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BOOKS


Can Human Papillomavirus DNA Testing Substitute for Cytology in the Detection of High-Grade Cervical Lesions?

To the Editor.—In a recent article, Lee et al.1 presented a review of 593 women who had undergone conventional Papanicolaou (Pap) tests and human papillomavirus (HPV) DNA typing. I would like to discuss 3 topics of this article: (1) diagnostic values of the liquid-based Pap test versus conventional smears; (2) diagnostic values of HPV DNA testing; and (3) whether HPV DNA testing can substitute for cytologic testing in the detection of high-grade cervical lesions.

Regarding the first point, the sensitivity and negative predictive value of cytologic testing can be up to 95.5% and 93.5%, respectively,2 with a liquid-based Pap test compared with 76.3% and 84.7%, respectively,1 for conventional smears.

Regarding the second point, the ASCUS (atypical squamous cells of undetermined significance) and LSIL (low-grade squamous intraepithelial lesion) Triage Study concluded that testing with the Hybrid Capture assay (version 2.0) (HCS II; Digene Corporation, Gaithersburg, Md) is a viable option in the treatment of women with ASCUS3 and high-grade squamous intraepithelial lesion (HSIL).1 In our study of 66 HSIL cytology cases,2 high-risk HPV DNA (22), low-risk HPV DNA (2), and both (41) were detected in 65 (98%) of the cases.

Regarding the third point, in their article, Lee et al.1 comment, “Therefore, HPV DNA testing can be highly recommended as a substitute method for detecting high-grade cervical lesions, especially for invasive cervical cancer [italics added].” This statement may need some qualification. The reason is that both high-risk and low-risk HPV DNA has been detected in a wide range of the uterine cervical dysplasia and normal to borderline lesions.2,3 In our review of 172 ASCUS cases,2 HPV DNA was detected in 32 (high-risk), 5 (low-risk), and 51 (high- and low-risk) cases. Also, the prevalence of HPV infection in patients with a normal Pap test result was 31 (16.2%) of 191 cases and was evident in high-risk and low-risk HPV DNA types.2

Testing with HCS II can be a “substitute method” for screening in a large population to detect high-risk women in a region or in countries where the services of cytopathology laboratories are not readily available. It can also serve as a valuable quality improvement index at a cytopathology laboratory.

Use of the Pap test plus concurrent HPV DNA assay of residual liquid-based samples is a reproducible, accurate, and cost-effective follow-up method for women with ASCUS and squamous intraepithelial lesions of the uterine cervix. The combination testing (liquid-based Pap test and HPV typing) is an objective and “gold standard” triage method of managing ASCUS and SIL.

Currently, the specificity of all triage methods for the diagnosis of “dysplasia” of the uterine cervix is relatively low. Therefore, one triage method cannot be substituted for the other method. To achieve an accurate diagnosis, it may require all of the triage methods for the early detection of uterine cervical lesions.

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A Review of Articles From Last Month’s
Archives of Pathology & Laboratory Medicine

Listed below are questions based on articles that appeared in last month’s print edition of the ARCHIVES. Registered continuing medical education participants should use the February 2005 answer sheet to answer these questions.

1. The determination of anti-phospholipid antibodies is used to evaluate the risk of thrombosis in patients with systemic lupus erythematosus.
   True or False?
   (from Prevalence and Clinical Correlation of Anti-Phospholipid-Binding Protein Antibodies in Anticardiolipin-Negative Patients With Systemic Lupus Erythematosus and Women With Unexplained Recurrent Miscarriages—Bizzaro et al)

2. According to a recent study on Wilms tumor gene protein (WT1) expression in ovarian carcinomas:
   a. 93% of serous carcinomas had a strong positive reaction for WT1 in more than 50% of the tumor cells
   b. a WT1 negative reaction does not exclude the diagnosis of a serous ovarian carcinoma
   c. mucinous and clear cell carcinomas also had a strong positive reaction
   d. a and b
   e. a and c
   (from Immunohistochemical Expression of Wilms Tumor Gene Protein in Different Histological Subtypes of Ovarian Carcinomas—Waldstrøm & Grove)

3. Primitive neuroectodermal tumor of the stomach may be immunoreactive for chromogranin A and synaptophysin.
   True or False?
   (from Primitive Neuroectodermal Tumor of the Stomach—Soulard et al)

4. In breast carcinoma:
   a. The cause of deregulated protein expression of c-erb-B2 and TOP2A is most frequently DNA amplification
   b. TOP2A is located at chromosome region 14q2–q19
   c. quantitative polymerase chain reaction using hybridization probes allows rapid analysis of gene copy numbers
   d. a and b
   e. a and c
   (from Copy Number Analysis of c-erb-B2 (HER-2/neu) and Topoisomerase IIα Genes in Breast Carcinoma by Quantitative Real-Time Polymerase Chain Reaction Using Hybridization Probes and Fluorescence In Situ Hybridization—Murthy et al)

5. Cyclin D1 is a cell-cycle protein that acts as a type of molecular roadblock in the passage of cells from G1 to S phase.
   True or False?
   (from Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma With Trisomy 12 and Focal Cyclin D1 Expression—O’Malley et al)

6. In patients with polycythemia:
   a. the peripheral venous hematocrit is lower than the total blood volume (body) hematocrit in normal subjects
   b. apparent polycythemia has been associated with such factors as obesity, hypertension, smoking, and alcohol consumption
   c. the majority of patients with apparent polycythemia have either a normal or increased red cell mass and a lower plasma volume
   d. a and b
   e. b and c
   (from Analysis of Red Cell Mass and Plasma Volume in Patients With Polycythemia—Lorberboym et al)
Seventh O.E.S.O. Congress
Gastroesophageal Reflux and Barrett Esophagus
Gregorio Chejfec, MD; Henry D. Appelman, MD

On September 3, 2003, a group of distinguished pathologists and clinicians gathered at the most recent meeting of the Congress of the O.E.S.O. in Paris, France. This issue of the Archives of Pathology & Laboratory Medicine includes the lectures given by each of the presenters at this meeting.

O.E.S.O. are the initials for the Organisation mondiale d’Études Spécialisées sur les maladies de l’œsophage, a French association dedicated to study of the esophagus. The English equivalent is World Organization for Specialized Studies on Diseases of the Esophagus. The initials of the French name are also the first 4 letters in the European spelled for the first part of the digestive tract, the oesophagus, so the acronym is very appropriate. This association was established in the late 1970s by an innovative French esophageal surgeon, Robert Giuli, who is still its guiding light. Dr Giuli had the foresight to recognize the need for bringing together physicians and scientists of many disciplines to exchange information on normal functions and diseases of the esophagus. He organized the first international O.E.S.O. Congress in Paris in 1984, and subsequent Congresses have been held in Paris in 1987, 1990, 1993, 1996, 2000, and 2003. These Congresses have brought together surgeons, gastroenterologists, pediatricians, radiologists, epidemiologists, pathologists, pharmacologists, biochemists, anatomists, and physiologists to discuss the most recent advances in esophageal disorders. The proceedings of each of these Congresses have been published as books, which are virtual encyclopedias of esophageal form and function, both normal and abnormal. The Congresses have become venues for pathologists to rub elbows with a variety of clinicians, and the pathologists feel welcomed and comfortable and are valuable contributors to this multidisciplinary activity. Pathologists who have been active participants and planners in these Congresses, many of whom are the most esteemed gastrointestinal pathologists in the world, include (from North America) Don Antonioli, Henry Appelman, Parakrama Chandrasoma, Greg Chejfec, Palayo Correa, Kim Geisinger, John Goldblum, Rodger Haggitt, Stanley Hamilton, Jeff Lee, Klaus Lewin, Liz Montgomery, Bob Adze, Bob Riddell, and Mamoun Younes. From Europe, the United Kingdom, and Japan, past participants and planners include Wladimir Bogomoletz, Mike Dixon, Nadine Ectors, Jean-François Flejou, Annie Galian, Daniel Gardiol, Bob Genta, Karel Geboes, David Hopwood, Francois Pottet, Neil Shepherd, Pentti Sipponen, Manfred Stolte, Kaiyo Takubo, Elizabeth Tschanz, and Michael Voith. In 4 of the past 6 Congresses, there have been symposia, ranging from 2 to 4 hours, devoted to the pathology of the esophagus, and many of the pathologists listed above have given lectures during these symposia.

The theme of the 2003 pathology symposium was metaplasia of the esophageal mucosa, whereby the squamous epithelium is replaced by mucosa lined with columnar epithelium and containing mucus-producing goblet cells. This condition is hereinafter referred to as Barrett metaplasia in honor of Norman Barrett, whose pioneer work first attracted the attention of investigators in this field.

The initial lectures at the 2003 symposium were devoted to gastroesophageal reflux disease, ultimately associated with the development of Barrett metaplasia. In keeping with previous O.E.S.O. meetings, each one of the presentations was a response to a specific question. Kaiyo Takubo (Tokyo Metropolitan Institute of Gerontology) answered the first question: “Is there a set of histologic changes that are invariably reflux associated?” The second question, “Has a histologic transition from GERD-damaged epithelium to columnar metaplasia ever been seen in humans?” was discussed by Robert Riddell (Mount Sinai Hospital, Toronto, Ontario).

Following these introductory discussions, attention was concentrated on the neoplastic potential of the metaplastic mucosa. The subject of dysplasia was the center of attention.

Appropriately, Henry Appelman (University of Michigan, Ann Arbor) opened this portion of the symposium with an eloquent dissertation, “What is dysplasia?” Interobserver variability in the diagnosis of dysplasia was thoroughly analyzed by Elizabeth Montgomery (Johns Hopkins, Baltimore, Md).

The wide variation in the incidence of adenocarcinomas associated with Barrett metaplasia was addressed by Elizabeth R. Tschanz (Hôpitaux Universitaires de Genève, Geneva) in collaboration with Robert Genta (Geneva).

The features distinguishing adenocarcinoma of the
esophagogastroduodenal junction from Barrett adenocarcinoma were discussed by Nadine Ectors (University Hospitals Leuven, Leuven, Belgium). The final presentation was by Mamoun Younes (Baylor College of Medicine, Houston, Tex), who discussed the role of cytokeratins in the identification of the mucosa of the gastric cardia and Barrett metaplastic mucosa.

The ARCHIVES is pleased to reproduce the text of the 2003 O.E.S.O. presentations. We hope our readers will benefit from the experience of these well-known investigators.
Is There a Set of Histologic Changes That Are Invariably Reflux Associated?

Kaiyo Takubo, MD; Naoko Honma, MD; Gopi Aryal, MD; Motoji Sawabe, MD; Tomio Arai, MD; Yasuo Tanaka, MD; Ken-ichi Mafune, MD; Katsuhiko Iwakiri, MD

Many histologic changes have been described in the esophageal squamous mucosa in patients with gastroesophageal reflux disease (GERD), including dilated intercellular spaces, balloon cells, intrapapillary vessel dilation, elongated papillae, basal cell hyperplasia, acanthosis, intraepithelial eosinophils, Langerhans cells, and p53 protein overexpression. To define a set of histologic changes that are invariably reflux associated, we examined the histologic changes in esophageal specimens from normal controls, patients with GERD, patients without GERD but with a suspicion of other pathology, and patients with esophageal carcinoma. We also examined biopsy specimens from sites with differing endoscopic features, including cloudy white and reddened mucosa. A definitive set of reflux-associated histologic changes could not be defined from the small number of biopsy specimens examined in the present study. Histologic changes indicative of GERD are likely to be found somewhere in the esophagus in all patients with GERD, but these changes are nonspecific. A set of histologic changes that are invariably reflux associated may exist, but these changes are nonspecific. To develop a set of characteristic reflux-associated features, endoscopists may perform targeted biopsies from several sites with various endoscopic features and at different stages of disease.

(Arch Pathol Lab Med. 2005;129:159±163)

Is there a set of histologic changes that are invariably reflux associated? This question was posed to us by Professors Appelman and Chejfec at a pathology symposium held in conjunction with the 7th World Congress of the International Organization for Specialized Studies on Diseases of the Esophagus (O.E.S.O.), in Paris, in September 2003.1 We undertook a study to attempt to answer this question. Because there have been many reports on the incidence of various histologic changes in the esophageal squamous mucosa of patients with gastroesophageal reflux disease (GERD), we examined esophageal squamous mucosa from 4 groups: patients with GERD, patients without GERD, patients with esophageal squamous cell carcinoma, and normal autopsy controls.

Longitudinal (palisaded) vessels, when seen at endoscopy, are always situated within the esophagus, never in gastric mucosa.2,3 Therefore, in Japan, the esophagogastric junction is now defined endoscopically as the lower limit of the longitudinal vessels.4 This definition has been approved by the Japanese Society for Esophageal Diseases (in June 2003, the Society changed its name to the Japan Esophageal Society).5,6 In patients with GERD with or without mucosal breaks, as defined by the Los Angeles Classification System,7 the longitudinal vessels may become difficult to see endoscopically through squamous mucosa, which has become cloudy white (opaque). Reddened lower esophageal squamous mucosa, with or without mucosal breaks, has also been reported in patients with GERD. This reddened mucosa differs from that seen in the central area of healing mucosal breaks, which consists of thin, regenerating epithelium. Cloudy white and/or reddened mucosa is classified in Japan as “discolored mucosa” or mucosa showing “minimal changes.” In the second part of our study, we evaluated the correlation between endoscopic discoloration (cloudy white and/or reddened mucosa) and histologic changes in biopsy specimens from patients with GERD but without mucosal breaks.

MATERIALS AND METHODS

Patient Groups

Biopsy specimens were obtained from 69 consecutive patients (mean age, 75 years) with endoscopically diagnosed GERD (Los Angeles Classification A through D). 49 patients (mean age, 73 years) with esophageal squamous cell carcinoma, and 38 patients (mean age, 72 years) without GERD or carcinoma. In the patients with GERD, biopsy sites were always from squamous epithelium-lined mucosa within 3 to 4 cm proximal to the esophagogastric junction, where longitudinal rather than arborescent vessels were observed endoscopically. No preoperative radiotherapy, laser ablation, or proton pump inhibitor therapy had been given prior to biopsy in any of the patients with GERD. In the patients with carcinoma, the biopsy specimens were taken from noncancerous mucosa adjacent (usually proximal) to the cancer. In the
8 patients without endoscopic evidence of GERD or carcinoma, the biopsy specimens were obtained because of a suspicion of other pathologic changes such as papilloma, glycolgenic atrophy, or candidiasis or because there were areas of mucosa that did not stain with Lugol iodine solution (in Japan, the esophageal mucosa is frequently stained with Lugol iodine during endoscopic procedures to detect unstained cancerous lesions). There was no endoscopic evidence of GERD in these 8 patients because no mucosal breaks were seen (as per the Los Angeles Classification System). Transverse sections of normal upper esophageal mucosa were also obtained from 16 autopsy subjects (mean age, 81 years) and used as controls. Five high-magnification fields of each control mucosa were examined.

**Histologic Markers**

We examined each specimen for the presence or absence of 9 previously reported reflux-associated histologic changes.

1. Dilation of intercellular spaces in the squamous epithelium, determined by viewing the specimens at a magnification of ×100.
2. Presence of balloon cells with pyknotic nuclei in the squamous epithelium, determined by viewing at a magnification of ×100.
3. Intrapapillary vessel dilation, as reported by Geboes et al.
4. Elongation of the lamina propria papillae to more than two thirds of the thickness of the epithelium. Such elongation, and an increase in the number of papillae, has been reported in the esophageal squamous mucosa of patients with GERD.
5. Basal cell hyperplasia, accounting for more than 20% of the thickness of the epithelium.
6. Acanthosis, which was defined as squamous epithelium with a thickness of greater than 0.4 mm.
7. Intraepithelial eosinophil infiltration. The presence of 1 or more intraepithelial eosinophils per histologic section was regarded as a positive result.
8. Langerhans cell infiltration. Langerhans cells and dendritic cells are present in the normal esophageal epithelium and in the epidermis. The number of Langerhans cells in the esophagus has been reported to be increased in patients with GERD. Langerhans cells and dendritic cells express CD1a (Dako, Glostrup, Denmark). For simplicity, both Langerhans cells and dendritic cells were regarded as Langerhans cells in this study. The presence of 3 or more Langerhans cells per microscopic field at a magnification of ×200 was regarded as a positive result.
9. Mutant type p53 protein overexpression (NCL-p53-D07, Novocastra Laboratories Ltd, Newcastle upon Tyne, United Kingdom). Positive staining of more than 5% of squamous epithelial cells with the p53 immunostain was regarded as a positive result, indicating p53 protein overexpression. The chi-square test was used to determine statistical significance.

**Correlation Between Minimal Endoscopic Changes and Histologic Findings**

Sections from squamous mucosa exhibiting minimal endoscopic changes were reviewed to evaluate the correlation between the endoscopic and histologic appearances. The biopsy specimens were embedded and sectioned using a dissecting microscope to obtain sections that were perpendicular to the epithelial basement membrane. Three to 5 biopsy specimens of cloudy white mucosa, through which the longitudinal vessels of the esophagus could not be seen, were examined from each of 11 patients. Three to 5 biopsy specimens of reddened mucosa from each of 10 patients were also examined.

**RESULTS**

**Incidence of Various Histologic Features**

The incidences of intercellular space dilation in the GERD, cancer, without-GERD, and autopsy control groups were 48% (33/69), 33% (16/49), 21% (8/38), and 0% (0/16), respectively. The incidence of intercellular space dilation was significantly higher in the GERD group than in the without-GERD group (P = .006) and the autopsy control group (P < .001). The incidence in the cancer group was significantly higher than that in the autopsy control group (P = .007). There was no significant difference between the GERD and cancer groups (P = .10).

The incidences of balloon cells in the GERD, cancer, without-GERD, and autopsy control groups were 49% (34/69), 24% (12/49), 47% (18/38), and 0% (0/16), respectively. The incidence of balloon cells in the mucosa was significantly higher in the 3 biopsy groups, with GERD (P < .001), with carcinoma (P = .03), and without GERD (P < .001), than in the autopsy control group. The incidence was significantly higher in the GERD group than in the cancer group (P < .001). The incidence in the GERD group was not significantly higher than that in the without-GERD group (P = .85).

The incidences of intrapapillary vessel dilation in the GERD, cancer, without-GERD, and autopsy control groups were 71% (49/69), 45% (22/49), 71% (27/38), and 19% (3/16), respectively. The incidence of intrapapillary vessel dilation was significantly higher in the GERD group than in the cancer group (P = .004) and autopsy control group (P < .001).

It was easy to prepare sections perpendicular to the epithelial basement membrane from the autopsy specimens, but correct orientation was much more difficult to achieve for the biopsy specimens. As a result, elongation of papillae, basal cell hyperplasia, and acanthosis were difficult to evaluate in many of the biopsy specimens. The incidences of elongated papillae in the GERD, cancer, without-GERD, and autopsy control groups were 61% (14/23), 44% (7/16), 50% (6/12), and 13% (2/16), respectively. The incidence of elongated papillae was significantly higher in the GERD group (P = .003) and the without-GERD group (P = .04) than in the autopsy control group. The incidence was not significantly higher in the GERD group than in the cancer group (P = .29) or the without-GERD group (P = .54).

The incidences of basal cell hyperplasia in the GERD, cancer, without-GERD, and autopsy control groups were 57% (13/23), 38% (6/16), 31% (4/13), and 19% (3/16), respectively. The incidence of basal cell hyperplasia was significantly higher in the GERD group than in the autopsy control group (P = .02) but was not significantly higher in the GERD group than in the cancer group (P = .24) or the without-GERD group (P = .18).

The incidences of acanthosis with or without keratinization in the GERD, cancer, without-GERD, and autopsy control groups were 41% (9/22), 24% (4/17), 33% (4/12), and 6% (1/16), respectively. The incidence of acanthosis in the GERD group was significantly higher than that in the autopsy control group (P = .02) but was not significantly higher in the GERD group than in the cancer group (P = .32) or the without-GERD group (P = .73).

The incidences of intraepithelial eosinophils in the GERD, cancer, without-GERD, and autopsy control groups were 23% (16/69), 20% (10/49), 21% (8/38), and 13% (2/16), respectively. Eosinophils were much more frequently observed in the papillae and in subepithelial stroma than in the epithelium. No significant difference in the incidence of intraepithelial eosinophil infiltration was seen between the GERD group and the other 3 groups: with cancer (P = .72), without GERD (P = .80), and autopsy control (P = .50).

The incidences of intraepithelial Langerhans cells (Fig-
Figure 1. Langerhans cells expressing CD1a101. Many dendritic processes and cell bodies are visible. The presence of 3 or more Langerhans cells and/or dendritic cells per microscopic field at a magnification of ×200 was considered a positive result (CD1a101 staining, original magnification ×200).

Figure 2. Endoscopic appearance of the normal esophagogastric junction. Longitudinal vessels are visible through the squamous epithelium and are always situated within the esophagus. In Japan, the esophagogastric junction is defined as the lower limit of the longitudinal vessels.

Figure 3. Endoscopic appearance of the esophagogastric junction in a patient with gastroesophageal reflux but without evidence of mucosal breaks. The longitudinal vessels are not visible through the cloudy white (opaque) squamous mucosa but are visible through the apparently normal squamous mucosa.

Figure 4. Biopsy specimen from a region of cloudy white mucosa. Acanthosis is evident (hematoxylin-eosin, original magnification ×40).

Figure 5. Biopsy specimen from a region of reddened mucosa. There is dilation of multiple intrapapillary vessels (hematoxylin-eosin, original magnification ×100).

The incidence of Langerhans cells in the GERD, cancer, without-GERD, and autopsy control groups were 48% (33/69), 31% (15/49), 32% (12/38), and 19% (3/16), respectively. The incidence of Langerhans cells in the GERD group was significantly higher than that in the autopsy control group (P = .049) but was not significantly higher in the GERD group than in the cancer group (P = .06) or the without-GERD group (P = .10).
No overexpression of p53 protein, as determined by immunostaining, was seen in any of the groups.

**Correlation Between Minimal Endoscopic Changes and Histologic Findings**

Examination of histologic sections of biopsy specimens from cloudy white mucosa, through which the longitudinal esophageal vessels could not be seen (Figures 2 and 3), revealed acanthosis (Figure 4) in all 11 patients and keratization in 3 of the 11. The acanthotic epithelium had lost its normal basal cells; the cells on the basement membrane were very similar to those in the middle layers of the normal esophageal squamous epithelium, with relatively abundant cytoplasm and small nuclei. Examination of the biopsy specimens from reddened mucosa revealed dilation of multiple intrapapillary vessels in 7 of the 10 patients.

**COMMENT**

The epidemiology of esophagitis in Japan is somewhat different from that in Western countries. Primary adenocarcinoma of the esophagus represents less than 3.1% of all esophageal malignancies in Japan, and this figure has not increased in the past 13 years. In contrast, the incidence of esophageal adenocarcinoma in Western countries has recently been increasing rapidly and is now very high in white men. Adenocarcinoma now accounts for more than 50% of all esophageal malignancies in some series. Although reflux esophagitis is still much less common in Japan than in Western countries, its incidence has increased during the past 20 years. Severe GERD (grades C and D in the Los Angeles Classification System) is rare in Japan.

Nine histologic changes that have been previously described in the esophageal squamous mucosa in patients with GERD were evaluated. These changes were seen individually in 23% to 71% of the GERD group, but no single change was seen in 100% of patients. Significant differences in the incidence of the various histologic changes were seen in different study groups, mainly between the GERD and the autopsy control groups. A relatively high incidence of most changes was seen in the GERD, non-GERD (without GERD or cancer but with some endoscopic changes), and cancer groups. When targeted endoscopic biopsy specimens were obtained, histologic changes were consistently correlated with the endoscopic changes.

Various other histologic changes not evaluated in the present study have also been reported in association with GERD. For example, positive reactions for Ki-67 have been reported in squamous mucosa from patients with and without GERD, although no significant difference was reported in the rate of positive reactions between patients with GERD and normal controls. Many reports on the histologic findings associated with GERD have been published, but the same changes have also been found in many other patients who did not have GERD. For example, Weinstein et al reported that elongation of papillae and basal cell hyperplasia is sometimes seen in the distal 2.5 cm of the esophagus in asymptomatic individuals, and Janisch et al reported that eosinophil infiltration of the esophageal mucosa is not a specific diagnostic feature of GERD.

In our study, there was a significant difference between the GERD and the autopsy control groups in the incidences of 7 of the histologic changes examined. However, we examined apparently normal mucosa in the autopsy control group, whereas endoscopists do not usually take biopsies of apparently normal mucosa. Therefore, differences in the incidences of various histologic changes among the 3 biopsy groups (GERD, without GERD, and cancer) is likely to be of greater significance in practice than differences between the GERD and the autopsy control groups. However, some patients in the without-GERD or cancer groups may have had GERD but without mucosal breaks.

Is there a set of histologic changes that are invariably reflux associated? Based on the small number of biopsy specimens examined in the present study, the answer to this question is no. Some histologic changes indicative of GERD are likely to be found somewhere in the esophagus in all patients with GERD, but these changes are not specific; the histologic changes observed in a patient with GERD may also be seen in mucosa adjacent to an esophageal cancer or in endoscopically abnormal mucosa from a patient without GERD.

Longitudinal vessels are often not visible at endoscopy through the cloudy white (opaque) epithelium in patients with GERD or through Barrett mucosa; in Japan, proton pump inhibitor therapy is recommended prior to endoscopy to help the endoscopist to visualize the longitudinal vessels. Mucosal redness may be observed in the lower esophagus of patients with GERD with or without mucosal breaks. Cloudy white mucosa and mucosal redness are often referred to as discolored mucosa or minimal GER changes. These changes may be seen in esophago-gastric junction mucosa even when there are no mucosal breaks. Endoscopists usually obtain biopsy specimens from various sites, including cloudy white areas, redened areas, regenerating mucosa, the edges of erosions, and necrotic debris at the base of erosions, to distinguish between malignant and benign lesions. Histologic findings from different sites do not necessarily demonstrate significant or consistent changes.

The endoscopic findings in reflux esophagitis have been classified into 3 main stages by Makuuchi et al (Table): active, healing, and scarring. Relapse and healing occur repeatedly in patients with GERD, and a mixture of the stages is often seen in biopsy specimens. The histologic changes in biopsy specimens correspond to the observed endoscopic changes and are not representative of all sites.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
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<tr>
<td>Active</td>
<td>Lesion is covered by a thick layer of necrotic debris, fibrin, and inflammatory cells, with no regenerating epithelium visible. The surrounding epithelium is edematous and elevated and stains brown with Lugol iodine.</td>
</tr>
<tr>
<td>Healing</td>
<td>Lesion is surrounded by regenerating epithelium, with a smaller area covered by necrotic debris, fibrin, and inflammatory cells. The regenerating epithelium does not stain with Lugol iodine.</td>
</tr>
<tr>
<td>Scarring</td>
<td>Lesion is covered by regenerated epithelium with no necrotic debris and with or without a reddened area. The epithelium stains brown with Lugol iodine.</td>
</tr>
</tbody>
</table>

* Makuuchi’s classification has been modified and simplified.
and stages of disease in the esophagus of a patient with GERD.

Cloudy white mucosa and small areas of mucosal redness were observed endoscopically in this study; these minimal changes correspond to areas of acanthotic squamous epithelium, with or without keratinization, and dilation of multiple intrapapillary vessels, respectively. Further studies are needed to establish a correlation between endoscopic findings and histologic changes in the esophagus.

For pathologists to define a set of histologic changes that are invariably reflux associated, endoscopists must obtain biopsy specimens from several sites with different endoscopic appearances and from areas showing all stages of GERD. Thorough sampling will make it easier to establish a correlation between endoscopic appearances and histologic changes.

Based on the findings of the present study, the following conclusions were reached: (1) a set of histologic changes that are invariably reflux associated was not found in the small set of biopsy specimens examined in this study; (2) a set of histologic changes that are invariably reflux associated may exist, but these changes are not specific to patients with GERD; and (3) to enable pathologists to find such a set of histologic changes, endoscopists should take targeted biopsies from multiple sites that have different endoscopic features.

We are grateful to Neil K. Lambie, FRCPA (Department of Anatomic Pathology, Canterbury Health Laboratories, Christchurch Hospital, Christchurch, New Zealand) for assisting with the preparation of this manuscript.

References

The Genesis of Barrett Esophagus

Has a Histologic Transition From Gastroesophageal Reflux Disease–Damaged Epithelium to Columnar Metaplasia Ever Been Seen in Humans?

Robert H. Riddell, MD, FRCPath, FRCPC

• Has a histologic transition from gastroesophageal reflux disease–damaged epithelium to columnar metaplasia ever been seen in humans? The answer to this question seems to be that it has but that we either do not readily recognize it or it is not readily recognizable with regular light microscopy. There are at least 3 possible mechanisms for the genesis of Barrett esophagus. The first is ulceration at the gastroesophageal junction with subsequent repair by an epithelium that differentiates into Barrett epithelium. The second is metaplasia through multilayered epithelium. The third is creeping columnar metaplasia at the Z-line proximally followed by intestinalization. These 3 hypotheses may not be mutually exclusive, and all may be operative, depending on the local circumstances, amount of inflammation, erosion, ulcers, healing, acid and alkaline reflux, and use of proton pump inhibitors. Any of the epithelial types involved could be stable and not progress. They might even be reversible, which may also in part explain the mosaic of epithelial types that typify Barrett esophagus, and may be modified by any of the molecular mechanisms that turn protein transcription on and off (eg, promoter methylation, mutations). These mechanisms ultimately may also be involved in the genesis of neoplastic transformation.

(Arch Pathol Lab Med. 2005;129:164–169)

Has a histologic transition from gastroesophageal reflux disease (GERD)–damaged epithelium to columnar metaplasia ever been seen in humans? This question in one sense asks whether one can ever observe a junction between squamous mucosa exhibiting features of GERD and glandular mucosa showing features of Barrett esophagus (BE), which is seen regularly. However, the real question is whether BE has ever been seen to “form before our very eyes” and if so what this formation looks like morphologically. In other words, has an actual morphological sequence of events been observed that starts as squamous mucosa (presumably at some point GERD damaged) and finishes as BE?

If a reductionist approach is taken, one can list all of the features that can be seen in GERD-damaged mucosa in one column and all of those known in columnar metaplasia in another column (Table 1). Assuming there are no other mucosal types about which we are unaware, the transition must be present among these features. Any of the features that can be seen in the squamous mucosa in GERD patients can obviously be teamed up with similar changes in columnar mucosa, including cardiac mucosa. Although we assume that this list includes goblet cells as part of either complete or incomplete intestinal metaplasia, this feature is not always found, even in patients with overt BE.

There are several epithelial types that probably play no role in any transition, including in squamous mucosa the pseudoeipitheliomatous hyperplasia that can occur when ulcers and granulation tissue become re-epithelialized by squamous mucosa (Figure 1), balloon cells1 that are a sequel of reflux-associated damage, pancreatic metaplasia at the cardia and in BE,2,3 and dysplasia and carcinoma that are well-known late complications of BE in glandular mucosa. There has long been a suspected relationship between GERD and esophageal squamous cell carcinoma,4,10 which is hardly surprising given the increased epithelial turnover associated with GERD in the squamous mucosa11 that particularly extends into the larynx and pharynx12–14 but is irrelevant to this discussion.

In Table 1, some pathologic changes are listed in both columns, which suggests that these changes might be involved in one (there may be more than one) of the potential mechanisms of the metaplastic process. This process begins in squamous mucosa but results in glandular mucosa that ultimately includes goblet cells, that is, it has also become intestinalized. Potential mechanisms include (1) ulceration at the gastroesophageal junction (the squamo-columnar junction [SCJ] or Z-line), with subsequent restitution by an epithelium that differentiates into Barrett epithelium, (2) metaplasia through multilayered epithelium, (3) creeping columnar metaplasia at the Z-line proximally followed by intestinalization, and (4) re-epithelialization following therapy, resulting in overgrowth of squamous epithelium over glandular/Barrett epithelium or regrowth of glandular mucosa from esophageal glands/ducts or from the cardia.
Table 1. Epithelial Changes in Squamous and Glandular Mucosa Associated With Gastroesophageal Reflux Disease (GERD) and Barrett Esophagus (BE)

<table>
<thead>
<tr>
<th>Squamous Mucosa in GERD</th>
<th>Columnar Mucosa in BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal squamous mucosa</td>
<td>Cardiac mucosa</td>
</tr>
<tr>
<td>Basal cell hyperplasia</td>
<td>Cardio-oxynetic (transitional) mucosa</td>
</tr>
<tr>
<td>Elongation of dermal papillae</td>
<td>Incomplete intestinal metaplasia</td>
</tr>
<tr>
<td>Balloon cells and dilated intercellular space</td>
<td>Complete intestinal metaplasia</td>
</tr>
<tr>
<td>Erosion, ulcers</td>
<td>Pancreatic metaplasia</td>
</tr>
<tr>
<td>Restituting/regenerating mucosa</td>
<td>Erosions, ulcers</td>
</tr>
<tr>
<td>Pseudoepitheliomatous hyperplasia</td>
<td>Restituting/regenerating mucosa</td>
</tr>
<tr>
<td>Multilayered epithelium</td>
<td>Multilayered epithelium</td>
</tr>
<tr>
<td>Subsquamous glandular mucosa</td>
<td>Subsquamous glandular mucosa</td>
</tr>
<tr>
<td>Dysplasia, carcinoma</td>
<td>Dysplasia, carcinoma</td>
</tr>
<tr>
<td>Intraepithelial and lamina propria inflammatory cells (neutrophil, eosinophils, excess lymphocytes)</td>
<td>Intraepithelial and lamina propria inflammatory cells (neutrophil, eosinophils, chronic inflammation)</td>
</tr>
</tbody>
</table>

ULCERATION FOLLOWED BY DEVELOPMENT OF BARRETT EPITHELIUM

GERD-associated ulceration at the SCJ is an indication of attempts by the squamous mucosa to increase proliferation so it can keep up with the damage caused to that epithelium. Increased epithelial turnover is reflected in the classical criteria (described by Ismail-Beigi and colleagues\textsuperscript{15,16}) of basal cell hyperplasia and papillary elongation, which are the morphological counterparts of increased turnover.\textsuperscript{11} Following ulceration at the SCJ, re-epithelialization can occur from the adjacent squamous mucosa but should not result in BE (at least immediately). However, if re-epithelialization occurs over an erosion, it can result in quite marked pseudoepitheliomatous hyperplasia with pseudoinvasion of the underlying granulation tissue (Figure 1, C and D). Repair by glandular mucosa can come from either the gastric cardiac mucosa directly (Figure 2) or, if the ulceration is in the squamous-lined esophagus, from the esophageal glands or the esophageal gland ducts. These ducts open directly into the lumen of the esophagus and therefore are available to initiate a regenerative process of both squamous mucosa from duct orifices lined by squamous mucosa or of glandular mucosa from ducts lined by columnar epithelium. Repair could also come from Z-line mucosa with subsquamous glands of cardiac type, which occasionally are found beneath squamous mucosa at the Z-line and likely represent a dynamic interplay at the Z-line by which it may shift cranially or caudally (up or down). This type of repair is reminiscent of the changes seen in BE that has regressed except that it is limited to a few glands (up to approximately 8) and by definition is nonintestinalized. Otherwise, this repair would be indistinguishable from regressed short-segment BE or intestinal metaplasia that occurred at the Z-line in cardiac mucosa followed by a minimal regrowth/overgrowth of squamous mucosa, pre-

![Figure 1. A, Restituting mucosa, presumably squamous, at an ulcer in the lower esophagus (hematoxylin-eosin, original magnification ×40). B, With maturation, the squamous nature of this mucosa is much more apparent (hematoxylin-eosin, original magnification ×100). C and D, Squamous pseudoepitheliomatous hyperplasia with elongated papillae often found when erosions are re-epithelialized (hematoxylin-eosin, original magnifications ×20 and ×200).]
sumably resulting from a decrease in gastroesophageal reflux. However, if regeneration comes from glandular mucosa, it still requires goblet cell metaplasia within the columnar mucosa to result in typical Barrett-type epithelium.

Although the hypothesis that BE occurs after ulceration at the Z-line is attractive, the major problem is the difficulty in proving that BE occurs at all. Patients presenting with Barrett ulcers (ulcers within or abutting onto Barrett mucosa) clearly already have BE. However, there have been no reports of patients who presented with an SCJ ulcer(s) (biopsied thoroughly to confirm no BE) and were then treated with proton pump inhibitors but were found subsequently to have BE, particularly at the site of the ulcer. Even if BE were found, the role that taking numerous biopsies around the edge may have played in potentiating the damage present and therefore any repair response would inevitably be raised. Several authors have hinted or speculated that repair at biopsy sites may be a mechanism, but compelling evidence is lacking. Theoretically, there is no reason why restituting and regenerating mucosa should not result in BE, the main hindrance being that restituting mucosa invariably reverts to the type of epithelium from which the restitution was initiated. Further, all restituting epithelium is flat and has no differentiation that is apparent with light microscopy; only when it redifferentiates into its originating phenotype (glandular or squamous) is the nature of the epithelium apparent.

If this mechanism is operative, then the initial morphological finding would be preulcerative hyperplasia in the squamous mucosa, which is rarely biopsied although it probably can also be seen in the regenerative phase that can appear endoscopically as red streaks. However, adjacent to this area we would expect to see columnar or Barrett mucosa. At least initially, columnar mucosa may be indistinguishable from cardiac mucosa and therefore may go unrecognized, whereas Barrett mucosa could be recognized as BE, but whether it had occurred in the last few days or had been there for years would be impossible to determine. Therefore, it may be possible to observe this sequence of changes morphologically without recognizing that it is neo-BE rather than pre-existing BE. Using this model, one could see any or all of these changes and still be unaware that the intestinal metaplasia observed is neo-BE because an easier alternative (just as plausible) interpretation of the same morphological change would be an ulcer at the squamo-Barrett junction in which the restituting epithelium is arising from BE mucosa.

Figure 2. Restituting columnar mucosa at the edge of an erosion at the Z-line, and adjacent mucin-depleted columnar epithelium appears largely undifferentiated so it is impossible to know whether it will differentiate into foveolar type mucosa or a metaplastic mucosa with goblet cells (hematoxylin-eosin, original magnification ×400).

Figure 3. A, Typical multilayered epithelium with apical mucin droplets overlying an inflamed lamina propria (hematoxylin-eosin, original magnification ×200). B, Multilayered epithelium with an apparent goblet cell (center), suggesting that this mucosa has the potential to revert directly to a columnar mucosa with goblet cells (hematoxylin-eosin, original magnification ×200).

Figure 4. A, Typical incomplete intestinal metaplasia seen in both Barrett esophagus and in incomplete intestinal metaplasia in native cardiac mucosa (hematoxylin-eosin, original magnification ×400). B, Goblet cells the intensely stained, and intervening foveolar type columnar cells are less intensely stained (columnar blues) (alcian blue, pH 2.5, original magnification ×200).

Figure 5. Subsquamous Barrett mucosa. Biopsy from squamo-Barrett junction showing Barrett mucosa that is intestinalized with overlying squamous mucosa typical of changes seen in regressing Barrett esophagus. This type of mucosa is not a serious contender for the genesis of Barrett mucosa (hematoxylin-eosin, original magnification ×40).
METAPLASIA THROUGH MULTILAYERED EPITHELIUM

Multilayered epithelium (MLE) is found almost exclusively at the SCJ and has features of squamous mucosa basally and glandular mucosa superficially (Figure 3). MLE has been proposed as a possible intermediate step between squamous and glandular mucosa. Although it is tempting to speculate that this process occurs in the uterine cervix, in fact the reverse is true. In the cervix, the glandular mucosa undergoes squamous metaplasia by this mechanism, so that it has more in common with regression in BE that with MLE. This situation suggests that such morphological changes can progress in either direction, in which case one would expect MLE to be found in regressing epithelium. Although I observed this type of epithelium in BE after therapy with endoscopic regression, at the time I interpreted it as a more dynamic change with both focal progression and regression occurring together, which in retrospect may not have been correct.

In the esophagus, MLE is frequently accompanied by intense inflammation and is relatively common in biopsies from the region of the cardia. The mucin component is rich in acidic mucopolysaccharides, including sulfomucins, which are also frequent in BE, whereas the cytokeratin profile shares similarities with BE. Occasionally, what appear to be goblet cells can be seen in MLE (Figure 3, B). However MLE seems to be rare in long-segment BE, especially in patients on long-term proton pump inhibitors (personal observation), so that the driving force for its formation is likely inflamed mucosa. MLE also is sometimes found isolated as an island within columnar mucosa. Whether there is (or was) also squamous mucosa nearby is unclear.

For MLE to be the precursor to BE, one would expect it to be seen throughout the esophagus and not just in inflamed mucosa, where it is most frequently observed. However, both the inflammation and MLE itself may be very dynamic. Inflammation can progress and regress both spontaneously and with therapy, but the natural history of MLE is unknown. The hypothesis that MLE has a short half-life and either regresses spontaneously when the driving force is removed or progresses rapidly to columnar or Barrett mucosa is consistent with what is actually seen. MLE is not reported frequently, possibly because pathologists are not trained to look for it (akin to Helicobacter pylori prior to 1983). However, even when present, adjacent BE is distinctly uncommon, which does not negate the possibility of an association between MLE and BE but makes it difficult to interpret an observation as evidence of such an association.

The hypothesis of an association of inflammation around the Z-line, multilayered epithelium, and BE assumes progressive transformation. The problem with this hypothesis is that a gradual transition through MLE to BE has not been observed commonly histologically, although this lack of observation does not preclude its occurrence. Because we have no idea of the transforming potential of this epithelium to change from squamous to columnar, if the transformation happened in just a few days or weeks, only a small amount might be required to catalyze a major change in epithelial type (metaplasia). However, even if the transformation were relatively slow and took months, most patients have had their reflux disease for years, and there is no reason to presume that this pathway does not exist. Our failure to see MLE frequently may simply reflect the fact that in most BE patients fail to progress after diagnosis, which may indicate a fairly stable epithelium.

CREeping METAPLASIA WITH AND WITHOUT GOBLET CELLS

One of the major difficulties in the diagnosis of BE is the dependence on goblet cells for the diagnosis, which creates several problems.

1. Even in patients with established BE, perhaps more than 20% of the time multiple biopsies can on any one occasion fail to demonstrate goblet cells. We know very little about the dynamics of goblet cells and whether phenotypes within a limited area of BE can change with time. Goblet cells do seem to be more common proximally. When no goblet cells are found in a patient for whom multiple biopsies clearly indicate extensive columnar metaplasia throughout much of the esophagus, we identify the tissue in question as one of the other epithelial types seen in BE (Table 1), or revert to a diagnosis of columnar-lined esophagus, perhaps just mention that these other mucosal types are well described in BE, and check prior reports or biopsies to ensure that they really were present.

2. The diagnosis of BE is a combined endoscopic and histologic diagnosis. Goblet cells must be present in an endoscopic scenario consistent with BE (ie, not a Z-line that is within normal limits, which renders a diagnosis of intestinal metaplasia in native cardiac mucosa) (Figure 4, A). However, where a Z-line stops being irregular and becomes consistent with BE is a decision with a distinctly subjective component, so that in some patients even when goblet cells are present the diagnosis of short-segment BE is still in doubt.

