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Periadventitial Adipose Tissue (Tunica Adiposa): Enemy or Friend Around?

To the Editor.—Recently, studies on the periadventitial adipose tissue (PAAT), a subfield of adipobiology, have attracted the attention of scientists because PAAT may indeed be a path to atherosclerosis.

In a recent issue of Archives of Pathology & Laboratory Medicine, Deborah Vela and colleagues1 presented a review on the role of PAAT in atherosclerosis.

In the accompanying editorial, Tellides2 highlighted the atherogenic potential of PAAT, discussing epicardial adipose tissue–associated, atherosclerosis-prone versus myocardium-associated, atherosclerosis-resistant segments of coronary arteries. Accordingly, he cited Scher’s article (Tellides’ reference 4), while failing, as did Vela et al, to mention the respective response to it, showing that PAAT is ‘‘another neglected phenomenon’’ in coronary atherosclerosis.3 Probably, a ‘‘space limitation’’ has not allowed Vela et al to cite many seminal papers in the field reviewed recently by our group.4

In our eyes, the article by Vela et al and the editorial by Tellides raise the important question of whether PAAT is an extravascular or, in nature, an ‘‘intravascular’’ compartment. Today’s paradigm holds that the vascular wall consists of 3 layers: the tunica intima, media, and adventitia. We suggest that PAAT may indeed be considered the fourth, outermost vascular layer, that is, tunica adiposa. Because (1) there is a lack of any fascia-like structure between the adventitia and the PAAT; (2) PAAT is a producer of a large number of bioactive molecules such as adipokines,1–4 which, in a paracrine way, may exert proinflammatory and smooth muscle cell growth/migration promoting, in fact, atherogenic effects; and (3) PAAT releases vasorelaxing factor(s),5 which may benefit the vascular biology, hence, a question whether PAAT is an enemy or a friend of the artery may emerge. Accordingly, new experiments, for example, PAAT-depleted mice and adipose-derived relaxing factor–deficient mice, as well as studies on animals and humans affected by cardiometabolic diseases such as atherosclerosis, hypertension, obesity, diabetes, and metabolic syndrome, should be developed in perivascular, ‘‘vasocrine’’ adipobiology. Briefly, the more we learn about PAAT (tunica adiposa), the more we may know about vascular and metabolic health and disease, including the role of an adipose dysfunction in cardiometabolic disease. Further studies should deal with both endothelial and adipose dysfunction in the field.

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In Reply.—In our manuscript,1 we critically reviewed the topics of vascular injury, atherosclerosis, and potential diagnostic and therapeutic implications of periadventitial fat and added some original work that suggests that periadventitial fat inflammation is not only associated with the presence but also with the phenotype (high risk vs low risk) of the underlying atherosclerotic plaque. Although 75 references were included, some relevant work was not listed, including contributions made and reviewed by Dr Chaldakov’s group.2 We believe that research in this field will lead to clinically relevant diagnostic and therapeutic advances.1–3

We would like to acknowledge the comments of Dr Chaldakov et al regarding our review article on aspects of periadventitial adipose tissue as well as the discussion of the innovative publications of Dr Chaldakov and colleagues. We definitively agree with the statement that the periadventitial adipose tissue ‘‘is another neglected phenomenon in coronary atherosclerosis.’’ This is even more puzzling considering that the field of adipose tissue, partially driven by the epidemics of obesity and diabetes in the United States, is actually advancing at good pace.

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The authors have no relevant financial interest in the products or companies described in this article.
Adequacy of Pathology Resident Training for Employment: A Survey Report From the Future of Pathology Task Group

To the Editor—I read with interest the article in the April 2007 Archives of Pathology & Laboratory Medicine concerning the adequacy of pathology residency training.1

It seems to me that if this article was included in the May 2007 Continuing Medical Education “Test Your Memory” Program offered by the College of American Pathologists, then it was an important article to read and to study.

What appears to me is that pathology programs are not being very effective if they produce some 31% of graduates with at least one major deficiency and if some 61% of personnel need guidance. Another glaring deficiency was that some 48% of graduates were deficient in “laboratory management/laboratory medicine.” Given these statistics, then, I think that there is a need for the data to be presented to the American Medical Association Graduate Council on Medical Education and that programs involved with these “deficient graduates” need to be closed. They are producing a “deficient product.” If you tout that the College of American Pathologists is “committed to excellence,” then unfortunately the training programs do not share that commitment. We want quality not quantity in graduate education.

If it is a “politics thing” and some programs have a few “prima donnas,” while having inadequacy in the rest of the department, then they should not be involved with training people. The trainees will not receive an adequate education. It is not fair to the trainees. There should be stricter requirements for keeping programs open. The American Medical Association Graduate Council on Medical Education should have stricter surveys of programs and inspections of programs. They should probably close at least one third to one half (33%–50%) of these outfits, especially the so called combined anatomic pathology–clinical pathology programs that may well be deficient in laboratory medicine.

What is also apparent to me is that the pathology groups who are hiring people also do not share a commitment to excellence. It leads me to believe that many groups thought they would “hire on the cheap.” They would hire a “defective graduate” because it might be cheaper to hire that individual rather than an experienced pathologist. They forgot the old adage by Diamond Jim Brady: “to make money . . . you have to spend money” or its corollary, “you get what you pay for.” Unfortunately, the groups may have saved a few dollars by hiring a graduate with deficiencies, but it will come back to haunt them with litigation and liability risk by those graduates, some 61% of whom need guidance. You cannot watch personnel all the time. Unfortunately, mistakes—which shall lead to litigation/malpractice with time—will eventually come back to haunt the group and leave some of them “penniless” in certain instances.

I think that training programs need more rigorous requirements. They need more inspections and surveys to maintain adequacy—especially in “clinical pathology” or laboratory management/laboratory medicine (48% inadequacy in graduates).

If some 33% to 50% of programs have to be closed, then so be it.

We want quality in training, not quantity.

Let us see a commitment to excellence in training as well as in the practice of pathology.

Groups should also be advised that hiring on the cheap is not recommended in the long run. The overall cost of guidance of these types of graduates and, in some cases, liability/malpractice far outweighs the savings versus the cost of an experienced pathologist. Do not be penny wise and dollar foolish in hiring. You may live to regret hiring such a graduate.

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In Reply.—We are in receipt of the letter from Dr Copeland regarding the effectiveness (or not) of pathology residency training programs. He offers several suggestions, including closure of one third to one half of combined anatomic pathology–clinical pathology (AP-CP) training programs that are deficient in “laboratory management/laboratory medicine” and hiring of more experienced pathologists by practice groups that are otherwise hiring “on the cheap.” These rather drastic suggestions are given to ensure that (1) the remaining pathology residency program graduates are free of such deficiencies; and (2) the practice marketplace is appropriately staffed with competent pathologists.

We take a somewhat less pessimistic view of the graduating classes of pathology residency training programs. First, identification of apparent deficiencies in graduates does not mean that they are incapable of practicing medicine as American Board of Pathology–certified pathologists. If anything, the first set of postgraduate tasks in what might be termed lifelong learning are clearly delineated. Grant-
ed, inadequate training in laboratory medicine/laboratory management may indeed qualify as a “major deficiency,” as stated by Dr Copeland. We concur that obtaining more uniform excellence of training in laboratory medicine is a worthy goal and note that the parent organization for this journal is taking a leadership role. Programming of the College of American Pathologists (CAP) includes the Virtual Management College, a complete set of practice management courses, and the CAP Residents Forum. All of these are benefits of membership, and the CAP offers free memberships to pathology residents. Moreover, the academic sector from which such pathology graduates emanate has not been idle on the need to enhance training in laboratory medicine—witness leading publications on this topic since acceptance of our manuscript by the Archives.

Dr Copeland makes the assumption that senior pathologists are readily available in the marketplace. This may not be true. On the one hand, experienced pathologists may not be “moveable,” either in the academic marketplace or in the private sector. On the other hand, and despite dire predictions to the contrary, our data suggest that there is indeed need in the marketplace. Bruce Alexander documents that the American Board of Pathology issued 608 certificates in 2005, of which 421 were combined AP-CP, 133 were AP, 35 were CP, and 19 were primary-plus-subspecialty certificates. However, the comprehensive 2006 Resident Survey conducted by the Residents Forum of the American Society of Clinical Pathology indicates that only 462 pathology residents from American medical schools to meet our country’s needs; closing existing training programs would only further exacerbate the shortage of pathologists from these schools.

Lastly, we consider dismantling of AP-CP programs to be counterproductive. Our survey gives no indication that the marketplace for hiring recent pathology graduates seeks single-board pathologists over AP-CP graduates. Rather, the ability of graduates to successfully handle the demands of both the anatomic pathology and laboratory medicine service sectors seems to be highly desired. Hence, we strongly advocate the strengthening of existing training programs.

The purpose of our report was to document the perceptions of both employers and recent pathology residency graduates regarding the adequacy of training. This was not an instrument to either pass judgment on the seriousness of perceived deficiencies, nor to make recommendations on how deficiencies might be corrected—in the overall administration of graduate medical education or in management of individual programs per se. The jurisdiction for these issues falls to the several agencies responsible for the oversight of graduate medical education. In the case of laboratory inspection and accreditation programs, we must be clear that such programs inspect the performance of a licensed laboratory, not the competencies of individual practitioners. The certification of competence of individual practitioners falls to professional bodies such as the American Board of Pathology.

For our part, the responsibility for the success of our profession falls to all of us: recruitment of outstanding medical students from a diverse spectrum of our society to the specialty of pathology; high-quality pathology residency training across the encyclopedic nature of our specialty; and positioning our graduates to lead our specialty into the future. In the last instance, the future of our specialty depends upon our graduates being able to be proactive in anticipating rapid evolution in the role of pathologists in patient care, and utilizing both emerging technologies and emerging practice models to the maximum benefit of the patients we serve.

We are optimistic that our specialty will make important adaptations and improvements in pathology residency training. We hope that our published study will provide specific guidance for doing so. We thank Dr Copeland for contributing to what is sure to be a lively follow-on discussion.

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Critical Values Reporting

A College of American Pathologists Q-Probes Survey of 163 Clinical Laboratories

Elizabeth A. Wagar, MD, FCAP; Richard C. Friedberg, MD, PhD, FCAP; Rhona Souers, MS; Ana K. Stankovic, MD, PhD, FCAP

Critical values reporting is the mechanism by which direct reporting of potentially life-threatening laboratory results is made to caregivers. First described by Lundberg more than 30 years ago, clinical laboratories identify critical results that may be life-threatening and contact physicians so that immediate clinical intervention may be initiated. Since this first report, the use of critical values reporting was adopted as a requirement in the Clinical Laboratory Improvement Amendments and regulatory agencies. The development of critical values often involves consultation with clinical services. Also, questions are frequently asked about how critical values compare between institutions.

Context.—Critical laboratory values are values that may be indicative of life-threatening conditions requiring rapid clinical intervention. Designation of critical values by clinical laboratories is required by the Clinical Laboratory Improvement Amendments and regulatory agencies. The development of critical values often involves consultation with clinical services. Also, questions are frequently asked about how critical values compare between institutions.

Objective.—To examine and compare critical value ranges for selected common critical value analytes. Additional specific questions addressed the source of these values, the inclusion of specific items on a critical values list, and the procedures for establishing such lists.

Design.—A total of 163 clinical laboratories provided critical values for potassium, calcium, magnesium, thyroid-stimulating hormone, hemoglobin, platelet count, and activated partial thromboplastin time. Collected data were subjected to analysis for statistical variation. A questionnaire regarding demographic characteristics, institutional practices, and critical values management was also completed by participants.

Results.—There was slight variation in pediatric and adult critical values used by the central 80% of study laboratories. Three areas of interest were noted: (1) 27% of laboratories allowed nonpractitioners to accept inpatient critical value reports, (2) there was nonconsensus regarding the handling of outpatient critical values during weekday versus evening/weekend hours, and (3) only 56% of respondents had a written critical values policy or procedure.

Conclusions.—Pediatric and adult critical values for the selected analytes were consistent in a comparison between the 163 clinical laboratories. Several weaknesses in current critical values management were identified. A consensus critical values list that may be of value to other institutions was assembled.

(Arch Pathol Lab Med. 2007;131:1769–1775)
MATERIALS AND METHODS

Study Design

This study was conducted according to the previously described Q-Probes study format. It relies on the voluntary participation of clinical laboratories that subscribe to the CAP Q-Probes Quality Management programs. A critical value analyte was defined as an analyte present on a participant’s current critical or panic value list and reported as a critical value because of an imminent threat to health status.

Only analyte values that require immediate notification to a patient caregiver were considered critical/action values for this study. Participants were asked to provide their high and low critical values for 7 analytes: potassium, total calcium, magnesium, thyroid-stimulating hormone (TSH), hemoglobin, platelet count, and activated partial thromboplastin time (aPTT). Adult critical values (assuming a 32-year-old male inpatient) and pediatric values (assuming an 8-year-old male inpatient) were collected. In addition, hemoglobin critical values were collected for female and male, adult and pediatric patients.

All specimens were specified as venous blood, either plasma or serum. For each analyte, the units of measure and analytic instrument manufacturer were also collected. The primary source used by the laboratory to determine critical low and high values for each analyte was also submitted. Current inpatient critical values as used in the primary clinical laboratory of the institution were included in this study. Critical values for point-of-care instruments and capillary, finger-stick, or earlobe blood collections were excluded. Also excluded were secondary laboratory sites, if more than one site was present and critical values differed between the sites.

Participants were additionally asked to provide demographic information consisting of occupied bed size, teaching status, pathology resident training status, government affiliation, institution location, institution type, and CAP and JC inspection status.

Laboratory Characteristics

A total of 163 institutions submitted data to this study: 97% (158) of these institutions were located in the United States, with the remaining institutions in Canada (2), Australia (1), Lebanon (1), and South Korea (1). A total of 31% of participating institutions were teaching hospitals, and 15% had a pathology residency training program. Within the past 2 years, CAP had inspected 78% of participating laboratories, and JC inspections had been performed at 66% of the institutions. Table 1 indicates the specific characteristics of the participating institutions, and Table 2 shows the types of analytic platforms used by participants to test each analyte. For chemistry testing, most institutions tested serum rather than plasma (ranging from 57% for potassium to 79% for TSH testing).

Statistical Analyses

The calcium units were standardized to milligrams per deciliter, and magnesium units were standardized to milliequivalents per liter (if they had been reported in millimoles per liter or milligrams per deciliter); potassium data were standardized to milliequivalents per liter. Prior to conducting statistical analyses, values were screened for outliers. Several participating institutions did not answer all the questions on the questionnaires about demographic characteristics, institutional practices, or critical values for particular analytes or age groups. These institutions were excluded from analyses that required these missing data elements. If demographics were available from previous Q-Probes studies, this information was added to the data set. Lows and highs for critical values were tested for association with the institutions’ demographic and practice variables. Individual associations were first tested using the nonparametric Kruskal-Wallis test. Variables with significant associations (P < .10) were then included in a multiple linear regression model. All remaining variables were significantly associated at the .05 significance level.

We used multivariate analysis of variance (MANOVA) to perform a joint analysis of the dependent variables: low and high adult and pediatric critical values. This approach simultaneously tests whether the mean low/high vectors are statistically different for the analyte-specific predictor variables. Because the cell counts for the predictor variables were not equal, the significance level was set at P < .01.

RESULTS

A composite critical values table demonstrates the mean ± SD for each of the 7 reported analytes for adult and pediatric critical values (Table 3). Adult and pediatric mean critical values ± SD were very similar. Critical values for hemoglobin did not vary by gender. SDs indicate the variability (square root of the variance) for each analyte in the comparison of participating clinical laboratories. The median and the 5th and 95th percentiles for adult and pediatric critical values, shown in Table 4, were also very similar. Thyroid-stimulating hormone was not listed as a critical value by more than 90% of the reporting laboratories. Low aPTT critical values were reported by only 17 institutions. High hemoglobin levels and high platelet counts were reported by 70% and 81% of participating institutions, respectively.

Table 5 lists the analytes present on the critical values list as reported by the participants. Multiple responses were allowed. More than 90% of the laboratories reported the following on their critical values lists, in descending frequency: (1) potassium, (2) sodium, (3) calcium, (4) platelets, (5) hemoglobin, (6) aPTT, (7) white blood cell count, and (8) prothrombin time (PT). More than 64% of laboratories additionally reported critical values from clinical microbiology (blood culture, Gram stain), bilirubin, carbon dioxide, magnesium, hematocrit, lithium, and fibrinogen. Critical values for blood gas analytes (pH, PCO₂, and PO₂) were reported as being on the critical values list of 91 laboratories (56%). Some of this variation may reflect differences in clinical service and satellite lab-

Table 1. Characteristics of Participating Laboratories

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institution type</td>
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<tr>
<td>Private, nonprofit</td>
<td>78</td>
<td>60.0</td>
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<tr>
<td>State, county, or city hospital</td>
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<td>13.8</td>
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<tr>
<td>Private, profit</td>
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<td>7.7</td>
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<tr>
<td>University hospital</td>
<td>6</td>
<td>4.6</td>
</tr>
<tr>
<td>Government, federal</td>
<td>5</td>
<td>3.8</td>
</tr>
<tr>
<td>Independent lab</td>
<td>5</td>
<td>3.8</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>6.2</td>
</tr>
<tr>
<td>No. of occupied beds</td>
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<td></td>
</tr>
<tr>
<td>0–150</td>
<td>53</td>
<td>42.1</td>
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<tr>
<td>151–300</td>
<td>41</td>
<td>32.5</td>
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<tr>
<td>301–450</td>
<td>19</td>
<td>15.1</td>
</tr>
<tr>
<td>451–600</td>
<td>10</td>
<td>7.9</td>
</tr>
<tr>
<td>&gt;600</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>Institution location</td>
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<td></td>
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<tr>
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<td>Suburban</td>
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<td>Rural</td>
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<td>Federal installation laboratory</td>
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<td>Governmental affiliation</td>
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<td>Nongovernmental</td>
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<tr>
<td>Nonfederal governmental</td>
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<td>16.2</td>
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<tr>
<td>Federal governmental</td>
<td>5</td>
<td>3.8</td>
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</table>

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Critical Values Comparison—Wagar et al
### Table 2. Manufacturer of Primary Test Systems*

<table>
<thead>
<tr>
<th>AnalYTE</th>
<th>Dade Behring (Deerfield, Ill)</th>
<th>Beckman Synchron (Fullerton, Calif)</th>
<th>Vitros (Rochester, NY)</th>
<th>Roche (Rochester, Ind)</th>
<th>Roche (Indianapolis, Calif)</th>
<th>Roche (Pittsburgh, Pa)</th>
<th>Abbott (Abbott Park, Ill)</th>
<th>Olympus (Center Valley, Pa)</th>
<th>Beckman Access (Fullerton, Calif)</th>
<th>Diagnostics Products Corp (Tarrytown, NY)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Potassium</td>
<td>53 (32.7)</td>
<td>37 (22.8)</td>
<td>26 (16.0)</td>
<td>17 (10.5)</td>
<td>14 (8.6)</td>
<td>7 (4.3)</td>
<td>4 (2.5)</td>
<td>4 (2.5)</td>
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<td>27 (17.4)</td>
<td></td>
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<tr>
<td>Calcium</td>
<td>54 (33.3)</td>
<td>36 (22.2)</td>
<td>26 (16.0)</td>
<td>17 (10.5)</td>
<td>14 (8.6)</td>
<td>7 (4.3)</td>
<td>4 (2.5)</td>
<td>4 (2.5)</td>
<td>...</td>
<td>5 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>53 (33.1)</td>
<td>36 (22.5)</td>
<td>25 (15.6)</td>
<td>17 (10.6)</td>
<td>13 (8.1)</td>
<td>8 (5.0)</td>
<td>4 (2.5)</td>
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<td>1 (0.6)</td>
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<tr>
<td>Thyroid-Stimulating Hormone</td>
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<td></td>
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<tr>
<td>Hemoglobin (Male)</td>
<td>33 (21.3)</td>
<td>4 (2.6)</td>
<td>14 (9.0)</td>
<td>8 (5.2)</td>
<td>11 (7.1)</td>
<td>34 (21.9)</td>
<td>18 (11.6)</td>
<td>18 (11.6)</td>
<td>...</td>
<td>92 (56.8)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (Female)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>90 (56.6)</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>92 (56.8)</td>
<td></td>
</tr>
<tr>
<td>Activated Partial Prothrombin Time</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<td></td>
</tr>
</tbody>
</table>

* Ellipses indicate the instrument does not perform the test; APTT, activated partial prothrombin time.

