synthetic water-soluble vitamin E homologue [59]. Studies show that α-CEHC had a protective effect against chromate- and thallium-induced nephrotoxicity in the rat model, caused by its antioxidant effect [60]. Therefore, vitamin E with its lipophilic and hydrophilic properties, could act as an effective antioxidant in vivo against hyperoxaluria-induced peroxidative damage in the kidney. In addition to the antioxidant properties of vitamin E, the absence of calcium oxide crystal deposition in group IV might be due to interference of calcium oxalate crystals with the carboxyl group of water-soluble CEHC. The possible existence of an interaction between calcium oxalate crystals and CEHC remains, at present, a matter of speculation and worth further investigation.

In conclusion, these findings present novel and direct evidence in vivo that hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface provides a favourable environment for individual calcium oxalate crystal attachment and subsequent development of kidney stones. Vitamin E treatment completely prevented calcium oxide crystal deposition in the kidney, by preventing hyperoxaluria-induced lipid peroxidation and tissue antioxidant imbalance. From these findings, vitamin E could therefore be considered in the therapy of hyperoxaluria-induced kidney stone formation, and this could benefit individuals with recurrent kidney stone disease. However, clinical trials with estimates of the dose–response effects of vitamin E are warranted in the prevention of calcium oxalate stone deposition.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

None declared.

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VITAMIN E AND HYPEROXALURIA-INDUCED CALCIUM OXALATE CRYSTAL DEPOSITION


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**Abbreviations:** SOD, superoxide dismutase; GTP, glutathione peroxidase; EG, ethylene glycol; MDA, malondialdehyde; GSSG, oxidized glutathione; GSH, reduced glutathione; G6PD, glucose-6-phosphate dehydrogenase; BUN, blood urea nitrogen; GGT, γ-glutamyl transpeptidase; LDH, lactose dehydrogenase; CEHC, 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman.
Treatment of pelvic fracture-related urethral trauma: a survey of current practice in the UK

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OBJECTIVE
To quantify experience of pelvic fracture-related urethral trauma (PFUT), a condition not often encountered and managed by urologists.

METHODS
The consultant urologists of the UK and Ireland were contacted informally to establish their experience with PFUT and its management, both immediate and delayed. In addition, particular individuals thought to have a specific interest in PFUT were targeted for more data.

RESULTS
The overall response rate was 49% (235 responders), representing 78% of urological departments, including all the targeted individuals. Of the responders, 129 (55%) had never seen PFUT in 1–25 years of consultant practice. Only four urologists (2% of responders) saw three or more cases a year. Another four (2%) saw one or two cases per year and the remaining 98 (41%) saw PFUT less frequently. Acutely, 69% of urologists who treated PFUT did so by placing a urethral catheter. Subsequent strictures were treated endoscopically for as long as this was possible. The other 31% inserted a suprapubic catheter and referred the patient for reconstructive surgery if needed. Those who used urethroplasty for strictures after PFUT were identified and targeted; half used urethral mobilization and spatulated anastomosis alone. Only three surgeons performed more than five procedures a year.

CONCLUSION
Whatever a specialist reconstructive unit might do, practice in the wider urological community is different. Even within specialized units, PFUT is rare and the surgical management is often significantly different from published ‘expert’ opinion.

KEYWORDS
pelvic fracture, urethral trauma, distraction defect, urethroplasty

INTRODUCTION
The annual incidence of pelvic fracture-related urethral trauma (PFUT) is not accurately documented. According to Hospital Episode Statistics (HES) data collected centrally by the Department of Health in England, and published on their website (www.doh.gsi.gov.uk/hes), there were, over the last 3 years, an annual mean of 6349 pelvic fractures in men (127 pelvic fractures per million) and annually 159 associated urethral injuries (2.5%) registered in England. This is a lower incidence of PFUT than generally quoted, at 5–10% [1,2]. These figures do not specify the severity and type
of associated urethral injuries, i.e. whether there was partial or complete disruption of the urethra. PFUT is therefore uncommon, and general urologists will not often see such injuries and manage them; in the present study we sought to quantify this experience.

METHODS

In all, 478 consultant urologists of the UK and Ireland were contacted informally by letter to establish their experience in PFUT and its management, both immediate and delayed. The overall response rate was 49% (235 replies), although the responding urologists represented 78% of departments of urology in the UK and Ireland. In addition, particular individuals regarded as having surgical experience of strictures after PFUT were targeted for more detail.

RESULTS

Of the 235 responders, 129 had never seen PFUT in 1–25 years of consultant practice. Only four urologists (2%), all of whom were particularly targeted for their experience, saw more than three cases per year, four (2%) saw one or two, 54 (23%) saw one every 1–5 years and 44 (18%) saw a case less frequently.

In the acute situation, 69% of consultant urologists manage PFUT by passing a urethral catheter, by whatever means (blindly, under endoscopic control or by open surgery) and continue to manage established strictures endoscopically, usually with self-catheterization between times thereafter. Only when this proves impossible or unreasonable would the patient be referred to a specialist unit for further management. Only 31% of consultant urologists treat PFUT by placing a suprapubic catheter and then advising urethroplasty 3 months later if necessary.

There are 27 surgeons in the UK and Ireland who have experience of urethroplasty for established strictures after PFUT. Only three of these surgeons do more than five posterior urethroplasties per year (Fig. 1). The surgical approach in repairing strictures after PFUT among those using a posterior urethroplasty in the UK and Ireland varied considerably (Table 1). Urethral mobilization and spatulated anastomosis alone or with corporal separation were used most often, particularly by surgeons who manage strictures less often after PFUT. Very few surgeons more extensively mobilize the urethra with inferior pubectomy and re-routing of the urethra. The surgical approach also varies amongst experts in posterior urethroplasty in the USA (Table 2).

DISCUSSION

The present study confirmed that PFUT is uncommon; in England the reported incidence, as calculated from HES data, is 2.5%. This amounts to about three stricture cases after PFUT per million of the population per year, of which one patient per year finally has an anastomotic urethroplasty. These figures suggest that PFUT is less common than generally reported. In the immediate management of PFUT, 31% of responding urologists in the UK and Ireland place suprapubic catheters and advise urethroplasty 3 months later if indicated. This is in line with the Consensus Report on Urological Trauma produced by the Société Internationale d’Urology in 2002 [3]. This report, having extensively surveyed the world publications and canvassed expert opinion, recommended that a patient with suspected urethral injury, or with blood at the meatus, should have an ascending urethrogram. If there is extravasation of contrast medium the patient should have a suprapubic catheter placed, either percutaneously with

![FIG. 1. The annual experience of the 27 surgeons using posterior urethroplasties in the UK and Ireland who do so at least once per year. Twenty surgeons operated on established PFU distraction defects; only three do more than five procedures per year.](image-url)
ultrasonographic control or by open cystotomy. Absence of blood at the meatus is not an indication that urethral injury is trivial, so if no blood is present at the external meatus but the patient cannot void, then gentle urethral catheterization by an experienced urologist may be attempted, but failure should be followed by a urethrogram and suprapubic catheter insertion.

Interestingly, in the acute situation, 69% of urologists would try to pass a urethral catheter by whatever means possible and manage any subsequent stricture endoscopically. We did not ascertain the usage and findings of ascending urethrography, and can only therefore speculate that in this group partial tears of the urethra were usual, as only a few urologists reported aggressive endoscopic manoeuvres or open ‘realignment’ to pass a urethral catheter. There are no accurate published data on the incidence of partial and complete urethral disruptions associated with pelvic fractures. These observations may explain why the incidence of PFUT from the HES data is three per million per year, whereas that of urethroplasty for an established distraction defect is only one per million per year.

The difference in managing PFUT might be of no importance if it did not affect the patient’s outcome adversely. Unfortunately, repeated instrumentation seems to affect the outcome adversely if a urethroplasty is subsequently necessary [4]. Whatever, a lifetime of instrumentation, albeit infrequent, does not compare well with a long-term stricture-free survival after urethroplasty of >90% [5].

There are several mechanisms of urethral injury in PFUT (e.g. crush, laceration, avulsion or distraction) and complete disruption of the membranous urethra is less common than partial injury [6]. In complex cases there may be additional injury of the bladder neck, pelvic floor or ano-rectum. Complete disruptions never heal without scar formation separating the distracted ends, and only partial urethral tears have the potential to heal spontaneously [7].

The surgical repair of an established stricture is described as a transperineal progression through four main technical stages, stopping at the stage which first gives a tension-free anastomosis [8,9]. Step one involves simple urethral mobilization with anastomosis of the spatulated healthy ends. Step two develops the intercural space to accommodate the mobilized urethra and thereby reduce the length of the defect. In step three the length of the defect is further reduced by resecting the inferior aspect of the pubic symphysis. If this still does not give a tension-free anastomosis then the final technical step is to re-route the urethra around one corpora cavernosum. The need for successive steps from a simple to a complex repair is determined by intraoperative findings. Imaging before surgery, e.g. with an ascending urethrogram and micturating cystogram, cannot predict the extent of urethral mobilization necessary to achieve a tension-free anastomosis [10].

Of the 27 surgeons who manage PFUT in the UK and Ireland, only three have a caseload of more than five per year and seven manage fewer than one case per year. The remaining 24 surgeons operate on strictures after PFUT only occasionally. The technical repertoire also varies among the 27 UK surgeons and among the five American expert surgeons who were asked about their experience. Only some use all surgical manoeuvres to mobilize the bulb urethra completely. Why there is such surgical variability is uncertain; possibly, once a surgeon is familiar with all the surgical techniques of urethral mobilization, the tendency might be to use them more than is actually necessary to achieve a tension-free spatulated anastomosis. However, someone not trained in more complex mobilization manoeuvres may have a different perception of the tension needed to bring the urethral ends together. Indeed, tension is a subjective assessment at the time of surgery. Another reason might also be relevant; it is very difficult to differentiate a partial urethral injury from a complete urethral disruption by a urethrogram alone, but the potential implication on loss of urethral length is important. In a complete urethral disruption there is no loss of urethral length and the fibrotic gap between the healthy ends is typically 1–2 cm (provided the pelvic fracture was reduced). Severe partial urethral injuries have less distraction and heal by fibrosis involving a variable length of bulbomembranous urethra. Therefore, perhaps different surgeons see a different proportion of complete and severe partial urethral injury, reflecting their need to mobilize the bulb urethra to a greater or lesser degree.

All this begs the question of how many surgeons should be doing this type of surgery. In the UK, at least 24 of the 27 surgeons using posterior urethroplasty are doing so fewer than five times a year; is this sufficient to maintain their skills? Certainly it is lower than the rates recommended by other disciplines for maintaining surgical competence. To maintain proficiency, a urethral surgeon managing strictures after PFUT should probably serve a population of ≥20 million, which would generate 20–30 procedures a year, i.e. the UK would need two or three specialist centres to service the national demand. Countries with a population of <5 million might have to pool resources or ‘buy in’ appropriate expertise.

In conclusion, whatever a specialist reconstructive unit might do, practice in the wider urological community is different. Even within specialized units PFUT is a rare injury and the surgical management is often significantly different from published expert opinion. Interestingly, two of the replies received commented that they were well aware of what the published expert opinion was, but they did not believe it, because experts clearly have a vested interest in their views!

CONFLICT OF INTEREST

None declared.

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Abbreviations: PFUT, pelvic fracture-related urethral trauma; HES, Hospital Episode Statistics.
Upper and lower urinary tract outcome after surgical repair of cloacal malformations: a three-decade experience

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OBJECTIVE
To report the urological outcome of the surgical correction of persistent cloaca, which is technically demanding and may require many procedures in an effort to preserve renal function and provide urinary continence.

PATIENTS AND METHODS
A retrospective chart review from 1971 to 2003 identified 23 patients with cloacal malformations (two posterior, 21 classical) that were reconstructed. The confluence of the urethra, vagina and rectum was noted to be high in 16, low in five and unknown in two; one patient was a conjoined twin.

RESULTS
The mean (range) follow-up was 9.3 (0.4–31.6) years. Urinary anomalies included 14 patients with renal anomalies (six solitary kidneys, four renal dysplasia, two pelviureteric junction, one each duplex and crossed fused) and two duplicated bladders. Vesico-ureteric reflux was present in 13 patients (57%), hydronephrosis at birth in 13, a bony vertebral abnormality in 14 and the VACTERL association in four. Total urogenital mobilization (TUM) was used in six patients and spinal cord untethering in four; a nephrectomy was required in three and partial nephrectomy in one. Upper tract dilation was still present in six patients. Age-adjusted creatinine levels were abnormal in four (18%) patients and borderline in another six (26%). In the nine patients with a solitary kidney (six solitary, three after nephrectomy), the age-adjusted creatinine level was abnormal in two and borderline in four. A vesicostomy was initially performed in 11 patients. The method of bladder emptying is known in 22; 10 void, 11 require clean intermittent catheterization (five abdominal stoma, six urethral) and one was diverted with a conduit. Of the 18 patients aged >47 months 15 were continent (14 complete >4 h, one partial 2–4 h), and three are wet (one conduit). Reconstruction of the lower urinary tract included four bladder augmentations (one ureteric, one ileal, two colon), five bladder neck procedures (two artificial sphincter, one each bladder neck repair, sling, bladder neck division) and six catheterizable channels (one now with a colon conduit). The ureters were re-implanted in 12 patients.

CONCLUSION
Although the surgical correction of this rare malformation is complex, the upper urinary tract is still in need of further improvement.
that were reconstructed. A classical cloaca was defined by a common perineal opening that drained the bladder, vagina and rectum. A posterior cloaca was defined by a very anteriorly placed rectum that entered proximal to the urogenital sinus and shared a common mucosal lining, as previously described by Pena and Kessler [19]. The level of confluence was defined by both cystoscopic and anatomical assessment at the time of reconstruction. A high confluence was defined by the vaginal takeoff being above the external sphincter mechanism if visualized, and/or near the bladder neck. A low confluence was defined as distal to any external sphincter mechanism and/or well separated from the bladder neck. The common channel length was not used as a factor to classify patients, because some had a masculinized phallic structure that gave them a long common channel but also a low vaginal confluence. In the present patients, the confluence was high in 16, low in five and unknown in two.

In terms of managing the cloaca, all patients initially were allowed to void. Intermittent catheterization of the cloaca was instituted in the setting of infections, urinary retention, worsening VUR, abdominal distension or metabolic acidosis. A vescostomy was created in patients in whom intermittent catheterization failed. Other congenital defects were present, as listed in Table 1.

Urinary continence was assessed in patients aged >47 months, and defined as complete if the voiding interval was >4 h, partial if 2–4 h and wet if <2 h.

Renal function was based on age-adjusted serum creatinine levels, although longitudinal values were not available for analysis. Age-adjusted normal creatinine values (μmol/L) were: 0–5 years, 17.7–53.2; 5–10 years, 26.6–62.1; 11–13 years, 35.5–71; 14–17 years, 35.5–79.8; >16 years, 62.1–133 [20]. Borderline values were considered as within 8.9 μmol/L of the highest accepted normal value.

RESULTS

The mean (range) follow-up from when initially seen to the time of review was 9.3 (0.4–31.6) years. The surgical approach was posterior sagittal while prone in 13 patients, while supine in two and combined posterior sagittal while prone and supine in eight. Total urogenital mobilization (TUM) was used in six patients and spinal cord untethering in four.

A nephrectomy was required in three patients and an upper pole nephrectomy in one, all for a poorly functioning renal unit. Upper tract dilatation remains in seven patients; in six it decreased in grade. One patient developed mild hydronephrosis and has had spinal cord untethering. The mean (range) serum creatinine level was 62 (26–283) μmol/L; the age-adjusted creatinine level was abnormal in four (18%) patients and borderline in another six (26%). In the nine patients with a solitary kidney (six solitary, three after nephrectomy), the age-adjusted creatinine level was abnormal in two and borderline in four.

A vesicostomy was initially created in 11 patients in whom clean intermittent catheterization of the cloaca failed, and in two in whom an initial ‘cutback’ of the phallic cloaca failed. The method of bladder emptying is known in 22 patients; 10 void, 11 require clean intermittent catheterization (five abdominal stoma, six urethral) and one was diverted with a conduit. Bladder neck competence before reconstruction was assessed in 12 patients and was defined as coapting in six and open in six. Three patients in both groups void and three intermittently catheterize.

In the 18 patients aged >47 months, 16 are continent diurnally (86%, 14 complete, two partial). Voiding with continence was present...
The reported initial and long-term approach to managing bladder emptying varies significantly; factors contributing to this include the need for temporary diversion, bladder augmentation, status of the spinal cord and the desire for continence. Temporary urinary diversion was required in nearly half the present patients because of the failure of a conservative treatment plan that included intermittent catheterization of the cloaca. A vesicostomy was shown to adequately decompress the cloaca, mainly the vagina, and allows delayed definitive reconstruction [21]. As we previously reported, antenatal urinary ascites may be a predictor of those patients in whom a vesicostomy may be required [22]. After formal reconstruction, most of the present patients empty via intermittent catheterization (52%). In the series of Hendren [11], 39 of 60 secondary cases had undergone diversion before reconstruction. Ultimately, 64% of the patients eventually voided spontaneously. The Great Ormond Street series [18] cited a spontaneous voiding rate of only 22% in their continent patients, an obvious reflection of the need for bladder reconstruction. Finally, Pena [8] reported a spontaneous voiding rate of 60% in his patients who were dry.

TUM was used in six of the present patients and was considered to enhance the reconstruction, similar to the original findings described by Pena [13]. As we previously described, TUM also allows for the sinus tissue to be used for a dorsal, ventral or lateral flap during urethral or vaginal reconstruction [16]. In addition, eight of the present patients required both prone and supine positioning. We consider that the prone position enhances the surgeon's ability to sight down the introitus when dissecting the plane between the bladder and the vagina, particularly when dealing with a high confluence. In the situation where supine positioning is also required, we have been able to reposition the patient with no difficulty. Similar to other centres that have used the TUM [15], the continence status is not yet available, but certainly is a concern. The use of TUM has facilitated reconstruction of the persistent cloaca.

Upper tract deterioration has been a concern in patients with persistent cloaca. The complexity and unique nature of these patients, including the presence of spinal cord tethering, persistent hydronephrosis, the need for bladder augmentation and the presence of reflux, make it extremely difficult to reach an overall conclusion about renal function in these patients. Although hydronephrosis was initially present in 57% of the present patients, it remained a persistent finding in 26%; in all but one the severity decreased. Hydronephrosis was of new onset in one patient who had spinal cord untethering. VUR was present initially in 57% and reimplantation required in 53%. Similar VUR findings were reported by other institutions, in 53–54% of patients [7,17]. It is imperative to understand the bladder dynamics after initial reconstruction of the cloaca, to prevent renal deterioration.

There are few reports of long-term renal function in patients with cloacal malformation. Although age-adjusted serum creatinine levels were used as a reflection of renal function in the present patients, we feel that this is not an entirely accurate assessment of renal function. Nadir creatinine and longitudinal values could not be obtained in the present patients. Also, inherent differences in patient size could affect age-adjusted values of serum creatinine. Nonetheless, it is important to include these data because they provide a gross assessment of renal function. The age-adjusted value was abnormal in 18% of the patients and there was a borderline increase in another 26%. In patients with a solitary kidney, there was an abnormal or borderline value in two-thirds. The Great Ormond Street group reported a higher incidence of renal disease, with chronic renal failure developing in half of their patients and progression to end-stage renal disease in 17%. All of the patients with a solitary kidney in their group had at least mild renal insufficiency [17]. This discrepancy is partly reflected by the renal function being based on age-adjusted serum creatinine levels.
in the present patients, rather than the more accurate GFR.

In conclusion, although the surgical correction of the persistent cloaca is complex, the upper and lower urinary tract outcome can be favourable, albeit with several reconstructive procedures. Spinal cord tethering and underlying neuropathic changes to the bladder affect the need for subsequent lower tract surgery. Up to half of the patients may ultimately need intermittent catheterization for bladder management. Urinary continence is achievable in most patients, but some will require bladder/bladder neck reconstruction to do so. TUM has emerged as the primary method to mobilize the vagina, but the long-term effects on the lower tract are awaited. Renal deterioration may occur in this population and therefore lifelong surveillance is mandatory. In particular, patients with a solitary kidney appear to be at high risk of renal insufficiency.

CONFLICT OF INTEREST

None declared.

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Abbreviations: TUM, total urogenital mobilization.
An objective assessment of the results of hypospadias surgery

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Presented at the XIV Annual Meeting of the European Society of Paediatric Urology, Madrid, 19–22 March 2003
Accepted for publication 14 March 2005

OBJECTIVE

To compare the cosmetic result of tubularized incised-plate urethroplasty (Snodgrass method) with that of two established techniques, the meatal-based flap and onlay island flap repair.

SUBJECTS AND METHODS

Photographs of the penis after hypospadias repair in 32 boys were assessed by a panel of five independent health professionals, including four surgeons with variable paediatric urological experience and a urology nurse. Twenty patients had a distal and 12 a proximal meatus. The Snodgrass technique was applied by one paediatric urologist for either distal (10) or proximal (six) hypospadias. A Mathieu repair was used for distal hypospadias (10) and an onlay preputial island flap for proximal hypospadias (six) by a second paediatric urologist. The panel was asked to grade cosmesis as poor, unsatisfactory, satisfactory or very good (points 1–4) for each of the following aspects of penile appearance: meatus, glans, shaft and overall appearance. Photographs were taken in a standard way, with a standard distance, lighting and two views, one of the dorsal surface and one ventral, for each patient. Signed written consent for the study was obtained from each family.

RESULTS

The mean assessment score for any aspect of cosmesis was significantly higher for the Snodgrass technique ($P < 0.05$). The mean score (95% confidence interval) for the meatus was 0.76 (0.4–1.1) points higher for the patients with a Snodgrass repair than those with a Mathieu or onlay island flap repair ($P = 0.002$). Correspondingly, the values for the glans were 0.67 (0.38–0.97) ($P = 0.003$), shaft 0.42 (0.16–0.69) ($P = 0.01$) and overall appearance 0.62 (0.24–1.0) ($P = 0.01$) points higher for the Snodgrass repair. The Snodgrass technique was more effective in producing a vertically orientated meatus (87.5%) than the Mathieu and Duckett onlay repairs (37.5%; $P = 0.009$).

CONCLUSION

The Snodgrass technique, as assessed by this panel, had a better cosmetic outcome than the Mathieu and Duckett onlay island flap repairs. The assessment of cosmesis in hypospadias surgery is potentially more objective when several health professionals, not involved in the surgery, compared the various methods of repair.

KEYWORDS

hypospadias, tubularized incised-plate urethroplasty, meatal-based flap, onlay island flap

INTRODUCTION

The traditional goals of hypospadias surgery have been focused on the functional aspects of the repair, which are a straight penis with a glanular meatus to permit voiding while standing, and to allow effective coitus in adulthood [1]. In recent years several techniques have been proposed aiming to improve cosmesis, which is the principal aim of hypospadias surgery in distal hypospadias. Snodgrass initially proposed tubularized-incised urethral plate urethroplasty as a surgical method of repairing distal hypospadias [2]. This method has become popular, as it claims to produce a vertically orientated, normal-looking meatus which is cosmetically superior to other techniques [3]. The complication rate was acceptable [4], and the technique was later recommended for proximal [5] and re-operative [6] hypospadias repair.

Most reviews which measure the cosmetic outcome of hypospadias surgery have been based on assessing the views of surgeons involved in the managing the patients. The patients’ view on the results of older techniques was analysed recently; there was a significant disparity of opinion between patients and surgeons about the cosmetic result [7]. In general, there is a lack of objective assessment of the cosmetic results of hypospadias repair by independent health professionals who have not been involved in the patients’ care. No direct comparison has been attempted between the Snodgrass repair and other established techniques.