3. Tongues of mucosa occur that are endoscopically indicative of short-segment BE, but multiple biopsies, sometimes on numerous occasions, can fail to reveal goblet cells. The morphological diagnostic options for diagnosis in this case are the same as those for problems 1 and 2.

4. When mucin stains are used to identify goblet cells, the results indicate that some of the foveolar cells do not produce only the neutral mucins expected but also may produce acidic mucins and so stain strongly with stains such as alcian blue (columnar blues) (Figure 4, B). These cells may represent the first stage in a process that results in some of them becoming goblet cells.

Although the mosaic nature of BE is well known, there are several potential mechanisms, either alone or in combination, that could explain both the initiation of BE, if it really does “creep,” and the mosaic that can occur. The first is a change in the extent of mucin-producing mucosa beginning at the Z-line, which is not detectable histologically unless it encroaches onto esophageal glands, although this mucosa can appear quite atrophic and lacking in mucous glands.

The second mechanism of BE is a change in the type of cells (eg, additional goblet cells or absorptive cells) and/or the accompanying potential changes in the mucin. Typically, the change is from neutral to acidic mucin in the foveolar mucin-producing cells, which appear as “columnar blues” after staining with alcian blue at pH 2.5 to 2.8 (Figure 4, B). Why these cells are produced, whether this change is reversible, and whether this process is really a precursor of BE is not entirely clear. However, the change occurs frequently; in one study acid mucin stains were
positive at the esophageal-gastric junction in 69.6% and in the cardia in 38.7% of patients with no evidence of BE.\textsuperscript{29} However, intestinal enzymes can be detected in these areas using sucrase isomaltase, dipeptidilpeptidase IV, and the intestinal transcription marker CDX2, suggesting that they already have at least a partial intestinal phenotype.\textsuperscript{30,31} The addition of goblet cells to a foveolar mucin type may produces the change recognized as incomplete intestinal metaplasia. Mucosa of this type (as well as full BE, including neoplasms) can be found following total gastrectomy and can precede the development of BE in the residual mucosa.

The third possibility (creeping mucous/columnar metaplasia) for BE development involves observing squamous mucosa, columnar mucosa, and metaplastic mucosa together and ideally sequentially. It is common to see these epithelial types together, especially in biopsies from around the cardia where intestinal metaplasia is frequently seen. This combination of mucosas is found in unremarkable cardia and extensively in BE, so it is a good candidate for an observable transitional mucosa for BE. In this respect, this mechanism is similar to the preceding model but dispenses with the need for an intermediate stage through MLE. The relative inability to think of these mucosal types dynamically may hinder our ability to consider them as part of the genesis of BE rather than as stable types. This problem of being closed minded applies to all of the hypotheses suggested here as potential pathways in the genesis of BE. A summary of these potential pathways is given in Table 2.

**Table 2. Potential Mechanisms of the Genesis of Barrett Esophagus (BE)**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
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<tbody>
<tr>
<td>Squamous mucosa $\rightarrow$ ulceration $\rightarrow$ restitution $\rightarrow$ BE or mucosa with an increased risk of BE</td>
<td></td>
</tr>
<tr>
<td>Squamous mucosa $\rightarrow$ inflammation $\rightarrow$ multilayered epithelium $\rightarrow$ ?BE</td>
<td></td>
</tr>
<tr>
<td>Squamous mucosa $\rightarrow$ creeping metaplasia with columnar mucosa $\rightarrow$ columnar metaplasia $\rightarrow$ Acid mucin $\rightarrow$ ?BE</td>
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cosa by squamous mucosa. Frequently, the subsquamous BE seems to revert to a complete intestinal metaplasia type, which may have less carcinogenic potential than incomplete intestinal metaplasia. If no dysplasia (or changes indefinite for dysplasia) or other major cell cycle disruption has occurred, the subsquamous BE may have little or no malignant potential, but the converse is also true. Nevertheless, if the 3 potential mechanisms described here can be invoked in the genesis of BE, then they can also be invoked in its regression. As in BE genesis, what one sees depends to a large extent on what one is looking for or is trained to look for.

**References**


Dysplasia in the gastrointestinal tract is considered both a carcinoma precursor and a marker of high cancer risk for the site at which it is found. Dysplasia is defined as unequivocally neoplastic epithelium, yet the specific criteria for making that determination are imperfectly defined. The current criteria actually include a mix of architectural and cytologic features, all of which occur in different intensities in different epithelia that are given the same diagnosis. Gastrointestinal dysplasias are divided into 2 grades, but there are problem areas in diagnosis at the lower end where low-grade dysplasias overlap with regenerating epithelia and in the middle where low- and high-grade dysplasias overlap. The diagnosis of dysplasia is too subjective with less than optimal reproducibility to be as useful a marker as needed. Pathologists need a dysplasia stain or a whole set of new markers of high cancer risk, presumably molecular and/or genetic, that are not dependent on pathologists’ diagnoses of dysplasia and their inherent subjectivity.

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What Is Dysplasia in the Gastrointestinal Tract?

Henry D. Appelman, MD

We have identified several models in the gastrointestinal tract in which there are postulated intermediates between normal mucosa and invasive adenocarcinoma. The most common of these models is typical or ordinary colorectal carcinoma in which the intermediate stage was given the name adenoma. Other such models are adenocarcinomas that arise in the esophagus in Barrett mucosa, in the stomach in atrophic gastritis, and in the colon and rectum in chronic inflammatory bowel diseases, mainly ulcerative colitis. In these cancer situations, the intermediate stages, which look very much like histologic adenomas, are not called adenoma but instead are called dysplasias. Why are there 2 different names for such histologically similar adenocarcinoma intermediates, and just what is this dysplasia thing anyway?

The answer to the first question is the easiest. The term adenoma had been used for benign nonsquamous epithelial neoplasms, such as those with adnexal differentiation in the skin and those in the salivary gland, the most prominent of which is the pleomorphic adenoma. These tumors are composed of mature epithelium, and the risk of cancer occurring within them is minute. It was no wonder that the same name, adenoma, was used for polypoid epithelial proliferations, mainly in the colon, that were considered neoplastic but not malignant. However, it was not known then that the things called adenomas in noncolonic sites were composed of mature epithelium, whereas the epithelium in the colonic lesions of the same name was mostly immature or at least less mature. In addition, at the time of this naming, the concept of dysplasia as a cancer precursor was not well established, and the fact that the epithelium contained in those colonic adenomas was the common colonic cancer precursor and identical to what we now call dysplasia was not appreciated. Adenomas of the gut are dysplasias and should be called by the dysplasia name. However, the term adenoma for noninvasive gut neoplasms is so much a part of our jargon that it will take an act of Congress or a natural catastrophe to get rid of it.

The answer to the second question, just what is this dysplasia thing, is more complex and is the major subject of this discussion. To be perfectly honest, the answer is elusive and may not even be obtainable. Certainly, many people have tried to tell us what dysplasia is; there is a huge body of literature devoted to discussing and describing dysplasia in various parts of the gut, and every textbook of gastrointestinal pathology devotes plenty of space to it. There are so many references to dysplasia, that I have limited the reference list here to only a few examples. Dysplasia was defined as unequivocally neoplastic epithelium by a group of supposedly experienced gastrointestinal pathologists who were studying a large number of epithelial changes associated with chronic colitis, mostly ulcerative colitis. The results of that study, copiously illustrated, were published in 1983; by now, it is old stuff. The definition they provided sounds fabulous, but exactly how do we know what is unequivocally neoplastic? This decision is made by pathologists. Those supposedly experienced gastrointestinal pathologists never said who was responsible for making the critical decisions in specific cases as to what changes constituted unequivocally neoplastic epithelium. How did those or any other pathologists learn how to make that decision? They were able to make the decision because of several factors. The most important factor is their training. Perhaps someone older and wiser taught them the truth during their residencies, and maybe they took postgraduate courses from alleged experts or sent cases to such experts. After all, the reason we have experts is to teach us stuff, to give courses,
Dysplasia in the Gastrointestinal Tract—Appelman

Dysplasia is said to be a cancer precursor, but how do we know that? We believe that it is a cancer precursor because we find invasive carcinomas in fields of dysplasia, mainly the high-grade type. Dysplasia is also a marker of high cancer risk, but how do we know that? We have lots of data indicating that once dysplasia develops, the likelihood of cancer occurring in that site in greatly increased over that for normal tissue. In addition, when we identify dysplasia, we find cancers not just at the sites of dysplasia but elsewhere in the same organ. There is a widespread belief that carcinomas throughout the body are the end of a sequence that begins with native epithelium altered by a combination of genetic and environmental factors. We preach that this in turn leads to the development of first low-grade dysplasia, which then gives rise to high-grade dysplasia and finally to invasive carcinoma. Perhaps much of this supposition is based on the fact that epithelia diagnosed as low-grade dysplasia at a particular site are less likely to be accompanied by invasive carcinoma than are epithelia diagnosed as high grade. Furthermore, when low-grade dysplasia is found, the future risk of developing invasive carcinoma at the specific site is less than that for high-grade dysplasia, and it takes longer for carcinomas to develop. However, there is virtually no information on what happens over time to a specific focus of low-grade or high-grade dysplasia. Thus, dysplasias are definitely risk indicators for carcinoma, but there is little proof that they are precursors.

Pathologists recognize many histologic alterations that are considered dysplastic changes. In the figures presented here, Barrett mucosa is used as a model. The alterations are both architectural and cytologic. The architectural changes involve differences from normal structure, such as clustering and crowding of tubules, an exaggerated villous surface pattern, or the appearance of bridges of cells that cross lumens. This last feature results in the classic cribiform pattern of growth, a form of architectural complexity. Other architectural changes involve the relation of the cells and their nuclei to other cells and are recognized as nuclear and cellular stratification (Figures 1 through 4). The cytologic features are more complex and involved both cytoplasm and nuclei. The cytoplasmic features mostly involve loss of maturation or specialization, such as decrease in or loss of mucus content. The nuclear changes include enlargement with increase in the ratio of nucleus to cytoplasm, pleomorphism (ie, variation in size and shape), hyperchromatism (ie, increase in nucleic acid content causing the nuclei to stain more darkly than normal), and increase in the number of mitotic figures and unusual or strange looking mitoses (Figures 1 through 8). These histologic changes are accompanied by numerous genetic variations, but at the moment, identification of such changes is not useful in routine diagnosis.

Dysplasias can be separated into as many histologic grades or subsets as pathologists are able to recognize. As an example, the squamous dysplasias in the uterine cervix, also known as cervical intraepithelial neoplasia, were divided into 3 grades. Gastrointestinal dysplasias involving columnar mucosa, including the metaplastic columnar mucosa of Barrett mucosa in the esophagus, have been divided into 2 grades, low and high, each of which leads to specific clinical responses for management of the patient. Thus, in the gastrointestinal tract dysplasia nomenclature, there is no such thing as mild, moderate, or severe dysplasia. The clinicians know this, and the pathologists must comply with this rigid nomenclature. To complicate things, these mucosae are subject to many punishments, which induce inflammation, epithelial damage, and concurrent regeneration. Regenerating epithelium shares some characteristics with dysplastic epithelium, including loss of maturation and increased proliferation, which means that regenerative and dysplastic epithelia can look very much alike microscopically. The difference between them is physiologic. Regenerating epithelium undergoes maturation and proliferation is reduced once it has accomplished its goal of repairing the damage. Dysplastic epi-
Figure 1. Dysplastic mucosa in which the tubules are more crowded toward the surface than at the base, and there is great variation in size and shape of the tubules (hematoxylin-eosin, original magnification ×100).

Figure 2. There is little difference in location of the crowded tubules, but they are even more architecturally complex (hematoxylin-eosin, original magnification ×100).

Figure 3. Villous architecture with covering epithelium that has cytoplasmic maturation, as indicated by apical mucin. The nuclei are hyperchromatic but not pleomorphic. The nuclear stratification is in the basal half to two thirds of the cells, except for a few foci where it approaches full thickness (hematoxylin-eosin, original magnification ×200).

Figure 4. Villous architecture with covering epithelium that has only focal cytoplasmic maturation. The nuclei are hyperchromatic as in Figure 3, but the stratification is almost all full thickness. However, there is little stratification (hematoxylin-eosin, original magnification ×200).

Figure 5. Complex tubular architecture with lining epithelium that has almost no cytoplasmic maturation. The nuclei are stratified full thickness, hyperchromatic, and pleomorphic (hematoxylin-eosin, original magnification ×200).

Figure 6. Variously complex tubular architecture with virtually no cytoplasmic maturation. There is also high variability in degree of nuclear stratification and hyperchromatism. Compare the highly stratified, uniform nuclei in the tubule at the lower left with the much less stratified but more pleomorphic and vesicular nuclei in the tubule at the right edge of the field (hematoxylin-eosin, original magnification ×200).

Figure 7. Tubules on the left and right sides have a similar shape, but the lining epithelia are different. The epithelium in the left tubule has more apical mucus and more pleomorphic, more vesicular, less hyperchromatic, and less stratified nuclei, whereas in the right tubule there is no apical mucus, and the nuclei are more uniform, more hyperchromatic, and more stratified (hematoxylin-eosin, original magnification ×200).

Figure 8. High variability in this single field of apical cytoplasmic mucin, vesicular nuclei, pleomorphism, hyperchromatism, and stratification. Two tubules with apical mucus in all the cells are not dysplastic, but are all the other cells dysplastic or are some of them regenerative, such as the syncytial epithelium with vesicular nuclei in the middle of the field (hematoxylin-eosin, original magnification ×200).

Thelium, in contrast, never matures. This overlap in histologic features between regenerating and dysplastic epithelium was recognized and led to the concept of epithelium that defied classification as to whether it was regenerative or dysplastic; such epithelium was named **indefinite for dysplasia**, one of the most honest inventions in histopathology. However, it is impossible to define this entity because, as the name indicates, it has no definition, and it is equally impossible to illustrate and teach it, because the diagnosis of indefinite for dysplasia lacks consistency. When I photograph some epithelium I think is indefinite for dysplasia and then look at the photographs
a few days later, that epithelium now looks either negative or low grade.

These cytologic and architectural changes do not all occur at the same rate in all epithelia. When a pathologist decides whether an epithelium is dysplastic and the grade of dysplasia, the decision is made not on individual features but on the aggregate of features. Therefore, not all low-grade dysplasias have the same changes, and the same is true for all high-grade dysplasias. Low-grade dysplasia, in simplest terms, has a greater degree of change than is acceptable for either normal or regenerative epithelium, but the changes are less intense than those associated with high-grade dysplasia. Changes associated with high-grade dysplasia are more intense than those associated with low-grade dysplasia. Because all low-grade and high-grade dysplasias contain epithelia with changes of variable intensity, the epithelia in both types are not homogeneous, but each type contains a group of different epithelia that have been given the same names.

There are some consequences inherent in this set of conclusions. First, because regenerating and low-grade dysplastic epithelia have so much in common, because the category of indefinite for dysplasia recognizes this overlap, and because there are so many histologic criteria involved in the diagnoses of these changes, the reproducibility for diagnosing regenerative, indefinite, and low-grade dysplastic epithelia is poor. In a number of studies of both colitis and Barrett mucosa, this hypothesis has been supported.11–14 However, because the changes increase in intensity as the grade of dysplasia increases, including the appearance of many features common to carcinomas, there is better reproducibility in the diagnosis of high-grade dysplasia. Second, because only 2 grades of dysplasia are recognized and each grade is a collection of epithelia with changes occurring at different rates in different epithelia of the same diagnosis, then there must be some overlap between the collection called low grade and that called high grade, and the distinction is not always going to be easy. This situation is exactly what occurs in practice. Some epithelia seem to have a mixture of low- and high-grade features. Unfortunately, the classification of dysplasia into 2 grades (low and high) does not allow for these mixed features, so we try to put such epithelia into one grade or the other.

To complicate this issue even more, when we read any article that deals with dysplasia of any grade, we must recognize that these dysplasias are not single entities but rather groups of entities, all of which have been given the same diagnosis. What the pathologist authors of one study have diagnosed as low- or high-grade dysplasia, regardless of the experience and/or reputation of those pathologists, will not necessarily be diagnosed the same way by the pathologist readers. Furthermore, pathologist authors of different studies of the same diagnostic category are not always talking about the same epithelia. Therefore, all articles dealing with dysplasia of any grade are not necessarily discussing the same entities, and such studies cannot be considered comparable. Thus, meta-analyses in this area may not be useful.

Now we can finally complete the definition of dysplasia. It is unequivocally neoplastic epithelium, an invention of pathologists, the diagnosis of which depends on a group of histologic features that vary in intensity from one case to the next and are therefore not as reproducible as we would like. The diagnosis of dysplasia, especially low grade, is just plain hard to make! In real life daily diagnostic pathology practice, how do we decide what is low-grade dysplasia? We evaluate all the architectural and cytologic features and decided whether they are intense enough for the low-grade diagnosis. Sometimes, we consult other people to see whether they agree. How do we diagnose high grade? Again, we try to determine whether those same architectural and cytologic features look worse than those seen in cases of low-grade dysplasia. Sometimes we consult other people to see whether they agree. If the diagnosis of dysplasia is fraught with so much subjectivity and so much inconsistency, isn't there some way to alleviate the difficulties and inconsistencies? The best solution would be for us to have a dysplasia stain or a specific dysplasia antibody, but these are not available. What we are really looking for is some definable, consistent, objective change that informs us that the risk of both concurrent and future carcinoma is high enough for some kind of clinical intervention. This is what we hoped dysplasia would be, but the inconsistencies get in our way. So we need a substitute, a surrogate for dysplasia. This substitute cannot be judged against our current diagnoses of dysplasia, or it will suffer from the same lack of objectivity. This substitute must be judged strictly by clinical outcome, and it will probably be molecular and/or genetic, incorporating reproducible measurements that are not subject to individual interpretation. Meanwhile, because we do not have such markers, we must plod along, doing the best we can, using old-fashioned histologic interpretation, and in the process we hope that we do not do too much harm to the patients.

References
Is There a Way for Pathologists to Decrease Interobserver Variability in the Diagnosis of Dysplasia?

Elizabeth Montgomery, MD

Many obstacles interfere with our efforts to screen patients with Barrett esophagus. Probably the largest is choosing the appropriate patient group for screening. Beyond this problem, sampling error on the part of endoscopists is probably more serious a problem than observer variation among pathologists reviewing patient samples. Pathologists agree well on lesions that merit close follow-up or other intervention (high-grade dysplasia and invasive carcinoma), although interobserver agreement between pathologists interpreting lesser lesions is not good. This lack of agreement is not likely to improve substantially, and many adjunct markers are being sought in an attempt to identify patients with lesions of lower grades that are most likely to progress, allowing doctors to identify patients who would benefit from upgraded surveillance.

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About 20 years ago, criteria for grading dysplasia in ulcerative colitis were developed by a group of observers. Cases were categorized as negative for dysplasia or as low-grade or high-grade dysplasia. However, these observers also noted that there was a subset of cases in which it was unclear whether the epithelial changes were truly neoplastic or simply the result of ongoing repair. Such cases tended to display abundant inflammation and were classified as indeterminate for dysplasia. When criteria were subsequently published for grading dysplasia in Barrett esophagus, the indeterminate category was conceptually retained but the preferred term has become indefinite. As such, currently Barrett esophagus cases are classified as negative for dysplasia; indefinite for dysplasia; dysplasia, low-grade; or dysplasia, high-grade.

To assess criteria for grading dysplasia in Barrett esophagus in 1988, 71 test cases were circulated twice to 10 observers, and percent agreement was calculated. There was about 60% agreement in separating cases interpreted as negative for dysplasia versus indefinite and low-grade dysplasia versus high-grade dysplasia and invasive carcinoma. The authors indicated that observer variation was a significant problem at the low end of the spectrum and suggested that such problems would be resolved by newer more objective techniques emerging at the time.

Using the criteria published in 1988 as a basis for our own review, my colleagues and I circulated 2 sets of 125 slides twice each to 12 observers. Between circulation of the 2 sets of slides, a consensus criteria meeting was held. With the benefit of previously published criteria, we were able to attain about 75% agreement in separating the same categories (negative vs indefinite and low grade vs high grade and cancer), but we continued to have difficulty with observer agreement at the lower end of the diagnostic spectrum. Our improvement after the consensus meeting was modest, and some observers had poorer agreement after the meeting. In evaluating the cases, we used \( \kappa \) statistics and percent agreement.

\( \kappa \) Statistics were initially developed to assess observations in psychiatric studies, which were believed to have the potential for subjectivity and for which there was a concern that any observed agreement might be accounted for by chance alone. \( \kappa \) Statistics were developed to correct for observer agreement due to chance alone. \( \kappa \) scores are quite unforgiving; a negative score can be attained. \( \kappa \) Scores range from negative values up to 1. Verbal scales have thus been developed together with the calculated numerical ones: poor, from any negative value to 0; slight, 0 to 0.2; fair, 0.2 to 0.4; moderate, 0.4 to 0.6; substantial, 0.6 to 0.8; and almost perfect, 0.8 to 1.0.

In our study, when \( \kappa \) scores were calculated by diagnostic category, our scores were 0.65 (substantial) for high-grade dysplasia/carcinoma and 0.58 (moderate to substantial) for Barrett esophagus without dysplasia, but the scores were 0.32 (fair) and 0.15 (slight) for low-grade dysplasia and indefinite for dysplasia, respectively. How do we obtain better \( \kappa \) scores?

Increases in \( \kappa \) scores probably are best accomplished by having fewer categories of readily separable entities, which is unrealistic for highly variable lesions such as Barrett esophagus dysplasia. For example, Cross et al. reported \( \kappa \) scores of 0.84 to 0.98 in separating hyperplastic and adenomatous rectal polyps. However, they noted that one inexperienced observer in their study had a \( \kappa \) score of 0.46, which improved after a tutorial. A similar study in which observers were asked to separate serrated adenomas, sessile serrated polyps, hyperplastic polyps, and tubular adenomas probably would yield considerably low-

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Figure 1. Barrett esophagus, negative for dysplasia. Surface maturation is present, and there are minimal nuclear alterations confined to the base of the glands (hematoxylin-eosin, original magnification ×40).

Figure 2. Case interpreted as indefinite for dysplasia. There is nuclear hyperchromasia focally extending onto the surface epithelium, but the surface nuclei are smaller than those beneath and appear degenerative, casting doubt as to whether this is a truly neoplastic process (hematoxylin-eosin, original magnification ×20).

Figure 3. Case interpreted as indefinite for dysplasia. Hyperchromatic stratified nuclei are present in deep glands extending onto the surface, but the process is accompanied by active chronic inflammation (hematoxylin-eosin, original magnification ×20).

Figure 4. Low-grade dysplasia. The lesion lacks surface maturation, and there is no inflammation (hematoxylin-eosin, original magnification ×20).

Figure 5. High-grade dysplasia. Nuclear polarity is lost, there is no surface maturation, and nuclear alterations are prominent (hematoxylin-eosin, original magnification ×40).

Figure 6. Classification of hypermucinous lesions is unclear. These lesions may share molecular features with low-grade dysplasia (hematoxylin-eosin, original magnification ×40).
In grading Barrett dysplasia, we developed an algorithm in which 4 overall categories were assessed: surface maturation, low-magnification architecture, cytologic features, and inflammation. Lesions classified as negative for dysplasia display surface maturation, ample lamina propria compared with glands, bland cytologic features, and typically little inflammation (Figure 1). Those lesions regarded as indefinite for dysplasia retain their architectural ratio of glands to lamina propria, have surface maturation (Figure 2), have nuclear alterations that are not particularly prominent, and tend to be complicated by inflammation (Figure 3). Low-grade dysplasia cases lose surface maturation but retain nuclear polarity (ie, the long axes of the nuclei remain perpendicular to the basement membrane in the pencillate fashion of tubular adenomas), displaying nuclear alterations, have minimal glandular crowding, and typically lack inflammation (Figure 4). In high-grade dysplasia, surface maturation is lost, glands become crowded (overrunning the lamina propria), nuclear alterations become striking (Figure 5), and abundant inflammation is not typical (but can be observed).

Outcome in cases graded using these categories is correlated closely with dysplasia grade, and at this point there is no better esophageal adenocarcinoma marker than high-grade dysplasia. In general, up to about 20% of patients with low-grade dysplasia and about 60% of those with high-grade dysplasia progress to invasive carcinoma, although these figures vary with the adequacy of screening and the prevailing trends for grading the dysplasia in any given institution. Despite advances in ancillary objective techniques, these techniques are not ready for real-time clinical use in all patients. However, several observers have found p53 protein quantification helpful as an adjunct to morphologic assessment in both improving observer reproducibility in assessment of low-grade dysplasia and in identifying which patients with low-grade dysplasia are likely to progress. In one laboratory, flow cytometry was useful in choosing which patients without high-grade dysplasia might benefit from more aggressive surveillance, but these data have not been reproduced by others.

Pathologists remain uncertain how to classify certain lesions, such as that depicted in Figure 6. Classification also tends to be institution specific, depending on the standard practice of clinical colleagues in the practice settings. For example, at our hospital, we diagnose focal high-grade dysplasia in cases in which striking nuclear alterations are found in only a small focus, with the knowledge that this interpretation will prompt aggressive surveillance and/or protocol treatments rather than esophagectomy.

Observer variation in diagnosing Barrett dysplasia–related lesions is not likely to improve, but this problem is not of great clinical import because pathologists perform extremely well when diagnosing lesions of the highest and lowest risk, and those in the intermediate categories are in the minority. Greater problems, such as identifying which patients will best benefit from careful screening and sampling, belong to the realm of our colleagues performing endoscopy.

References
Do 40% of Patients Resected for Barrett Esophagus With High-Grade Dysplasia Have Unsuspected Adenocarcinoma?

Elizabeth R. Tschanz, MD

Results of studies conducted in the last 2 decades suggest that the detection of high-grade dysplasia in patients with Barrett esophagus is the harbinger of a synchronous adenocarcinoma, which remains undetected even by rigorous biopsy protocols but is discovered during resection of the esophagus. The reported prevalence of synchronous carcinomas ranges from 0% to 75%. Other researchers maintain that appropriate surveillance programs can be used to detect carcinomas at a curable stage and to prevent unnecessary esophagectomies. Both logistical difficulties and potential methodological pitfalls have plagued many studies designed to investigate this issue. A large multicenter study that would stratify participants for hitherto unexplored variables (eg, age, gender, and ethnic background) may be required before the 40% occult cancer prevalence can be either confirmed or refuted. However, the large scale needed for such a study to provide reliable data and new developments in endoscopic imaging (eg, magnification endoscopy and optical coherence tomography) and endoscopic therapy (eg, mucosectomy) are likely to make such a study both ethically unacceptable and logistically and financially unfeasible. Future research should utilize the combination of new endoscopic technologies with the continuing search for validated biomarkers that help predict the biological behavior of Barrett epithelium in individual patients, with a particular focus on the possible development of preneoplastic and neoplastic lesions. Pathologists who chose to shift their focus from the traditional morphological investigation of dysplasia to the search for usable biomarkers can position themselves at the center of innovative research projects that could radically modify the management of patients with Barrett esophagus.

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Results of several studies conducted in the last 2 decades suggest that the detection of high-grade dysplasia in patients with Barrett esophagus is the harbinger of a synchronous adenocarcinoma. According to such reports, these carcinomas, variously defined as occult, unexpected, or unsuspected, remain undetected even by the most rigorous biopsy protocols, only to be discovered when the esophagus is resected and thoroughly sampled. The reported prevalence of such tumors, which I prefer to call synchronous, previously undetected carcinomas rather than unexpected, ranges from 0% to 75%.

Other researchers, based on the careful initial endoscopic and biotopic screening of Barrett patients, maintain that an appropriate surveillance program will detect carcinomas at an early and still curable stage, thus preventing unwarranted esophagectomies.

NO EVIDENCE IN THE LITERATURE

In the presence of conflicting data, particularly when the evidence is provided by numerous studies that each involve a small number of cases, meta-analysis is now considered the approach of choice. By selecting published articles according to rigorous pre-established criteria, usable data are sifted from the unusable, and a reasonable evidence-based answer is ultimately extracted.

Attempts to apply meta-analytic methods to evaluate the literature concerning the simultaneous presence of high-grade dysplasia and adenocarcinoma in cases of Barrett esophagus would likely prove futile; the number of published cases found in resection-based studies is just more than 300, with more than half of the series consisting of fewer than 20 cases. The reported biopsy protocols that led to the resection are widely different, the methods used to examine the resected specimens are often incompletely described, and the endpoint (invasive vs superficial or intramucosal carcinoma) is not always clearly stated. Only a handful of the reports provide sociodemographic, ethnic, and medical information that would allow the stratification of patients in categories with potentially different progression rates.

The study that could address the issue of the 40% occult cancer prevalence has not yet been carried out, and a constellation of methodological, ethical, and logistical issues and new developments in interventional endoscopy will probably prevent it from ever being performed.
THE STUDY THAT NEVER WILL BE

Two methodological approaches can be used to determine the prevalence of synchronous carcinoma in patients with high-grade dysplasia in Barrett mucosa: the biopsy-based and the resection-based study. The former includes an initial extensively mapped biopsy protocol to characterize each patient's Barrett mucosa as accurately as possible at enrollment. Participants are then followed by endoscopy and systematic biopsies at intervals whose length may be adjusted depending on the presence of lesions of increasing neoplastic potential. The detection of high-grade dysplasia may lead to more intense surveillance and more aggressive biopsy protocols, but esophagectomy is performed only when carcinoma is detected. Resection-based studies have similar foundations but differ in the indication for esophagectomy, which in this case is triggered by the finding of high-grade dysplasia.

The prudent attitude of biopsy-based studies may benefit some patients by avoiding unnecessary esophagectomies, but resection-based studies are the only accurate way to determine the true prevalence of adenocarcinomas synchronous with high-grade dysplasia. The ideal study should, therefore, follow the resection-based model.

The detection of the dysplasia that prompts esophageal resection relies by necessity on the histopathologic examination of biopsy specimens obtained through extensive biopsy mappings. Even under the best currently available circumstances, however, this method is far from perfect. The accuracy of jumbo esophageal biopsies has been recently calculated by Boyce. The area of a 2-cm segment of esophageal mucosa is estimated to be approximately 14 cm²; a single jumbo biopsy samples 0.125 cm². Thus, a 4-quadrant 2-cm protocol (eg, the Seattle protocol) will yield 0.5 cm² of mucosa, equivalent to a sample of only 3.5% of the surface of that segment (Figure 1). Cameron and Carpenter found the total surface of Barrett mucosa in 9 resected patients to be around 32 cm², of which a little more than 1 cm² consisted of high-grade dysplasia and adenocarcinoma, respectively. Because the Barrett area usually consists of a distal segment of completely metaplastic mucosa and more proximal tonguelike projections mixed with native squamous epithelium, 3 to 4 2-cm segments should be biopsied to map the entire metaplastic surface. This protocol would consist of 12 or 16 biopsy specimens, with a total surface of 1.5 to 2 cm² of surface mucosa available for microscopic examination or 5% of the estimated average extension of the Barrett epithelium. The chances of biopsying an endoscopically undetectable focus of dysplasia would be less than 1 in 3, and if a modified protocol were to be used (with 4-quadrant biopsies for every 1-cm segment), the chances would increase to 2 in 3, a sensitivity still far below the level acceptable for any medical test.

Having detected one or more foci of high-grade dysplasia (and none of invasive carcinoma, because in that case the patient could not be included in the occult cancer study), an esophageal resection is performed. The resected specimen would have to be examined systematically in its entirety, according to a system similar to the one illustrated in Figure 2. Fixation, sampling, and embedding of the blocks could be standardized and performed at each participating site, as could the diagnostic work. However, a predetermined number of serial sections from each block should be sent to a central pathology laboratory, where uniform staining (including special and immunohistochemical stains as needed) should be performed. All specimens should then be examined by the same group of experienced pathologists, who should apply rigorous criteria for dysplasia and carcinoma and avoid interobserver variation by reading all slides together and generating a consensus report for each specimen.

How many resection specimens should be examined to generate usable results? If the working hypothesis is that simultaneous cancer is present in 40% of specimens re-
have to be studied. Because more than 96% of patients with a synchronous carcinoma is at stake. The inclusion of 200 patients would improve the 95% confidence limits to 33.3% to 46.9%. If the actual percentage of patients with Barrett esophagus under surveillance never progress to carcinoma, even the largest specialized centers can rarely enroll more than a few patients a year into this type of study, and to be completed in a reasonable period of time the study should be multicentric. This type of design carries a number of variables, including the recruitment biases, the attitudes and skills of the endoscopists and surgeons, and the characteristics of the participants. The population studied is crucial to the interpretation and even more to subsequent utilization of the results. If all patients were, for example, white men between the ages of 55 and 65 years previously treated with proton pump inhibitors, no inference could be made regarding the behavior of Barrett dysplasia and carcinoma in men of African origin, in women, in older patients, in nonmedicated individuals, or in any other group different from the study population. Thus, several relevant groups of participants, stratified according to race, age, gender, and medication history, should be evaluated before data can be applied to a wide heterogeneous population.

IN VIVO MICROSCOPY?

The preceding description of a hypothetical study is based on the use of traditional endoscopic methods. It assumes that the visualization of subtle mucosal lesions in the Barrett mucosa is not reliably achieved and that blind mapping protocols must be used. In the last few years, however, 2 endoscopic techniques have emerged that might change our approach to the detection of gastrointestinal mucosal lesions. Magnification endoscopy, tested mostly in Japan for a decade, is now becoming increasingly available to gastroenterologists worldwide. Endoscopes that provide optical magnification up to ×100 and digital enhancements up to ×200 have become relatively simple to operate and are now being marketed at affordable prices. Combined with the uncomplicated instillation of 1.5% acetic acid or various dyes, an expert operator using magnification endoscopy can detect lesions otherwise invisible; thus, targeted biopsy specimens can be obtained from the lesions rather than from predetermined criteria. Results of recent studies in Barrett esophagus patients suggest that this technique enhances the ability to detect small or indistinguishable remnant islands of columnar epithelium and allows the detection of even microscopic foci of high-grade dysplasia and carcinoma.

Another technique that might do for endoscopy what the computerized axial tomography has done for radiology is optical coherence tomography (OCT). Initially developed for eye imaging, OCT is analogous to ultrasound, but it measures the echo time delay and magnitude of light rather than sound and provides cross-sectional images of structures below the tissue surface in real time. The images are generated in black and white or in computer-enhanced false colors and have such a resemblance to histopathologic sections that enthusiasts frequently refer to this technique as in vivo microscopy. Standard-resolution OCT can achieve axial resolutions of 10 to 15 μm. Recently, using state-of-the-art lasers as light sources, ultrahigh-resolution imaging with axial resolutions as fine as 1 to 2 μm have been obtained. Although still at an investigational stage, OCT may one day become the state of the art in the endoscopy suite. Even if it is unlikely to replace mucosal biopsy, as some of its most enthusiastic supporters suggest, OCT may well become an important tool for the in vivo detection of microscopic lesions, which would allow endoscopist’s to obtain targeted samples and increase the biopsy’s diagnostic yield.

INTERVENTIONAL ENDOSCOPY

Even before the startling technological improvements for image acquisition were introduced, endoscopists had developed techniques that allow accurate surgical manipulation of the gastrointestinal mucosa by means of devices (eg, wire loops, laser beams, and microstiches) passed and operated through the endoscope. The earliest of these techniques, polypectomy, showed for the first time that a tumor could be completely and safely removed without open surgery. Furthermore, specimens of satisfactory quality could be obtained for histopathologic diagnosis and evaluation of the resection margins. Now it is possible to lift mucosal lesions by submucosal injection of saline...
line and removal of the desired portion of mucosa in one or more fragments, which can be marked to provide pathologists with orientation clues. The specimens, reconstructed like a puzzle, can then be evaluated for margins as accurately as a surgically obtained resection.24 Mucosectomy, as this technique is called, can be safely performed in many patients who could never endure an esophagectomy because of their poor surgical risk.25 Once magnification endoscopy and OCT are perfected and widely used, mucosectomy, which is also evolving in accuracy, safety, and the development of new possibilities, will undoubtedly become the standard of care for the treatment of many early neoplastic lesions of the esophagus.26,27

THE PATHOLOGIST'S OUTLOOK

The 40% figure proposed in the title question represents the mode of the available resection-based study results. Before interventional endoscopy became available, one might have adopted this figure to formulate provisional risk-assessment guidelines for the management of Barrett esophagus patients in whom high-grade dysplasia was detected. I say “provisional” because of the final study likely to settle the question could have been expected to be forthcoming. Realistically, such a study will never take place. The costs associated with such large long-term observational studies are enormous, and funding agencies do not look at them favorably. When high-resolution endoscopy becomes widely available, tested, and established and increasing numbers of patients can benefit from the early detection of small lesions amenable to endoscopic removal, there will be little justification for carrying out blind biopsy protocols and even less for the performance of questionable esophagectomies prompted only by the statistical probability of coexistence with an invisible tumor.

Initially, pathologists faced with poorly oriented irregular fragments of endoscopically aspirated mucosa will feel as if they were receiving only the crumbs of the real surgical specimens. However, as techniques evolve, larger fragments of mucosa will be extracted intact, and the accurate reconstruction and orientation of specimens that still need piecemeal removal will be made possible by a combination of endoscopic imaging and specimen markings. Already experienced in the use of such techniques, Japanese pathologists achieve accurate evaluations of both lateral and deep resection margins of most mucosectomy specimens, as illustrated in many meticulously detailed figures that appear regularly in Japanese journals.

Another area in which pathologists can make important contributions is the study of potential biomarkers for Barrett esophagus and its potential progression to cancer. The histologic changes leading to adenocarcinoma are accompanied (and most likely preceded) by alterations at the molecular level, including the accumulation of gene mutations and changes in gene expression.28 The ultimate goal is to identify and validate biomarkers that may eventually serve as surrogate endpoints in clinical studies. None of the many biomarkers proposed and investigated to date has been evaluated in long-term prospective clinical studies.29 Initially, potential biomarkers are evaluated using a variety of laboratory tools that allow the detection of subtle alterations of the cell-cycle clock apparatus and the activation, inactivation, and loss of genes. Eventually, simple techniques such as immunohistochemistry can be developed to reliably detect most of these changes. At that point, pathologists may be able to make the transition from debating the reliability of a diagnosis of dysplasia to assessing objectively the presence of molecular alterations with a validated predictive value.

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References

What Is the Role of Cytokeratins in Barrett/Cardia Differentiation?

Mamoun Younes, MD

The importance of distinguishing between Barrett metaplasia and intestinal metaplasia of the gastric cardia is now accepted, and the management of each entity is quite different. Patients with Barrett metaplasia are enrolled in surveillance programs, consisting of periodic endoscopy and biopsy, because of the known risk of developing adenocarcinoma of the esophagus. Patients with intestinal metaplasia of the gastric cardia, however, are not currently enrolled in such programs, because this condition carries a low risk of developing adenocarcinoma of the gastric cardia. The distinction between both conditions by morphologic examination of routine histologic sections of endoscopic biopsies is extremely difficult if at all possible. A group of investigators proposed the use of immunostains for cytokeratin (CK) 7 and CK20 to overcome such difficulty. They concluded that the Barrett CK7/CK20 pattern was a highly sensitive and specific marker for Barrett metaplasia. Their observations, however, were not confirmed by other investigators. However, because it may be associated with premalignant lesions elsewhere in the gastric mucosa, we propose that intestinal metaplasia of the gastric cardia may have the same clinical implication as Barrett metaplasia.

Subjectivity in the interpretation of results also may play a significant role; careful examination of illustrations of the examples that Ormsby et al deemed weakly reactive could be easily interpreted by other experienced investigators as negative. This weak positive staining was present in 44% of their BM cases. When a high percentage of cases is weakly positive, slight variation in the methodology, such as type of fixative, duration of antigen re-

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The observations by Ormsby et al regarding the use of the Barrett cytokeratin (CK) 7/CK20 pattern to distinguish Barrett metaplasia (BM) from intestinal metaplasia of the gastric cardia (IMGC) has generated considerable interest among gastroenterologists. They defined the pattern as “band-like CK20 staining of the surface epithelium and the superficial glands,” with a diffuse and weak CK7 staining of superfi
cial and deep glands in both complete and incomplete types of intestinal metaplasia.

The pattern was found in 100% of BM cases and was completely absent in IMGC cases. Conversely, the gastric CK7/CK20 pattern was present in 100% of IMGC cases and was totally absent in BM cases. Subsequently, Ormsby et al determined that the Barrett CK7/CK20 pattern was present in 98% of patients with long-segment BM and 82% of those with short-segment BM. None of the IMGC cases displayed the Barrett CK7/CK20 pattern. These findings could not be duplicated by other investigators, who concluded that the pattern could not reliably distinguish between these 2 conditions.

WHY ARE THE RESULTS AND CONCLUSIONS DIFFERENT?

There are several possible reasons why all investigators have not obtained the same results: (1) differences in methods and reagents used (eg, fixatives, antigen retrieval methods, antibodies, and detection kits); (2) differences in the endoscopic and clinical definitions of Barrett metaplasia, such as the location of the squamous columnar junction; and (3) differences in interpretation of immunostained materials.

Effect of Tissue Fixation

Ormsby et al did not report the fixative they used. In previous reports, these investigators had used Hollande fixative, a modified Bouin fixative, for esophageal biopsies. The other investigators used formalin. To my knowledge, there have been no comparison studies of the effects of these different fixatives on CK7 or CK20 immunoreactivity.

Definitions, Criteria, and Interpretation of Immunostaining Results

Ormsby et al defined positive results as “when greater than 5% of goblet cells displayed immunoreactivity regardless of intensity.” However, it is not clear how the percentage of positive cells was determined. For example, they did not state whether positive and negative cells actually were counted in part or all of a tissue section and the percentage calculated, which would be more accurate, or whether the percentage of positive cells was estimated, in which case a cutoff value of 5% leaves a wide margin for interpretation error.
metaplasia was present at the cardia unless concurrently the cardia was involved by atrophy. No incomplete intestinal diffuse patterns were associated with a significant risk of distinguishing, in our opinion, between Barrett intestinal of the esophagus in patients with BM.

**CLINICAL IMPLICATIONS OF IMGC**

IMGC is associated in a significant number of cases with intestinal metaplasia elsewhere in the gastric mucosa. Cassaro et al identified 4 patterns of gastric atrophy based on the distribution of intestinal metaplasia in the stomach. The first pattern was described as focal, consisting of scattered foci mainly in the lesser curvature near the incisura angularis. The second pattern was called antrum predominant, involving most of the antrum. The third pattern was called magenstrasse, involving the lesser curvature from the cardia to the pylorus and the prepyloric antrum on the greater curvature side. The fourth pattern was diffuse, which involved all the gastric mucosa except the fundus. Of these 4 patterns, only the magenstrasse and diffuse patterns were associated with a significant risk of gastric adenocarcinoma, and in both patterns the gastric cardia was involved by atrophy. No incomplete intestinal metaplasia was present at the cardia unless concurrently present in the antrum. Of 24 prevalent adenocarcinomas associated with the magenstrasse pattern, only 1 was located in the gastric cardia, and none of 13 prevalent adenocarcinomas associated with the diffuse pattern was located in the gastric cardia.