### Table 3. Adult and Pediatric Mean Critical Values

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Low Critical Value</th>
<th>Mean SD</th>
<th>High Critical Value</th>
<th>Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult Critical Values Summary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>2.8</td>
<td>0.21</td>
<td>6.1</td>
<td>0.27</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>6.42</td>
<td>0.49</td>
<td>12.84</td>
<td>0.90</td>
</tr>
<tr>
<td>Magnesium, mEq/L</td>
<td>0.88</td>
<td>0.13</td>
<td>4.06</td>
<td>1.07</td>
</tr>
<tr>
<td>Hemoglobin, male patients, g/dL</td>
<td>6.9</td>
<td>0.85</td>
<td>19.9</td>
<td>1.52</td>
</tr>
<tr>
<td>Hemoglobin, female patients, g/dL</td>
<td>6.9</td>
<td>0.85</td>
<td>19.9</td>
<td>1.54</td>
</tr>
<tr>
<td>Platelet count, ×10^9/μL</td>
<td>383.164</td>
<td>16.4</td>
<td>936.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Activated partial thromboplastin time, s</td>
<td>16.2</td>
<td>4.7</td>
<td>92.9</td>
<td>35.8</td>
</tr>
</tbody>
</table>

| **Pediatric Critical Values Summary** | | | | |
| Potassium, mEq/L      | 2.8 | 0.23 | 6.1 | 0.27 |
| Calcium, mg/dL        | 6.40 | 0.48 | 12.84 | 0.92 |
| Magnesium, mEq/L      | 0.88 | 0.13 | 4.07 | 1.10 |
| Hemoglobin, male patients, g/dL | 7.0 | 0.89 | 20.1 | 2.02 |
| Hemoglobin, female patients, g/dL | 7.0 | 0.89 | 20.1 | 2.02 |
| Platelet count, ×10^9/μL | 39.4 | 16.8 | 935.1 | 117.0 |
| Activated partial thromboplastin time, s | 16.6 | 4.6 | 91.7 | 35.0 |
Table 4. Adult and Pediatric Median Critical Values

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No. of Institutions</th>
<th>5th</th>
<th>50th (Median)</th>
<th>95th</th>
<th>No. of Institutions</th>
<th>5th</th>
<th>50th (Median)</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Critical Values Summary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>162</td>
<td>2.5</td>
<td>2.9</td>
<td>3.1</td>
<td>162</td>
<td>5.9</td>
<td>6.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>160</td>
<td>6.0</td>
<td>6.1</td>
<td>7.1</td>
<td>161</td>
<td>12.0</td>
<td>13.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Magnesium, mEq/L</td>
<td>124</td>
<td>0.7</td>
<td>0.8</td>
<td>1.1</td>
<td>125</td>
<td>2.5</td>
<td>4.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Hemoglobin, male patients, g/dL</td>
<td>157</td>
<td>5.0</td>
<td>7.0</td>
<td>8.0</td>
<td>153</td>
<td>18.0</td>
<td>20.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Hemoglobin, female patients, g/dL</td>
<td>155</td>
<td>5.0</td>
<td>7.0</td>
<td>8.0</td>
<td>153</td>
<td>18.0</td>
<td>20.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Platelet count, ×10^9/μL</td>
<td>162</td>
<td>20</td>
<td>31</td>
<td>70</td>
<td>131</td>
<td>700</td>
<td>999</td>
<td>1000</td>
</tr>
<tr>
<td>Activated partial prothrombin time, s</td>
<td>17</td>
<td>5</td>
<td>18</td>
<td>22</td>
<td>154</td>
<td>42</td>
<td>90</td>
<td>150</td>
</tr>
<tr>
<td>Pediatric Critical Values Summary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>144</td>
<td>2.5</td>
<td>2.9</td>
<td>3.1</td>
<td>143</td>
<td>5.9</td>
<td>6.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>142</td>
<td>6.0</td>
<td>6.1</td>
<td>7.1</td>
<td>143</td>
<td>12.0</td>
<td>13.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Magnesium, mEq/L</td>
<td>109</td>
<td>0.7</td>
<td>0.8</td>
<td>1.1</td>
<td>109</td>
<td>2.5</td>
<td>4.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Hemoglobin, male patients, g/dL</td>
<td>143</td>
<td>5.0</td>
<td>7.0</td>
<td>8.1</td>
<td>98</td>
<td>18.0</td>
<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Hemoglobin, female patients, g/dL</td>
<td>143</td>
<td>5.0</td>
<td>7.0</td>
<td>8.1</td>
<td>98</td>
<td>18.0</td>
<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Platelet count, ×10^9/μL</td>
<td>146</td>
<td>20</td>
<td>31</td>
<td>70</td>
<td>115</td>
<td>600</td>
<td>999</td>
<td>1000</td>
</tr>
<tr>
<td>Activated partial thromboplastin time, s</td>
<td>16</td>
<td>5</td>
<td>18</td>
<td>22</td>
<td>135</td>
<td>40</td>
<td>90</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 5. Analytes Included on Critical Values List

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>160</td>
<td>98.8</td>
</tr>
<tr>
<td>Sodium</td>
<td>158</td>
<td>97.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>158</td>
<td>97.5</td>
</tr>
<tr>
<td>Platelets</td>
<td>157</td>
<td>96.9</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>154</td>
<td>95.1</td>
</tr>
<tr>
<td>Activated partial thromboplastin time, s</td>
<td>153</td>
<td>94.4</td>
</tr>
<tr>
<td>White blood cells</td>
<td>149</td>
<td>92.0</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>147</td>
<td>90.7</td>
</tr>
<tr>
<td>Blood culture</td>
<td>142</td>
<td>87.7</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>137</td>
<td>84.6</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>136</td>
<td>84.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>132</td>
<td>81.5</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>122</td>
<td>75.3</td>
</tr>
<tr>
<td>Lithium</td>
<td>121</td>
<td>74.7</td>
</tr>
<tr>
<td>Gram stain</td>
<td>108</td>
<td>66.7</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>105</td>
<td>64.8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>104</td>
<td>64.2</td>
</tr>
<tr>
<td>pH*</td>
<td>91</td>
<td>56.2</td>
</tr>
<tr>
<td>PCO₂*,</td>
<td>91</td>
<td>56.2</td>
</tr>
<tr>
<td>PO₂*,</td>
<td>91</td>
<td>56.2</td>
</tr>
<tr>
<td>Creatinine</td>
<td>86</td>
<td>53.1</td>
</tr>
<tr>
<td>Malaria smear</td>
<td>80</td>
<td>49.4</td>
</tr>
<tr>
<td>Troponin</td>
<td>79</td>
<td>48.8</td>
</tr>
<tr>
<td>Chloride</td>
<td>59</td>
<td>36.4</td>
</tr>
<tr>
<td>Blast cells</td>
<td>53</td>
<td>32.7</td>
</tr>
<tr>
<td>D-dimer</td>
<td>41</td>
<td>25.3</td>
</tr>
<tr>
<td>Fungal stain</td>
<td>37</td>
<td>22.8</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>29</td>
<td>17.9</td>
</tr>
<tr>
<td>Atypical/immature cells</td>
<td>19</td>
<td>11.7</td>
</tr>
</tbody>
</table>

* Blood gas analytes.

Results. The test is designed to convert the differences (or deviations) between the two into the probability of their occurring by chance, taking into account both the size of the sample and the number of variables (degrees of freedom). This hypothesis was supported with the exception of adult and pediatric aPTT sources, which were significantly (P = .003 and P < .001 for adult and pediatric aPTT sources, respectively) different from this hypothesis.

The aPTT test was selected for this study as a marker for coagulation critical values. Approximately 28% to 29% of all respondents used published literature or textbooks as a primary source for both pediatric and adult aPTT critical values. A total of 26% and 27% (39 and 43 institutions, respectively) used nonlaboratory medical staff recommendations to establish these values. When aPTT is compared with the 5 other analytes examined in this survey (Table 6), a higher percentage of institutions (12% and 15%, 16 and 23, respectively) performed internal studies of healthy individuals for pediatric and adult critical values. This may be a reflection of annual practices at some clinical laboratories that perform annual control lot analysis. Similarly, compared with the other analytes, a higher percentage (16% and 14%) of laboratories reported an “other” category for pediatric and adult aPTT critical values, respectively; this is perhaps also related to differences in reagent lot management.

Because critical values are increasingly scrutinized by regulatory and accrediting agencies, several questions were asked on the questionnaire regarding critical values policies (Table 7). A total of 56% had a written policy for establishing, revising, and updating their critical values. A total of 67% of reporting laboratories indicated that they kept a unique range for distinct populations by age. Only 16% of reporting laboratories, however, indicated that they had unique critical values for distinct populations by location, and only 7.5% of laboratories indicated they had unique critical values based on provider or practice group. A total of 10% of laboratories reported a unique critical value based on disease type. No laboratory reported a unique critical values set based on ethnicity.

The general questionnaire provided a variety of inter-
Table 6. Comparison of Primary Sources for Critical Values

<table>
<thead>
<tr>
<th></th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult values</td>
</tr>
<tr>
<td></td>
<td>n = 159</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Values H11005</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
</tr>
<tr>
<td></td>
<td>Magnesium</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin, Male</td>
</tr>
<tr>
<td></td>
<td>Platelet Count</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.6</td>
</tr>
<tr>
<td></td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>33.8</td>
</tr>
<tr>
<td></td>
<td>27.9</td>
</tr>
<tr>
<td>Published literature/textbooks</td>
<td>39.6 36.7 40.0 33.3 33.3 33.8 27.9</td>
</tr>
<tr>
<td>Nonlaboratory medical staff recommendations</td>
<td>33.3 32.3 28.6 33.3 34.0 33.8 27.3</td>
</tr>
<tr>
<td>Manufacturer’s recommendation/package inserts</td>
<td>8.8 10.8 10.0 3.8 3.9 4.5 8.4</td>
</tr>
<tr>
<td>Internal study of healthy individuals</td>
<td>6.3 7.0 5.7 10.3 9.8 10.8 14.9</td>
</tr>
<tr>
<td>Other laboratories (adopted with internal validation)</td>
<td>2.5 3.8 4.3 7.1 7.2 6.4 5.8</td>
</tr>
<tr>
<td>Other laboratories (adopted without internal validation)</td>
<td>2.5 2.5 4.3 1.3 1.3 1.3 1.9</td>
</tr>
<tr>
<td>Other</td>
<td>6.9 7.0 7.1 10.9 10.5 9.6 13.6</td>
</tr>
<tr>
<td>Published literature/textbooks</td>
<td>40.7 38.4 41.3 35.9 37.3 35.9 29.1</td>
</tr>
<tr>
<td>Nonlaboratory medical staff recommendations</td>
<td>33.6 32.6 31.4 33.1 33.1 34.5 26.1</td>
</tr>
<tr>
<td>Manufacturer’s recommendation/package inserts</td>
<td>9.3 10.1 9.9 3.5 3.5 4.2 9.0</td>
</tr>
<tr>
<td>Internal study of healthy individuals</td>
<td>3.6 4.3 4.1 5.6 5.6 4.9 11.9</td>
</tr>
<tr>
<td>Other laboratories (adopted with internal validation)</td>
<td>1.4 2.9 3.3 5.6 4.9 4.9 6.0</td>
</tr>
<tr>
<td>Other laboratories (adopted without internal validation)</td>
<td>3.6 2.9 2.5 4.9 4.2 3.5 1.5</td>
</tr>
<tr>
<td>Other</td>
<td>7.9 8.7 7.4 11.3 11.3 12.0 16.4</td>
</tr>
</tbody>
</table>

Table 7. Characteristics of Critical Values Policies

<table>
<thead>
<tr>
<th>Critical Values Policies</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written policy for establishing, revising, or updating</td>
<td>Yes 88</td>
<td>56.1</td>
</tr>
<tr>
<td>No 69</td>
<td>43.9</td>
<td></td>
</tr>
<tr>
<td>Unique ranges for distinct populations by age</td>
<td>Yes 106</td>
<td>66.7</td>
</tr>
<tr>
<td>No 53</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Unique ranges for distinct populations by location</td>
<td>Yes 26</td>
<td>16.3</td>
</tr>
<tr>
<td>No 134</td>
<td>83.8</td>
<td></td>
</tr>
<tr>
<td>Unique ranges for distinct populations by provider or practice group</td>
<td>Yes 12</td>
<td>7.5</td>
</tr>
<tr>
<td>No 148</td>
<td>92.5</td>
<td></td>
</tr>
<tr>
<td>Unique ranges for distinct populations based on disease type</td>
<td>Yes 16</td>
<td>10.1</td>
</tr>
<tr>
<td>No 142</td>
<td>89.9</td>
<td></td>
</tr>
<tr>
<td>Unique ranges for ethnicity</td>
<td>Yes 0</td>
<td>0.0</td>
</tr>
<tr>
<td>No 159</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Testing responses (Table 8). The vast majority of laboratories use either technical personnel exclusively (81%) or a mixture of technical and nontechnical personnel (18%) to deliver the critical values notification (81% + 18% = 99%). Who was permitted to receive the critical values varied somewhat for inpatients as opposed to outpatients, although most laboratories clearly required notification to a physician or licensed caregiver. Administrative personnel could accept critical values more frequently for outpatients (48%) than for inpatients (27%).

Only 11% of institutions had multiple tiers for a critical values laboratory response that were dependent on the time of day (ie, call back within 1 hour vs 8 hours; call only during normal working hours vs call at any time). However, special criteria for calling critical values to outpatients in evenings, nights, or on weekends existed for 43% of the participants. A total of 98% of institutions did, however, expect to make the first attempt to contact a caregiver for a critical value for inpatients within the first hour. Only 5% of reporting laboratories permitted an answering service or fax machine to receive critical values after repeated failure to contact a caregiver qualified to receive a result. If acceptable at all, electronic means of communication was generally acceptable only after direct verbal communication. The JC-mandated read-back policy was largely enforced by the laboratory, although 12% of reporting laboratories did not maintain documentation of the read-back record (Table 8). Nearly 1 (18%) in 5 laboratories permitted physicians to request exceptions to their critical values notification policy.

**COMMENT**

Critical value notification procedures are essential for clinical laboratories. They are required by the CAP Laboratory Accreditation Program (LAP) and by standards provided by the JC.3 Periodically, every clinical laboratory experiences a review of its critical values list either in response to an internal policy review or for clinical purposes. Each time a critical values list or an individual critical analyte is evaluated, laboratories desire an improved definition of critical values and search for further documen-
### Table 8. Characteristics Relating to Critical Values Practices

<table>
<thead>
<tr>
<th>Routine notification of critical values to caregivers by:</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical personnel</td>
<td>130</td>
<td>80.7</td>
</tr>
<tr>
<td>Nontechnical personnel</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>Both technical and nontechnical personnel</td>
<td>29</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Who can receive critical values for inpatients?

<table>
<thead>
<tr>
<th>Who can receive critical values for inpatients?</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any licensed caregiver</td>
<td>146</td>
<td>91.8</td>
</tr>
<tr>
<td>Ordering physician</td>
<td>142</td>
<td>89.3</td>
</tr>
<tr>
<td>On-call physician/resident</td>
<td>117</td>
<td>73.6</td>
</tr>
<tr>
<td>Fax, only as follow-up to direct verbal communication</td>
<td>59</td>
<td>37.1</td>
</tr>
<tr>
<td>Administrative personnel (ie, ward clerk)</td>
<td>43</td>
<td>27.0</td>
</tr>
<tr>
<td>Fax, without direct verbal communication</td>
<td>4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Who can receive critical values for outpatients?

<table>
<thead>
<tr>
<th>Who can receive critical values for outpatients?</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordering physician</td>
<td>150</td>
<td>93.8</td>
</tr>
<tr>
<td>Any licensed caregiver</td>
<td>137</td>
<td>85.6</td>
</tr>
<tr>
<td>Fax, only as follow-up to direct verbal communication</td>
<td>81</td>
<td>50.6</td>
</tr>
<tr>
<td>Office personnel (ie, receptionist)</td>
<td>76</td>
<td>47.5</td>
</tr>
<tr>
<td>Answering service</td>
<td>6</td>
<td>3.8</td>
</tr>
<tr>
<td>Fax, without direct verbal communication</td>
<td>4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Multiple tiers for critical value laboratory response dependent on the time of day (ie, call back within 1 h vs 8 h vs 24 h; call only during normal working hours vs call at any time)

<table>
<thead>
<tr>
<th>Multiple tiers for critical value laboratory response dependent on the time of day</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>18</td>
<td>11.2</td>
</tr>
<tr>
<td>No</td>
<td>143</td>
<td>88.8</td>
</tr>
</tbody>
</table>

Special criteria for calling critical values to outpatients on weekends, evenings, or nights?

<table>
<thead>
<tr>
<th>Special criteria for calling critical values to outpatients on weekends, evenings, or nights?</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>69</td>
<td>43.1</td>
</tr>
<tr>
<td>No</td>
<td>91</td>
<td>56.9</td>
</tr>
</tbody>
</table>

Laboratory’s time expectations for the first attempt to contact a caregiver for a critical value result from the time the result is available?

<table>
<thead>
<tr>
<th>Laboratory’s time expectations for the first attempt to contact a caregiver for a critical value result from the time the result is available?</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 h</td>
<td>157</td>
<td>98.1</td>
</tr>
<tr>
<td>1–3 h</td>
<td>3</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Electronic communication of critical values

<table>
<thead>
<tr>
<th>Electronic communication of critical values</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, after speaking with someone qualified to receive a result</td>
<td>63</td>
<td>39.4</td>
</tr>
<tr>
<td>Yes, after repeated failure to contact someone qualified to receive a result</td>
<td>8</td>
<td>5.0</td>
</tr>
<tr>
<td>No</td>
<td>89</td>
<td>55.6</td>
</tr>
</tbody>
</table>

If you electronically communicate critical value results without speaking to someone qualified to receive the result, do you have a process to confirm receipt?