SUBJECTS AND METHODS

A panel of five health professionals assessed the photographs of the penis after hypospadias repair in 32 boys. The operations were performed by two paediatric urologists who applied different techniques for comparable groups of patients. The Snodgrass technique was used by one surgeon for either distal (10) or proximal (six) hypospadias. A Mathieu repair was used by the second surgeon for distal hypospadias (10), and an onlay preputial island flap for proximal hypospadias (six). Photographs of each
patient were taken in a standard way, with a standard distance, lighting and two views, one of the dorsal surface and one of the ventral. Signed written consent for the study was obtained from each family. The visual presentation was in one sitting lasting \( \approx 1 \) h, in which slide images were projected onto a standard lecture-theatre screen. The slides were presented for assessment in two consecutive groups of distal and proximal hypospadias repair. The sequence in each group was according to alphabetical order of the patients’ names.

The panel (three men and two women) included three paediatric surgeons with variable experience, an adult urologist and a urology nurse. They had not been involved in the clinical management and were not aware of the identity and type of repair of the individual patients. The aspects of penile appearance that were assessed were the meatus, glans, shaft and overall appearance. Cosmesis was graded as poor, unsatisfactory, satisfactory or very good (points 1–4). The panel was also asked to guess which type of repair was used in each case. Finally, one of the authors assessed the incidence of a vertical slit-like meatus in each group.

The number of patients included in each group was limited because the Snodgrass technique has only recently been applied in our hospital. Consequently this number was defined by the availability of hypospadias cases with the most recent type of repair. Twenty-seven patients who had had incised-plate urethroplasty (Snodgrass technique) [2,3] from 1998 to 2001 were invited for review in the clinic. Sixteen of them (group S) presented at the follow-up to have the patients’ names.

### RESULTS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mean difference (95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Meatus</td>
<td>0.76 (0.45–1.10) [0.25]</td>
<td>0.002</td>
</tr>
<tr>
<td>Glands</td>
<td>0.67 (0.38–0.97) [0.24]</td>
<td>0.003</td>
</tr>
<tr>
<td>Shaft</td>
<td>0.42 (0.16–0.69) [0.21]</td>
<td>0.01</td>
</tr>
<tr>
<td>Overall</td>
<td>0.62 (0.24–1.00) [0.30]</td>
<td>0.01</td>
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</tbody>
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The mean assessment score of the panel for each cosmetic variable is shown in Table 1; score for any aspect of cosmesis was significantly higher for the Snodgrass technique (Table 2). Examples of photographs are shown in Figs 1–6. (The number of points corresponds to the average ranking by the panel).

<table>
<thead>
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<td>0.01</td>
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The incidence of vertically orientated meatus, as assessed by one of the authors, was significantly higher \( (P = 0.009) \) in group S (88%) than in group M/D (38%). The configuration of the meatus in the remaining patients was stenotic, circular or irregular. Inflammation and oedema from a recent repair contributed to the irregularity of the meatus in two cases in the M/D group. The transversely lying, mouth-like meatus which is commonly reported after a Mathieu repair (3 days) than after Snodgrass repair (6 days, range 4–7; \( P < 0.05 \)). There was no difference between the groups in proximal hypospadias repair (median catheterization 7 days, range 5–10). The median (range) duration of follow-up (and time of photography) was 21 (1–120) months and not significantly different between the groups.

### Table 1 The mean assessment scores for the S and M/D groups

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<tr>
<th>Assessor</th>
<th>Meatus</th>
<th>Glands</th>
<th>Shaft</th>
<th>Overall</th>
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</thead>
<tbody>
<tr>
<td>S</td>
<td>3.56</td>
<td>2.94</td>
<td>3.43</td>
<td>2.88</td>
</tr>
<tr>
<td>M/D</td>
<td>3.38</td>
<td>2.88</td>
<td>3.38</td>
<td>2.88</td>
</tr>
<tr>
<td>S</td>
<td>3.00</td>
<td>2.44</td>
<td>3.13</td>
<td>2.69</td>
</tr>
<tr>
<td>M/D</td>
<td>3.00</td>
<td>2.67</td>
<td>2.81</td>
<td>2.67</td>
</tr>
<tr>
<td>S</td>
<td>3.38</td>
<td>2.38</td>
<td>3.25</td>
<td>2.56</td>
</tr>
<tr>
<td>M/D</td>
<td>3.00</td>
<td>2.38</td>
<td>2.94</td>
<td>2.38</td>
</tr>
<tr>
<td>S</td>
<td>2.81</td>
<td>2.25</td>
<td>2.94</td>
<td>2.31</td>
</tr>
<tr>
<td>M/D</td>
<td>2.88</td>
<td>2.60</td>
<td>2.88</td>
<td>2.31</td>
</tr>
<tr>
<td>S</td>
<td>3.63</td>
<td>2.56</td>
<td>3.63</td>
<td>2.56</td>
</tr>
<tr>
<td>M/D</td>
<td>3.53</td>
<td>2.87</td>
<td>3.64</td>
<td>2.57</td>
</tr>
</tbody>
</table>

The mean assessment score for the panel of the S and M/D onlay techniques was obtained from each family. The visual presentation was in one sitting lasting \( \approx 1 \) h, in which slide images were projected onto a standard lecture-theatre screen. The slides were presented for assessment in two consecutive groups of distal and proximal hypospadias repair. The sequence in each group was according to alphabetical order of the patients’ names.

The incidence of vertically orientated meatus, as assessed by one of the authors, was significantly higher \( (P = 0.009) \) in group S (88%) than in group M/D (38%). The configuration of the meatus in the remaining patients was stenotic, circular or irregular. Inflammation and oedema from a recent repair contributed to the irregularity of the meatus in two cases in the M/D group. The transversely lying, mouth-like meatus which is commonly reported after a Mathieu repair (3 days) than after Snodgrass repair (6 days, range 4–7; \( P < 0.05 \)). There was no difference between the groups in proximal hypospadias repair (median catheterization 7 days, range 5–10). The median (range) duration of follow-up (and time of photography) was 21 (1–120) months and not significantly different between the groups.

The mean assessment score of the panel for each cosmetic variable is shown in Table 1; score for any aspect of cosmesis was significantly higher for the Snodgrass technique (Table 2). Examples of photographs are shown in Figs 1–6. (The number of points corresponds to the average ranking by the panel).

The incidence of vertically orientated meatus, as assessed by one of the authors, was significantly higher \( (P = 0.009) \) in group S (88%) than in group M/D (38%). The configuration of the meatus in the remaining patients was stenotic, circular or irregular. Inflammation and oedema from a recent repair contributed to the irregularity of the meatus in two cases in the M/D group. The transversely lying, mouth-like meatus which is commonly reported after a Mathieu repair was not found in any patient.

The results of the panel’s guess about which technique was used for each patient are shown in Table 3. The higher percentage of successful guesses by assessors 1, 2 and 3 implies that the general appearance of penis
and, in particular, the configuration of the meatus, is distinctly different in most cases, to permit recognition of the technique. Most of the wrong guesses applied to the cases with M/D onlay repairs which produced a vertical meatus, giving the impression of a Snodgrass technique. Some of the failures also occurred in the few patients in whom the Snodgrass repair produced an abnormal or stenotic meatus. The failure of assessor 4 (a nurse) to have a successful guess and the total inability of assessor 5 to guess are ascribable to their vague knowledge of the type of techniques under consideration.

DISCUSSION

Previous studies have stressed the advantage of the Snodgrass repair in producing a vertically orientated, slit-like normal-looking meatus [2,5,10]. Nevertheless, most reviews of the outcome of hypospadias surgery were by
and 13.8%, respectively). This implies that the common causes of concern (11.2%, 10.3% scars and penile size, were much more other aspects of cosmesis, like circumcision, improvement in only a very few (1.7%) [14].

The nonspecialist members of the panel, a nurse and a surgeon with no experience with the techniques under consideration, were least likely to guess the type of repair from the appearance alone. A study which evaluated the view of the patients on various aspects of appearance is important before concluding the surgeon. The opinion of patients and parents, although far from objective, is of paramount importance when assessing outcome. The present patients were too young to comment on appearance. On direct questioning at the follow-up all parents were satisfied with the appearance.

The Mathieu and Duckett onlay techniques can produce a vertical meatus in about a third of patients. A deep cleft in the urethral plate is most likely to lead to a vertical meatus, irrespective of the technique. Although the meatus was circular or irregular in most cases in the Mathieu group, no case of horizontally orientated configuration was identified. This implies that it is technically possible to avoid this complication, which is regarded as the main disadvantage of this technique. In 1989, Rich et al. [11] modified the meatal-based flap and onlay island flap procedures by hinging the urethral plate, with no increased morbidity and a significant improvement in meatal cosmetic results. Modifications of the Mathieu repair, by incising the urethral plate, have recently been claimed again to improve the cosmetic result [12,13]. A longer follow-up is necessary to show that these modifications do not increase the incidence of stricture.

The nonspecialist members of the panel, a nurse and a surgeon with no experience with the techniques under consideration, were least likely to guess the type of repair from the appearance alone. A study which evaluated the view of the patients on various aspects of penile appearance after hypospadias repair by older techniques showed that the configuration of the meatus was a motive for improvement in only a very few (1.7%) [14]. Other aspects of cosmesis, like circumcision, scars and penile size, were much more common causes of concern (11.2%, 10.3% and 13.8%, respectively). This implies that the appearance of the meatus may not be as significant for the patient or the parent as for the surgeon. The opinion of patients and parents, although far from objective, is of paramount importance when assessing outcome. The present patients were too young to comment on appearance. On direct questioning at the follow-up all parents were satisfied with the appearance.

TABLE 3 The panel’s guess (%) as to the method of repair

<table>
<thead>
<tr>
<th>Assessors</th>
<th>Successful</th>
<th>Wrong</th>
<th>Unable to judge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>19</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

The issue of stricture after the Snodgrass technique may be a problem in the long term, considering that most patients with this complication after various types of hypospadias surgery have been reported to ultimately require open urethroplasty [15]. The concern that incising the urethral plate could cause scarring and stricture led to the application of a dorsal inlay graft to the defect [16]. Others have recommended a more careful selection of patients and modifications of the Snodgrass procedure to reduce the complication rate in distal hypospadias [17]. We showed previously that the Mathieu repair is safe and reliable for correcting distal hypospadias with no chordee. It has the advantage of a complication rate of <5% and can be achieved with limited hospital stay and 2 days of postoperative urinary drainage [18]. The Snodgrass repair required longer urethral stenting afterward in the present study. Nevertheless, in a recent report, the absence of a urethral catheter after incising the urethral plate in distal hypospadias did not seem to increase the morbidity [19].

We think that an objective evaluation of appearance is important before concluding that a new technique is better than previous ones. An ideal study would be a multicentric randomized prospective trial involving several surgeons. The greatest obstacle in such a study is the need for participating surgeons to be prepared to use both surgical techniques in randomized fashion, otherwise any differences in the results might merely reflect differences in the inherent quality of the procedures. This clearly is not feasible. Most surgeons are fully committed to certain types of repair and they are not prepared to do anything other than what in their hands they have found to be safe and effective.

In the present pilot study assessing whether a surgeon or other health professional in a panel can tell the difference in the cosmetic outcome between the techniques under consideration, incised-plate urethroplasty had a better cosmetic result than the two older techniques, but we cannot draw general conclusions. A method of assessing cosmetic outcome in hypospadias surgery is proposed.

ACKNOWLEDGEMENTS

The authors express their thanks to Mr Aivar Bracka for his invaluable help for the construction of the study, to the departments of Medical Illustration of Royal Manchester Children’s Hospital and Wythenshaw Hospital, to Mr Andy Vail for assistance with statistical analysis and to the State Scholarship Foundation of Greece (IKY) for funding the research.

CONFLICT OF INTEREST

None declared.

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NOTE FROM THE EDITOR

Unfortunately and sadly, between acceptance of this paper and its publication, David Gough has died, aged only 57, after a very short illness. He was a most enthusiastic person who brought this characteristic to his chosen speciality of paediatric urology. He developed a special expertise in congenital abnormalities of the lower urinary tract, particularly bladder exstrophy. He was an enthusiastic proponent and founder member of the British Association of Paediatric Urologists, established in 1992. He was also a Board Member of the European Society of Paediatric Surgeons. A graduate of Liverpool University, he subsequently took up his appointment at the Royal Manchester Children’s Hospital. He was a very fine surgeon who made important contributions to research and the literature, and was a first-rate teacher. He will be greatly missed.

JOHN M. FITZPATRICK
Editor-in-Chief
Myogenic bladder decompensation in boys with a history of posterior urethral valves is caused by secondary bladder neck obstruction?

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Department of Paediatric Urology, ‘Aghia Sophia’ Children’s Hospital, Athens, Greece
Accepted for publication 2 February 2005

OBJECTIVE
To investigate whether myogenic bladder decompensation in patients treated for congenital posterior urethral valves (PUV, the most serious cause of infravesical obstruction in male neonates and infants) may be secondary to bladder neck obstruction, as despite prompt ablation of PUV these patients can have dysfunctional voiding during later childhood or adolescence, the so-called ‘valve bladder syndrome’.

PATIENTS AND METHODS
The study comprised 18 boys (mean age 14 years, range 6.2–18.5) who had had successful transurethral ablation of PUV between 1982 and 1996, and had completed a follow-up which included serial assessment of serum creatinine, completion of a standard voiding diary, ultrasonography with measurement of urine before and after voiding, a urodynamic examination with simultaneous multichannel recording of pressure, volume and flow relationships during the filling and voiding phases, coupled with video-cystoscopy at least twice. The mean (range) follow-up was 9.3 (6–17) years.

RESULTS
Urodynamic investigation showed myogenic failure with inadequate bladder emptying in 10 patients; five with myogenic failure also had unstable bladder contractions. On videocystoscopy the posterior bladder neck lip appeared elevated in all patients but in those with myogenic failure it was strongly suggestive of hypertrophy, with evidence of obstruction. At the last follow-up one patient with myogenic failure who had had bladder neck incision and four others who were being treated with α-adrenergic antagonists had a significant reduction of their postvoid residual urine.

CONCLUSION
Despite early valve ablation, a large proportion of boys treated for PUV have gradual detrusor decompensation, which may be caused by secondary bladder neck obstruction leading to obstructive voiding and finally detrusor failure. Surgical or pharmacological intervention to improve bladder neck obstruction may possibly avert this course, but further studies are needed to validate this hypothesis.

KEYWORDS
posterior urethral valves, valve bladder, urodynamics, puberty

INTRODUCTION
PUV have a broad spectrum of clinical presentation; currently, most present in utero with a variable effect on the upper and lower urinary tract [1–4]. Their effect is generally but not specifically related to the severity of valvular obstruction. The effect on bladder function may be evident long after valve ablation, leading to a variety of abnormal urodynamic findings, with a tendency to myogenic decompensation as these boys reach adolescence and adulthood [5,6]. De Gennaro et al. [6] suggested that myogenic decompensation develops secondary to chronic increased detrusor pressure when younger. This progressive deterioration leads to poor bladder emptying with large postvoid residual volumes (PVRs). In some cases this deterioration may be secondary to obstruction at the level of the bladder neck. To examine this hypothesis, we retrospectively reviewed a cohort of adolescent and pubertal boys who previously had had PUV ablated; these boys had had many urodynamic and video-endoscopic evaluations.

PATIENTS AND METHODS
This study included 18 patients treated for PUV in our department between 1982 and 1992 (mean age 14 years, range 6.2–18.5; Table 1) and who fulfilled the following criteria: early diagnosis (<3 months old), treatment started and continued in our hospital, and at least a 6-year follow-up (mean 9.3, range 6–17) with at least two urodynamic and video-cystoscopic studies during their routine follow-up evaluations. Urodynamic studies were part of our follow-up protocol, provided the child was cooperative; this is why no urodynamic studies were used before the age of 5 years.

The diagnosis of PUV was based on UTI in 10 patients, a palpable abdominal mass in three, electrolyte imbalance with dehydration in three and prenatal ultrasonography (US) in two. At diagnosis, seven patients had unilateral (five) or bilateral (two) VUR (nine ureteric units); there was moderate hydronephrosis in 10 patients on the basis of renal-bladder US. Treatment consisted of endoscopic valve ablation in all patients. The serum creatinine at the initial presentation was compromised in 10 patients (>88 μmol/L) and it was normal in the remaining eight.

All patients were evaluated throughout their follow-up according to the following protocol used in our department: (a) At 2 months after treatment; voiding cysto-urethrography and...
BLADDER-NECK OBSTRUCTION IN PUV

TABLE 1 The demographic characteristics of the patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Age at valve ablation, months</th>
<th>Age at last urodynamic and videocystoscopic follow-up, years</th>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>7</td>
<td>3</td>
<td>12.2</td>
</tr>
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<td>8</td>
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</tr>
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<td>14</td>
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<tr>
<td>18</td>
<td>1.4</td>
<td>11.6</td>
</tr>
</tbody>
</table>

**FIG. 1.** Videocystoscopic view of the bladder neck in a boy with myogenic failure. The bladder neck is very thick and fixed in a semi-closed position.

The Bcap expected for age (Bcap$_E$) was calculated using Koff’s formula, (age in years x 30) + 30) mL; Bcap$_{max}$ was measured and compared to Bcap$_E$ in children aged ≤12 years, and in those aged >12 years to 450 mL [9]. PVR was considered pathological if >15% of Bcap$_{max}$ during the filling phase of the cystomanometric procedure. The BC was calculated by dividing the volume by P$_{res}$ at several intervals throughout filling. We graded BC as proposed by Misseri et al. [10], i.e. impaired when the volume/pressure relationship was <30 mL/cmH$_2$O and not when it was >30 mL/cmH$_2$O at Bcap$_{max}$. Detrusor instability was defined as an uninhibited contraction of any magnitude causing incontinence or significant urgency, or any uninhibited contraction of >10 cmH$_2$O during the filling phase, even with no leakage [10].

Myogenic failure (overdistended bladder) was diagnosed when there was an increased Bcap$_{max}$ associated with a P$_{det}$ during voiding of <20 cmH$_2$O, inability to generate a sustained detrusor contraction and a PVR of >15% of Bcap$_{max}$ [11].

At the last follow-up four boys were in chronic renal failure, two had already received a kidney transplant and the other 12 had normal renal function (plasma creatinine <88 µmol/L). VUR had resolved in six of nine ureters, one ureter had been successfully reimplanted and two were still refluxing.

**RESULTS**

Four patients had two, four had three and the remaining 10 more than three urodynamic and video-cystoscopic studies. There was some urodynamic abnormality in all the patients; an unstable bladder in four (three after the follow-up), a low BC in five and myogenic failure in nine; one patient with instability and hypercontractility developed myogenic failure with detrusor decompression during the follow-up, and thus had myogenic failure with inadequate bladder emptying.

During videocystoscopy the posterior lip of the bladder neck appeared elevated in the patients with no myogenic failure, but in the 10 (9 + 1) boys with myogenic failure there was videocystoscopic evidence strongly suggestive of secondary bladder neck obstruction, i.e. the bladder neck was overall very thick and fixed in a semi-closed position (Fig. 1). One of these boys was treated with transurethral bladder neck incision (BNI) and four were given α1-adrenergic antagonist medication (tamsulosin). When re-evaluated, their bladder neck appeared more open, and they felt their flow had improved, although there were no formal measurements of flow rate. In the patient after BNI, the PVR was <15% of Bcap$_{max}$ and in the other four on tamsulosin it decreased but not to <15% of Bcap$_{max}$.

Two patients with a low-compliance bladder who had a nonfunctioning kidney caused by unilateral renal dysplasia had a nephrectomy and ureteric-bladder augmentation, after which they used clean intermittent catheterization; in these two patients, US showed that the hydronephrosis initially present had resolved. There was no correlation between the type of urodynamic...
disorder with renal function, the presence or absence of VUR or hypertension.

**DISCUSSION**

Even after prompt valve ablation, a significant proportion of boys with a history of PUV present during late childhood and adolescence with voiding dysfunction and renal failure [12,13]. The reported incidence of voiding dysfunction in patients treated for PUV is 13–38% [12,14–22]. In a previous study, compiling the results of seven previous series of PUV, an average rate of 21% was reported [23]. Persistent bladder dysfunction has been implicated as a cause of deterioration of the upper urinary tract and kidney function, and this has lead to the urodynamic investigation of boys with a history of PUV as part of their regular follow-up [1]. In 1979, Bauer et al. [1] reported retrospectively on the specific urodynamic findings in nine boys with voiding disturbances after valve treatment, and soon after the concept of the ‘valve bladder syndrome’ emerged to define the spectrum of urodynamic disorders found in these patients [2,3].

It was suggested that the different patterns of urodynamic disorders found in patients treated for PUV are variations of the same basic bladder dysfunction. Myogenic failure is one of the three major urodynamic abnormalities in these patients [24–26], but the definition of myogenic failure and its reported incidence vary greatly among studies [27–29]. Misseri et al. [10] defined myogenic failure as either an acontractile detrusor or one that cannot generate a sustained contraction sufficient to empty the bladder adequately. They considered a PVR > 30% of Bcapmax to be significant and indicative of myogenic failure, but others think the threshold should be > 20% [11]. In the present study we considered 15% of Bcapmax as the critical limit for defining significant PVR. However, apart from a critical threshold of PVR for defining myogenic failure, we also think that urodynamic evidence of poor detrusor contractility, reflected in a Pdetrmax of <20 cmH2O during voiding, is an important criterion for its diagnosis [10].

The incidence of myogenic failure and bladder decompensation varies among studies. Misseri et al. [10], in their retrospective uncontrolled study of 51 patients, noted myogenic failure in three (6%), all anticholinergically induced. In the present study there was myogenic failure in nine of 18 patients but five of them also had evidence of bladder instability, thus leaving only four with pure myogenic failure. Others [5,25,30] suggested that the pattern of urodynamic disorder may change with age, with a tendency to bladder hypocontractility and detrusor decompensation as patients with a history of PUV reach puberty, hence the incidence of myogenic failure may be greater as patients approach adulthood.

However, a more crucial question is the possible cause leading to bladder decompensation. In older studies the theory most widely accepted was that detrusor instability remaining after valve treatment has an obstructive effect on voiding, from the attempt to maintain continence by tightening the pelvic floor and external sphincter during the unstable contractions [29]. Another possible explanation for progressive bladder overdistension and decompensation could be the constant presence of significant pseudo-residual urine caused by defective urine concentration and the production of large urine volumes, gradually leading to bladder overdistension [3,28].

However, DeGennaro et al. [26] found no clear relation between bladder function and impairment of renal function, and the present results also show no correlation between the type of urodynamic disorder and kidney function. In their retrospective study, Misseri et al. [10] expressed the view that myogenic failure is unlikely to develop when patients with abnormal urodynamic findings are treated with anticholinergics when young, but three of their patients developed myogenic failure while on anticholinergics. The authors concluded that it was iatrogenic, secondary to anticholinergic therapy administered in boys treated for PUV who had symptoms of day-time wetting resulting from unstable bladder contractions, rather than a preordained consequence of valve disease; this should not be overlooked. However, they also acknowledged that in some boys detrusor decompensation with time could be a consequence of some persistent obstructive process secondary to bladder neck dyskinesia, where a very thickened and fixed bladder neck hinders the flow of urine.

The present videocystoscopic results, when correlated with the respective urodynamic findings, seem to support this latter view. Although the finding of a prominent posterior lip of the bladder neck was common in all the patients, boys with myogenic failure had anatomical evidence of genuine bladder neck obstruction (Fig. 1). Despite videouroynamics not being included in the present study, the endoscopic evidence of not just a prominent but also totally hypertrophic, semi-closed and fixed bladder neck permits the hypothesis that voiding under such circumstances may be obstructive at the level of bladder neck, and cause detrusor decompensation by a prolonged and increased bladder outlet resistance. That BNI (one patient) or tamsulosin (four) had a positive effect on bladder emptying, substantially lowering the PVR and changing the bladder neck appearance, supports of this view, and is in accordance with the observations of Misseri et al. [10], who also treated three boys with secondary bladder neck obstruction with tamsulosin. Furthermore, hydronephrosis decreased in three of the five patients, but the lack of urodynamic data in these patients after treatment is a limitation of the study.