Thus, although IMGC carries no significant risk for the development of adenocarcinoma of the cardia per se, it is associated with patterns of gastric atrophy, the magenstrasse and diffuse types, that carry a significant risk for adenocarcinoma in other areas of the stomach.

Currently, there are no guidelines for surveillance in patients with IMGC. However, a small series of 93 patients with an initial diagnosis of intestinal metaplasia were followed prospectively with endoscopy and biopsy. Ten of the patients developed gastric adenocarcinoma. These patients had significantly better survival than did patients who were found to have gastric cancer at initial examination but were outside the surveillance program. Although these results must be confirmed by larger prospective studies, they suggest that the risk of adenocarcinoma of the stomach in patients with intestinal metaplasia may not be too different from the risk of adenocarcinoma of the esophagus in patients with BM.

**SUMMARY AND CONCLUSIONS**

Cytokeratins 7 and 20 immunostaining do not reliably distinguish, in our opinion, between Barrett intestinal metaplasia and IMGC. When no reliable diagnosis of BM or IMGC can be made on an initial biopsy from the gastroesophageal junction showing intestinal-type goblet cells, the following approach is suggested. The gastroenterologist should map the stomach with adequate sampling of the antrum, corpus, and cardia. If there is no intestinal metaplasia elsewhere in the stomach then the diagnosis is likely BM, and the patient should be followed-up accordingly. If the patient is found to have the magenstrasse or diffuse types of gastric atrophy, then the patient should be followed because of the high risk of gastric, not cardia, adenocarcinoma. It should be noted though that BM, even long segment, and gastric intestinal metaplasia can coexist. Including biopsies of the gastroesophageal junction in the surveillance routine for gastric atrophy will ensure that all bases are covered.

**References**


Is Adenocarcinoma of the Esophagogastric Junction or Cardia Different From Barrett Adenocarcinoma?

Nadine Ectors, MD, PhD; Ann Driessen, MD, PhD; Gert De Hertog, MD; Toni Lerut, MD, PhD; Karel Geboes, MD, PhD

Over time the relative distribution of cancers of the proximal digestive tract has changed. Squamous cell carcinomas of the esophagus have become less common, while numbers of adenocarcinomas have greatly increased. This shift most likely reflects an increase in the incidence of gastroesophageal reflux. Moreover, there is a decline in the incidence of distal gastric cancer, which in turn may be related to Helicobacter pylori eradication. Simultaneously, there is a time trend toward a more proximal localization of gastric cancer. If the above-mentioned etiopathologic links are correct, this could indicate that the so-called cardia adenocarcinomas are not related to H pylori infection and that they may instead be related to gastroesophageal reflux and eventually may not be considered to be “gastri” cancers. The rapidly growing quantity of literature on this subject is, however, confounding. A major source of discordance would seem to be a Babylonian confusion of tongues concerning the terms cardia and cardiac carcinomas. Unfortunately, this confusion is also apparent in the classification systems available for staging of cancer, thus closing the “vicious” circle.

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Gastric cancer and esophageal cancer, respectively the fourth and the eighth most common cancers in the world, are a major health problem.1 During the past 50 years there has been a steady decline in gastric cancer worldwide, in particular in the intestinal-type adenocarcinomas. There has also been a change in the anatomical localization of gastric cancer, in which there is a trend toward a more proximal localization of the gastric cancer, namely the cardia. In contrast, there has been a decline in the incidence of cancer of the distal stomach. In parallel with gastric cancer, the incidence of esophageal adenocarcinomas has increased by 5% to 10% per year, as a result of which nearly half of the esophageal cancers nowadays are adenocarcinomas. Gastric cancer as well as esophageal cancer have a poor prognosis, with respective mortality rates of 647,000 and 338,000 cases annually. Compared to cancer of the distal stomach, cardiac cancer carries an even worse prognosis. The overall 5-year survival after surgery for esophageal as well as gastric adenocarcinomas is less than 20%.

GASTRIC CARDIA

Strangely enough, the location, extent, and even the existence of the gastric cardia are controversial. Anatomists have applied the term cardia to that part of the stomach that lies around the orifice of the tubular esophagus. The esophagogastric junction (EGJ) is localized at the level of the angle of His. This is the point at which the tubular esophagus joins the saccular stomach. The American Joint Committee on Cancer describes the EGJ as the first part of the stomach, which is located immediately below the diaphragm and is often called the cardia. The most reliable endoscopic landmark for the EGJ is the proximal margin of the longitudinal gastric mucosal folds. However, there is no anatomical landmark for the distal margin of the so-called cardia. The squamocolumnar junction (SCJ or Z-line) is the endoscopically visible line formed by the juxtaposition of squamous and columnar epithelia. This junction may occur at or above the EGJ. When the SCJ is located above the EGJ, there is a columnar-lined segment of the esophagus. Histologists describe the cardiac mucosa to be composed of shallow, occasionally cystic, loosely packed coiled glands that are almost exclusively lined by mucus-secreting epithelium. However, the histologic finding of cardiac mucosa does not necessarily identify the cardia, since it can be found in the esophagus. Based on a morphologic study of autopsy material from embryos, fetuses, and infants we concluded that cardiac mucosa was present in all cases, but varied in length throughout gestation. Cardiac mucosa is present at birth as a normal structure. If the angle of His is taken as a landmark for the EGJ, the cardiac mucosa is located in the distal esophagus.2 The same material was further analyzed by means of immunohistochemical stains for cytokeratins.3 The reported adult cytokeratin expression patterns of the esophagus and stomach were seen to be established fairly early in gestation. Our finding of Barrett cytokeratin 7/20 pattern (cytokeratin 7 positivity in absence of cytokeratin 20 expression) in the cardiac mucosa in approximately 50%
of fetuses indicates that this pattern may be a normal finding in the adult EGJ in the absence of intestinal metaplasia (Table). We speculate that the finding of the Barrett cytokeratin 7/20 pattern in adult cardiac mucosa is not an argument for metaplasia. If, as we believe, the cardia exists, neoplasms may develop herein.

**Etiopathogenesis**

Corley and Buffer performed an analysis on regional variation for esophageal and gastric cardia adenocarcinomas using the Cancer Incidence in Five Continents Database. They found different rate distributions and thus concluded that these data support the hypothesis that these cancers may represent biologically different malignancies. Although esophageal and gastric cardia carcinomas have a similar male-to-female ratio, median age at diagnosis of disease, and survival rate, some studies indicate that epidemiologic and molecular differences may exist between these cancers. Esophageal adenocarcinomas are strongly associated with gastroesophageal reflux and obesity and are inversely associated with Helicobacter pylori and antioxidant intake. In contrast, gastric cardia cancers show no really convincing association with gastroesophageal reflux and obesity and have no association with Helicobacter pylori, as there is a lack of epidemiologic and molecular differences between these cancers. Esophageal adenocarcinomas are strongly associated with gastroesophageal reflux and obesity and are inversely associated with Helicobacter pylori and antioxidant intake. In contrast, gastric cardia cancers show no really convincing association with gastroesophageal reflux, may show a weak link to obesity, have no association with antioxidant intake, and have a dubious association with Helicobacter pylori. Inflammation has been proposed as a potential risk factor and thus as the inhibitor of a carcinogenesis cascade. It has been associated with premalignant changes of the esophagus; however, studies linking gastric cardia inflammation with Helicobacter pylori, gastroesophageal reflux, or gastric cancer have been contradictory. Our data concerning the association of chronic gastritis (and its parameters) reveal similarities between esophageal and gastric cardia adenocarcinomas on the one hand and statistically significant differences with distal gastric cancer on the other. Both cancers are thought to have a similar precursor lesion (ie, intestinal metaplasia). However, immunohistochemical studies have revealed some subtle differences between esophageal intestinal metaplasia (Barrett mucosa) and gastric cardia intestinal metaplasia. These differences in patterns would tend to indicate that cardia cells are derived from a different cell line than esophageal metaplastic cells. More recently, a “specific” cytokeratin pattern (cytokeratin 7 positivity in the absence of cytokeratin 20 expression) has been proposed as diagnostic for an esophageal origin of cancer. However, in our hands and in those of others, this does not prove to be the case. Moreover, this hypothesis is not corroborated by our study of the cardiac mucosa in fetuses.

**Surgical Pathology—Classification**

All these findings on esophageal and gastric cardia adenocarcinomas tend to indicate that esophageal and gastric cardia adenocarcinomas are highly similar but not, properly speaking, identical. The vast majority of esophageal and gastric cardia adenocarcinomas are, unfortunately, still diagnosed and treated in an advanced stage of disease. Clinicians and pathologists are very often confronted with adenocarcinomas that straddle the EGJ. Various criteria have been used to categorize tumors situated at the EGJ. In most of these classification systems, the anatomic location of the epicenter or predominant mass of the tumor is used to determine whether the neoplasm is esophageal or gastric (cardia) in origin. From personal experience we all know that tumors tend to be roughly spherical, which fits with the idea of the onset of proliferation in one aberrant cell and thus, eccentric growth. However, to the best of our knowledge, nobody has ever shown that in a given location, such as the EGJ, tumors will grow to the same extent in a proximal and distal direction, allowing us to conclude that the epicenter is the origin.

The TNM (tumor, node, metastases) classification of the International Union Against Cancer, identical to that of the American Joint Committee on Cancer (AJCC), is the most widely used system for staging of carcinomas, and its applicability in the treatment and prognostication of esophageal and gastric cancer has been convincingly validated. The Atlas of Tumor Pathology of the Armed Forces Institute of Pathology includes a figure that has been taken from Spiessl et al. The primary sites for gastric cancer are defined as cardia (site: C16.0), fundus, corpus, antrum, and pylorus. The AJCC Cancer Staging Manual describes the first part of the stomach as the EGJ, which is located immediately below the diaphragm and is often called the cardia (site: C16.0 Cardia, NOS). The Japanese Research Society on Gastric Cancer divides the primary sites of stomach cancer into 3 regions (ie, the proximal one third as the cardia, the central region of the stomach, and the distal one third as the antrum). Each region is defined by one third of the length along the lesser and the greater curvature. None of these classification systems addressed the problem of the definition of the EGJ and of the cardia. Neither did they solve the problem of the lesion that straddled the junction. In conclusion, both of the most important (gastric) cancer staging systems (TNM/AJCC and Japanese Research Society on Gastric Cancer) acknowledge the existence of the cardia; they do not, however, agree on its delimitations. In fact, there simply is a lack of international consensus about the definition of cancer of the gastric cardia.

In 1994 Siewert and Stein proposed a topographic classification for the cardiac carcinomas. This classification was approved at the consensus meetings of the Seventh International Society of Diseases of the Esophagus in 1995 and the Second International Gastric Cancer Congress held in 1997. According to the authors, epidemiologic, clinical, and pathologic data support a subclassification of adenocarcinomas arising in the vicinity of the EGJ into adenocarcinoma of the distal esophagus, which usually arises from an area with specialized intestinal metaplasia (ie, Barrett esophagus) and may infiltrate the EGJ from above (type I); true carcinoma of the cardia arising immediately at the EGJ (type II); and subcardial carcinoma that infiltrates the EGJ and distal esophagus from below (type III). In contrast to the previously described classification system, Siewert and Stein attempt to solve the problem of splitting up EGJ tumors into esophageal and gastric tumors by creating a third entity.
In 2000, the World Health Organization Classification of Tumours published *Pathology and Genetics of Tumours of the Digestive System.* This book includes a chapter on adenocarcinoma of the EGJ. The authors formulate diagnostic criteria based on the following definition of the EGJ (ie, the EGJ is the anatomical region at which the tubular esophagus joins the stomach). The guidelines specify the following: Adenocarcinomas that cross the EGJ are called adenocarcinomas of the EGJ, regardless of where the bulk of the tumor lies. Adenocarcinomas located entirely above the EGJ, as defined above, are considered esophageal carcinomas. Adenocarcinomas located entirely below the EGJ are considered gastric in origin. The use of the ambiguous and often misleading term *carcinoma of the gastric cardia* is discouraged—depending on their size—these should instead be referred to as carcinoma of the body of the stomach.

Nothing has changed in the most recent recommendations of the International Union Against Cancer (TNM, 6th ed., 2002) as far as EGJ tumors are concerned. However, according to the advice formulated in the TNM supplement, adenocarcinomas situated at the gastroesophageal junction are to be classified into esophageal, esophagogastric junction, or cardiac adenocarcinomas according to a single major criterion (ie, the localization of the bulk of the tumor). If more than 50% of the mass of the tumor is situated in the cardia, the tumor should be considered to be of cardiac origin; if the mass of the tumor is predominantly found in the esophagus, it is to be classified as an esophageal tumor. Furthermore, it is specified that a tumor situated on the gastroesophageal junction is likely to be of esophageal origin when the neoplastic lesion was associated with a Barrett esophagus of the specialized or intestinal type. However, the description of how to handle these tumors in the most recent *Cancer Staging Manual* may not always be compatible. The chapter on stomach (chapter 9) indicates that “tumors arising within the EG junction and gastric cardia that have minimal (2 cm or less) involvement of the esophagus are considered primary gastric cancers.”

**COMMENT**

A continuing increase in the incidence of cardia cancer has been reported since the mid 1970s. The output of scientific publications on cardia and cardiac cancer has evolved in parallel. This story of the cardia and cardiac carcinoma bears, in its evolution in time, some resemblance with that of dysplasia. Dysplasia, as a preneoplastic lesion in the gastrointestinal tract, was described in the early 1970s. Some 25 years later, during the World Congresses of Gastroenterology in Vienna (1998), the problems of diagnosing and handling dysplasia were acknowledged. Moreover, a new classification system has been designed, taking these difficulties into account in order to unify classification, terminology, and diagnostic criteria internationally.

Today, the vast majority of the data available on the cardia and cardiac cancer are not comparable because of lack/variability of diagnostic criteria. Uniformity in classification, terminology, and diagnostic criteria is needed urgently. Only then will it be possible to analyze data on epidemiology and etiopathogenesis, to clarify the issue of cardia, carditis, and cardiac cancer. Only then will it be possible to answer with certainty the question “Is adenocarcinoma of the esophagogastric junction or cardia different from Barrett adenocarcinoma?” At present, in our experience, it would appear that the similarities between adenocarcinoma of the EGJ or cardia and Barrett adenocarcinoma outnumber the dissimilarities.

**References**

Detection and Correction of Systematic Laboratory Problems by Analysis of Clustered Proficiency Testing Failures

Gerald A. Hoeltge, MD, FCAP; Mari Gina Phillips, MT(ASCP); Patricia E. Styer, PhD; Peter Mockridge, PhD

Context.—The Laboratory Accreditation Program of the College of American Pathologists monitors the performance of its subscribers in proficiency testing (PT). Failure to perform as expected prompts the program to query the laboratory.

Objective.—To determine whether laboratories are correcting apparent problems when contacted by the program about repeatedly unacceptable performance in a diagnostic test.

Design.—Retrospective analysis of 1 year’s records (2002–2003) from the College's Proficiency Testing Exception Summary correspondence, which identifies clusters of PT failures. The analysis focused on those laboratories in which the Proficiency Testing Exception Summary algorithm identified repeated failures over 3 or 4 testing events; PT performance is monitored as a condition of accreditation. During 1 survey year, approximately 6300 accredited laboratories collectively tested approximately 1,205,000 analytes and submitted results to their PT providers on more than 3,500,000 PT challenges. During the period of observation, 14,085 Proficiency Testing Exception Summary reports were mailed to participants. Educational materials were included to help laboratories identify and correct each PT failure.

Results.—There were only 1304 cases of repeated PT failures after the initial correspondence from the accreditation program (9.3%). Of these, there were only 119 cases of unsatisfactory results on the subsequent PT event (9.1%). All systematic problems were resolved by the conclusion of the third round of correspondence.

Conclusions.—Accredited laboratories generally perform well in proficiency testing. Identification of clusters of PT failures by the accreditation provider can help those laboratories having analytic difficulties to investigate and correct the problems.

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not a regulatory tool. It is an internal routine used exclusively by the LAP. Table 1 compares the scope of the PTES with that of the Clinical Laboratory Improvement Amendments of 1988.

3. Performance data from any approved PT provider may be included. The college recognizes PT programs other than its own. Among the requirements for recognition by CAP is that the provider submit data to the LAP in a format that can be read by the PTES algorithm.

4. Clusters of unacceptable results are extracted for review by the LAP. Repeated identification of an analyte by PTES prompts closer review.

Identification of a cluster of unacceptable results by PTES prompts a round of correspondence between the LAP and the laboratory’s director. Any response from the laboratory that describes the results of the laboratory’s investigation closes the initial dialogue.

Should the laboratory report unacceptable results in a subsequent testing event, the PTES program continues with a second round of correspondence. Because the expectation is that corrective action has been implemented, the LAP views repeated unsuccessful performance as a serious situation that requires definitive resolution.

At the end of each evaluation period, PTES also compares PT results with the menu of testing activities for that laboratory. Failure to have submitted results is considered an unsuccessful testing event that initiates PTES correspondence.

We reviewed the results from recent experience to determine whether the PTES program is achieving its stated goal: determine whether laboratories are correcting significant problems as identified by PTES.

### MATERIALS AND METHODS

A 12-month survey year beginning in 2002 was studied. During that time, the CAP accredited approximately 6300 laboratories. CAP-accredited laboratories collectively tested about 1205000 analytes and reported on approximately 3524000 annual PT challenges.

The portion of the PTES algorithm that monitors PT scores was run 7 to 10 days after those scores were submitted by the PT provider. In most cases, the CAP was the PT provider, but the algorithm was applied to data submitted by any provider approved by the LAP. Each PTES report was mailed to the laboratory director with a request for a written reply.

In each round of correspondence, the laboratory director was asked to describe the apparent cause of the PTES event and the corrective actions taken. Instructions for categorizing the problem were provided. Cases that offered no useful data (eg, enrollment error, wrong laboratory identification number, or test no longer performed) were excluded from the analysis.

Three rounds of correspondence were compared. The first round followed a reported unsatisfactory PT event. Included with the LAP letter to the laboratory was a brochure entitled “How to Respond to a Proficiency Testing Exception Summary.” The brochure includes a list of suggestions for investigating the event as adapted from an NCCLS guideline and helpful examples. Each response was scored only for completeness. Reminder letters were sent if the laboratory did not respond or if the response omitted required data.

Correspondence from the second round was reviewed by one of us (M.G.P.) for content. Items looked for at this stage were a description of how the problem was investigated, whether the cause of the repeated unsuccessful performance was identified, specific corrective action to prevent recurrence, and—ideally—evidence that the problem was corrected. Letters to gather additional documentation were sent as necessary.

For the third round, documentation was expected to indicate that the problem was corrected or that the laboratory had ceased testing for that analyte. All correspondence at this level was reviewed by 2 of us (M.G.P. and G.A.H.) for adequacy. By policy, inadequate responses to third-round correspondence are referred to the CAP Commission on Laboratory Accreditation for action.

Responses to the third round of correspondence were tallied as to category of apparent cause and whether the problem had been resolved on subsequent testing events.

### RESULTS

There were 14085 PTES letters generated in the first round of correspondence, 1304 in the second round, and 119 in the third round. Table 2 details the totals. Regulated tests are those tests for which PT (3 challenges per year, 5 samples per challenge) is required by the Centers for Medicare and Medicaid Services for all laboratories performing tests on such analytes. The total for this category includes all results for such analytes, whether or not they were reported to the Centers for Medicare and Medicaid

### Table 2. Decrement in the Annual Number of Proficiency Testing Exception Summaries Following Repeated Correspondence

<table>
<thead>
<tr>
<th>Round 1</th>
<th>Round 2</th>
<th>Round 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated tests</td>
<td>7549</td>
<td>573</td>
</tr>
<tr>
<td>Nonregulated tests</td>
<td>5804</td>
<td>654</td>
</tr>
<tr>
<td>Subspecialty tests</td>
<td>713</td>
<td>75</td>
</tr>
<tr>
<td>Toxicology</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Gynecologic cytopathology</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>14085</td>
<td>1304</td>
</tr>
</tbody>
</table>

* CLIA indicates Clinical Laboratory Improvement Amendments of 1988; LAP, Laboratory Accreditation Program; and CLA, Commission on Laboratory Accreditation.

† Or 1 or more for immunohematology.
Services for regulatory purposes. Round 2 correspondence occurred only 7.6% as frequently as round 1 correspondence for regulated tests, and 99.0% of the PT performance problems were resolved before a third round of correspondence was triggered.

Second-round correspondence for nonregulated tests occurred 11.3% as frequently as first-round correspondence; 99.0% of the problems were resolved before reaching the third round.

Subspecialty testing (principally microbiology results, which are grouped and regulated by the Centers for Medicare and Medicaid Services according to subspecialty), toxicology, and gynecologic cytopathology results were counted separately. The number of PTES letters decremented similarly over time.

Of the 119 cases from all categories that reached a third round of correspondence, 115 were resolved. In 106 cases, subsequent PT performance was acceptable, and the laboratory chose to discontinue testing in 9 cases. Four cases remained unresolved; these were all international PT subscribers. Deterioration of the PT material in transit was presumed. Of the third-round cases, the results of 41 were attributed to methodologic problems, 42 to technologic problems, 28 to clerical issues, and 8 to problems with the survey material. No case lacked an explanation after investigation.

**COMMENT**

Proficiency testing is one way to measure laboratory quality. As a metric for interlaboratory variation, it is a useful tool to ensure comparability of results between testing laboratories. Originally designed as an educational tool for self-evaluation,6 external PT took on new meaning after adoption by regulatory agencies. Accrediting programs such as the college's LAP must require their subscribers to participate in PT activities as defined by law.

The LAP endeavors to meet all regulatory requirements while maintaining laboratory improvement as its primary goal. Laessig et al3 and many others have commented on the difficulty in reconciling a quality-improvement goal with a regulatory mandate. Participants in the CAP LAP have elected external review beyond any regulatory mandate. Of the 423 graded analytes offered by LAP-approved PT providers, only 97 are required by Centers for Medicare and Medicaid Services regulation. Participation in PT for nonregulated analytes is a condition of accreditation, and laboratories that have chosen CAP as their accreditation provider have agreed to the expanded list.

The PTES program identifies potentially correctable problems. None of the performance difficulties flagged by a PTES report was unknown to the laboratory at the time of PTES correspondence. A PTES report presents data in a format that may focus a busy laboratory director's initial attention on an important problem. Educational materials are provided to assist laboratory workers' investigation and resolution of the problems. In addition, the scientific resource committees of the college stand ready to answer participants' questions about the CAP Surveys Program.

The LAP adds on-site inspection to the laboratory's tools for quality improvement. Data from PTES are available to the on-site inspector. Follow-up may include recommendations to the laboratory for further opportunities to improve care. When repeated problems as flagged by PTES are evident, the LAP has the information needed for intervention. A laboratory may, for example, be directed by the CAP Commission on Laboratory Accreditation to discontinue clinical testing until the problem has been fully resolved.

The limitations of PT as a stand-alone tool for laboratory improvement were summarized in a review by Shanagian,8 who concluded that 4 issues challenge any PT program: (1) the inability of PT to address the total testing process; (2) the inevitable differences between PT materials and fresh biological specimens; (3) inadequacies of PT evaluation criteria; and (4) the matrix effects unique to a particular PT system. Combining on-site inspection with the monitoring of PT addresses the first and second of these limitations. The LAP's criteria for approval of a PT provider's program targets the third. All PT providers seek to minimize matrix effects as they are identified.

Klee and Forsman7 noted that external PT programs from each of 3 different providers introduced errors that were artifacts of the PT process itself. Such attributions decrease in frequency with subsequent rounds of correspondence with participating laboratories. Experience with the survey tool and improvements in the survey process both seem to contribute to this improvement. Even so, making errors in reconstituting PT specimens, reporting results in the wrong units, coding for the incorrect peer group, and similar problems persist even to the third round of correspondence. Such errors must be resolved for the value of interlaboratory comparison to be realized.

Parts of the improvement in external PT scores are attributable to better control of intralaboratory analytic processes. For this reason alone, PT is a legitimate component of the accreditation routine. Studies using CAP data have demonstrated that careful attention to analytic linearity (as evidenced by participation in the CAP Linearity Survey) is positively associated with fewer PT failures.8,9 Similarly, participation in the college's LAP was demonstrated by Lawson et al12 generally to correlate with fewer unacceptable PT scores. The present study illustrates how interpretation of these 2 measures of laboratory quality—proficiency testing plus inspection and accreditation—combine to target specific opportunities for analytic improvement.

The categorization of the causes of PT failures used in this study was proposed in 1987.13 With each PTES letter, the list reproduced in Table 3 is provided. The list of categories is provided to help a laboratory think through the problem. The category 'no explanation after investigation' is unreasonable for repeatedly unsuccessful results (rounds 2 and 3).

In 41 cases (34% of the third round) a systematic problem in the method was detected. The cause was usually identified as a faulty instrument (20 cases). In 42 cases (35%), 1 or more technical errors were revealed. Twelve of these were repeated misidentifications of clinical microscopy or parasitology images. The need for remedial training in these and other technical cases was clear.

In a CAP Q-Probes study of 665 laboratories, Steinidel et al14 reported that the rate at which the problems with the survey material contributed to PT failures varied from 4.3% to 29.7% among 8 PT providers, according to the participants in those programs. The rate of unexplained PT failures in that study ranged from 22.9% to 49.3%. Repeated unsuccessful performance demands an explanation. The present study provides at least indirect evidence that laboratories do identify contributory causes. Much of the improvement followed technologic or methodologic
This study represents about 1% of analytic testing by accredited laboratories (14085 first-round PTES reports among 1205000 analytes). The data show that laboratories are successful at correcting systematic problems within their LAP experience. Moreover, these are the problems that may have remained overlooked had they not been identified by the PTES program.

To document an investigation for external review like this requires firsthand involvement by the laboratory director. Spotlighting a problem in performance helps focus remedial resources on an issue that may have gone unobserved. Accredited laboratories perform consistently well on PT surveys. Their documentation of problem resolution in those exceptions that reflect systematic problems is evidence of the value of these CAP programs to laboratory improvement.

**Table 3. Categories of Causes of Unacceptable Results in Proficiency Testing (PT) as Used by Participants in the Laboratory Accreditation Program**

<table>
<thead>
<tr>
<th>Methodologic Problems</th>
<th>Technical Problems</th>
<th>Clerical Errors</th>
<th>Problems With PT Materials</th>
<th>No Explanation After Investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument problem identified</td>
<td>Misinterpretation/misidentification</td>
<td>Transcription error</td>
<td>Hemolized specimen</td>
<td>No Explanation After Investigation</td>
</tr>
<tr>
<td>Instrument repaired or replaced</td>
<td>Dilution error or incorrect pipetting</td>
<td>Transposition error</td>
<td>Bacterial contamination</td>
<td>Instrument problem identified</td>
</tr>
<tr>
<td>Faulty standard or other reagent</td>
<td>Time delay between reconstitution and analysis</td>
<td>Wrong peer-group code entered</td>
<td>Perceived survey bias</td>
<td>Instrument repaired or replaced</td>
</tr>
<tr>
<td>Incorrect calibration</td>
<td>Calculation error</td>
<td>Failure to submit results</td>
<td>Poor growth in culture</td>
<td>Incorrect calibration</td>
</tr>
<tr>
<td>Other method problem</td>
<td>Run accepted in nonlinear range</td>
<td>Sample mix-up</td>
<td>Unstable PT material</td>
<td>Other method problem</td>
</tr>
<tr>
<td>Other method problem</td>
<td>Run accepted even though controls were out of range</td>
<td>Other technical problem</td>
<td>Matrix effect incompatible with method</td>
<td>Other method problem</td>
</tr>
<tr>
<td></td>
<td>Sample mix-up</td>
<td>No comparable peer group</td>
<td>No comparable peer group</td>
<td>Other technical problem</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acceptable range too low</td>
<td>Acceptable range too low</td>
<td>Other technical problem</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late shipment</td>
<td>Late shipment</td>
<td>Other technical problem</td>
</tr>
</tbody>
</table>

Changes. A substantial number of laboratories decide to discontinue performing a problematic test when they realize that their difficulties are tied to the infrequency with which they perform the test.

The PTES program provides no novel information to the laboratory participant. By the time PTES correspondence is received, the laboratory should already have investigated and corrected the problem. The LAP expects each unacceptable response—including those that do not cluster to the level of unsatisfactory performance—to be investigated. Isolated PT failures are approximately 14 times more common than PTES clusters (unpublished observation). Accredited laboratories are investigating many more PT failures than are reflected in PTES statistics.

**References**

Phenotype Matching of Donor Red Blood Cell Units for Nonalloimmunized Sickle Cell Disease Patients
A Survey of 1182 North American Laboratories

Melanie Osby, MD; Ira A. Shulman, MD

• Context.—The transfusion of donor red blood cell units (RBCs) that lack certain red cell antigens (such as C, E, and K) when the corresponding antigens are absent from the recipient’s red cells has been shown to reduce the risk of red cell alloimmunization in sickle cell disease patients. However, data are limited regarding the extent to which transfusion services routinely perform red cell antigen phenotype testing of nonalloimmunized sickle cell disease patients, and then use that information to select donor RBCs lacking 1 or more of the red cell antigens that the patient’s red cells do not express.

Objective.—To determine the extent to which transfusion services routinely perform red cell antigen phenotype testing of nonalloimmunized sickle cell disease patients, and then use that information to select donor RBCs lacking 1 or more of the red cell antigens that the patient’s red cells do not express.

Design.—An educational subsection of a College of American Pathologists Proficiency Testing Survey (J-C 2003) assessed transfusion service practices regarding performance of red cell antigen phenotype testing of nonalloimmunized sickle cell disease patients and how transfusion services use this information for the selection of donor RBCs. The data analysis of the survey included 1182 North American laboratories.

Results.—Data from 1182 laboratories were included in the survey analysis, of which the majority (n = 743) reported that they did not routinely perform phenotype testing of sickle cell disease patients other than ABO and D. The other 439 laboratories reported that they did routinely perform phenotype testing of sickle cell disease patients for antigens other than ABO and D. The majority of these 439 laboratories (three fourths; n = 330) reported that they used these patient data for prophylactic matching with donor RBCs when sickle cell disease patients required transfusion. When phenotype-matched donor RBCs were used, the antigens most commonly matched (85% of the time) were C, E, and K.

Conclusions.—The majority of North American hospital transfusion service laboratories do not determine the red cell antigen phenotype of nonalloimmunized sickle cell disease patients beyond ABO and D. Those laboratories that do determine the red cell antigen phenotype of nonalloimmunized sickle cell disease patients beyond ABO and D most commonly match for C, E, and K antigens when phenotype-matched donor RBCs are used.

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nonalloimmunized sickle cell disease patients and then select donor RBCs that lack 1 or more red cell antigens that the patient’s red cells do not express.

MATERIALS AND METHODS

The CAP Proficiency Testing Survey J-C 2003 was distributed to 4251 participants to assess their proficiency in testing the following analytes: ABO, Rhesus (Rh), antibody detection, antibody identification, and crossmatching.

A subset of the survey participants (n = 1360) also subscribed to an educational module through which they were surveyed for their routine practice to provide (or not to provide) phenotype-matched donor RBCs for the transfusion of nonalloimmunized sickle cell disease patients. The survey consisted of a case history, a series of short vignettes that pertained to the case history, and follow-up questions that allowed for the survey of laboratory practices (see “Results”). By the data collection deadline (set as 10 working days after receipt of survey materials), participants had submitted their survey responses. The survey focused on whether or not laboratories routinely performed red cell phenotype testing of nonalloimmunized sickle cell disease patients, and if so, whether they used that information for prophylactic matching of donor RBCs for antigens other than ABO and D. No effort was made to stratify the data according to the category of the transfusion (eg, red cell exchange, response to a life-threatening event). In addition, no questions were directed at the participants to determine how they discover that a particular patient has sickle cell disease.

RESULTS

The survey participants were to answer a number of questions based on the following history: “Your blood bank is made aware of a newly diagnosed sickle cell disease patient who will require a chronic transfusion therapy regimen. Your tests show that the patient is group A, Rh positive, and has a negative antibody screen. The physician orders a unit of red blood cells to be crossmatched for a transfusion later that day.” The responses to the survey questions are displayed in Tables 1 through 9.

COMMENT

A total of 1182 North American laboratories (most located in the United States) participated in the educational module of the CAP J-C 2003 survey. The majority of laboratories participating (n = 743; 62.9% of all participants) followed a policy that did not require them to perform red cell antigen phenotype testing beyond ABO and D for nonalloimmunized sickle cell disease patients. Slightly more than 37% (n = 439) of participants routinely determined a red cell antigen phenotype beyond ABO and D for nonalloimmunized sickle cell disease patients. Of these 439 laboratories, 75% (n = 330) reported using the patient’s phenotype to select ABO/D-compatible donor RBC units lacking 1 or more of the following red cell antigens (in descending order of frequency): K, E, and C (about one third of all laboratories); c, e (about one fifth of all laboratories); and Jk(a), Jk(b), Fya*, and Fyb* (about one seventh of all laboratories).

Clinical evidence that the transfusion of phenotype-matched donor RBCs benefits sickle cell disease patients is provided by several studies.

Phenotype Matching in Sickle Cell Disease—Osby & Shulman

Table 1. Which of the Following Most Closely Approximates Your Hospital’s Standard Operating Procedure for Selection of Donor Red Blood Cell Units for Transfusion of Sickle Cell Disease Patients?*

<table>
<thead>
<tr>
<th>Choice</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crossmatch an ABO/Rh(D)–compatible unit and issue it for transfusion.</td>
<td>743</td>
<td>62.9</td>
</tr>
<tr>
<td>Determine the patient’s baseline phenotype for commonly immunogenic antigens other than ABO and D (eg, C, c, E, e, K, etc); crossmatch an ABO/Rh(D)–compatible unit of red cells that, in addition to being ABO/Rh(D) compatible, is matched for additional antigens for which the patient was typed, and issue it for transfusion.</td>
<td>330</td>
<td>27.9</td>
</tr>
</tbody>
</table>

* n = 1182.

Table 2. Which of the Following Antigens if Negative in the Patient Would You Require to Be Absent From the Transfused Unit?*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>747</td>
<td>64.5</td>
</tr>
<tr>
<td>K</td>
<td>394</td>
<td>34.0</td>
</tr>
<tr>
<td>E</td>
<td>383</td>
<td>33.1</td>
</tr>
<tr>
<td>C</td>
<td>373</td>
<td>32.2</td>
</tr>
<tr>
<td>c</td>
<td>265</td>
<td>22.9</td>
</tr>
<tr>
<td>e</td>
<td>245</td>
<td>21.1</td>
</tr>
<tr>
<td>Jk(b)</td>
<td>163</td>
<td>14.1</td>
</tr>
<tr>
<td>Fya*</td>
<td>157</td>
<td>13.6</td>
</tr>
<tr>
<td>Jk(c)</td>
<td>145</td>
<td>12.5</td>
</tr>
<tr>
<td>Fyb*</td>
<td>129</td>
<td>11.1</td>
</tr>
<tr>
<td>S</td>
<td>95</td>
<td>8.2</td>
</tr>
<tr>
<td>k</td>
<td>86</td>
<td>7.4</td>
</tr>
<tr>
<td>s</td>
<td>74</td>
<td>6.4</td>
</tr>
<tr>
<td>M</td>
<td>7</td>
<td>0.6</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>Le(a)</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>P1</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>Le(b)</td>
<td>4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* n = 1159.

Table 3. In a Nonemergent Situation, and Based on Your Standard Operating Procedure, Would a Donor With the Phenotype Ror, K+, Jk(a+b) Be Acceptable for Transfusion of a Sickle Cell Disease Patient (Antibody Screen Negative) With the Following Phenotype? D+, C–, E–, c+, e+, Le–, Le+, M+, N+, P+, K–, k+, Fya–, Fyb–, Jk+a, Jk+b, S–, s+*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>721</td>
<td>62.1</td>
</tr>
<tr>
<td>No</td>
<td>440</td>
<td>37.9</td>
</tr>
</tbody>
</table>

* n = 1161.

Table 4. In a Nonemergent Situation, and Based on Your Standard Operating Procedure, Would a Potential Donor With the Phenotype Ror, K–, Jk(a+b) Be Acceptable for Transfusion of a Sickle Cell Disease Patient (Antibody Screen Negative) With the Following Phenotype? D+, C–, E–, c+, e+, Le–, Le+, M+, N+, P+, K–, k+, Fya–, Fyb–, Jk+a, Jk+b, S–, s+*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>956</td>
<td>82.1</td>
</tr>
<tr>
<td>No</td>
<td>208</td>
<td>17.9</td>
</tr>
</tbody>
</table>

* n = 1164.
alone. Another study, by Vichinsky et al, showed that from patients who had not developed alloantibodies, as compared with 70.9% under the protocol of matching for ABO and D antigens, 87.5% of sickle cell patients in the study would be compatible. When using the protocol that matched for at least C, E, and K antigens, the majority of patients would be compatible. However, arguments in favor of choosing not to provide donor RBC units that are matched for at least C, E, and K antigens for sickle cell patients might be that at most only about 25% of sickle cell disease patients will develop alloantibodies if phenotype-matched donor RBCs are not provided. In other words, if phenotype matching of the nonalloimmunized patient is not done and if donor RBCs are not selected to match the phenotype of the patient, then the majority of sickle cell disease patients (up to or approximately 75%) do not develop alloimmunization. However, arguments in favor of the routine use of at least C, E, and K-matched donor RBC units are seen in the data published by Castro et al. and by Vichinsky et al., which show the greatest reduction in alloimmunization risk when donor RBC units are selected to avoid expressing C, E, or K when the recipient is capable of making anti-C, anti-E, or anti-K. A reduction in alloimmunization risk can translate into a reduction in morbidity and mortality of sickle cell disease patients that might otherwise occur if alloantibodies developed, including the cost to treat complications of alloimmunization, such as acute and delayed hemolytic transfusion reactions. In addition, one might also consider the cost and risks associated with a delay in locating compatible blood for necessary transfusions in the event that alloimmunization occurs.

The CAP survey data presented in this report showed

<table>
<thead>
<tr>
<th>Table 5. Assume the Patient From the Question in Table 4 Underwent Several Transfusion Events During the Next Year (Not All at Your Institution), and Developed Anti-Jk. Based on Your Identification of the Antibody, Which of the Following Antigens Would You Now Generally Require to Be Absent From the Donor Red Blood Cells?*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Jk⁰</td>
<td>1161</td>
</tr>
<tr>
<td>K</td>
<td>440</td>
</tr>
<tr>
<td>E</td>
<td>292</td>
</tr>
<tr>
<td>C</td>
<td>289</td>
</tr>
<tr>
<td>Fy⁺</td>
<td>104</td>
</tr>
<tr>
<td>S</td>
<td>91</td>
</tr>
<tr>
<td>Fy⁻</td>
<td>77</td>
</tr>
<tr>
<td>c</td>
<td>31</td>
</tr>
<tr>
<td>e</td>
<td>28</td>
</tr>
<tr>
<td>Jk⁺</td>
<td>24</td>
</tr>
<tr>
<td>Le⁺</td>
<td>13</td>
</tr>
<tr>
<td>S</td>
<td>10</td>
</tr>
<tr>
<td>k</td>
<td>8</td>
</tr>
<tr>
<td>None</td>
<td>3</td>
</tr>
<tr>
<td>Le⁻</td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>1</td>
</tr>
<tr>
<td>PI</td>
<td>1</td>
</tr>
</tbody>
</table>

* n = 1178.

<table>
<thead>
<tr>
<th>Table 6. In a Nonemergent Situation, and Based on Your Standard Operating Procedure, Would a Donor With the Phenotype Ror, K⁺, Jk⁻ Be Acceptable for Transfusion of a Sickle Cell Disease Patient With Anti-Jk and the Following Phenotype? D⁺, C⁻, E⁻, c⁺, e⁻, Le⁻, Le⁰⁺, M⁺, N⁺, P⁺, K⁻, k⁺, Fy⁺, Fy⁻, Jk⁺, Jk⁻, S⁻, s⁺*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Yes</td>
<td>577</td>
</tr>
<tr>
<td>No</td>
<td>588</td>
</tr>
</tbody>
</table>

* n = 1165.

<table>
<thead>
<tr>
<th>Table 7. In a Nonemergent Situation, and Based on Your Standard Operating Procedure, Would a Potential Donor With the Phenotype Ror, K⁻, Jk⁰⁻ Be Acceptable for Transfusion of a Sickle Cell Disease Patient With Anti-Jk, and the Following Phenotype? D⁺, C⁻, E⁻, c⁺, e⁻, Le⁻, Le⁰⁺, M⁺, N⁺, P⁺, K⁻, k⁺, Fy⁺, Fy⁻, Jk⁺, Jk⁻, S⁻, s⁺*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Yes</td>
<td>1110</td>
</tr>
<tr>
<td>No</td>
<td>56</td>
</tr>
</tbody>
</table>

* n = 1166.

dropped 90%), whereas the alloimmunization rate dropped from 3% to 0.5%.

Despite the evidence that phenotype matching of donor RBCs for C, E, and K antigens (in addition to ABO and D) reduces the risk of alloimmunization of sickle cell disease patients, the data presented in the CAP survey under discussion indicate that the majority of North American hospital transfusion service laboratories do not appear to perform phenotypic testing for antigens other than ABO and D for these patients. The justification for choosing not to provide donor RBC units that are matched for at least C, E, and K antigens for sickle cell patients might be that at most only about 25% of sickle cell disease patients will develop alloantibodies if phenotype-matched donor RBCs are not provided. In other words, if phenotype matching of the nonalloimmunized patient is not done and if donor RBCs are not selected to match the phenotype of the patient, then the majority of sickle cell disease patients (up to or approximately 75%) do not develop alloimmunization. However, arguments in favor of the routine use of at least C, E, and K-matched donor RBC units are seen in the data published by Castro et al. and by Vichinsky et al., which show the greatest reduction in alloimmunization risk when donor RBC units are selected to avoid expressing C, E, or K when the recipient is capable of making anti-C, anti-E, or anti-K. A reduction in alloimmunization risk can translate into a reduction in morbidity and mortality of sickle cell disease patients that might otherwise occur if alloantibodies developed, including the cost to treat complications of alloimmunization, such as acute and delayed hemolytic transfusion reactions. In addition, one might also consider the cost and risks associated with a delay in locating compatible blood for necessary transfusions in the event that alloimmunization occurs.