<table>
<thead>
<tr>
<th>If you electronically communicate critical value results without speaking to someone qualified to receive the result, do you have a process to confirm receipt?</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>12</td>
<td>63.2</td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>36.8</td>
</tr>
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</table>

Physicians/clinicians permitted to request exceptions to your critical values notification policy (ie, “no call hours”)

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<th>Physicians/clinicians permitted to request exceptions to your critical values notification policy (ie, “no call hours”)</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>29</td>
<td>18.2</td>
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<tr>
<td>No</td>
<td>130</td>
<td>81.8</td>
</tr>
</tbody>
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Approximate percentage of critical values that are “read back” by the receiving personnel

<table>
<thead>
<tr>
<th>Approximate percentage of critical values that are “read back” by the receiving personnel</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
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</thead>
<tbody>
<tr>
<td>100</td>
<td>69</td>
<td>42.9</td>
</tr>
<tr>
<td>90–99</td>
<td>70</td>
<td>43.5</td>
</tr>
<tr>
<td>50–89</td>
<td>10</td>
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<tr>
<td>&lt;50</td>
<td>12</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Location of “read back” record

<table>
<thead>
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<th>Location of “read back” record</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>In laboratory records (ie, laboratory information system)</td>
<td>139</td>
<td>85.8</td>
</tr>
<tr>
<td>Someplace other than the laboratory</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Not kept</td>
<td>20</td>
<td>12.3</td>
</tr>
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How is reporting of a critical value documented in laboratory?

<table>
<thead>
<tr>
<th>How is reporting of a critical value documented in laboratory?</th>
<th>No. of Institutions</th>
<th>Percentage of Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comment in the computer system</td>
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<td>73.9</td>
</tr>
<tr>
<td>Written on the result form</td>
<td>6</td>
<td>3.7</td>
</tr>
<tr>
<td>Both of the above</td>
<td>36</td>
<td>22.4</td>
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</tbody>
</table>

A review of the analytes listed on institutional critical values lists in this study shows that most laboratories (90%) include 8 key analytes on their critical value list: (1) potassium, (2) sodium, (3) calcium, (4) platelets, (5) hemoglobin, (6) aPTT, (7) white blood cell count, and (8) PT. Additional analytes, such as blood gas analytes, that are not in this primary grouping probably represent differences in laboratory services (since only the primary lab-
laboratory service was evaluated) or different institutional clinical requirements. It might also be noted that there are advantages to a relatively limited list of critical values. Long, complex lists require significant laboratory personnel investment for calling the results. Also, increased list complexity does not necessarily reflect "life-threatening" results and may diffuse the perceived importance of critical values contacts. Table 5 provides an important guide to laboratories devising a critical values list for their health care facilities providing cumulative information from 163 institutions.

The variability of critical values and the mean and median between institutions are provided in Tables 3 and 4. From these tables, a laboratory can judge how far its analyte value varies from the mean and median and the variability in the survey between clinical laboratories for each of the representative analytes reported. Individuals can review the results for potassium, for example (median low: 2.9 mEq/L; median high: 6.0 mEq/L), and benchmark their position. An institution finding itself below the 5th or above the 95th percentile may wish to review a given analyte for appropriateness in their institutional setting.

The 3 most important findings of this study are: (1) only 56% of the participating laboratories have a policy or procedure for managing critical values, (2) 27% of the laboratories allow non–health care professionals to accept critical value results for inpatients, and (3) there is a nonconsensus regarding how to handle outpatient critical values and who should receive results for such patients on nights and weekends.

Somewhat surprisingly, only a small majority of participants reported an existing written policy for establishing, revising, or updating the critical values list (Table 7). This finding should serve as a reminder to review current procedures and ensure that a procedure or policy exists for the reporting of critical value results. Establishing and reviewing written policies for critical values is increasingly important as regulatory agencies focus on critical value reporting mechanisms.

Reporting critical laboratory results to an appropriate and responsible health care professional has been shown to improve critical value reporting in a recent review of a Q-Tracks study.11 In this study, 27% of participating laboratories allowed reporting to nonpractitioner staff for inpatients, and 48% laboratories allowed reporting to nonpractitioner staff for outpatient critical values. Clinical laboratories may wish to review their policies of reporting to licensed versus unlicensed caregivers, given that laboratories showing the most improvement in critical value reporting over time report only to licensed health care professionals.

The nonconsensus regarding how to handle outpatient reporting at night and on weekends is perhaps not a surprising finding. It probably reflects some uncertainty in the clinical laboratory community regarding how best to manage this important patient care process. A more detailed analysis in a future study may allow further explanation and possible solutions. It is also apparent that most clinical laboratories still rely on voice contact with readback for the reporting of critical values. These procedures may change, as electronic innovations provide better management for confirmation of receipt of a critical result report.12,13 Systems such as the telephone critical value reporting system Veriphy, by Vocada Inc (Dallas, Tex), may be adapted for clinical laboratory reporting. Perhaps our finding that 11% of laboratories have tiered responses will provide impetus for these electronic developments. Many clinicians and laboratories would look forward to a time when laboratories could develop critical values lists designed for a specific clinic or physician.

The expansion of critical and alert values into other diagnostic areas, such as radiology and anatomic pathology, has accentuated the need to clearly define critical, life-threatening results as distinguishable from other alert values.14,15 Silverman and Pereira,16 for example, reported several criteria to consider for anatomic pathology critical value reporting. The question of critical values continues as a high-profile topic in anatomic pathology.17 The CLIA definition identifies a test result that indicates an "imminently life-threatening condition," yet uses alternative terms such as panic or alert values, when referring to these test results.1 A consensus of terms and definitions should be a high priority for clinical laboratories and physicians as concerns regarding patient safety continue to influence new requirements and recommendations.18 Constructive discussion of critical values provides an integral pathway by which laboratories can participate in patient safety initiatives.

The authors thank Christine Bashleban for her administrative support and Bushra Yasin, PhD, for her detailed review of the manuscript.

References

Vasculogenic mimicry (VM)—the development of fluid-conducting pathways by highly invasive and genetically dysregulated tumor cells—appears in 2 forms. In VM of the tubular type, non–endothelial cell-lined tubes resembling blood vessels are identified. In VM of the patterned matrix type, sheaths of extracellular matrix rich in laminin, collagens IV and VI, fibronectin, and heparan sulfate proteoglycan form loops surrounding packets of tumor cells. The extracellular matrix connects to endothelial cell lined blood vessels and transmits fluid, forming a fluid-conducting meshwork. Vasculogenic mimicry has been described in many tumors including melanoma, inflammatory and ductal breast carcinoma, ovarian carcinoma, prostatic carcinoma, synovial sarcoma, rhabdomyosarcoma, osteosarcoma, and pituitary tumors. The presence of VM has been associated with more aggressive tumor biology and increased tumor-related mortality.

There are limited data regarding VM in hepatocellular carcinoma (HCC). Tumor “vasularity” was partly attributed to VM in a transgenic mouse model of HCC. Two studies described the presence of “vasculogenic mimicry” in human HCC, although the endothelial cell markers used identified the tubular type but do not detect patterned matrix VM. Consequently, patterned matrix VM has not been systematically evaluated in human HCC lesions. This pilot study was designed to determine whether VM of the patterned matrix type occurs in HCC, and if so, to evaluate for an association between VM and HCC recurrence after orthotopic liver transplantation (OLT).

MATERIALS AND METHODS

Tissue blocks from 20 consecutive patients who underwent OLT for HCC were studied. Tissue specimens from 5 normal livers and 5 patients with hepatitis C–related cirrhosis served as controls. The study protocol was approved by the institutional review board at the University of Illinois at Chicago (UIC).

Evaluation of Tumor Tissue in Liver Explants

Liver explants were sectioned horizontally at 1-cm intervals. A histologic section was obtained from cirrhotic tissue and any
nodules that differed in size or color from the cirrhotic liver tissue. The physical characteristics of the tumor including size, location, appearance, and number were recorded. Hematoxylin-eosin–stained slides were reviewed for evaluation of tumor type, grade, and stage following the guidelines of the World Health Organization (WHO) and International Union Against Cancer (UICC).Areas of dysplasia were characterized as groups of cells containing nuclei with abnormal sizes, inconsistent configurations, and occasional multinucleation in regions with partial or complete nodular replacement and normal liver cell plate thickness.22

**Immunohistochemical Studies**

Serial 4-μm sections were obtained from formalin-fixed and paraffin-embedded tumor tissue. Hematoxylin-eosin staining was performed to evaluate histologic features of HCC. Slides were examined for VM by 3 independent observers who were blinded to outcome. Laminin staining was used as the primary indicator of VM.14 Vascularogenic mimicry patterns were identified by the detection of laminin-positive loops surrounding clusters of 3 to 15 tumor cells. Red blood cells were variably present within these patterns. There was agreement among observers in all cases. Hematoxylin-eosin–stained slides were reviewed for confirmation of tumor type, grade, and stage.24 Standard immunohistochemical staining was performed on paraffin-embedded tumor blocks and control tissues for laminin, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and CD31.

**Procedure for Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissue samples were sectioned at 4 μm thickness and mounted on Superfrost/Plus slides. Slides were deparaffinized in xylene and rehydrated through a decreasing ethanol gradient. Slides were rinsed in distilled water followed by antigen unmasking utilizing a 10× concentrated retrieval solution (Target Retrieval Solution, Dako, Carpinteria, Calif) according to manufacturer’s instructions and then rinsed in phosphate-buffered saline for 5 minutes. For the demonstration of laminin, EGFR, VEGF, and CD31, blocking solution ( Peroxidase Blocking Reagent, Dako) was applied for 10 minutes at room temperature. Slides were pretreated with proteinase K (Dako) for 5 minutes. Slides were treated with protein blocking solution (Protein Block Serum-Free, Dako) for 10 minutes at room temperature, then rinsed and incubated with monoclonal mouse anti-laminin antibody (L8271, clone LAM 89, Sigma Aldrich, St Louis, Mo) at a titer of 1:200; mouse anti-EGFR (clone 2-18C9, Dako) at a titer of 1:200; anti-human VEGF antibody (555036, BD Pharmingen, San Diego, Calif) at a titer of 1:50; or monoclonal mouse anti-human CD31 antibody (M0823, Dako) at a titer of 1:25; for 30 minutes at room temperature. Slides were rinsed, then treated with EnVision+–labeled polymer (Dako) for 30 minutes at room temperature. Immunohistochemical staining for the different antibodies was detected by DAB+ (Dako) for 10 minutes. Slides were rinsed in distilled water, counterstained, and dehydrated through an alcohol gradient and mounted with Permount.

**EGFR or VEGF Staining Intensity Index**

Membranous staining for EGFR and cytoplasmic staining for VEGF and CD31 were considered positive. The intensity of staining for EGFR and VEGF was graded as 0 for absent immunoreactivity, 1 for weak, 2 for moderate, and 3 for intense positivity. The percentage of positivity within the tumor was assessed. A staining intensity index was calculated by multiplying the percentage of the entire tumor sample staining positively and the intensity grade (eg, 80 × 2 = staining intensity index of 160).25

**Surveillance for HCC Recurrence**

**After Liver Transplantation**

Liver transplant recipients underwent cross-sectional imaging on a 3- to 6-month basis to assess for evidence of posttransplant recurrence. Time to tumor recurrence was based on the interval from transplantation to detection of characteristic features of HCC on contrast enhanced computerized tomography or magnetic resonance imaging.

**Statistical Analysis**

Normally distributed variables were compared between patients with and without VM expression using a Student t test, and categorical variables were compared using the Pearson χ2 test or Fisher exact test when appropriate. Time to HCC recurrence was estimated by the Kaplan-Meier product-limit method. Survival distributions were compared between patients with and without VM by univariate analysis using the log-rank test. Multivariate analysis was then performed using the Cox proportional hazards model. Only 2 variables were entered into the multivariate model at a time due to the small sample size. Statistical analysis was performed using SPSS (version 12.0, Chicago, Ill).

**RESULTS**

**VM in HCC and Control Specimens**

Patterned matrix vascularogen mimicry was identified in HCC tissue from 11 (55%) of 20 liver explants including 8 of 9 cases that subsequently recurred posttransplant (Figure 1). Nine primary HCC lesions showed no evidence of VM (Figure 2). None of the control tissues including specimens from 5 normal livers and samples from 5 cirrhotic livers without HCC (Figure 3, A and B) contained VM.

**Comparison of VM-Positive and VM-Negative HCC Cases**

There was no difference in demographic features including sex, age, or cause of liver disease (viral or other) between subjects with VM-positive and VM-negative tumors (Tables 1 and 2). The tumor size, grade, stage, and presence of lymphovascular space tumor invasion did not distinguish VM-positive and VM-negative cases (Table 1). The α-fetoprotein serum level (20 ng/mL [P = .10]) at the time of diagnosis did not correlate with the presence of VM.

**VM Expression and Time to Recurrent HCC**

Patients found to have HCC in liver explants were followed for a median of 711 days (range, 108–2163 days) after OLT. Nine (45%) of 20 patients developed evidence of recurrent HCC, with a median time to recurrence of 191 days (range, 40–988 days). Eight of 11 patients with expression of VM in explanted tumor tissue developed recurrent HCC posttransplant, whereas only 1 of 9 patients without VM had recurrent HCC. The expression of VM in primary HCC lesions was associated with a shorter time to posttransplant recurrence (P = .01) (Figure 4). In multivariate analysis using Cox regression, potentially confounding factors were analyzed individually in conjunction with VM. The association between VM expression and more rapid tumor recurrence persisted when controlling for age, sex, cause of liver disease, tumor size, grade, and stage.

**VM Expression and EGFR, VEGF, and CD31 Immunoreactivity**

There were no significant associations among the following parameters: VM and EGFR staining intensity index (270 [100–300]; P = .36) or VM and VEGF staining intensity index (240 [100–300]; P = .89) (Figure 5, A and B). All hepatocytes in HCC lesions and control tissues were negative for CD31 (Figure 6, A and B).
COMMENT

The current study provides the first evidence of patterned matrix VM in human HCC. We found VM in 55% of HCC specimens from 20 liver explants. Patterned matrix VM was associated with aggressive HCC, reflected by more rapid HCC recurrence after OLT. These retrospective findings suggest that staining for VM could have prognostic value in OLT candidates with HCC.

In the normal liver, laminin is present in the basement membranes of bile ducts, bile ductules, and vessels. In this pilot study we found that VM was not identified in normal hepatocytes, or in the hepatic parenchyma in chronic liver disease or cirrhosis. However, patterned matrix VM was easy to identify in HCC specimens by laminin immunostaining. Patterned matrix VM was distinct from angiogenic vessels on light microscopy because endothelial cell lined vessels do not form back-to-back loops in 2-dimensional histologic sections.

We performed immunostaining for EGFR, VEGF, and CD31 to further evaluate whether changes of VM in HCC were associated with cell growth and cell survival or with endothelial markers. Epidermal growth factor receptor is a 170-kd transmembrane glycoprotein receptor with intrinsic tyrosine kinase activity that regulates cell growth, differentiation, and survival. Vascular endothelial growth factor stimulates proliferation of endothelial cells through specific tyrosine kinase receptors, flt-1 and flt/KDR, and is a central regulator of the angiogenic process. CD31 is a platelet endothelial cell adhesion protein molecule and is an indicator for angiogenesis in many tumors including hepatocellular carcinoma. In the current study, there were no significant associations between VM and expression of EGFR or endothelial cell markers VEGF and CD31. These results provide further evidence that VM expression in HCC is distinct from endothelial lined vessels.

The physical features of HCC that have been conventionally associated with HCC recurrence include the size and number of tumor nodules, microvascular/macrovascular invasion, and high serum α-fetoprotein levels. In a previous study, we found a significant association between
Table 1. Comparison of Clinical Factors as a Function of Vasculogenic Mimicry (VM) Expression in Hepatocellular Carcinoma

<table>
<thead>
<tr>
<th>Factor</th>
<th>VM Positive (n = 11)</th>
<th>VM Negative (n = 9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>5/6</td>
<td>2/7</td>
<td>.27</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>52 ± 13</td>
<td>57 ± 13</td>
<td>.95</td>
</tr>
<tr>
<td>Cause of liver disease (viral/other)</td>
<td>9/2</td>
<td>5/4</td>
<td>.34</td>
</tr>
<tr>
<td>Tumor size (≤5 cm/&gt;5 cm)</td>
<td>8/3</td>
<td>7/2</td>
<td>.43</td>
</tr>
<tr>
<td>Tumor grade (low [1]/high [2 or 3])</td>
<td>3/8</td>
<td>1/8</td>
<td>.38</td>
</tr>
<tr>
<td>Tumor stage (low [1]/high [2 or 3])</td>
<td>8/3</td>
<td>4/5</td>
<td>.65</td>
</tr>
<tr>
<td>Lymphovascular space tumor invasion (−/+)</td>
<td>9/2</td>
<td>9/0</td>
<td>.29</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Cause of Liver Disease and Vasculogenic Mimicry (VM) Expression and Tumor Recurrence

<table>
<thead>
<tr>
<th>Cause of Liver Disease</th>
<th>VM Positive</th>
<th>VM Negative</th>
<th>Tumor Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune hepatitis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Alcohol-induced cirrhosis</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chronic hepatitis B virus cirrhosis</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chronic hepatitis C virus cirrhosis</td>
<td>7</td>
<td>5</td>
<td>6*</td>
</tr>
<tr>
<td>Chronic hepatitis C virus and alcohol-induced cirrhosis</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nonalcoholic steatohepatitis</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

* Recurrences were positive for VM in 5 of 6 cases.

Figure 4. Kaplan-Meier plot showing time to recurrence in patients with vasculogenic mimicry (dashed line) and without vasculogenic mimicry (solid line) expression by laminin stain. Patients with vasculogenic mimicry expression had a more rapid hepatocellular carcinoma recurrence after liver transplantation (P = .01).

A serum α-fetoprotein level greater than or equal to 100 ng/mL at time of diagnosis pretransplant and shorter time to recurrent HCC (P = .003). In the current study, there was no significant association between VM expression and serum α-fetoprotein level at the time of diagnosis or physical characteristics of the tumor. Moreover, the cause of liver disease (viral vs nonviral) was not associated with VM. Therefore, the formation of VM does not appear to be dependent on the cause of liver disease. It is likely that the formation of VM in biologically aggressive HCC is a host survival mechanism and has a dual effect of containing the tumor in a biological “cocoon,” thereby slowing its growth and metastasis, but could also potentially curtail the effectiveness of chemotherapy.
This pilot study was retrospective and the sample size was relatively small, limiting the power to detect differences between groups. The posttransplant recurrence rate was quite high. The recurrence rate appears to be related in part to the inclusion of 10 living donor recipients with HCC who were fast-tracked to transplantation. As a point of comparison, 42% of patients who underwent living donor liver transplantation for HCC in the multicenter adult-to-adult living donor liver transplantation (A2ALL) cohort study developed recurrence at 3 years. Nevertheless, our study population was followed in a consistent manner for tumor recurrence, which provides a clear clinical endpoint.

It is likely that VM in HCC was observed as early as 1982 by Sell and Ruoslahti in an immunofluorescence study that addressed the distribution of laminin in F344 rat livers. They described that laminin was predominantly observed in the basement membrane of blood vessels and bile ducts and inconsistently seen in sinusoids. Following treatment with carcinogens, laminin staining was associated with newly formed ductlike structures and oval cells, now known to be hepatic stem cells.

In the current study, we identified vasculogenic mimicry in HCC and that it was associated with a more aggressive course of HCC after OLT. These data, subject to validation in larger patient samples, suggest that the histologic detection of VM could be added to the list of parameters used to predict the clinical course of HCC.

Supported by grant EY10457, National Institutes of Health, Bethesda, Md.