However, few patients have been treated and to validate our hypothesis we intend to report further results of α1-adrenergic antagonist in more boys treated for PUV with secondary bladder neck obstruction and myogenic failure, with detailed urodynamic data before and after treatment. If α1-adrenergic antagonists are effective in improving uncoordinated voiding in patients with a history of PUV, their use after valve ablation could possibly avert myogenic failure and bladder decompensation.

**CONFLICT OF INTEREST**

None declared.

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valve disease: real or imagined?

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Pediatric Urology


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Williams DI, Whitaker RH, Barratt TM, Dillon MJ et al.

Peters CA, Bauer SB.

Capozza N et al.

De Gennaro M, Capitanucci ML, Silveri M et al.

De Gennaro M, Capitanucci ML, Mosiello G, Gatti C, Lais A.


Koff SA, Mutabagani KH, Jayanthi VR.


Misseri R, Combs AJ, Horowitz M et al.

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Peters CA, Bolkier M, Bauer SB.

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e-mail: dimkaram@medscape.com

Abbreviations: PVR, postvoid residual volume; US, ultrasonography; Bcap, bladder capacity; BC, bladder compliance; Pdetmax, maximum detrusor storage pressure; BNI, bladder neck incision.
Expression of Ki-67 in squamous cell carcinoma of the penis

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Accepted for publication 3 February 2005

OBJECTIVE

To investigate the Ki-67 labelling index (LI) as a prognostic factor for the outcome of penile carcinoma, as in squamous cell carcinoma (SCC) of the larynx the expression of this marker correlates with histological features indicative of prognosis.

PATIENTS AND METHODS

We retrospectively analysed the records of 44 patients in whom primary SCC of the penis was treated with amputation and bilateral lymphadenectomy (pT1, in 24, pT2 in 20, pN+ in 10; G1 in 12, G2 in 28 and G3 in four).

RESULTS

During a mean follow-up of 35.6 months, four patients had disease progression. Tumour tissue was stained immunohistochemically using the streptavidin-biotin method. The mean Ki-67 LI was defined as the percentage of total tumour cells that were Ki-67-positive. The results were compared with pathological tumour stage, grade, nodal status and clinical disease progression.

CONCLUSIONS

The Ki-67 LI is correlated with tumour grade in penile cancer, and may indicate a greater risk of nodal metastasis.

KEYWORDS

penile carcinoma, Ki-67, lymph nodes
differentiated tumours (was significantly associated with poorly (rate was categorized as low (range) LI of 40.5 (6.4–93)%. The proliferation grade SCC of the penis (original staining for Ki-67 in most tumour cells of a high-

FIG. 1. (A) Diffuse nuclear immunohistochemical staining for Ki-67 in most tumour cells of a high-grade SCC of the penis (original × 100). (B) Weakly positive immunostaining for Ki-67 in a well-differentiated penile carcinoma (original × 100).

(range) LI of 40.5 (6.4–93)%. The proliferation rate was categorized as low (<40.5%) or high (≥40.5%), from the mean Ki-67 LI. A high LI was significantly associated with poorly differentiated tumours (P < 0.005, Fig. 1). The mean LI was higher in patients with regional lymphatic spread (51.4%) than in those with localized disease confined to the penis (37.6%), but this difference was not statistically significant (P = 0.02). There was also a tendency for high Ki-67 expression with advanced local tumour stage and disease progression, but these correlations were not statistically significant either (P = 0.07 and 0.06).

DISCUSSION

Ki-67 is a non-histone nuclear matrix protein expressed in all cell-cycle phases except G0. An assessment of Ki-67 protein expression by immunohistochemistry is a reliable means of evaluating tumour cell proliferation [11]. To our knowledge, there are no previous reports on the correlation between the Ki-67 LI and clinicopathological variables for SCC of the penis. Studies show that for SCC of the head and neck there is a correlation between the proliferation rate determined by Ki-67 and tumour de-differentiation, nodal involvement or disease progression [12–14]. In the present study there was a significant association only between the Ki-67 LI and tumour grade, but there was a tendency towards a higher LI with tumour stage, nodal metastasis and clinical disease progression at follow-up. The lack of correlation for these factors might be because there were too few patients.

In a recent report, Martins et al. [15] examined the proliferative activity in penile carcinoma using immunostaining for proliferating cell nuclear antigen (PCNA); the PCNA LI had a significant correlation in univariate analysis with the presence of nodal metastasis. In contrast to the present findings for Ki-67 LI, there was no correlation between the PCNA LI and tumour grade.

Emerson et al. [8] found that the depth of stromal tumour invasion, measured by a computerized micrometer, and vascular invasion were predictive for cancer progression in patients with penile SCC. In two other studies the immunohistochemical overexpression of tumour-suppressor gene product p53 in penile carcinoma was correlated with disease progression [15,16].

Further investigations of molecular markers to predict tumour behaviour in penile carcinoma, and cohorts with more cases, are needed for this relatively rare tumour. Besides known prognostic factors like pT stage and grade, they might be helpful in selecting patients who would benefit from inguinal lymphadenectomy.

CONFLICT OF INTEREST

None declared.

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Abbreviations: SCC, squamous cell carcinoma; LI, labelling index; PCNA, proliferating cell nuclear antigen.
Accuracy of the routine detection of mutation in mismatch repair genes in patients with susceptibility to hereditary upper urinary tract transitional cell carcinoma

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INTRODUCTION

Upper urinary tract (UUT) TCCs are rare tumours which account for <5% of all urothelial carcinomas [1]. UUT-TCCs belong to the spectrum of hereditary nonpolyposis colorectal carcinomas (HNPCC) [2,3], an autosomal dominant syndrome predisposing to colorectal carcinoma but sometimes also to extracolonic tumours such as UUT-TCCs [5% of cases] [4]. HNPCC is caused by germ-line mutations affecting one or several mismatch repair genes, i.e. hMLH1 (50% of the time), hMLH1 (30%) and hMLH6 (5–8%) [4–6]. More recently, genes such as hMLH3, hPMS1, hPMS2, TGFBrII and EX01 have also been implicated [7,8]. Together, deleterious mutations of these other genes, account for <5% of cases. Tumour microsatellite instability (MSI) indicates probable mutations or epigenetic alterations in these mismatch repair genes [4,9]. High MSI levels are detected in nearly 15% of patients with UUT-TCC [10,11]. We already established that high MSI status is useful to indicate a hMLH2 mutation in these patients [12]. To avoid overlooking a hereditary cancer, we showed that patients with a high MSI level and a history of HNPCC-associated cancer or aged <60 years should be tested for hMLH2 mutation [12]. The aim of the present study was to establish the clinical benefits of systematic testing for hMSH6 and hMLH1 mutations in the very rare patients with UUT-TCC, a clinical predisposition for hereditary nonpolyposis colorectal-related cancer (three colorectal). There were only mutations in hMSH2 gene detected, with none in hMSH6 and hMLH1.

CONCLUSION

For the rare patients with UUT-TCC who are suspected of carrying mismatch repair gene mutations if no hMLH2 mutation is found by genetic testing, complementary DNA sequencing for hMLH1 and hMSH6 mutation does not seem to contribute and should not be recommended in daily practice.

KEYWORDS

microsatellite instability, germline mutation, ureter, HNPCC, TCC

OBJECTIVE

To establish the clinical benefits of systematic testing for hMSH6 and hMLH1 mutations in the very rare patients with upper urinary tract transitional cell carcinomas (UUT-TCCs), a clinical predisposition for hereditary tumour and no mutation detected in hMSH2 gene.

PATIENTS AND METHODS

In all, 164 UUT-TCC specimen blocks were screened for microsatellite instability (MSI); 27 (16%) had high MSI levels. Eight patients (30%) had clinical criteria suspicious of hereditary tumour; in three a mutation in hMSH2 was detected. For the other patients, clinical data were collated, and DNA gene sequences analysed to detect mutations in hMLH1 and in hMSH6 genes.

RESULTS

Five patients were assessed (mean age at the diagnosis of UUT-TCC 65.2 years, 80 years, range 54–71; two aged <60 years). Three patients had a personal history of hereditary nonpolyposis colorectal-related cancer (three colorectal). There were only mutations in hMSH2 gene detected, with none in hMSH6 and hMLH1.

PATIENTS AND METHODS

The files of 164 patients treated for sporadic UUT-TCC over 12 years were reviewed; all tumour blocks retrieved were screened for MSI. Paired DNA from tumours and normal tissues were amplified by PCR using five microsatellite markers from the Bethesda consensus [13], any pair of samples of normal DNA and tumour DNA that had instability at two or more of these five loci was scored as having high-frequency MSI, whereas a sample pair with no instability at these five loci was scored as having MSI. Any sample pair having instability at one of the five loci was tested again at that locus to exclude artefact. If MSI was confirmed additional loci were tested to determine whether the phenotype of the sample was low-frequency (1–4 loci) or high-frequency MSI (five or more loci). For additional loci, we used markers that we had already tested in UUT-TCC [10,14]: MFD15 (1q23), APC (5q22), BAT40 (1p13.1), d18s58 (18q22), D18S569 (18q21), d10s197 (10p12), MYC1L (1p34), UTS320 (8q24), ACTBP2 (6q13), CFS1R (5q33–q35), D20S82 (20p12), d11s488 (11q24) and D9S242 (9q33). PCR amplification was carried out with ≈10 ng of DNA in a 20-μL final volume of reaction mixture (0.25 mmol/L dNTP in 1 mol/L Tris, 0.01 mol/L EDTA, 20 pmol of each primer (MWG Biotech, Ebersberg, Germany), 0.75 μL of DMSO, and 1 U Taq Polymerase (Qiagen, Illkirch, France). Cycling parameters...
were described previously [10]; 1 μL of PCR product was added to 1 μL blue Dextran and 3 μL formamide. After a 2-min denaturation step at 94°C, the mixture was immediately immersed in an ice bath. The amplified fragments were separated by denaturing gel electrophoresis in Tris-borate-edetic acid buffer/4% polyacrylamide (acryl-to-bisacryl 29 : 1), 6 mol/L urea (gel) using an PRISM 377 Genetic Analyser (Applied Biosystems, Palo Alto, California); GeneScan 3.1 Fragment Analysis software (Applied Biosystems) was used to analyse the data.

Twenty-seven patients (16%) had high MSI levels; the following data were collated: age, personal or family history of a HPNCC-associated tumour, history of other cancers, tumour stage (TNM 1997) and grade. None of these 27 patients had a family history of HPNCC. Twenty-seven patients (16%) had high MSI levels; the following data were collated: age, personal or family history of a HNPCC-associated tumour, history of other cancers, tumour stage (TNM 1997) and grade. None of these 27 patients had a family history of HNPCC. Two hundred and seventy patients (16%) had high MSI levels; the following data were collated: age, personal or family history of a HNPCC-associated tumour, history of other cancers, tumour stage (TNM 1997) and grade. None of these 27 patients had a family history of HNPCC.

RESULTS

Of the 27 patients with high MSI levels, eight (30%) met the clinical criteria for hereditary UUT-TCC. Their gender, age, personal and family history, and tumour characteristics are given in Table 1. Of these eight patients, five were included in the present study (mean age at the diagnosis of UUT-TCC 65.2 years, SD ± 8, range 54–71; two aged <60 years). Three patients had a personal history of cancer related to the HPNCC spectrum; UUT-TCC was never the first cancer in their personal history. No patient had a family history of HPNCC-associated cancer. Of the five patients, two developed UUT-TCC in the renal pelvis and three in the ureter. No patient had metastases when the UUT-TCC was diagnosed. Of the five tumours, two were superficial (pT1) and three were invasive (pT2, pT3). (pT4). All five patients had a radical nephroureterectomy; two had a recurrence (one bladder cancer, one cancer of the contralateral upper urinary tract). As reported in Table 1, only mutation in hMSH2 was detected, with no mutation on MLH1 and MSH6 after DNA sequencing in these patients.

DISCUSSION

This study of patients suspected of having hereditary UUT-TCC caused by germline mutation of mismatch repair genes and previously explored for hMSH2 gene [12] provides no support for the hypothesis that DNA sequencing of MLH1 and MSH6 might be useful to detect hereditary disease among these rare cases of UUT-TCCs. Undoubtedly some hereditary cancers, whether of the colon or UUT-TCC, are misclassified as sporadic and their incidence is underestimated [3,4,12]. In addition, the incidence of de novo mutations is not negligible, especially in hMSH2 [17,18]. Moreover, in half of patients, UUT-TCC shows the presence of HPNCC and, conversely, the relative risk of UUT-TCC in HPNCC patients is 14 [3]. The daily practice of diagnosis in hereditary cancers must be improved; in such cases, when gene mutations are detected, the patient and his family benefit from multidisciplinary management [5,19]. The presence of other HPNCC-associated cancers is sought and patients closely monitored. Genetic counselling is provided to the patient’s family. So that a hereditary cancer is not overlooked, we already suggested changing screening strategies in UUT-TCCs based on the above tests [12]. Screening for MSI is now warranted as routine in all patients with UUT-TCC, as for colorectal cancers, irrespective of age at diagnosis. A panel of five microsatellite markers is usually used to determine MSI; when the discrimination is poor, a further 10 markers or more are used [13]. In our choice of markers, we applied the criteria of the 1998 consensus [13] and used markers that were relevant in our earlier studies on UUT-TCC [10,14]. In future, the more precise 2004 criteria need to be implemented [20]. MSI screening identifies a further 5% of hereditary cancers than when applying the stringent clinical criteria for diagnosing HPNCC (Amsterdam criteria) [4,12]. Immunohistochemistry can be a useful tool to identify MSI.
additional test to indicate which mismatch repair gene might be involved [12]; some authors even consider that the results are sufficiently well correlated with MSI phenotype to act as a surrogate for MSI determination, especially as it is quicker [21,22]. However, tumour MSI phenotype determined by PCR is more specific for changes in DNA repair genes and is still the standard method [12,20,23].

DNA sequencing, as the last step for a positive search systematically for microsatellite instability analysis. Not all tumours, only five of the present 164 rare tumours, is not currently always [4,5,8]. Furthermore, detecting mutations in two genes is not always currently available [24]. As sporadic UUT-TCCs are very rare tumours, only five of the present 164 patients were included in this study, and the final result was of little practical value. The reported risk for all HNPCC-related tumours is significantly lower in MSH6 or in MLH1 than in MSH2 mutation carriers [5,8]. Consequently, there is no doubt that searching systematically for hMLH1 and hMSH6 are involved in hereditary tumours in only 30% and <8% of cases, respectively [4,5,8]. Furthermore, detecting mutations in these two genes is not currently always available [24]. As sporadic UUT-TCCs are very rare tumours, only five of the present 164 patients were included in this study, and the final result was of little practical value. The reported risk for all HNPCC-related tumours is significantly lower in MSH6 or in MLH1 than in MSH2 mutation carriers [5,8]. Consequently, there is no doubt that searching systematically for hMLH1 and hMSH6 mutations by genetic testing is not cost-effective and is unwarranted in daily practice for managing UUT-TCC.

CONFLICT OF INTEREST
None declared.

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Abbreviations: UUT, upper urinary tract; HNPCC, hereditary nonpolyposis colorectal carcinoma; MSI, microsatellite instability.
Mesenchymal cells infiltrating a bladder acellular matrix gradually lose smooth muscle characteristics in intraperitoneally regenerated urothelial lining tissue in rats

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OBJECTIVE
To characterize serial long-term histological changes in mesenchymal cells infiltrating a collagen-based matrix, as in a hollow organ with differentiated urothelial lining created intraperitoneally by grafting cultured urothelial cells, mesenchymal cells with smooth-muscle immunohistochemical characteristics infiltrated into the scaffold, despite no mesenchymal cells being seeded into the scaffold before grafting.

MATERIALS AND METHODS
To regenerate a urothelial lining tissue intraperitoneally, rat urothelial cells were cultured and seeded with the feeder-layer technique onto bladder acellular matrix (BAM). After 7 days of cultivation to attach urothelial cells on the BAM, the matrix was folded with the urothelial cells inside and grafted onto the mesentry of the previously partially cystectomized rat.

RESULTS
The formed urothelial cells on the BAM, which formed a monolayer before grafting, stratified into three to four layers as early as 4 days after grafting. Although the regenerated urothelium became thinner with time, there was urothelial stratification and a peculiar angular appearance on the apical surface of the regenerated urothelium even after 56 days. The mesenchymal cells infiltrating the BAM showed positive immunohistochemical staining to α-smooth muscle actin or desmin at 7 days. Subsequently, the number of actin- or desmin-positive cells gradually decreased with time. On transmission electron microscopy, the infiltrating mesenchymal cells were characterized as myofibroblasts at 7 days. Smooth muscle-like cells were identified at 14 and 28 days, and fibrocytes were the main population at 56 days.

CONCLUSIONS
Although epithelial–mesenchymal interactions have been assumed to be one of the most critical factors in smooth-muscle development, mesenchymal cells infiltrating the scaffold in this intraperitoneal regeneration model gradually lost smooth muscle characteristics with time. These results suggest that interactions between cultured urothelial cells and infiltrating mesenchymal cells alone could not maintain the smooth muscle character of infiltrating mesenchymal cells.

KEYWORDS
urothelial cells, regenerated tissue, mesenchymal cells, smooth muscle

INTRODUCTION
Various techniques have been developed for bladder reconstruction using native tissues other than the urinary tract. Among them, current approaches favour the use of gastrointestinal segments to augment the bladder. However, the use of gastrointestinal segments can lead to various complications including chronic infection, electrolyte derangement, mucus secretion and stone formation [1].

Recent advances in tissue-engineering techniques have enabled the regeneration of functional bladder wall from a scaffold with or without bladder cells. These techniques have allowed the reconstitution of normal bladder wall consisting of mucosa, muscularis mucosa, detrusor muscle and serosa at the site where the scaffold was grafted. Urodynamic studies have shown that this composite bladder can function as a low-pressure reservoir and empty efficiently in neurologically intact animals. These regenerated tissues have a luminal surface covered with differentiated urothelium, which avoids the disadvantages of gastrointestinal grafts [2–6].

Nonetheless, the mechanism of bladder regeneration remains unclear. In the regeneration of smooth muscle and bladder wall, it was speculated that mesenchymal cells, which were derived from de-differentiation of mature bladder smooth muscle and migrated into an acellular matrix, would re-differentiate into bladder smooth muscle via diffusible growth factors that might be produced from the urothelium [7,8].

It was also shown that smooth muscle development is facilitated by placing the epithelium onto the surface of the matrix. Thus, epithelial–mesenchymal interactions are necessary for the development of bladder smooth muscle. Recent data showed that by implanting embryonic urothelium seeded on bladder acellular matrix (BAM) into the immunodeficient mouse, fibroblastic cells differentiated into smooth muscle cells [9]. These results suggest that the epithelium is important for smooth muscle regeneration from migrating mesenchymal cells into the scaffold.

We previously reported that cultured autologous urothelial cells on a collagen-based matrix were successfully implanted into the peritoneal cavity of the rat to create a hollow-like tissue with differentiated
urothelium [10]. In that created tissue, mesenchymal cells infiltrating the matrix showed smooth muscle characteristics [10]. The aims of the present study were to characterize the serial histological changes in mesenchymal cells infiltrating a scaffold grafted with urothelial cells.

MATERIALS AND METHODS

Female Wistar rats (7–8 weeks old) were used; the primary culture of urothelial cells was as previously reported with slight modifications [10]. Briefly, the apical two-thirds of the rat bladder was harvested and the epithelial layer peeled. Isolated urothelial cells obtained from the epithelial layer by trypsinization were cultured with the feeder-layer technique in a humidified, 37°C, 95% air/5% carbon dioxide environment. The primary cultures became confluent in 10–12 days, when there was a mean (range) of 2.9 (1.5–5.1) × 10⁶ cultured urothelial cells per sample of bladder tissue.

BAM was prepared as previously reported, with slight modifications [4]; briefly, the bladder tissues after de-epithelialization for urothelial culture were treated with 10 mmol/L PBS and 0.1% sodium azide, then in 1 mol/L sodium chloride containing 60 units/mL DNase (Takara, Osaka, Japan). After digestion, they were digested twice in 4% sodium deoxycholic acid (Sigma, St Louis, MO, USA) containing 0.1% sodium azide (Sigma). Each process was used for 10–14 h with serial agitation at a room temperature. After this chemical digestion, the BAM was opened by cutting from the edge to the dome (Fig. 1A,B) and placed in six-well plates with the inner surface facing upwards. The opened BAM was fixed on the plate with fibrin made from 10 mL of 40 mg/mL fibrinogen solution and the same aliquot of 3 U/mL thrombin solution (Fujisawa Pharmaceutical, Osaka, Japan). They were incubated overnight under the same conditions as the cell culture.

After making a feeder layer on the BAM fixed in the six-well plate, urothelial cells cultured from each sample of bladder tissues were seeded onto each BAM in a culture medium containing 0.15 TIU/mL aprotinin (Sigma) (Fig. 1C). After 7 days of culture the BAM was removed from the well and folded with the urothelial layer inside. The edges were running-sutured with 9–0 Nylon to form a closed pouch. Then the BAM was grafted into the previously cystectomized rat as it was wrapped with the mesentery of the terminal ileum. The grafted BAMs were harvested after 4, 7, 14, 28 and 56 days.

The harvested BAMs were fixed in 10% buffered formalin and processed for paraffin-wax embedding. Immunohistochemistry was used with antibodies for α-smooth muscle actin (Sigma) and desmin (DAKO, Carpinteria, CA, USA) using commercially available kits (Nichirei, Tokyo, Japan). Paraffin-embedded sections of the rat bladder were used as controls.

For transmission electron microscopy (TEM), specimens were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer at 4°C, and post-fixed with 1% OsO₄ solution for 2 h. They were then stained with 2% aqueous uranyl acetate for 2 h at 4°C, dehydrated in a graded ethanol series, cleared in propylene oxide and embedded in Epon 812. Ultra-thin sections were cut and examined with a transmission electron microscope (H7100, Hitachi, Katsuta, Japan).

RESULTS

There was urothelial lining on all matrices at 14 days or earlier after grafting but it was also identified in four of five matrices at 28 days, and in three of four at 56 days. There was no stone formation on the suture materials.
Urothelial cells, which formed as a monolayer on the BAM before grafting (Fig. 2A), were stratified into three or four layers as early as 4 days after grafting (Fig. 2B–D). While grafted urothelial cells became flatter and the regenerated urothelium became thinner with time (Fig. 2E,F), TEM showed the presence of a peculiar angular appearance, which is specific to the urothelium, on the apical surface of the regenerated urothelium even at 56 days (Fig. 3).

There were infiltrating mesenchymal cells in the outer half of the matrices at 4 days (Fig. 4A) and throughout the matrices at 7 (Fig. 4B) and 14 days. The matrices on which urothelial cells were identified at 28 and 56 days had two layers histologically. The inner layer, just below the urothelial lining, had numerous mesenchymal cells, and the outer layer had few mesenchymal cells (Fig. 4C). In each matrix on which urothelial cells did not survive at 28 and 56 days only a few mesenchymal cells infiltrated into the matrix.

Mesenchymal cells infiltrating the BAM had positive immunohistochemical staining to actin and/or desmin at 7 days (Fig. 5A/A'). Subsequently, the number of actin- or desmin-positive cells gradually decreased with time (Fig. 5B/B', C/C'), and were identified mainly underneath the regenerated urothelium at 56 days (Fig. 5D/D').

Infiltrating mesenchymal cells were characterized mainly as myofibroblasts at 7 days, and smooth muscle-like cells were apparent from 14 days, with fibrocytes present from 28 days, forming the main population in the BAM at 56 days (Fig. 6).