The CAP survey data presented in this report showed
that about 37% of laboratories perform red cell phenotype testing for antigens other than ABO and D to obtain baseline phenotype data that can be used for donor RBC unit selection. Most, but not all, of the laboratories that do this phenotype testing use this patient phenotypic information to provide prophylactic matching of donor RBCs with the red cell phenotype of the patient. This suggests that the actual practice of the larger “community” favors not performing antigen phenotyping of sickle cell disease patients beyond ABO/D until the patients become alloimmunized. These data are different than those published in a smaller survey by Afenyi-Annan and Brecher,11 which surveyed 50 academic medical centers. In the Afenyi-Annan and Brecher survey, the authors found that 66% of academic hospital transfusion services routinely provided ABO/D-compatible donor RBCs that were matched for 1 or more additional red cell antigens, in order to reduce the risk of red cell alloimmunization. This suggests that the actual practice of academic centers favors performing antigen phenotyping of sickle cell disease patients beyond ABO/D before the patients become alloimmunized. Importantly, both surveys clearly show that there is not a consensus among community hospitals and the academic centers regarding provision of phenotype-matched RBCs to prevent (or reduce) the risk of red cell alloimmunization in sickle cell disease patients who require transfusion. The difference between the current data set and the data from Afenyi-Annan and Brecher11 might be due to the method employed to collect the CAP data subset shown in this report. The responding hospitals in the CAP survey data subset were participating in an educational module, so that there might have been self-selection in favor of laboratories unfamiliar with phenotyping sickle cell disease patients and then matching donor RBC phenotypes to reduce their risk of red cell alloimmunization. However, both the Afenyi-Annan and Brecher11 and CAP survey data suggest there is no consensus to provide phenotype-matched donor RBCs for sickle cell disease patients. Between one third and two thirds of laboratories routinely do not provide ABO/D-compatible donor RBCs that are matched for 1 or more additional red cell antigens, in order to reduce the risk of red cell alloimmunization in patients with sickle cell disease. One might argue that a more standardized approach should be encouraged for the pretransfusion selection of donor RBCs for nonalloimmunized sickle cell disease patients. A more standardized approach would increase the likelihood that sickle cell disease patients would receive a similar standard of care with respect to transfusion therapy.

This study’s data demonstrate a lack of consensus for the use of red cell phenotype information in the selection of donor RBCs for nonalloimmunized sickle cell disease patients. It is likely that this lack of consensus is due to a combination of cost and cost-benefit considerations for the management of sickle cell disease in general and transfusion therapy in particular. For example, one study showed that the financial impact of chronic transfusion therapy for sickle cell disease patients is substantial, with charges approaching $400,000 per patient-decade for patients who require deferoxamine chelation.12 A lack of universal use of phenotype-matched donor RBCs for the transfusion of sickle cell disease patients is likely to continue as long as cost-containment pressures exist and there is a lack of clear evidence that the use of phenotype-matched donor RBCs will improve overall cost-effectiveness of therapy. We believe that there is a need for well-designed studies that can provide objective evidence.

References
Estrogen Receptor Expression in Papillary Urothelial Carcinoma of the Bladder and Ovarian Transitional Cell Carcinoma

Philip R. Croft, MD; Sarah L. Lathrop, DVM, PhD; Richard M. Feddersen, MD; Nancy E. Joste, MD

Context.—Relatively little is known about estrogen receptor (ER) expression in papillary urothelial carcinoma (PUC) of the bladder. Greater understanding of this feature of PUCs could aid with the treatment and identification of the origin of metastases, particularly with relation to the morphologically similar entity of ovarian transitional cell carcinoma (TCC).

Objective.—To assess the presence of ERs in PUC of the bladder, its metastases, and ovarian TCC.

Design.—Formalin-fixed, paraffin-embedded archival tissue from 92 primary bladder PUCs, 11 PUC metastases, and 11 primary or metastatic ovarian TCCs was immunostained with a monoclonal antibody against the human ER β-molecule. The ER-positive and ER-negative tumors were compared by the patients’ sex and age, tumor grade, and the presence or absence of invasion. Statistical analysis was performed on the PUC results, first defining a positive result as staining of at least 10% of nuclei and then repeated using any percentage of staining as a positive result.

Results.—By the 10% criterion, 11% of PUCs of the bladder were ER positive. Invasive PUCs were more likely to be ER positive (P = .10). Women with ER-positive PUCs were older than their male counterparts (P = .03). By the second criterion, 22% of all PUCs were ER positive, and both higher grade and the presence of invasion were significantly associated with ER expression (P = .004 and .01, respectively). All 11 PUC metastases were totally ER negative. Ten of the 11 ovarian TCC cases exhibited strong and diffuse ER expression.

Conclusion.—Depending on the criterion used, up to 22% of bladder PUCs were ER positive. Higher grade and the presence of invasion were significantly associated with ER expression in these bladder carcinomas. In contrast, most all of the ovarian TCCs marked strongly for ERs, a characteristic that may help differentiate these lesions from PUCs metastatic to the ovary.

(Arch Pathol Lab Med. 2005;129:194–199)

Materials and Methods

Patients and Specimens

Ninety-two patients with PUCs of the bladder treated from 1991 to 2001 at the University of New Mexico (UNM) Health Sciences Center were identified through a search of the UNM Department of Pathology surgical pathology computer database. In an effort to minimize the effect that any prior treatment for the carcinoma may have on hormone receptor status, only the initial patient specimen identified in the selected timeframe was used. Eleven specimens of metastatic PUC from 9 patients were identified in the same timeframe. Eleven cases of primary or metastatic ovarian TCCs from 5 patients (2 primary ovarian TCCs and the following sites of metastasis: colon [3], omentum [2], peritoneum, small intestine, uterus, and uterine cervix) were included for comparison with the results of the bladder cases. No

Driven in part by questions regarding the pathophysiology of urinary incontinence, several studies performed during the past few decades have attempted to elucidate the distribution of steroid hormone receptors within the human lower urinary tract. Although the findings have not always been consistent, most investigators have identified at least small concentrations of estrogen receptors (ERs) in the trigone, posterior bladder neck, and proximal urethra in women; although less numerous, studies of men have demonstrated ERs in the prostatic urethra but not in the bladder.

In contrast, relatively little is known about the presence of ERs in carcinomas that arise in the lower urinary tract, in particular papillary urothelial carcinoma (PUC) of the bladder. A few studies have addressed this issue with frozen tissue; another study used paraffin-embedded tissue and immunohistochemical analysis. In the current study, formalin-fixed, paraffin-embedded tissue sections of bladder PUCs and their metastases were evaluated for ER expression using immunohistochemical analysis. The results were analyzed for significant associations with the sex and age of the patients, tumor grade, and presence or absence of invasion. The ER status of primary and metastatic ovarian transitional cell carcinomas (TCCs) was also assessed to compare the characteristics of these 2 morphologically similar lesions.
cases of PUC metastatic to the ovaries were identified in the database in the same timeframe. The hematoxylin-eosin-stained slides of all 114 cases were reviewed, and a slide and paraffin block representative of the original diagnosis were selected for each. Where patients had multiple concurrent bladder specimens of differing histologic grades, the tissue that demonstrated the highest-grade lesion was selected.

All primary PUC cases were then organized by sex, grade, and presence or absence of invasion. Of the 92 primary bladder PUC specimens reviewed, 32 were from women and 60 were from men. The ages of the patients ranged from 30 to 93 years (mean, 65 years; median, 64 years). Most cases were originally graded using the World Health Organization (WHO) 1973 grading system; for the few cases that were evaluated using either the Bergkvist or the 1998 WHO/International Society of Urological Pathology classification schemes, the grades were translated into the WHO 1973 grading system. Twenty-one cases were grade I, 29 were grade II, and 42 were grade III. Invasive PUCs accounted for 49 of the 92 primary cases.

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue sections made from the blocks selected were immunostained for ERs using β-ER primary antibody clone 6F11 (Ventana Medical Systems Inc, Tucson, Ariz) per the manufacturer's instructions. The entire sequence of this automated immunohistochemical package will not be reiterated here. Antigen retrieval was accomplished via a standard pH 6 citrate buffer; the slides were brought to 120°C at 20 psi in a Biocare-dedicated Decloaker pressure oven for 2.5 minutes, followed by a 15-minute depressurization and a 10-minute cooldown period. Immunohistochemical analysis was performed using the standard Ventana reagent package on the Nexus immunostainer, including the amplification sequence (after application of the primary antibody, rabbit anti-mouse heavy and light chain followed by mouse anti-rabbit heavy chain). Sections of human uterus were stained in the same manner for use as a positive control. Negative-control slides were also made following the same procedure, but using a negative reagent control instead of the primary antibody.

Analysis

All of the resulting slides were randomly assigned identification numbers before review. Without knowledge of the site of origin or grade, 2 pathologists (N.E.J., P.R.C.) independently evaluated the stained tissue sections for ER immunoreactivity, and the percentage of positive cells was scored semiquantitatively. Only nuclear staining in urothelial carcinoma cells was assessed; neither cases with only cytoplasmic staining nor cases that exhibited staining in stromal cells or nonneoplastic urothelium were considered positive. Analogous to breast carcinoma, a case was considered ER positive when the tissue examined was judged to have at least 10% of its nuclei staining with the ER antibody. Subsequently, the definition of a positive result was revised to include any degree of positive ER staining.

The results obtained using both criteria were statistically analyzed using χ² tests, Fisher exact tests when expected counts were less than 5, and t tests when comparing mean ages of patients with ER-positive and ER-negative PUCs and mean ages of ER-positive men and women. Analyses were performed using SAS version 8.02 statistical analysis software for Windows (SAS Institute Inc, Cary, NC). Results were considered statistically significant when P ≤ .05, nonsignificant if P > .10, and marginally significant when P > .05 and ≤ .10.

RESULTS

Twenty (22%) of the 92 cases demonstrated at least a modicum of ER expression. Ten cases had between 1% and 9%, 7 had between 10% and 24%, and 3 had greater than 75% of PUC nuclei staining positively with the ER antibody. Thus, using the 10% criterion, 10 (11%) of the 92 PUCs were classified as ER positive, including 6 (10%) of 60 specimens from the male patients and 4 (13%) of 32 specimens from the female patients (Figure 1). No significant difference in the proportion of ER-positive PUCs was found between male and female patients (P = .73). One case showed significant cytoplasmic staining in addition to nuclear staining.

None (0%) of the 21 grade I PUCs were ER positive, 3 (10%) of the 29 grade II PUCs were ER positive, and 7 (17%) of the 42 grade III PUCs were ER positive (Figures 2 and 3). No significant difference was identified in the proportion of ER-positive cases between all 3 grades of PUCs (P = .13). Subsequent analysis grouping grade II and III lesions together (10 [14%] of 71 ER-positive cases) and comparing this combination to grade I cases alone yielded a result of only marginal significance (P = .10). Eight (16%) of 49 invasive PUCs and 2 (5%) of 43 noninvasive PUCs were ER positive. Comparison of the proportion of ER-positive tumors in these 2 groups identified a difference of only marginal statistical significance (P = .10).

The mean (±SD) age of patients with ER-positive specimens was 66.3 (±14.9) years, whereas the mean (±SD) age of patients with ER-negative specimens was 64.7 (±12.1) years. No statistically significant difference between these 2 groups was found (P = .71). However, when the ages of women (mean ± SD age, 78.3 ± 13.8 years) and men (mean ± SD age, 58.3 ± 9.6 years) with ER-positive PUCs were compared, a statistically significant difference was identified (P = .03).

These statistical analyses were performed again, redefining a positive result as any percentage of staining, which effectively doubled the number of ER-positive cases to 20 (22%) of 92 (Figure 4). By this approach, 6 (19%) of 32 female patients and 14 (23%) of 60 male patients had ER-positive PUCs; however, the difference in ER-positive cases between the sexes remained nonsignificant (P = .79).

The mean ± age of patients with ER-positive specimens increased slightly (67.4 ± 12.9 years) and decreased for ER-negative patients (64.2 ± 12.2 years), but these changes did not result in statistical significance (P = .32). By grade, none (0%) of the 21 grade I lesions, 6 (21%) of 29 of grade II lesions, and 14 (33%) of 42 grade III lesions were classified as ER positive. Sixteen (33%) of 49 cases of invasive PUCs were ER positive, and 4 (9%) of 43 cases of noninvasive PUCs were ER positive. With the additional ER-positive cases, both grade (P = .004) and invasion (P = .01) became statistically significant variables. The addition of these cases resulted in a nonsignificant difference between the ages of ER-positive male and female patients (P = .47).

Of the 11 distant PUC metastases evaluated, 8 were from 7 male patients and 3 were from 2 female patients. The sites of metastases included lung (2), bone (2), lymph node (2), spleen, colon, perineum, vagina, and peritoneum. Six of the 7 male patients had their primary bladder PUC included in this study. In all 6 cases, the primary PUC was considered ER negative by the 10% staining criterion, although 2 of the cases had between 1% and 9% of PUC nuclei staining ER positive. All 8 male tissue samples were ER negative, with no appreciable nuclear staining. Both of the female patients had their primary bladder PUCs included in this study, and both primary tumors were considered ER negative, with no appreciable staining identified. All 3 of these metastases were also ER negative.
Figure 1. Estrogen receptor staining characteristics of papillary urothelial carcinomas when a positive result is defined as 10% nuclear staining.

Figure 2. Grade III invasive papillary urothelial carcinoma (hematoxylin-eosin, original magnification ×20).

Figure 3. Grade III invasive papillary urothelial carcinoma (β-estrogen receptor antibody, original magnification ×20).

Figure 4. Estrogen receptor staining characteristics of papillary urothelial carcinomas when a positive result is defined as any nuclear staining.

Figure 5. Ovarian transitional cell carcinoma (hematoxylin-eosin, original magnification ×20).

Figure 6. Ovarian transitional cell carcinoma (β-estrogen receptor antibody, original magnification ×20).
By way of comparison, all but 1 of the 11 ovarian TCC specimens were strongly ER positive, with 1 case exhibiting between 50% and 74% and the remainder demonstrating greater than 75% positivity (Figures 5 and 6). The eleventh case showed staining in less than 10% of PUC nuclei. This case, a biopsy specimen from a sigmoid colon metastasis, was one of a series of 4 from a single patient; this patient's other 3 sites were the ovary, rectum, and small intestine, all of which were strongly ER positive.

**COMMENT**

Several studies have shed light on the presence of ERs in the human lower urinary tract. Most studies of human female bladders have detected ERs in the trigone and posterior bladder neck, increasing in concentration as one moves distally to the urethra. In contrast, ERs generally have not been found elsewhere in the bladder. This disparity is thought to be a consequence of the differing embryologic origins of the trigone, which is the same as that of the upper part of the vagina, and the rest of the bladder, which derives from the urogenital sinus. Men also have a difference in ER expression between the prostatic urethra, where ERs are detectable, and the bladder, which is generally devoid of ERs; however, this difference is more puzzling, because both structures arise from the urogenital sinus.

In contrast, few investigators have addressed this question as it relates to urothelial carcinomas of the bladder. In the early 1980s, Iosif and coworkers examined tissue from 4 female patients in an effort to find ERs in the urethra and bladder. Three of the patients had concurrent urothelial carcinoma of the bladder, whereas 1 patient did not. This patient, as well as one of the patients with carcinoma, had no detectable nuclear or cytosolic ERs in the bladder specimens. At the time, this raised the possibility that the positive results were an artifact of contamination by the carcinoma cells, but the investigators had no reason to suspect that urothelial carcinoma but not normal urothelium would have ERs.

Several years later, Noronha and Rao examined urothelial carcinoma specimens from 6 patients, 4 women and 2 men, for the presence of ERs in the cytosol. These included 3 “superficial bladder cancer” biopsy specimens and 3 cases of advanced bladder and renal pelvic carcinomas. They did not detect ERs in any of the localized bladder tumors but did detect them in 2 of the 3 patients with “advanced” disease. Few conclusions could be drawn from such a small study population, and the authors cited the need for additional, larger studies to address the question.

Larger studies of ERs and human urothelial carcinoma were undertaken in the late 1990s. Shan and coworkers used an enzyme-linked histochemical stain (E2-HRP) on frozen sections of 38 urothelial carcinomas of the bladder. These cases included 11 grade I, 19 grade II, and 8 grade III lesions. A positive result was defined as greater than 20% staining in the cells of interest. These investigators realized an overall ER positivity rate of 34.2%; by grade, the ER positivity rates were 36% for grade I, 42% for grade II, and 13% for grade III cases.

Kaufmann and colleagues gathered tissue from 185 PUCs of the urinary bladder, including 84 men (mean age, 68.2 years; range, 29–88 years) and 101 women (mean age, 68.4 years; range, 38–94 years). Using conventional immunohistochemical analysis and then a tyramide amplification technique, they were able to detect ER in 18% and 25% of all PUCs, respectively. With the tyramide staining amplification technique, these investigators found no significant difference in ER detection between male and female patients or different age groups. They did find ER-positive cases to be significantly higher among invasive lesions and higher-grade lesions (grade 2/3 vs grade 1).

The current study also used paraffin-embedded tissue and immunohistochemical analysis, with similar results to the study by Kaufmann et al. Using a threshold of 10% to define a positive lesion, similar to that used in evaluation of breast carcinoma, 11% of the 92 primary bladder carcinomas expressed ERs. When stratified by the WHO 1973 grading system, the percentage of cases considered positive increased from 0% for grade I to 17% for grade III, but this trend did not achieve statistical significance. Because some investigators and some grading systems do not consider grade I lesions to be true carcinomas, but rather “papillary neoplasms of low malignant potential,” the analysis was repeated grouping all grade II and III lesions together; this resulted in a difference of only marginal statistical significance. There were no statistically significant differences between ER-positive and ER-negative tumors when comparing the age and sex of the patients, but women with ER-positive bladder carcinomas were significantly older than their male counterparts when using the 10% positive staining threshold. The presence or absence of invasion was at most of only marginal significance, with invasive PUCs more likely to be ER positive.

With so little known about the significance of the presence of ERs in PUCs, it was difficult to establish a meaningful threshold to define a positive result. Recognizing the relatively arbitrary selection of 10% staining as a cutoff, the statistical analyses were performed again using any percentage of ER staining as a positive result. Reclassification effectively doubled the number of positive cases to 22%, a result similar to that realized in the study by Kaufmann et al. This increase translated into statistical significance for the independent variables of grade and invasion. These findings mirrored those of the study by Kaufmann et al, in which the authors also found that ERs were significantly more likely to be detected in higher-grade PUCs and invasive PUCs. In addition, a smaller study of ER expression in superficial urothelial carcinomas of the bladder also demonstrated an increased likelihood of ER-positive staining in high-grade lesions, although it failed to demonstrate any relation between ER expression and prognosis. The reason for increased ER expression in high-grade urothelial carcinomas is unclear, with some authors speculating that the heterogeneity of bladder carcinoma, the degree of tumor undifferentiation, or a possible functional role for estrogen may be involved. Despite the increase in positive cases, the variables of age and sex again failed to achieve statistical significance.

All 11 metastatic PUCs were ER negative, including 8 cases from men and 3 from women. These 11 tissue samples came from 9 patients, of whom 8 had their primary lesion analyzed in this study. Only 2 of these corresponding primary PUCs exhibited any ER staining, and both were less than 10%. Although these patients’ metastases demonstrated no appreciable ER staining, it is conceivable that other metastases from these lesions could demon-
strate ERs, a potentially important consideration in the evaluation of metastasis from an unknown primary.

Although ERs were found only in a few cases, the presence of ERs in primary and metastatic PUC may have implications for treatment, specifically with regard to the nonsteroidal antiestrogenic compound tamoxifen. As early as the 1970s, experimentation with mice suggested the possibility that sex hormones could inhibit the growth of bladder tumors. In the early 1980s, Lau and colleagues observed a significant decrease in the recurrence rate of bladder carcinomas in men whose prostate carcinomas were treated with hormone manipulation. More recently, researchers at National Taiwan University Hospital showed that the introduction of tamoxifen into a cocktail of chemotherapeutic agents enhanced cytotoxicity within vitro bladder carcinoma cell lines, but these results failed to translate to a limited trial involving bladder carcinoma patients. A recent case report also describes a male patient whose cutaneous deposits of metastatic bladder urothelial carcinoma vanished after 3 months of daily treatment with 10 mg of tamoxifen for coincident gynecomastia. However, neither this report nor the studies from Taiwan evaluated the neoplastic cells for the presence of ERs. Nevertheless, some institutions have initiated routine immunohistochemical ER evaluation of all urothelial neoplasms, achieving at least anecdotal success with tamoxifen therapy in those patients whose lesions exhibit ER staining of 10% or greater.

Potential confounding factors not addressed in this study include the effects of each patient’s hormone status and the area of the bladder from which the tissue samples were obtained. At least one previous study suggested that the bladders of premenopausal and postmenopausal women may exhibit different amounts of ERs, but the results did not achieve statistical significance. Hormone status may play a role in male patients as well. Whether the female patients were premenopausal or postmenopausal or whether they were undergoing hormone replacement therapy was not documented in this study. Hormone status may account for some of the statistically significant difference in the ages of male and female patients with ER-positive PUCs identified in this study.

As previously discussed, male and female bladders have varying ER distributions. The part of the bladder from which a neoplasm arises may influence the ER status of that neoplasm. The location of the tissue samples used in this study was not recorded, because in most cases that information was not available. Unfortunately, even if that information were extant, the many inconsistencies between the previous research into the presence and distribution of ERs in the lower urinary tract would severely limit its utility. Some of these inconsistencies are due in part to small sample sizes, varying receptor detection techniques, and differing notions of what was considered a positive result. In addition, these studies were often unclear as to which were the cells of interest: urothelium, stroma, or both. In the current study, the cells of interest were neoplastic urothelium only. The ER expression in stroma or nonneoplastic urothelium was ignored.

Ovarian TCC is a recently recognized subtype of ovarian surface epithelial-stromal tumor morphologically similar to bladder urothelial carcinoma, differing from malignant Brenner tumor by its lack of a benign or borderline Brenner tumor component. The resemblance of ovarian TCC to urothelial carcinoma may lead to questions about the origin of an ovarian lesion composed of transitional-type epithelium. Immunohistochemical analysis can be helpful in answering these questions. Although cyto-keratin 7 has been identified in both ovarian and bladder TCCs, ovarian TCCs also are often positive for vimentin, CA 125, and Wilms tumor protein, whereas bladder TCCs generally exhibit cytokeratin 20 staining and sometimes are positive for thrombomodulin and uroplakin III. Significant cyto-keratin 20 expression in an ovarian lesion with transitional features supports a metastatic urinary tract lesion over a primary ovarian neoplasm. Eleven tissue samples of primary and metastatic ovarian TCCs were captured in this study, and all but one were strongly ER positive. This is in stark contrast to the PUCs, nearly 90% of which were ER negative by the 10% criterion. These results suggest that ER status may be a useful tool in identifying the site of origin for a TCC found in an ovary, particularly when used in combination with cytokeratin 20.

In summary, a few PUCs of the bladder expressed ERs. Depending on the criterion used to define a positive result, up to 22% of these lesions were ER positive, a proportion that corresponds with the findings of a previous, similar study. Higher grade and invasion were significantly associated with ER expression in these bladder carcinomas, whereas age and sex were not. However, women with ER-positive PUCs were significantly older than their male counterparts. The 11 metastatic PUC specimens obtained from 9 patients were all ER negative; 8 of these patients had their primary lesion included in this study, 6 of which were completely negative, with the remaining 2 showing between 1% and 9% of nuclei staining positive for ER. Ten of the 11 primary or metastatic ovarian TCC specimens obtained from 5 patients were strongly and diffusely positive, with the eleventh exhibiting less than 10% ER-positive staining. These findings suggest that ER expression, or more specifically a lack of ER expression, may help differentiate ovarian TCCs from PUCs metastatic to the ovary.

We thank Artemis Chakerian, PhD, and David Viswanatha, MD, from the UNM Department of Pathology Experimental Pathology Laboratory, and Laurie Lundmark from TriCore Reference Laboratories for their assistance with this project.

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Rapid Quantitative Analysis of Human Cytomegalovirus DNA by the Real-Time Polymerase Chain Reaction Method

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Context.—Human cytomegalovirus (CMV) infection is a progressive and life-threatening complication in immunocompromised patients even now. Therefore, early and accurate treatment based on rapid and certain detection is needed to prevent fatal CMV infection diseases.

Objective.—To study a quicker, simpler, and less expensive method of quantitative analysis using real-time polymerase chain reaction based on the SYBR Green I method of CMV detection for appropriate treatment of CMV infection in immunocompromised patients.

Design.—We quantified 50 samples tested by direct immunoperoxidase staining of leukocytes with peroxidase-labeled monoclonal antibody (C7-HRP test), 30 samples from healthy persons, and 47 samples from 7 patients suspected of having CMV infection diseases. We used the primer set in the pp65 gene of CMV and whole blood without a preparatory process. The setting for the study was the First Department of Pathology, Kurume University School of Medicine, St Mary’s Hospital, and the Gene Section of the Clinical Laboratory at St Mary’s Hospital, Fukuoka, Japan.

Results.—The results obtained with this method corresponded well with conventional C7-HRP tests and demonstrated excellent reproduction. Additionally, the results were better correlated with the clinical course than were C7-HRP tests.

Conclusions.—This method was more useful than the C7-HRP test as a rapid diagnostic test for early treatment of CMV infection. This test also demonstrated its usefulness for monitoring CMV infection during treatment using ganciclovir. Moreover, it was quicker, simpler, and cheaper than other real-time polymerase chain reaction methods.

CYTOMEGALOVIRUS (CMV) infection in immunocompromised patients has increased in incidence with advances in transplantation and chemotherapy.1,2 An early diagnosis is especially necessary for the accurate treatment of CMV pneumonia, because the symptoms are so serious and the death rate remains high.3,4

The C7-HRP test is the most-used method for diagnosing CMV infection, and it correlates well with the clinical course in pneumonia.2,5 However, it is a complicated procedure with a false-negative rate problem.6 On the other hand, polymerase chain reaction (PCR) is becoming a common method for detecting CMV infection, because it is both quick and sensitive. Although it is currently thought that real-time PCR using probes is the best method7 and many studies have been carried out, this method seems to be too expensive for many laboratories.8

Moreover, the diagnostic usefulness of PCR has not yet been accepted, because it is necessary to distinguish between CMV infection disease and abortive infection.9,10 Therefore, we studied a quicker, simpler, and more cost-saving method of quantitative analysis using real-time PCR based on the SYBR Green I method for appropriate treatment of CMV infection. Additionally, we investigated its usefulness as a marker in treatment with ganciclovir.

MATERIALS AND METHODS

Specimens

We tested whole blood categorized into the following 3 groups.

1. The first group included 50 samples of patients tested with the C7-HRP test from August 28 to September 26, 2001. Of these 50 patients, 32 were men and 18 were women, with an average age of 64 years.

2. The second group included 30 samples from healthy persons who underwent medical examination at our hospital. We selected 5 persons in each decade of life from the 20s through the 70s. Of these, 16 persons were men and 14 were women.

3. The third group included 47 samples from 7 patients with suspected CMV infection diseases after November 2001 that were tested with the C7-HRP test. Of these patients, 3 were men and 4 were women. The average age was 59 years. Their clinical diagnoses were adult T-cell leukemia (3 cases), aplastic anemia (2 cases), acute myelogenous leukemia (1 case), and chronic myelogenous leukemia (1 case).
Controls

Genomic DNA from CMV AD-169 was isolated using the QIAamp blood mini kit (QIAGEN, Hilden, Germany), and the pp65 gene of CMV AD-169 was amplified by the usual PCR method using the primer sets designed by Shibata et al. Their sequences were as follows: forward primer: 5’-AgACTATCAAC TTAATTTCTgATCA-3’, reverse primer: 5’-CCTTCCCTCTTTT TgATTTTgTTT-3’. Polymerase chain reaction products were in 139 bp. This PCR was carried out in a total volume of 50 μL, containing 1.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphates, 1.25 U Taq DNA polymerase, 5 μL 10× buffer, 0.5 μM of each primer, and 0.5 μg genomic DNA of CMV AD-169.

The 139-bp PCR products of CMV AD-169 were purified using the Wizard PCR Preps DNA purification system (Promega, Madison, Wis). The concentration of purified products was measured. The positive controls were 10-fold–diluted solutions of purified products; the negative control was distilled water.

Real-Time PCR

Genomic DNA from whole blood was also isolated using the QIAamp blood mini kit (QIAGEN). The pp65 gene of CMV was amplified from genomic DNA by the real-time PCR method using LightCycler (Roche, Indianapolis, Ind).

Polymerase chain reaction was carried out in a total volume of 20 μL, containing 2 μL genomic DNA, 0.5 μM of each primer (the same primer set used for making the control), 4.0 mM MgCl₂ and 2 μL LightCycler FastStart DNA Master SYBR Green I (Roche). The PCR was performed as follows: there was an activation step of Taq polymerase at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for 0 second, annealing at 65°C for 5 seconds, and extension at 72°C for 10 seconds and at 87°C for 3 seconds. We created the final step (87°C for 3 seconds) to measure the fluorescence signal. After the PCR reaction, the temperature was decreased to 65°C and then gradually raised to 95°C at a rate of 0.2°C/s. The fluorescence signal was continuously monitored during this process for melting curve analysis.

The results were judged positive or negative by the presence or absence of the melting temperature peak of 88°C and by the effectiveness of the quantitative score.

Additionally, 10 μL of PCR products were visualized by 2% agarose gel electrophoresis and ethidium bromide staining.

RESULTS

Correlation and Reproducibility

We tested between 1 and 10⁸ copies/μL of the positive control. The amplification plots are shown (Figure 1). Primer dimers were not obtained during 35 cycles. The target peak of 88°C was obtained between 10² and 10⁶ copies/μL (Figure 2), and a positive band on the electrophoresis occurred at the same point (photo not shown). The correlation of cycle numbers (cycle thresholds) and log concentration was excellent (y = -3.321x + 38.88, R² = 0.998), as shown in Figure 3.

On the other hand, the 3 sample concentrations we chose were different, and these were quantified 3 times each. The average coefficient of variation in simultaneous reproducibility was 0.92% (range, 0.44%–1.50%), and the average coefficient of variation in reproducible day difference during 5 days was 2.17% (range, 1.74%–2.99%).

Comparison With the C7-HRP Test

Fourteen of 50 samples were positive, and 36 samples were negative. Their quantitative scores were as high as 2.089 × 10⁴ copies/μL, and as low as 4.428 × 10³ copies/μL, and a positive band was obtained in all 14 cases for electrophoresis. The matching rate with C7-HRP tests was 82%, but in the 4 samples for which only real-time PCR was positive, the quantitative scores were low (5.860 × 10³ copies/μL, 4.428 × 10³ copies/μL, 5.370 × 10³ copies/μL, and 5.325 × 10³ copies/μL). On the other hand, positive cells of C7-HRP tests were 1 cell in 5 samples, and only the C7-HRP test was positive.

Healthy Persons

Two of 30 cases were positive, but their quantitative scores were low (5.040 × 10³ copies/μL and 9.370 × 10³ copies/μL).

Case Reports

In our cases, the quantitative scores were matched with the results of the C7-HRP tests and decreased after treat-
Figure 2. The melting peak of polymerase chain reaction products in positive control in the range of 1 to 10⁸ copies/µL. The tight melting temperature at the expected T_max of 88°C is shown.

Figure 3. Logarithmic graph of concentration (x axis) and cycle number of cycle threshold (y axis). In the range of 10⁵ to 10⁸ copies/µL, there was a linear correlation.

Treatment with ganciclovir. The range of their quantitative scores was from 5.400 × 10³ copies/µL to 1.129 × 10⁴ copies/µL. We showed a case in which real-time PCR more closely matched the clinical course than the C7-HRP test did. The patient was 36 years old and had been diagnosed with chronic myelogenous leukemia. After the allo–bone marrow transcription, the patient developed fever, and the C7-HRP test was positive. He was diagnosed with CMV infection disease, and treatment with ganciclovir was started. The positive cells of the C7-HRP test and the quantitative score of real-time PCR decreased gradually. The C7-HRP test was negative on day 14, and treatment was stopped. However, the quantitative score was still high (1.435 × 10³ copies/µL). The C7-HRP test done 2 days later was positive again, and treatment was restarted (Figure 4).
COMMENT

Cytomegalovirus is a common opportunistic infection, but CMV infections in immunocompromised patients are progressive and remain fatal.\(^1\)\(^-\)\(^4\) For this reason, preventive treatment using antiviral drugs may be used to protect against occurrences even without confirmation of CMV presence in patients who have undergone organ transplantation.\(^2\)\(^-\)\(^3\) However, antiviral drugs must be used initially for infectious diseases, and they also must be used very carefully to avoid side effects and an outbreak of a resistance virus.

Additionally, in the hematologic case, after bone marrow transplantation, for example, preventive treatment is impossible because of the “bone marrow inhibitory” side effect. Therefore, a rapid and certain method is needed to diagnose CMV infections for early and accurate treatment.\(^3\)\(^,\)\(^9\)

In our hospital, the C7-HRP test is used for diagnosis and monitoring of treatment using ganciclovir for CMV pneumonia, because the test is covered by insurance and the results became positive even before the occurrence of symptoms, correlating with the clinical course.\(^2\)\(^,\)\(^5\) But the C7-HRP test procedure is complicated as a routine test at the laboratory in our hospital. Therefore, we entrust another institution with the test, which takes at least 2 days to be reported.

On the other hand, quantitative PCR is becoming a common method for detecting CMV infection because it is quick and sensitive and has good singularity. Additionally, real-time PCR is faster, as indicated by many reports.\(^7\)\(^,\)\(^10\) Furthermore, the LightCycler system (Roche) used in this study is very simple to use and can save time in the PCR process, observing the real-time amplifying circumstances of PCR products. By analysis of the melting curve of PCR products, we can confirm the target products without electrophoresing on agarose gels.\(^13\)\(^,\)\(^14\) However, although many reports about real-time PCR of CMV have been published, there is no consensus regarding material or the gene amplified. In previous studies, some have used whole blood as material,\(^10\)\(^,\)\(^13\)\(^,\)\(^14\)\(^,\)\(^15\) but most have used plasma\(^10\)\(^,\)\(^13\)\(^,\)\(^14\),\(^15\) or leukocytes.\(^4\)\(^,\)\(^10\)\(^,\)\(^12\) At present, the most commonly used real-time method uses a specific fluorescent probe and can detect CMV in a few hours.\(^7\)\(^,\)\(^16\) Although it is reported to provide better results,\(^4\)\(^,\)\(^16\)\(^,\)\(^17\) this method has not become the norm in laboratory practice because the probes are expensive.\(^8\) Although the SYBR Green I method we used measures dsDNA and is thought to allow many nonspecific reactions, it is simpler, quicker, and less expensive than the probe method.\(^8\)

Additionally, in this study we used whole blood without a sample preparation process.\(^15\)\(^,\)\(^16\) Therefore, the time to reporting from specimen introduction was less than 1 hour.

We had believed that we could quantify only the target PCR products because using hot-start PCR inhibited the nonspecific product and added the fluorescent measuring step after the extension. In fact, primer dimers were not obtained, and quantitative analysis using the real-time PCR method in this study showed excellent correlation and reproducibility.

We also examined 2 other primer sets in the MIE and IE genes besides the pp65 gene in the initial stage of the study. However, a good result was not obtained with these primers. Besides, the primer set we used in the pp65 gene did not cross-react with other microbes (Table).

Some previous studies have compared real-time PCR assay with the C7-HRP test and have reported that the result of PCR was better than that of the C7-HRP test.\(^10\)\(^,\)\(^14\),\(^15\) We also acquired a result almost equal to that of the C7-HRP test, and the result was obtained very rapidly compared with the C7-HRP test.
Moreover, it is well known that the C7-HRP test returns a false-negative result in cases with a marked decrease of white blood cells. Also in this study, there were some cases suspected of being falsely negative for the C7-HRP test in patients with decreased white blood cell counts, because the result did not correlate with the clinical course. However, in our method using LightCycler (Roche), it was more possible to achieve a result that correlated well with the clinical course than with the C7-HRP test in patients with a marked decrease of white blood cells.

We obtained positive results from healthy persons in some cases, but the copy numbers were very low, and there seemed to be no problem in diagnosis after careful consideration of the clinical course. Although the PCR method is a good method with very high sensitivity, usefulness should be confirmed by carrying out comparative examinations, not only in patients but also in healthy people, in order to distinguish simple infections from CMV infection diseases. In our study, a cutoff value of $10^2$ copies/µL was considered appropriate.

Therefore, it is suggested that this method is quicker, simpler, and more cost-efficient than the C7-HRP test or real-time PCR using probes. Furthermore, it is useful not only for rapid diagnosis and early treatment but also as an exact monitor during treatment using ganciclovir in CMV infection diseases. Additionally, it was considered that it could be easily performed in any laboratory as a routine test.

We thank Professor Kouhei Harada, PhD, of the Department of Economics, Kurume University, for statistical advice.

References

List of Microbes Examined for Cross-Reaction

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<td>Aspergillus fumigatus</td>
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Association of Drowning and Myocarditis in a Pediatric Population
An Autopsy-Based Study

Gino R. Somers, MD, PhD; Charles R. Smith, MD; Gregory J. Wilson, MD; Maria Zielenska, PhD; Raymond Tellier, MD; Glenn P. Taylor, MD

Context.—Drowning is a frequent cause of accidental death in childhood, but the association of myocarditis and drowning has only rarely been reported.

Objective.—To report 5 cases of drowning in children with coexistent myocarditis.

Design.—A retrospective review of autopsy records of patients 0 years to 18 years of age was performed during a 6-year period (1998–2003, total cases reviewed = 1431).

Results.—Twenty-two drownings were identified, in 14 male and 8 female children. Five patients (23%), 3 female and 2 male children, had coexistent myocarditis. The 5 patients ranged in age from 23 months to 13 years (mean, 7 years 2 months). None of the patients had antecedent symptomatology suggestive of myocarditis. In all patients, the myocarditis was focal mild or moderate, and the inflammatory infiltrate comprised lymphocytes with smaller numbers of neutrophils. All 5 patients had foci of myocyte necrosis. One patient had histologic evidence of myocar-dial hypertrophy but no evidence of a cardiomyopathy. Microbiologic studies, including culture, immunohistochemistry, polymerase chain reaction, and reverse transcriptase polymerase chain reaction, revealed Mycoplasma pneumoniae DNA in 1 case.

Conclusions.—The finding of myocarditis in a significant proportion of drowning victims in this series highlights the importance of a thorough autopsy examination in apparently straightforward cases and has clinicopathologic significance.

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Drowning is the second leading cause of accidental death in the pediatric population.¹⁻⁴ At autopsy, findings are often nonspecific, and the diagnosis rests on a combination of circumstances and clinicopathologic correlation.⁵⁻⁶ Some authors consider the diagnosis to be one of exclusion.⁵⁻⁷ Occasionally, after careful review of the history and analysis of the laboratory studies, additional findings are uncovered that suggest a more complex series of events resulting in death. Usually, such findings are related to central nervous system or cardiovascular pathology,⁷⁻⁸ but the coexistence of myocarditis is rare.⁹⁻¹⁰ This study describes the occurrence of myocarditis in 5 children whose deaths were thought to be straightforward cases of drowning.

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The authors have no relevant financial interest in the products or companies described in this article.

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MATERIALS AND METHODS

Autopsy Review

Autopsy records for the period January 1, 1998, to November 30, 2003, were examined for cases of drowning (total number 1431: 739 forensic cases, 692 hospital cases).

Histologic Analysis

Routine sections of heart taken at autopsy included right ventricular inflow and outflow tracts, left ventricular anterior and posterior papillary muscles, and interventricular septum. Sections were fixed in 10% buffered formalin overnight and processed for routine histologic analysis. Sections were cut at 4 μm, mounted on glass slides, and stained with hematoxylin-eosin using routine methods.

Sections of myocardium were reviewed, and the myocarditis was classified using the Dallas criteria.¹¹ However, these criteria were designed for endomyocardial biopsies. In addition, the definitions of focal, diffuse, mild, moderate, and severe myocarditis are not clearly defined by the Dallas criteria. Therefore, for the purposes of this study, an arbitrary working classification of myocarditis severity was designed. Mild myocarditis was defined as fewer than 4 clusters of interstitial lymphocytes per ×40 field; moderate myocarditis was defined as between 4 and 7 clusters of lymphocytes per ×40 field; and severe myocarditis was defined as 8 or more clusters of lymphocytes per ×40 field. All cases required the presence of myocyte necrosis, and the myocarditis was classified on the most severely affected field. For the myocarditis to be classified as diffuse, all sections needed to be involved, and some degree of confluence of the infiltrate was required.

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RESULTS

Autopsy Review

During the 6-year period, 1431 autopsies were performed at our institute in infants and children 0 years to 18 years of age. Of these, 739 were forensic autopsies, which constituted 25% of all forensic autopsies performed in children younger than 18 years in the province of Ontario. Twenty-two drownings were identified in 14 male and 8 female children whose ages ranged from 6 months to 17 years (mean, 4 years 3 months). Five patients (23%) had coexistent myocarditis; 3 of these were female and 2 were male children. The 5 patients ranged in age from 23 months to 13 years (mean, 7 years 2 months). None of the 5 patients had antecedent symptomatology suggestive of myocarditis.

Clinical Details

Patient 1.—A previously well girl, 12 years 5 months old, was swimming in shallow lake water (approximately 90 cm deep) with friends. There was no adult supervision, and she was described as a good swimmer. She complained of feeling “dizzy” and then floated on her back for a short period of time. Approximately 5 minutes later, she was found face down and unresponsive. Her friends pulled her out of the water. Upon arrival of emergency personnel, she had no vital signs and was pronounced dead after 30 minutes of resuscitative efforts. 

Patient 2.—A previously well boy, 4 years 7 months old, was swimming in a stream with a moderate flow of water at a public park. He was with his 11-year-old sibling and under adult supervision. He was swept under tree branches and carried further downstream. The supervising adult was unable to find him in the stream, and his body was recovered approximately 2 km downstream, 1 hour after having disappeared. He was pronounced deceased after 30 minutes of resuscitative efforts.

Patient 3.—A previously well girl, 4 years 2 months old, was left on the dock by a cottage while her older siblings took a boat out onto the lake. Approximately 1 hour later she was found head down in the water. Resuscitative attempts by family at the scene and by emergency personnel were unsuccessful, and she was pronounced deceased 2 hours later.

Patient 4.—A previously well 23-month-old girl was found face down and unresponsive in a steep-banked
A previously well 13-year-old boy was attempting to swim to a raft on a lake. He was described as not a strong swimmer. He began to struggle, and a bystander (approximately 15 years old) attempted to come to his aid but had to kick him away to avoid being pulled underwater. His body was recovered a few hours later, and he was pronounced deceased at the scene.

Autopsy Findings

The cardiac anatomy and gross appearance of the myocardium was unremarkable in all patients. The heart weights of the 2 oldest patients (patients 1 and 5) exceeded the standard for body length by 14% and 34%, respectively (Table). In both patients, the weights of the systemic organs (liver, kidneys, pancreas) were also proportionately increased above the standards for body length (re-
sults not shown). Furthermore, there was no gross evidence of a cardiomyopathy or a second disease process.