References
5. Massi D, Franchi A, Paglierani M, et al. Vasculogenic mimicry has no prog-

Vasculogenic Mimicry in Hepatocellular Carcinoma—Guzman et al
Vimentin Reactivity in Renal Oncocytoma

Immunohistochemical Study of 234 Cases

Ondrej Hes, MD, PhD; Michal Michal, MD, PhD; Naoto Kuroda, MD; Guido Martignoni, MD; Matteo Brunelli, MD, PhD; Yi Lu, MD; Brian P. Adley, MD; Isabel Alvarado-Cabrero, MD; Ximing J. Yang, MD, PhD

• Context.—The expression of vimentin in benign renal oncocytomas has been controversial. However, this is of clinical significance because immunostains may be used in differential diagnosis of renal tumors on limited biopsy specimens. Using different staining and analysis methods, we studied vimentin immunoreactivity in a large series of renal oncocytomas with a special emphasis on the immunoreactivity patterns.

Objective.—Immunohistochemical expression of vimentin has been used in the differential diagnosis of renal epithelial neoplasms. Although typically expressed in most renal cell carcinomas, the immunoreactivity of this intermediate filament in renal oncocytomas has been controversial.

Design.—We studied vimentin immunoreactivity in a large series of 234 renal oncocytomas using 2 staining methods as well as manual and automated imaging analyses.

Results.—We found that the focal vimentin immunoreactivity can be seen in most (72.6%) renal oncocytomas with vimentin-positive tumor cells usually found in the edge of a central scar or in small clusters scattered throughout the tumor. Computer-aided imaging analysis using ChromaVision Automatic Cellular Imaging System II confirmed the difference in vimentin immunoreactivity between oncocytoma and other renal neoplasms.

Conclusions.—Our study of vimentin immunohistochemistry in a series of renal oncocytomas, which to our knowledge is the largest ever published, showed focal vimentin positivity detected in most oncocytomas. Because the vimentin staining patterns in renal oncocytomas are different from those seen in clear cell or papillary renal cell carcinomas, we consider vimentin staining to be helpful in the differential diagnosis of oncocytoma from other renal tumor mimics. Furthermore, strong vimentin positivity in a renal cell neoplasm does not exclude the diagnosis of renal oncocytoma, particularly in a limited biopsy specimen.

(Arch Pathol Lab Med. 2007;131:1782–1788)

Renal cell carcinoma (RCC) is one of the few epithelial cell neoplasms that consistently express the intermediate filament vimentin. This finding is useful to differentiate clear cell RCC from other potential mimics. The presence of both vimentin and keratin in a benign tumor is uncommon, limited to pleomorphic adenoma, adrenal cortical adenoma, and a few others. The presence of vimentin expression in renal oncocytoma, a benign tumor sometimes difficult to distinguish from subtypes of RCC with eosinophilic cytoplasm, has been a controversial issue. A number of authors claimed negative vimentin staining in oncocytomas.1,4 Therefore, positive vimentin immunoreactivity in a renal neoplasm was often considered to be one of the diagnostic features of RCC. Although focal vimentin positivity has been observed in renal oncocytomas in several other reports,3,7–10 none of the previous studies have focused on the vimentin staining patterns in oncocytomas. Furthermore, many of these previous studies analyzed a relative small number of oncocytomas, typically ranging from 7 to 18 cases per series. In the current study, we analyzed the intensity and percentage of vimentin positivity detected in most oncocytomas in a large series of 234 renal oncocytomas. Additionally, we evaluated and described the patterns of vimentin immunoreactivity in tumors to determine if this parameter may have diagnostic importance.

MATERIALS AND METHODS

Case Selection

The surgical pathology files and consultation files of the Department of Pathology, Charles University in Pilsen, Czech Republic, were searched for tumors of the kidney coded as renal oncocytomas. A total number of 210 cases were obtained from nearly 11 000 tumors of the kidney in this registry, representing approximately 1.9% of all renal tumors. Original hematoxylin-eosin slides were reviewed. Eighteen cases were excluded from this study because the morphologic features of the tumors were not definitive of renal oncocytoma. An additional 8 cases were excluded because of lack of additional material for immunostaining. One hundred eighty-four cases of renal oncocytoma from 1

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

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Immunohistochemistry

For immunohistochemical studies, formalin-fixed, paraffin-embedded tissue sections were stained with an antibody against vimentin Ab-2 (clone V9, monoclonal, 1:800; Neomarkers, Fremont, Calif). In brief, 5-μm-thick sections were deparaffinized. Sections were microwave-pretreated in 10mM citrate buffer solution (pH 6.0) at 750 W for a 3-minute cycle repeated 3 times. The sections were treated with 0.3% hydrogen peroxide in methanol for 30 minutes and washed with phosphate-buffered saline solution. Endogenous biotin was blocked using a biotin blocking kit (Vector Laboratories, Burlingame, Calif). Thereafter, the sections were exposed to primary antibody overnight (approximately 12 hours) at 4°C. Sections were cooled in phosphate-buffered saline buffer in each step of immunohistochemistry. All slides were visualized using a supersensitive streptavidin-biotin system (BioGenex, San Ramon, Calif).

For comparison, another staining method using a biotin-free detection system was used as follows. Antigen retrieval was carried out in citrate buffer (10mM, pH 6) for 15 minutes at 100°C in a microwave oven. The slides were incubated with a primary mouse monoclonal anti-vimentin antibody (clone Vim 3B4, Dako, Carpinteria, Calif) at 1:100 dilution for 1 hour at room temperature. Sections were then incubated with the secondary anti-mouse immunoglobulin G antibody for 30 minutes. A subsequent reaction was performed with biotin-free horsedarsoxid peroxidase enzyme-labeled polymer from EnVision + detection system (Dako). A positive reaction was visualized with diaminobenzidine solution followed by counterstaining with hematoxylin. Immunohistochemical vimentin positivity in the vascular walls was used as an internal positive control. Only cases with strong vimentin staining were counted as positive. For conventional sections, the positive staining was graded as focal (greater than 1%, less than or equal to 5% cells) or diffuse (greater than 5% cells). For the TMA sections, positivity was defined as any case with positive tumor cells. Tumors with weak focal or weak diffuse staining were counted as negative.

Automatic Imaging Analysis

An automated Cellular Imaging System II (ACISII, ChromaVision Medical System Inc, San Juan Capistrano, Calif) was used to compare the intensity and percentage of vimentin staining in 2 cases of renal oncocytoma with that of adjacent benign renal cortical and medullary tissue in the same section. In addition, 2 papillary RCCs (positive controls) and 2 chromophobe RCCs (negative controls) were studied for comparison. With ACISII, positive staining was calculated by applying 2 thresholds with one recognizing blue background (hematoxylin-stained) cells and another recognizing brown positive cells. The percentage of positivity was the area detected by the brown threshold (positive cells) divided by the sum of the area detected by the brown and blue thresholds (total positive and negative cells). The intensity was calculated by masking out all areas not selected by the brown threshold and calculating the integrated optical density of brown within the remaining area. This value was divided by the area in pixels of the brown mask to calculate an average intensity of a selected area. The mean values from nonneoplastic kidneys (22 areas), oncocytomas (75 areas), chromophobe RCCs (27 areas), and papillary RCCs (20 areas) were obtained and analyzed by using the ChromaVision ACISII.

RESULTS

Each tumor was classified according to the predominant histologic growth pattern identified on hematoxylin-eosin–stained sections. There was no difference in the vimentin staining patterns when comparing the biotin-based method (77.1% positive) or the biotin-free method (75% positive) (Table 1). Among the 234 cases examined, 64 (27.4%) renal oncocytomas were entirely negative for vimentin. The remaining 170 (72.6%) tumors showed focal vimentin positivity representing a small subset of tumor cells. Among the conventional sections, 77% (157/204) of oncocytomas showed focal vimentin positivity, whereas among the TMA sections, 43% (13/30) of oncocytomas showed vimentin positivity. We evaluated the vimentin positivity in 7 different growth patterns of oncocytomas. The vimentin-positive rates ranged from solid-alveolar pattern (91/129, 70.5%), small cell pattern (4/5, 80%), solid tubular pattern (10/12, 83.3%), tubular pattern (23/24, 95.8%), prominent stromal component (12/12, 100%), and pseudopapillary pattern (2/2, 100%). Essentially, all the patterns may show vimentin positivity in more than 70% of cases. Results are summarized in Table 2.

Three staining patterns of vimentin were identified: clustered, tubular, and mosaic. The clusters of vimentin-positive oncocytoma cells were often seen in areas next to fibrotic and hyalinized scars of the tumors (Figure 1, A through D). Vimentin-positive tumor cells forming small tubules were also noted in the central scars or in viable parenchyma of the tumors (Figure 2, A and B). The mosaic pattern was characterized by scattered tumor cells with vimentin-positive cytoplasmatic staining and negative nuclear staining (Figure 2, C and D). Sometimes vimentin immunoreactivity was observed in
Figure 1. Vimentin staining in oncocytoma. Oncocytoma stained with hematoxylin-eosin (original magnifications ×40 [A] and ×400 [C]) and vimentin (original magnifications ×40 [B] and ×400 [D]). Vimentin reactivity in the vascular networks in scar tissue is highlighted (B). In the area next to the hyalinized central scar (A), there are clusters of vimentin-positive tumor cells (B). The clusters of oncocytoma cells (D) show strong vimentin staining in the cytoplasm.

the neoplastic cells showing multinucleation and degenerative nuclear changes that can occur in renal oncocytomas. Vimentin usually stained the cells with copious cytoplasm. We also observed scattered foci of vimentin-positive tumor cells without any relation to hyalinization and fibrous changes or degenerative changes especially in the peripheral parts of the tumors. Other stromal elements associated with the neoplastic epithelial cells showed vimentin positivity including delicate vascular networks and fibrous tissue and were used as internal positive controls.

Forty-eight of 50 chromophobe RCCs tested for comparison were vimentin negative. The 2 vimentin-positive chromophobe RCCs demonstrated diffuse but weak vimentin staining, different from the strong but focal vimentin reactivity observed in a portion of oncocytomas.

The mean vimentin staining intensities and percentages of immunoreactive cells measured using ACISII were as follows. Nonneoplastic kidneys showed intensities of 150.5 and 143.7 and percentages of 61.2% and 54.0%, respectively. Oncocytomas were divided into 2 groups: one with focal vimentin reactivity (1A and 2A intensities = 159.1 and 156.7, percentages = 89% and 83.7%, respectively) and one without vimentin reactivity (1B and 2B intensities = 31.6 and 33.5, percentages = 0.1% and 0.1%). The papillary RCCs demonstrated intensities of 158.3 and 157.1 and percentages of 81.7% and 98.4%, and the chromophobe RCCs demonstrated intensities of 31.8 and 32.3 and percentages of 0.1% and 0.1%. The results of vimentin immunoreactivity represented by the product of intensity and percentage (intensity × percentage) are summarized in Table 3.

COMMENT
Renal oncocytoma and chromophobe RCC may share several overlapping morphologic and immunophenotypic features. Efforts to distinguish difficult cases of renal oncocytoma and chromophobe RCC using different immunohistochemical markers have been made.11–14 Tickoo et al15 reported the utility of antimitochondrial antibody 113-1 reactivity in distinguishing renal oncocytoma and chromophobe RCC from granular variants of clear cell RCC and papillary RCC.15 However, in some cases of renal tumors that had overlapping morphologic features between renal oncocytoma and chromophobe RCC, the immunohistochemical staining for antimitochondrial antibody

Vimentin Reactivity in Oncocytoma—Hes et al
Figure 2. Other vimentin staining patterns in oncocytomas. The vascular networks in renal stromal tissue provide a positive internal control. Tubular patterns of vimentin positivity observed in the center of an oncocytoma (original magnification ×100 [A]) and within the hyalinized central scar of another oncocytoma (original magnification ×100 [B]). Mosaic patterns of vimentin positivity seen in areas with scattered tumor cells showing cytoplasmic staining in a background of tumor cells with negative nuclear staining (original magnifications ×400 [C] and ×100 [D]).

Table 3. Vimentin Immunoreactivity in Renal Tumors and Renal Tissue Controls Measured by Using Automatic Imaging Analysis (ChromaVision ACISII)*

<table>
<thead>
<tr>
<th>Case</th>
<th>Fields Examined, No.</th>
<th>Mean Intensity</th>
<th>Mean Percentage</th>
<th>Immunoreactivity (Intensity × Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign kidney 1</td>
<td>11</td>
<td>150.5</td>
<td>61.2</td>
<td>92.1</td>
</tr>
<tr>
<td>Benign kidney 2</td>
<td>11</td>
<td>143.7</td>
<td>54.0</td>
<td>77.6</td>
</tr>
<tr>
<td>Oncocytoma 1A</td>
<td>20</td>
<td>159.1</td>
<td>89.0</td>
<td>141.6</td>
</tr>
<tr>
<td>Oncocytoma 1B</td>
<td>27</td>
<td>31.6</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Oncocytoma 2A</td>
<td>22</td>
<td>156.7</td>
<td>83.7</td>
<td>131.6</td>
</tr>
<tr>
<td>Oncocytoma 2B</td>
<td>6</td>
<td>33.5</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Chromophobe RCC case 1</td>
<td>12</td>
<td>31.8</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Chromophobe RCC case 2</td>
<td>15</td>
<td>32.3</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Papillary RCC case 1</td>
<td>10</td>
<td>158.3</td>
<td>81.7</td>
<td>129.3</td>
</tr>
<tr>
<td>Papillary RCC case 2</td>
<td>10</td>
<td>157.1</td>
<td>98.4</td>
<td>154.6</td>
</tr>
</tbody>
</table>

* RCC indicates renal cell carcinoma. Oncocytoma 1A: case 1, vimentin-positive areas; 1B: case 1, vimentin-negative areas; 2A: case 2, vimentin-positive areas; 2B: case 2, vimentin-negative areas.

113-1 gave confusing results. Castrén et al. reported that immunohistochemical staining with cathepsin H could be used to differentiate renal oncocytomas (positive) from subtypes of RCCs (negative). However, both studies have not been substantiated by other investigators.

Many malignant tumors resemble their cell of origin, expressing only one type of intermediate filament. Some types of cancers, particularly RCC, may express more than one type of intermediate filament including vimentin as well as keratin. Vimentin, a 15-kd protein that is one of
atrophy, possibly contributing to an enlarging central scar. The expression may indicate a process of tumor cell death, with occasional vimentin-positive cells bordering a central scar. They theorized that vimentin staining might be due to the presence of small, often lipid and lipochrome-containing vacuoles in renal oncocytomas. The reactivity was restricted to intracellular vimentin. Several other articles described strong vimentin positivity in oncocytomas. Castrén et al.\(^4\) mentioned 13 of 16 cases of renal oncocytoma to be weakly positive, although they did not specify the percentage of cells showing staining. In our series, we excluded all cases of oncocytic tumors with atypical morphology. In our experience, these oncotic tumors with atypical morphology may represent oncocytic papillary RCCs, especially when stained strongly and diffusely with antibody to vimentin. McNutt et al.\(^5\) suggested that renal oncocytoma and low-grade clear cell RCC seldom show positive reaction with vimentin antibody and that the expression of vimentin becomes more intense in RCC of higher grades, especially in sarcomatoid RCC. This opinion is in agreement with a published study of sarcomatoid components arising in chromophobe RCC in which vimentin staining was focal in the carcinomatous components and diffusely in the sarcomatoid components. We obtained similar results in our series of 13 sarcomatoid components arising in chromophobe RCC (unpublished data).

Chromophobe RCC and oncocytoma show some overlapping genetic, biochemical, and morphologic features. However, chromophobe RCC is usually vimentin negative but positive for keratin and epithelial membrane antigen, which may help to distinguish chromophobe RCC from clear cell or papillary RCC (vimentin positive and epithelial membrane antigen negative).\(^6\) In our study, we confirmed the vimentin-negative nature of chromophobe RCC and validated our immunostaining methods. Although occasional vimentin positivity ranging from diffuse weak to moderate staining was seen in chromophobe RCC, the patterns were different from those oncocytomas, which showed strong but focal vimentin immunoreactivity.

Other technical issues may contribute to the variation of vimentin immunoreactivity in renal tumors. It is well known that kidney cells including renal epithelial tumors contain a high level of endogenous biotin-binding proteins.\(^7\) Therefore, nonspecific staining using a biotin-

### Table 4. Previous Studies of Vimentin Immunoreactivity in Renal Cell Carcinoma (RCC)*

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Types of Sections</th>
<th>Clear Cell RCC, No. (%)</th>
<th>Papillary RCC, No. (%)</th>
<th>Chromophobe RCC, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skinnider et al,(^{21}) 2005</td>
<td>Large sections</td>
<td>13/15 (87)</td>
<td>15/15 (100)</td>
<td>1/15 (7)</td>
</tr>
<tr>
<td>Bazille et al,(^{9,10}) 2004</td>
<td>TMA</td>
<td>40/75 (54.5)</td>
<td>76/89 (85)</td>
<td>0/50 (0)</td>
</tr>
<tr>
<td>Cameron et al,(^{22}) 2003</td>
<td>Large sections</td>
<td>7/14 (50)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Young et al,(^{21}) 2003</td>
<td>Large sections</td>
<td>19/23 (82.6)</td>
<td>6/7 (85.7)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Moch et al,(^{22}) 1999</td>
<td>TMA</td>
<td>194/383 (51)</td>
<td>35/57 (61)</td>
<td>1/23 (4)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>273/510 (53.5)</td>
<td>132/168 (80.4)</td>
<td>2/96 (2.1)</td>
</tr>
</tbody>
</table>

* TMA indicates tissue microarray; NA, not available.

### Table 5. Comparison of Reported Vimentin Immunoreactivity in Oncocytomas

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Cases, No.</th>
<th>Positive Cases, No. (%)</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cochard-Prilliet et al,(^{11}) 1997</td>
<td>103</td>
<td>0 (0)</td>
<td>Not specified</td>
</tr>
<tr>
<td>Morell-Quadreny et al,(^{14}) 1996</td>
<td>14</td>
<td>0 (0)</td>
<td>Not specified</td>
</tr>
<tr>
<td>Shimazaki et al,(^{1}) 2001</td>
<td>1</td>
<td>0 (0)</td>
<td>Not specified</td>
</tr>
<tr>
<td>Khoury et al,(^{6}) 2002</td>
<td>16</td>
<td>0 (0)</td>
<td>Not specified</td>
</tr>
<tr>
<td>Lyzak et al,(^{7}) 1994</td>
<td>7</td>
<td>1 (14.3)</td>
<td>Focal</td>
</tr>
<tr>
<td>Bonsib et al,(^{8}) 1991</td>
<td>18</td>
<td>7 (39)</td>
<td>Focal</td>
</tr>
<tr>
<td>Castrén et al,(^{14}) 2002</td>
<td>16</td>
<td>13 (81)</td>
<td>Strong and diffuse</td>
</tr>
<tr>
<td>Hes et al (current series), 2007</td>
<td>234</td>
<td>170 (72.6)</td>
<td>Strong and focal</td>
</tr>
</tbody>
</table>
based immunostaining detection system may be interpreted as positive if endogenous biotin-binding activity in renal tumors is not sufficiently blocked. Therefore, the results from biotin-based staining in renal tissues would preferably be verified by using a biotin-free method. To exclude the possibility of false-positive vimentin staining in oncocytomas, we compared 2 staining methods and found that vimentin immunoreactivity in oncocytomas is not affected by using either of the staining methods used in this study (Table 1).

Furthermore, high-throughput TMA s are widely used to evaluate protein expression in tissues. Our study demonstrates the validation of TMA s in evaluating vimentin expression, although TMA s specimens typically show lower sensitivity (43%) compared with that using large conventional sections (75%) when staining is focal. On the other hand, it is more difficult to be certain whether the positivity in cells represents a focal or diffuse process. Although computer-aided automatic imaging systems are believed to provide a more objective analysis, they are also operator-dependent. As demonstrated in Table 3, unlike chromophobe RCC or papillary RCC, which will be negative or positive for vimentin immunostaining, respectively, regardless of the areas of selection, oncocytomas could be either positive (1A and 2A in Table 1) or negative (1B and 2B in Table 1) for vimentin depending on the areas of selection. Therefore, the results obtained using these automatic imaging analysis systems, which could also be subjective, should be interpreted with caution.