DISCUSSION

We characterized histologically the fate of mesenchymal cells infiltrating the BAM that was used for the intraperitoneal regeneration of urothelial lining tissues. BAM is one of the best matrices for bladder regeneration when grafted to a partially cystectomized bladder. The excellent histological and functional properties of the regenerated bladder were reported in normally voiding animals [4–7] and in animals with a diseased bladder [11]. In our previous study in which a collagen-based matrix was used as a scaffold for urothelial cell implantation, the 4-week survival rate of autologous urothelial cells was low [10]. Therefore in the present study, we used BAM as a scaffold for the implantation of intraperitoneal urothelial cells, expecting better survival of the urothelial cells.

However, urothelial cells failed to survive in one of five and one of four matrices at 28 and 56 days, respectively. As collagenase is synthesized via keratinocyte growth factor [12], which is secreted via epithelial-mesenchymal interactions [13], it seems that the degradation of BAM through the remodeling process of the extracellular...
matrix by collagenase at least partly affected the urothelial survival in the present model.

Mesenchymal cells infiltrating the BAM showed positive staining for actin and/or desmin at 7 days after grafting. These actin- and/or desmin-positive cells gradually decreased with time and finally localized mainly underneath the regenerated urothelium. TEM showed serial changes in the character of infiltrating mesenchymal cells, from myofibroblasts (7 days) and smooth muscle-like cells (14 and 28 days), to fibrocytes thereafter. Thus, in the present study, infiltrating mesenchymal cells

FIG. 4. Representative micrographs of implanted matrices. Infiltrating mesenchymal cells were apparent in the outer half of the matrices at 4 days (A) and throughout the matrices at 7 days (B). The matrices on which urothelial cells were identified at 56 days (C) had two layers histologically; the inner layer (i) had numerous and the outer layer (o) had fewer mesenchymal cells. Bar 20 μm.

FIG. 5. Representative micrographs of infiltrating mesenchymal cells on immunohistochemistry for α-smooth muscle actin (A–D) and desmin (A’–D’); the cells showed positive staining to actin and/or desmin at 7 days. The number of actin- or desmin-positive cells gradually decreased, but were identified underneath urothelium at 56 days. A/A’, 7 days; B/B’, 14 days; C/C’, 28 days; D/D’, 56 days. Bar 100 μm.
gradually lost smooth muscle characteristics with time. One possible explanation for these results might be that regenerated urothelium which became flatter and thinner with time was insufficient to maintain epithelial-mesenchymal interactions.

In previous studies using a bladder augmentation model, mesenchymal cells infiltrating BAM became smooth muscle cells via a diffusible growth factor that might be produced from the urothelium, and formed muscle bundles [4–7]. Although mesenchymal cells infiltrating in a bladder augmentation model are thought to originate from the host bladder [7], even the heterotypic epithelial-mesenchymal interactions can induce mesenchymal cells in smooth muscle [14]. Recruitment and transdifferentiation of fibroblasts into smooth muscle were reported in BAM after implanting embryonic rat urothelium under the renal capsule or skin of the immunodeficient mouse [9]. These results indicate that the existence of mesenchymal cells originating from the native bladder is not critical for smooth muscle regeneration.

There are some differences between previous studies [4–7,9] and the present study in the source of the grafted urothelium (embryonic or adult rat urothelium), grafting methods (grafting into immunodeficient animals or autologous implantation) or grafting sites (bladder augmentation or ectopic implantation onto the mesentery). These differences in the materials and methods might affect the fate of infiltrating mesenchymal cells. In the present study, we attempted to create a cystic tissue with a luminal surface covered with regenerated autologous urothelium in an ectopic site (mesentery) with no continuity with the native bladder, which we considered would be the ideal method of bladder regeneration and would have the potential for neobladder creation. The present study showed that the existence of cultured epithelium seeded onto BAM with no continuity with the native bladder is not the sole definitive factor for infiltrating mesenchymal cells to maintain their smooth muscle characteristics. Soon after grafting, epithelial-mesenchymal interactions seem to be important for the infiltrating mesenchymal cells to maintain smooth muscle characteristics. However, epithelial-mesenchymal interactions alone could not maintain these characteristics in infiltrating mesenchymal cells.

When the bladder is augmented with BAM, mesenchymal cells migrating into BAM are thought to be derived from bladder smooth muscle cells [5,7]. However, Badylak et al. [15] showed that ≈70% of cells migrating into subcutaneously implanted acellular matrix were derived from bone marrow. A rigorous demand for tissue repair may recruit circulating or resident stem cells and trigger them to undergo differentiation into various cells [16]. These bone marrow-derived cells are capable of maintaining, generating and replacing terminal differentiated cells, including smooth muscle cells [17,18]. In this regard, cells in the regenerated bladder in an augmentation model could be derived not only from native bladder tissue but also from bone marrow. Fibrocytes, which were apparent after 56 days in the present study, were reported to have features of haematopoietic cells and are considered to be derived from bone marrow. Accordingly, mesenchymal cells infiltrating BAM in the present model might have been derived from bone marrow and from surrounding tissues (mesentery). Further studies are needed to clarify the possible role of various factors, including bone marrow-derived and circulating stem cells, in bladder regeneration.

In conclusion, epithelial-mesenchymal interactions have been assumed to be one of the most critical factors in smooth muscle development. In the present intraperitoneal regeneration model, mesenchymal cells infiltrating the scaffold gradually lost smooth muscle characteristics with time. The present results suggest that interactions between cultured adult urothelial cells and infiltrating mesenchymal cells alone could not maintain the smooth muscle characteristics of infiltrating mesenchymal cells.

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CONFLICT OF INTEREST

None declared.

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Abbreviations: BAM, bladder acellular matrix; TEM, transmission electron microscopy.
Apoptosis: a key effector mechanism of lymphocyte action in human nonseminomatous testicular carcinoma?

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INTRODUCTION

Human testicular tumours, especially seminomatous tumours, are typically infiltrated by numerous lymphoid cells [1,2]. According to concepts of tumour immunology, tumour-infiltrating lymphocytes (TILs) are believed to attack and eliminate tumour cells, thus being significant determinants of outcome for a variety of malignancies [3–5]. TILs also seem to be a favourable prognostic factor of seminomas, although the functional role of TILs in seminomas was questioned [1,2,6,7]. Only recently was significant lymphoid infiltration reported in nonseminomatous germ cell tumours (NSGCTs) [8,9].

The mechanism of action of TILs is not clear; triggering apoptosis in tumour cells may be one possible way [10]. There is considerable evidence that apoptosis is a relevant factor of tumorigenesis and tumour progression in several tumours, including human testicular GCTs [11–14]. This was shown for several tumours, e.g. colorectal carcinoma, carcinoma of the breast, or lymphomas [15–17]. In previous studies of the apoptotic index (AI) of tumour cells and lymphocytes in testicular cancer, AIs of both cell types were significantly higher in the tumour region than in tumour-associated tissue. It was suggested that there is a lymphocyte-tumour cell interaction restricted to the tumour region, thus supporting the hypothesis of apoptosis being a major biological effect of lymphoid tumour infiltration [8]. Others questioned the capability of TILs to induce apoptosis in testicular tumour cells, as they showed a low activity of perforin and FasL [18].

To date the degree of lymphoid infiltration has not been correlated with the degree of apoptosis in human testicular cancer. Thus the objective of the present study was to determine whether the number of TILs correlates with the extent of apoptosis in testicular GCTs. As in previous investigations there was a discrepancy in the number of cells with the two main apoptotic features in human testicular tumours, DNA fragmentation and chromatin condensation [19,20], and thus TIL infiltration was correlated separately with cells showing one or both of these features.

MATERIALS AND METHODS

Tissue samples obtained from 47 patients with nonseminomatous and 15 with seminomatous GCTs were examined under the guidance of an experienced pathologist. The cases were selected from files of diagnosed surgical specimens at the authors’ institution, and selected for evaluation by the pathologist according to the quality of the histological sections. All investigations had the approval of the local Human Investigations Committee.

In all, 26 NSGCT were pure embryonal cell carcinoma (ECC) and 21 were different tumour entities; there were no metastases in 24 patients (12 each pure ECC and 12 mixed GCT) and 23 had metastatic disease (14 pure ECCs and nine mixed).

OBJECTIVE

To correlate the number of tumour-infiltrating T lymphocytes (TILs) with the extent of apoptosis in testicular germ cell tumours, as TILs are considered to be a favourable prognostic factor of human testicular tumours, especially of seminomas, but the mechanism by which TIL contribute to an improved outcome is unclear.

MATERIALS AND METHODS

Tissue samples from 47 patients with nonseminomatous germ cell tumour (NSGCT) and 15 with seminomatous GCT were investigated immunohistochemically for lymphocyte infiltration and apoptosis. The apoptotic index (AI) was assessed in various categories (DNA condensation and fragmentation) using in-situ end-labelling to identify typical apoptotic DNA strand breaks, and nuclear staining to identify typical apoptotic morphology.

RESULTS

In seminomatous GCT there was no correlation between the number of TILs and any AI. In NSGCT there was only a relationship between lymphoid infiltration and those AIs showing morphological criteria of apoptosis in a small subgroup of NSGCT, i.e. metastasized embryonal cell carcinomas. Only 1.2% (AI, chromatin condensation) and 0.8% (AI, fragmentation and condensation) of all tumour cells showed these features of apoptosis. The overall AI in NSGCT was 7.9%.

CONCLUSIONS

TILs do not seem to induce apoptosis in testicular tumours. Embryonal cell carcinomas might be susceptible to lymphocyte attack, resulting in apoptosis of the tumour cell. The mechanisms of interaction between lymphocytes and testis tumour cells need further investigation.

KEYWORDS

apoptosis, testicular cancer, tumour infiltrating lymphocytes

LYMPHOCYTE INFILTRATION AND APOPTOSIS IN HUMAN TESTICULAR CARCINOMA

All NSGCT patients with no signs of metastasis on CT of the abdomen (clinical stage, CS, I) had a retroperitoneal lymph node dissection, confirming pathological stage I in 24; six had pathological stage IIA after investigating histological sections of the lymph node dissection. Initially 17 patients had retroperitoneal metastases on CT of the abdomen (CS IIA in seven, CS IIB in four, CS IIC in one) or of the lung (CS III in five). All received at least three cycles of polychemotherapy with cisplatin, etoposide and bleomycin or cisplatin, etoposide and ifosfamide.

Ten patients with pure seminoma showed no metastasis at the time of primary diagnosis and five did (CS IIA in two, CS IIB in two, CS IIC in one). All patients with CS I seminoma received radiotherapy to the para-aortic/paracaval field as initial adjuvant treatment. Patients with metastatic disease were treated with three cycles of polychemotherapy with cisplatin, etoposide and bleomycin.

Microdissection was not used for NSGCT because there were too few evaluable cases of each subtype to detect significant differences between the histological components (ECC in 17, yolk sac in six, choriocarcinoma in five, seminoma in five, mature teratoma in three, and immature teratoma in six). There were even fewer evaluable cases because in some of the tumours the percentage of the specific region was too small to investigate enough microscopic fields to get a representative cell count for the specific tumour entity. However, to have some impression of possible differences among histological subtypes, pure ECCs were investigated separately.

All patients were followed regularly at intervals according to the European Germ Cell Cancer Consensus Group [21]. All patients are still alive and relapse-free after a median follow-up of 91.9 months.

To evaluate apoptosis, the in-situ end-labelling (ISEL) method and DNA counterstaining was used. Paraffin-embedded tissues were fixed in 4% buffered formalin and processed by standard methods; 5 µm consecutive sections were mounted on coated slides (Superfrost/Plus, Menzel Gläser, Munich, Germany). The ApoTaq Plus Kit (Oncor, Appligene, Heidelberg, Germany) was used for ISEL as reported previously [19]. In brief, after deparaffinisation, tissue sections were incubated with 20 µg/mL of proteinase K (Boehringer Mannheim, Germany) diluted in distilled water for 8 min at 37 °C, washed in distilled water (four times), incubated with TdT mix according to the manufacturer’s protocol for 60 min at 37 °C in a humidified chamber, washed three times in distilled water and incubated with antidig-fluorescein isothiocyanate (FITC) for 30 min at room temperature in a humidified chamber. The DNA specific dye 4',6-diamidino-2-phenylindole (DAPI, final concentration 1.0 µg/mL; Serva, Heidelberg, Germany) was added to examine nuclear apoptotic morphology and incubated for 5 min. Slides were washed three times with distilled water, dried at room temperature and mounted in glycerol/paraphenylene diamine (anti-fading agent; final concentration 1 mmol/L, Aldrich, Steinheim, Germany). HL-60 cells (as the positive control) were treated similarly, only omitting the first treatment step with proteinase K. For negative controls, TdT was omitted from the reaction mixtures. The method was optimized (e.g. duration of proteinase K exposure) in several preliminary experiments (not shown). Cells with a FITC signal in the nucleus were considered to contain fragmented DNA.

Morphologically, cells were differentiated into non-apoptotic cells, characterized by a homogeneous distribution of DNA in the normal-sized nucleus and therefore considered to represent normal cells, and apoptotic cells (condensed DNA) using DAPI as a DNA-specific fluorescent dye.

T-cells were visualized in the same tissue sections as used to determine the AI, using a double-immuno-fluorescence technique with a CD45RO mouse antibody (clone OPD4 Fa, Dako, Hamburg, Germany) using a rhodamine-conjugated goat anti-mouse secondary antibody (Fa.Dako, Hamburg, Germany), using the standard protocol published recently [9]. Tissue sections of activated lymph nodes were used as positive controls.

The amount of apoptosis was quantified as the AI, i.e. the percentage of apoptotic cells in all investigated cells of the tumour. A recent report showed a significant difference in the AI in testicular tumours depending on the detection method used [20]. Therefore, the AIs were obtained with ISEL and by counting typical apoptotic morphology. The slides were scored for the two methods using an epifluorescence microscope (×400; Orthoplan, Leica, Wetzlar, Germany) equipped with a filter block for DAPI excitation (excitation 270–380 nm; emission 410–580 nm). When changing the filter block with the filter wheel, the same cells were examined for FITC signals (excitation 450–490 nm; emission ≥ 520 nm, long-pass filter) enabling differentiation of morphologically normal or apoptotic cells which either FITC signals (DNA fragmentation) or not. T-lymphocytes were also visualized with the epifluorescence microscope using a filter for rhodamine signals (excitation 546–555 nm; emission 570 nm). This gave three different AIs, i.e. of morphologically normal cells showing DNA fragmentation (AI_DNAfrag), of morphologically apoptotic cells with no DNA fragmentation (AI_cond) and of morphologically apoptotic cells with DNA fragmentation (AI_sim-cond). The sum of these AIs was considered to represent all apoptotic cells that could be detected in the tissue section, and therefore termed AI_total.

The main aim was to assess whether TILs induce apoptosis in tumour cells; clinically it is relevant to correlate the number of TILs with the degree of apoptosis separately for metastatic and non-metastatic NSGCTs, to have some impression of whether there is any clinical significance of TILs in NSGCT warranting further investigation. This was not done for the seminomas as all patients in CS I received radiation therapy. All AIs were correlated with the size of the tumour, to exclude the possibility that a large tumour volume is associated with greater apoptosis, thus falsifying other correlation data.

For statistical analysis, 15 microscopic fields were scored per testis tumour tissue sample, with a mean (SD) of 1517 (365) cells examined. Significance levels for the difference between groups were calculated using Student’s t-test, or if the equal-variance test failed, with the Mann-Whitney rank sum test. The AI and degree of TIL was expressed as the percentage of apoptotic cells from all investigated cells of the tumour, if not indicated differently; values shown are the mean (SD). Non-apoptotic TILs were correlated with the AI of tumour cells using Spearman rank order correlation.

RESULTS

The AI_DNAfrag, AI_cond and AI_sim-cond differed significantly in testicular tumours, independent of the histological subtype, as reported recently [20]. Briefly, in all NSGCT investigated the AI_DNAfrag at 7.37 (10.4)% was...
lymphoid infiltration and AI

Only lymphocytes which showed no feature of apoptosis were used for Spearman rank correlation with apoptotic tumour cells, as they were considered to be unable to induce apoptosis in tumour cells. In NSGCT, 81.7 (21.03)% and in seminomas, 89.8 (6.65)% of all TILs showed no features of apoptosis; the proportion of TILs was significantly higher in seminomas, at 25.3 (12.41)% than in NSGCT, at 5.0 (5.83)% (P < 0.001). There was no difference in proportion of TILs in metastatic and non-metastatic ECC, at 6.38 (8.81)% and 5.01 (4.77)% (P = 0.875) or in mixed GCTs, at 4.76 (2.68)% and 3.57 (4.21)% (P = 0.465).

The mean diameter of the tumour was similar in all subgroups, i.e. metastatic and non-metastatic, respectively, pure ECC, mixed GCT and seminoma, at 2.37 (1.9), 3.06 (2.0), 3.4 (2.2), 3.00 (1.5) and 3.49 (1.7) cm. There was no significant difference between metastatic and non-metastatic tumours within one entity nor among these tumour types. There was also no correlation between tumour size and AI (Table 1) or in metastatic, respectively, pure ECC, mixed GCTs and non-metastatic tumours. However, the overall AI did not differ between metastatic and non-metastatic tumours (data not shown), in agreement with previous results [8]. Therefore the clinical significance of this correlation seems questionable.

In NSGCT the situation differed; for all NSGCT independent of their metastatic status or histological subtype, there was a significant positive correlation between the degree of lymphoid infiltration and $A_{\text{Il}}$ and $A_{\text{Il,cond}}$ (Fig. 1A). There was no correlation for both either $A_{\text{Il,frac}}$ ($r = 0.075$, $P = 0.613$) or $A_{\text{Il,cond}}$ ($r = 0.055$, $P = 0.710$).

Further differentiation of all NSGCT into pure ECC and mixed GCTs showed a highly significant correlation in pure ECC (Fig. 1B), whereas in mixed germ cells this correlation could not be confirmed (Fig. 1C). Considering metastatic status, there was the same significant correlation for metastatic ECC only (Fig. 1D). All other subgroups showed no correlation between lymphoid infiltration and any AI. Thus the significant correlation of lymphoid infiltration and $A_{\text{Il,cond}}$ and $A_{\text{Il,frac}}$ in all NSGCTs is a consequence of this correlation in metastatic pure ECC.

DISCUSSION

Lymphocytic infiltration is common in testicular carcinoma, both for seminomatous [7] and NSGCT [8,9]. The degree of lymphocytic infiltration in testicular cancer, at least in seminomas, seems to correlate with a favourable outcome [7]. However, we could not confirm these findings for NSGCTs in the present study; there was no difference in TILs between metastatic and non-metastatic tumours.

The mechanism by which TILs interact with tumour cells is not fully understood [10,18]; a possible mechanism might be the induction of apoptosis. If apoptosis is a key biological effect, mechanism of cytotoxic action of TILs [10] a correlation would be expected between TILs and apoptosis in testicular GCTs.

The equivalence of methods for detecting apoptosis in human testicular tumours is questionable; only 30% of all apoptotic cells could be detected using typical apoptotic morphology, compared to 80% using an ISEL method [20]. Thus we correlated the degree of lymphocytic infiltration separately with cells showing one or both of these features.

For NSGCT there was no correlation between TILs and the AI in the tumour tissue, except in the subgroup of metastasized ECCs, in which there was a correlation between TILs and $A_{\text{Il,cond}}$ and $A_{\text{Il,frac}}$ but not with $A_{\text{Il,frac}}$. From the clinical perspective these results are surprising, as a correlation would be expected between the AI and lymphoid infiltration in non-metastatic rather than metastatic tumours. However, the AIs showing this correlation ($A_{\text{Il,cond}}$ $A_{\text{Il,frac}}$) make only a very small contribution to the overall AI. Indeed the overall AI did not differ between metastatic and non-metastatic tumours (data not shown), in agreement with previous results [8]. Therefore the clinical significance of this correlation seems questionable.

Nevertheless, these data suggest the selective activation of a distinct apoptotic programme in ECC. DNA fragmentation and the typical apoptotic morphology in testicular cancers might be a consequence of two independent genetic programmes [8]. Evidence for this was based particularly on the finding that most of the apoptotic bodies representing the final stage of the apoptotic process had no DNA fragmentation; at least in this stage the occurrence of both apoptotic features should be expected [8,19]. In other systems the morphological features of apoptosis also occur in the absence of DNA fragmentation and vice versa [22,23].

That only in a distinct subgroup of NSGCT was there a significant correlation between TILs and the AI implicates tumour-specific changes that make tumour cells susceptible to lymphocytic attack, resulting in apoptosis of the tumour cell. Indeed, seminomas have a different gene expression pattern than do NSGCT [24]; it cannot be excluded that a

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>$A_{\text{Il,frac}}$</th>
<th>$A_{\text{Il,frac}}$</th>
<th>$A_{\text{Il,cond}}$</th>
<th>$A_{\text{Il,cond}}$</th>
</tr>
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<tr>
<td>Seminoma</td>
<td>0.352 0.600 0.233 0.700</td>
<td>0.683 0.300 0.683 0.075</td>
<td>0.067 0.200 0.450 0.500</td>
<td>0.450 0.050 0.233 0.700</td>
</tr>
<tr>
<td>metastatic</td>
<td>0.631 0.158 0.404 0.286</td>
<td>0.535 0.213 0.838 0.067</td>
<td>0.512 0.213 0.838 0.099</td>
<td>0.445 0.254 0.377 0.275</td>
</tr>
<tr>
<td>non-metastatic</td>
<td>0.444 0.219 0.659 0.124</td>
<td>0.856 0.049 0.727 0.075</td>
<td>0.572 0.171 0.869 0.05</td>
<td>0.434 0.075 0.520 0.233</td>
</tr>
<tr>
<td>ECC</td>
<td>0.067 0.200 0.450 0.500</td>
<td>0.450 0.050 0.233 0.700</td>
<td>0.783 0.200 0.500 0.050</td>
<td>0.450 0.050 0.233 0.700</td>
</tr>
<tr>
<td>metastatic</td>
<td>0.067 0.200 0.450 0.500</td>
<td>0.450 0.050 0.233 0.700</td>
<td>0.783 0.200 0.500 0.050</td>
<td>0.450 0.050 0.233 0.700</td>
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<tr>
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<td>0.783 0.200 0.500 0.050</td>
<td>0.450 0.050 0.233 0.700</td>
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$P < 0.001$. The distribution was similar for seminomatous tumours, with respective values of 2.30 (1.7)% 0.49 (0.4)% and 0.39 (0.3)% (P < 0.001). $A_{\text{Il,cond}}$ was 7.99 (6.8)% in NSGCTs and 3.18 (2.0)% in seminomas.
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FIG. 1. Spearman rank correlation between TILs and Al in: A, NSGCT (metastatic and not); B, pure ECC (metastatic and not); C, mixed GCTs (metastatic and not); and D, metastasized pure ECC. In A, B and D there was a significant correlation for $A_{\text{chrom cond}}$ and $A_{\text{fr+cond}}$ but not in C for either. In each plot, a shows $A_{\text{chrom cond}}$ and b $A_{\text{fr+cond}}$.

Although there were too few seminomas treated by surveillance to allow an assessment of metastatic and non-metastatic seminomas in the present study, it was clear that the number of TILs did not correlate with the AI in this entity. There might be several reasons for this lack of correlation in most of the NSGCT subgroups and in seminomas. One could be the underestimation of the AI as a result of rapid phagocytosis. Irrespective of the initiating insult, apoptosis is quick [26], thus in any statistical analysis, very few apoptotic cells would be apparent at any given instant, reflecting a considerable contribution to cell turnover [27], thus failing to detect any correlation between lymphocyte infiltration and the degree of apoptosis.