Histologically, all 5 patients had focal myocarditis. Two patients were classified as mild (patients 1 and 5) and 3 as moderate (patients 2, 3, and 4) using the definitions outlined in the “Materials and Methods” section. All patients had an infiltrate comprising lymphocytes and occasional neutrophils, as well as foci of myocyte necrosis (Figure 1). Microscopic foci of myocardial hypertrophy and interstitial fibrosis were present in patient 1; however, neither patient 1 nor patient 5, with the proportionately heavier hearts, had evidence of myofiber disarray, endocardial fibroelastosis, or diffuse fibrosis and alternate fiber hypertrophy and atrophy suggestive of a cardiomyopathy. No viral inclusions, infective organisms, or evidence of a vasculitic process were present in any of the sections examined. Significant unexpected extracardiac pathology was not seen.

Immunohistochemistry, PCR, and Enteroviral RT-PCR

No staining for adenovirus, parvovirus B19, measles virus, or cytomegalovirus early antigen was present in any of the myocardial samples. DNA was successfully extracted from all cases, 1 of which (patient 2) was positive for \textit{M. pneumoniae} DNA (Figure 2). RNA was successfully extracted from all cases, none of which was positive for enterovirus transcripts (Table).

COMMENT

Drowning is the second most common cause of accidental death in children in the developed world.\textsuperscript{1-4} Post-mortem examination of drowning victims may reveal positive findings, such as a white frothy exudate from the mouth and nose and bulky edematous lungs. Although characteristic, these findings are not specific for drowning,\textsuperscript{5-6} and an accurate account of the events leading up to death are necessary for the diagnosis to be made with any certainty.\textsuperscript{5,7} Occasionally, review of the history and post-mortem examination reveals additional factors that may have contributed to death. In 2 published series of childhood drownings, 9 (14%) of 65 patients had other significant factors: 6 had epilepsy, 1 had an intellectual handicap, 1 suffered a subarachnoid hemorrhage while in the water, and 1 was found to have a severely hypoplastic right coronary artery.\textsuperscript{7,8}

In the present series, 23% of patients who drowned were found to have coexistent myocarditis. An infectious cause was established in patient 2, where PCR revealed \textit{M. pneumoniae} DNA in cardiac tissue. In the other 4 patients, no infectious cause was established by immunohistochemistry, PCR, or RT-PCR. There was no unexpected significant extracardiac pathology. Cardiac output was not re-established in any of the patients, and all patients were pronounced deceased within a few hours of the drowning episode. Interestingly, 2 patients had heart weights above the expected weight based on body length. Detailed gross and microscopic examination of the heavier hearts did not reveal evidence of a cardiomyopathy or a second disease process, and no cause for the increased heart weight could be established. However, the weights of the systemic organs (liver, kidneys, pancreas) were also proportionately increased above the standard for body length in both patients.

Subclinical myocarditis is a well-described cause of sudden unexpected death in children and adults.\textsuperscript{21} Because many patients are asymptomatic, clues as to its true prevalence have come from autopsy studies. Such studies have identified myocardial inflammation in 1% to 9% of otherwise routine autopsies.\textsuperscript{21-24} However, many of these studies were performed prior to the introduction of the strict Dallas criteria\textsuperscript{11} for the diagnosis of myocarditis; thus, comparison with present-day studies is difficult. Etiologies of myocarditis include viral infection, particularly enteroviruses such as coxsackie virus; bacterial infections, including \textit{M. pneumoniae} (as in patient 2); drugs and toxins; and autoimmune diseases, especially systemic lupus erythematosus, scleroderma, and sarcoidosis.\textsuperscript{21} \textit{Mycoplasma}-associated myocarditis is an uncommon complication of a common pathogen. In a recent review of 21 cases of \textit{Mycoplasma}-associated myocarditis, the clinical features ranged from an absence of symptoms to rapid-onset cardiac failure and death.\textsuperscript{22} Most patients were adults, but the youngest patient was 4 years of age (as in the present patient).
The finding of incidental myocarditis in accidental and traumatic deaths has been reported previously in both adults and children. For example, myocarditis has been described in motor vehicle and building site accident victims, homicide victims, and pilots involved in fatal aircraft accidents. However, the co-occurrence of myocarditis and drowning is rare. Two cases of myocarditis have been reported in the German-language literature, both occurring in adult women while swimming. A similar case has been published in the English-language literature, that of a previously well 7-year-old boy who died while immersed in a whirlpool. Autopsy showed no positive features of drowning. Histologic analysis revealed isolated foci of lymphocytes within the myocardium, and RT-PCR for coxsackie B3 virus on RNA extracted from formalin-fixed cardiac tissue was positive.

Myocarditis is a well-known cause of sudden death. However, when other potential causes of death are also present, the significance of myocarditis is more difficult to determine. This is not an unusual phenomenon in autopsy examination; when more than 1 lesion known to cause death is present, determining the precise role of each in the fatal episode may be problematic. Nevertheless, given that both myocarditis and swimming increase the risk of arrhythmias, the combination of both may predispose the individual to sudden cardiac death. Furthermore, in a mouse model of coxsackie myocarditis, mice that were forced to swim after infection showed increased levels of virus during the acute stages of the infection and had significantly higher mortality rates compared with mice that were rested. The circumstances surrounding the death of patient 1 seem to suggest a role for myocarditis in causing death: a good swimmer, in shallow water, complaining of feeling “dizzy.” However, in the other 4 cases, there was insufficient eyewitness information to draw firm conclusions as to the role of myocarditis in the fatal episode.

The present study indicates that cardiac pathology sufficient to raise a competing hypothesis for the cause of death may be seen in apparently straightforward cases of drowning. This has obvious implications for the families involved and for death certification and highlights the need for thorough autopsy examination and clinicopathologic correlation.

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References
Clinical Diagnoses and Autopsy Findings

A Retrospective Analysis of 252 Cases in Greece

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Context.—Despite medical and technologic advances, clinicians may misdiagnose a patient’s situation and the cause of death. Autopsy may be valuable in uncovering the most frequent diagnostic pitfalls and helping clinicians to learn and to develop the medical art and science.

Objective.—To compare the clinical diagnoses with postmortem findings and evaluate the frequency of diagnostic errors assessed by autopsies.

Design.—Retrospective analysis of the protocols of 252 consecutive cases of adult patients autopsied in the Department of Forensic Medicine and Toxicology of Athens Medical School during the period 1999–2003. The outcome measures included concordance between diagnosis before death and at autopsy, sex, age, and length of hospitalization of the patient.

Results.—In 73 cases (29%), the autopsy findings confirmed the clinical diagnosis and the cause of death suggested by the clinicians. In 45 cases (19%), the clinical diagnosis and the cause of death suggested by the clinicians were discordant with the autopsy findings. In 105 cases (42%), the autopsy requests did not include any suggestion about the cause of the patient’s death. In 7 cases (3%), several diagnoses were suggested by the clinicians, and in 16 cases (6%), the comparison between clinical and postmortem diagnosis was not possible. The most frequently misdiagnosed diseases were coronary disease and pulmonary embolism.

Conclusions.—It is concluded from this study that autopsies may reveal unexpected findings that are of critical importance and that a continued emphasis on autopsy evaluation is necessary to improve the quality of patient care.

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The importance of autopsy in clinical practice has long been the subject of discussion, and it has recently attracted even more interest. Autopsy is a valuable tool for evaluating the accuracy of clinical diagnosis, investigating and discovering unsuspected diseases, and recognizing causes of death. Moreover, it is an essential element of medical auditing and teaching, as well as for the study of cause, natural history, and course of diseases. However, the relative number of autopsies performed over recent decades has been declining worldwide.1–4 This fact has been attributed to increased costs, fear of malpractice litigation, and advances in medical technology.5–8

Undoubtedly, the recent technologic advancements in medical areas have allowed more sensitive and reliable methods for clinical diagnosis during life; nevertheless, significant discrepancies between clinical diagnosis and autopsy findings for patients who died in the hospital have been reported, and it is possible that autopsy may reveal unexpected data that, had the information been known before death, may have changed patient management.7,11

MATERIALS AND METHODS

Autopsies in Greece are performed by forensic pathologists only after a juridical order. Autopsy is mandatory in case of sudden or violent death. Most cases concern patients whose situation or short stay in the hospital did not allow the establishment of a clear cause of death. In a smaller number of cases, the order for an autopsy may arise as a consequence of a legal procedure for medical negligence.

The aims of the present study were to compare the clinical diagnosis with the autopsy findings of cases referred to the Department of Forensic Medicine and Toxicology of Athens Medical School during the period 1999–2003 and to highlight the most common discrepancies. It is worth mentioning that, to our knowledge, it is the first such study conducted in Greece.

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A total of 252 cases of patients who were hospitalized and died in a hospital and whose postmortem examination was referred to our department during the period 1999–2003 were retrospectively analyzed. The cases were consecutively selected according to the registration number of the autopsy records. The criteria for exclusion were as follows: (1) age of patient younger than 15 years old, (2) time of stay in hospital less than 1 hour, and (3) violent death. Data concerning the clinical diagnosis established during life, as well as the cause of death suggested by the clinicians, were collected from the autopsy requests.

Complete autopsy was performed within 24 hours of death, and the procedure included macroscopic examination and histopathologic investigation of the internal organs. Toxicologic analysis was also performed when indicated. Causes of death were classified according to the 9th edition of the International Classification of Diseases.
Cases were categorized into 5 groups: group 1, cause of death suggested by the clinicians and confirmed by the autopsy findings: concordant cases; group 2, cause of death suggested by the clinicians but not confirmed by the autopsy findings: discordant cases; group 3, cause of death not suggested by the clinicians; group 4, several causes of death suggested by the clinicians; and group 5, comparison between clinical and postmortem diagnosis not possible.

Statistical Classification of Diseases and Related Health Problems,12 which is still in use in Greece.

Data were grouped into 5 categories: (1) cause of death suggested by the clinicians and confirmed by the autopsy findings: concordant cases; (2) cause of death suggested by the clinicians but not confirmed by the autopsy findings: discordant cases; (3) cause of death not suggested by the clinicians; (4) several causes of death suggested by the clinicians; and (5) comparison between clinical and postmortem diagnosis not possible.

Age, sex, and length of stay in hospital were also analyzed. Statistical analysis was done using a 2-tailed $x^2$ test.

**RESULTS**

Of the 252 patients who underwent autopsy, 148 (59%) were men and 104 (41%) were women. Eighty-nine percent (224 cases) of patients were 60 years old or older, and 11% (28 cases) were younger than 60 years old (average age, 75 years; SD, 13 years).

In 73 cases (29%), the autopsy findings confirmed the clinical diagnosis and the cause of death suggested by the clinicians. In 49 cases (19%), the clinical diagnosis and the cause of death suggested by the clinicians were discordant with the autopsy findings. In 106 cases (42%), the autopsy requests did not include any suggestion about the cause of the patient's death. In 8 cases (3%), several diagnoses were suggested. Finally, in 16 cases (6%), the comparison between clinical and postmortem diagnosis was not possible. These last cases concerned patients whose death was due to senile myocardium degeneration and old age. The results are shown in the Figure.

The numbers of concordant cases according to the sex, age, and length of hospitalization of the patient are shown in Table 1.

Deaths due to complications of coronary disease were the great majority in our study, 105 cases (42%). Autopsy revealed a myocardial infarction in 69 cases. Coronary artery occlusion was the cause of death in 32 cases and ischemic cardiac disease in 4 cases. In 19 cases (18%), a diagnosis of coronary artery disease had successfully been established during life. In 24 (23%) of these cases, a discrepancy between the autopsy findings and the clinical diagnosis was observed (Table 2). A cause of death was not mentioned by the clinicians in 60 (57%) of these cases.

Several clinical diagnoses were suggested in 2 (2%) of these cases.

The clinical diagnosis of acute myocardial infarction was wrongly established in 2 cases, which were proved to be aneurysm rupture of thoracic and abdominal aorta, respectively.

The presence of coronary artery disease as the cause of death was suspected at the same percentage (16%) for both sexes (11/67 cases for men, and 6/36 cases for women).

Pulmonary embolism was found in 23 cases. In only 2 (7%) cases had this diagnosis been suspected during life. In 2 cases, the existent malignancy was regarded as the cause of death, which undoubtedly was the underlying cause of death, although the immediate cause was pulmonary embolism. In 16 cases, no potential cause of death was suggested by the clinicians, whereas in 2 cases a diagnosis of aspiration pneumonia was made. There was also 1 case for which 3 diseases were listed in the differential diagnosis: respiratory infection, acute cholecystitis, and mesenteric obstruction.

Pneumonia was found in 16 cases. Clinical diagnosis of pneumonia was established before death in 7 cases (44%), whereas in 5 cases a potential cause of death was not suggested. There were discrepancies in 3 cases; in these cases, pulmonary embolism, heart disease, and sepsis were the presumed causes of death. In 1 of the pneumonia cases, more than 1 cause of death was suggested (pneumonia and ischemic heart disease).

A clinical diagnosis successfully addressed the cause of death in only 2 of 4 cases of patients who died of peritonitis. In 1 case, the patient was hospitalized because of ileum, but the death was attributed by the clinicians to pulmonary embolism or respiratory infection. For the last of the 4 cases, no cause of death was suggested.

The autopsy revealed malignancy as a new finding in 10 (34%) of 29 cases. In the rest of the cases, the clinicians were aware of the malignancy.
COMMENT

Our study has demonstrated poor agreement between the clinical diagnosis before death and postmortem findings in patients who died during their hospitalization. A clinical diagnosis successfully addressed the cause of death in a relatively low number of cases (29%). Disagreement between clinical diagnosis and postmortem findings was observed in 18% of the total number of cases. Moreover, our study revealed a considerable number of cases (42%) for which a potential cause of death was not mentioned by the clinicians. Our goal was simply to draw attention to some of the most common misdiagnoses and omissions and to highlight the need for clinical physicians to request autopsies even in cases in which they consider themselves certain of diagnoses.

Reported studies concerning patients who died in hospitals have shown a 12% to 37% rate of discordance.\textsuperscript{10,13–16} In intensive care units this rate ranges from 20% to 34%.\textsuperscript{11,17–19} Evaluating the results of these studies is rather difficult, because various factors may influence the rate of correctly diagnosed causes of death, such as the rate of autopsy,\textsuperscript{20} the patient’s age,\textsuperscript{21,22} the length of hospitalization of the patient,\textsuperscript{23–25} the distance between location of death and site of examination,\textsuperscript{26} and the degree of clinical confidence.\textsuperscript{27,28}

It must be pointed out that in Greece, every case of sudden death must be subjected to autopsy. The definition of sudden death is rather unclear and varies according to the authority and the convention. The World Health Organization defines it as a death that occurs within 24 hours from the onset of symptoms, although many clinicians and pathologists believe that this is too long. In Greece, this definition is generally accepted. Moreover, another common reason for medicolegal investigation is the “unexplained” death, in which the clinician is unable to determine a cause of death, although the patient was under medical care. In such cases, because a medicolegal investigation has been requested, the Greek clinician is not obliged to report a possible cause of death, and the clinician may prefer to obscure his or her opinion. Other reasons for not completing the autopsy request include 3 already described in the literature: the lack of time, the urgent need of medical care for patients who are still alive, and the insufficient number of available physicians.\textsuperscript{14}

In 6% of the total number of cases, no specific findings were discovered except a general senile atrophy of most organs and old age. Moreover, the history was unhelpful as to a specific mode of death, and any unnatural cause was excluded. According to the World Health Organization system for classification of deaths, it is quite legitimate to ascribe the death to “myocardial degeneration due to senility,” although many cardiac pathologists seem reluctant to agree to the existence of this condition.\textsuperscript{29} Senile myocardial degeneration is classified in both the \textit{International Classification of Diseases}, 9th edition (429.1),\textsuperscript{29} and the \textit{International Classification of Diseases}, 10th edition (I51.5).\textsuperscript{30}

Another matter that deserves discussion is the possible bias towards lower levels of concordance because of the selection of cases. We reasonably consider that the most autopsy requests are obtained for those cases with diagnostic problems. Thus, we can presume that many of the unstudied cases include those in which clinicians were more confident of their diagnoses. An autopsy in these cases would improve the concordance rate. This hypothesis is supported by the data described by Hartveit\textsuperscript{26} and Britton,\textsuperscript{27} who reported that autopsies changed clinical diagnoses in only 19% to 25% of the cases in which the clinician was certain about the cause of death, but that this percentage increased to as much as 35% to 45% of the cases in which the clinician was uncertain. Nevertheless, other studies have shown that the confidence of the clinicians does not alter the rate of misdiagnosis and that there is no way to identify from the clinical data the autopsies likely to have discrepant findings.\textsuperscript{22,23,32}

Our observation that the concordance rate was higher among the patients younger than 60 years old compared with the patients 60 years old and older indicates that patient age could be an influencing factor. However, the small number of cases (n = 28) in the first group does not allow us to fully support this hypothesis. Positive correlation between increasing age and incorrect diagnoses has been reported by Cameron et al\textsuperscript{33} and Veress and Alafuzoff.\textsuperscript{34} Goldman et al\textsuperscript{21} noted a higher percentage of misdiagnoses among patients younger than 40 years old, as well as in those older than 65 years. Nevertheless, in a considerable number of previous studies, no correlation with the age or the sex of the patient has been found.\textsuperscript{17,23–36} No differences between the 2 sexes were observed in our study.

The rate of discrepancies did not differ between the group of patients hospitalized for 24 hours or less and the group hospitalized for more than 1 day. The relevant results in the literature are conflicting. Pobregar et al\textsuperscript{35} and Battle et al\textsuperscript{36} did not find any correlation between the length of hospitalization and the rate of discrepancies in an intensive care unit and a community hospital, respectively. Drexler et al\textsuperscript{24} reported an increased frequency of misdiagnosis related to a decrease in the duration of hospitalization. Increased rate of misdiagnosis with increased duration of hospitalization has frequently been reported,\textsuperscript{21–23} which may be attributed to the diversion of the staff’s attention toward newly admitted patients and their acute disturbances, as well the higher rate of undiagnosed infections.\textsuperscript{23,37}

The most commonly missed clinical diagnoses in our study were pulmonary embolism and coronary disease, at rates of 93% and 84%, respectively. Malignancy as a new finding was also revealed in a considerable percentage (34%) of the cases. Pulmonary embolism has been the most common misdiagnosed condition in various studies, with 23% to 87% of the cases of pulmonary embolism revealed only in the autopsy.\textsuperscript{16,28,38,39} The literature has also shown 17% to 24% of the myocardial infarctions,\textsuperscript{14,35} 26% to 44% of the neoplasias,\textsuperscript{16,40–42} and 67% of the pneumonias\textsuperscript{43} to be first diagnosed at the autopsy.

In 2 cases of aortic aneurysm rupture, a myocardial infarction had been presumed. It is a fact that patients with aortic dissection may present symptoms mimicking acute myocardial infarction.\textsuperscript{43–45} Thrombolytic treatment given to these patients seems to lead to fatal outcomes. In patients with chest pain symptoms without typical history and electrocardiographic changes, the diagnosis should be considered with echocardiographic findings. Furthermore, quick, accurate diagnosis in aortic dissection can be lifesaving.

Except for aortic dissection, many life-threatening disease processes may share similar symptoms with myocardial infarction, because of the fact that chest pain may arise from any structure located in the thoracic cavity.
cases that require immediate attention and intervention are pericarditis, pulmonary embolism, pneumothorax, and pneumonia. Respiratory problems as the cause of death in cases of coronary disease were the most common diagnostic errors in our study. In 7 cases of coronary disease, a diagnosis of cerebral stroke had been suggested by the clinicians. Myocardial infarction presenting solely as an acute, severe headache is underdiagnosed in elderly patients. We might suppose that the manifestation of a myocardial infarction as a headache could be a justification for its misdiagnosis as cerebral stroke. However, the high frequency with which this is reported in our study is in contradiction with the limited number of cases reported in the literature. In 2 cases, the diagnosis of peritonitis was missed. Peritonitis may have different causes, the most frequent ones being acute appendicitis and perforating ulcer. Related infections, when present, amplify, modify, or, on the contrary, make the symptoms confused. Mortality has been reported to be high (25%–50%).

In our study, we found significant discrepancies between clinical diagnosis and autopsy findings. Autopsy has double roles: it is both a method by which to detect diagnostic errors and a source of knowledge to be applied to future cases, influencing learning and adding data on local epidemiology of diseases and quality control for technical investigations. In a large cross-sectional study and a review of published studies, higher autopsy rates in teaching hospitals than in nonteaching hospitals were associated with fewer diagnostic errors. This reinforces the significance of autopsy in detecting unexpected diagnoses, and it should encourage clinicians to remember the value of postmortem examination in case of doubtful cause of death. Furthermore, autopsies can provide valuable information for quality control of the health care system and the full development of medical art and science. Accepting imperfections, detecting errors, and learning from them are essential attitudes in order to improve the patient care given by clinicians.

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Primary Localized Laryngeal Amyloidosis

Report of 3 Cases With Long-term Follow-up and Review of the Literature

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Context.—Localized laryngeal amyloidosis is an uncommon condition with limited long-term follow-up studies. The precise etiology and pathogenesis are not entirely clear.

Objective.—To further characterize the histopathologic features and possible pathogenesis of localized laryngeal amyloidosis.

Design.—Three cases of primary localized laryngeal amyloidosis were identified at our institutions from 1980 to 2003. The clinical features and histologic and immunohistochemical patterns were evaluated. Systemic workups were pursued during the long-term follow-up.

Results.—The common presentation of the patients was hoarseness. The lesions involved vocal cords, anterior commissure, and ventricle. Microscopically, the amyloid was present within the submucosa with an adjacent lympho-plasmacytic infiltrate. The plasma cells and amyloid demonstrated monoclonal light chain restriction in all 3 cases (2 λ, 1 κ). No evidence of systemic amyloidosis or an overt B-cell lymphoma was found in these patients. Two patients with long-term follow-up underwent subsequent surgical removals for multiple recurrences, which occurred within 1 year of the initial diagnosis.

Conclusions.—The demonstration of monoclonal light chain expression in the plasmacytic infiltrate and amyloid component in the absence of systemic lymphomas indicates that localized laryngeal amyloidosis may represent a form of benign monoclonal plasma cell dyscrasia. A close follow-up of the patients may be indicated for early detection of recurrences.


Amyloidosis is a group of disorders in which an extracellular deposition of an abnormal amount of proteins occurs in a variety of organs, including the larynx. Von Rokitansky first described such deposits in 1842, but it was not until 1851 that Virchow applied the term amyloid to describe this deposition. Clinically, amyloidosis is divided into 2 categories: systemic and localized. Approximately 9% to 15% of amyloidosis is of the localized type. Localized amyloidosis in the head and neck is a rare and benign disease. The larynx is the most common site of involvement and accounts for 0.2% to 1.5% of benign laryngeal tumors. Within the larynx, the vocal folds and ventricle are more commonly affected. Presenting symptoms include hoarseness, sometimes cough, and sensation of fullness. Stridor and dyspnea may occur in patients with extensive involvement. Laryngeal involvement could be either diffuse subepithelial deposition (diffuse type) or discrete tumor nodules (nodular type). Histologically, the presence of amyloid can be confirmed by the characteristic Congo red staining under polarized light microscopy, through immunohistochemical stains, or by electron microscopic findings of laryngeal biopsy specimens. In this report, we examined 3 cases of localized laryngeal amyloidosis in our institutions from 1980 to 2003 and reviewed clinical, pathologic, histochemical, and immunohistochemical aspects of the disease.

REPORT OF CASES

Patient 1 (Table) was a 36-year-old man who presented with several months of hoarseness at the end of 1989. He was neither a smoker nor drinker. His medical history was unremarkable. Laryngoscopic examination at initial presentation revealed small nodular lesions that involved the anterior two thirds of both vocal cords and the anterior commissure. Microdirect laryngoscopy with forceps excision was performed. The lesion was biopsied and diagnosed as laryngeal amyloidosis. All other laboratory findings, including chest x-ray examination, blood workup, and serum and urine protein electrophoresis, were normal. The first recurrence was 1 year later in the beginning of 1991, involving the same location. Later there were multiple recurrences, and the patient underwent subsequent microdirect laryngoscopy and forceps excisions in 1992, 1993, 1997, and 1999. With each additional procedure, the size of the lesion increased from 1.5 to 3.0 cm in greatest dimension. In 2000, the patient care was transferred to one of the authors (S.B.L.), and laryngoscopic examination revealed that the endolarynx was filled with multiple large polypoid lesions that involved the entire supraglottis and anterior commissure, with a compromised endolaryngeal lumen. The lesions were treated with a combination of forceps excision and carbon dioxide laser. Two additional similar procedures were performed in 2000 and again in 2002. During the last procedure, the patient experienced an intraoperative pneumothrax that was treated with a thoracostomy tube and tracheotomy. Six weeks later, the lesion was debulked using a powered laryngeal microdebrider, permitting the surgeon to achieve a more extensive debulking than had previously been performed. The tracheotomy...
was decannulated, and the site healed without incident. A second debulking with the microdebrider and standard endotracheal intubation was performed 5 months later. No further procedures have been performed in the subsequent 12 months. Despite the repeated recurrences, his disease remains limited to the larynx only (Figure 1).

Patient 2 (Table) was a 50-year-old man who presented with a 2-year history of hoarseness, which was worsening with use and unresponsive to steroid therapy. His medical history included mitral valve prolapse, status post appendectomy, and bilateral nephrolithotomy. Medication included aspirin. He did not smoke and was only a social drinker. He had no radiation or major trauma to this site. Indirect laryngoscopy showed thickened and lobulated vocal cords, particularly on the right side, with normal movement of the vocal cords. The lesions were removed during direct laryngoscopy with a carbon dioxide laser, and he was diagnosed as having laryngeal amyloidosis in 1986. Results of urine and serum protein electrophoresis performed in the same year were negative. Serum antinuclear antibody test results were negative. A year later, a large amount of amyloid was seen in the subglottic pads (bilateral) and anterior commissure. Most of the amyloid was removed at that time. The amyloid in the anterior commissure was excised a few months later. In 1988, the patient was noted to have subglottic amyloid anteriorly on the left side and posteriorly on the right side, which was again removed with a carbon dioxide laser. The patient tolerated the procedures well and was followed up by a speech therapist. In 1993, his follow-up laryngoscopy showed a small white mass on the right vocal cord, which did not require excision. Since then, he was lost to follow-up.

Patient 3 (Table) was a 56-year-old man who presented with a 5-month history of hoarseness. Past medical history included hypertension and hypercholesterolemia. He was treated with steroid and antibiotics with no improvement. Laryngoscopic examination revealed a 1.0-cm polypoid lesion in the right false vocal cord and ventricle. The extensive search for other locations of amyloidosis was negative. No lymphoproliferative disorder was detected. The polypoid lesion was removed by a carbon dioxide laser.

**PATHOLOGIC FINDINGS**

Grossly, the specimens from all 3 patients were similar and consisted of multiple friable, gray to tan soft tissue fragments, ranging in size from 1.0 to 3.0 cm. Histologic examination of the specimens from all 3 patients revealed diffuse submucosal globular deposition of amorphous, acellular, eosinophilic material on hematoxylin-eosin-stained slides (Figure 2, A). Sparse mixed chronic inflammatory infiltrate, which consisted predominantly of mature plasma cells and lymphocytes, was present at the periphery of the amyloid deposits (Figure 2, A). Some of the amyloid material was observed around the stromal blood vessels (Figure 2, B) and the residual submucosal glands (Figure 2, C). The overlying squamous mucosa occasionally showed reactive changes and mucosal ulceration with secondary microthrombi in submucosal vessels (patient 3; Figure 2, D). Foreign-body giant cell reactions were easily identified adjacent to the amyloid deposits (patient 1; Figure 2, E). Congo red stains revealed apple-green birefringent material under polarized light (Figure 2, F), confirming the diagnosis of amyloidosis.

Immunohistochemical staining for lambda and kappa demonstrated light chain restriction in the infiltrating plasma cells and the amyloid substance in all patients, indicating the nature of the amyloid deposits being monoclonal immunoglobulins. Patients 1 and 3 showed solely lambda light chain staining (data not shown), whereas patient 2 displayed kappa light chain restriction (Figure 3). Electron microscopy was performed on the specimen from patient 1 and revealed characteristic interlacing meshwork of nonbranching fibrils (data not shown).

**COMMENT**

The first case of localized laryngeal amyloidosis was documented by Borow in 1873. Until 1990, only approximately 300 cases of upper airway amyloidosis were reported in the literature. This condition most commonly occurs in the fourth to sixth decades of life, with a male-female predominance of 3:1.

In 1980, Glenner proposed the following classification system for amyloidosis: (1) the type of amyloid protein, such as AL (immunoglobulin light chain amyloid) and AA (secondary amyloidosis); (2) the protein precursor, such as kappa or lambda light chains, trans-thyretin, or beta2-microglobulin; and (3) clinical presentations, such as primary, secondary, myeloma associated, familial, localized, aging, or hemodialysis associated. Laryngeal amyloidosis is usually a localized and primary disease and is classified as AL/κ or λ/primary. In agreement with previously reported series, all cases of our laryngeal amyloidosis occurred as local-
Figure 2. Deposition of amyloid in the submucosa with surrounding lymphoplasmacytic infiltrate (A, hematoxylin-eosin, original magnification ×20). Some of the amyloid material is located around small blood vessel walls (B, hematoxylin-eosin, original magnification ×40) and the residual mucosal glands (C, hematoxylin-eosin, original magnification ×40). Reactive changes in the overlying squamous mucosa (D, hematoxylin-eosin, original magnification ×20) and foreign-body giant cell reactions (E, hematoxylin-eosin, original magnification ×20) are sometimes present. The amyloid deposits display characteristic apple-green birefringence by polarized light microscopy (F, Congo red stain, original magnification ×40).

Figure 3. Immunohistochemical stainings of the lymphoplasmacytic infiltrate and amyloid deposits (patient 2) using κ (A, original magnification ×40) and λ (B, original magnification ×40) demonstrates κ light chain restriction.

ized lesions without systemic involvement. The disease occurred in true and false vocal cords, subglottis and supraglottis, and anterior commissure, which are the common locations in laryngeal amyloidosis. Even in patients with multiple recurrences (patients 1 and 2), the disease is still limited to the larynx, indicating the benign nature of the disease.

The precise etiology and pathogenesis of laryngeal amyloidosis remain unknown. There is no definite established link with smoking, drinking, vocal abuse, or recurrent infection. In the 1980s, Preud’Homme et al. demonstrated immunoglobulin light chains in some of their amyloid specimens. Since then, restricted light chain staining (λ or κ) in both plasma cells and the amyloid has been observed in more cases of laryngeal amyloidosis. Furthermore, Westermark et al. and Berg et al. discovered several unusual amino acid substitutions in κ or light chain from cases of laryngeal amyloidosis. Some of these substitutions are located at highly conserved region of light chain and therefore could substantially change the configuration of the protein and result in the amyloid formation. This evidence further elucidates the pathogenic mechanism of localized amyloidosis. Compared with κ chain, λ light chain restriction was reported with greater frequency in the literature, because the variable region of λ light chain is known to be the most amyloidogenic. Perhaps certain amino acid residues that occur at particular positions in the λ sequence render them particularly amyloidogenic. All of our cases showed a population of plasma cells intimately associated with amyloid deposits. Immunohistochemical studies revealed κ (n = 1) or λ (n = 2) light chain restrictions for both amyloid deposits and plasma cells, suggesting a localized plasma cell dysfunction or dyscrasia. Our findings support the speculation that monoclonal plasma cells at this location are probably responsible for producing excessive or structurally abnormal immunoglobulins, which our body fails to remove.

Although it is rare, laryngeal amyloidosis may occur in patients with an underlying lymphoid neoplasm or in association with systemic amyloidosis. A number of reports...
have demonstrated a relationship between extramedullary plasmacytoma or multiple myeloma and localized laryngeal amyloidosis. Pribitkin et al described 2 patients who experienced systemic involvement (rectum, lung, and heart) among 16 patients with upper aerodigestive tract amyloidosis. Therefore, most authors believe that an evaluation for systemic disease is necessary because of the morbidity of systemic disease. Complete blood cell count, liver and renal function tests, urinary test for Bence-Jones protein, chest radiography, electrocardiogram, echocardiogram, and abdominal ultrasound may be warranted. A rectal biopsy or simple needle biopsy of abdominal fat may be performed to exclude systemic involvement. During the long period of follow-up, none of our patients developed plasmacytoma or multiple myeloma or systemic amyloidosis, although all of them had monoclonal expression of the plasmacytic infiltrate and amyloid component in the laryngeal lesion. In this regard, localized laryngeal amyloidosis may represent a form of benign monoclonal plasma cell dyscrasia.

Currently, the most popular and highly effective treatment available is microdirect laryngoscopy with a carbon dioxide laser excision. The goal is to maintain an adequately patent airway with as few procedures as possible, since each procedure potentially creates additional intra-laryngeal scar and webbing. In nearly half of the cases reported in literature, the surgery had to be repeated due to localized recurrent or large lesions. Therefore, regular follow-up with laryngoscopy is crucial for early diagnosis of recurrence. Adjuvant therapies, such as irradiation, chemotherapy, and steroids, have shown no proven benefit in the treatment of this disorder.

Recently, an experimental small-molecule drug called CPHPC was developed by Pepys et al. CPHPC depletes serum amyloid P component, which binds to and stabilizes amyloid fibrils. Therefore, the decrease of serum amyloid P component in amyloid fibrils leads to the degradation of amyloid fibrils. Human trials for this molecule are currently under way.

References
Evaluation of Mast Cells in Myeloproliferative Disorders and Myelodysplastic Syndromes

Cherie H. Dunphy, MD

• **Context.**—Mast cells may be increased as a reactive mastocytosis in various hematologic disorders and malignant neoplasms, as well as in systemic mast cell disease (SMCD). There are no statistical differences in mast cell numbers in reactive mastocytosis and SMCD; however, SMCD usually reveals dyspoietic mast cells and other dyspoietic bone marrow elements. In addition, SMCD is frequently (45%) associated with myeloproliferative disorders (MPDs) (17%) and myelodysplastic syndromes (MDSs) (28%). Thus, it has been suggested that SMCD may represent one aspect of a hematologic disorder that involves multiple bone marrow lineages.

**Objective.**—To perform a systematic evaluation of MPDs and MDSs without SMCD for dyspoietic mast cells.

**Design.**—A total of 55 MPDs or MDSs were reviewed, including 20 cytogenetically proven chronic myeloid leukemias, 6 essential thrombocythemas, 2 polycythemia veras, 21 cytogenetically proven MDSs, and 6 chronic myelomonocytic leukemias. Cases of idiopathic myelofibrosis were not included due to lack of spicules. The bone marrow aspirates were reviewed for an increase in mast cells

**Results.**—All cases, except 2 MDSs, had evaluable bone marrow spicules. Of interest, the MPDs were significantly more associated with increased and dyspoietic mast cells (57% and 61%, respectively) than were the MDSs (11% and 4%, respectively). The 2 polycythemias and 6 chronic myelomonocytic leukemias did not reveal increased or dyspoietic mast cells.

**Conclusions.**—These findings indicate that MPDs (chronic myeloid leukemia and essential thrombocythemia) frequently contain neoplastic mast cells as the spectrum of abnormal bone marrow cells. This feature, in conjunction with other parameters, may possibly be useful in the differential diagnosis of MPDs and MDSs. Our findings, compared with the previously reported findings in SMCD, suggest that SMCD may be more closely related to MPDs than to MDSs.

(Arch Pathol Lab Med. 2005;129:219–222)

Systemic mast cell disease (SMCD) is a rare disorder characterized by the accumulation of mast cells in a variety of sites, including the bone marrow, spleen, liver, and skin. However, mast cells may also be increased as a reactive mastocytosis in various hematologic disorders and malignant neoplasms. The SMCD cannot be distinguished from reactive mastocytosis based solely on the quantity of mast cells, since it has been determined that there are no statistical differences in mast cell numbers in reactive mastocytosis and SMCD. However, SMCD usually reveals dyspoietic mast cells and other dyspoietic bone marrow elements (ie, erythroid, myeloid, and megakaryocytic cells), which aid in the distinction between reactive mastocytosis and SMCD.

In addition, SMCD frequently occurs (45%) in association with myeloproliferative disorders (MPDs) (17%), myelodysplastic syndromes (MDSs) (28%), and acute myeloid leukemia. Thus, it has been suggested that SMCD may represent one aspect of a hematologic disorder that involves multiple bone marrow lineages. A systematic evaluation of MPD and MDS cases without SMCD for the approximate quantitation of mast cells and the presence of dysplastic mast cells has not been performed. Such a study is of interest to determine whether dysplastic changes are present in the mast cells of these lesions and whether they occur as frequently as in SMCD. In addition, such a study may reveal findings that are useful for the differential diagnosis of MPDs and MDSs.

**MATERIALS AND METHODS**

A total of 55 well-established MPD or MDS cases were retrospectively reviewed. These cases included 20 cytogenetically proven chronic myeloid leukemias, 6 essential thrombocythemas, 2 polycythemia veras, 21 cytogenetically proven MDSs, and 6 chronic myelomonocytic leukemias. The MDSs included refractory anemia with ringed sideroblasts (3), sideroblastic anemia (1), refractory cytopenias (5), refractory cytopenias with excess blasts (3), RAEB-I (1), MDS not otherwise specified (2), high-grade MDS (2), MDS with fibrosis (2), and secondary MDS (1). Cases of idiopathic myelofibrosis were not included due to the uniform lack of spicules for review in these cases. The bone marrow aspirates were reviewed for the presence of spicules, an increase in mast cells (1+, average of 3–5 mast cells per 60× oil-immersion field; 2+, >5–10; and 3+, >10), dyspoietic features within mast cells (ie, significantly decreased cytoplasmic granularity and un-
Figure 1. A, A case of chronic myeloid leukemia, demonstrating the common finding of increased mast cells (scattered darkly staining basophilic cells) seen in this myeloproliferative disorder (Wright stain, original magnification ×200). This disorder is also characterized by the common finding of fusiform and dyspoietic mast cells. B, Fusiform (elongated) mast cells (arrows) are commonly seen in chronic myeloid leukemia (Wright stain, original magnification ×600). Dyspoietic mast cells are characterized by uneven, abnormal localization of basophilic granules (as seen in the fusiform mast cell on the left) (B) and decreased basophilic granulation (arrow) (C) (Wright stain, original magnification ×600).

Figure 2. A, Although increased, dyspoietic, and fusiform cells are much less commonly seen in myelodysplastic syndrome, this case reveals increased mast cells (scattered darkly staining basophilic cells) (Wright stain, original magnification ×200). B, The typical case of myelodysplastic syndrome does not reveal increased mast cells as seen in this case (Wright stain, original magnification ×400). C, None of the chronic myelomonocytic leukemia cases revealed increased mast cells, as in this smear of a chronic myelomonocytic leukemia case (Wright stain, original magnification ×500).
mast cells are characteristic of SMCD. However, as mentioned previously, SMCD frequently occurs in association with other hematologic disorders, including MPDs, MDSs, and acute myeloid leukemias. This association has led to the suggestion that SMCD represents one aspect of a hematologic disorder that involves multiple bone marrow lineages. In addition, some MPDs or MDS cases may have a mild, diffuse increase in mast cell numbers, unassociated with SMCD, which may be a part of the clonal bone marrow proliferation. However, a critical cytomorphic analysis of the mast cells in these conditions has not been previously described. Therefore, we decided to systematically review cases of MPDs or MDSs without SMCD to determine whether dysplastic changes are present in the mast cells of these lesions and whether they occur as frequently as in described cases of SMCD.

We have demonstrated that the chronic MPDs (ie, chronic myeloid leukemia and essential thrombocytopenia) frequently contain neoplastic mast cells as the spectrum of abnormal cells in the bone marrow. In fact, in chronic myeloid leukemia, a higher percentage (80%) of cases reveal dyspoietic mast cells than merely increased mast cells (70%). A lesser percentage of essential thrombocytopenia cases revealed increased mast cells (33%) and dyspoietic mast cells (17%), when compared with the chronic myeloid leukemia cases. Of interest, none of the polycythemia vera cases revealed increased or dyspoietic mast cells. Thus, the finding of increased and dyspoietic mast cells, in combination with additional clinical and laboratory data, may be useful in excluding polycythemia vera.

In addition, we have shown that increased and dyspoietic mast cells are significantly more characteristic of MPDs (ie, chronic myeloid leukemia and essential thrombocytopenia) than MDSs. Of note, the relatively rare findings of increased or dyspoietic mast cells in MDSs did not correlate with any specific subtype of MDS. Since no cases of chronic myelomonocytic leukemia were associated with increased or dyspoietic mast cells, these findings, in combination with additional clinical and laboratory data, may aid in exclusion of this diagnosis and differentiation from a chronic MPD.

Lastly, our findings, compared with the previously reported findings in SMCD, suggest that SMCD may be more closely related to chronic MPDs than to MDSs. Perhaps SMCD arises in a background of a chronic MPD, such as chronic myeloid leukemia or essential thrombocytopenia, since they are frequently associated and share the findings of increased and dyspoietic mast cells. Based on our findings, it is not clear how one would distinguish between a chronic MPD and an early SMCD associated with one of these chronic MPDs. The clinical significance of such a distinction is also unclear.

**REFERENCES**

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Lung Transplantation Biopsy Specimens With Bronchiolitis Obliterans or Bronchiolitis Obliterans Organizing Pneumonia Due to Aspiration

Aya Miyagawa-Hayashino, MD; John C. Wain, MD; Eugene J. Mark, MD

Context.—Bronchiolitis obliterans (BO) is generally thought to be a marker of chronic airway rejection in patients who have undergone lung transplantation. Bronchoscopic biopsy specimens, by virtue of their small size, may sample only BO and not a lesion of bronchiolitis obliterans organizing pneumonia (BOOP). A role for ongoing chronic infection or aspiration has also been suggested, and the distinction of these etiologies may be difficult clinically and pathologically.

Objective.—To investigate the etiology of BO and BOOP in lung transplantation patients who had chronic aspiration.

Design.—This is a clinicopathologic study of 7 patients who had undergone lung transplantation in which biopsy findings suggested the possibility of chronic airway rejection but in which aspiration was subsequently proven as a cause of the bronchiolar disease.

Results.—All patients were men, who ranged in age from 19 to 57 years. A clinical diagnosis of aspiration was considered based on history, acid reflux testing, and radiographic findings in all 7 patients. Three patients had BO and 4 patients had BOOP. Histiocytic giant cells or foreign material was absent. The interval from transplantation to BO ascribed to aspiration ranged from 2.5 months to 7 years. The patients were treated aggressively with medication for gastroesophageal reflux disease. Their respiratory function and chest radiography results improved.

Conclusion.—Although BO may be a manifestation of rejection, it may also be a manifestation of aspiration. Because the latter is potentially correctable, aspiration should be considered etiologically in lung transplantation patients with either BO or BOOP. Reliable distinction between aspiration-related or rejection-related BO and BOOP cannot be made on morphologic grounds alone. Clinical and radiologic correlations are indicated to establish the distinction.