Our results indicate that focal vimentin positivity does not rule out a diagnosis of renal oncocytoma, particularly when one is evaluating a small needle core biopsy of a renal mass. It is also important to note that vimentin immunoreactivity patterns in oncocytomas are different from those observed in RCC, with the exception of chromophobe RCC. Other markers or additional tissue may be necessary for a definitive diagnosis. The immunohistochemical application of vimentin may be a supportive tool in distinguishing chromophobe RCC from other RCCs with the combination of other antibodies including E-cadherin, N-cadherin, or antimitochondrial antibody 113-1.30

It is important to realize the clinical implication of focal vimentin positivity in oncocytoma. Currently, there is an increasing number of preoperative needle core biopsies of renal masses, because a therapeutic decision is often based on the pathologic classification of the renal mass. A diagnosis of oncocytoma or RCC may lead to totally different approaches and management plans to the patient. In the case of needle core biopsy, limited material containing strong vimentin-positive cells may lead to a misdiagnosis of RCC in a patient with renal oncocytoma. Several options can be considered in such an uncertain situation: (1) morphologic features as the key for distinction; (2) vimentin staining patterns for clear cell RCC (diffuse), papillary RCC (diffuse), and chromophobe RCC (negative); and (3) additional immunostaining using markers for clear cell RCC (CD10, RCC, glutathione S-transferase α), papillary RCC (cytokeratin 7, α-methylacyl CoA racemase), or chromophobe RCC (epithelial membrane antigen, c-Kit, RON, kidney-specific cadherin).31–33 Distinction of oncocytoma from chromophobe RCC can still be a challenge because most markers are not highly specific.

In summary, we analyzed vimentin immunoreactivity in 234 renal oncocytomas and found that 72.6% of these tumors showed focal vimentin positivity. The vimentin reactivity patterns observed in oncocytomas were strong and focal, very different from those of chromophobe (negative or weakly diffuse), clear cell (diffuse and strong), or papillary (diffuse and strong) RCCs. Therefore, when focal vimentin immunoreactivity is encountered in a renal cell neoplasm, oncocytoma should be considered in the differential diagnosis, particularly in the case of a limited specimen.

We are grateful to William Laskin, MD, for his critical review of the manuscript.

References


**Polymorphisms in TGFβ and TNFα Are Associated With the Myelodysplastic Syndrome Phenotype**

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**Context.**—Myelodysplastic syndromes (MDSs) are characterized by ineffective hematopoiesis, excessive apoptosis, and the aberrant expression of a number of cytokines. The genes encoding these cytokines are significantly polymorphic. It is unknown whether these cytokine polymorphisms are associated with, and may therefore be playing a role in the pathogenesis of, MDS.

**Objective.**—To determine if certain polymorphisms in the tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β) cytokines are overrepresented in a cohort of patients with MDS.

**Design.**—DNA was isolated from the peripheral blood or bone marrow aspirate of 21 patients with MDS. The genotypes for 4 different polymorphisms, 2 in TNFα and 2 in TGFβ1, were determined using single-specific-primer polymerase chain reaction. The allele and genotype frequencies were compared with similar populations in the National Cancer Institute SNP500 database.

**Results.**—In our MDS population, the −308 A/A genotype of the TNFα gene and the TGFβ1 allele +29T and genotype +29T/T, each associated with higher levels of expression, were overrepresented in our MDS population.

**Conclusions.**—Polymorphisms associated with increased expression in the cytokines TNFα and TGFβ1 are overrepresented in the MDS population suggesting that increased TNF-α and TGF-β activity may contribute to the susceptibility and/or pathogenesis of MDS. Further studies with larger sample sizes are warranted to confirm our observation.

(Arch Pathol Lab Med. 2007;131:1789–1793)

The myelodysplastic syndromes (MDSs) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis, multi-lineage dysplasia, peripheral cytopenias, and susceptibility to leukemia.1 Demonstrating a varied spectrum of biologic, genetic, morphologic, and clinical characteristics, MDS has a natural history ranging from enduring years of indolent disease to an accelerated progression toward acute leukemia. The diagnosis of MDS, particularly the low-grade varieties such as refractory anemia, is difficult in the absence of supportive findings (ie, clonal cytogenetic abnormalities), and early stages may be underdiagnosed. The only known curative treatment available is bone marrow transplantation. Only with a full characterization of the pathobiology of MDS will better and targeted therapies be developed and earlier diagnosis possible.

The etiology and pathophysiology of myelodysplasia is poorly understood. Mechanisms of disease include pluripotent stem cell damage and abnormalities in proliferation, differentiation, maturation, and apoptosis leading to an ineffective hematopoiesis.1 Like most malignancies,2 MDS is presumed to develop from multiple genetic aberrations generating the developmental phenotypes seen in the hematopoietic lineage. Except for a polymorphism in the granulocyte colony-stimulating factor receptor that is associated with high-risk MDS,3 the genetic risk factors that predispose to the development of MDS are unknown.

Deregulation of immunomodulatory cytokines in the bone marrow stroma and hematopoietic cells has been suggested to play an important role in the pathogenesis and phenotypes (ie, cytopenia) of MDS. Tumor necrosis factor α (TNF-α), one of the best characterized immunomodulatory cytokines, has been shown to strongly inhibit hematopoiesis.4-6 Several studies report up-regulation of TNF-α in the hematopoietic cells and stroma of MDS patients.7-12 Other cytokines and cytokine-related signaling molecules reportedly involved in the processes leading to ineffective hematopoiesis in MDS include transforming growth factor β (TGF-β), interferon-γ, interleukin 1β (IL-1β), and the TNF-related signaling molecules TRADD/FADD (TNF receptor 1–associated protein via death domain/Fas associated via death domain), RIP (receptor interacting protein), and TRAIL (TNF-related apoptosis–inducing ligand).12-18 Allampallam et al12 showed that TGF-β levels are increased in MDS marrows, correlate with the level of TNF-α, and are associated with an increased number of blasts. These data strongly suggest that cytokines...
are critical to the pathogenesis and progression of MDS, but previous studies have only examined the cytokines at one time in the course of the disease and have not provided evidence if the cytokine changes are the causes or consequences of MDS.

The cytokine gene families are highly polymorphic within their coding sequences and in sequences regulating their expression including a significant number of single nucleotide polymorphisms (SNPs), some of which are known to modify cytokine activity. For example, polymorphisms have been identified in the upstream regulatory regions of TNFα and within the leader peptide sequence of TGFβ. Under certain experimental situations, the −308A and −238A polymorphisms in TNFα and the polymorphisms that encode a leucine at amino acid 10 and an arginine at amino acid 25 in TGFβ are associated with increased expression. If increases or decreases in cytokine activity promote the development of MDS, it is likely that polymorphisms in such cytokine genes that modify their expression and/or activity will be associated with an increased risk of disease and therefore overrepresented in an MDS population. Previous studies have associated a polymorphism in TGFβ with more severe anemia in patients with refractory anemia, but this study and one other have not identified an association between polymorphisms in TNFα and MDS using a limited number of samples.

Using single-specific-primer polymerase chain reaction, the current study counted the frequencies for 2 SNPs in TGFβ and 2 SNPs in TNFα known to be associated with variations in cytokine activity in a cohort of patients with a primary MDS phenotype. We compared the frequencies observed in our cohort with the expected frequencies available in a public SNP database. We found that 2 polymorphisms, each associated with increased expression of TNFα or TGFβ, respectively, were overrepresented in our MDS population suggesting that increased TNFα and TGFβ activity may contribute to either the susceptibility and/or pathogenesis of MDS.

**MATERIALS AND METHODS**

Archived peripheral blood or bone marrow aspirate samples were obtained from 20 patients with MDS according to institutional review board approval provided by The Methodist Research Institute. One additional sample was provided by Cherie Dunphy, MD, University of North Carolina, Chapel Hill. A summary of all patients is provided in Table 1. Myelodysplastic syndrome patients were defined by the World Health Organization classification scheme. The myeloid stem cells were excluded from the DNA analysis with magnetic beads coupled to anti-CD34 antibodies, and the lymphocytes were enriched with anti-CD3/anti-CD19 coated magnetic beads (MACS Technology, Miltenyi Biotech, Bergisch Gladbach, Germany). Cell counting using Wright-Giemsa staining confirmed greater than 90% enrichment for lymphocytes in the CD34-negative, CD3- or CD19-positive cell population. DNA was isolated from the lymphocytes with the TRIZOL reagent (Invitrogen, Carlsbad, Calif), chloroform extraction, and ethanol precipitation according to the manufacturer’s protocol. DNA was genotyped for 22 different SNPs in 13 different cytokine genes using single-specific-primer polymerase chain reaction with the PEL-FREEZ cytokine genotyping kit (Invitrogen).

Allele frequencies were compared with the allele frequencies available in the National Cancer Institute SNP500 database of the Cancer Genome Anatomy Project (http://snp500cancer.nci.nih.gov/snp.cfm) as of November 1, 2006. This database has the genotype frequencies for more than 375 random subjects of mixed ethnicity. We specifically focused on 4 polymorphisms: (1) dbSNP ID rs1982073 TNFα Ex1-327C>T (nucleotide +29) P10L, (2) dbSNP ID rs1800471 TNFα Ex1-282G>C (nucleotide +75) R25P, (3) dbSNP ID rs1800629 TNFα −487A>G (aka −308), and (4) dbSNP ID rs361525 TNFα −417A>G (aka −238). Fisher exact test was used to look for significant differences in the allele and genotype frequencies with the VassarStats Web site (http://faculty.vassar.edu/lowry/VassarStats.html) accessed repeatedly between September 2006 and April 2007.

**RESULTS**

The genotyping results for TNFα and TGFβ are summarized in Table 2. Briefly, the A/A genotype at TNFα −308 was overrepresented 14-fold in our MDS population (P = .02), but neither of the other TNFα genotypes (A/G or G/G) were underrepresented or overrepresented. For TGFβ, the T/T genotype (2.0-fold) and the T allele (1.4-fold) at TGFβ +29 were each overrepresented (P = .01 for each comparison). The C/C and C/G genotypes are each underrepresented, but neither of these comparisons was statistically significant. The A allele at TNFα −308 has previously been associated with increased TNFα expression, and the T allele at nucleotide +29 (leucine at amino acid 10) of TGFβ has been previously associated with increased TGFβ expression. These results suggest that increased TGFβ and TNFα expression are associated with the MDS phenotype. Conversely, no alleles at −238 in TNFα, of which the A allele is associated with increased expression of TNFα, and no alleles at nucleotide +75 (amino acid 25) in TGFβ, of which the G allele (encoding an arginine at amino acid 25) is associated with increased expression, showed any statistical association with our MDS population. We also compared the 18 other polymorphisms typed by the PEL-FREEZ cytokine genotyping kit with the available information in the SNP500 database. Three of these polymorphisms did not have comparable information in the SNP500 database (IL1R, p51 1970 C/T, ILIRA mspAI11 11000 C/T, Gamma-IFN UTR 5644) and were not analyzed further. Of the remaining polymorphisms, most did not show a significant difference when compared with the SNP500 database. A borderline statistical association was seen with the heterozygous genotype

| Table 1. Clinical Characteristics of Myelodysplastic Syndrome (MDS) Patients |
|---------------------------------|---|
| **Diagnosis** | **No. (n = 21)** |
| MDS-NOS | 4 |
| RA | 2 |
| RARS | 2 |
| RAEB | 2 |
| RCMD | 8 |
| CMML | 1 |
| AML transformed from MDS | 2 |
| Ethnicity | |
| White | 10 |
| Black | 2 |
| Hispanic | 1 |
| Other/unknown | 8 |
| Transfusion dependent | 8/9 |

* MDS-NOS indicates myelodysplastic syndrome not otherwise specified; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; CMML, chronic myelomonocytic leukemia; and AML, acute myeloblastic leukemia.
at position −590 in IL-4 and with the C allele at position −511 in IL-1β. No definitive conclusions can be drawn from these data given both the relatively small sample size and the lack of clinical information in the SNP500 database.

**COMMENT**

Myelodysplastic syndrome is characterized by multilineage cytopenias and abnormal hematopoiesis.1 The cytokines TNF-α and TGF-β have been shown to inhibit various stages of hematopoiesis,4,5 and previous studies have associated differences in TNF-α, TGF-β, and other cytokines’ expression in the pathology of MDS.7–13,15,16 Our analyses demonstrate a strong association between 2 polymorphisms known to be associated with increased expression of TNFα and TGFβ and the MDS phenotype. Therefore, it is likely that these 2 polymorphisms directly contribute to the pathogenesis of MDS either by increasing the likelihood that a patient will develop the genetic changes that cause MDS or by increasing the likelihood of expressing the MDS phenotype (dyspoiesis and/or cytopenia) once MDS initiating genetic changes have occurred. The latter may be favored given the known myelosuppressive function of these 2 cytokines. Because these SNPs have only 2 alleles, it is possible that the overrepresentation of 1 allele with 1 phenotype may actually reflect more the association of its converse allele with the opposite phenotype. However, this is unlikely as the association of the homozygous genotypes with MDS was the statistically strongest association, as opposed to the heterozygous or converse homozygous genotype being underrepresented in MDS. Nevertheless, a protective role for the converse allele cannot be completely ruled out.

The same allele [Ex1-327C>T (nucleotide +29) P10L] in TGFβ has been previously associated with MDS in a population of refractory anemia patients with severe anemia,6 suggesting that TGFβ may contribute to a more severe MDS phenotype. The same allele [−487A>G (aka −308)] in TNFα has not been shown to have an association with MDS in 2 different studies.26,27 However, one of these studies only examined patients with refractory anemia, and the other study had a poorly matched control population including a number of younger patients that may or may not develop MDS in the future. Our cohort contained predominantly higher grades of MDS including a large proportion of patients with refractory cytopenia with multilineage dysplasia suggesting that these polymorphisms may only be overrepresented in certain subtypes and/or severities of MDS. Therefore, it is still likely that the TNFα and TGFβ polymorphisms contribute to MDS, although they may not contribute in all ethnicities, genetic backgrounds, or MDS subtypes. Of note, 2 patients in our cohort with homozygous A/A in TNFα position −308 were classified as MDS not otherwise specified and refractory cytopenia with multilineage dysplasia, respectively (Table 3). However, we did not have enough cases of any one subtype to test the association between the polymorphisms and MDS subtype. Furthermore, we intentionally depleted the myeloid cells that may have accumulated genetic changes such as deletions, point mutations, and loss of heterozygosity. This may have contributed partially to the differences observed between our and the previous studies.

In our current study, we only had transfusion information available for 9 of the 21 patients (of whom 8 were transfusion dependent, Table 1), and we only had cyto-

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**Table 2. Summary of Results**

<table>
<thead>
<tr>
<th>Polymorphism: TGFβ Nucleotide +29 (Amino Acid 10), No. (%)</th>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/C</td>
<td>C/T</td>
</tr>
<tr>
<td>MDS* (n = 20)</td>
<td>2 (10)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Control (n = 310)</td>
<td>67 (22)</td>
<td>159 (51)</td>
</tr>
<tr>
<td>P = .01</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Polymorphism: TGFβ Nucleotide +75 (Amino Acid 25), No. (%)</th>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/C</td>
<td>C/G</td>
</tr>
<tr>
<td>MDS (n = 17)</td>
<td>1 (6)</td>
<td>3 (23)</td>
</tr>
<tr>
<td>Control (n = 102)</td>
<td>0</td>
<td>12 (12)</td>
</tr>
<tr>
<td>P = .10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polymorphism: TNFα –308, No. (%)</th>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/A</td>
<td>A/G</td>
</tr>
<tr>
<td>MDS (n = 18)</td>
<td>2 (11)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Control (n = 376)</td>
<td>3 (1)</td>
<td>74 (20)</td>
</tr>
<tr>
<td>P = .02</td>
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</table>

<table>
<thead>
<tr>
<th>Polymorphism: TNFα –238, No. (%)</th>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/A</td>
<td>A/G</td>
</tr>
<tr>
<td>MDS (n = 18)</td>
<td>0</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Control (n = 102)</td>
<td>0</td>
<td>19 (19)</td>
</tr>
<tr>
<td>P = .35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* MDS indicates myelodysplastic syndrome.
alleles in the diagnosis of TNF-α. We have strengthened the evidence that MDS may be associated with inherited polymorphisms that modify the TNF-α cytokine activity. However, we cannot rule out if certain polymorphisms are associated with any particular cytokigenic abnormality.

Identifying an association between a genetic marker and a disease does not guarantee causation. Because SNPs are inherited in blocks of linkage disequilibrium, it is also possible that an identified genetic marker is only linked with a nearby genetic change that actually causes the phenotype. This is especially true for TNF-α as it is a class III gene of the major histocompatibility complex, and certain alleles in TNF-α may be associated with specific HLA class I or class II alleles. Therefore, further studies are needed to better define if an allele directly explains the linkage seen. However, given the known direct roles of TNF-α and TGF-β in inhibiting hematopoiesis, and the known increases in TNF-α and TGF-β in MDS, we think it likely that polymorphisms such as −308A and 10L, which are associated with a constitutive increase in TNF-α and TGF-β, respectively, would predispose those who inherit them to myelodysplasia.

Previous studies only measured the level of cytokines in the bone marrow or plasma of patients with already-diagnosed MDS. This static, postdiagnosis view suggests that such cytokines are involved in the pathogenesis but do not define if they have a primary causative role or are secondary changes associated with the disease. By associating MDS with inherited polymorphisms that modify cytokine activity, we have strengthened the evidence that TGF-β and TNF-α are primarily involved in the pathogenesis of the MDS phenotype.

Numerous pharmaceuticals useful in treating MDS modify cytokine activity. Additionally, direct inhibitors of TNF-α have been used in MDS patients in an effort to improve hematopoiesis. TNF-α inhibitors improve the cytokenia associated with chronic inflammatory states (anemia of chronic disease). Early clinical trials with a soluble TNF receptor fusion protein or an anti–TNF-α monoclonal antibody have shown modest improvements in hematologic indices. It would be of great interest to know if the response to such treatment correlates with inherited variations in TNF-α or other cytokine genes. In the future, genetic analyses such as this study along with other novel biomarkers may better predict who responds to each of these drugs and new pharmaceuticals as they are developed.

In conclusion, polymorphisms associated with the overexpression of TNF-α and TGF-β are overrepresented in patients with MDS strongly suggesting that high levels of expression of TNF-α and TGF-β are direct causes of MDS or its cytopenic phenotype. In future studies, we plan to further characterize the associations with these alleles and neighboring alleles in a larger cohort and to correlate the genotypes we see with expression of cytokines, with different MDS phenotypes, and with other biomarkers for MDS. We anticipate using this information to develop new and better biomarkers that diagnose MDS, predict its progression, and guide future therapeutic developments.

This study was supported with funds provided by The Methodist Hospital Research Institute.

### Table 3. Genotyping Results by Myelodysplastic Syndrome (MDS) Subtype

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>TGF-β Nucleotide +29 (Amino Acid 10), No. (n = 20)</th>
<th>TNF-α −308, No. (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/C</td>
<td>C/T</td>
</tr>
<tr>
<td>MDS-NOS</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>RA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RARS</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>RAEB</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RCMD</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CMML</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AML transformed from MDS</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* MDS-NOS indicates myelodysplastic syndrome not otherwise specified; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; CMML, chronic myelomonocytic leukemia; and AML, acute myeloblastic leukemia.