Another reason might be a disturbed interaction between both cell types; Bols et al. [18] found CD4+ T cells, CD8+ T cells and B cells present within the infiltrate in similar proportions, while others found B cells were more sparse [6]. Seminoma cells do not express MHC class I molecules [28] and therefore they are not thought to be susceptible to conventional T cell attack. Moreover CD8+ T cells within the infiltrate show low levels of activity, as measured by their expression of perforin [18]. This contradicts the findings of Yakirevich et al. [29], who found activated granzyme B+ lymphocytes to correlate strongly with the tumour cell AI in testicular seminoma. They suggested that apoptotic tumour cell death in this neoplasm is triggered by this cytotoxic granule effector.

The Fas/FasL system is a further possible mechanism of interaction [30,31]. FasL in lymphocytic cell lines is induced by re-stimulation of previously activated T cells, thus executing the organism’s antitumour response by inducing apoptosis in Fas-releasing tumour cells [32]. Recently Fas and FasL expression in tumour cells was shown in human seminoma and NSGCT, but there was no correlation with the AI. It was suggested that the Fas/FasL system is unlikely to be responsible for immune escape of the tumour in testicular cancer [9,33,34].

The AIs may depend on the size of the tumour, as with increasing size oxygen and nutritional supply may decrease [25]. Therefore the AIs were compared with tumour size, but they were not correlated. Taken together, these results further suggest that subgroups of NSGCT may have distinct genetic programmes that can be executed according to apoptotic stimuli. However, the clinical significance in testicular tumours seems questionable.

Similar correlation would be found in other pure NSGCTs, and therefore further investigations on a larger cohort of pure NSGCT are necessary.

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This supports the findings of other groups, that apoptosis does not seem to be the key effector mechanism for lymphocyte-tumour cell interaction. If it is confirmed that the number of TILs correlates with a more favourable outcome in patients with seminoma, other mechanisms than apoptosis should be responsible.

Thus the present results contradict the hypothesis that apoptosis is a biological key effector of lymphoid infiltration in testicular tumours in most of NSGCT or seminomas. In NSGCT there might be a subgroup that is susceptible to lymphocyte attack, resulting in apoptosis of the tumour cell, but the clinical significance of this finding is questionable. The mechanisms of interaction between lymphocytes and testis tumour cells need further evaluation.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

None declared. Source of funding: German Ministry of Defense.

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Abbreviations: TIL, tumour-infiltrating lymphocytes; (nonseminomatous), germ cell tumour; AI, apoptotic index; ECC, embryonal cell carcinoma; CS, clinical stage; ISEL, in-situ end-labelling; FITC, fluorescein isothiocyanate; DAPI, 4',6-diamidino-2-phenylindole.
In vivo and in vitro response of corpus cavernosum to phosphodiesterase-5 inhibition in the hypercholesterolaemic rabbit

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OBJECTIVE
To investigate the effects of hypercholesterolaemia (HC) on rabbit corpus cavernosa in vivo and in vitro, and evaluate the efficacy of vardenafil and sildenafil in normal and HC rabbits, as the phosphodiesterase-5 (PDE-5) inhibitors vardenafil and sildenafil are widely used for treating erectile dysfunction (ED) and most organic causes of ED are associated with vascular risk factors like HC.

MATERIALS AND METHODS
Male New Zealand White rabbits were randomly divided into two groups; 11 HC rabbits were fed a 2% cholesterol diet, and 12 age-matched control rabbits received a regular diet. After 12–14 weeks, erectile responses to intravenous sodium nitroprusside (SNP) and PDE-5 inhibitors were evaluated for 2 h in conscious rabbits. Penile length was measured and the area under the curve calculated. Relaxant responses of corpus cavernosal strips to electrical-field stimulation (EFS) were measured before and after exposure to PDE-5 inhibitors and the nitric oxide synthase inhibitor N’-nitro-L-arginine methyl ester.

RESULTS
HC rabbits had a lower erectile response to SNP than controls; in both control and HC rabbits there was a greater erectile response after simultaneous exposure to SNP and vardenafil, or SNP and sildenafil. However, the responses of the HC rabbits were still significantly less than those of the controls.
Corpora from control rabbits responded to EFS with greater relaxations at all frequencies, except 1 Hz. Corpora from both HC and control rabbits had greater responses to EFS after exposure to vardenafil and sildenafil; N’-nitro-L-arginine methyl ester diminished the response to EFS.

CONCLUSIONS
There was a significantly lower in vivo and in vitro erectile response in HC rabbits than in controls; erectile function measured in conscious rabbits can be used to assess quantitatively the efficacy of different agents, e.g. sildenafil and vardenafil, in pathological animals. In addition, both agents improve in vitro responses of erectile tissue from HC rabbits to EFS.

KEYWORDS
erectile dysfunction, hypercholesterolaemia, nitric oxide, rabbit

INTRODUCTION
Penile erection depends on smooth muscle relaxation affected by endothelial and neural factors [1]. The main neurotransmitter responsible for the erectile response is nitric oxide (NO), produced by innervated endothelium in the corpora cavernosa [2]. In addition, other neurotransmitters, e.g. noradrenaline, have been shown to be involved in erectile function [3]. By stimulating the formation of the intracellular second messenger cGMP, a cascade of events is initiated, which results in smooth muscle dilatation and ultimately the erectile response [2]. Conversely, the erectile response is eventually terminated when cGMP-specific phosphodiesterases (PDEs) catalyse the hydrolysis of cGMP to 5’-GMP, thus halting the cascade of reactions and leading to smooth muscle contraction with concomitant detumescence [2].

There are several causal factors involved in male erectile dysfunction (ED), i.e. psychogenic, organic, pharmacological and vasculogenic. Most cases of ED are associated with vascular risk factors, e.g. hypertension, hypercholesterolaemia (HC), diabetes and smoking [4]. These factors have been shown to cause atherosclerotic changes in penile arteries, which in turn result in poor arterial inflow [5]. In addition, these factors impair endothelium-mediated relaxation of blood vessels [6,7]. Recent studies showed that HC inhibits endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle [8], and that changes in the formation of arachidonate and cyclooxygenase products, or in cGMP-dependent (endothelium-independent) relaxation of corporal smooth muscle, were not responsible for the HC-induced impairment of erectile function [8].

With greater knowledge of the physiology of penile erection, the management of ED changed dramatically about a decade ago with the advent of the selective PDE-5 inhibitor, sildenafil [9]. The efficacy of sildenafil was confirmed in vivo and in vitro, in both animal and human studies [9,10]. Newer agents, which include vardenafil and tadalafil, have also been approved [11–15]. Recent studies comparing the efficacy of sildenafil and vardenafil show that the latter was significantly more effective than sildenafil in facilitating erection in anaesthetized rabbits [10]. In addition, using normal animals, a conscious-rabbit penile-erection model was designed for the same purposes of evaluating...
FIG. 1. The length of uncovered penile mucosa measured after intravenous (A) SNP (0.2 mg/kg), (B) sildenafil (5 mg/kg) and SNP, and (C) vardenafil (0.5 mg/kg) and SNP in conscious control (green closed circle) and HC rabbits (red closed square). *P < 0.05 vs control.

**TABLE 1.** RESPONSES OF CORPUS CAVERNOSUM FROM HC RABBITS TO PDE-5 INHIBITION

<table>
<thead>
<tr>
<th>Time after SNP (min)</th>
<th>Penile length (mm)</th>
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<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
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**MATERIALS AND METHODS**

Adult male New Zealand White rabbits (Millbrook Breeding Laboratories, Amherst, MA) were randomly divided into two groups fed normal control diets or diets containing 2% cholesterol (Purina LabDiet, St. Louis, Missouri). For the in vivo study, after 12–14 weeks on the diets, rabbits were randomly chosen and placed in a restraining device. Three rabbits from each group received intravenously (via the lateral ear vein) one of: sodium nitroprusside (SNP, 0.2 mg/kg) followed immediately by saline, SNP followed immediately by sildenafil (5 mg/kg), or SNP followed immediately by vardenafil (0.5 mg/kg) [17]. The volumes introduced intravenously were 0.1 mL/kg for SNP and 2.5 mL/kg for the PDE-5 inhibitors.

The length of uncovered penile mucosa was measured with callipers at 0, 2.5, 5, 7.5, 10, 15, 20, 30, 60 and 120 min, as described by Bischoff and Schneider [17].

For the in vitro study, rabbits were anaesthetized with intravenous ketamine (25 mg/kg) and blood drawn from the inferior vena cava to measure plasma cholesterol, using the Cholesterol-5-L and Triglyceride-5-L assay kits (Diagnostic Chemicals Ltd, Charlottetown, Prince Edward Island, Canada). The entire penis was then removed, the corpus cavernosa dissected into two or three strips of 2 × 10 mm, and the strips then suspended in organ chambers filled with 10 mL Krebs solution (containing indomethacin 10 μmol/L) and equilibrated with 95% O_2/5% CO_2 at 37°C.

Each strip was attached by a 3-0 silk ligature to a force-displacement transducer (FT0.03, Grass Instruments, Quincy, MA). The changes in muscle tension were recorded on a polygraph (7E, Grass Instruments). For the in vivo study, SNP-stimulated erections were expressed as penile length (mm) × time of measurement (min). Relaxant responses to EFS are expressed relative to the pre-contraction response to noradrenaline.

All data are expressed as the mean (SEM); the area under the curve (AUC) for the erectile response was calculated as penile length (mm) × time of measurement (min). Relaxant responses to EFS are expressed relative to the pre-contraction response to noradrenaline.

**RESULTS**

HC rabbits weighed significantly less than controls after 12–14 weeks on the 2% cholesterol diet, at a respective mean (SEM) of 3.04 (0.12) and 4.21 (0.05) g, and had significantly higher blood cholesterol, at 6284 (677) and 193 (10) mg/L, and triglyceride levels, at 2034 (304) and 779 (110) mg/L, respectively. Two rabbits in the HC group died during the study (one at 9 and the other at 10 weeks) secondary to severe aortic occlusion resulting from marked atherosclerotic disease, and were excluded from the study.

In the in vivo study, SNP-stimulated erections were <6 mm long and lasted <10 min. The erectile response of the HC group to SNP was significantly less than that of the control group (Fig. 1A). Intravenous vardenafil (0.5 mg/kg) with SNP caused a significant increase in erectile response in both groups (Fig. 1). The maximum was at 2.5 and 5 min.
and thereafter penile length gradually decreased over the 2-h period. The erectile response of the HC group to SNP and vardenafil was significantly less than that of the control group (Fig. 1B). The AUC was significantly greater in control rabbits receiving SNP and vardenafil than in those receiving SNP alone, and significantly greater than that of HC rabbits receiving SNP and vardenafil (Table 1).

Intravenous sildenafil (5 mg/kg) with SNP caused a significantly greater amplitude of response and AUC than in rabbits given SNP alone (Fig. 1A,C and Table 1). Controls had a significantly greater amplitude and AUC than HC rabbits (Figs 1C and Table 1). The onset of action and time course of erection were similar to those with vardenafil (Fig. 1B,C). The maximum amplitude, similar to that with vardenafil, was at 2.5 and 5 min, with erections waning over the 2-h period.

In the in vitro study, there was no difference in contractile response to noradrenaline between corporal strips from the HC and control groups (data not shown). Relaxation of corpus cavernosum smooth muscle in response to EFS was significantly less in strips from the HC group than controls at all frequencies except 1 Hz (Fig. 2). There was no significant effect of time on the relaxant response of strips to EFS in either group; repeating the frequency-response curve with no PDE-5 inhibitors caused similar responses to the first curve (data not shown). Corporal strips from control rabbits had significantly greater relaxant responses to EFS after incubation with both sildenafil and vardenafil at all frequencies (Fig. 3). There were no significant differences between responses in the presence of the PDE-5 inhibitors. L-NAME caused a significant decrease in the relaxant response of corpora from controls at 16 and 32 Hz (Fig. 3).

The effects of PDE-5 inhibition were qualitatively similar in corpora from HC rabbits, but the changes were significantly smaller. Incubation with sildenafil and vardenafil increased the relaxant response significantly at most frequencies (Fig. 4); there was no significant difference between the PDE-5 inhibitors. L-NAME decreased the relaxant response at 16 Hz only (Fig. 4).

**DISCUSSION**

The aim of the present study was two-fold: first, to determine if there was a difference in *in vivo* and *in vitro* erectile responses of control and HC rabbits to PDE-5 inhibition, and second, to determine whether these agents could stimulate erections measured in conscious HC rabbits. HC rabbits had

**TABLE 1** The effects of intravenous SNP, sildenafil and vardenafil on the duration of the erectile response, calculated as the AUC of erectile response

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HC</th>
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<tbody>
<tr>
<td>SNP</td>
<td>22.08 (3.25)</td>
<td>2.19 (2.19)*</td>
</tr>
<tr>
<td>+ sildenafil</td>
<td>567.9 (49.4)†</td>
<td>176.3 (21.9)*</td>
</tr>
<tr>
<td>+ vardenafil</td>
<td>616.3 (10.3)†</td>
<td>181.3 (16.35)*</td>
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*P < 0.05 vs control; †P < 0.05 vs SNP alone.
significant lower in vitro and in vivo erectile responses than the controls, and treatment with the PDE-5 inhibitors improved both in vitro and in vivo erectile responses in controls and, to a significantly lesser extent, HC rabbits.

Relaxation of the corpus cavernosum smooth muscle requires an intact penile endothelium [8,19–21]. Recent studies showed that HC impairs endothelium-dependent relaxation in the corpus cavernosum smooth muscle [8]. The mechanism is postulated to be multifactorial. The main attributed factor is the impaired ability of the endothelium to produce NO, a key component in the pathway of erectile response [2,8]. Other studies showed that atherosclerotic, ischaemia-induced corporal veno-occlusive dysfunction may result in alterations in corpus cavernosum smooth muscle relaxation or changes in structural properties of erectile tissue [20,21]. As the erectile response relies on intact endothelium and its production of NO, we attempted to show the efficacy of different PDE-5 inhibitors, both in vitro and in vivo, in HC rabbits and the influence of L-NAME, a NOS inhibitor that decreases NO formation.

As expected, the in vitro studies showed a lower relaxant response of corpus cavernosum smooth muscle strips in the HC group than in controls. There was no significant difference in the contractile response of corpus cavernosum smooth muscle from control or HC rabbits to noradrenaline, as reported previously [8]. Confirming these studies, there were no significant differences in the relaxant responses of corpus cavernosa of control rabbits after treatment with sildenafil and vardenafil [8]. Despite an impaired ability to relax in response to EFS under control conditions, which was consistent with the findings of Azadzoi et al. [8] in their study showing impaired endothelium lacking the appropriate NO production as the causative factor, we showed a significant increase in relaxant responses of corpus cavernosum smooth muscle from HC rabbits after treatment with sildenafil or vardenafil. Similar to the present findings in the control animals, there were no significant differences between the effects of vardenafil and sildenafil on responses to EFS.

For the in vivo studies we used the conscious-rabbit model described by Bischoff and Schneider [17], to bypass the complicated surgery required to monitor intracavernosal pressure, and the potential inhibitory effects of anaesthesia on erectile function. Although the efficacy of sildenafil and vardenafil had been tested using this method in normal rabbits, the efficacy had not been confirmed in a pathological animal model. In the absence of sexual stimulation the method requires a source of NO, provided by SNP. There was a significant potentiation of the erectile response in control rabbits when given either sildenafil or vardenafil with SNP, and a greater overall duration of erectile response (measured as the AUC) with the combined treatments. Sildenafil (5 mg/kg) and vardenafil (0.5 mg/kg) were equally effective in terms of the timing of the greatest amplitude of the erectile response and AUC in control rabbits.

In HC rabbits there was also a significantly greater amplitude of erectile response and AUC after treatment with sildenafil or vardenafil with SNP. As in the control group, there were no significant differences between the chosen doses of sildenafil and vardenafil when comparing the time of greatest erectile amplitude or AUC in HC rabbits. However, we confirmed the utility of the conscious-rabbit penile erection model for evaluating the efficacy of different agents in animals in this pathological state.

Thus we successfully used the conscious-rabbit penile erection model to test the efficacy of different agents for improving erectile function, in both normal and HC rabbits. The ability to use this test with consistent reproducibility on HC rabbits, showing significant differences from control rabbits, allows experiments with other controlled disease states that may impair erectile function. We showed that in vivo, erectile function is probably affected by atherosclerotic disease (as reduced blood flow and hampered innervation), and in vitro, secondary to decreased NO from an impaired endothelium. With this knowledge the present findings in the HC rabbit can be applied to the current management of ED in humans with atherosclerotic disease secondary to HC.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

M.D. White was the recipient of a North-eastern Section AUA Grant for research support.

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Abbreviations: SNP, sodium nitroprusside; AUC, area under the curve; NO(S), nitric oxide (synthase); PDE, phosphodiesterase; ED, erectile dysfunction; HC, hypercholesterolaemia(ic); EFS, electrical-field stimulation; L-NAME, N’-nitro-L-arginine methyl ester.
Effect of bladder ischaemia/reperfusion on superoxide dismutase activity and contraction

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OJECTIVES
To correlate the effect of bilateral in-vivo bladder ischaemia/reperfusion on superoxide dismutase activity (SOD) and then to correlate this with contractile responses to various forms of stimulation.

MATERIALS AND METHODS
Twenty mature male New Zealand White rabbits were divided into five equal groups: group 1 (controls); group 2, 2 h of in-vivo bilateral bladder ischaemia; and groups 3–5, 2 h of in-vivo ischaemia followed by 1, 7 or 14 days of reperfusion (recovery). At the end of the treatment period, bladder strips were incubated and placed in isolated baths for contractile studies. The contractile responses to field stimulation, carbachol (10 μmol/L), ATP and KCl were determined. The balance of the bladder body was separated into muscle and mucosa sections and analysed for SOD activity.

RESULTS
There were few effects on contraction either directly after ischaemia or after 1 day of reperfusion. However, all contractile responses were significantly reduced at 7 and 14 days after ischaemia. SOD activity of the detrusor muscle was reduced significantly immediately after ischaemia and at 7 and 14 days of reperfusion. SOD activity of the mucosa was significantly greater than that of the muscle, and was significantly reduced by both ischaemia and all times of reperfusion.

CONCLUSIONS
These studies show clearly that both ischaemia and reperfusion result in significantly lower activity of SOD, and in contractile dysfunctions, and that reperfusion results in greater decreases in both SOD activity and contractile responses than ischaemia alone.

KEYWORDS
bladder, superoxide dismutase, ischaemia, reperfusion

INTRODUCTION
The function of the lower urinary tract is collecting and storing urine at low pressures and expelling it by maintaining adequate intravesical pressures [1,2]. Normal bladder function depends on the integrity of its autonomic innervation, cellular structure and metabolism [1,2]. Currently it is thought that various bladder disorders, including obstructive bladder dysfunction, hyperactivity, hypercholesterolaemia and diabetes, may in part be caused by ischaemic reperfusion (I/R) injury [3–7]. For partial BOO, Greenland et al. [5,6] showed clearly that in normal pigs, bladder contraction during micturition results in cyclical decreased blood flow and simultaneous decreased tissue-oxygen tension, both of which recovered after voiding. In pigs with partial BOO the ischaemia and tissue hypoxia were significantly and substantially greater. Reperfusion after the cyclical periods of ischaemia/hypoxia would also be significantly greater in these pigs. Decreased blood flow (ischaemia) has also been shown in rat, rabbit and dog models of partial BOO [8–10]. In addition to partial BOO, acute overdistension (retention) has also been shown to result in ischaemia [11]. Also, rabbits fed a high cholesterol diet developed severe ischaemia and contractile dysfunction of the lower urinary tract [7,12].

A recent study indicated that reperfusion may cause a more severe injury than ischaemia alone [13]. Further evidence for an ischaemic cause of obstructive and ischaemic bladder disorders comes from studies showing that phytotherapeutic products rich in antioxidants have significant protective effects on rabbits subjected to both partial BOO and in vivo ischaemia [14–16].

Superoxide dismutase (SOD) is the cell’s chief defence against activated-oxygen free radicals; it comprises a family of enzymes, of which three unique members have been described [17,18]. They all act by converting superoxide to peroxide which is then further broken down by catalase to oxygen and water. Because I/R is a major causal factor in the pathological response of the rabbit to partial BOO and other disorders [3–12], activation of SOD would be important to the recovery of the bladder. We have direct evidence (unpublished observations) that partial BOO results in the generation of free radical damage to detrusor smooth muscle proteins, which supports the importance of SOD within the bladder.

The specific aim of the present study was to determine the effects of bilateral ischaemia (in a model of pure I/R) on SOD activity and to correlate it with the effects on contractile responses to various forms of stimulation.

MATERIALS AND METHODS
Twenty mature male White New Zealand rabbits were divided into five equal groups: group 1 (controls); group 2, 2 h of in vivo bilateral ischaemia; and groups 3–5, 2 h of ischaemia followed by 1, 7 or 14 days of reperfusion (recovery), respectively. At the end of the experimental period each rabbit was anaesthetized and the bladder rapidly excised; two bladder strips were prepared from the bladder body for contractility studies. The
balance of the bladder body was separated by blunt dissection into muscle and mucosa sections, frozen in liquid nitrogen, and stored at –70 °C for biochemical analysis of SOD.

To create ischaemia, each rabbit was anaesthetized with isofluorane (1–3%) and the bladder base exposed. The vesical arteries were isolated and clamped with microvascular clamps for 2 h, after which the bladder was either immediately excised (group 2) or the clamps removed and the wound closed with 2–0 silk in layers for 1, 7 or 14 days (groups 3–5).

Each strip was mounted in a separate 15-ml bath containing Tyrode’s solution (in mmol/L; 124.9 NaCl, 2.6 KCl, 23.8 NaHCO3, 0.5 MgCl2, 0.4 NaH2PO4, 1.8 CaCl2, and 5.5 dextrose) at 37 °C. Tissues were equilibrated with a mixture of 95% O2 and 5% CO2 at 2 g tension for 120 min to allow for full recovery of ATP after ischaemia. In a previous study we correlated the rate of recovery of contractile function with the rate of recovery of the intracellular ATP concentration after 60 min of in-vitro anoxia. Maximal contractile and ATP was recovered after 60 min, thus we were confident in using 120 min to allow for full recovery [13].

One end of each strip was connected to a force-displacement transducer and contractile responses recorded using a Model D Polygraph (Grass Instruments, Quincy, MA). The signal was then digitized using the Polyview A/D (Grass Instruments) computer analytical system.

Field stimulation (FS) was applied using platinum electrodes set on each side of the muscle strip, using a stimulator (S-88, Grass Instruments) delivering square-wave pulses of 80 V and 1 ms duration at 2, 8 and 32 Hz. FS was maintained for 20 s, the tension recorded and calculated as grams tension/100 mg tissue.

Following FS, maximal responses were determined sequentially for 1 mmol/L ATP, 20 μmol/L carbachol and 120 mmol/L KCl. Between the pharmacological stimulations, each strip was washed three times with fresh Tyrode’s solution at 15 min intervals.

SOD (total) activity was determined by the method of Flohe and Otting [19], using a cytochrome C reduction test. In this model, oxygen free radicals are generated by xanthine oxidase reactions with ferricytochrome C. SOD activity is calculated from the degree of inhibition of this reaction and recorded as the change in optical density (mOD) at 550 nm (using a spectrophotometer) per milligram of protein.

Specifically, bladder tissue was homogenized in a 50-mmol/L phosphate buffer (pH 7.8) at 200 mg/mL. The homogenate was centrifuged at 18,000 g for 10 min. The pellet was eliminated and the supernatant used for the following assay: 2 mL of solution A (0.76 mg xanthine in 10 mL of 1 mmol/L NaOH, added to 50 mg cytochrome C + 3.7 mg EDTA in 100 mL 50 mmol/L phosphate buffer) at 25 °C was incubated with 50 μL of the tissue sample or SOD standards in a 3-ml cuvette; 200 μL of solution B (5.63 μL xanthine oxidase in 1 mL 0.1 mmol/L EDTA) was used to start the reaction. After mixing, the absorbance change indicating cytochrome C reduction was measured in a spectrophotometer at 550 nm for 2 min. The change in absorbance with time over the first 2 min for all preparations was linear, and used in the plots shown.