(March Pathol Lab Med. 2005;129:223–226)

Materials and Methods

Seven patients who had undergone lung transplantation underwent biopsies to assess rejection and had biopsy findings of BO or BOOP. Clinical records, chest x-ray films, and computed tomograms were reviewed in all cases. Bronchoscopic biopsies were performed in 6 patients and thoracoscopic biopsy in 1 patient (patient 7). Each of the biopsy specimens had been stained with hematoxylin-eosin, Verhoeff-van Gieson for elastic tissue, Mallory trichrome stain for location and age of collagen, and methenamine silver stain for exclusion of organisms and in particular Pneumocystis carinii. Immunohistochemical staining for cytomegalovirus and herpesvirus was performed to exclude these infections in 4 patients. In situ hybridization for Epstein-Barr virus-encoded RNA was performed in 1 patient. The clinical and pathologic features were tabulated.

We defined the histologic features of BO as proliferation of submucosal or intraluminal fibrous tissue, which was confined to respiratory bronchioles or alveolar ducts with luminal occlusion. We used the term BO as a histologic pattern different from organizing pneumonia (OP), where there was fibromyxomatous granulation tissue in alveoli. We diagnosed BOOP when there was both BO and OP.24,25,26 Rejection was graded according to the revised grading scheme for pulmonary allograft rejection.

Results

The clinical, radiographic, and pathologic findings are summarized in the Table. All 7 patients were men, who ranged in age from 19 to 57 years. Three biopsy specimens...
The interval from transplantation to the first episode of BO ranged from 1 month to 3.3 years, and the interval from transplantation to BO ascribed to aspiration ranged from 2.5 months to 7 years. No histiocytic giant cells or foreign material was identified. Neutrophils and eosinophils were absent. Special stains for bacteria, fungi, and *P. carinii* in all cases were negative. No viral inclusions were seen, and there was no immunochemical evidence of herpesvirus or cytomegalovirus infection at the time of aspiration with BO in 4 cases in which immunohistochemical analysis was performed. Cytomegalovirus had been cultured within the first year after transplantation in 2 patients. In situ hybridization for Epstein-Barr virus in 1 patient was negative.

One of the biopsy specimens had coexistent acute cellular rejection of grade 3/4 in the contralateral lung at the time of the biopsy that showed BOOP. The other 6 patients had no acute cellular rejection at the time. Three patients had acute cellular rejection at earlier times and 1 at a later time. No chronic vascular rejection was present.

**COMMENT**

Chronic allograft rejection remains an important cause of postoperative mortality in lung transplantation. The histologic hallmark of chronic airway rejection is BO, an organizing inflammation centered on the respiratory and terminal bronchioles, resulting in fibrotic occlusion of the lumen or obliteration by extrinsic compression. Bronchiolitis obliterans is assumed to be an expression of chronic rejection due to immune-mediated injury. However, there are many causes of BO, including collagen-vascular disease, sequel to infection, drug reaction, inhalation of toxic or irritant materials, or aspiration of particulates. In the transplantation setting, hypoperfusion with ischemia is another possibility. Organizing pneumonia is not necessarily an accompanying feature of BO. When both BO and OP are present and thus the condition

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**Clinical and Pathologic Findings in 7 Patients With Bronchiolitis Obliterans (BO) Following Lung Transplantation (LT)**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at LT, y/Sex</th>
<th>Underlying Disease</th>
<th>Radiographic Features</th>
<th>Interval From LT to Diagnosis of BO</th>
<th>Interval From LT to Diagnosis of BO Caused by Aspiration</th>
<th>Other Complications</th>
<th>Clinical Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19/M</td>
<td>Cystic fibrosis</td>
<td>Nodules in lower lobes</td>
<td>8 mo</td>
<td>2 y</td>
<td>No</td>
<td>Alive, 2y</td>
</tr>
<tr>
<td>2</td>
<td>19/M</td>
<td>Cystic fibrosis</td>
<td>Opacification of the right lower lobe</td>
<td>3.3 y</td>
<td>3.3 y</td>
<td>Yes Grade 3/4 acute rejection (3.3 y)</td>
<td>Alive, 3.5 y</td>
</tr>
<tr>
<td>3</td>
<td>22/M</td>
<td>Cystic fibrosis</td>
<td>Opacification of the right upper lobe</td>
<td>1 mo</td>
<td>2.5 mo</td>
<td>Yes Grade 1/4 acute rejection (3 mo)</td>
<td>Alive, 6 mo</td>
</tr>
<tr>
<td>4</td>
<td>31/M</td>
<td>Bronchiectasis</td>
<td>Nodular opacity</td>
<td>2 y</td>
<td>6 y</td>
<td>No</td>
<td>Alive, 9 y</td>
</tr>
<tr>
<td>5</td>
<td>47/M</td>
<td>Emphysema (α₁-antitrypsin deficiency)</td>
<td>Consolidation at the apices</td>
<td>1 y</td>
<td>7 y</td>
<td>Yes</td>
<td>Alive, 8 y</td>
</tr>
<tr>
<td>6</td>
<td>53/M</td>
<td>Bronchiectasis</td>
<td>Ill-defined opacities at lung bases</td>
<td>3 y</td>
<td>3 y</td>
<td>No</td>
<td>Alive, 5 y</td>
</tr>
<tr>
<td>7</td>
<td>57/M</td>
<td>Emphysema</td>
<td>Patchy opacity</td>
<td>9 mo</td>
<td>9 mo</td>
<td>Yes Grade 1/4 acute rejection, CMV infection</td>
<td>Dead, 5 y; autopsy showed DAD, bronchopneumonia, and no rejection</td>
</tr>
</tbody>
</table>

* OP indicates organizing pneumonia; CMV, cytomegalovirus; and DAD, diffuse alveolar damage.

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came from the right upper lobe, 1 from the right lower lobe, 1 from the left upper lobe, and 2 from the left lower lobe. Two patients received single lung allografts, and 5 patients received double lung allografts. Four patients received a cadaveric donor lung transplant, and 3 patients received a living donor lung transplant. In all 7 patients, BO was present, and OP additionally was present in 4 cases. The 3 biopsy specimens with BO and not OP were obtained by bronchoscopy.

**Radiographic Features**

Computed tomography of the chest at the time of biopsy that showed BO or BOOP attributed to aspiration revealed pulmonary nodular or patchy opacification in 6 patients and bilateral apical consolidation in 1 patient. The radiographic findings suggested inflammatory consolidations in all cases, with the radiographic differential diagnosis including infectious pneumonia or aspiration pneumonia.

**Clinical Features**

A clinical diagnosis of aspiration was considered based on history, acid reflux testing, and radiographic findings in all 7 patients. After lung biopsy, the patients were treated aggressively for gastroesophageal reflux disease with medication that included ranitidine and esomeprazole. The therapy resulted in symptomatic and radiographic improvement in all cases. The patients were not treated at this time for cellular rejection. None of the patients have developed chronic rejection.

**Pathologic Features**

The BO was manifested as branching tongues of myxoid fibrous tissue in respiratory bronchioles and alveolar ducts (Figure 1). The anatomic location was confirmed in all cases with histochemical staining for collagen and elastica (Figure 2). In 4 patients, organizing fibrosis and histiocytic inflammation in alveoli were also present (Figures 3 and 4).
is BOOP, the etiologic differential diagnosis might include infection or aspiration. Aspiration has been known to cause acute bronchiolitis and BO in animal models and in humans. The histopathologic distinction between BO and BOOP is not always evident, and the clinical BO syndrome could include either.

Histologically, the distinction between chronic rejection and aspiration as a cause of the BO can be facilitated if one distinguishes BO from BOOP. In a proposal for the standardization of the nomenclature in the diagnosis of pulmonary rejection, the most diagnostic lesion is concentric fibrous scarring with formation of a fibrous plaque in the wall of the airway. The scarring process is usually confined to the submucosal and intraluminal parts of membranous and respiratory bronchioles and rarely extends into the alveoli. The loose edematous fibrous tissue in BOOP can be distinguished from the denser scarring of BO in chronic rejection. In this sense, BOOP does not necessarily imply chronic rejection. Distinction of BO and constrictive forms of bronchiolitis from BOOP due to chronic infection or other causes may be difficult.

Although transbronchial lung biopsy is a common surveillance procedure for transplantation patients, the sensitivity and specificity of this procedure remain uncertain, including the distinction of BOOP from BO in small samples. In fact, in our cases, BO alone was found in 3 of the bronchoscopic biopsy specimens, and the smaller size of these specimens might have made the identification of adjacent OP less probable. Clinical correlation is thus necessary for interpretation of biopsy findings, and the clinical correlation in our cases was obtained when the clinical and radiographic features were noted to be atypical for rejection and then a detailed history was obtained to implicate aspiration. Foreign-body reaction to aspirated particulates is much less likely to be found in a transbronchial biopsy specimen than in a thoracoscopic biopsy specimen and not to be expected if the aspirated material is gastric acid alone, since gastric acid can cause severe lung injury, including edema, bronchiolitis, interstitial pneumonitis, and diffuse alveolar damage.

Patients who undergo lung transplantation are subjected to a variety of mechanical procedures and clinical considerations. Although aspiration generally involves the lower lung zones, upper zone disease can occur, and the biopsy specimens of 4 of our cases came from the upper...
lobes. Clinically, there is controversy regarding the relationship between gastroesophageal reflux with aspiration and various forms of obliterative bronchiolar disease.\textsuperscript{17-19,23,38} But gastroesophageal reflux has been reported as a cause of BO in some patients who have undergone heart-lung transplantation.\textsuperscript{14-16} Mechanical factors involved in the development of BO in lung transplantation patients include post-transplantation derenervation of the lung, reduction of mucociliary clearance, and aspiration.\textsuperscript{14-20} The inflammatory response induced by aspiration has been postulated to augment rejection by facilitating the encounter of lymphocytes with foreign tissue and antigens.\textsuperscript{15}

In our cases, it is unclear whether biopsy specimens taken before the time of defined aspiration that showed BO or BOOP were due to aspiration, infection, or rejection. Bronchiolitis obliterans due to chronic rejection typically first appears around the end of the first postoperative year.\textsuperscript{21} Either BO or BOOP due to aspiration in our patients appeared both earlier and later than this interval of 1 year, and other authors have reported BO or BO syndrome due to aspiration as early as 1 month and as late as 4 years after transplantation.\textsuperscript{14-17,19,20} Since it is possible that all these changes might be related to persistent aspiration from the beginning and because aspiration may be correctable, one should consider aspiration as a potential cause of BO or BOOP, particularly if there are clinical and radiographic features atypical for rejection.

References


Sodium Status of Collapsed Marathon Runners

Alexander Kratz, MD, PhD, MPH; Arthur J. Siegel, MD; Joseph G. Verbalis, MD; Marvin M. Adner, MD; Terry Shirey, PhD; Elizabeth Lee-Lewandrowski, PhD, MPH; Kent B. Lewandrowski, MD

• **Context.**—Recommendations for prevention and treatment of medical emergencies in participants in marathon races center on maintenance of adequate hydration status and administration of fluids. Recently, new recommendations for fluid replacement for marathon runners were promulgated by medical and athletic societies. These new guidelines encourage runners to drink ad libitum between 400 and 800 mL/h as opposed to the previous “as much as possible” advice.

**Objective.**—To assess the sodium and hydration (plasma osmolality) status of collapsed marathon runners after the promulgation of new hydration guidelines.

**Design.**—Plasma sodium and osmolality values of runners who presented to the medical tent at the finish line of the 2003 Boston Marathon were measured.

**Results.**—Using reference ranges derived from the general population, of 140 collapsed runners, 35 (25%) were hypernatremic (sodium, >146 mEq/L) and 6 (12%) were hyperosmolar (osmolality, >296 mOsm/kg H₂O), whereas 9 (6%) were hyponatremic (sodium, <135 mEq/L) and 8 (16%) were hypo-osmolar (osmolality, <280 mOsm/kg H₂O). Compared with a population of marathon runners who had experienced no medical difficulties, 9% of the runners were hypernatremic, 5% were hyponatremic, 8% were hypo-osmolar, and none were hyperosmolar.

**Conclusions.**—Our findings indicate a significant incidence of hypernatremia with hyperosmolality and hyponatremia with hypo-osmolality among collapsed runners despite the new fluid intake recommendations, suggesting that either further educational measures are required or that the new guidelines are not entirely adequate to prevent abnormalities in fluid balance. Furthermore, the immediate medical management of hypernatremia and hyponatremia is different. Administration of fluids to severely hyponatremic patients may result in fatal cerebral edema. Our findings caution against institution of treatment until laboratory tests determine the patient’s sodium status.

(Arch Pathol Lab Med. 2005;129:227–230)

Many modern-day marathon races are mass events with tens of thousands of participants. Most of these contestants are not professional athletes but recreational runners who are physically challenged by a 42.2-km (26.2-mile) race. In some marathons, hundreds of these amateurs collapse and require immediate medical attention, necessitating the establishment of medical support services at these events.1 Losses of water and electrolytes have historically been assumed to be the dominant cause of collapse in marathon runners. Consequently, the administration of intravenous fluids is frequently the first line of treatment for exercise-associated collapse.1

The best strategy to prevent the development of medical emergencies in marathon runners has been the subject of a longstanding debate centered on the ideal recommendations for fluid intake. From antiquity until the late 1960s, athletes were advised not to drink during exercise.2 This recommendation changed after a series of articles published after 1969 stressed the dangers of dehydration during marathon running. By 1996, various medical and athletic societies had issued forceful guidelines promoting vigorous fluid intake, usually encouraging runners to drink “as much as possible.”1,3,4 Data obtained from healthy runners who did not experience any adverse medical events suggest that by 2001 these recommendations were achieving the desired effect and that the biochemical markers of dehydration could be largely mitigated by these recommendations.5 However, although these recommendations may have reduced the prevalence of dehydration among marathon runners, overhydration with hyponatremia has become an increasingly important problem.2,6,7 At least 250 cases of cerebral edema, 7 of them fatal, have been reported in the literature.2 Between 1989 and 1999, there were 190 hospitalized cases of water intoxication in the US Army alone, leading to a revision of the fluid replacement guidelines in the military in 1999.8

To protect runners from the effects of overhydration, the International Marathon Medical Directors Association (IMMDA), representing medical experts in the field, and USA Track and Field, the national governing body for long-distance running, also issued new fluid replacement guidelines in 2003. These recommendations advise runners to drink ad libitum between 400 and 800 mL/h, as opposed to the previous “as much as possible” recommendation.1

In view of the controversies and changes surrounding...
recommendations for fluid replacement for marathon runners, there is a clear need for up-to-date information on the fluid status of collapsed marathon runners. This need arises from the desire to know if the latest recommendations are effective and from the need for data to provide the best diagnostic tools and treatment to collapsed runners. We therefore investigated the incidence of hyponatremia and hypernatremia in a subset of participants in the Boston Marathon of 2003, which took place after the new fluid replacement guidelines were announced.

MATERIALS AND METHODS

Specimens

The study participants included 140 runners who took part in the 107th Boston Athletic Association Marathon in 2003 and who collapsed during or immediately after the race and for whom physicians at the medical station ordered a chemical blood analysis. To participate in the Boston Marathon, runners were required to have run a qualifying time at a certified marathon within the last 18 months. Qualifying times were age and sex specific and between 3 hours 10 minutes and 5 hours 30 minutes. The study participants included 140 runners who took part in the Boston Marathon of 2003, which took place after the new fluid replacement guidelines were announced.

Sample Analysis

Whole blood samples were obtained by trained phlebotomists using lithium heparin collection tubes. Samples were analyzed on Stat Profile M7 (Nova Biomedical, Waltham, Mass) instruments by trained personnel provided by the instruments' manufacturer. The Stat Profile M7 measures blood pH, PCO2, PO2, SO2, hematocrit, Na+, K+, Cl−, Ca++, glucose, blood urea nitrogen (BUN), creatinine, and lactate directly and calculates the osmolality based on the following formula: osmolality = 1.86[Na] + [glucose]/18 + [BUN]/2.8 + 9. The osmolality of 50 randomly selected samples was also directly measured with a Vapro Vapor Pressure Osmometer (Wescor Inc, Logan, Utah).

RESULTS

The 2003 Boston Marathon was run at a temperature of 14.4°C to 21.6°C with a light northeastly wind of 1 to 5 mph, near perfect conditions for a race. Of the 17,548 runners who entered the marathon, 17,030 (97.1%) made it to the finish line.

Physicians who provided emergency medical services in an aid station set up at the finish line ordered blood analysis for 140 collapsed runners. Direct measurement of the whole blood sodium levels of these patients indicated that one quarter (n = 35) were hypernatremic relative to the reference range for the general population in use at our hospital (sodium, 135–146 mEq/L) (Table 1). Nine (6%) of the patients were hyponatremic relative to this standard reference range; one of these patients was severely hyponatremic, with a sodium level below 125 mEq/L. Hypernatremia appeared to be a larger concern for runners who finished within the first 4.5 hours of the race, during which time 10 of the 35 hypernatremic runners were sampled. The first of 9 hyponatremic runners was sampled at 4 hours 35 minutes. Seven of the 9 hyponatremic runners were sampled between 5 hours 50 minutes and 7 hours. The calculated osmolality derived by the instrument from the sodium, glucose, and BUN levels provided similar information as the sodium determination (Table 1 and Figure 1).

To obtain a direct measurement of the osmolality, 50 randomly selected samples were analyzed with an osmometer. Nonparametric (Spearman) correlation between the measured and the calculated osmolality was performed, and a positive association between the 2 variables was observed (r = 0.744, P < .001). A 1-tailed t test indicated that the difference between the calculated and the measured osmolality was not significantly different from 0 (P = .30). These findings confirmed that the calculated osmolality obtained from the Stat Profile M7 correlated with the measured osmolality. The proportions of hyperosmolar and hypo-osmolar runners were similar according to the calculated method and to the direct measurement (Figure 2 and Table 1).

Our group has recently described modified reference ranges (or expected ranges) for a variety of hematologic

<table>
<thead>
<tr>
<th>Variable</th>
<th>Below Reference Range</th>
<th>Within Reference Range</th>
<th>Above Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>9 (6)</td>
<td>96 (69)</td>
<td>35 (25)</td>
</tr>
<tr>
<td>Osmolality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated</td>
<td>17 (12)</td>
<td>92 (66)</td>
<td>31 (22)</td>
</tr>
<tr>
<td>Measured</td>
<td>8 (16)</td>
<td>36 (72)</td>
<td>6 (12)</td>
</tr>
</tbody>
</table>

* Reference ranges for the general population are 135 to 146 mEq/L for sodium and 280 to 296 mOsm/kg H2O for osmolality.
and biochemical parameters in marathon runners derived from 37 athletes who had completed the Boston Marathon of 2001 without medical problems. At 134 to 149 mEq/L for sodium and 273 to 318 mOsm/kg H2O for osmolality, these ranges are considerably wider than for the general population. Comparison of the sodium and osmolality levels of the collapsed runners of the present study with these reference ranges still showed notable proportions of hyponatremic and hypo-osmolar collapsed runners (Table 2).

### COMMENT

The new hydration guidelines of the IMMDA are intended to minimize the risk of overhydration and dehydration and represent a major change in the recommendations given to marathon runners regarding fluid intake before and during the race. We have determined the hydration status of collapsed marathon runners after the promulgation of these new guidelines and found that, when compared with the general population, approximately one third showed abnormalities in sodium levels and osmolality. Although hyperosmolar, hypernatremic runners represented most of these patients, a substantial fraction was hyponatremic and hypo-osmolar, findings consistent with overhydration. The proportion of hyponatremic and hypo-osmolar collapsed runners (relative to hypernatremic and hyperosmolar runners) was even larger when compared with reference ranges derived from runners who had uneventfully completed a similar race. The reference ranges for marathon runners have been derived from a relatively small sample size and are wider than the ranges for the general population. This explains the absence of hyperosmolar runners when reference ranges from marathon runners are used.

A number of earlier studies on the fluid status of collapsed athletes has focused on forms of exercise more physically challenging than a 42.2-km marathon race and were performed before the new fluid replacement guidelines went into effect. Speedy and colleagues studied athletes participating in the 1996 and 1997 New Zealand Ironman ultradistance triathlons (consisting of a 3.8-km swim, a 180-km cycle race, and a 42.2-km run) at temperatures of approximately 21°C. They found that between 9% and 23% of the athletes who required medical care after the competition were hyponatremic. In a study on participants in the Hawaii Ironman Triathlon, O’Toole et al found that hyponatremia occurred in 30% of athletes who required race-day medical care. Hyponatremic athletes had significantly lower postrace osmolality (276 vs 290 mOsm/kg H2O) than did normonatremic athletes. In this study, some of the participants had never before taken part in a triathlon; the temperature was between 22°C and 31°C. Holtzhausen and coworkers described the biochemical characteristics of collapsed participants in the 56-km Two Oceans Marathon held at 13°C to 19°C in 1990 and found an incidence of hyponatremia (sodium, <135 mEq/L) of 8% among collapsed runners. Interestingly, none of their study participants were hypernatremic or hyperosmolar, indicating that the fluid replacement guidelines in effect at that time were effective at addressing the problem of dehydration.

In a previous study on collapsed runners in the 2000 and 2001 Boston Marathons, which were run at temperatures between 20°C and 22°C, only 1 hyponatremic patient (1.2%) was present in a group of 86 collapsed runners. This relatively low incidence of hyponatremia may have resulted from selection bias introduced by testing predominantly faster runners (ie, those with finishing times between 3 and 5 hours), in whom the incidence of hyponatremia is lower. Three other groups have described the incidence of hyponatremia in participants in regular mar-

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### Table 2. Sodium and Osmolality Levels in Collapsed Marathon Runners Relative to the Reference Ranges for Marathon Runners*

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of Runners</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Below Reference Range</td>
</tr>
<tr>
<td>Sodium</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Osmolality Calculated</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Measured</td>
<td>4 (8)</td>
</tr>
</tbody>
</table>

* Reference ranges for marathon runners shortly after a race are 134 to 149 mEq/L for sodium and 273 to 318 mOsm/kg H2O for osmolality.
marathon races. In contrast to our study, these investigations were focused on hyponatremia and took place before the new fluid replacement guidelines were announced. Hsieh and colleagues15 found an incidence of 5.6% of hyponatremia in marathon runners who required medical treatment in a race run at temperatures between 65°F and 86°F and of 0.1% among all entrants in a race held in 2000. The authors noted that the weather on the race day was particularly hot and humid and may not have been representative of all marathons. Hew and colleagues16 found that among runners who sought medical care after the 2000 Houston Marathon, which was run at temperatures between 16.6°C and 25°C, 9% had hyponatremia; this represented 0.31% of the entrants. Similar to our findings, hyponatremic runners had longer finishing times. Reporting on the 1998 Suzuki Rock ‘N’ Roll Marathon in San Diego, Calif, Davis and coworkers17 found that of 19,978 runners, 21 (0.1%) presented to area emergency departments with hyponatremia after a race that took place at a maximum air temperature of 22°C. Eleven of these patients were severely hyponatremic (sodium, ≤125 mEq/L). All of these runners had attempted to drink “as much fluid as possible.” In contrast, our study, which took place after the recommendations were revised to drink ad libitum, only found 9 hyponatremic runners in a starting field of similar size as in the study of Davis and colleagues; only one of our runners had a sodium level below 125 mEq/L. The incidence of hyponatremia associated with collapse among entrants in the marathon (0.05%) in our series was lower than in any of the previous studies. This may indicate that the new fluid replacement guidelines have succeeded in reducing the incidence of hyponatremia in marathon runners.

Although our findings are consistent with a decrease in the incidence of hyponatremia due to the new fluid replacement recommendations, they also indicate that despite these new guidelines, both dehydration and fluid overload continue to occur among participants in marathon races that require medical attention. It is therefore possible that the latest recommendations are not optimal and will need to be adjusted further. Alternatively, it is conceivable that due to the short period between the promulgation of the new recommendations and the race, some runners were still following the old guidelines and drank “as much as possible,” causing them to become fluid overloaded. Due to concerns for patient confidentiality, our study design did not allow us to investigate patient compliance with the new fluid replacement guidelines. Further studies, which will have to correlate fluid intake with hydration status, will be needed to address this question.

The clinical differentiation of dehydration from overhydration in a collapsed athlete can be difficult.18 This distinction is critical, since the treatment for the 2 conditions is fundamentally different. Dehydration is treated by the administration of fluids. In contrast, hyponatremia with fluid overload requires the opposite approach. In severe cases, the mistaken treatment of an dehydrated patient with intravenous fluids can be life threatening, resulting in cerebral edema and death. Our finding of a significant incidence of hyponatremia among collapsed marathon runners emphasizes the need for rapid measurement of sodium in the emergency workup of the collapsed runner. In most cases, laboratory testing must be immediately available at the point of care. If point-of-care testing had not been available for the workup of the collapsed runners in our study, some of the hyponatremic patients may have received unnecessary administration of intravenous fluids. However, as shown by Davis and colleagues,17 determination of sodium levels or osmolality is not always part of the medical workup of collapsed marathon runners; in their series, only 37 of 50 runners who presented to area emergency departments after a marathon had electrolyte panels performed (as mentioned, 21 of these individuals were hyponatremic).17

In conclusion, we have reevaluated the incidence of dehydration and fluid overload in collapsed marathon runners. Given our findings, it is obvious that either the new guidelines are inadequate or athletes are not always following the guidelines. The significant incidence of both hyponatremia and hypernatremia emphasizes the need for point-of-care testing devices for electrolytes or osmolality at the race site. Further studies on education of athletes are needed to address whether the new fluid replacement recommendations are optimal for all marathon runners.

References
Recurrent and De Novo Glomerular Immune-Complex Deposits in Renal Transplant Biopsies

James Gough, MB; Asli Yilmaz, MD; Serdar Yilmaz, MD; Hallgrimur Benediktsson, MD

Context.—Recurrent and de novo glomerulonephritis is an important cause of renal allograft failure, but estimates of its prevalence vary widely. One reason for such variability is the inconsistency with which electron microscopy and immunofluorescence are used in assessing renal allograft biopsies.

Objective.—To determine the prevalence of immune-complex deposits in all renal allograft biopsies performed during a 1-year period and to correlate their presence with clinical data.

Design.—Our center accessioned a total of 118 renal allograft biopsies during 1 year from 88 patients. All biopsies were examined by both electron microscopy and immunofluorescence in addition to conventional light microscopy. Patient and donor characteristics were obtained as well as follow-up data for a minimum of 26 months after the index biopsy.

Immune-complex-mediated glomerulonephritis is considered 1 of the 3 leading causes of renal allograft loss (after chronic rejection and death with a functioning graft). Reports of its prevalence vary from 6% to 19.4%. This variation may be due to the restricted populations studied (eg, those with recurrent glomerulonephritis only or with proteinuria only) or to the inclusion of patients with metabolic glomerulopathies. Incomplete graft biopsy examination has not been emphasized as a cause of such disparities, and the aim of our present study was to determine the prevalence and significance of graft glomerulonephritis as detected in unselected biopsies submitted for electron microscopy (EM) and immunofluorescence (IF).

MATERIALS AND METHODS

One hundred eighteen consecutive renal allograft biopsies from 88 patients, performed during a 1-year period (July 1999 to July 2000) at a single renal transplant center, were processed in a standard fashion and examined by light microscopy as well as by EM and IF. Glomerular electron-dense deposits, in nonscle-

Results.—Eight cases of immunoglobulin (Ig) A nephropathy were found (recurrent in 7 and de novo in 1). There were 9 instances of what we designate “IgM-positive immune deposits” without specific features of a recognized glomerulonephritis. To the best of our knowledge, the latter has not hitherto been described and may be part of a heterogeneous group of glomerulopathies. Other unexpected findings included de novo fibrillary glomerulonephritis and de novo membranous glomerulonephritis, the latter occurring at 3 months after engraftment.

Conclusions.—A high proportion (19.5%) of unselected renal allograft biopsies show immune-complex deposits both with and without a recognized glomerulopathy. These require both electron microscopy and immunofluorescence for detection. IgM-positive deposits of uncertain etiology are relatively frequent.

(Arch Pathol Lab Med. 2005;129:231–233)
Nine Cases of Immunoglobulin M-Positive Glomerular Immune-Complex Deposits in Renal Allograft Biopsies*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Length of Time Between Transplant and Biopsy, mo</th>
<th>Latest Follow-up²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Graft loss due to chronic allograft nephropathy at 20 mo after transplant</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>Creatinine, 1.69 mg/dL</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>Creatinine, 1.30 mg/dL</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>Creatinine, 0.57 mg/dL</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Creatinine, 2.24 mg/dL</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Graft loss due to BK nephritis at 14 mo after transplant</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>Creatinine, 1.41 mg/dL</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>Creatinine, 2.68 mg/dL; nephrotic syndrome with recurrent focal segmental glomerulosclerosis</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>Death due to disseminated cytomegalovirus at 14 mo after biopsy</td>
</tr>
</tbody>
</table>

* The indication for biopsy was an unexplained rise in serum creatinine in all cases except the biopsy of patient 4, which was a surveillance biopsy. Proteinuria was an additional indication in patients 8 and 9.

² Follow-up was a minimum of 26 months after index biopsy.

with respect to the following: number of rejection episodes, age, HLA matching, and cadaveric versus living (related and unrelated) donor. Female sex and previous transplantation were slightly more common in the IC group, but this did not reach the level of statistical significance. After a minimum follow-up period of an additional 26 months, the mean serum creatinine level for the IC group was significantly higher than in the non-IC group ($P < .001$, using analysis of variance). There were 2 graft losses in the “IgM” group, 1 from BK nephritis and 1 from chronic allograft nephropathy. One other patient died from disseminated cytomegalovirus with a functioning graft. In the non-IC group, there were 4 deaths with functioning grafts and 3 graft failures due to rejection.

COMMENT

In the largest study of recurrent glomerulonephritis to date, EM was not used in approximately 50% of graft biopsies, and an unspecified number of cases did not have IF performed.¹ This may have led to underestimation of both recurrent and de novo glomerulonephritis, such as recurrent IgAN. Without EM and IF, making the distinction between membranoproliferative glomerulonephritis and transplant glomerulopathy is very difficult. The utilization rates of IF and EM are particularly low in early graft biopsies, so the timing of onset of glomerulonephritis in allografts is also hard to estimate. In our biopsy-based study, on the other hand, all graft biopsies were submitted for light microscopy, EM, and IF.

Our results confirm the high rate of recurrent IgAN and show its early onset (4/8 patients were biopsied at less than 12 months after transplantation). In addition, we found 1 case each of de novo fibrillary glomerulonephritis and membranous glomerulonephritis, the latter occurring at 3 months after transplantation. There were also 9 patients who had IgM-positive mesangial electron-dense immune deposits, in some cases with C3 positivity also (Table). On EM, the size of these deposits varied from small to very large (Figure) and resembled the deposits seen in IgAN. Only 1 of these cases resembled a recognized clinicopathologic entity (ie, recurrent focal segmental glomerulosclerosis, which was diagnosed in a second biopsy). The presence or subsequent development of viral infection (BK, cytomegalovirus, and hepatitis C and B) in 3 of the IgM group raises the possibility of antiviral antibody/viral antigen immune-complex deposits. Three biopsies from the IgM group showed only mesangial expansion by light microscopy, and the remainder showed no diagnostic light microscopic feature. Seven of the 9 occurred less than 1 year after transplantation. Four showed proteinuria greater than 500 mg/L/d. The etiology of the transplant-associated IgM deposits is not clear. Possibilities include T-cell deficiency or mesangial overloading by antigen-antibody complexes (the antibody possibly directed against an unidentified microorganism). Rejection-related immune events are another possibility. However, we found no case of transplant glomerulopathy or of “glomerulitis” (which can be associated with acute humoral rejection) with glomerular immune deposits. We are now studying all our cases of IgM-positive glomerular IC deposits in allografts over a 10-year period and its association with graft function, infection, and recurrent disease (particularly IgAN).

In summary, the results of this comprehensive cross-

Electronic micrograph of large glomerular mesangial immune-complex deposits in a renal allograft. Immunofluorescence was positive for immunoglobulin M and C3 in a mesangial distribution (original magnification x8000).
sectional analysis of renal allograft biopsies suggests a high prevalence of immune-complex deposition (19.5% of all biopsies and 26% of all patients biopsied), often occurring early. We also report on a group of IgM-positive glomerular immune-complex deposits that, to our knowledge, has not been previously described.

References
Mixed Hepatoblastoma in an Adult

Hans-Udo Kasper, MD; Thomas Longerich, MD; Dirk L. Stippel, MD; Michael A. Kern, MD; Uta Drebber, MD; Peter Schirmacher, MD, PhD

We report a case in an elderly adult of a highly malignant liver tumor with blastoid features that resembled hepatoblastoma. A liver tumor with a diameter of 23 cm was removed in a 78-year-old woman. The tumor showed highly differentiated epithelial hepatocellular and poorly differentiated epithelial and mesenchymal components. The blastoid nature and pluripotent differentiation potential were supported by immunohistologic analysis and suggest an origin of a poorly differentiated pluripotent hepatic cell with the potential to mature. We believe that this case of a mixed hepatoblastoma in an adult should be added to the growing number of presumed hepatic precursor cell neoplasms in adults.

(REPORT OF A CASE

Clinical History

A 78-year-old woman was admitted to the hospital with severe weight loss, pain in the right upper abdomen, and heartburn that had lasted for 8 weeks. Mild edemas were present in both legs. Her medical history included hepatitis A 50 years ago and chronic hepatitis B, which was treated successfully with alpha interferon, leading to seroconversion 7 years ago. Computed tomography showed a tumor of approximately 20 cm in diameter located in liver segments V, VI, and VII. The liver function parameters were within the reference range. Serologically, antibodies against hepatitis A virus and hepatitis B virus surface and core antigens were present. The test results for hepatitis B surface antigen and hepatitis C antibodies in the serum were negative. The following tumor markers were determined: alpha-fetoprotein, more than 121 000 ng/mL; CA 19-9, 16 IU/mL; and carcinoembryonic antigen, 0.9 ng/mL. Serum electrophoresis showed a monoclonal gammapathy type IgG lambda.

The tumor was removed surgically by a nonanatomical resection of liver segments V, VI, and partially VII. The immediate postoperative course was uneventful, and all liver function parameters remained within the reference range. Twenty-two days after surgery, the patient experienced a prolonged, reversible ischemic neurologic deficit and was admitted to an outside neurologic clinic. She developed cardiovascular failure on day 67 postoperatively. A thrombosis of the intrahepatic veins was diagnosed, and tumor recurrence was suspected. The patient died 2 days later. An autopsy was not performed.

Pathomorphologic Findings

The partial liver resection specimen from the right lobe contained a single, partially necrotic tumor with a maximal diameter of 23 cm. The cut surface was slightly lobular and whitish and showed cystic spaces and areas of hemorrhage. The tumor extended through the liver capsule and infiltrated into the diaphragm.

The tumor was mainly composed of a poorly differentiated, epithelial small cell component (approximately 70%), intermingled with a mesenchymal spindle cell component of approximately 30% (Figure 1, A). The small cell component showed a solid growth pattern and a high tendency for necrosis. The tumor cells revealed a high nuclear-cytoplasmic ratio, poorly defined cytoplasm, and significant hyperchromatic and pleomorphic nuclei. A high frequency of atypical mitoses was seen. Partly, this tumor component resembled intermediate-type small cell carcinoma of the lung. A few foci of ductular differentiation of the epithelial component (<1%) were present in areas of dense collagen deposition. The spindle cell component mainly showed fi-
tumor cells that resemble hepatoblastoma (⇐; hematoxylin-eosin, original magnification ×300). C. There is no sharp demarcation to malignant spindle cell areas with transition of spindle cells (△) in epithelial cells (▼; hematoxylin-eosin, original magnification ×400). D. Focal osteoblast-like differentiation and osteoid formation occurred within the tumor (hematoxylin-eosin, original magnification ×400).

Both main cellular constituents showed signs of maturation: the small cell component showed multiple foci of changes into a moderately differentiated, medium- to large-sized hepatocyte-like cell population with round to ovoid, mildly hyperchromatic nuclei and a broad, well-defined, more eosinophilic cytoplasm. Here, the mitotic activity was reduced. No obvious tendency for necrosis was seen. There were no signs that indicated a replacement of the higher differentiated hepatocyte-like cell population by the small cell population (Figure 1, C). The spindle cell component showed a focal hemangiopericytoma-like pattern with broad arborizing capillary structures. Focal osteoblast-like differentiation and osteoid formation occurred (Figure 1, D).

Although the different histologic pattern could be well identified, tumor cells of intermediate phenotypes were present throughout the tumor, suggesting transitional maturation steps. The nonneoplastic liver showed mild fibrosis without signs of cirrhosis or hepatitis.

Immunohistologic Analysis

The small cell epithelial component showed moderate cytokeratin (CK) 8 and CK19 reactivity and was negative for vimentin and CK7 (Figure 2, D). A partial neuroendocrine phenotype of these tumor cells was suggested by the focal dot-like expression of CK19, CK8, and neuron-specific enolase. The spindle cell component was positive for vimentin and negative for CK7, CK8, and CK19, demonstrating mesenchymal differentiation. In addition, the spindle cells were focally positive for CD34 and strongly for CD56 (neuronal cell adhesion molecule). Both main constituents (small cell and spindle cell components) showed a high proliferative activity of up to 50% Ki-67 positivity, whereas proliferative activity in the more differentiated areas was lower.

Markers of hepatocellular differentiation showed the following reactivity: α-Fetoprotein reacted with hepatocyte-like cells but also cells forming osteoid (Figure 2, B). α1-Antitrypsin reactivity was observed in all cell populations with extremely high reactivity in the spindle cell component. HepPar-1 (OCH1E5) was strongly but not uniformly expressed by the hepatocyte-like cells (Figure 2, C). Small cells showed only focal moderate positivity. All cells with mesenchymal phenotype, including osteoid-forming cells, were negative. Albumin showed reactivity with hepatocyte-like small cell and to a lesser extent with the spindle cell component. Ferritin showed a unique reaction pattern with single disseminated but strongly positive cells in the spindle cell and small cell compartment (Figure 2, A). Hepatocyte-like and oste-
Figure 2. Immunohistologic analysis of highly malignant hepatic blastoma in an adult. A, The epithelial tumor cells are strongly positive for ferritin (original magnification ×400). B, The tumor cells of the epithelial and spindle cell components express α-fetoprotein (original magnification ×200). C, They are positive for HepPar-1 (original magnification ×200). D, They are also focally positive for cytokeratin 8 (original magnification ×400). E, Expression of p53 (original magnification ×200) is seen. F, Expression of β-catenin is also seen (original magnification ×200).

oid-forming cells were strongly positive. CD10 was present with some heterogeneity in all cellular compartments with predominance in the spindle and hepatocyte-like cells. A canalicular pattern, however, was not seen. Thus, immunoreactivity clearly shows the hepatic phenotype of the hepatocyte-like cell population but also partial hepatocellular expression phenomena in all other cellular components of the tumor (Figure 2).

E-cadherin was weakly membranous positive in the highly differentiated hepatic tumor areas and completely negative in all other parts of the tumor. Nuclear β-catenin expression occurred only in the epithelial component of the tumor, whereas p53 showed strong nuclear expression in all tumor cells (Figure 2, E and F). All tumor cells lacked p16 or cyclin D1 expression. The main immunohistologic findings are summarized in the Table.

COMMENT

We describe a case of a highly malignant primary liver tumor in an elderly woman. The tumor was primary to the liver, since no other tumor site was detected by extensive preoperative staging. In addition, the tumor contained areas of definite hepatocellular differentiation as shown by morphologic and immunohistologic analysis. The highly malignant potential of the tumor was confirmed by the predominance of poorly differentiated or undifferentiated morphologic features, a high frequency of atypical mitoses, and a high rate of positivity for the proliferation marker Ki-67.

In the differential diagnosis, the tumor has to be compared with several entities, including HCC with sarcomatoid dedifferentiation, carcinosarcoma, hepatoblastoma, and hepatic precursor cell (oval cell) neoplasms.

In dedifferentiated HCCs, an originally highly or moderately differentiated HCC gives rise to a dedifferentiated sometimes sarcomatoid component that usually marginates or overgrows the better differentiated component during tumor progression due to its more aggressive behavior.2 Usually, a peripheral area of higher differentiation in an otherwise dedifferentiated tumor is detected. In the presented tumor, there was no indication for dedifferentiation. The observed maturation phenomena are generally not observed in dedifferentiated HCC.

One may favor calling this tumor a hepatic carcinosarcoma, but it lacks the typical strict dichotomy of the carcinomalous and sarcomatous elements. Instead, a smooth gradual transition that involves various cells of intermediate phenotype is found throughout the tumor.

Recently, several so-called oval cell or precursor cell tumors have been described. They are characterized by their reactivity with antibodies that typically recognize oval cells or by their histomorphologic features. Robrechts and coworkers3 described a highly malignant, monomorphic, poorly differentiated hepatic neoplasm that strongly reacted with the antibody OV-6. Neoplasms of hepatic differentiation that showed areas that closely resembled the undifferentiated morphologic structure of oval cells have also been reported.5 In both reports, the tumors differed significantly and are also different by morphologic features when compared with the present tumor.

Hepatoblastoma can be subclassified into epithelial (fetal, embryonal and fetal, macrotrabecular, and small cell) and mixed epithelial-mesenchymal (with or without teratoid features) types.11 The present tumor certainly resembles mixed-type hepatoblastoma without teratoid features. Maybe a comparable histogenesis exists. For hepatoblastoma, an origin from a pluripotent precursor cell, the hepatoblast, has been suggested. We do call the presented tumor a hepatoblastoma because of morphologic features and not because of the patient’s age. Hepatoblastoma is a tumor of newborn and young children and is extremely rare even in adolescents and young adults. Although a few cases of mixed-type hepatoblastoma have been reported in adults older than 30 years,7–10 existence of hepatoblastoma in older adults has been refused by others.2

In regard to its molecular pathogenesis, the detection of
nuclear β-catenin accumulation suggests an oncogene alteration of the wnt/β-catenin pathway. Furthermore, nuclear p53 accumulation indicates that a p53 mutation is also involved in the molecular pathogenesis of this tumor. Frequent alterations of both pathways have been described in HCCs and hepatoblastomas.

The polymorphic phenotype of the presented tumor may indicate evolution from a pluripotent liver cell with the potential for further maturation. This is supported by the proximity, even intermingling, of all differentiation phenomena. Multiple cell complexes with hepatocyte-like differentiation are embedded into areas of small cell differentiation without any sign of replacement. Cells that represent intermediate differentiation were found in close proximity. Comparable maturation phenomena are frequent findings in childhood tumors. Furthermore, all components, even including spindle and osteoid-forming cells, showed partial hepatocellular differentiation by immunohistologic analysis. Few tumors with comparable morphologic features have been more or less well documented in adults older than 30 years. They have been called mixed-type hepatoblastoma, hepatic embryonal mixed tumor, or malignant mixed tumor of the liver. We believe that this case of a mixed hepatoblastoma in an adult should be added to the growing number of presumed hepatic precursor cell neoplasms in adults.

Dr Kasper was supported partly by the Center for Molecular Medicine of the University of Cologne. For all surgical procedures, consent was obtained from the patient. We are very thankful to Ms S. Sattler for her excellent technical assistance and to Mr Y. A. Weidemann for his language review.