### References


Patterns of Add-on Tests for Hospitalized and for Private Patient Populations

Nadia N. Naumova, MD; Joseph Schappert, MD; Lawrence A. Kaplan, PhD

- **Context.**—The policy of storing clinical samples in a pathology laboratory is based on long-standing practice rather than on objective data regarding the actual use of the stored samples.

- **Objective.**—To determine the time after initial order that requests for add-on tests are submitted to the laboratory. These data might be useful for improving the efficiency of sample storage.

- **Design.**—Two hundred sixteen add-on requests evenly divided between inpatients and private practice patients were reviewed for types of tests added on and the time of the requests.

- **Results.**—Ninety-five percent of add-on test requests for inpatients were made by 0.75 day after the initial order (range, 0.01–4.3 days). However, the 95th percentile for private practice patients’ add-on requests did not occur until 5.6 days later (range, 0.01–7.0 days). The pattern of

    initial receipt of a sample at which an add-on test request was made and the time point by which 95% of the add-on requests were made. Because the availability of a sample for an add-on request has different clinical and economic implications for inpatients and private practice patients, we separated the data by source of origin.

- **STUDY DESIGN**

Beth Israel Medical Center serves as a core laboratory/receiving facility for 5 New York City hospitals (Continuum Health Partners) and for private physician practices in and around New York City (Outreach). The hospitals include intensive care units, emergency departments, and clinics; test orders from these areas are designated as inpatients. The laboratory runs approximately 5,805,000 billable tests per year; approximately 1,600,000 of these are for Outreach patients. We reviewed 595 consecutive add-on requests that were listed in a computer-generated “add-on pending list” for samples received at Beth Israel Hospital and the other Continuum Hospitals for the first 2 weeks of August 2005. This list, which included requests for add-on tests that were received by telephone and requisitions from inpatient and Outreach locations, contained the time the add-on request was ordered and the tests ordered. We excluded tests on the pending list within 20 minutes of the initial order; these were manual accessioning errors resulting from incomplete or erroneous initial order-entry and had no further add-on requests. Laboratory protocol requires that add-on requests should be rejected when the patient cannot be accurately identified, when the appropriate sample tube is not available for the

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

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Table 1. Characteristics of Ordered Tests and Add-on Requests*

<table>
<thead>
<tr>
<th>Ordered Tests</th>
<th>Frequency, No. (%)</th>
<th>Add-on Tests</th>
<th>Frequency, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inpatient Population</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF (protein, glucose)</td>
<td>3 (1.3)</td>
<td>CMP</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Culture, urine</td>
<td>3 (1.3)</td>
<td>Acetaminophen</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>Thyroid (panels, individual tests)</td>
<td>3 (1.3)</td>
<td>Amylase</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>Urine analysis</td>
<td>5 (2.1)</td>
<td>Digoxin</td>
<td>6 (4.1)</td>
</tr>
<tr>
<td>Liver profile</td>
<td>8 (3.3)</td>
<td>Phosphorus</td>
<td>7 (4.8)</td>
</tr>
<tr>
<td>Urine toxicology (DAU)</td>
<td>8 (3.3)</td>
<td>BMP</td>
<td>9 (6.2)</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>14 (5.8)</td>
<td>CBC</td>
<td>9 (6.2)</td>
</tr>
<tr>
<td>CMP</td>
<td>15 (6.3)</td>
<td>Liver profile</td>
<td>9 (6.2)</td>
</tr>
<tr>
<td>Cardiac (panels, individual tests)</td>
<td>17 (7.0)</td>
<td>Mg</td>
<td>12 (8.3)</td>
</tr>
<tr>
<td>Mg</td>
<td>19 (7.9)</td>
<td>PT/PTT</td>
<td>13 (9.0)</td>
</tr>
<tr>
<td>PT/PTT</td>
<td>19 (7.9)</td>
<td>Thyroid (panels, individual tests)</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>Others f &lt; 2</td>
<td>21 (8.8)</td>
<td>Urine analysis</td>
<td>9 (6.2)</td>
</tr>
<tr>
<td>BMP</td>
<td>48 (20.0)</td>
<td>Urine toxicology (DAU)</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>CBC</td>
<td>57 (23.8)</td>
<td>TDM</td>
<td>8 (5.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hCG</td>
<td>5 (3.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipase</td>
<td>4 (2.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osmolarity</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others f &lt; 2</td>
<td>16 (11.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiac (panels, individual tests)</td>
<td>21 (14.5)</td>
</tr>
<tr>
<td></td>
<td><strong>Total Tests</strong></td>
<td><strong>240 (100)</strong></td>
<td><strong>Total Tests</strong></td>
</tr>
<tr>
<td></td>
<td><strong>(Individual or Panels)</strong></td>
<td></td>
<td><strong>(Individual or Panels)</strong></td>
</tr>
<tr>
<td><strong>Outreach Population</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPYL</td>
<td>3 (1.0)</td>
<td>ANA</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>PT/PTT</td>
<td>4 (1.3)</td>
<td>Creat/BUN</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>ESR</td>
<td>4 (1.3)</td>
<td>EBV</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>Hemoglobin A1c</td>
<td>4 (1.3)</td>
<td>HIV</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>SPEP</td>
<td>4 (1.3)</td>
<td>PRL</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>Urine toxicology (DAU)</td>
<td>5 (1.6)</td>
<td>Lipid profile</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>ANA</td>
<td>6 (1.9)</td>
<td>CMP</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>RPR, Gc/Chlam,Trep</td>
<td>10 (3.2)</td>
<td>SPEP</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>ESRUrinary protein electrophoresis</td>
<td>12 (3.9)</td>
<td>HIV</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>Liver profile</td>
<td>14 (4.5)</td>
<td>Hemoglobin A1c</td>
<td>4 (3.0)</td>
</tr>
<tr>
<td>TORCH serologies</td>
<td>15 (4.8)</td>
<td>HPYL</td>
<td>4 (3.0)</td>
</tr>
<tr>
<td>Urine analysis</td>
<td>15 (4.8)</td>
<td>Liver profile</td>
<td>4 (3.0)</td>
</tr>
<tr>
<td>Basic metabolic profile</td>
<td>18 (5.8)</td>
<td>PT/PTT</td>
<td>5 (3.8)</td>
</tr>
<tr>
<td>Hepatitis A,B,C serology</td>
<td>20 (6.4)</td>
<td>TORCH serologies</td>
<td>5 (3.8)</td>
</tr>
<tr>
<td>Lipid profile</td>
<td>28 (9.0)</td>
<td>CBC</td>
<td>6 (4.5)</td>
</tr>
<tr>
<td>Comprehensive metabolic profile</td>
<td>33 (10.6)</td>
<td>FERR</td>
<td>7 (5.3)</td>
</tr>
<tr>
<td>Thyroid (panels, individual tests)</td>
<td>34 (10.9)</td>
<td>Urine analysis</td>
<td>7 (5.3)</td>
</tr>
<tr>
<td>ESR</td>
<td>40 (12.9)</td>
<td>Hepatitis A,B,C serology</td>
<td>12 (9.0)</td>
</tr>
<tr>
<td>Others f &lt; 2</td>
<td>42 (13.3)</td>
<td>Thyroid (panels, individual tests)</td>
<td>13 (9.8)</td>
</tr>
<tr>
<td></td>
<td><strong>Total Tests</strong></td>
<td>Reference laboratory</td>
<td>14 (10.6)</td>
</tr>
<tr>
<td></td>
<td><strong>311 (100)</strong></td>
<td>Others f &lt; 2</td>
<td>10 (22.7)</td>
</tr>
<tr>
<td></td>
<td><strong>Total Tests</strong></td>
<td><strong>132 (100)</strong></td>
<td><strong>(Individual or Panels)</strong></td>
</tr>
</tbody>
</table>

* CSF indicates cerebral spinal fluid; CMP, comprehensive metabolic profile; DAU, drugs of abuse urine; BMP, basic metabolic profile; CBC, complete blood count; Mg, magnesium; PT/PTT, prothrombin time/partial thromboplastin time; TDM, therapeutic drug monitoring; hCG, human chorionic gonadotropin; Cardiac, creatine kinase-MB isoenzyme, and creatine kinase and troponin; HPYL, Helicobacter pylori; ANA, antinuclear antibody; Creat/BUN, creatine/blood urea nitrogen; ESR, erythrocyte sedimentation rate; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; SPEP, serum protein electrophoresis; PRL, prolactin; RPR, rapid plasma reagin; Gc/Chlam,Trep, Gonococcus/Chlamydia, Treponema pallidum; UTPR, urine total protein; TORCH, Toxoplasma, rubella, cytomegalovirus, herpes simplex virus; and FERR, ferritin.

The add-on requests were sorted by code for site of origin; all orders from private physicians were designated to the Outreach category and all other patients were included in the Inpatients category. We then took the first 108 add-on requests originating from inpatient locations and the first 108 add-on requests from Outreach locations for further investigation. Prior to the add-on requests, the original requests contained 240 and 311 orders, respectively (Table 1). The Inpatients category included 6.5% patients from clinics, 44.4% from inpatient beds, and 49.1% from emergency departments.

Serum samples at Beth Israel Medical Center are routinely refrigerated for 3 days in a Beckman automated laboratory system (Beckman-Coulter, Inc, Fullerton, Calif). When the add-on requests for these samples are accessed, the laboratory information system will automatically retrieve the samples and convey them either to an analytical workstation or to a site for manual processing. After 3 days, serum samples are removed from the automated storage site and placed into standard laboratory refrigerators, where all other manually processed samples (such as urine, plasma, and cerebral spinal fluid) are stored. Samples at the other Continuum locations are stored in laboratory refrigerators. Retrieval of all samples stored in the laboratory refrigerators must be performed manually and hand-carried to a testing or processing site. The time of initial ordering, the time of the add-on request order, and the type of tests being ordered were re-
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—Naumova et al

Figure 1. Pattern of initial test orders for inpatients. CSF, cerebral spinal fluid; Thyroid, thyroid-stimulating hormone, free and total thyroxine-T4, free and total triiodothyronine-T3; DAU, drugs of abuse urine; CMP, comprehensive metabolic profile; Cardiac, creatine kinase-MB isoenzyme, and creatine kinase and troponin; Mg, magnesium; PT/PTT, prothrombin time/partial thromboplastin time; BMP, basic metabolic profile; and CBC, complete blood count.

Figure 2. Pattern of initial test orders for Outreach patients. HPYL, Helicobacter pylori; PT/PTT, prothrombin time/partial thromboplastin time; ESR, erythrocyte sedimentation rate; SPEP, serum protein electrophoresis; DAU, drugs of abuse urine; ANA, antinuclear antibody; RPR, Ge/Chlam, Trep, rapid plasma reagin, Gonococcus/Chlamydia, Treponema pallidum; TORCH, Toxoplasma, rubella, cytomegalovirus, herpes simplex virus; BMP, basic metabolic profile; CMP, comprehensive metabolic profile; Thyroid, thyroid-stimulating hormone, free and total thyroxine-T4, free and total triiodothyronine-T3; and CBC, complete blood count.

corded. For these samples, the time required to complete the add-on tests was assessed. Furthermore, we investigated the reason for the ordering of additional tests by speaking directly to a small number (19) of requesting physicians in hospital settings during a single week in September 2005. We were unsuccessful in contacting Outreach physicians for this purpose.

The Beth Israel Medical Center Institutional Review Board does not require approval for this type of study. Statistical differences were determined by t test as performed by the Microsoft Excel 2003 program (Microsoft Corporation, Redmond, Wash).

RESULTS

During this study period, there were approximately 48,400 tests requested from Outreach clients and 76,430 tests ordered for hospitalized patients; approximately 39% of the ordered tests were from Outreach clients. For the 108 add-on requests for inpatients, there were 145 additional tests requested, either as individual tests or panels of tests. For the 108 samples originating from Outreach clients, there were 132 additional tests requested, either as individual tests or as panels. The test patterns for the inpatient and Outreach samples are listed in Table 1 and Figures 1 through 5.

As one might expect, the patterns of both the original tests and add-on tests also differed between Outreach and inpatient areas (Table 1), with Outreach physicians ordering more general care tests (ie, thyroid and lipid panels, serology) and inpatient physicians ordering more tests needed for acute care (ie, cardiac, magnesium, coagulation), as seen in Figure 5.

The mean time between the initial order and the add-on requests was 0.35 days for inpatients (range, 0.01–4.3 days; 95th percentile, 0.75 days) and 1.3 days (range, 0.01–7 days; 95th percentile, 5.6 days) for Outreach clients; the mean times were statistically different (P < .001).

The distributions of the time for add-on requests are shown in Figures 6 and 7. The mean add-on time for the clinic orders was not statistically different from the other orders in the Inpatients category (P = .21), but the mean times for add-on requests originating from emergency departments were significantly different from the overall mean time for inpatients (emergency mean time of 0.12 days vs overall mean time of 0.35 days; P < .001).

The times needed to produce results for the add-on tests were also statistically different (P < .001), with the inpatient add-on test results available a mean of approximately 0.16 days after the request (95th percentile by ~0.9 days) and the Outreach add-on test results available a mean of approximately 1.3 days after the request (95th percentile by 5.9 days).

In order to determine why tests were being added on, we spoke directly with 19 physicians who requested inpatient add-on tests. All of these physicians were resident...
Figure 3. Pattern of add-on requests for inpatients. CMP, comprehensive metabolic profile; Thyroid, thyroid-stimulating hormone, free and total thyroxine-\(T_4\), free and total triiodothyronine-\(T_3\); DAU, drugs of abuse urine; hCG, human chorionic gonadotropin; TDM, therapeutic drug monitoring; CBC, complete blood count; Mg, magnesium; PT/PTT, prothrombin time/partial thromboplastin time; and Cardiac, creatine kinase-MB isoenzyme and creatine kinase and troponin.

Figure 4. Pattern of add-on requests for Outreach patients. ANA, antinuclear antibody; Creat/BUN, creatinine/blood urea nitrogen; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; PRL, prolactin; CMP, comprehensive metabolic profile; SPEP, serum protein electrophoresis; UTPR, urine total protein; HPYL, Helicobacter pylori; T/F, prothrombin time/partial thromboplastin time; TORCH, Toxoplasma, rubella, cytomegalovirus, herpes simplex virus; CBC, complete blood count; FERR, ferritin; and Thyroid, thyroid-stimulating hormone, free and total thyroxine-\(T_4\), and free and total triiodothyronine-\(T_3\).

Figure 5. Comparison of initial orders. ◆, inpatients; ■, Outreach; and TDM, therapeutic drug monitoring.
physicians. The reasons for the add-on requests are listed in Table 2.

**COMMENT**

The question, “How long should a sample be stored after analysis?” is not simply an academic exercise. Aside from questions of sample stability, there are economic and medical factors that must be considered. Storage refrigerators, particularly automated refrigeration systems, are expensive and consume valuable laboratory space. In Beth Israel Medical Center, approximately 3.7 full-time employees per week are required to maintain the sample storage system. Because of the economic impact of storing samples, we investigated the pattern of add-on requests that originated from both hospitalized and Outreach patients.

We found that the patterns for add-on requests were statistically different, with mean times for ordering the add-on tests of 0.35 days versus 1.3 days for inpatient and Outreach physicians, respectively, with 95% of the add-on requests received by 0.75 and 5.6 days, respectively (Figures 6 and 7). These differences are most likely the result of different clinical requirements for laboratory results; that is, the acute care hospitalized setting versus ambulatory care provided in office-based practices. The need to discharge a hospitalized patient as rapidly as possible certainly requires close attention to laboratory results.

The difference in the time required to produce the add-on test result is also based on the differences in physician practice. The Outreach physicians ordered a larger number of tests that are not performed as frequently as the tests needed for acute care within the hospital (Table 1), and these tests take a longer time to obtain results. For example, approximately 10.6% and 12.8% of add-on tests

### Table 2. Reasons for Add-on Test Requests for Inpatients

<table>
<thead>
<tr>
<th>Explanation</th>
<th>No. of Requests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forgot to order</td>
<td>7</td>
</tr>
<tr>
<td>Not sure</td>
<td>4</td>
</tr>
<tr>
<td>Order error</td>
<td>1</td>
</tr>
<tr>
<td>Response to patient symptoms</td>
<td>3</td>
</tr>
<tr>
<td>Response to laboratory tests</td>
<td>4</td>
</tr>
</tbody>
</table>
originating from Outreach physicians were for reference laboratory work and TORCH (Toxoplasma, rubella, cytomegalovirus, herpes simplex virus)/hepatitis serologies, respectively, and hospital-based physicians ordered none of these tests. On the other hand, 14.5% and 8.3% of add-on requests from hospital doctors were examinations for acute myocardial injury and magnesium, respectively, and Outreach physicians rarely requested these tests (eg, creatine kinase-MB, <1% of total).

Another reason for the longer delay in result times for Outreach add-on tests was the impact of the automated storage-retrieval unit in Beth Israel Hospital, which has the capacity to store samples for 3 days. After 3 days, samples are transferred to refrigerators. Because 95% of inpatient add-on requests were made within 1 day of the initial request, 95% of the samples could be retrieved rapidly from the automated storage unit. For Outreach requests made 3 or more days after the initial request for analysis (14%), samples had to be retrieved manually.

In a previous study of add-on requests, 60.6% and 89.4% of the requests for inpatients were made within less than 4 and 8 hours, respectively, after the initial order; 100% of the add-on requests were completed by 18.6 hours. The pattern observed for the inpatient add-on tests in our study was similar, with 60.6% and 89.4% of the add-on tests requested less than 6 and 12 hours, respectively, after the initial order, and 95% of the add-on orders were made by 18 hours (Figure 6). The similarity of results most likely reflects the similar patient populations, that is, hospitalized patients. The similarities are striking because the report by Melanson et al considers only “chemistry” tests and our study includes all aspects of laboratory medicine, including test requests that are referred to a reference laboratory.

However, the time within which add-on requests were made for the Outreach patients was noticeably longer than that for the inpatient orders in the current and the previous study. In our study, for the Outreach service, the mean time for add-on requests was 1.3 days, and 95% of the add-on requests were made by 5.6 days (Figure 7). Despite the fact that the previous study included a separate outpatient category, it did not identify a private practice–based outreach clientele, so no direct comparison can be made. The difference in time may be attributable to the electronic reporting of patient results to the patient care areas in the hospital. Outreach clients are serviced by an Internet-based result reporting system, but the primary result-reporting mechanism used by most Outreach physician clients is the paper-based laboratory report.

The small survey of inpatient add-on requests from Beth Israel Hospital suggests that only a small number (7/19, 37%) of requests are based on actual acute patient needs (Table 2). The remainder of the requests was either clerical in nature (“forgot to order”) or the resident was not sure why the order was being made. The approach by the Harvard group to approve add-on requests would be useful for screening inappropriate requests. In our study, 26% (5/19) of the add-on requests were canceled after discussion with the resident. The Melanson et al study also suggests that increased laboratory-physician communication could lead to a decrease in the number of unnecessary add-on requests. Because our study indicates that add-on tests from the Outreach community represent a different dataset than that observed in the Melanson et al study, this approach may not be effective when dealing with an Outreach practice.