Responses were compared quantitatively as the concentration of enzyme (protein) that inhibited the reaction by 25% (IC25); although the IC25 is used more widely, several of the preparations did not reach 50% inhibition. For comparison the reaction curve of pure SOD is also given.

Purified SOD (Sigma Chemical Co., St Louis, MO) was prepared at 25 ng/mL and diluted 1:1 to 0.39 ng/mL for a standard curve, with specificity confirmed by heating both preparations for 10 min at 90 °C, which eliminated all activity.

Data were assessed using ANOVA followed by a Bonferroni test for individual differences, with P < 0.05 taken to indicate statistical significance.

FIG. 1. Contractile responses of the control bladder. Each bar is the mean (SEM) of four individual bladders.

RESULTS

Figure 1 shows the maximum responses to the various forms of stimulation used, and Fig. 2a the effect of bilateral ischaemia on the contractile responses to FS. Data are presented as the percentage of the control response. The response to FS at 2 Hz was decreased by ischaemia alone; there was no significant difference in the responses after 1 day of reperfusion but after 7 days all were significantly less, and further reduced at 14 days. Fig. 2b shows the effect of bilateral ischaemia on the contractile responses to ATP, carbachol and KCl. The response to ATP was decreased by ischaemia alone and after all periods of reperfusion; there were no significant differences in the responses after ischaemia alone or after 1 day of reperfusion for carbachol or KCl. The responses at 7 days after ischaemia for carbachol and KCl were significantly lower and further reduced at 14 days. In general, the responses to FS were significantly more sensitive to reperfusion than to either carbachol or KCl.

Figure 3 shows the SOD activity curve for purified SOD; the IC25 was 0.5 ng/mL. The SOD activity curves for detrusor smooth muscle and mucosa are shown in Fig. 4a,b with a comparison of the IC25 in Fig. 5. In both muscle and mucosa the control bladders had the highest SOD activity (the greater the rate of mOD decrease the greater the activity). The activity of the control mucosa was significantly greater than that of the control muscle. Ischaemia alone resulted in significant decreases in SOD activity in both muscle and mucosa. In the muscle, the activity increased to control levels at 1 day of...
The SOD activity of the muscle decreased to very low levels at 7 days of reperfusion and recovered somewhat by 14 days, although still significantly low. The SOD of the mucosa remained low at 7 days and was reduced further at 14 days.

**DISCUSSION**

Ischaemia and reperfusion have been implicated as important causes in deteriorating bladder function [3–13,20–25]. Whenever the bladder contracts, the increased intra-wall pressure results in compression of the blood vessels (probably the veins) and results in decreased blood flow and tissue hypoxia. As mentioned above, this has been shown best in studies on the obstructed pig. Partial BOO resulted in significant cyclical ischaemia-hypoxia. When the bladder empties, the contraction results in significant ischaemia and tissue hypoxia, followed by a rapid increase in blood flow (reperfusion) during bladder filling [3,5,6].

In both animal models and man, bladder overdistension (retention) results in significant decreases in blood flow. Decompression (catheterization and rapid emptying) of the bladder results in a rapid and significantly increased blood flow for a period [22,23]; it is this rapid increase in blood flow that results in the generation of reactive oxygen and nitrogen species that can then cause oxidative damage to lipids, proteins and DNA. Similarly, in the present model of bilateral ischaemia the clamped arteries induce a period of ischaemia which is then followed by a period of reperfusion when the clamped arteries are released [13].

Previous studies have indicated that after bilateral ischaemia, injury caused by reperfusion is worse than ischaemic injury alone, by the increase in free oxygen radicals [13]. These studies support the importance of SOD in the response of the bladder to I/R insults.

Hypercholesterolaemia, induced by feeding rabbits a high-cholesterol diet, results in significant atherosclerosis, which mediates lower urinary tract ischaemia and hypoxia; the result is compromised contractile function [7,12]. Partial BOO and in vivo ischaemia result in significant increases in...
cytosolic free Ca\(^{2+}\) that then activate specific calcium-activated hydrolytic enzymes, including calpain and phospholipases A\(_2\) [24,25]. Both these enzymes result in significant cellular and subcellular membrane damage, that participates in the disruptive effects of partial BOO and ischaemia on bladder function.

Another line of evidence that free radical damage is important in obstructive and ischaemic injury comes from studies showing that natural products high in antioxidants protect the bladder against both functional and biochemical damage induced by partial BOO and ischaemia [14–16]. These products and agents include Tadenan (Pygeum africanum), Kohki tea and grape products [14–16]. Perhaps the strongest evidence is that the antioxidant vitamin E was one of the most potent protective agents against obstructive damage [26]. In addition to this indirect evidence, there is now direct evidence (unpublished observations) that partial BOO results in oxidative damage directly to smooth muscle protein.

An important question about which there is little information is the effect of I/R on natural antioxidant mechanisms. The present study assessed the effect of bilateral I/R on SOD activity, SOD being the cell’s chief defence against activated oxygen free radicals. SOD1 contains copper and zinc, and is located in the cytoplasm, SOD2 contains manganese and is located in mitochondria, while SOD3 exists extra-cellularly [17,18]. They all act by converting superoxide to peroxide \((2\mathrm{O}_2^- + 2\mathrm{H}^+ \rightarrow \mathrm{H}_2\mathrm{O}_2 + \mathrm{O}_2)\) which is then further broken down by catalase to oxygen and water. The intermediate peroxide is itself a dangerous molecule and could cause damage to the cell if its production exceeds the catalytic ability of catalase. The importance of SOD in attenuating I/R injury was shown clearly using transgenic animal models [27,28], and that in acute ischaemic events, the level of intracellular SOD decreases, facilitating further injury [29].

The present study clearly shows that the SOD activity of the bladder mucosa is significantly greater than that of the detrusor smooth muscle. Direct ischaemia resulted in a reduction of SOD activity within the mucosa but not in the smooth muscle; however, reperfusion resulted in a progressive decrease in SOD activity. In the muscle this reached a maximum at 7 days but was still reduced at 14 days, whereas in the mucosa the activity was reduced at 7 days and further reduced at 14 days of reperfusion. The progressive decreases in contractile responses during this period correlate very well with the lower SOD activity; there was a significant decrease at 7 days and a slightly smaller decrease at 14 days. This corresponds with the previous study by Bratslavsky et al. [13].

These studies show that I/R result in significant decreases in SOD activity in both the bladder mucosa and muscle, and the time course observed is consistent with the hypothesis that reperfusion is more damaging to the bladder than ischaemia alone. They also show the importance of antioxidants and products high in antioxidants, as they protect the bladder from free radical (reperfusion)
damage in relation to ageing and obstructive bladder disease. This idea is supported by the multicentre study currently undertaken by the National Institutes of Health, comparing the effectiveness of natural products (*Pygeum africanum* and saw palmetto) in the treatment of obstructive bladder disease. Further studies will be needed to characterize more completely the importance of cellular defence mechanisms against free radical damage in the pathogenesis of specific bladder diseases.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

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Abbreviations: SOD, superoxide dismutase; I/R, ischaemia/reperfusion; FS, field stimulation; mOD, change in optical density.
Allopurinol provides long-term protection for experimentally induced testicular torsion in a rabbit model

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OBJECTIVE

To assess the effect of five antioxidants on exocrine function of rabbit testes retained in situ for 24 h and 3 months after experimental torsion.

MATERIALS AND METHODS

The left testes of peripubertal rabbits were clamped for 60 min, after which the clamps were removed and the testes allowed to reperfuse. The right testes served as internal controls. There were eight rabbits in each of the following experimental groups: (a) sham; (b) 60-min ischaemia followed by reperfusion; (c) 60-min ischaemia followed by left orchiectomy. In five further groups, rabbits were exposed to 60-min ischaemia followed by reperfusion, but received one of the following antioxidants before reperfusion: acetyl salicylic acid, ascorbic acid, allopurinol, quercetin or superoxide dismutase. Both testes were excised at 24 h or 3 months. The degree of lipid peroxidation, a measure of free radical damage, was assessed in testicular tissue homogenates by measuring the tissue levels of malondialdehyde (MDA). The Johnsen score was used to assess the morphological damage at 24 h and 3 months for each group.

RESULTS

At 3 months twisted viable testes allowed to reperfuse had higher MDA levels than controls; the left testes of rabbits treated with allopurinol had significantly lower MDA levels than untreated rabbits and rabbits given other antioxidants. Rabbits given quercetin, ascorbic acid or superoxide dismutase had lower (but not significantly) left testicular MDA levels than untreated rabbits, while rabbits given acetyl salicylic acid had even higher levels. Allopurinol-treated rabbits had a Johnsen score of >7.6 and those given other antioxidants had scores of <7.6 at 3 months.

CONCLUSION

The twisted viable testis treated by orchidopexy contains high free radical levels at 3 months. Of the antioxidants studied, only allopurinol had a beneficial long-term effect, by significantly reducing testicular MDA levels at 3 months.

KEYWORDS

testicular torsion, antioxidants, treatment, long-term results.

INTRODUCTION

The current view is that the sequelae of testicular torsion can be explained on the basis of ischaemia/reperfusion (I/R) injury [1–6]. Previously the sequelae were attributed to an autoimmune reaction or a sympathetic mechanism, among other factors [7,8]. In agreement with the I/R injury hypothesis and the role of free radicals in the disease process, numerous experimental animal studies have confirmed the efficacy of antioxidants like superoxide dismutase (SOD), catalase, allopurinol and aspirin in reducing the short-term damaging effect of torsion of the testis [1–6]. However, nearly all previous studies reporting beneficial effects of antioxidants were based on short-term observations. Thus beneficial effects were documented in <24 h [3,4,6], in <168 h [1] and in <4 weeks [2]; Only one study to date reported long-term protective effects of antioxidants on both the twisted testis and the contralateral testis at 6 weeks [5]. In humans, torsion of testes is common in the peripubertal age group (12–25 years) [9–12]. The sequelae of the disorder may take years to develop, e.g. reduced fertility is not apparent until the patient attempts paternity, perhaps 5–10 years after the initial torsion of the testis [9]. Hence there is a need to assess the long-term protective effects of antioxidants on testicular exocrine and endocrine function. In the present study, we assessed the effect of five antioxidants on testes retained in situ for 3 months after experimental torsion in a rabbit model.

MATERIALS AND METHODS

Conventionally reared New Zealand white male peripubertal rabbits (aged 3–6 months, weight 3–4.5 kg) were used; they were cared for according to Kuwait University Animal Resources Centre guidelines, reared in divided cages at 18–25 °C, a humidity of 50%, and allowed food and water ad libitum.

In all experiments the right testis served as an internal control and the left as the experimental side. The rabbits were anaesthetized using intravenous pentobarbitone sodium (Sagatal; Rhone Merieux, Dublin, Ireland, 26 mg/kg body weight). The left testis was delivered to the operating field via a longitudinal scrotal skin incision. Wounds were closed using 4/0 chromic catgut sutures. Ischaemia of the left testis was produced by clamping the spermatic cord structures using surgical spring clips (Atraumax™, Applied Vascular Devices, California, USA). The small blood supply to the testis via the remnant of the gubernaculum was cut during the period of ischaemia by applying mosquito artery
forceps to the gubernaculum. The left testis was subjected to 60 min of ischaemia. In control, sham-operated rabbits (no ischaemia) the left testis was exposed for 60 min but cord structures were not clamped. Reperfusion was established by removing the clips at the end of the period of ischaemia. The following groups of rabbits were studied; (A) sham-operated; (B) 60-min ischaemia followed by reperfusion; (C) 60-min ischaemia followed by left orchidectomy (no reperfusion); (D) 60-min ischaemia followed by reperfusion, but given the following antioxidants before reperfusion (eight rabbits each): (D1) acetyl salicylic acid (ASA) 50 mg/kg body weight intravenously 10 min before reperfusion; (D2) ascorbic acid, 2 mmol/kg body weight intraperitoneally 30 min before reperfusion; (D3) allopurinol 200 mg/kg body weight intraperitoneally 30 min before reperfusion; (D4) quercetin 30 mg/kg body weight intraperitoneally 50 min before reperfusion; (D5) SOD 3 mg/kg body weight intravenously 50 min before reperfusion.

ASA was obtained from Laboratories Synthelabo Groupe Le Pleisis Robinson, France; ascorbic acid, allopurinol, quercetin and SOD were obtained from Sigma Chemical, St Louis, Missouri, USA.

The dosage and timing of each antioxidant were based on our preliminary data on the metabolism of the drugs, and on published data, which determined the peak serum concentrations of the drugs at the time reperfusion was initiated [3,13–18]. In each group, both testes were harvested in four rabbits after 24 h of reperfusion, and after 3 months in the remaining four rabbits. At the end of the experiments the rabbits were killed by an overdose of pentobarbitone sodium. After harvesting the testes, each was divided into three equal parts for measuring the level of testicular malondialdehyde (MDA), histological examination and storage for possible later use. MDA was used as a measure of free radical damage, to assess the degree of lipid peroxidation in each testis. Details of the MDA assay and quality control are as described previously [19].

Testicular tissue was homogenized with 1.5% KCl to make a 10% homogenate, using a glass PTFE homogeniser. The degree of lipid peroxidation in tissue homogenate was assessed by the method of Ohkawa et al. [20], which measures MDA levels as the concentration of a pink chromogen compound formed when MDA couples with thiobarbituric acid. The protein content of the homogenate was determined according to the procedure of Lowry et al. [21] and values expressed as nmol MDA/mg protein.

A portion of the harvested testis was fixed in Bouin’s solution, processed routinely into paraffin wax and stained with haematoxylin and eosin. To minimize intra-observer variation in using the scoring system, only one histopathologist (J.T.A.) assessed all the testicular specimens while unaware of origin. The mean testicular biopsy score (Johnsen score, [22]) was used to compare the histology of testes exposed to 24 h of reperfusion with those exposed to 3 months of reperfusion. The Johnsen score (Table 1) is based on the premise that with testicular damage there is successive disappearance of the most mature cell type, with progressive degeneration of germinal epithelium, with the disappearance of spermatozoa and spermatids, then spermatocytes and finally Sertoli cells, in that order [22,23].

Testicular MDA was expressed as the mean (SD) and the experimental groups compared by ANOVA, followed by Student’s t-test, with P < 0.05 considered to indicate statistically significant differences.

RESULTS

The rabbits had a mean (SD, range) age of 4.3 (0.7, 3–6) months and weighed 3.45 (0.31, 3–4.5) kg; there were no significant differences in the mean weights and ages among the various treatment groups. Table 2 shows the left testicular MDA levels of rabbits whose left testes were subjected to 60 min of ischaemia followed by 24 h reperfusion, but given various antioxidants before reperfusion. Table 2 also shows values for rabbits given no antioxidants. The mean MDA level in the left testes was lower in rabbits given ASA, ascorbic acid, quercetin and allopurinol before reperfusion than in rabbits not given antioxidants, but the difference was not significant, although much lower in rabbits given allopurinol (P = 0.093) and ascorbic acid (P = 0.084). Conversely, rabbits given SOD had a higher MDA level than control rabbits. There was no significant difference in the right testicular MDA levels among the various groups.

Table 2 also compares the testicular MDA levels in the right and left testes at 3 months (long-term reperfusion). Twisted but viable testes allowed to reperfuse (detorsion/orchidopexy) had higher MDA levels after 3 months than controls. Allopurinol-treated rabbits had the lowest right and left (both P < 0.001) testicular MDA levels compared to controls. Allopurinol-treated rabbits also had lower right and left testicular MDA levels than rabbits given other antioxidants. Quercetin-, ascorbic acid- and SOD-treated rabbits had lower left testicular MDA levels than control rabbits, but these differences were not statistically significant; ASA-treated rabbits had even higher left testicular MDA levels.

Figure 1 shows the morphological damage scoring in the testes and Table 2 the mean testicular biopsy scores (Johnsen score). Table

<table>
<thead>
<tr>
<th>Score</th>
<th>Description of Scoring System</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Complete spermatogenesis with many spermatozoa (determined by head form). Germinal epithelium organized in regular thickness leaving an open lumen.</td>
</tr>
<tr>
<td>9</td>
<td>Many spermatozoa present but germinal epithelium disorganized with marked sloughing or obliteration of lumen.</td>
</tr>
<tr>
<td>8</td>
<td>Only a few spermatozoa present (&lt; 5–10)</td>
</tr>
<tr>
<td>7</td>
<td>No spermatozoa but many spermatids present.</td>
</tr>
<tr>
<td>6</td>
<td>No spermatozoa and only a few spermatids present (&lt; 5–10)</td>
</tr>
<tr>
<td>5</td>
<td>No spermatozoa and no spermatids but several or many spermatocytes present.</td>
</tr>
<tr>
<td>4</td>
<td>Only a few spermatocytes (&lt; 5) but no spermatids or spermatozoa present.</td>
</tr>
<tr>
<td>3</td>
<td>Spermatagonia are the only germ cells present.</td>
</tr>
<tr>
<td>2</td>
<td>No germ cells, but Sertoli cells are present.</td>
</tr>
<tr>
<td>1</td>
<td>No cells in tubular section.</td>
</tr>
</tbody>
</table>

*All currently used testicular biopsy scoring systems are modifications of the Johnsen score [23].
was little histological damage in the left testes of all rabbits when the reperfusion period was limited to 24 h. However, at 3 months rabbits given SOD, ASA and allopurinol had Johnsen scores of >6, while those given ascorbic acid and quercetin had scores of <6, indicating poor protection of exocrine function at 3 months by quercetin and AA.

**DISCUSSION**

The ideal treatment for torsion of testis remains elusive; 20–40% of patients treated by bilateral orchidopexy after unilateral torsion eventually develop some atrophy of the twisted testis, with reduced fertility [12,24–27]. Factors determining these sequelae include the duration and the degree of torsion [26,27]. While the duration of torsion can be shortened by health education, or by asking young men with testicular pain to report promptly to a hospital, the degree of torsion cannot be influenced. Torsion of the testis causes ischaemia of the testis, and treatment by untwisting and orchidopexy results in reperfusion. As with any I/R injury, free radicals released during reperfusion contribute to the damage. Numerous experimental animal studies confirm that torsion of the testis is associated with free radical production, and that the effects of free radicals can be mitigated by using antioxidants [3,4,6]. Most experiments have assessed the short-term effects of antioxidants; to our knowledge, only one [5] has assessed the effect of antioxidants on experimentally produced torsion of the testis over 6 weeks.

In the present study, 3 months was chosen to assess the long-term effects of antioxidants because, under laboratory conditions, these rabbits have a life-expectancy of 36–48 months [28]. They attain puberty at ≈3 months and become sexually active at ≈6 months [28]. Observations in our Animal Resources Centre over a 15-year period also confirm these earlier observations about the life-cycle of laboratory reared New Zealand White rabbits [13]. Thus, extrapolating to humans, 3 months in the life of the laboratory rabbit corresponds to 6–8 years, the approximate time humans are estimated to marry after torsion of testis, and attempt to have a family, when the sequelae of torsion (atrophy and reduced fertility) become apparent [9,12,24,25].

The present data confirm the beneficial effect of ASA, ascorbic acid, quercetin and allopurinol in reducing free radical damage within 24 h of initiating reperfusion in experimental animals. Allopurinol was associated with the lowest testicular MDA levels, but SOD was not very effective at protecting the twisted testis from free radical damage, as the level of testicular MDA in rabbits given SOD did not differ much from controls. The assessment of long-term (3 month) effects of antioxidant treatment shows that allopurinol-treated rabbits had significantly lower right and left testicular MDA levels than controls or rabbits given the other antioxidants. Morphologically, allopurinol-treated rabbits at 3 months had Johnsen scores of >7.5, while scores were <7.5 for rabbits given the other antioxidants. Possible explanations include that in different tissue systems, different antioxidants are effective in preventing free radical damage; e.g. aspirin (ASA) provides better protection for myocardial infarction [29], while ascorbic acid is more effective in preventing ischaemic damage to the intestine [18]. SOD and quercetin have been shown to prevent free radical damage in the transplanted kidney [15,17], and others have confirmed that allopurinol also prevents cardiac I/R injury during coronary artery bypass graft surgery [30], colonic I/R injury [31] and I/R injury of the testis [3]. SOD, together with catalase, has also been shown to prevent I/R injury when administered to animals whose testes were subjected to 60 min of ischaemia, but not for animals given >60 min of ischaemia [5]. These findings are similar to the present results. Antioxidants do not appear to be of benefit after prolonged ischaemia, as the organs are likely to become atrophic with time as reperfusion fails [5,32]. Riaz et al. [31] also...
reported that in experimental colonic I/R injury, 30 min of ischaemia is followed by adequate reperfusion and a response to allopurinol and SOD, whereas ischaemia of >30 min is associated with poor reperfusion and a poor response to antioxidants. These findings confirm the importance of determining the point of reperfusion failure in experimental I/R injury [32].

We examined the long-term benefits of five antioxidants that can be given intravenously, and are thus of potential value in an emergency, and which have previously been shown to offer protection in short-term experimentally induced torsion of the testis [2–6,32]. The field of I/R injury of the testis is ever-expanding, with new antioxidants being discovered regularly, e.g. caffeic acid phenethyl ester [33], pentoxifylline [34], and vasoactive intestinal peptide [35]. The beneficial effects of inhibiting nitric oxide synthase in I/R of the testis is also emerging as an important therapeutic strategy in minimizing the sequelae of torsion of the testis [36]. An assessment of the long-term benefits of these newer antioxidants is warranted.

The dose of allopurinol (200 mg/kg) used in the present experiments is large and might limit its use in humans; this dose was also found to prevent I/R injury of the rat testis [3] and rat intestine [31], but a lower dose (50 mg/kg) was reportedly effective in protecting the rat liver against I/R injury [37]. From experience of using allopurinol in the treatment of urological diseases like hyperuricaemia, doses of 100–300 mg (three times daily) gave a serum concentration of 37–50 μmol/L [38]. We also measured the serum concentrations of allopurinol and oxypurinol in the rabbits given 200 mg/kg of the drug intraperitoneally, and found peak mean (50) levels for allopurinol of 41 (13.4) μmol/L and of 70 (16.4) μmol/L for oxypurinol. These data indicate that giving humans up to 300 mg of allopurinol should produce serum levels that provide antioxidant effects with no toxicity. Currently, allopurinol 300 mg (three times daily) can be safely prescribed for patients with hyperuricaemia [38]. Hopefully, clinical trials will reveal a dose of allopurinol that has antioxidant effects in humans with little or no toxicity.

We used the Johnson scoring system to assess the damage in the present testes; this is an objective grading system that uses morphometric and cellular characteristics to produce a quantitative description of a testicular biopsy specimen [23], and is one of the most cited scoring systems [23]. The Johnsen score [22] is based on the premise that in testicular damage there is progressive degeneration of germinal epithelium: spermatozoa and spermatids, then spermatocytes and finally Sertoli cells [22,33]. For experimentally induced damage to the testis, this premise has been found to be largely correct [22,39], and these earlier

FIG. 1. Photomicrograph of a testicular biopsy to show different stages of spermatogenesis according to the Johnsen scoring system. (A) Johnsen score 10, showing normal spermatogenesis, with numerous spermatozoa lining the central lumen. (B) Johnsen score 9, showing sloughing into the lumen, adequate number of spermatozoa present. (C) Johnsen score 8, showing only few spermatozoa. (D) Johnsen score 7, showing only spermatids on the luminal side of the tubules. (E) Johnsen score 6, showing only few spermatids. (F) Johnsen score 5, showing only spermatocytes with no spermatids. (G) Johnsen score 4, showing only few spermatocytes and spermatagonia. (H) Johnsen score 2, showing only Sertoli cells lining the tubules. Haematoxylin and eosin × 400.
observations are supported by the experimental data in Table 2.