References

Primary Renal Synovial Sarcoma Confirmed by Cytogenetic Analysis

A Lesion Distinct From Sarcomatoid Renal Cell Carcinoma

Beverley A. Shannon, PhD; Ashleigh Murch, PhD; Ronald J. Cohen, FRCPA

Primary synovial sarcoma rarely originates in the renal parenchyma. When this occurs, origin of this unusual tumor type has been the subject of debate in the literature, with a suggestion that previously reported cases may be more correctly described as renal cell carcinoma with sarcomatoid dedifferentiation. Synovial sarcoma and sarcomatoid renal cell carcinoma may be indistinguishable on pure histologic and immunohistochemical grounds, but these tumors contain distinctly different sets of chromosomal abnormalities. Most previous cases of primary renal synovial sarcoma were confirmed by molecular biology techniques, which detected the SYT-SSX gene fusion transcript typical of this tumor, but no details of the other chromosomal anomalies have been published. We report a case of primary renal synovial sarcoma confirmed by standard cytogenetic analysis, showing the characteristic t(X;18)(p11.2;q11.2) translocation and other chromosomal aberrations that are typical of synovial sarcoma as opposed to sarcomatoid renal cell carcinoma.

Arch Pathol Lab Med. 2005;129:238–240

Primary renal synovial sarcoma is a rare tumor type first described in 1999 and further characterized by 2 separate studies in 2000. Many of these tumors were initially diagnosed as embryonal sarcoma of the kidney or adult Wilms tumor but were subsequently found to harbor the t(X;18)(p11.2;q11.2) translocation that is specific for synovial sarcoma (SS). Although SS is most commonly found adjacent to joints and tendons in the limbs of children and young adults, this tumor type has occasionally been identified in more uncommon sites, including head and neck, lung, heart, peritoneum, and prostate. To date, there are 16 cases of genetically confirmed primary renal SS reported in the English-language literature. More recently, however, some doubts have been raised as to whether these tumors should be more correctly classified as sarcomatoid renal cell carcinomas (SRCCs). The argument for this classification is based on the fact that mixed malignant renal tumors have historically been designated as adenocarcinomas with divergent sarcomatoid differentiation (sarcomatoid carcinoma). In contrast, SSs are generally thought to arise from mesenchymal stem cells capable of stromal and/or epithelial differentiation.

If primary renal SS is indeed a form of sarcomatoid differentiation that arises from renal cell carcinoma (RCC), then it is reasonable to expect that the t(X;18)(p11.2;q11.2) translocation associated with the SS phenotype would occur within a cytogenetic profile characteristic of the underlying RCC subtype. Fifteen of the 16 previous cases of primary renal SS were diagnosed using reverse-transcription polymerase chain reaction to detect the SYT-SSX gene fusion transcript caused by the t(X;18) translocation, whereas in the one remaining case a t(X;18) translocation was identified by cytogenetic analysis but no further karyotypic details were published. Therefore, the background cytogenetic profile of these tumors was unknown. We present a case of primary renal SS assessed by standard cytogenetic analysis, which confirms a karyotype that is characteristic of SS as opposed to RCC with or without further genetic aberrations.

REPORT OF A CASE

Following an episode of macroscopic hematuria, a 60-year-old man underwent radical nephrectomy for a radiologically confirmed right-sided renal mass. After receipt of the pathology report, computed tomograms of the chest, abdomen, and pelvis showed no other lesions or evidence of metastatic disease. The patient made an uneventful recovery but developed pulmonary metastases 6 months later. Despite an initial response to chemotherapy, which included imatinib plus 5 cycles of alternating vincristine, adriamycin, and cyclophosphamide or cisplatinum and etoposide, he died 12 months after initial diagnosis.

MATERIALS AND METHODS

Routine tumor samples were fixed in 4% buffered formaldehyde and embedded in paraffin wax. Four-micrometer sections were assessed by routine hematoxylin-eosin stains and immunostains for vimentin (3B4, 1:50; Dako Corporation, Carpinteria, Calif), cytokeratin (AE1/AE3, 1:50, and CAM 5.2, 1:100; Dako), desmin (D33, 1:100; Dako), S100 protein (Z0311, 1:100; Dako), and CD117 (c-Kit) (M7140, 1:100; Dako). A fresh tumor sample was subjected to routine cytogenetic analysis. Chromosome analyses were conducted on 20 G-banded metaphases.
RESULTS

Gross and Microscopic Findings

A 38 × 29 × 32-mm, ill-defined tumor mass was identified in the renal medulla. The tumor was associated with an area of central necrosis and extended focally into the renal pelvis, which contained an abundant blood clot (Figure 1). Histologic examination revealed a hypercellular monomorphic sarcoma composed of fascicles of spindle cells (Figure 2). No evidence of epithelial differentiation was noted. Mitotic activity was high, with 3 to 5 mitotic figures per high-power field. Tumor infiltration of the renal pelvis and renal parenchyma was noted, along with extension through the renal capsule into the renal sinus and invasion into the main renal vein.

Immunohistochemical Results

Immunostains for S100 protein, desmin, and cytokeratin (Figure 3) were negative. Positive immunoreactivity was observed for vimentin and CD117 (c-kit oncogene product), which showed diffuse cytoplasmic staining.

Cytogenetic Analysis

Cytogenetic analysis of 20 metaphases revealed 2 distinct but closely related karyotypes (Figure 4): minor cell line: 44,t(X;18)(p11;q11),?dic(Y;14)(p11;q11),add(1)(p13),−Y,−14,−22 identified in 14 of the 20 metaphases studied is characteristic of synovial sarcoma. There are no complex anomalies typical of sarcomatoid carcinoma or the more usual anomalies of renal carcinoma.
[6]; and major cell line: 43,t(X;18)(p11;q11),–Yadd(l)(p13),−14,−22[14]. Both were near diploid and showed the t(X;18) translocation characteristic of SS plus a rearrangement of the short arm of one chromosome 1 and monosomy for chromosome 22. The major cell line appeared to have arisen from the minor line, losing the Y;14 translocated chromosome and thus becoming monosomic for chromosome 14 and lacking Y.

COMMENT

Morphologically, this tumor was consistent with the monophasic spindle cell (fibrous) subtype of SS (MFSS). Although classic biphasic SS is composed of glandular or solid epithelial structures admixed with a population of spindle cells, MFSSs have spindle to ovoid cells with only immunohistochemical or ultrastructural evidence of epithelial differentiation.7 The immunohistochemical profile of this tumor also fell within the accepted range for MFSSs, of which 63% to 70% are reported to have focal positive staining for cytokeratin AE1/AE3, 83% for vimentin, 4% to 35% for S100 protein, 11% to 100% for CD117 (c-Kit), and 2% for desmin.7,9 However, the morphologic and immunohistochemical profiles do not distinguish this tumor from SRCC, which can appear as a purely spindle cell tumor through overgrowth of the underlying RCC subtype.10 Also, SRCC stains positively for cytokeratin in most cases and for vimentin in 33% to 100% of cases but is negative for desmin and S100 protein.10,11 To date, there are no reports of c-Kit-positive SRCC or any response of these renal tumors to tyrosine kinase inhibition. It is also unknown whether SSs positive for c-Kit will respond to imatinib therapy. Although the present case showed an initial positive response to chemotherapy including imatinib, the patient succumbed to the disease 12 months after initial diagnosis. Recent research indicates that patients with metastatic SS may respond well to combined chemotherapy with doxorubicin and ifosfamide.12,13

Although the morphologic and immunohistochemical profiles do not clearly distinguish this tumor from SRCC, the cytogenetic profile in this current case is highly characteristic of SS. Most SSs have a near-diploid karyotype, with more than 90% of cases showing the t(X;18)(p11.2:q11.2) translocation, as seen in the present case.1 Furthermore, the most common additional aneuploidies in SSs include monosomy for chromosomes 14 and 22 and rearrangements on chromosome 1,14 all of which were observed in the present case. This differs significantly from the cytogenetic analyses available for SRCC to date. Of 10 SRCCs investigated, 7 had highly complex karyotypes, whereas the remaining 3 cases had several deletions, gains, and/or losses, but a t(X;18) translocation was not found in any case.15-17 Gross karyotypic complexity is a characteristic of SRCC and various spindle cell sarcomas, including leiomyosarcoma and malignant peripheral nerve sheath tumor, but it is extremely rare in SS.18 Although our results have established that the current case shows cytogenetic features more characteristic of SS than the complex karyotype of SRCC, it is conceivable that acquisition of the t(X;18)(p11.2;q11.2) translocation in a typical RCC could lead to evolution of a renal carcinoma to SS-like tumor. However, in such a case, we would then expect to also identify the specific abnormalities typical of the progenitor tumor, for example, deletion of 3p in conventional (clear cell) RCC, trisomies of chromosomes 3q, 7, 12, 16, 17, and 20 as associated with papillary RCC, or the hypodiploid karyotype with multiple monosomies (chromosomes 1, 2, 6, 10, 13, 17, and 21) associated with chromophobe RCC.19 We and other investigators15,17,20 have shown this frequent preservation of progenitor tumor karyotype in SRCC. Finally, because the tumor contained no epithelial component, we conclude that this case represents a monophasic SS rather than a RCC or SRCC with SS differentiation.

We acknowledge Philip Allen, MD, Flinders Medical Centre, for his assistance.

References
High-Grade Pelvic Osteosarcoma With Intravascular Extension to the Right Side of the Heart

A Case Report and Review of the Literature

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We describe a rare case of high-grade osteosarcoma with intravascular extension to the right atrium and right ventricle in a 23-year-old woman. Osteosarcomas rarely metastasize to the heart, and only a few cases have been reported in the literature thus far. Diagnoses in some of these cases were made during investigation for severe cardiac failure and in most of these cases at autopsy. We describe a unique case of intravascular extension of the tumor embolus in a cordlike fashion from the left femoral vein to the right side of the heart that morphologically resembled a chondrosarcoma.

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The tumors that commonly invade the inferior vena cava (IVC) include Wilm tumor, testicular tumors, renal cell carcinoma, adrenal carcinoma, hepatocellular carcinoma, and uterine leiomyomatosis. Among sarcomas, chondrosarcoma and leiomyosarcoma are the most common. We report a rare case of an osteosarcoma with intravascular extension from the left femoral vein to the right side of the heart.

REPORT OF A CASE

A 23-year-old woman presented with complaints of leg weakness for 1 month. At the time of presentation, the patient was noted to have a large pelvic mass with extension into the lumbosacral region. Her legs were swollen and tender. A biopsy of the sacrum was performed via an L4 through L5 laminectomy, and the specimen was diagnosed as sarcoma.

The patient was then referred to our institution, where T2-weighted magnetic resonance imaging (MRI) was subsequently performed, which demonstrated a soft tissue mass that invaded the sacrum and bilateral iliac wings. The mass extended into the L4 epidural space. The tumor involved the lower spine and pelvis, and there were right para-aortic, iliac, and right aortic bifurcation nodes seen. Furthermore, venous thrombosis was a concern. The patient was admitted to the hospital and underwent a subsequent debulking procedure that consisted of reexposure of L4-S1 and limited tumor resection. Another biopsy was performed, and the specimen revealed osteosarcoma, chondroid variant.

Shortly after surgery, the patient underwent venography of the IVC, which demonstrated that the entire length of the IVC from the infrarenal area to the right atrium was thrombosed. A transesophageal echocardiogram demonstrated a 4.5 × 1.5-cm mass that originated in the IVC and extended into the right atrium with extension into the right ventricle. The cardiothoracic surgery department was then consulted, and the patient underwent a resection of the mass in the IVC and right atrium (Figure 1). She was subsequently referred for postoperative radiation therapy and chemotherapy. Shortly after the radiation therapy and before the start of chemotherapy, the patient died. Computed tomography of the abdomen and lung immediately before her death showed liver and lung metastases. There was no autopsy performed.

PATHOLOGIC FINDINGS

Grossly, the specimen obtained from the lumbar spine tumor consisted of multiple fragments of tan-pink soft tissue that measured 4.5 × 4.5 × 1.1 cm in aggregate. Microscopically, the tumor was highly cellular, formed by sheets of round to oval to spindle-shaped cells. The primitive-appearing cells had scant cytoplasm, mild pleomorphic nuclei and small nucleoli, and brisk mitotic activity at 15 per 10 high-power fields (Figure 2, A, inset). There were some islands of osteoid (Figure 2, A) and some filigree form of osteoid. Some of the osteoid islands were densely mineralized and lacked osteoblastic rimming (Figure 2, B); rimming osteoblasts are most commonly observed in reactive and benign bone-forming lesions (eg, callus, myositis ossificans, osteofibrosis dysplasia, osteoma, and osteoblastoma). Immunohistochemical analysis for vimentin, S100 protein, smooth muscle antigen, pan-keratin (AE1/AE3), and CD99 showed no reactivity in the tumor cells.

A subsequent specimen from the right ventricular mass grossly consisted of fragments of tan-white firm rubbery lobulated tissue, with cartilaginous consistency that measured 5 × 4 × 2 cm. Histopathologic examination revealed lobules that contained abundant chondroid and myxoid matrix. The cells in the matrix were composed of neoplastic cartilage cells (chondrocytes) that showed severe atypia indicative of a high-grade chondrosarcoma (grade 3). The neoplastic cells in the cartilaginous matrix were...
high grade with binucleated forms, pleomorphism, nucleomegaly (Figure 3), and hyperchromasia. The periphery of the lobules was hypercellular, with hyperchromatic, pleomorphic, spindled, and stellate cells, few mitoses, and focal necrosis (Figure 3, inset). There were some areas of necrosis. The tumor cells were diffusely positive for vimentin and focally positive for S100 protein, and proliferating marker Ki-67 showed 60% staining. A diagnosis of high-grade osteosarcoma with intravascular extension to the right side of the heart was rendered.

COMMENT

Conventional osteosarcoma is a malignant tumor of unknown etiology that most frequently involves the appendicular long bones of young adults within the second decade of life, with 60% of cases occurring when they are younger than 25 years.1 It is the most common nonhematopoietic primary malignant tumor of the bone, with an estimated incidence of 4 to 5 per million. Radiographic findings, particularly on plain x-ray films and MRIs, are an important element in the diagnostic workup. Histopathogic features show a varied degree of cellularity, with the presence of osteoid as the key diagnostic feature.1,2 Osteosarcoma appears to be a highly anaplastic, pleomorphic tumor in which the tumor cells are diverse and may be epithelioid, plasmacytoid, fusiform, ovoid, small round cells, clear cells, mononucleated or multinucleated giant cells, or spindle cells. Most cases are a mixture of 2 or more of these cell types. Osteosarcoma is further subdivided based on the production of varying amounts of cartilage and/or fibrous tissue into osteoblastic (50%), chondroblastic (25%), and fibroblastic (25%) var-
iants. The chondroblastic variant is predominant in the head and neck region.3 There is tendency for metastases to mimic the primary tumor, but exceptions (such as in the current case) are frequent; there is a higher than expected incidence of fibroblastic differentiation in metastases.4,5 Other histologic subtypes include malignant fibrous histiocytoma-like, telangiectatic, low-grade central, giant cell rich, and epithelioid.1,5

The lack of specificity of immunoperoxidase and electron microscopy in osteosarcoma limits their use in diagnosis. Osteocalcin, osteonectin, osteopontin, and alkaline phosphatase have been demonstrated in osteosarcoma.5,6 It has been shown that S100 protein, osteocalcin, and proliferating cell nuclear antigen were highest in osteoblastic and stromal areas and lowest in chondroblastic areas.8

Osteosarcoma of the pelvis constitutes less than 10% of all osteosarcomas.7 The predominance of chondroblastic osteosarcoma in the pelvis has been previously documented.7 Also emphasized in this study is the distribution of primary chondrosarcoma, which commonly involves the pelvis.7 In the absence of specific immunohistochemical markers, it is a great challenge to differentiate these 2 entities, as was the case in our patient.

Untreated osteosarcoma is universally fatal. Its course is marked by its aggressive local growth and rapid hematogenous spread. Pulmonary metastases are the most common site of clinical significance followed by bone metastases. Neoadjuvant therapy (chemotherapy and/or radiotherapy) is typically administered before resection of the primary tumor. Although local therapy (local excision and/or radiotherapy) would likely be performed in a similar fashion for both pathologic types, systemic therapy (chemotherapy) may be different between osteosarcoma and chondrosarcoma, heightening the importance of establishing an accurate diagnosis.

Tumor thromboembolism by osteosarcoma is extremely rare. To our knowledge, only 7 cases of osteosarcoma that causes pulmonary embolism have been reported in the literature thus far.8 The primary sites in these cases were the distal femur in 5 cases, the sacrum in 1 case, and the humerus in 1 case. The primary histologic type was osteoblastic in 2 cases, chondroblastic in 4 cases, and unknown in 1 case. Only 1 reported case involved a tumor that arose from the sacrum and extended as cord to the pulmonary vasculature. However, unlike our case, the histologic type in the vasculature tree was not known. In 2 reported cases,10 the tumor emboli were entirely cartilaginous and no osteoid element was found, whereas the primary site was distal femur in both cases and the histologic type was chondroblastic in one case and osteoblastic in another. Another case8 describes a 65-year-old woman with primary chondroblastic osteosarcoma of the distal femur; the pulmonary emboli showed only cartilaginous differentiation but no osteoid. In a case11 of hepatocellular carcinoma with cartilaginous differentiation, there was intravascular growth. It seems therefore that chondroid or chondromyxoid patterns are associated with intravascular growth. In a previous report, 2% of patients with soft tissue or bone sarcomas showed intravascular thrombi, with associated poor prognosis12; however, most of these tumors were soft tissue sarcomas.

The unique feature in our case was its primary location and the unusual intravascular and intracardiac extension. Although prior reports have documented such a presentation in the pediatric population,13 the constellation of features described is, to our knowledge, the first such report in an adult patient.

References


Primary Broad Ligament Cystadenocarcinoma With Mucinous Component

A Case Report With Immunohistochemical Study

Karima Mrad, MD; Maha Driss, MD; Salma Abdelmoula, MD; Samia Sassi, MD; Monia Hechiche, MD; Khaled Ben Romdhane, MD

Primary cystadenocarcinoma that arises in the broad ligament is extremely rare, especially when it is mucinous. We report the case of a 59-year-old woman with a cystic mass of the right broad ligament who underwent a complete excision of the mass (7 × 7 × 3 cm) with hysterectomy, right salpingo-oophorectomy, omentectomy, appendicectomy, and peritoneal biopsies. Pathologic examination showed a low-grade cystadenocarcinoma with a mucinous component limited to the broad ligament. Despite the chemotherapy (cisplatinum and cyclophosphamide) performed, early recurrence occurred after approximately 6 months. Our observation revealed an abundant mucin production with pools of mucin similar to those of pseudomyxoma peritonei and an inflammatory infiltrate with prominent lipid phagocytosis. Immunohistochemical analysis demonstrated a strong and diffuse positivity for both cytokeratin 7 and epithelial membrane antigen. A less extensive staining with carcinoembryonic antigen and a focal unequivocal positivity with cytokeratin 20, particularly in mucin-secreting cells, were also observed. This finding could indicate a metaplastic process toward colonic phenotype similar to primary ovarian tumors.

Although the secondary involvement of the broad ligament by malignant tumors is common, primary tumors in this site are rare, especially primary malignant tumors, either epithelial or nonepithelial. In fact, nearly all reported cases are benign tumors, either epithelial or nonepithelial (1-4). In fact, nearly all reported cases are benign tumors, either epithelial or nonepithelial (1-4). We report a case of a cystadenocarcinoma with a mucinous component in the broad ligament of a 59-year-old woman.

REPORT OF A CASE
A 59-year-old, para 6, gravida 4, postmenopausal woman with an uneventful gynecologic history was referred to our institution with a diagnosis of a right adnexal tumor revealed by a right leg thrombosis. The etiologic workup, which was composed of an abdominal ultrasonographic examination and a computed tomography study, showed a cystic mass posterior to the uterus with a secondary dilatation of the renal pelvis and ureter. Because the patient was obese, the uterus and ovaries could not be palpated clearly at pelvic examination. However, the pelvic lymph nodes were not enlarged and no ascites was detected. CA 125 and carcinoembryonic antigen levels were within normal limits, whereas the CA 15.3 level was slightly above normal (45 U/mL).

At laparotomy, a right 7 × 7 × 3-cm cystic tumor was found in the right mesosalpinx, which was completely separated from the ovary, fallopian tube, uterus, colon, and bladder. A thorough examination of the abdominal and pelvic cavities at the time of operation revealed no evidence of tumor spread beyond the mesosalpinx. A complete removal of the cystic tumor was performed successfully with no rupture or spillage. The patient then underwent a total hysterectomy with bilateral salpingo-oophorectomy followed by omentectomy, appendicectomy, random peritoneal biopsies, and peritoneal washings. Direct palpation allowed the exclusion of a colonic tumor.

The pathologic examination showed a multilocular cystic mass with smooth inner and outer surfaces filled with a hemorrhagic fluid. Microscopic examination revealed large cysts lined by simple to multistratified, cuboidal to columnar epithelium with a more complex pattern of papillary fronds and coalescent tufts. In solid areas, between the large cysts, we noted glands of variable size and shape with round and angular contours that displayed disordered growth, which indicate a stromal invasion. Most of the glands were filled with mucus and lined by one cell layer that was focally discontinuous. Some nuclei were bland looking and others showed frank atypia with mitotic figures, but in the whole nuclear atypia were moderate (Figure 1). Large pools of mucus were dissecting the stroma and were associated with an abundant polymorphous inflammatory infiltrate that consisted of giant cells with cholesterol clefts alongside lymphocytes, histiocytes, and plasma cells (Figure 2). Immunohistochemical analysis demonstrated a strong and diffuse positivity for both cytokeratin 7 (Figure 3) and epithelial membrane antigen (80%-90% of the cells). Less extensive staining with carcinoembryonic antigen (approximately 60% of tumor cells) and a focal unequivocal positivity with cytokeratin 20, specifically in mucin-secreting cells (5% of tumor cells) (Figure 4), were also observed. Both ovaries and the fallopian tube were grossly and histologically normal. Neither pelvic endometriosis nor metastases in the omentum, peritoneum, and/or appendix were noted. Peritoneal washings were negative for malignant cells.

We diagnosed low-grade cystadenocarcinoma with a mucinous component of the broad ligament. Postoperative chemotherapy was conducted for 6 months using cisplatinum and cyclophosph-
phamide. During the last month of treatment, a pelvic scan revealed a recurrent tumor, which was irradiated (30 Gy).

COMMENT

In 1977, Gardner et al. proposed criteria for primary carcinoma of the broad ligament, namely a primary location within or on the surface of the broad ligament and a complete separation of the tumor from the uterus, ipsilateral ovary, and fallopian tube. When these stringent criteria are applied, primary carcinoma of the broad ligament becomes extremely rare; to our knowledge, only 18 cases have been reported in the literature. In approximate order of frequency, endometrioid, serous, and clear cell carcinoma are the most reported carcinomas, whereas only one case of mucinous cystadenocarcinoma was previously reported by Clark et al. and revised by Aslani and Scully. The mean age of patients with these neoplasms is 46 years (range, 29–70 years). Unlike ovarian carcinoma, which occurs mainly during the sixth and seventh decades of life, these tumors tend to be diagnosed during the reproductive period.

Clinical manifestations are similar to those of ovarian tumors. Although most of the tumors are well demarcated, pelvic adhesions and distant metastasis are possible at the time of diagnosis.

The tumors, which vary in size from 5 to 13 cm in diameter, are always unilateral and can be solid, cystic, or both. Although there is little experience with the therapy of the broad ligament, follow-up data of the reported cases indicate that prognosis of these tumors is favorable, with an 80% to 90% 5-year survival rate and rare metastases. Their proximity to the ovary and the similar histologic features of the 2 types of tumor justify a management similar to that of an ovarian equivalent tumor. The one exception, however, relates to the differing laterality of ovarian and broad ligament carcinoma. In fact, ovarian
carcinomas are bilateral in one third to half of the cases, whereas all the reported carcinomas of the broad ligament are unilateral. Therefore, for young women for whom preservation of reproductive function is desired, a wide local excision of a carcinoma confined to the broad ligament without removal of the adnexa or the uterus may be performed. An adjuvant chemotherapy regimen should be the same as the one used for ovarian carcinomas of similar histologic types. In our case, the early recurrence could be related to the mucinous type of the tumor. The extreme rarity of broad ligament carcinoma could be related to a misdiagnosis in advanced cases, where the whole adnexa is invaded by the tumor, which looks like an ovarian carcinoma. However, nonextensive tumors are also rare, which raises questions about their origin.

Although an accessory ovary or fallopian tube may be postulated as being the origin of these tumors, most authors agree that these and paraovarian tumors are presumed to originate from the wolfian (mesonephric), mullerian (paramesonephric), or mesothelial tissues. Some authors relied on the existence of smooth muscle cells to prove the mullerian origin of these tumors. In our observation, because smooth cells were absent, we presume that they could be invaded by tumor cells or otherwise embedded in the stroma reaction. As described in previous reports, we believe that mullerian and wolfian tumors are morphologically distinct and that glandular and cystic growths are particularly characteristic of wolfian tumors. The originality of our observation lies in the fact that the mucinous component with abundant mucus production mimics the one observed in pseudomyxoma peritonei, whereas both the ovary and appendix were normal. Colonic metastasis was excluded because of the absence of putative primary organs at the operation and the normality of the carcinoembryonic antigen level. The second striking point in our observation, as reported by Rojansky et al, is the extensive stroma reaction with lipid phagocytosis. Immunohistochemical analysis results were typical of a mullerian-type tumor with positivity of epithelial membrane antigen and carcinoembryonic antigen, which contrasts with their negativity in wolfian tumors. To our knowledge, cytokeratins 7 and 20 have not been studied in previous reports. Diffuse positivity of cytokeratin 7 is expected in this tumor, whereas focal positivity of cytokeratin 20 in mucin-secreting cells, an antigen usually positive in intestinal-type cells, could indicate a metaplastic process toward colonic phenotype and therefore explain the extreme rarity of the primary mucinous broad ligament.

References

Extensive Aortic Thromboembolism Due to Acquired Hypercoagulable State
An Autopsy Case Report

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Aortic thrombosis rarely occurs without severe atherosclerosis, aneurysm, or cardsurgical or traumatic state. Arterial thrombosis is commonly related to an inherited and/or acquired hypercoagulable state. A 50-year-old woman presented with diffuse abdominal pain. One day after her admission, she experienced bloody stools. Computed tomography showed multiple extensive thromboses in the aorta and superior mesenteric arteries. She underwent a partial jejunooileostomy and colectomy for extensive bowel infarction. Following surgery, her condition deteri- and she died on the fourth hospital day. Autopsy, gross examination showed 2 large thrombi (7 and 8 cm in length) in the proximal and descending (thoracic) aorta, with mild atherosclerosis. A mesenteric artery thromboembolus with extensive bowel infarction was present. Post-mortem laboratory studies revealed an elevated anticardioli- pin immunoglobulin G antibody level. The thrombotic state in this patient was considered multifactorial second- ary to acquired risk factors, including obesity, mild aortic atherosclerosis with coronary artery disease, and presence of a high titer anticardiolipin antibody.

Report of a Case

A 50-year-old woman presented to the emergency department with abdominal pain that extended from the xiphoid to the umbilicus and had lasted for 4 hours. She complained of stabbing pains, exacerbated with urination, and nausea, vomiting, urinary urgency, and loose bowel movements. There was no history of illicit drug use, alcohol abuse, or smoking. Physical examination revealed normal vital signs, but respiratory effort was decreased...
phenylmethyl-iminodiacetic acid (HIDA scan) was scheduled on for an intra-abdominal process. Technetium Tc 99m dimethyl-pival for further investigation. A CT after admission was negative. Inlet x-ray films showed dilated loops of small bowel with air.

She had chills and diaphoresis. Abdominal examination revealed diffuse and shallow. Tenderness and guarding in the right lower and left lower quadrants were noted on physical examination. No costovertebral angle tenderness was elicited. Laboratory data included the following values: white blood cells, 18,000/μL with 87.3% granulocytes; hemoglobin, 15.9 g/dL; hematocrit, 47.8%; and platelets, 240,000/μL. Coagulation screening test results were within reference ranges, with a prothrombin time of 10.4 seconds with an international normalized ratio of 1.1 and an activated partial prothrombin time of 25.8 seconds. An elevated blood sugar level was found, with a blood glucose level of 215 mg/dL (11.9 mmol/L). Serum electrolyte levels and liver function test results were within normal limits. Erythrocytes, protein, and bacteria were detected by microscopic examination of urinary sediment. An abdominal CT demonstrated a small focus of calcification in the left pelvic-urethral area and chronic pyelonephritis. Abdominal plain x-ray films showed no bowel obstruction or perforation. After receiving morphine and supportive treatment, the patient improved and was discharged. Two days later, she re-presented to the emergency department with increased epigastric and right upper quadrant abdominal pain. She was unable to lie down and had chills and diaphoresis. Abdominal examination revealed diffuse tenderness without rebound pain, guarding, and decreased bowel sounds. Laboratory studies again revealed leukocytosis, with a white blood cell count of 16,000/μL. Subsequent abdominal x-ray films showed dilated loops of small bowel with air-fluid levels suggestive of an ileus. She was admitted to the hospital for further investigation. A CT after admission was negative for an intra-abdominal process. Technetium Tc 99m dimethylphenylmethyl-iminodiacetic acid (HIDA scan) was scheduled on hospital day 2. Maroon blood was passed per rectum during days 1 and 2, and on hospital day 3, her temperature rose to 37.8°C with a white blood cell count of 21,000/μL and bandemia. Pulse was 141/min and blood pressure was 99/46 mm Hg. She was hypoxic with abdominal distention. An abdominal CT performed in the afternoon of day 3 revealed thrombi in her descending aorta and occlusion of her superior mesenteric artery (Figure 1). An immediate exploratory laparotomy was undertaken, and 254 cm of infarcted small bowel and right colon were resected. After surgery, her condition did not improve, and the patient developed mottled legs (ecchymosis). Therapy with intravenous unfractionated heparin was started. Based on her wishes, only comfort measures were instituted. She died on the fourth day of admission.

**AUTOPSY FINDINGS**

Postmortem examination showed a well-developed, moderately obese, white woman who weighted 119 kg and was 1.78 m tall, giving a body mass index of 38.4. A 37-cm recent surgical incision was present in the middle abdomen. Diffuse erythematous mottled areas were identified on the left leg and right thigh, and ecchymoses were present on the feet. Internal examination revealed a 500-g heart with left ventricular concentric hypertrophy and focal cardiac lipomatosis. There was focal occlusion (75%) of the proximal left anterior descending coronary artery and recent blood clots in the left atrium. No vegetations or thrombi were noted on the cardiac valves. No remote or recent myocardial infarctions were present on gross or microscopic examination. Mild aortic atherosclerosis was present, along with organized aortic thrombi: one (7.0 × 1.5 × 1.0 cm) in the first portion of the descending aorta, another (8.0 × 1.0 × 0.8 cm) at the level of the middle descending aorta (Figure 2, A), and another (0.8 × 0.6 × 0.3 cm) just before the abdominal bifurcation. A large, fresh thrombus (6.0 × 0.7 × 0.6 cm) was seen in the superior mesenteric artery. Examination of major veins revealed no antemortem clots. Lungs were diffusely congested and edematous bilaterally. The liver was 2500 g with diffuse congestion, and the gallbladder was unremarkable. Focal fatty infiltration was seen in the pancreas. A 1.5-cm cortical depression in her right kidney was associated with focal dense fibrosis. No renal or urethral calculi were found. The remaining small bowel appeared dark purple and gangrenous with yellow-green pseudomembranous mucosa. The remaining large intestine was unremarkable. No pathologic changes were seen in the genital organs. The cause and mechanism of death was determined to be septic shock due to bowel infarction, secondary to thromboembolic disease. Because no tests for a hypercoagulable state were performed during hospitalization, a limited profile was completed as part of the postmortem examination. Whole blood specimens obtained during the patient’s hospitalization and retained in the chemistry laboratory were sent for molecular studies to include factor V Leiden, prothrombin mutation G20210A, and mutation of methylene tetrahydrofolate reductase (MTHFR). No genetic mutations of prothrombin (G20210A) or factor V Leiden were found. A single copy of the mutation A1298C of the MTHFR gene was detected. Whole blood obtained at autopsy could have also been used for these studies. A serum sample, also drawn during the patient’s hospitalization and retained in the chemistry department, was assayed for anticardiolipin antibodies by an enzyme-linked immunosorbent assay method (Bindazyme, The Binding Site, San Diego, Calif). An elevated titer of anticardiolipin IgG of 82 GPL units was
Figure 2. A, Extensive thrombosis in the proximal and middle portion of the descending aorta (7.0 × 1.5 × 1.0 cm and 8.0 × 1.0 × 0.8 cm). B, Thrombus firmly adhered to intima of thoracic aorta (hematoxylin-eosin, original magnification ×20). C, Aortic thrombosis with hemosiderin-laden macrophage, fibrin deposition, and fibrosis (hematoxylin-eosin, original magnification ×100).

Figure 3. Extensive hemorrhage, necrosis, and sloughing of surface epithelium of small bowel indicated a recent infarct (hematoxylin-eosin, original magnification ×20).

Figure 4. Microscopic view of cross-section of thromboembolism in superior mesenteric artery (hematoxylin-eosin, original magnification ×20).

found (reference range, <10 GPL units). Anticardiolipin IgM and IgA were 8 MPL units and less than 6 APL units, respectively (reference range, <12 MPL units and <15 APL units).

The significant postmortem microscopic findings included recent, extensive small bowel hemorrhagic infarctions (Figure 3) and aortic thrombi with foci of fibrosis, macrophage infiltration, and hemosiderin deposits (Figure 2, B and C). The mesenteric arterial emboli appeared fresh with focal organization (Figure 4). Additional findings were mild myocardial hypertrophy and focal lipomatosis (fatty dysplasia) that involved the right atrial septum and left ventricle; focal 75% arteriosclerotic occlusion of the left anterior descending coronary artery; pulmonary congestion and edema with focal consolidation and mild pleural effusion; sinusoidal congestion of the liver and fatty infiltration of the pancreas; chronic pyelonephritis; global cerebral hypoxia; and a pituitary chromophobe microadenoma (4 mm).

COMMENT

Factor V Leiden, which leads to an activated protein C resistance, is the most common inherited risk factor of venous thromboembolism. Prothrombin (G20210A) mutation is usually associated with venous thromboembolism but may play a role in arterial thrombosis, since the mutation in the 3’-nontranslated code region may cause an accumulation of messenger RNA and high circulating levels of prothrombin. MTHFR participates in regulating homocysteine metabolism, and a mutation of MTHFR may be a marker for possible elevated homocysteine levels when the serum folate level was lower than the reference range. High levels of homocysteine are thought to damage endothelium, producing arterial thromboses in arteries and veins. Currently, no causal role for hyperhomocysteinemia in venous or arterial thrombosis is yet established. Two common mutations involving the MTHFR gene have been identified: C667T and A1298C. A recent 772-case study demonstrated that the prevalence of the C677T and A1298C polymorphisms did not differ among individuals with coronary atherosclerosis disorders, with deep venous thromboses, or without vascular diseases. Finding an MTHFR mutation, therefore, is considered to be an indicator for measurement of homocysteine levels and possibly B vitamins or folic acid therapy. Therefore, a heterozygous mutation (A1298C) in the MHTFR gene without an accompanying fasting homocysteine level in our patient could not be evaluated as a contributor to her hypercoagulable state.

Antiphospholipid antibodies are the most common acquired blood protein defect associated with either venous or arterial thromboses. These antibodies include lupus anticoagulants, anticardiolipin antibodies, and newly recognized subgroups of antiphospholipid antibodies (eg, β2-glycoprotein, β2-glycoprotein-1, phosphatidylserine). Studies have shown that anticardiolipin antibodies play a
major role in arterial and venous thrombosis. Anticardiolipin antibodies interfere with hemostasis and coagulation by such mechanisms as blocking release of prostacyclin from endothelium, inactivating thrombomodulin, the anticoagulant function of activated protein C and its cofactor protein S, and interacting with platelet membrane phospholipid, leading to activation of platelets. Clinically, anticardiolipin antibodies can occur in many situations, including inflammatory and infectious disorders, malignancies, immune thrombocytopenia purpura, leukemia, and medication use. The antibodies may be transient, which may or may not cause thrombotic events. However, persistence of the anticardiolipin antibodies (2 positive test results at an interval of 6 weeks or greater) is considered clinically significant. In addition, the finding of a positive anticardiolipin IgG antibody at medium or high titer on a single occasion may help identify patients at risk for thrombosis. The high level of anticardiolipin antibodies on a single occasion may help identify patients at risk for thrombosis. Anticardiolipin antibodies are independent risk factors for thromboses. Anticardiolipin antibodies interfere with hemostasis and coagulation by such mechanisms as blocking release of prostacyclin from endothelium, inactivating thrombomodulin, the anticoagulant function of activated protein C and its cofactor protein S, and interacting with platelet membrane phospholipid, leading to activation of platelets. Clinically, anticardiolipin antibodies can occur in many situations, including inflammatory and infectious disorders, malignancies, immune thrombocytopenia purpura, leukemia, and medication use. The antibodies may be transient, which may or may not cause thrombotic events. However, persistence of the anticardiolipin antibodies (2 positive test results at an interval of 6 weeks or greater) is considered clinically significant. In addition, the finding of a positive anticardiolipin IgG antibody at medium or high titer on a single occasion may help identify patients at risk for thrombosis.

Testing for lupus anticoagulants is an additional way to detect antiphospholipid antibodies. It is less sensitive but more specific for detecting antiphospholipid antibodies than anticardiolipin antibodies. Screening tests for lupus anticoagulants are functional assays, as are the screening tests for protein C, protein S, and antithrombin, and cannot be performed on postmortem or unfrozen stored plasma. Thus, these important studies were not available for our patient.

Prothrombotic and antithrombotic balance in the body depends on an intact endothelium, normal blood flow, and balanced coagulation elements. In the described patient, the balance was disrupted by an acquired circulating anticardiolipin antibody level, obesity with occult metabolic syndrome, and other cardiovascular problems. She especially had cardiomegaly, lipomatosis in her atria and ventricles, and occlusion of her left anterior descending coronary artery, which caused an abnormal blood flow. Patients with coronary artery disease and damaged myocardium can experience decreased fibrinolytic activities and a hypercoagulable state due to increased factor VIII activity and fibrinogen levels (acute-phase reactants). In addition, aortic atherosclerosis, even mild, can damage an intact endothelium. In this case, moderate obesity was a potential cause of dyslipidemia, insulin resistance, and diabetes mellitus (metabolic syndrome). The patient, however, lacked primary care and had not undergone laboratory investigation for these disorders, although an elevated fasting glucose level was documented during hospitalization. Her increased body mass index and occult metabolic syndrome were acquired risk factors associated with hypercoagulability, as was her atherosclerosis, coronary artery disease, and elevated anticardiolipin antibody level, making her thrombotic tendency multifactorial. It is very likely that the anticardiolipin antibody was an initiator of the aortic-artery thromboses, which led to bowel infarction and sepsis. A high clinical index of suspicion for hypercoagulability could offer the opportunity to avoid such catastrophes. This index of suspicion can be carried over to an autopsy, and laboratory tests for a hypercoagulable state, using retained blood samples or postmortem whole blood for molecular studies, may be included as part of the postmortem examination.

References

Polyclonal B-Cell Lymphocytosis Mimicking Malignant Lymphoma in a Newborn

Kit Fai Wong, MD; Hui Leung Yuen, MBBS; Jennifer N. S. Leung, MBBS; John K. C. Chan, MBBS

- We describe a 17-day-old newborn with fever and peripheral blood lymphocytosis. The circulating lymphocytes were large with lobulated and nucleolated nuclei. Their immature and uniform appearance raised the possibility of malignant lymphoma in the leukemic phase. Immunophenotypic study, however, showed that the lymphocytes were CD19+ B cells with expression of both κ and λ light chains. Molecular biology study confirmed a polyclonal nature of the immunoglobulin heavy-chain gene. Cytogenetic analysis showed a normal karyotype, and viral cultures and serologic studies yielded negative results. The polyclonal lymphocytosis was self-limiting and disappeared within a month.

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Persistent polyclonal B-cell lymphocytosis is a rare disorder that occurs predominantly in women and is characterized by circulating, proliferated polyclonal binucleated B cells. This is a benign condition with a chronic and stable clinical course. Reactive lymphocytosis in children is not uncommon and is almost always due to polyclonal CD8+ T-cell response toward viral infections. In this report, we describe an unusual example of polyclonal B-cell lymphocytosis that occurred in a newborn.

REPORT OF A CASE

A 17-day-old male newborn who was born full term by normal delivery presented with fever for 1 day. There was a history of neonatal jaundice (serum bilirubin level up to 13.2 mg/dL) managed by phototherapy. Clinical examination showed a febrile newborn with no rash, lymphadenopathy, or organomegaly. Peripheral blood examination showed the following values: hemoglobin, 13.5 g/dL; platelets, 480 000/μL; and leukocytes, 22 600/μL. A manual differential cell count revealed 19% neutrophils, 37% lymphocytes, 4% monocytes, 2% eosinophils, and 38% atypical mononuclear cells. The atypical mononuclear cells were large (2 to 3 times the size of a small lymphocyte) and had irregularly folded to lobulated nuclei, open to partially clumped chromatin, distinct nucleoli, and lightly basophilic agranular cytoplasm (Figures 1 and 2). Bone marrow examination showed a normocellular marrow with adequate megakaryocytes, normoblastic erythropoiesis, normal granulopoiesis, and the presence of 12% abnormal mononuclear cells similar to those observed in the peripheral blood. Immunophenotypic study using the labeled streptavidin-biotin technique on cytopsin preparation of Ficoll-concentrated mononuclear cells showed that the circulating abnormal mononuclear cells were B cells, expressing CD19 and CD20 but not CD3, CD5, CD10, CD23, and CD56. These B cells showed polyclonal staining for κ and λ light chains. The CD3/CD19 ratio was 1.2, and the CD3+ cells were almost invariably small lymphocytes. Cytogenetic analysis performed by fluorodeoxyuridine-synchronized unstimulated and 12-O-tetradecanoylphorbol-13-acetate stimulated cultures of the marrow mononuclear cells showed a normal karyotype of 46,XY. Polymerase chain reaction using primers against immunoglobulin heavy-chain gene (framework 2 and framework 3) showed a polyclonal pattern. Viral cultures of cerebrospinal fluid, urine, and upper respiratory tract secretions and serologic studies for Epstein-Barr virus, respiratory syncytial virus, influenza virus, parainfluenza virus, adenovirus, cytomegalovirus, herpes simplex virus, and varicella-zoster virus were all negative. Microbiologic culture of the blood was also negative. The patient was treated empirically with ampicillin and gentamycin. His fever subsided 1 day after initiation of antibiotic therapy, and the lymphocytosis gradually disappeared within a month.