Once each laboratory has determined how long samples can be usefully stored, the answer to the question, “How long should a sample be stored after analysis?” depends on the population the laboratory is serving. For hospitalized populations, the results from the aforementioned study and this study indicate that sample storage for 2 to 3 days after initial sample receipt would be acceptable in terms of both clinical and regulatory considerations. This recommendation is based on the data presented here and on the empirical observation that, for hospitalized patients, with the exception of timed samples and/or sterile body fluids, adding the order onto a specimen that will be collected subsequently is usually not a clinical issue. For Outreach practices, because the patient is not easily accessible and does not have other routine blood collections scheduled, collecting a new sample can be a burden on the practice and on the patient. For an Outreach population, we recommend maintaining the current, common practice of a 7-day storage period. Given the ability of automated archiving instrumentation to interact with a laboratory information system, separating samples by type for shorter-term storage (inpatients) and longer-term storage (Outreach) is probably the most efficient solution to the sample storage problem. Decisions regarding analytic stability must still be made for individual add-on requests.

It has been suggested that institutions might need to modify their sample retention policy to account for the medical needs of transplant programs or for the legal medical needs of a medical examiner’s office. In our experience, the protocols for transplant programs are closely followed, obviating the need for add-on requests. Although medical examiners do request stored samples, such requests do not have an urgency that would require modification of a sample retention policy based on patient medical needs.

This study was limited to a representative portion of the calendar year, and future investigations might look to see if the patterns of add-on requests fluctuate with time. In addition, it might be interesting to determine if the pattern of add-on requests is similar for institutions that are not resident teaching hospitals.

References
Novel Criteria for Parathyroid Hormone Levels in Parathyroid Hormone–Guided Parathyroid Surgery

Robert de Vos tot Nederveen Cappel, MD; Nicole Bouvy, MD, PhD; Wouter de Herder, MD, PhD; Yolanda de Rijke, PhD; Hans van Toor; Jaap Bonjer, MD, PhD

Parathyroidectomy is the only cure for hyperparathyroidism. The traditional surgical approach involves a bilateral neck exploration to identify all parathyroid glands and remove grossly enlarged ones. Using this approach allows experienced endocrine surgeons to cure 97% to 99% of patients undergoing their first parathyroidectomy.1

Primary hyperparathyroidism is generally caused by a solitary adenoma, although 15% of patients have multiple gland disease.2 Using localizing methods such as technetium-99m sestamibi and ultrasonography, minimally invasive surgery can be performed in the case of a single hyperfunctioning adenoma.3–6 Bilateral surgery is required when multiple gland disease is present in primary hyperparathyroidism and inevitable in cases of secondary or tertiary hyperparathyroidism or multiple endocrine neoplasia (MEN) I/IIa syndromes. Most of these disorders are caused by 4 hyperplastic glands, but variations of 2 to 8 glands have been reported.7 Preoperative and perioperative discrimination between uniglandular disease and multiglandular disease remains the key problem in parathyroid surgery.

Measuring parathyroid hormone (PTH) levels perioperatively (quick PTH [qPTH]) was introduced in the early 1990s as a tool to determine whether resection of parathyroid tissue was sufficient.8,9 A 50% reduction of qPTH value 5 minutes after removal of all hyperfunctioning parathyroid tissue is generally assumed to indicate complete parathyroidectomy. Hence, further exploration of the neck would be unnecessary. However, various authors have reported postoperative hypercalcemia in spite of an apparent sufficient drop of qPTH levels during surgery of the neck.10–12

The purpose of this consecutive study was to determine whether novel criteria for decrease of perioperative qPTH levels can improve the accuracy of perioperative qPTH-guided parathyroidectomy.

PATIENTS AND METHODS

From February 2000 to February 2003, all consecutive patients eligible for parathyroidectomy for hyperparathyroidism at our institution were included in this prospective study. Patients with primary hyperparathyroidism, as well as patients with secondary or tertiary hyperparathyroidism or MEN I/IIa disease, were entitled to qPTH measurement during surgery.

Sequential sestamibi scintigraphy was done routinely. Planar images were collected at 15, 90, and 150 minutes after intravenous administration of 370 MBq sestamibi. Single photon emission computed tomogram images were documented at 30 minutes after injection of the radiofarmacon. Ultrasonography was only performed in some patients undergoing first explorations of the neck. In patients with either persistent or recurrent hyper-
Results of 110 quick parathyroid hormone (qPTH)–guided surgical interventions for hyperparathyroidism on 100 patients. PHP indicates primary hyperparathyroidism; SHP, secondary hyperparathyroidism; THP, tertiary hyperparathyroidism; and MEN, multiple endocrine neoplasia.

RESULTS
A total of 100 patients underwent 110 parathyroid surgeries with qPTH monitoring. Of these 100 patients, 72 had primary hyperparathyroidism and 28 had secondary or tertiary hyperparathyroidism or MEN I/IIa disease. The average age of all patients was 53 years (range, 10–91 years), and 70 (70%) were female. The Figure presents the results of surgical interventions performed in both groups.

Primary Hyperparathyroidism (n = 72)
Sixty-three patients did not have a history of neck surgery. Fifty-eight (92%) of them were cured after the first surgical intervention, most (46/58) by unilateral exploration. Furthermore, 12 of 58 patients were cured by 1 surgical procedure but needed bilateral exploration. Reasons for bilateral surgery in these 12 patients were misdirection or lack of detection by preoperative imaging techniques (n = 5); concomitant contralateral thyroid enlargement, thyroid nodules, or thyroiditis (n = 4); double adenoma bilaterally (n = 1); absence of preoperative imaging (n = 1); or intrathyroidal parathyroid location (n = 1).

Five (8%) of the 63 primary explorations were unsuccessful due to undetected multiple gland disease, and...
these patients were cured by second intervention (n = 2) or are still being followed.

Nine patients (of 72, 13%) previously underwent surgery for primary hyperparathyroidism at another hospital. Seven of them (78%) were subsequently cured by 1 unilateral exploration, and 2 patients needed 2 and 3 procedures, respectively, to achieve normocalcemia at our institution.

Table 1 shows the results of preoperative imaging techniques for patients with unilobar and multiglandular disease. Seventy-four preoperative sestamibi scans revealed a single positive lesion suggesting parathyroid hyperactivity in 61 patients (82%). Ultrasonography was less distinctive with 9 positive localizations (26%) of 35 studies.

Measurement of qPTH based on criteria of solely greater than 50% decrease of PTH showed 69 true-positive assay declines (Table 2). Multiple gland disease was found in 8 patients (13%). In these 8 patients, 4 neck explorations were completed after a drop of greater than 50% of qPTH. However, hypercalcemia persisted in these patients who should have been cured according to conventional criteria. These 4 procedures with false-positive results were performed in 3 patients (Table 2, patients 1–3). Subsequent successful reexplanation (with true-positive qPTH results) took place in 2 of them, and 1 patient will be operated on in the near future. In 4 patients (6%) there was a decrease of less than 50% (at \( t = 5' / t = 10' \)) of the qPTH level after removal of 1 enlarged parathyroid (patients 4–7). However, the exploration was ended because the surgical team considered the resection of hyperparathyroid tissue to be sufficient. These patients were subsequently found to have another hyperfunctioning gland and were considered to have true-negative results. If surgical strategy had been based on the results of qPTH measurement, further exploration in the same procedure had been performed with good reason. Measurement of qPTH in 1 patient with multiple gland disease (cured by 1 bilateral exploration) was performed after resection of the second (and last) adenoma, and results were interpreted as true positive.

The average weight of the removed parathyroid of all 77 procedures was 2656 mg (range, 23–27000 mg) with a median weight of 870 mg. No permanent laryngeal nerve injury occurred.

### Secondary and Tertiary Hyperparathyroidism and MEN I/IIa Disease

Thirty-two neck explorations with simultaneous qPTH measurement were performed in 28 patients. Five patients had undergone previous neck surgery for hyperparathyroidism. Three of those 5 patients had positive preoperative imaging results, so a reexploration could be performed unilaterally. All but those 3 patients in this group underwent bilateral neck surgery.

Nine sestamibi scans were performed in 7 patients, and all but 1 of them showed locations (varying from 1 [n = 8] to 2 \( n = 1 \)) of hyperactive parathyroid (89%). Only 4 patients had preoperative ultrasound imaging, which revealed enlarged parathyroid glands in 3 of them (75%). The average weight of the removed parathyroids was 1042 mg (range, 30–12850 mg), with a median value of 398 mg. No permanent laryngeal nerve injury occurred.

Table 1. Results of Sestamibi Scanning and Ultrasonography in Primary Hyperparathyroidism Patients

<table>
<thead>
<tr>
<th>Explorations</th>
<th>Unilateral Explorations</th>
<th>Positive Sestamibi Scanning, No./Total (%)</th>
<th>Positive Ultrasonography, No./Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniglandular disease ( n = 64 )</td>
<td>64</td>
<td>52</td>
<td>53/62 (85)</td>
</tr>
<tr>
<td>Multiglandular disease ( n = 8 )</td>
<td>13</td>
<td>0</td>
<td>8/12 (67)</td>
</tr>
<tr>
<td>Total ( n = 72 )</td>
<td>77</td>
<td>52</td>
<td>61/74 (82)</td>
</tr>
</tbody>
</table>

Table 2. False-Positive and True-Negative Results Based on Greater Than 50% Fall of Quick Parathyroid Hormone (qPTH) Measurement in 77 Primary Hyperparathyroidism Procedures*

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Patients</th>
<th>qPTH Level (ng/L)</th>
<th>% Fall</th>
<th>Cured</th>
</tr>
</thead>
<tbody>
<tr>
<td>False-positive procedures by criterion</td>
<td>1 (Reexploration)</td>
<td>74.6</td>
<td>24.5</td>
<td>NA</td>
</tr>
<tr>
<td>&gt;50% ( n = 4 )</td>
<td>2 (Reexploration)</td>
<td>370</td>
<td>338</td>
<td>162</td>
</tr>
<tr>
<td>&gt;50% ( n = 4 )</td>
<td>3 (Reexploration)</td>
<td>355</td>
<td>111</td>
<td>202</td>
</tr>
<tr>
<td>True-negative procedures by criterion</td>
<td>4</td>
<td>347</td>
<td>316</td>
<td>218</td>
</tr>
<tr>
<td>&gt;50% ( n = 4 )</td>
<td>5</td>
<td>359</td>
<td>244</td>
<td>NA</td>
</tr>
<tr>
<td>&gt;50% ( n = 4 )</td>
<td>6</td>
<td>213</td>
<td>193</td>
<td>123</td>
</tr>
<tr>
<td>&gt;50% ( n = 69 )</td>
<td>7</td>
<td>388</td>
<td>303</td>
<td>330</td>
</tr>
</tbody>
</table>

\* 5’ and 10’ indicate 5 and 10 minutes; NA, not available.

† Average results of 69 procedures.
The decline of more than 80%. Table 5 shows results of false-positive results occurred when postexcision qPTH levels of more than 200 ng/L were combined with a serum qPTH measurement revealed a fall of more than 70%, no false-negative results occurred. Furthermore, no false-positive results occurred when postexcision qPTH levels of more than 200 ng/L were combined with a serum qPTH decline of more than 80%. Table 5 shows results of false-positive, true-negative, and true-positive qPTH measure-
ment by conventional (>50%) criteria versus our novel criteria.

**COMMENT**

Perioperative qPTH measurement was introduced by Irvin and colleagues. This technique is used to determine whether multiple parathyroid tumors are present. An inappropriate fall of qPTH requires further exploration to identify and remove hyperactive parathyroid tissue. Rapid PTH assessment has been accepted by many as a useful tool during parathyroid surgery. Due to the use of this technique, surgeons have become increasingly tempted to perform limited neck explorations when a qPTH fall of more than 50% is established. However, false-positive results have been reported, and therefore the specificity of this technique demands improvement. Another disadvantage is that results of blood testing are reported to the surgeon 20 to 30 minutes after sampling, which is an expensive way of passing time. A mobile qPTH system inside the operating room can reduce these delays but is not available in most hospitals. It is still unclear whether major benefits such as reduction of operating time and diminution of postoperative events of hypoparathyroidism and recurrent laryngeal nerve injuries can be accomplished by performing PTH-guided parathyroidectomy.

In this study 7 patients with primary hyperparathyroidism had unsuspected multiple gland disease. Four of them could have been cured by further exploration during the same session when we would have complied with the qPTH outcome. However, in 4 procedures (3 patients), qPTH measurement revealed a fall of more than 50% at t 10', suggesting no further need for exploration based on conventional criteria. In these patients, hyperparathyroidism persisted, and therefore qPTH results should be considered as false-positive.

False-positive and true-negative qPTH results have been reported with use of different criteria and can be due to various factors. Using other criteria as suggested by Carneiro et al may reduce false-positive events, but the rate of false-negative predictions would increase. Manipulation of parathyroid glands can increase PTH levels, causing “spikes,” which gives a false-negative result and a prolonged surgical time. Therefore, baseline levels should be achieved at skin incision time (before mobili-

### Table 3. False-Positive and True-Negative Results Based on Greater Than 50% Fall of Quick Parathyroid Hormone (qPTH) Measurement in 32 Secondary Hyperparathyroidism (SHP), Tertiary Hyperparathyroidism (THP), and Multiple Endocrine Neoplasia (MEN) I/IIa Procedures (28 Patients)*

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Patients</th>
<th>qPTH Level (ng/L)</th>
<th>% Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>False-positive</td>
<td>8 (SHP)</td>
<td>Preop</td>
<td>t 5'</td>
</tr>
<tr>
<td>procedures by</td>
<td>9 (SHP) (Reexploration)</td>
<td>3100</td>
<td>3430</td>
</tr>
<tr>
<td>criterion</td>
<td>10 (SHP)</td>
<td>566</td>
<td>467</td>
</tr>
<tr>
<td>&gt;50% (n = 4)</td>
<td>11 (MEN)</td>
<td>216</td>
<td>NA</td>
</tr>
<tr>
<td>True-negative</td>
<td>9 (SHP) (Reexploration)</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>procedures by</td>
<td>(Reexploration)</td>
<td>2409</td>
<td>2283</td>
</tr>
<tr>
<td>criterion</td>
<td>(Reexploration)</td>
<td>2141</td>
<td>2383</td>
</tr>
<tr>
<td>&gt;50% (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True-positive procedures by criterion &gt;50% (n = 25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1044 (93–2500)</td>
<td>191.1 (5–2015)</td>
</tr>
</tbody>
</table>

* 5' and 10' indicate 5 and 10 minutes; NA, not available. † Average results of 25 procedures.

### Table 4. Novel Criteria for Adequate Discrimination Between Unilgandular Disease and Multiglandular Disease in Primary Hyperparathyroidism and Predicting Cure in All Hyperparathyroid Disorders

<table>
<thead>
<tr>
<th>t 5'/t 10' Level (ng/L)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;100</td>
</tr>
<tr>
<td>2</td>
<td>100–200</td>
</tr>
<tr>
<td>3</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

### Table 5. Effect of Introduction of Novel Quick Parathyroid Hormone Criteria Versus Conventional Criteria With Regard to Surgical Results in 77 Cases of Primary Hyperparathyroidism

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>50% Criterion</th>
<th>Novel Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>False-positive declines</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>True-negative declines</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>True-positive declines</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>
zation of suspected glands). In our study, sampling at the time of manipulation was not performed routinely. Nonetheless, we did not find any false-negative results.

Another cause of a false-positive qPTH test is suppression of an enlarged parathyroid gland in multiple gland disease. Although questioned previously, experience with qPTH assay has provided evidence for parathyroid suppression.

In order to increase the specificity of this procedure, we tried to find a solution for diminishing false-positive results. In this report we introduced new criteria for adequately interpreting the peroperative fall of qPTH (Table 4) measured by the Immulite assay, especially for patients with primary hyperparathyroidism. These criteria may not apply to other assays that do not measure the 7-84 PTH fragment.

In our series, false-positive qPTH outcome was associated with postexcision levels of at least 100 ng/L in 3 of 4 procedures in patients with primary hyperparathyroidism as well as in 3 of 4 procedures in patients with secondary or tertiary hyperparathyroidism or MEN 1/IIa disease. We believe that this is suggestive for remnance of hyperactive parathyroid tissue. For revealing true-positive results we propose t 10’ qPTH levels between 100 and 200 ng/L combined with a decline of more than 70% and t 10’ levels of more than 200 ng/L combined with a drop of 80% of qPTH to be interpreted as a measure for adequate surgery. Preexcision PTH levels of all 72 primary hyperparathyroidism patients may seem quite high (329 ng/L combined with a decline of more than 70% and t 10’ 1804 Arch Pathol Lab Med—Vol 131, December 2007 Novel Criteria for Parathyroid Hormone Levels—de Vos tot Nederveen Cappel et al

References
Tissue Handling for Genome-Wide Expression Analysis

A Review of the Issues, Evidence, and Opportunities

Fabiola Medeiros, MD; C. Ted Rigl, PhD; Glenda G. Anderson, MBA; Shawn H. Becker, MD; Kevin C. Halling, MD, PhD

Context.—Molecular diagnostic applications that use microarrays to analyze large numbers of genes simultaneously require high-quality mRNA. As these genome-wide expression assays become more commonly used in medical practice, pathologists and oncologists will benefit from understanding the importance of obtaining high-quality RNA in order to generate reliable diagnostic and prognostic information, especially as these relate to cancer.

Objective.—To review the effects that different tissue preservation techniques have on RNA quality and to provide practical advice on changes in tissue acquisition and handling that may soon be needed for certain clinical situations.

Data Sources.—A review of recent literature on RNA quality, tissue fixation, cancer diagnosis, and gene expression analysis.

Conclusions.—Studies have consistently shown that frozen tissue yields more intact RNA than formalin-fixed, paraffin-embedded tissue. The chemical modification, cross-linking, and fragmentation caused by formalin fixation often render RNA unsuitable for microarray analysis. Thus, when expression analysis involving hundreds or more than 1000 gene markers is contemplated, pathologists should consider freezing a specimen within half an hour (preferably within minutes) of surgical resection and storing it at −80°C or below. In coming years, pathologists will need to work closely with oncologists and other clinicians to determine when saving frozen tissue for microarray expression analysis is both practical and necessary. In select cases, the benefit of implementing a few extra tissue-handling steps may improve diagnostic and prognostic capability.

(Arch Pathol Lab Med. 2007;131:1805–1816)
Tissue Handling for Gene Expression Analysis—Medeiros et al

The early rapid evolution in microarray technology was accompanied by a lack of coherency and standards among the different platforms. While this created some doubts about the reliability of results, many studies have now confirmed the reliability of microarray platforms. For example, the US Food and Drug Administration–initiated MicroArray Quality Control study recently demonstrated that microarray results show good reproducibility within and between test sites, as well as good comparability between different platforms.

Although current expression microarrays vary widely in design, the essential reaction at the heart of all microarray technology is identical: the hybridization of patient-derived mRNA or copy mRNA to complementary DNA on a solid support. Thus, as outlined next, any degradation of the patient’s input mRNA will tend to obscure the overall expression profile. The impact of the degradation will depend in part on the design of the assay. For example, microarrays using oligo-dT primers for reverse transcription may be more sensitive than non-oligo-dT methods to the loss of certain full-transcript RNA messages. Also, those platforms that do not rely on either amplification or reverse transcription (such as the DNA branch method) are likely to have entirely different requirements in terms of nucleic acid quality in the starting material.