Randomized placebo-controlled clinical trials should be undertaken using prophylactic antioxidants like allopurinol, because, as our experimental studies show, a testis subjected to 60-min ischaemia will reperfuse and remain viable at 3 months, but remains very high in free radical levels. We suggest that part of the deleterious effects of torsion might be caused by the high free radical levels in the twisted testis. This provides a rational explanation for earlier observations that patients with testicular torsion treated by orchidectomy had less reduction of fertility than those treated by orchidopexy, the current standard treatment. Thus, the removal of a twisted testis effectively prevents significant damage to the contralateral testis [24,39,40]. However, orchidectomy is not always possible, for obvious emotional reasons, so there is a need for agents that reduce the damage that occurs to both testes after orchidectomy. This will prevent the present unfortunate situation in which the salvaged twisted testis are eventually a liability to the patient’s contralateral testis and ultimate fertility.

The present data indicate that the twisted viable testis treated by orchidopexy contains high free radical levels in the long term. Administration of allopurinol before reperfusion of the twisted testis is associated with prolonged reduction of free radical level and maintenance of a good Johansen score. However, quercetin, ascorbic acid, ASA and SOD have no significant long-term effect on testicular free radical levels, although in the short term all these antioxidants except SOD were associated with lower testicular MDA levels. We propose that the outcome of treating testicular torsion can be improved by using prophylactic and effective antioxidants like allopurinol. Clinical trials involving allopurinol or other effective antioxidants in patients with torsion of the testis are warranted.

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CONFLICT OF INTEREST

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Abbreviations: SOD, superoxide dismutase; MDA, malondialdehyde; ASA, acetyl salicylic acid; I/R, ischaemia/reperfusion.
The era of ESSTIs is slowly approaching?

Oh no, you say, not another acronym to add to the lexicon of BPH, LUTS, OAB, UI, ED, PDEs, IIEFs, IVELTs, PE, etc! What’s this one about? Is it more pharmaceutical industry hype? Will it matter to me, or my patients or my budget? With my usual degree of certainty, I predict that the arrival of dapoxetine within the next year will usher in the decade of the ejaculoselective serotonin transport inhibitor (ESSTI). The issue for patient, physician, healthcare provider and scientist is whether dapoxetine (or any similar drug) merits this label. As a veteran of the ‘uroselective α-blocker’ wars I am anxious to avoid some of the confusion created by pharmaceutical industry advertising. To help the reader decide on the appropriateness of this (or any other) descriptor some of the more relevant data and assumptions are provided below.

At the risk of alienating the reader I will start with the basic science. As seen from research reports, including a plethora of AUA 2005 abstracts, dapoxetine is a potent (nanomolar) inhibitor of the serotonin re-uptake system in human brain slices. The serotonin re-uptake system is also described (somewhat less precisely) as the serotonin transport system; so we can accurately describe dapoxetine as a serotonin transport inhibitor (STI).

Furthermore, as dapoxetine inhibits the monoamine transporter systems at much higher (micromolar) concentrations, the drug can be considered as ‘selective’, i.e. warranting a description of an SSTI.

Ultimately (cf the α-blocker story) the clinical profile of a drug should be more important than any scientific nuances particularly relating to test-tube findings. On this premise then we must turn to the credentials of dapoxetine as a selective agent in the clinic; in particular the relative activity on ejaculatory function relative to other physiological, pathophysiological or disease processes. The most relevant definition of clinical selectivity as far as the patient is concerned is the ability to produce benefit with no obtrusive side-effects.

Examination of early reports shows that dapoxetine was removed from development as an antidepressant by Lilly and re-positioned for ejaculatory dysfunction, with development initially being taken over by Detlef Albrecht’s group within Alza/J&J and subsequently by Usman Azam’s late-stage development group. Certainly at doses three to four times higher than those being used in premature ejaculation (PE), there was no antidepressant activity in short- and long-term studies. In part this may have been due to the relatively short half-life (<6 h) of dapoxetine. Ironically, this apparent pharmacokinetic ‘deficiency’ was considered by the subsequent developers to be nearly ideal for ‘on demand’ use by patients with PE, i.e. patients wanted a drug with a relatively rapid onset that is quickly cleared thereby reducing the propensity for systemic side-effects.

Turning now to efficacy; there is no agreed regulatory route for approval of a drug for PE. However, the clinical development group within J&J have shown convincing efficacy using a variety of clinically relevant endpoints, including ejaculation latency, patient control and patient/partner satisfaction. This has been shown in both short-term studies and long-term open-label extensions. The effect is apparent within the first few doses and is maintained, i.e. there is no evidence of tachyphylaxis or tolerance. The drug appears well tolerated with a discontinuation rate remarkably low (at <3% in all clinical studies).
In general the side-effect profile is consistent with the primary mechanism of action. Overall, dapoxetine is effective at doses and situations (on demand) in which it is not effective as an antidepressant, and is well tolerated. QED ‘ejaculo-selective’?

Dapoxetine is, therefore, an sSTI and is ‘ejaculo-selective’. It is well known to students of the art of algebra that two lower cases equal one upper case. Dapoxetine can be reasonably considered as the first example of an ESSTI. As the drug is also potent (P), it could be referred to as a PESSTI, particularly on 25 January, a date of great significance to followers of Rabbie Burns. Let us hope that the discussions with the FDA do not result in ‘the best laid plans of mice and men, going agley’.

None of the above should in any way detract from the significance of the arrival of dapoxetine as the first drug ‘fit for purpose’ for the treatment of PE. The development of dapoxetine exemplifies the culmination of a rational programme based originally on the chance observation of the ejaculation-retarding activity of the original selective serotonin re-uptake inhibitors such as fluoxetine and sertraline. A combination of relatively poor benefit-risk ratios and pharmacokinetics essentially excluded them from approval for treating PE, although there is some degree of ‘off-label’ use.

We assume that at some stage Karl Thor within Lilly rationalised that a relatively short-acting agent would result in a drug that would minimise side-effects. Furthermore, an on-demand use would be consistent with ‘normal’ sexual activity which is (as much as) 49 times per annum. The rest is soon to be history.

The arrival of dapoxetine on the marketplace is unlikely to be the end of the story. As in the case of PDE inhibitors in the ED market, several ‘copy-cat’ drugs will follow. This market, by way of benchmarking for potential investors, is likely to be similar to that for PDE inhibitors. The epidemiologists tell us that the incidence, prevalence and bother of PE is equivalent to that of ED. Therapy will be considered to be ‘lifestyle’ and will therefore be subject to the same constraints on reimbursement, and pricing is likely to be the same. The major difference from the ED market is that dapoxetine is unlikely to have the 5-year market exclusivity that was afforded to sildenafil, as several similar agents are already in development.

Almost certainly this will not represent the end of the story of drug development in PE. There is a considerable amount of research into the serotonin receptor subtype involved in producing the increased ejaculation observed with dapoxetine; the elevated synaptic serotonin must act on or via a receptor. The major issue is that there is a considerable degree of species variation with animal ‘models’, often producing confusing and contradictory data. In essence research into PE is where ED research was before our understanding of peripheral nitric oxide systems and the action of PDE inhibitors in the 1990s.

We appear to be on the cusp of another revolution in sexual medicine. Hopefully, with or without the ESSTI acronym, dapoxetine will come to the market within the next year.

Next month I will do my traditional update on tales of derring doo from the AUA 2005.

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Laparoscopic O’Conor’s repair for vesico-vaginal and vesico-uterine fistulae

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INDICATION

Vesico-vaginal fistula (VVF) has been a social and surgical problem for centuries. In the developed world >90% of cases are caused by inadvertent injury to the bladder during surgery [1]. Obstetric VVF related to prolonged labour remains a major medical problem in many underdeveloped countries with a low standard of obstetric care [2]. In 1852, Sims reported a successful repair of VVF in female slaves [3]; since then, many surgical techniques have been developed to correct this abnormality, including transabdominal, transvaginal and endoscopic approaches [4–9]. The selected route of repair depends mostly on the training and experience of the surgeon. The best approach is probably the one with which the surgeon feels most experienced and comfortable. The main disadvantages of the abdominal approach include the requirement for laparotomy, splitting of the bladder, and its associated morbidity with longer recovery. For women who have a VVF during or after recovery from a gynaecological procedure, the prospect of undergoing further surgery and recovery can also be stressful, especially if laparotomy is required.

Ever since its inception, laparoscopy has become increasingly popular in urology, reducing the invasiveness of treatment and shortening the period of convalescence. Most ablative and reconstructive surgery in urology can be accomplished with the laparoscope, and laparoscopic repair of VVF may offer the patient less morbidity and quicker recovery than with the traditional transabdominal approach. We present our retrospective results of laparoscopic transabdominal transvesical repair, as described by O’Conor et al. [10–12] for managing supratrigonal VVF in the last 4 years.

METHOD

We analysed retrospectively eight consecutive patients with VVF and vesico-uterine fistula who had a laparoscopic transabdominal transvesical repair at our institute between January 2000 and April 2004. Of these, six patients had VVF after abdominal hysterectomy and the other two a vesico-uterine fistula after Caesarean section.

The patients with VVF presented with urinary incontinence at 3–16 days after their surgery; all had a pelvic examination, IVU and cystoscopy before repair to confirm the VVF and exclude associated ureteric injury. Two of the six patients had a recurrent VVF, having had a previous transvaginal repair 10 and 12 weeks after abdominal hysterectomy. On cystoscopy all patients had a supratrigonal fistula, with a mean (range) diameter of 12 (8–20) mm, and had a laparoscopic repair 6–8 weeks after their initial surgery.

The two patients with vesico-uterine fistula presented with cyclical menouria and...
amenorrheoa of 4 and 6 months' duration; they had normal urinary continence. Both patients had had a Caesarean section at 8 and 9 months previously. On genital examination no fistula was palpable vaginally; the diagnosis was made by hysterosalpingography (Fig. 1). Cystoscopy showed a supratrigonal fistula of 8 and 10 mm diameter.

For the repair, the patients were given an intubated general anaesthesia and placed in the lithotomy position. Initially they had cystoscopy with bilateral ureteric catheterization using 5 F ureteric catheters to help identify and protect the ureters during surgical dissection. In patients with a VVF a catheter was placed vaginally through the fistula when possible. A nasogastric tube and urethral Foley catheter were also placed. Gentle traction was applied on the urethral Foley catheter to mechanically block the bladder neck and prevent leakage of air through the bladder neck. A sponge stick was inserted in the vagina for manipulating the vaginal vault. A primary 10-mm port was inserted with Hassan’s technique in the midline infra-umbilically. The laparoscope was introduced and two 5-mm secondary ports were created in both iliac fossae under laparoscopic vision, with care taken to avoid inferior epigastric vessels by transillumining the abdominal wall. Adhesions were lysed in the pelvis, and the uterus and bladder were identified. Another 5-mm port was then created suprapublically in the midline to aid in retracting the bladder during suturing. The bladder was distended with 300 mL of normal saline.

Gentle traction on the Foley catheter in the fistula and urethrally helped to retain saline in the bladder. The peritoneum between the bladder and vagina was incised with cautery. Using laparoscopic scissors and gentle counter-traction, a plane was developed between the bladder and vagina. The sponge stick inserted in the vagina greatly aided in the dissection. Than a vertical cystotomy was created with laparoscopic scissors, starting at the dome and continuing down to the fistula site posteriorly, as described by O’Conor et al. [10–12] (Fig. 2). Flaps of bladder wall were dissected free from the vagina, until the fistula was separated completely from the vagina (Fig. 3). The catheter placed through the fistula communication was then removed and adequate bladder margins exposed on all sides of the fistula by further dissecting the bladder from underlying vagina (Fig. 4). The edges of the fistula opening in vagina were trimmed back to healthy tissue and sutured with interrupted 3-0 polyglactin sutures in a single layer. Once the vagina was sutured omentum [13], peri-otic or mesenteric fat [14] was mobilized and anchored to the vagina with two more sutures, so that it covered the vaginal suture line (Fig. 5). These provide an additional layer of separation with improved lymphatic drainage. The bladder was then sutured in a single layer with 3-0 polyglactin intracorporeal sutures (Figs 6 and 7). The laparoscope was then withdrawn, the cannula removed and the 10-mm port incision closed with 2-0 polyglactin sutures.
The ureteric catheter and Ryle’s tube were removed at the end of procedure. A 16 F urethral Foley catheter was left in place. We do not place a suprapubic catheter after surgery for bladder drainage. The vagina was packed with a betadine ointment-soaked roller gauze at the end of procedure. The operative steps during the laparoscopic repair of the vesico-uterine fistula were similar.

Patients were encouraged to take food after 6 h, and the vaginal pack was removed after ~12 h. Patients were given anticholinergic agents to prevent bladder spasms and diclofenac sodium to manage pain. All the patients were fit for discharge 3 days after surgery. At 14 days after surgery the patients were assessed by cystography to confirm complete bladder integrity, during which methylene blue was injected into the bladder and any leakage into the vagina detected by inserting a gauze. If there was doubt about the healing of the bladder, the urethral catheter was maintained for one more week.

The laparoscopic procedure was successfully completed in seven of the eight patients, with conversion to open surgery in the first, where after dissecting the vesico-uterine fistula from the uterus there was technical difficulty while suturing the cystotomy incision. The mean (range) operative duration was 220 (190–280) min. There were no other complications during surgery and no patient required a blood transfusion. All but two patients were fit for discharge and fit for ambulatory 1 day after surgery and fit for return home with a catheter and hence were kept in the ward for 15 days. There were no complications after surgery. The urethral catheter was removed after 2 weeks in seven patients and was maintained for 3 weeks in one. In all patients the fistula was cured after removing the urethral catheter, and were asymptomatic during a follow-up of 3–40 months.

**COMPARISON WITH OTHER METHODS**

The appearance of iatrogenic urogenital fistula is one of the most devastating complications of surgery. The emotional distress to the patient and surgeon is high because there is little hope offered by conservative therapy and most cases need a second operation to correct the problem. In our department we favour the transabdominal transvesical approach for surgical repair of supratrigonal VVF, as described previously [11,12]. This approach can be used for all fistulae, including complicated VVF, with advantages of high success rates, optimal surgical access to the fistula and ureters, and the ability to add an interposition graft with this procedure. As stressed by the original authors, the key is to bisect and widely mobilize the bladder from the vagina to produce a closure with separate tension-free layers. It was their extensive studies, with success rates of >85%, that popularized the suprapubic technique. Among the successful cases of repaired VVF were patients with complex and difficult repairs, e.g. radiation-associated cases. Nesrallah et al. [15] investigated the clinical efficacy of the O’Conor transperitoneal supravesical technique for repairing supratrigonal VVF in 29 patients. They found the technique to be successful in all patients, with no significant bladder dysfunction or decrease in bladder capacity after repair. They suggested that the O’Conor technique be considered the standard surgical method for repairing supratrigonal VVF. Blaivas et al. [16] in a study of 24 patients with VVF found that, once acute local inflammation had subsided, there is no benefit to be derived from delaying the surgery. Delay can have a devastating impact on quality of life and ability to function, which cannot be underestimated. Considering all these factors we favour early intervention for the surgical repair of VVF.

We introduced the laparoscopic technique in our department in 1999; after using it for ablative procedures, we gradually extended the indications to incorporate various reconstructive procedures. The technique of laparoscopic repair of cervicovesical fistula after Caesarean section was described in detail by Hemal et al. [17], who used an extravesical technique, with excision of the fistulous tract using the Nd:YAG laser. Of the two patients described the procedure required conversion to open surgery in one because of a technical problem. In the other patient in whom the procedure was successful laparoscopically there was an inadvertent bladder tear during dissection that was repaired intracorporeally. We used the transvesical technique of repair in the present patients with Youssef’s syndrome, and had the advantages of optimum access to the fistula site and tension-free closure of the bladder, as in the classical O’Conor repair. The conversion in the first patient and difficulty in intracorporeal suturing was attributed to early inexperience. However both patients were treated successfully and were disease-free during the follow-up. Although vesicouterine fistulae are less common than other urogenital fistulae, worldwide the prevalence of disease is increasing with the frequent use of Caesarean section [18]. Surgery is the mainstay and definitive treatment of vesicouterine fistulae; from our experience laparoscopy should be offered to patients who require surgical treatment for Youssef’s syndrome. Laparoscopy has advantages over open surgery in producing less pain, shorter hospitalization, better cosmesis and quicker recovery.

Nezhat et al. [4] first reported the laparoscopic repair of a VVF, and later assessed the laparoscopic closure of intentional and unintentional bladder lacerations in a series of 20 cystotomies [19]. In that study the only complication was one VVF that required reoperation, successfully repaired laparoscopically with a single-layer closure. The authors concluded that in experienced hands, the endoscopic management of complex VVF might be an alternative to the traditional abdominal approach. Von Theobald et al. [6] used an omental J-flap interposition during the laparoscopic repair of VVF. Recurrent VVF was similarly successfully repaired laparoscopically by Miklos et al. [7]. Their patient had previous two failed Latzko partial colpocleisis, and closing the vagina and bladder with an interposed omental flap using a laparoscopic approach ultimately repaired the persistent fistula. Similar success with the
laparoscopic approach was described by others [8,9]. The results in the present six patients with VVF confirmed the feasibility of the laparoscopic approach to VVF, with a good outcome.

ADVANTAGES AND DISADVANTAGES

The advantages of laparoscopy are well known and already discussed; there are few case reports [4–9] showing the feasibility of the laparoscopic repair of VVF and vesicouterine fistulae. It seems to offer patients a shorter hospital stay, quicker convalescence, better cosmesis and equal efficacy. Technically, laparoscopy provides better visualization through magnification, but is more difficult to learn, as is intracorporeal suturing. Another difficult technical challenge is treating sufficient patients to stay proficient. Larger studies with a comparison of outcome of the laparoscopic approach with that of other open approaches are warranted to define the exact role of laparoscopy in managing VVF. At present, based on our experience, it appears to be a viable alternative for managing VVF and Youssef’s syndrome for surgeons experienced with laparoscopic suturing techniques.

CONFLICT OF INTEREST

None declared.

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Abbreviations: VVF, vesico-vaginal fistula
Tied and tested: a cheap and simple method for transurethral resection

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INDICATIONS

Transurethral endoscopic procedures are common in urology; the minimum set-up for transurethral resection (TUR) requires a light source and its cable, irrigation fluid and its delivery tube, a camera and its cable, and if resection is required, a diathermy cable. These cables are not fixed and their mobility can lead to various problems. It is not uncommon for one of these cables to be displaced from the instrument or the surgeon’s hand and fall to the floor, resulting in contamination or even damage. We describe a technique where a simple braided flat elastic band is used to keep these cables together to prevent these mishaps, and allow increased freedom of movement for the operating urologist.

METHOD

A braided flat elastic band found on the side of oxygen masks or bought from a sewing store is tied to form a band of ~20 cm long. A ring–hitch knot (tête d’alouette) [1] is made around all three leads (Fig. 1) and looped over a knob protruding from the theatre light (Fig. 2) or a drip-stand hook. The length of the leads is adjusted so that they can be attached to the resectoscope with the minimum of slack (Fig. 3). This prevents the potential contamination and knotting of the cables that may occur because they are mobile. It is also useful when the resectoscope needs to be changed, as all the cables are suspended in one place. At the end of the procedure the cable tie is removed and sent for sterilization.

ADVANTAGES

(i) The elastic band is cheap, can be bought easily or taken from oxygen masks; 100 m of six-cord (5 mm wide) black braided elastic costs £3.88 [2]; this is much cheaper than the prototype of the Skyhook® introduced in 1996, and costing £275 [3]. (ii) The elastic band can be autoclaved along with other urology instruments and therefore re-used. (iii) The light-source cable is prevented from coming into contact with the drapes, thus avoiding fire and patient injury (Fig. 4). Fire caused by igniting surgical drapes with the light-source cable resulting in serious injuries was reported previously [4]. (iv) The elastic band allows for freedom of movement of the instruments and the hand of the operating urologist. (v) The added control allows for less participation of the scrub nurse and therefore the operation can be done with no assistant. (vi) The operating field is cable-free, thus preventing clutter at the site of surgical activity.
DISADVANTAGES

(i) Snapping of the elastic band; although this has not been our experience. (ii) Loss of elasticity of the band on repeated sterilization; we have noticed that the elastic band can be sterilized and used up to 10 times on average before discarding it. (iii) The irrigation fluid needs to be at a higher level than the apex of the cables.

In conclusion, this technique of holding the cables together with an elastic band is simple and something that other urologists can adopt at virtually no expense. It makes endoscopic resection procedures easier, neater and, most importantly, safer for the patient.

CONFLICT OF INTEREST

None declared.

REFERENCES


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BASIC AND ADVANCED TECHNIQUES IN PROSTATE BRACHYTHERAPY

Edited by AP Dicker, GS Merrick, FM Waterman, RK Valicenti and LG Gomella

Martin Dunitz, 2005; pp 451, £125; ISBN 1 84184 298 2

Brachytherapy is now increasingly used as a curative treatment option for prostate cancer. It was therefore only a matter of time before such a definitive textbook was produced. The editors of this book are to be congratulated with the ultimate 'Who's Who' of brachytherapy with this book. Their list of authors stretches to 91, with 50 chapters in eight sections. This book describes brachytherapy from a historical perspective, patient selection, the rationale behind its choice and even a chapter from a patient detailing his journey from the beginning to the end of his treatment. There is much detail about the technique itself, including variations, often subtle, between institutions around the world. The dosimetry, morbidity and biochemical outcome are also described. Much of the text is frank and provides a balanced view of outcome and current controversies surrounding brachytherapy for prostate cancer from different units.

Each chapter is well written with subheadings, and with a useful summary at the end. They are concise and contain an abundance of excellent diagrams, photographs and imaging that has been reproduced to the highest quality. Although each chapter provides adequate detail, there is also a bibliography for those left hunger for more information. There is a detailed index that allows the reader to target areas on interest, and the book also highlights the multidisciplinary oncology team approach to prostate cancer. However the book is unfortunately let down much repetition between chapters.

Urologists, oncologists, medical physicists, and pathologists involved with brachytherapy will find this book very useful. In addition, interested trainees and general practitioners may also gain a detailed insight into the basics of this growing field of uro-oncology. This book deserves a place in the reference library of every urological department and could be described as the 'Campbell's of brachytherapy'.

PROFESSOR STEPHEN LANGLEY

TRANSURETHRAL RESECTION

By John Blandy, Richard Notley and John M. Reynard

Taylor & Francis, November 2005, Hardback, ISBN 184184408X; £65.00, 236 pp

This latest monograph is a worthy successor to previous editions of what, justifiably, has become the reference handbook on TUR. The treatise is comprehensive, its scope including history, equipment selection and maintenance, indications for operation, technical points and pitfalls with TURP and TUR of bladder tumour (TURBT), postoperative care and complications, then finally leading to alternatives to TURP and medico-legal aspects.

The text is easy to read and is very matter-of-fact, in keeping with previous editions. Indeed, with one's eyes closed, it is possible to recall the most senior author speaking through some of the anecdotes which are cited, such as 'an expensive tennis racket does not guarantee victory at Wimbledon' and 'it is no good buying a Rolls Royce if their nearest agent is in Ruritania'. The references are selective and illustrate the text aptly, as do the illustrations, particularly the line drawings, which are outstanding.

Our criticisms are few. However, one surprising omission is a failure to advocate current evidenced-based recommendations which strongly support the use of a single instillation of cytotoxic drug to minimise recurrent tumour formation after TUR of superficial bladder tumours. The line drawing of a patient smoking a pipe with a bladder irrigation in place is an anachronism, especially given current official stance on the use of tobacco in public places. Throughout the book, there is repeated use of the word 'regime' instead of 'regimen', which may reflect more an inevitable acceptance of this term as a result of its widespread misuse, rather than anything else.