COMMENT

Reactive lymphocytosis is common in children during viral infections. A notable example is the atypical lymphocytosis associated with infectious mononucleosis. In infectious mononucleosis, the Epstein-Barr virus primarily infects B lymphocytes. During the first week of illness, the infected B lymphocytes proliferate but constitute only 1% to 2% of the peripheral blood lymphocytes. Subsequently, CD8+ T lymphocytes are produced to control the B-lymphocyte response, and they account for the bulk of circulating atypical lymphocytes characteristic of infectious mononucleosis. In fact, it has been shown that all cases of atypical lymphocytosis, irrespective of etiology, are characterized by a marked increase of activated CD8+ T lymphocytes in the peripheral blood with no increase in B lymphocytes. The activated T lymphocytes often show a marked degree of morphologic pleomorphism, which has led Downey to categorize the atypical lymphocytes of infectious mononucleosis into different subtypes.

In this report, we describe a transient reactive lymphocytosis of unknown etiology in an infant. The circulating proliferated lymphocytes have more irregular and segmented nuclei than the Downey type III cells. Their morphologic features are alarming in that they exhibit an immature or open chromatin pattern and marked nuclear

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foldings, raising the possibility of lymphoma because of the atypia. The cytologic features are not in keeping with acute lymphoblastic leukemia or acute myeloid leukemia. It is understandable that lymphoid neoplasms other than lymphoblastic type are extremely rare in this age group, but anaplastic large cell lymphoma and large B-cell lymphoma are certainly possible. On immunophenotyping, the atypical lymphocytes are surprisingly shown to represent B rather than T cells. The polytypic pattern of immunoglobulin staining suggests a reactive process, a fact further confirmed by the lack of clonal population on polymerase chain reaction analysis of immunoglobulin genes, absence of cytogenetic abnormality, and clinical evolution. Persistent polyclonal B lymphocytosis is an uncommon disorder that occurs almost exclusively in female patients, usually cigarette smokers, and is characterized by a stable but persistent expansion of polyclonal binucleated CD5~CD23~ lymphocytes and elevated serum IgM levels. An association with HLA-DR7 phenotype and multiple bcl-2-immunoglobulin gene rearrangements has been previously reported. This condition has not been described in children and is certainly different from the case described herein.

To the best of our knowledge, only a single case of polyclonal B-cell lymphocytosis has been reported in an infant. In that case, the abnormal lymphocytes were mature CD23~ HC2~ B lymphocyte with a single small nucleus and condensed chromatin and scanty cytoplasm, thus making it morphologically different from our case. The association with a febrile illness and the transient nature of the lymphocytosis suggest a viral etiology, although the cause remains elusive despite exhaustive virologic studies.

References
Pathologic Quiz Case

An 86-Year-Old Man With a Painless Right Tongue Mass

Ronald M. Angeles, MD; Jonathan Vasquez, MD; Oliver Kim, MD

A n 86-year-old man with a significant history of smoking and alcohol intake presented with a painless right tongue mass that had been present for an unknown period of time. Physical examination revealed a pedunculated polypoid mass measuring 0.5 cm in greatest dimension involving the midanterior two thirds of the right tongue blade. The rest of the physical examination and the laboratory studies were unremarkable. The patient subsequently underwent an outpatient excisional biopsy of the tongue mass.

Grossly, the specimen consisted of an irregular, round fragment of tan to light yellow rubbery soft tissue measuring 0.3 × 0.2 × 0.2 cm. Histologically, the specimen was a well-circumscribed mass lined by squamous epithelium with mild parakeratosis (Figure 1). The submucosa was expanded by variably sized adipocytes interspersed within a fibrous stroma supported by a fibrovascular stalk. There were monovacuolated and multivacuolated lipoblast-like cells that showed scalloped nuclei (Figures 2 and 3). Immunohistochemical stains were positive for S100 protein (lipocytes and lipoblast-like cells) and CD34 (spindle cells, focal). The CD31 stain highlighted the blood vessels. Cytokeratin stains (AE1/AE3 and MAK-6) were negative.

What is your diagnosis?
Liposarcoma is the most common soft tissue sarcoma, accounting for approximately 20% of all mesenchymal malignancies encountered by practicing surgical pathologists. The World Health Organization classification currently recognizes 5 types of malignant adipocytic tumors, including atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLPS). ALT/WDLPS represents 40% to 45% of all liposarcomas and tends to occur in the retroperitoneum and the limbs. It is very rare in the head and neck region and even rarer in the oral cavity. Fewer than 50 cases appear to have been described in the literature. The most commonly reported histologic subtype in this location has been myxoid liposarcoma, followed by ALT/WDLPS. Some authors believe that reports of the former cases may represent examples of the well-differentiated subtype, with pronounced stromal myxoid changes as a consequence of chronic local trauma to the area. The most commonly reported site in the oral cavity is the cheek, followed by the tongue. It can occur in the floor of the mouth, soft palate, lip, and gingiva.

In the study by Nascimento and colleagues, the median age at time of presentation was 49.5 years. There was a slightly greater frequency in men. The most common symptom was a slowly growing, painless mass or swelling. The initial impressions were mostly benign lesions, including lipoma or fibroma. Treatment consisted of excisional biopsy, followed by wider re-excision because of positive margins.

Grossly, these tumors are usually well-circumscribed, lobulated masses. Encapsulation is uncommon. The appearance can vary from white-tan to yellow depending on the proportion of adipocytic, fibrous, and myxoid areas. Necrosis and hemorrhage are common in larger specimens.

Microscopically, infiltration to the surrounding muscle is typically seen. Some cases may show diffusely infiltrative lesions with entrapment of muscle, nerves, or both. All tumors show variation in adipocyte size, which is sometimes the most striking feature. There are hyperchromatic, atypical stromal cells present in the fibrous septa. A varying number of monovacuolated or multivacuolated lipoblasts may be seen. Lipoblasts are defined by the presence of single or multiple sharply margined cytoplasmic vacuoles scalloping an enlarged hyperchromatic nucleus. It should be emphasized that the presence of lipoblasts does not confirm a diagnosis of liposarcoma, because some benign lesions, such as lipoblastoma, chondroid lipoma, spindle cell lipoma, and pleomorphic lipoma, contain lipoblasts. Additionally, the presence of lipoblasts is not required for the diagnosis of liposarcoma if atypical stromal cells are present.

The major differential diagnoses are mostly benign lesions such as lipoma, fat necrosis, and spindle cell lipoma. Lipomas are lobulated and are composed of mature fat cells, with minimal variability in adipocyte size and no cytologic atypia. Trauma and ischemia can lead to fat necrosis. A thin fibrous capsule surrounds most of these tumors. Spindle cell lipomas are characterized by varying admixtures of adipose tissue, multinucleated giant cells, and bland spindle cells associated with bands of ropy collagen, often within a fibromyxoid matrix containing mast cells. Fibrous septa with atypical stromal cells and numerous lipoblasts should not be seen.

ALT/WDLPS is characterized by the presence of supernumerary ring and giant chromosomes composed of amplified sequences from the 12q14–15 chromosome region. Several genes are present in this region, including MDM2 and CDK4. These oncogenes act as positive regulators of cell cycle progression at the G1-S checkpoint. Hostein and colleagues used real-time polymerase chain reaction to detect MDM2 and CDK4 gene amplification in 36 cases of lipomas and 48 cases of ALT/WDLPS and dedifferentiated liposarcomas. They found that MDM2 and CDK4 gene amplifications were seen in 98.2% and 82.4%, respectively, of liposarcomas and in only 2.8% and 5.6%, respectively, of lipomas. Co-amplification of the 2 genes was observed more in dedifferentiated liposarcomas than in ALT/WDLPS. This is a fast and reliable method that can be used with paraffin-embedded tissues when distinguishing between lipoma and liposarcoma proves to be difficult histologically. Amplification of chromosome 1 sequences is also seen in liposarcomas. Nilsson and colleagues showed that candidate oncogenes COA51, COA52, and COA53 (ie, chromosome one amplified sequence) originating in 1q21–23 were amplified in 13 of 21 cases (2 lipomas and 11 liposarcomas). The most common location of extra COA5 signals was in supernumerary ring and giant marker chromosomes.

In summary, we present a rare case of an ALT/WDLPS of the tongue. The preferred term is an atypical lipomatous tumor, because the oral cavity is generally a surgical amenable site and the tumor's excision with clear margins usually results in cure. One should be aware of this type of tumor so that the proper diagnosis and treatment can be given accordingly to patients.

We thank Sharon Weiss, MD, of Emory University for confirming our diagnosis.

References
A 52-year-old woman presented with jaundice, weight loss, and generalized pruritus. Her past medical history was significant for necrotizing pancreatitis with pseudocyst formation 10 years prior to the current presentation and a 2-year history of a cyst in the head of the pancreas. A computerized tomographic scan demonstrated a large, calcified, 10-cm mass in the head of the pancreas that involved the duodenum. There was also marked dilatation of the entire biliary tree, including the intrahepatic ductal system as well as the common bile duct. No radiologic evidence of vascular invasion by the pancreatic mass was noted. An ultrasound-guided fine-needle aspiration was performed using a 22-gauge Franseen needle. Smears were air dried and stained with Diff-Quik or were wet fixed in ethanol for subsequent Papanicolaou staining.

On gross examination, the aspirate had a smooth, glistening appearance. Cytopathologic examination showed abundant mucinous material, degenerated inflammatory cells, and rare 3-dimensional fragments of benign-appearing epithelium (Figure 1). Higher magnification showed cytologic atypia with enlarged, crowded, hyperchromatic nuclei (Figure 2). Numerous single cells with large solitary intracytoplasmic vacuoles consistent with mucin were also noted (Figure 3). These cells occasionally had indented nuclei simulating signet ring cells.

The patient was taken to the operating room and was found to have a large cystic pancreatic lesion extending to the retroperitoneum and encasing visceral vessels. A biopsy of the mass was performed, which revealed scattered loosely cohesive groups of epithelium and cells with large cytoplasmic vacuoles in a mucinous background (Figure 4).

What is your diagnosis?
Primary colloid carcinoma (CC) is a rare subtype of adenocarcinoma of the pancreas characterized by production of extensive mucin. Although CC of other organs such as the breast, colon, and prostate have been studied extensively, there are only a limited number of reported accounts on CC of the pancreas.1-3

Although the clinicopathologic characteristics of the pancreatic CC (location, gender distribution, age, and clinical presentation) are similar to those of conventional-type pancreatic adenocarcinoma, recent studies have suggested that it may have a better prognosis.4,7 Grossly, CCs are often large cystic masses with copious mucoid material. Fine-needle aspiration is an extremely useful adjunct to radiology in the evaluation of cystic lesions of the pancreas.5 Cytologically, abundant mucin is seen admixed with fragments of benign-appearing glandular epithelium or single cells. Cytopathologic distinction from other mucinous neoplasms (such as an intraductal pancreatic mucinous neoplasm [IPMN]) can be difficult. Incidentally, on initial cytopathologic evaluation, the current case was interpreted as consistent with a mucinous cystic neoplasm with focal marked atypia, and the possibility of adenocarcinoma was suspected. Subsequent evaluation, however, revealed cytopathologic features (marked nuclear enlargement, hyperchromasia, and signet ring–type cells) consistent with adenocarcinoma. Histologically, the cystic areas in CC are lined either by flattened mucin-producing cells or by simple connective tissue strands. The tumors have well-defined pools of mucin and free-floating mucinous epithelial cells. Occasionally, signet ring cells (as seen in our case) may be identified in the mucin.

The differential diagnosis of CC on fine-needle aspiration includes mucinous cystadenocarcinoma, IPMN, conventional-type ductal adenocarcinoma with mucinous features, and signet ring adenocarcinoma. The clinicopathologic features of CC of the pancreas have not yet been well characterized, because cases of CC are often associated with mucinous cystic neoplasm or IPMN.6 On histopathologic examination, the current case also revealed an associated IPMN. There are conflicting reports as to whether colloid differentiation may be an independent variable in survival studies.6,7

Adsay et al7 examined 17 cases of CC, 9 in men and 8 in women. The mean age of the patients was 61 years. Of these cases of CC, 10 were originally classified as mucinous ductal adenocarcinoma and 4 as mucinous cystadenocarcinoma. The tumors had abundant mucin, with malignant cells amid the mucin pools. Regional lymph node metastasis was seen in 8 cases. Molecular studies showed K-ras mutations (codon 12) in 4 of 12 cases.7

In the study by Adsay et al,7 the 5-year survival of CC patients was 57%. The authors compared the CC cases with 82 cases of typical ductal adenocarcinoma and found that although the patients with CC presented with larger tumors, they presented at a lower stage (P = .01). The authors also reported that the prognosis of CC cases was significantly better than that of the typical ductal-type pancreatic adenocarcinomas. At 5 years, CC patient survival was 57% versus 12% for the typical ductal adenocarcinoma.7 Other studies have also shown that CC arising in association with IPMN has a favorable prognosis.9

In contrast to the study by Adsay et al,7 other authors have reported that colloid differentiation was not an independent predictor of patient survival.4 Seidel et al described 39 cases of pancreatic and periampullary carcinoma with colloid differentiation. Colloid carcinoma patients had 2- and 5-year survival rates of 69% and 29%, respectively. The authors reported that in a multivariate model, colloid differentiation was not an independent predictor of patient survival. Therefore, CC of the pancreas and periampullary region does not necessarily have a better prognosis than carcinomas without colloid differentiation. Instead, the authors reported that other factors, including location, perineural and vascular invasion, and margins, may be more important.6

In conclusion, CC of the pancreas may occur with or without an IPMN and mucinous cystic neoplasm component. Because of asymptomatic presentation or nonspecific symptoms, diagnosis may not be made until a very advanced stage. Imaging studies play a pivotal role in the diagnosis of CC, and the best modalities include computed tomography and magnetic resonance imaging. The outcome for patients with CC is dependent on the tumor location and the extent of metastatic spread. Overall, CC of the pancreas may be associated with a better prognosis than ordinary ductal adenocarcinoma. Therefore, an accurate and early diagnosis is very important and can make a difference in successful outcome. Fine-needle aspiration is a useful diagnostic modality and can be used to provide rapid and accurate diagnosis in cases of CC.
Pathologic Quiz Case

A 24-Year-Old Woman With Abnormal Hemoglobin and Thrombocytopenia

Lizmarie Andino, MD; Semyon A. Risin, MD, PhD

The patient, with a 1-year history of idiopathic thrombocytopenic purpura, was admitted to the hospital because of a severely decreased platelet count (9000/µL) found during follow-up complete blood count testing. She did not have any significant complaints. There was no history of bleeding from any source. However, on questioning, she mentioned a few spontaneous ecchymoses on the arms. No other significant findings were obtained on physical examination. Her complete blood count was remarkable for moderate anemia (hemoglobin, 8.5–9.6 g/dL), microcytosis (mean corpuscular volume of 60.8 fl, mean corpuscular hemoglobin of 19.7 pg, and mean corpuscular hemoglobin concentration of 32.4 g/dL), and elevated red cell distribution width (19.1%). Her red blood cell count was within the normal range. The platelet count was 11,000/µL. Mild neutrophilia with a mild left shift was also present. The patient was admitted with a diagnosis of a relapse of idiopathic thrombocytopenic purpura, and treatment with steroids and intravenous immunoglobulin was initiated. Because of the microcytic anemia, further workup was performed, and hemoglobin studies were ordered. Hemoglobin electrophoresis was performed at alkaline (8.6) and acidic (6.2) pH. The corresponding gels are presented in Figure 1, A and B, respectively. Arrows indicate the index case in lane 3 of both gels. Lanes 1 and 5 are markers (hemoglobin A, S, C and hemoglobin A, F). Figure 2 represents the scan of the alkaline gel, and Figure 3 the high-performance liquid chromatography results.

What is your diagnosis?

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Pathologic Diagnosis: Compound Heterozygosity for Hemoglobin S and E

S/E hemoglobinopathy occurs in a double-heterozygous individual with 2 different mutations in the beta-chain genes located on the p arm of chromosome 11. It is quite a rare entity. The prevalence of S/E carriers among all individuals with major hemoglobinopathies is not defined, because only a few cases have been reported so far. As is well known, the S mutation causes substitution of valine for glutamic acid in the 6 position, and the E mutation causes substitution of lysine for glutamic acid in the 26 position in the hemoglobin beta chain. As a result of mutations in both beta chains, the patient does not have any hemoglobin A. Approximately 30% to 35% of the total hemoglobin is hemoglobin E, and about 60% to 65% is hemoglobin S. The hemoglobin F level is usually normal or might be slightly elevated. The hemoglobin A2 level cannot be assessed from the high-performance liquid chromatography data, because hemoglobin E has the same retention time as hemoglobin A2 and overlaps the A2 pick (Figure 3, the shaded area). Isoelectric focusing and its modification could be used for separation of these hemoglobins.

S/E hemoglobinopathy is usually associated with mild anemia, microcytosis, and the presence of few target cells. As a rule, it does not cause sickle cell formation, and there are no complications associated with sickling. In general, these are healthy individuals with no significant pathologic changes related to the hemoglobinopathy. However, there are some reports that describe manifestations of a sickling disorder in affected individuals. To the best of our knowledge, there is no information suggesting higher incidence of idiopathic thrombocytopenic purpura in individuals with S/E or any other hemoglobinopathy. Most likely, in our case, these conditions occurred coincidentally.

The S mutation is the most common hemoglobin mutation in the African American population as well as in black populations worldwide. The E mutation is most common in Southeast Asia. This implies a higher probability of appearance of such double-heterozygous individuals from interracial relationships between individuals belonging to these ethnic groups. Nevertheless, S/E individuals have been reported in families where both biologic parents belong to the same ethnic group. These are cases of Saudi Arabian, Indian, Pakistani, Turkish, and African American origin. Our patient is of African American background.

References

Pathologic Quiz Case

A Woman With Human Immunodeficiency Virus With Right Lower Quadrant Pain and Ascending Colon Mass

Jigna C. Jani, MD; Russell Brown, MD; Andre Kajdacsy-Balla, MD, PhD; Grace Guzman, MD

A 44-year-old African American woman with a past medical history of acquired immunodeficiency syndrome and hepatitis C cirrhosis presented to our institution with complaints of right lower quadrant pain for 2 months. The pain occurred daily and was constant, with acute exacerbations from food consumption and mild relief from ibuprofen and lying down. She had history of anorexia but no history of nausea or vomiting. She also had a history of diarrhea for 2 months, with 2 episodes daily. There was no history of melena or hematochezia. She was taking a number of antiretroviral medications.

Her physical examination revealed mild abdominal distension and marked tenderness in the right lower quadrant, with guarding but without rebound. Hepatomegaly to 2 to 3 cm below the right costal margin was noted. A digital rectal examination revealed external hemorrhoids and a 2-cm palpable rectal mass that was soft, mobile, and somewhat tender. The stools were heme positive and brown in color.

Computed tomographic scan of the abdomen showed an irregular thickening of the cecum and ascending colon with mild fat stranding. The thickening was abrupt and had sharp margins, with adjacent colon appearing normal. There was no evidence of abscess. Para-aortic and mesenteric lymphadenopathy were seen measuring 2 cm in greatest dimension; retrocrural lymphadenopathy was seen as well.

Colonoscopy demonstrated a large, polypoid, ulcerated, and malignant-appearing tumor in the ascending colon. There was no active bleeding noted or stigmata of recent hemorrhage (Figure 1). The mass was biopsied and sent for histology and viral cultures. A similar but smaller lesion was seen in the rectum, and it was also biopsied. Multiple sessile polypoid lesions were also found in the transverse, descending, and sigmoid colon.

The specimen received in the surgical pathology laboratory consisted of biopsy of ascending colon and rectal masses. Biopsy of the ascending colon mass consisted of few irregular fragments of tan soft tissue measuring 0.7 × 0.3 × 0.3 cm, whereas the rectal lesion consisted of a cobblestone, polypoid, brown tan structure measuring 0.7 × 0.6 × 0.3 cm.

Microscopically, both biopsies showed colonic mucosa with florid histiocytic infiltrates within the lamina propria containing multiple organisms (Figures 2 and 3). Special stains were performed, including periodic acid–Schiff and Gomori methenamine silver (shown in Figure 4). Giemsa, acid-fast bacilli, and mucicarmine stains were negative.

What is your diagnosis?
Pathologic Diagnosis: Histoplasmosis of the Colon

*Histoplasma capsulatum* is a ubiquitous dimorphic fungus. It grows as mold in nature and as budding yeast in host tissue and on enriched agar. The fungus usually grows in soil enriched with accumulations of bat or bird droppings. The disease is acquired via inhalation of spores (conidia) from soil contaminated with bat or bird droppings.

Primary gastrointestinal infection is an uncommon manifestation of histoplasmosis. It is almost always associated with disseminated disease and often occurs in an immunocompromised host. Gastrointestinal histoplasmosis is an uncommon complication of acquired immunodeficiency syndrome, but it is occasionally seen, particularly in some endemic areas of North America. Histoplasmosis is more common in the southeastern and south-central United States, especially along major waterways (eg, the watershed areas of the Ohio and Missouri rivers). An association with a disseminated mycotic infection is common. The ileum and cecum are the most common sites involved. When the mycologic study is not performed or is negative, only morphologic and immunohistochemical methods can establish the diagnosis and eliminate other mycotic diseases occurring during acquired immunodeficiency syndrome. Depending on the layer of bowel wall involvement and the extent of the disease, the manifestations of histoplasmosis vary and can include gastrointestinal bleeding, perforation, peritonitis, and malabsorption syndrome. Perforation most commonly occurs in the small intestine, although colonic perforation is also reported. Human immunodeficiency virus disease is often associated with diarrhea, which may be accompanied by enteric infection or gastrointestinal tumor. Patients with CD4 counts of less than 50 cells/mm³ and with low albumin levels are more likely to have a primary infectious diagnosis.

Four different types of intestinal histoplasmosis have been described. First is the silent infection, with no gross abnormalities but presence of fungus in the lamina propria. Macrophages positive for periodic acid–Schiff in the small intestine biopsy are described in histoplasmosis, and so it is an important element in the differential diagnoses of Whipple disease, *Mycobacterium avium intracellulare* infection, and macroglobulinemia. Small pseudo-polyps and plaques characterize the second form. The third type involves discrete ulcerations in the mucosa. Fourth, rarely, thickening of bowel leads to obstructive symptoms mimicking Crohn disease or malignancy. This fourth type is the extremely rare category of gastrointestinal histoplasmosis. Our patient had both the second type and this rare fourth type.

Tissue culture or histologic identification on biopsy specimen establishes the diagnosis. The important message we wish to convey in this report is that the diagnosis of gastrointestinal histoplasmosis should be considered in any immunocompromised patient presenting with a colorectal mass, multiple polyps, or symptoms of obstruction. The diagnosis is particularly challenging, because serology and skin testing are not reliable in immunocompromised patients, emphasizing the need for accurate histologic identification.

The patient was started on itraconazole, metronidazole, ceftriaxone, and doxycycline. Later, she was able to tolerate oral intake, and her abdominal pain improved. She will require chronic treatment with itraconazole to prevent recurrence unless her CD4 count improves.

References

Leukemic Ascites

Liron Pantanowitz, MD; Richard Steingart, MD; Kenneth B. Miller, MD; Jonathan B. Kruskal, MD; German Pihan, MD

A diagnosis of myeloid sarcoma (granulocytic sarcoma or chloroma) usually refers to a tumor arising from the extramedullary infiltration of leukemic cells that (1) precedes or occurs concurrently with acute myelogenous leukemia (AML) of the bone marrow, or (2) heralds the blastic transformation of a chronic myeloproliferative disorder. The infiltration of leukemic cells into serous effusions is unusual. Extramedullary infiltration of effusions has been reported most often in cases of AML with monocytic differentiation, including M4 and M5 AML in the French American British classification.1,2 We present a case of leukemic ascites that illustrates how unexpected extramedullary sites of relapse in AML, such as the paranasal sinuses, female genital tract, and breast, are being recognized with increasing frequency as the long-term survival of leukemic patients improves.3

A 27-year-old man diagnosed with acute myelomonocytic leukemia (M4 AML) underwent induction chemotherapy followed by a female sibling matched allogeneic stem cell transplant. His karyotype at this time was 46,XY,–18,+mar[4]. Almost 2 years after his transplant he presented with an occipital subcutaneous myeloid sarcoma. Although immunohistochemical studies were not performed on this scalp mass, cytogenetic studies showed more complex abnormalities including t(2;7;7;5) and t(6;16). A bone marrow biopsy did not reveal recurrent acute myelomonocytic leukemia (M4 AML) and yielded a 46,XX karyotype consistent with successful engraftment. His mass was successfully treated with local radiation therapy. One month later he presented with dyspnea, weight gain, abdominal distention, and pedal as well as scrotal edema. On examination, he had tense ascites with prominent shifting dullness. His peripheral white blood cell count was 9200/μL with 90% neutrophils and no circulating blasts. A chest x-ray film revealed bilateral pleural effusions. Axial postoral contrast computerized tomographic scans obtained through the upper abdomen (Figure 1) and
pelvis demonstrated a large amount of ascitic fluid (indicated by stars) throughout the peritoneal cavity. Apart from some peripancreatic lymphadenopathy, there was no thickening of the peritoneal lining, and no masses or peritoneal implants were present. There was no evidence of another site of solid tissue involvement.

A diagnostic and therapeutic paracentesis was performed. Analysis of the patient's ascitic fluid showed an exudate with a white blood cell count of 288 cells/μL; red blood cell count of 72 cells/μL; total protein of 1.3 g/dL; glucose, 99 mg/dL (serum, 107 mg/dL); lactate dehydrogenase, 168 U/L (serum, 609 U/L); and albumin, 0.7 g/dL (serum, 3.5 g/dL). Cytologic examination of Wright-stained cytocentrifuged preparations showed a markedly cellular fluid (Figure 2) composed of immature granulocyte precursors including 85% myeloblasts. The blasts had a high nuclear to cytoplasmic ratio, with immature and folded nuclei containing fine granular chromatin and prominent nucleoli (Figure 3). They also had a deeply basophilic cytoplasm with numerous small vacuoles and were strongly myeloperoxidase positive. Coarse and sparse cytoplasmic granules were clearly evident in some of the differentiating immature myelocytes. Auer rods were not seen. Mitotic figures were abundant. Megakaryocytes and erythroid precursors were not apparent, and no microorganisms were identified. Flow-cytometric analysis of the ascitic fluid demonstrated that the majority of the cells (82% of total gated events) expressed CD34 and HLA-DR; myeloid-associated antigens CD13, CD33, CD117, CD11c, and CD15; as well as a subset that coexpressed monocytic markers CD14 and CD64. These findings are diagnostic of acute myelomonocytic leukemia (M4 AML). Cytogenetic studies were not performed on the ascites. The patient died within 1 month of his presentation with ascites.

The development of leukemic ascites at initial presentation and also as a late extramedullary relapse following bone marrow transplantation for AML, of monocytic differentiation as well as other subtypes, is rare. Infectious peritonitis in such cases needs to be excluded. The finding of large immature neoplastic cells in ascitic fluid can be seen with large cell lymphoma, natural killer cell lymphoma, poorly differentiated carcinoma, sarcoma, and melanoma. Cytochemistry to demonstrate peroxidase-positive leukemic cells and diagnostic immunohistochemical studies may help. If available, flow cytometry and cytogenetics can be used to further classify AML, particularly in cases such as this one, where the bone marrow did not reveal evidence of disease.

References

Concurrent Papillary and Medullary Thyroid Carcinoma

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A 67-year-old man presented with a thyroid nodule and an elevated calcitonin level. He underwent subtotal thyroidectomy.

A follow-up computed tomographic scan of the neck a few weeks later showed 2 enlarged cervical and superior mediastinal lymph nodes suspicious for tumor involvement. Chest radiograph, chest computed tomographic scan, whole-body bone scan, and magnetic resonance imaging of the abdomen revealed no evidence of metastatic disease. Completion thyroidectomy and bilateral cervical lymph node dissection were subsequently performed.

A solitary focus of papillary thyroid carcinoma (PTC), 1.2 cm in diameter, in the left lobe, and multifocal medullary thyroid carcinoma (MTC) in both right and left lobes and isthmus, with the largest tumor focus measuring 0.7 cm in diameter, were identified. The histomorphology and immunoprofile of the primary PTC and MTC were typical. The fingerlike projections of the PTC were lined by cuboidal cells with overlapping grooved nuclei and often with ground-glass ("Orphan Annie" eye) appearance (Figure 1, a). The MTC component consisted of nests of predominantly round cells with ample, finely granular amphiphilic cytoplasm and ovoid to round nuclei (Figure 1, b). The chromatin material was stippled, and the nucleoli were rarely prominent. Amyloid deposit was admixed with MTC tumor cells. The MTC component extended into the perithyroidal soft tissue.

Immunohistochemically, the lesional cells of the PTC were positive for thyroglobulin (Figure 1, a, inset) and negative for calcitonin, carcinoembryonic antigen, and chromogranin. The MTC cells were positive for calcitonin (Figure 1, b, inset), chromogranin, and carcinoembryonic antigen and negative for thyroglobulin.

There were multiple MTC metastases identified in cervical soft tissue and in 8 of 20 cervical and superior mediastinal lymph nodes. Two lymph nodes had both PTC and MTC metastases, and in each of these 2 lymph nodes the PTC and MTC metastases were closely associated (Figures 2 and 3). Amyloid material was also seen intermingled with metastatic MTC cells (Figure 2).

Cases of concurrent or mixed papillary and medullary thyroid carcinoma are rare.1-3 These patients may have RET proto-oncogene mutation.1 In some of these patients,
the tumor spread to cervical lymph nodes and distant organs, causing death from metastatic disease.\textsuperscript{1}

Concurrent PTC and MTC neoplasms appear to occur in 2 circumstances: (1) PTC and MTC, being morphologically and immunophenotypically distinct tumors, are believed to arise from embryologically different cells, follicular cell (endodermal anlage) origin for PTC and C cell (ultimobranchial body) origin for MTC. In this setting, PTC and MTC may be regarded as collision tumors. (2) A common stem cell derivation for follicular and C cells is considered by others, resulting in a true follicular-parafollicular cell carcinoma.\textsuperscript{3}

References

A 63-year-old man with a history of extensive asbestos exposure and cigarette smoking presented with shortness of breath. The asbestos exposure occurred while the patient was employed as a pipe insulator during the years 1959 to 1973. A computed tomographic scan of the lungs demonstrated bilateral pleural effusions, bilateral pleural plaques, and a 1.3-cm mass in the left lower lung lobe. A left lower lobectomy was performed, and sectioning of the lung demonstrated a 1.3-cm firm, tan-pink mass which was representatively frozen. Microscopically, the frozen section showed adenocarcinoma (Figure 1) associated with ferruginous bodies (Figure 1, arrow). Higher magnification of the ferruginous bodies demonstrated characteristic golden-brown, beaded rods with bulbous tips (Figure 2). Permanent sections showed numerous ferruginous bodies in multiple hilar lymph nodes in addition to the lung parenchyma.

Ferruginous bodies form when mineral particle fibers such as asbestos become coated with iron protein complexes presumed to be derived from phagocyte ferritin. A Prussian blue stain for iron may be helpful in their identification; however, their golden-brown color makes them easily recognizable on hematoxylin-eosin-stained sections when present in significant numbers.

Bronchogenic carcinoma and mesothelioma develop with increased frequency in workers exposed to asbestos. The risk of bronchogenic carcinoma is increased 5-fold for asbestos workers, and the relative risk of mesothelioma is increased more than 1000-fold. Cigarette smoking in this setting greatly increases the risk of bronchogenic carcinoma but not of mesothelioma. A latency period of 20 years usually exists between exposure to asbestos and the development of bronchogenic carcinoma.
Embryo & Fetal Pathology: Color Atlas With Ultrasound Correlation


This is a gem of a book. The title of the book does not do justice to the contents, because this is more than just an atlas. The wealth of information in this textbook is substantial. Embryology is covered, as well as fetal autopsy, genetics, and a wide spectrum of fetal abnormalities. The chapters are arranged in a logical “developmental” fashion from early to late embryonic abnormalities to placental pathology to chromosomal abnormalities. The chapter on terminology of errors of morphogenesis is excellent. The latter part of the book concentrates on malformation syndromes, dysplasias, and pathology of given organ systems, including the skin, and ends with chapters on intrauterine death, multiple and conjoined twins, and metabolic diseases. Each chapter is well written and concise. The chapters are richly illustrated with tables, charts, diagrams, and color photographs. The gross pictures are of high quality and good examples of the given entity or abnormality discussed. The appendices contain many useful charts and tables of embryonic and fetal development, which is often difficult to find all in one textbook. The ultrasound chapter is divided into parts from general concepts to organ system malformations, with a section devoted to current advances in ultrasound. This section is also well written in understandable language with good illustrations.

This textbook is the best I have seen on the topic of embryonic and fetal pathology. As an atlas, it far surpasses expectations. As a reference book and bench book, it should be on the bookshelf of everyone who has an interest in this area. I highly recommend this book to my surgical and pediatric pathology colleagues, neonatologists, and obstetricians. As much as one can enjoy a pathology textbook, I enjoyed this book very much and found it a pleasure to read.

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Pathology and Law: A Practical Guide for the Pathologist


Of the several excellent handbooks written on the subject of medical malpractice, Pathology and Law is the first primer that is written by pathologists and targeted to pathologists. The soft-cover edition is largely written by Dr. Gregory G. Davis with additional supplemental material provided by Dr. Gregory J. Davis (no relationship) and Dr. Margie A. Scott. The 2 Davises have backgrounds in forensic pathology, whereas Dr. Scott has contributed in areas of clinical pathology. The book is well written in a conversational style and is peppered with many malpractice scenarios well recognized by in-the-trenches anatomic and clinical pathologists. Its brevity (208 written pages) and low price also make this an extremely attractive book. As stated by the authors, the goal of their book is to sufficiently educate pathologists about the legal system and the courtroom so that we can avoid confusion and make a better impression in our courtroom defense.

The book is divided into 10 chapters and contains an appended glossary of frequently used legal terms. The first chapter provides an introduction that articulates the similarities and differences between lawyers and physicians. Just as lawyers feel out of sorts when they are patients, so too do physicians feel uncomfortable and confused in court, because they have been coerced into a court appearance and their professional reputations may be on the line.

Chapter 2 explains the theory and operation of the adversarial American legal system. The text disabuses the notion that verdicts always are the result of a correct interpretation of evidence. Rather, our legal system is described as a clash of opposing sides, each championed by its attorney, where the valid testimony of a truthful witness can be rendered useless if that witness flounders in the heat of cross-examination. Sitting in the witness seat under cross-examination is more stressful than any surgical pathology “hot seat” for reading frozen sections. The remainder of the chapter describes the cast of characters that appear in the legal battlefield: jury, judge, plaintiff’s lawyer, defense lawyer, fact witnesses, and expert witnesses. Although we as surgical pathologists pride ourselves on being “100%” certain a biopsy specimen demonstrates carcinoma, many of us are surprised that in court the burden of proof in civil malpractice cases is based on only the “predominance of evidence,” in that an expert’s beliefs and opinions need only be medically probable, being more likely true than not (ie, 51%).

Chapter 3 discusses the impact of law on pathology as exemplified by case studies of what are everyday occurrences to us. Covered in this chapter are such essential topics as the treatment of surgical pathology specimens of forensic worth (eg, bullets, other foreign objects, traumatically ruptured spleens), the liberal use of photography specimens, rules of chain of custody, and the treatment of specimens with civil implications (especially potential malpractice cases). Detailed discussions follow regarding the proper processing of a lymph node biopsy specimen (eg, flow cytometry, immunohistochemical analysis). Articulated is the need to always focus on the breadth of di-
agnostic possibilities to guide the tissue studies appropriately based on those possibilities that the differential diagnosis would entertain and the need to document all ancillary studies performed (with both positive and negative results). The authors adhere to the old adage that “if it wasn’t documented, it wasn’t done.” Likewise, they advise never to render a “final diagnosis” if ancillary studies or consultative reports are still pending.

Because of their roots in forensic pathology, the authors give an impassioned plea for the performance of autopsies, especially in cases that might be considered clinical failures, with the far greater likelihood that the information generated from such autopsies, even if uncovering diseases not clinically suspected when the deceased was alive, will exonerate a defendant clinician. The unofficial 11th commandment of pathologists is articulated unequivocally: neither change your postmortem findings once a final report has been issued nor by omission fail to document adverse clinical and surgical events that contributed to the death of the individual. The chapter also details the ground rules of legal autopsy practice: know who is allowed to sign the permit, correctly identify bodies, have respect for the limitation of dissection, and save body fluids and tissues for potential future toxicologic analysis. The reminder of the chapter is an encyclopedia of pathologic issues to which we all have been exposed: potentiality of false-positive results in testing for drugs of abuse; admissibility of hospital blood alcohol testing for medical purposes without the usual chain of custody expected of criminal evidence; issues of hospital employee drug testing (or contract testing for other companies’ employees); issues in transfusion medicine; laboratory errors, including computer entry errors, specimen mix-ups, and specimen mishandling; reporting of critical values in clinical laboratory testing (and its documentation); disciplinary actions against laboratory employees; legal status of informal (curbside) consultations; the practice of intradepartmental consultations in difficult surgical pathology cases; and implementation of total quality improvement plans. The text is enlivened by “case studies” of real pathology malpractice cases and the references given are pertinent and up-to-date.

Chapter 4 covers the details of a malpractice case. Articulated in the text are the 4 postulates that the plaintiff must fulfill to successfully mount a malpractice action: (1) the named physician’s duty to the patient; (2) a breach by that physician of the “standard of care”; (3) identifiable damages (physical, economic, or psychological) experienced by the patient; and (4) a proximate relationship between the alleged negligent acts on the part of the physician and the identifiable damages. Quoted in the text is the full legal definition of the physician’s standard of care as written in the 1898 malpractice decision Pile v. Honsinger. The text details the reasons that patients (or their families) sue. The authors posit that you should not underestimate anger and revenge as the 2 most common denominators. The remainder of the chapter is a fictionalized biography of a lawsuit (against you), the importance of keeping your mouth shut, and, most interesting, the liabilities of a pathologist practicing in a group or a professional corporation in regard to the professional misconduct of a colleague.

Chapter 5 is a primer for the pathologist who wants to become an expert witness. Lest you think that only authors of standard texts in pathology can or should qualify as medical experts, the legal definition of an expert (in any field) is any individual who has training or experience beyond that of everyday experience. As such, the expert witness is a teacher to the court who is called on to provide to the judge and jury the benefit of his or her special training and experience so that the best possible judgment can be rendered. The expert is not to view himself or herself as an advocate for one side or the other. Expounded in the text are what the court’s expectations are of a good expert witness.

Chapter 6 is a detailed natural history of a medical negligence lawsuit, beginning with the initial episode of alleged malpractice and ending in settlement. Statistically, only 7% of filed lawsuits ever go to trial. The other 93% are either dropped or are settled prior to a trial. Of the 7% that go to trial, 4% are settled during the course of a trial, with only 3% actually going to jury verdict, 80% of which are won by the defense.

Chapter 7 is an interesting discussion regarding unethical expert witness testimony. Based on the Daubert v Merrell Dow Pharmaceutical case ruling (1993), the courts have now been given the parameters by which a judge can determine the validity, reliability, and subsequent admissibility into court of scientific methods or theories. The Daubert ruling gives judges the authority to dismiss testimony as “junk science” even before it is offered in court. The chapter also details the proposed mechanisms for handling unethical medical testimony and the so-called medical experts who give such testimony, including the important role of medical societies and licensing boards in maintaining the integrity of physicians who provide expert medical testimony. Cited are the ground-breaking steps taken by the American Association of Neurological Surgeons in flushing out unethical expert witnesses within its own specialty.

Chapter 8, in brief, deals with laboratory issues of quality assurance and record keeping—document! document! document!

Chapter 9 is an impassioned plea to pathologists that we as physicians have an ethical obligation to attempt to constructively influence legislation that will affect the quality of health care offered to our patients. Who among us will be the next US Senate majority leader?

The last chapter is a fascinating discussion regarding the legal implications of the information age. A discussion of the Health Insurance Portability and Accountability Act of 1996 requirements is disconcerting. Also discussed are the disadvantages of telemedicine, especially in pathology, in regard to state licensure, qualifications, accountabilities, and individual requirements for hospital accreditation. The chapter ends with a discussion of the requirements for storage of digital images and the safeguards against the digital manipulation of such images.

All told, this is an excellent source book for the attending pathologist who is a neophyte in the medical-legal world and for residents who have the world to learn about legal medicine. An especially valued audience
would be international medical school graduates in any stage of their careers who are not as sophisticated about the legal jurisprudence system in the United States as those of us who grew up watching "Perry Mason."

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Forensic Pathology Reviews: Volume 1


This is the first in a review series of forensic pathology, starting with the premise that "over the last decade, the field of forensic science has expanded enormously, with the rapid emergence of new autopsy and laboratory techniques, and the identification of many new markers for specific pathological conditions." This book is a good start in attempting to define these recent forensic pathology advances.

The first chapter is a review of burned bodies, with black-and-white photographs that show charring with "puppet organs" and tables that compare grading schemes for thermal injury. Chapter 2 reviews the blunt force trauma experience in Germany from "stomping" and assault. Chapter 3 deals with timing of traumatic brain injury through immunohistochemical analysis. New to me was the extensive text and tables that summarize all the markers and the earliest and average times they are found after injury. Chapter 4 reviews the effects of drug abuse on the central nervous system, with 31 pages of 505 references. Chapter 5 is an up-to-date review of sudden cardiac death, and chapters 6 and 7 deal with the problems and pitfalls of neonaticide and sudden infant death syndrome, respectively.

Chapters 8 and 9 review rare fatalities from Mycoplasma pneumoniae and Waterhouse-Friderichsen syndrome. Chapter 10 is a review of autoerotic deaths, with the history of the practice the most informative. The chapter on hypothermic deaths concentrates on the pitfall of misdiagnosing paradoxical undressing as homicide and the process of crawling into enclosed spaces during hypothermia called the hide and die syndrome, which is believed to be related to a hibernation reflex. The text, photographs, and concise tables in the review of maternal death from HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) are excellent.

The most encyclopedic chapter is the review of the forensic aspects of postmortem alcohol interpretation, with all the biochemical calculations, pitfalls, and strengths in interpreting alcohol values thoroughly discussed. Cardiopulmonary resuscitation injuries are also reviewed. The last chapter on iliopsoas muscle hemorrhage deals with this rare autopsy finding, most likely related to sepsis, DIC, or trauma.

Generally, the references at the end of the chapters are extensive, but unfortunately many to most in each chapter are in German. This reflects the different perspectives of the European practice environment of the editor and his mainly German coauthors. Some authors also seem to place a heavy emphasis on microscopic studies over the gross findings, which is different from the American forensic pathologist. I appreciated the summary sections at the end of each chapter. Forensic pathology is a visual discipline, and more photographs in the subsequent volumes would be beneficial.

Finally, this volume contains timely, comprehensive reviews for the selected specialized topics. Although these reviews do not define many new advances in forensic pathology techniques or practices, this may reflect the field of forensic pathology itself rather than any deficit of the authors. I would recommend the first book in this series to the practicing forensic pathologist who wishes to fill in some gaps of the standard forensic pathology texts or as a starting point to begin to master the art of traumatic forensic neuroimmunohistochemistry.

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