However, with these few reservations, there is no doubt that this is an excellent book which thoroughly deserves a place on the shelf of every trainee and in every Urology Unit's library. Although the three reviewers are at quite different stages in terms of experience, all found the book to be very informative, with the two trainees advocating that reading the book should start before commencing TUR, as soon as that person is au fait with cystoscopy.

We anticipate that the fifth edition of Transurethral Resection will have global appeal and utility. We recommend it wholeheartedly.

DC DANGERFIELD
E McLARTY
RA GARDINER
TECHNICAL CHARACTERIZATION OF AN ULTRASOUND SOURCE FOR NONINVASIVE THERMOABLATION BY HIGH-INTENSITY FOCUSED ULTRASOUND

Sir,

Dr Leonid Gavrilov, a member of the Andreyev Acoustic Institute at the Russian Academy of Sciences, drew our attention to the calculation of the used intensities published in your journal in 2002 [1]. Those computed by us were incorrect; we apologise for the mistake and provide the following corrections.

The following table on page 250 had incorrect values; the correct values are shown under the incorrect:

TABLE 1 Acoustic power ($P_{ac}$), spatial averaged intensity without tissue penetration ($I_{SAL}(0)$) and at 5 cm ($I_{SAL}(5)$) and 10 cm ($I_{SAL}(10)$) of tissue penetration calculated for a given electric power ($P_{el}$) of the generator

<table>
<thead>
<tr>
<th>$P_{ac}$, W</th>
<th>$P_{ac}$, W</th>
<th>$I_{SAL}$, W/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorrect</td>
<td>Correct</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>55</td>
<td>2148</td>
</tr>
<tr>
<td>200</td>
<td>110</td>
<td>4296</td>
</tr>
<tr>
<td>300</td>
<td>165</td>
<td>6443</td>
</tr>
<tr>
<td>400</td>
<td>220</td>
<td>8591</td>
</tr>
</tbody>
</table>

Consequently, the $I_{SAL}$ values in Table 2 were also incorrect, and are shown here with the correct values:

TABLE 2 Dimensions of the HIFU lesions depending on intensity in the focal zone. These intensities are held for $2s$ ($t_{ave}$) at 1 MHz

<table>
<thead>
<tr>
<th>$I_{SAL}$ W/cm²</th>
<th>coagulation</th>
<th>necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2148 0.16 0.16 0.16</td>
<td>0.11 0.11 0.82</td>
<td></td>
</tr>
<tr>
<td>4296 0.89 0.89 3.84</td>
<td>0.85 0.85 2.74</td>
<td></td>
</tr>
<tr>
<td>6443 1.56 1.56 7.44</td>
<td>1.28 1.28 4.29</td>
<td></td>
</tr>
<tr>
<td>8591 2.26 2.26 8.82</td>
<td>1.91 1.91 7.9</td>
<td></td>
</tr>
</tbody>
</table>

We apologise for this error.

The formulae used were:

$P_{ac} = X P_{el}$

$\frac{I_{SAL}}{W/cm²} = 0.867 \frac{P_{ac}}{D_{hub}²}$

$X = 0.55$ for the system used; $D_{hub} = 0.4$ cm; $\mu_{source} = 0.5$ dB/cm

We apologise for this error.

Conventional and alternative methods for providing analgesia in renal colic

Sir,

We read with interest this article [1] on conventional and alternative medications for renal colic. The paper is very useful in situations where conventional medications cannot provide analgesic relief or in patient with allergies to these drugs. However, we draw attention to an untapped line of management that may prove useful in ureteric colic. Even though histamine has been confirmed as an active mediator of ureteric contraction, the use of antihistamines in a clinical setting of ureteric colic has not been documented. In-vitro experiments have shown histamine to be one of the most potent stimulators of ureteric peristalsis [2,3]. Histological and immunohistochemical studies show a uniform and abundant distribution of histamine receptors along the entire ureter, in particular H1 receptors [4,5]. Electron microscopic studies of human ureter exposed to urine have shown degranulation of mast cells with release of histamine, producing forceful peristaltic contractions simulating renal colic [6]. In-vitro studies again showed the inhibitory effects of H1 antagonists in abolishing the contractions induced by histamines [2,6]. A randomized trial involving an H1 receptor antagonist vs placebo will be interesting and may provide yet another alternative medication for renal colic.


Conventional and alternative methods for providing analgesia in renal colic

Most studies to date have used similar inclusion criteria: Patients are generally <80 years old and with biopsy-confirmed early prostate cancer in at least six cores (with at least four cores being positive for cancer [3,7]); early cancer is typified with a clinical staging of T1–T2NOMO [1,7–10]; the PSA level is <10 ng/mL [2,8] (although <15 ng/mL has also been used [2,3,11]); the Gleason score is £7 [2]; and the maximum prostate size 30–40 mL [2]. Patients were either unsuited for radical prostatectomy or were unwilling to undergo a potentially more dangerous procedure [1,5,7–10]. All patients signed consent forms and had a preoperative assessment with TRUS, a DRE, bone scan with or without MRI.

The follow-up included regular PSA assays (from 1 day after surgery, and then at 3–monthly intervals), a DRE and TRUS. Randomized sextant biopsies were taken at regular intervals, e.g. at 6 and 36 months. Bone scans and CT/MRI were used to check for metastatic disease in patients with a rising PSA level, while complications and symptoms in patients were assessed by patient-based questionnaires (IPSS and quality-of-life measures) although complaints were also recorded. The median follow-up times ranged from a mean of 17.6 months [10] up to 3 years.

Patients were treated in one to three sessions. Blana et al [2] reported a negative biopsy rate of 93% at 22 months of follow-up. They also quoted a median PSA level of 0.15 ng/mL and stated that 87% of patients had a constant PSA level of <1 ng/mL. This contrasted with 77% of patients with negative biopsies at 27 months in another study [8]. The latter group also found a significant difference between patients with a preoperative Gleason score of 2–6 (85%) or with a score of 7–10 (61%). Gelet et al [9] reported a much lower overall success rate of 66% at a mean follow-up of 19 months, but confirmed a significant difference in outcome with different Gleason scores. There was no statistically significant change in urinary symptom scores after the procedure.

Most studies reported a better side-effect profile than conventional operative interventions [6]. The rates of symptomatic UTI were <5%, while chronic pain occurred in 1–2%, and infravesical obstruction in 10–15%. For this reason some studies assessed an elective TURP at the time of the procedure, with decreased urinary catheterization time and better urinary symptoms after treatment. Mild (Grade 1) stress incontinence occurs in 4–5% of cases but tends to resolve after a few weeks. Other less common side-effects include recto-urethral fistulae. Sexual function was intact in 73% of patients [2].

In summary, despite some good results from HIFU, which are comparable to non-surgical treatments [12,13] most of the evidence still supports radical prostatectomy as the best curative treatment for prostate cancer, particularly in younger patients. However, there is a role for HIFU in those patients not eligible for radical prostatectomy [2]. There is also an advantage in that the treatment is repeatable and has lower morbidity [3,14]. It can also be used in combination with radiotherapy or chemotherapy [14]. In addition, TURP at the time of the procedure can reduce urinary retention, which is one of the main side-effects of the treatment. It may also be possible to use HIFU in patients with recurrent prostate cancer who have previously undergone radical prostatectomy.


Poissonier L, Gelet A, Chapelon J et al. Results of transrectal focused ultrasound for the treatment of localised prostate cancer (120 patients with PSA < or +10 ng/ml). Prog Urol 2003; 13: 60–72


VESICO-VAGINAL FISTULA

Sir,

It was with pleasure I read the Surgery Illustrated article describing the diagnosis and surgical management of vesico-vaginal fistulae [VVF] [1]. Although most of the ‘tricks’ in diagnosis and surgical steps have been described, I would like to highlight the difficult diagnosis of a ureterovesico-vaginal fistulae [2] masquerading as a simple VVF. These patients have the injury at the level of the intramural portion of the ureter, where an injury at one level will injure both the ureter and the bladder. The patient presents with a urinary leak after a pelvic surgical procedure. The vaginal findings show an opening in the anterior wall of the vagina. The three-swab test is positive for a VVF, i.e. the swab stains blue with leakage of urine from the bladder. The ureteric urine leak (of unstained urine) into the swabs is masked by the coloured leakage from the bladder, from the vesical component of the fistula. IVU may show the ureteric obstruction, but not uncommonly may show a normal ureter. This happens if the ureter empties completely into the fistula, and thus appears undilated and normal. Thus IVU can be absolutely normal in a patient with a ureterovesico-vaginal fistula. The cystoscopy findings show a bladder opening in the supratrigonal region of the bladder, which is in the line of the ureter and communicating with the vagina. It is only if a ureteric catheter is passed retrogradely that the delay is detected. Both the ureter and the bladder open into a single opening in the vagina through a complex tract.

The treatment of such a fistula involves surgical management, as for a complex VVF, i.e. repair by the abdominal approach, with bivalving the bladder up to the fistula. In addition, the involved ureter is dissected to the juxtavesical region. The dissection in this region is sometimes difficult, so if an adequate length of the ureter is secured for reimplantation, the ureter can be transected at its distal most point, after the lower end is ligated. After the vaginal opening is closed and the posterior wall of the bladder is closed, the ureter is reimplanted with an adequate submucosal tunnel. A psoas hitch is a useful adjunct, and interposition of omentum between the posterior wall of the vagina and the bladder is helpful.

In the event that a uretero-vesico-vaginal fistula is operated without a complete diagnosis (which in a difficult case would only be by conducting a retrograde ureteric dye study in a patient with a normal IVU, and no evidence of an obstructed/injured ureter), the fistula would recur [2]. This recurrence of fistula would be attributed to improper repair of the vesical fistula, but in reality would be due to the hidden ureteric component of this complex fistula. To avoid this frustrating situation, meticulous care is necessary during the diagnosis of all VVF.

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MANAGING PATIENTS WITH AN OVERACTIVE BLADDER AND GLAUCOMA: A QUESTIONNAIRE SURVEY OF JAPANESE UROLOGISTS ON THE USE OF ANTICHOLINERGICS

In their paper discussing the potential adverse effects of anticholinergics prescribed for an overactive bladder in patients with glaucoma, Kato et al. [1] rightly present a balanced argument of avoiding undue caution in prescribing effective treatment, against underestimating the risk of iatrogenic angle-closure. Recent advances in the understanding of variations in the clinical characteristics of angle-closure glaucoma, especially in Asian nations, render some of their comments and recommendations incorrect.

Most cases (75%) of angle-closure in Asians are asymptomatic [2]. Consequently, relying on symptoms such as pain or a red eye as the sole method of identifying a rise in intraocular pressure is inadequate. Furthermore, pupil-block is not the only mechanism responsible for angle-closure. While 38% of Asian people suffer from angle-closure solely caused by pupil-block, it is estimated that over half of all Asian people (54%) have mixed-mechanism disease (pupil-block combined with anterior, non-pupil-block or ‘plateau iris’ syndrome) [3]. An iridotomy will therefore not prevent angle-closure occurring in all cases. Kato et al. also emphasize the association of greater age with both glaucoma and bladder instability. The proportion of east-Asian people at risk of angle-closure rises from 1.5% to 2% in the 40–49-year age-group to >10% in people aged ≥70 [4]. Chinese ethnicity seems to be associated with a considerably greater risk than in other Asian groups [2].

It would seem more appropriate to recommend caution in the use of anticholinergics in cases of glaucoma, rather than saying there is a contraindication. However, in Asia, where angle-closure glaucoma is a leading cause of blindness, these agents should only be started in patients with established glaucoma after the involvement of their ophthalmologist. All ethnic Chinese patients aged ≥40 years should have an ophthalmic assessment before
using anticholinergic agents. Consideration should be given to a similar examination for all other Asian people.

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*Glaucoma Service, Moorfields Eye Hospital, †International Centre for Eye Health, London School of Hygiene and Tropical Medicine, and ‡Division of Epidemiology, Institute of Ophthalmology, University College London, UK


2 Foster PJ. The epidemiology of primary angle closure and associated glaucomatous optic neuropathy. Semin Ophthalmol 2002; 17: 50–8


Surgical Atlas

Transureteroureterostomy

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The Oregon Health & Science University, Portland, Oregon, USA

ILLUSTRATIONS by STEPHAN SPITZER, www.spitzer-illustration.com

INDICATIONS

The indication for transureteroureterostomy is to bypass a distal ureter without compromising the recipient ureter with disease from the donor renal unit. It is useful in patients who have had previous pelvic surgery that would make a ureteroneocystostomy with a psoas hitch, with or without a bladder flap procedure, difficult or inadvisable. The procedure is not recommended under the following circumstances: chronic pyelonephritis, renal calculus disease, previous ureteric trauma with scar, idiopathic retroperitoneal fibrosis, fibrosis following previous aortoiliac vascular surgery, high-dose radiation therapy, urosepsis, uroepithelial tumours, pelvic visceral tumours with ureteric involvement, or inadequate ureteric length for a tension-free anastomosis. The procedure is useful when the better of the two ureters is reimplanted into the bladder. When a normal ureter remains after nephrectomy, a transureteroureterostomy will provide drainage for the remaining kidney when its ureter is diseased. The procedure is of value when the smaller of two ureters is anastomosed to the larger one, which is then used to bridge the abdominal wall and form a stoma.

SPECIFIC EQUIPMENT/MATERIALS

- Basic Bookwalter table-fixed retractor.
- Basic laparotomy set.
- Headlight.
- Magnification if the patient is small.
- Morse-Andrews suction tube.
- DeBakey 'Atraugrip' vascular tissue forceps.
- Lahey/Sweet gallbladder duct forceps, 19 cm.
- Potts-style scissors with short blades and semi-sharp tips.
- Thin vascular needle holders.
- 5/0 double-armed monofilament absorbable sutures.
- Soft suction drain.
- Y-connector system for intraoperative bladder irrigation and drainage.
- Double-pigtail stent with thread left on bladder/distal end.
- Cysto-urethroscopy set-up with two open-ended ureteric catheters to accept guidewires (optional).
- Foley catheter that will comfortably fit the urethra after calibration with bougie à boule.
- Sequential calf-compression devices.

SPECIFIC PATIENT PREPARATION

- Treat urinary tract infection if present.
- Bowel preparation if the patient has bowel dysfunction, infrequent bowel movements, or prior abdominal surgery.
- Antibiotic administration within 30 min of procedure.
- Calf sequential compression devices to prevent deep venous thrombosis.

SPECIFIC PATIENT POSITIONING

- Lithotomy position for cystoscopy and bilateral ureteric catheterizations (optional).
- After cystoscopy and bilateral ureteric catheterization, extended supine position with a break in the table just above the iliac crest.
Figure 1

Cystoscopy is performed and open-ended ureteric catheters placed. The ureteric catheters are brought out alongside the Foley catheter and each connected to its own drainage system; they will be removed later. The patient is placed supine, slightly hyperextended, sequential calf-compression devices applied (not shown), and the Foley catheter attached to a Y-connector, connected to an irrigation/drainage system so that the anaesthetist can drain and fill the bladder during surgery. The patient is prepared and draped for a vertical midline incision that will be extended as much as necessary to comfortably perform the procedure.
Figure 2

Enough adhesions are taken down to allow the Bookwalter retractor to be placed; the Bookwalter ring is positioned 4–5 cm above skin level. When the abdominal wall retractors are placed, this creates intra-abdominal space to pack the intestines out of the way. The posterior peritoneum is incised as would be done for a retroperitoneal lymphadenectomy. This will expose both ureters. If they are not easily seen, suspicious structures can be plucked and observed for peristalsis, or the previously placed ureteric stents palpated.
The donor ureter is traced into the pelvis, and as much peri-ureteric tissue as possible is left with the ureter to provide blood supply. The catheter in the donor ureter is withdrawn from below. The donor ureter is ligated distally and divided proximal to the ligature. The ureter is spatulated on its medial surface to create a 2-cm opening and tagged with a stay suture that will be used for gentle traction. The donor ureter is dissected proximally. The gonadal vessels are divided between ligatures so they will swing medially with the donor ureter. (If the patient has had previous vasectomies, he will probably develop testicular atrophy on the side of the donor ureter.)

The donor ureter is swung over the great vessels towards the recipient ureter. The donor ureter is passed cephalad or caudad to the inferior mesenteric artery depending on which will bring the donor ureter closer to the recipient ureter with no tension. If necessary, a plane lateral to the recipient ureter is opened, and the ureter is teased medially towards the donor ureter until they meet with no tension.
**Figure 4**

**A,** 5/0 monofilament stay sutures are placed side-by-side in the recipient ureter at the proposed longitudinal ureterotomy.

The recipient ureter is incised with a #15 blade and the incision is extended with Potts-style scissors to match the opening in the donor ureter; 5/0 absorbable monofilament sutures are placed at either end of the recipient ureterotomy and into the heel and toe of the donor ureter.

**B,** The posterior wall of the donor ureter is sewn to the medial wall of the recipient ureter from inside the lumen. The recipient ureteric catheter is withdrawn by an unscrubbed assistant until the open end appears in the half-completed anastomosis. A guidewire is passed into the end of the recipient ureteric catheter and withdrawal of the ureteric catheter is completed.

A double-pigtail stent is passed over the wire into the bladder. A suture is left on the distal curl in case the curl later retracts up the ureter. The wire is removed and the position of the distal curl confirmed when the anaesthetist fills the bladder through the Y-connector hook-up by clamping the outflow catheter and opening the inflow tube.

A guidewire is passed through a side hole in the double-pigtail stent to straighten the proximal curl, and the proximal stent is passed up the donor ureter into the renal pelvis. The guide wire is removed.

**C,** The ureteric anastomosis is completed with the running 5/0 monofilament absorbable suture.
Figure 5

A soft suction drain is placed in the retroperitoneum and brought out lateral to the colon through a stab wound lateral to the abdominal incision. The posterior peritoneal incision is closed with running 3/0 absorbable suture. The intestines are allowed to return to their natural positions. The midline incision is closed with interrupted far-far-near-near 0 monofilament absorbable sutures. If epidural catheter analgesia will not be used, the wound is injected with a long-acting local anaesthetic such as ropivacaine. Scarpa’s fascia is closed with running 3/0 absorbable suture. The skin is closed with a running 4–0 absorbable subcuticular suture. Adhesive strips are applied across the suture line. Dry dressings are placed over the wound and the drainage tube. The Y-connector is removed and the Foley catheter is connected to a urine drainage bag.
CARE AFTER SURGERY

Patient-controlled intravenous analgesia is used. The Foley catheter is removed in a day or two when the patient can void or resume intermittent catheterization. If epidural analgesia is used, urethral catheter removal is delayed for ≥6 h after the epidural has been discontinued.

The wound dressing is removed 2 days after surgery. The adhesive strips will come off several days later with a bath or shower. The sequential calf compression devices are removed when the patient is ambulating. The suction drain is removed when the drainage is <50 mL/24 h. In ~6 weeks, the patient has baseline renal ultrasonography and the double-pigtail ureteric stent removed via flexible cystoscopy in the clinic 10 min after instilling a urethral anaesthetic.

SURGEON TO SURGEON

The Y-connector system can be used to fill the bladder to consider the option of ureteroneocystostomy with or without a psoas hitch or bladder flap procedure, instead of a transureteroureterostomy. Some surgeons will prefer to avoid ureteric catheterization for ureteric identification and rely on simple observation and diuresis to identify the ureters.

Dissecting clamps like the Lahey/Sweet gallbladder duct forceps have longitudinal rather than cross serrations and do not get caught on tissues during dissection.

Skin closure with an absorbable subcuticular suture is more comfortable for the patient than skin staples, clips or nonabsorbable sutures any of which must be removed later.

One of our more disappointing cases was donor ureteric obstruction that occurred as a child grew and the ureter became trapped under the inferior mesenteric artery.

CLOSING COMMENTS

Material has been used freely, and with permission, from a previous publication [1]. The procedure was described in dogs and cadavers nearly 100 years ago [2], and reported in a patient 70 years ago [3].

Although transureteroureterostomy may seldom be indicated, it is a good procedure to have available.


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   e-mail: barryj@ohsu.edu

REFERENCES

In [1], the following error was published on page 909.

Author Listing

JOE PHILIP and RAMASWAMY MANIKANDAN, Department of Urology, Royal Liverpool University Hospital, Liverpool, UK

The text was incorrect and should have read:

Author Listing

JOE PHILIP, RAMASWAMY MANIKANDAN and PALANISWAMY VISWANATHAN*, Department of Urology, Royal Liverpool University Hospital, Liverpool, UK, and *Department of Urology, Royal Cornwall Hospital, Truro, UK

REFERENCE

1 Philip J, Manikandan R. Prostate cancers in the transition zone: Part 2; clinical aspects. BJU Int 2005; 95: 909
### 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>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AUA</td>
<td>American Urological Association</td>
</tr>
<tr>
<td>BAUS</td>
<td>British Association of Urological Surgeons</td>
</tr>
<tr>
<td>BCG</td>
<td>bacille Calmette-Guérin</td>
</tr>
<tr>
<td>BPH</td>
<td>benign prostatic hyperplasia</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<tr>
<td>BOO</td>
<td>bladder outlet obstruction</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<td>CNS</td>
<td>central nervous system</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DMSA</td>
<td>dimercapto-succinic acid</td>
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<tr>
<td>DRE</td>
<td>digital rectal examination</td>
</tr>
<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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<tr>
<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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<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
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<tr>
<td>GnRH</td>
<td>gonadotrophin-releasing hormone</td>
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<td>GP</td>
<td>general practitioner</td>
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<td>hCG</td>
<td>human chorionic gonadotrophin</td>
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<td>HIV</td>
<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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<tr>
<td>IgX,</td>
<td>immunoglobulin (class X, subclass x)</td>
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<td>International Prostate Symptom Score</td>
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<td>intravenous urography</td>
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<tr>
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<td>luteinizing hormone-releasing hormone</td>
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<td>lower urinary tract symptoms</td>
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<tr>
<td>MAG</td>
<td>mercapto-acetylglycine</td>
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<tr>
<td>MAG3</td>
<td>mercapto-acetyltrimycolic acid</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>National Health Service</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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<td>PCR</td>
<td>polymerase chain reaction</td>
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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>posterior urethral valves</td>
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<td>RCC</td>
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<td>sodium dodecyl sulphate</td>
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<tr>
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<td>transitional cell carcinoma</td>
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<tr>
<td>TGF</td>
<td>transforming growth factor</td>
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<td>TNF</td>
<td>tumour necrosis factor</td>
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<td>TRUS</td>
<td>transrectal ultrasonography</td>
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<td>TURP</td>
<td>transurethral resection of the prostate</td>
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<tr>
<td>UTR</td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>VUR</td>
<td>vesico-ureteric reflux</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
XXV Biannual Congress of the Urological Association of South Africa, Sun City, Pilanesberg, South Africa.

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W http://www.urosa.co.za

35th Annual Meeting of the International Continence Society, Montreal, Canada.

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E vicky@icsoffice.org
W http://www.icsoffice.org

35th Annual Meeting of the International Continence Society, Montreal, Canada.

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

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

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
November 9-11
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
Comprehensive Urological Laparoscopy: An Intermediate Level Training Course incorporating 'Different Techniques of Nephrectomy'. Course Director: Mr. A. Rané. Venue: Aesculapium, Tuttlingen, Germany.
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

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 1-5
VIII Congress of the Latinamerican Society for Sexual and Impotence Research, Punta del Este, Uruguay, Dr. Miguel Alfredo Rivero VIII Congress President, Dr. Luiz Otavio Torres SLAIS President.
T: 54 11 4325 1273/4325 1290
F: 54 11 4326-8517
E: info@slaiss2005.org
W: www.slaiss2005.org

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: www.essm.org/index.cfm

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