Information obtainable by techniques such as immunohistochemistry (IHC) or expression analysis of a limited number of genes by RT-PCR is composed of only a limited number of data dimensions; however, the information sought with microarray-based expression analysis is much more complex and highly multidimensional. This goal of “high multiplexy” with the microarray explains why higher-quality input RNA is essential. Simply put, more data points are required to evaluate the numerous clinical scenarios and outcomes under consideration. Although real-time RT-PCR techniques have proven capable of salvaging an mRNA signal from heavily degraded FFPE tissues, these techniques are generally limited to analysis of a panel of 10 to 200 carefully selected genes. This RT-PCR approach can, in fact, be extremely valuable in certain clinical situations. The OncoType DX Assay illustrates one strategy wherein microarray was used to discover some of the relevant genes, and then the RT-PCR–based commercial assay is developed for use on FFPE tissues. A similar strategy has been used to identify a 2-gene ratio (ratio of homeobox 13 [HOXB13] to interleukin 17B receptor [IL17BR]) that may predict recurrence and survival in women receiving adjuvant tamoxifen. Given the ready availability of FFPE specimens, the practicality of such a 2-step approach to test development is clear; however, the complexities and potential limitations of this approach, including a possible sacrifice of sensitivity and accuracy, have also been described.

One of the chief technical limitations of the RT-PCR technique involves the need to create primers and probes specific for all of the mRNAs of interest. This requirement places a logistical limit on the number and type of expressed genes that can be analyzed. Such a narrow analysis of expression may be perfectly adequate in clinical situations in which only simple and specific binary classifications are required (eg, as with the OncoType DX Assay that asks, “What is the likelihood of systemic disease recurrence in node-negative estrogen receptor–positive breast cancer?”). However, only a genome-wide expression microarray can measure several hundreds or thousands of genes simultaneously to answer the intrinsically more complex, multiclass questions that are common in oncology. For these genome-wide microarrays to perform optimally, the highest-quality input mRNA will likely be required and, as discussed
in the next section, the standard formalin-fixed tissue is often inadequate.

**EFFECT OF FORMALIN FixATION ON mRNA QUALITY**

Formaldehyde was first used to fix tissues in the 1890s. Unlike alcohol fixatives, it produced only slight shrinkage and distortion of tissues.11,12 Today, formaldehyde as 10% neutral-buffered formalin is still the most common fixative used by pathologists. Unfortunately, the chemical properties that make formalin an ideal fixative for preserving tissue structure for visual or antigenic evaluation also cause it to degrade mRNA macromolecules in a severe and often random manner.25,26,63 The main impacts involve the addition of monomethylol groups to RNA bases, cross-linkage of nucleic acids to proteins, and fragmentation (Figure 1). These formalin-induced impacts are discussed after the following brief discussion of the potential for mRNA degradation due to delay in tissue preservation.

**The Danger of Delay: Enzymatic Degradation**

Messenger RNA is, partly by design, an ephemeral molecule. Regulation of cytoplasmic RNA degradation is one of the cell’s mechanisms for controlling gene expression, and it can be triggered by alterations such as changes in nutrient or hormone levels, warm ischemia, or tissue hypoxia.84–87 Although relatively stable thermodynamically, RNA is rapidly digested by RNase enzymes, which are ubiquitous in most tissues. This is why mRNA begins to degrade within the first hour after surgical excision of tissue if the tissue is not frozen.87,88 The RNA loss is greatest in tissues harboring high levels of endogenous RNases and proteases, such as the pancreas, gall bladder, and skin.89 Many eukaryotic mRNAs have half-lives of 30 minutes or less.90 Certain mRNAs, such as those for cytokines and proto-oncogenes, contain sequences that render them highly labile to enzymatic degradation—again, a likely regulatory mechanism for governing expression in the natural cell environment.91,92 For the pathologist, prevention of this degradation of RNA by RNases is the prime goal of rapid temperature reduction after tissue collection.

The extensive degradation of mRNA that can occur with a delay in fixation or preservation has been well documented. In one study of decades-old archived tissue blocks obtained at autopsy, Mizuno et al93 found that the integrity of the mRNA in liver and thyroid tissue was diminished in those cases with the longest postmortem time before collection. Several other studies have correlated the length of time before completion of tissue fixation and embedding with the amount of enzymatic degradation in the RNA.33,34,83,88,94,95 In one of these studies, Florell et al96 directly evaluated the impact of alternative tissue handling procedures on the quality of RNA. Freshly excised normal skin was taken from patients during Mohs surgery. One portion of the tissue was placed immediately into RNA-later (Ambion, Inc, Austin, Tex) for 24 hours and then stored at 4°C for 2 to 6 weeks. Another portion of the tissue was held for 30 minutes at room temperature during dissection in surgical pathology before being flash frozen at −20°C. Purification and analysis of the total RNA in these specimens showed that the tissues preserved immediately after biopsy produced distinct ribosomal bands of high-molecular-weight RNA, whereas the tissues held at room temperature resulted in smearing of lower–molecular weight RNA species typical of extensive RNA deg-

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**Figure 1.** How formalin fixation damages mRNA. The timing and method of tissue preservation have a direct impact on mRNA quality and, hence, the potential for more accurate diagnosis and prognosis based on genome-wide expression analysis.
The Nature of the Formalin Damage: Methylation, Cross-linking, Fragmentation

Formalin preserves tissue structure mainly by creating cross-links between proteins. While protein-protein cross-links are the predominant reaction, extensive intermolecular cross-linking of proteins with nucleic acids also occurs. Specifically, formaldehyde reacts with the nucleic acid bases to form an N-hydroxymethyl (methylol) group (–CH2OH), followed by a slower electrophilic attack of this N-methylol group on amino acid bases to form methylene bridges between two amino groups. While this action makes neutral-buffered formalin (10%) ideal for hardening tissues for later microscopic and IHC analysis, the hydroxymethylene cross-linking between proteins and nucleic acids also makes it difficult to extract intact mRNA from formalin-fixed tissues.

The adverse effect on nucleic acid quality is dramatic. In one study, nearly 40% of the adenine bases in synthetic RNA acquired monomethylol additions after fixation in formalin for several days. Proteinase K digestion can relieve some elements of cross-linking, and heating with guanidinium can disrupt some of the noncovalent interactions that prevent RNA solubilization and isolation; both methods are used routinely to isolate RNA, but neither can correct the other major impact of fixation (ie, fragmentation). RNA extracted from FFPE tissue is most often present in fragments less than 200 or 300 bases in length.

The results from one animal study clearly illustrate the detrimental impact of formalin fixation on RNA quantity and transcript size. While the yield of total RNA from frozen liver was 625 μg/100 mg tissue, the yield in formalin-fixed tissue was only 30 μg/100 mg. Further, analysis of RNA length showed that the frozen tissue reflected the expected distribution of sizes, including the classic peaks at the ribosomal 18S and 28S equivalents, whereas the formalin-fixed tissue was extremely degraded, with the major peak reflecting a predominant molecular size of only 200 bases.

In addition, RT-PCR analysis showed a significant decrease in the amount of β-actin mRNA in formalin-fixed and Carnoy-fixed tissues but not in frozen samples. A more recent study showed that an optimized RNA extraction technique could improve the RNA yield from FFPE tissue to 30% of the amount of RNA extracted from fresh tissue. Although pretreatment with RNAlater be-

NS, not significant (as reported; no value given); P values as reported.
Adapted from Benchekroun et al, with permission from Lippincott Williams & Wilkins, copyright 2004.

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Figure 2. Preservation of total RNA: freezing versus formalin fixation. Electropherograms from an Agilent 2100 Bioanalyzer of total RNA extracted from frozen (A) and formalin-fixed (B) rat liver tissue stored for 3 months after sample acquisition. Note the presence of ribosomal 18S and 28S peaks and the full range of RNA sizes in frozen tissue; note shift to left (smaller molecular weight with major peak at 200 bases) in formalin-fixed tissues. Adapted from Benchekroun et al, with permission from Lippincott Williams & Wilkins, copyright 2004.

Figure 3. Freezing versus 3 methods of fixation. Percent template detection for β-actin transcript (244-base pair amplicon) in commercial rat liver reference. Analysis by quantitative polymerase chain reaction assays shows significantly decreased template detection in Carnoy- and formalin-fixed samples versus the reference. NA, not applicable; P values not calculated because fewer than 3 observations.

Figure 4. Fragmentation of RNA increases with storage time in formalin-fixed tissue. Analysis of RNA (with Agilent 2100 Bioanalyzer, RNA 6000 Nanochip) from 12 breast cancer tissue specimens collected during a 17-year period in one institution using a consistent formalin fixation protocol. Note larger molecular weights of RNA in tissue archived for just 1 year versus sizes seen in those archived for 6 or 17 years, suggesting time-dependent fragmentation of formalin-fixed, paraffin-embedded tissue even after dehydration and embedding in wax. (Lanes M1 and M2 contain reference RNA.) Adapted from Cronin et al, with permission from the American Society for Investigative Pathology, copyright 2004.
fore fixation further boosted the yield to approximately 80% of that in fresh tissue, the quality of the RNA in that pretreated FFPE remained poor (ie, highly fragmented).

The Extent of the Damage

How much mRNA is lost by chemical fixation? This depends primarily on the methods used for tissue preservation and RNA isolation. Total RNA quality can be assessed by determining the size distribution of the extracted RNA with agarose gel electrophoresis or an automated microcapillary electrophoretic analyzer (eg, Agilent 2100 Bioanalyzer, Agilent Technologies, Quantum Analytics, Foster City, Calif). The quantity and quality of mRNA can be estimated by attempting to detect expression levels of known transcripts of various sizes with RT-PCR. For example, a study of fixative effect on RNA integrity in endometrial tissues on which RT-PCR was performed for various amplicon sizes of 2 genes (glyceraldehyde-3-phosphate dehydrogenase [GAPDH] and β-globulin) showed that longer mRNA fragments were present in much lower quantities than shorter mRNA fragments in fixed tissues. The investigators found that the RNA fragmentation was much more extensive in formalin-fixed tissues than in tissues that were fixed in acetone or RNA Later (Table 1).

The reduced ability to detect changes in expression of key genes in fixed tissues has also been documented in studies that used oligonucleotide assays to monitor expression. Bibikova et al. for example, showed that formalin-fixed tissues allowed detection of about half as many genes as could be detected with matched frozen tissues. A more recent microarray-based analysis focused on the detection of genes regulated by p38 inhibitor treatment to gauge the impact of preservation methods on expression analysis. In the frozen samples, a total of 799 genes exhibited at least 1.5-fold change, whereas in the formalin-fixed samples, a total of only 356 genes were detected with a 1.5-fold change. Laser capture microdissection and/or novel RNA purification techniques have very recently been shown to improve the quality of the input RNA from FFPE tissues, but even in these studies the RNA quality was lower than that in frozen specimens. Coulry and coworkers reported a significant concordance between expressed genes in FFPE and frozen samples, with only a slight loss of sensitivity in the formalin-fixed specimens. However, in the Penland et al study of specially processed FFPE specimens, only 24% were suitable for microarray analysis; this low yield is unacceptable for any microarray-based diagnostic test intended for clinical use. These authors also observed a significant loss of gene sig-

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* Table shows ratio of long transcripts to shortest glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript (121 bp) in 3 fixatives as detected by real-time polymerase chain reaction; note that higher ratio indicates more fragmentation. Adapted from Paska et al with permission from Lippincott Williams & Wilkins, copyright 2004.

Thus, using both overall and gene-specific measures of RNA quality, several investigators have documented the relative impact of various tissue fixation methods. Practically all published studies indicate that formalin fixation produces the greatest degree of RNA degradation (Table 2). While non-cross-linking preservatives improve RNA quality relative to that obtained with formalin fixation, many of them contain solvents that may also destroy the integrity of certain tissues and thereby preclude further histologic analysis of the tissue. At present, although formalin remains the unquestionable gold standard for most diagnostic human histopathology, flash freezing by immersion in liquid nitrogen seems to produce the highest mRNA quality for certain microarray-based analyses of gene expression.

The Implications of mRNA Damage

Many factors affect the extent of the formalin damage to RNA. The degree of formalin-induced modification may, for example, vary with the concentration, temperature, pH, and penetration time of the formalin. The types, amount, and shape of the tissue will also affect overall fixation results, whereas the duration of fixation and the choice of embedding method including the choice of buffer in the formalin, may directly affect RNA preservation.

Some of these factors, such as prefixation delay, fixation period, and storage time, are somewhat predictable and generalized in their impact on the tissue content. For example, Figure 4 shows increased RNA fragmentation with increased archive storage time. In addition, Cronin et al reported that the loss of average real-time RT-PCR signal was approximately 90% between samples collected in 2001 and those collected in 1985 (data not shown). The mechanism of this RNA loss in older blocks is unknown. Although the amount of mRNA lost was extensive, the investigators still found that they could quantify relative levels of mRNA expression with RT-PCR by normalizing the expression level of the gene of interest to a reference set of genes. Even when the FFPE samples showed a 75% loss of intact amplicon template compared with paired frozen samples, use of reference genes to correct for the deterioration produced a good correlation in expression profiles for 42 test genes.

Although sample-handling variables that lead to RNA degradation can be ameliorated with normalization techniques, this assumes that the fixative has an equivalent impact on all mRNA species. However, several groups have shown that fixation can disproportionately increase or decrease the level of an mRNA of interest relative to a so-called housekeeping gene mRNA that is being used to normalize the data. The apparent variable sensitivity of different mRNA species to fixation implies that quantitation of mRNA expression from fixed tissue may provide erroneous results and provides further evidence for the importance of obtaining frozen tissue if performance of genome-wide expression analysis is anticipated.

It must be emphasized, however, that all expression assays will require their own process of validation before they can be applied clinically. If a test is designed based on the assumption that formalin has a uniform effect on all mRNA species, then the actual effect may be quite different. Therefore, it is important to validate the test for each mRNA species other than the reference gene before using it for diagnostic purposes.
on microarray data derived from frozen specimens, for example, then any derivative RT-PCR test that employs FFPE to analyze a subset of the discovered genes cannot be assumed to have valid normalization and quantitation characteristics until large numbers of new patient specimens are tested with that platform and protocol in rigorous clinical trials. Although the selected genes may be the same in each assay, the differences in specimen preservation, in addition to the differences in RNA extraction, amplification, and analysis, may produce greatly altered ratios of detectable genes and thereby lead to poor results.

In summary, formalin fixation of tissues results in a significant chemical alteration of the RNA. RNA isolated from fresh tissues can sometimes produce reliable expression data for selected genomic markers when heavily amplified using specific primers and short amplicons or when assayed with novel expression test platforms such as the branched-DNA assay. However, in many tests that require amplification, labile or low-level mRNA transcripts from certain key genes may be rendered unamplifiable and therefore undetectable by the harsh fixation procedures. In microarray procedures, any formalin damage to the poly-A tail of transcripts (the short chain of adenine nucleotides found on the end of all intact mRNA molecules) makes reverse transcription and amplification extremely difficult. Extensive fragmentation is also a problem, since the oligo-dT primers used for amplification will not amplify fragments that have been broken off from their poly-A tail (the target of the oligo-dT primer). Because conserving transcript abundance is critical to expression test results, the loss of any mRNA due to delays in processing, formalin fixation, or fragmentation may severely limit test accuracy. Again, although amplification makes it possible to generate results from extremely small samples or from degraded samples, it is likely that many moderate- to low-abundance transcripts will not be preserved in their relative distribution—meaning that the chances for misleading data are high. Freezing of specimens soon after biopsy may help deliver the higher-quality RNA that is required for genome-wide expression analysis.

### PRACTICAL ISSUES AND NEW OPPORTUNITIES

What do these facts about the comparative quality of mRNA in formalin-fixed versus frozen tissues mean for the pathologist and the oncologist? On a very practical level, they suggest a compelling reason to consider saving extra or separate tissue samples for genome-wide expression analysis. In addition, several of the studies just reviewed indicate the types of clinical situations where these relatively simple changes in sample acquisition and handling may lead to new opportunities for improved diagnosis and prognosis.

#### Recommendations on Freezing Tissue for Expression Analysis

With growing use of genome-wide microarrays in the pathology laboratory, pathologists and oncologists will increasingly need to consider saving separate samples during certain biopsy procedures: one fixed for standard morphology and IHC, and the other frozen for expression analysis. While such an extra step can easily be accomplished in high-volume cancer centers and other institutions with the proper facilities for freezing specimens (e.g., liquid nitrogen), oncologists and pathologists in small offices or community practice may admittedly have difficulty implementing this recommendation. Other practical issues related to this recommendation to freeze tissue for microarray analysis involve biopsy timing and methods.
In terms of biopsy timing, in cases where genome-wide expression analysis is a consideration, the separate or split samples may need to be taken during the initial biopsy procedure. Capturing both samples during the initial biopsy procedure allows for future access to the tissue (assuming adequate quantities are available), is easier on the patient, and also avoids the delay and expense of a possible follow-up biopsy. Of course, in some cases the frozen sample may not need to be evaluated, and this needs to be considered in workflow and cost-effectiveness analyses. Overall, however, saving 2 tissue samples during the initial biopsy may be worth the minimal up-front effort and expense because it keeps the door open for later genome-wide expression analysis. For pathologists, the practical challenge will be remaining alert for cases in which microarray-based testing might have an impact on patient management.

Based on this review of the literature, immediate freezing in liquid nitrogen appears to be the current best choice for preserving tissue for genomic analysis. Specifically, to prevent tissue ischemia and hypoxia and the related RNA degradation, researchers have recommended that approximately 0.1 cm$^2$ of the tissue specimen, which should yield sufficient mRNA for most studies, be snap frozen in liquid nitrogen within half an hour (preferably within minutes) of surgical resection and stored at −80°C or below. This conclusion was also reached by the Tumor Analysis Best Practices Group, a group of investigators using Affymetrix oligonucleotide microarrays, the commercial platform with the widest usage in current clinical trials. In terms of tissue handling, the Tumor Analysis Best Practices Group recommends the following:

All tissue samples should be flash frozen within minutes of surgery and stored at −80°C or below. Samples should also be kept in small, airtight containers and kept from drying out during frozen storage by placing fragments of ice in with the sample.

While freezing of tissue within minutes of biopsy is the preferred method, this admittedly may not be practical in every setting. In particular, as already mentioned, smaller office practices may not have access to adequate freezing facilities. Clinicians in such settings will undoubtedly need to weigh the clinical value of cancer microarray results from larger academic centers to determine whether adding freezing facilities and changing biopsy protocols for certain clinical situations are justifiable. In the interim, compromise solutions to preserve quality mRNA may be considered. For example, since brief transport of tissue on ice before fixation and processing appears to have minimal impact on RNA quality and expression, a practical approach in the clinical setting where patients are often physically dispersed is for clinicians to send the samples by routine iced transport for centralized processing. A recent study showed that tumor samples can be frozen and thawed at least 3 times without compromising the RNA integrity and genetic expression profile. Another potential compromise solution for settings without access to adequate freezers is, as discussed below, saving a portion of the biopsy in an RNA-friendly preservative.

For those cancer centers where freezing of specimens is already routine, other questions will need to be addressed. There are, of course, many different protocols for freezing tissues, with subtle and not-so-subtle variations in parameters (e.g., the delay before exposure to freezing temperature, the possible addition of a nonformalin preservative or additive, the sizing of tissue, the storage container, the speed of freezing, and the final temperature). The impacts of these variables on mRNA quality and expression results are not yet clear. The need for standardization in tissue collection and handling has been emphasized by the many regulatory, scientific, and commercial groups with interests in full-genome expression testing (e.g., US Food and Drug Administration, National Cancer Institute, Affymetrix, Inc). While specific recommendations are still evolving, there is already strong consensus that warmer storage environments permit RNA degradation and that samples slanted for nucleic acid microarray analysis should be rapidly frozen and then stored at −80°C in a mechanical freezer or in liquid nitrogen.

Based on such consensus, most clinicians planning to use expression analysis are developing simple institutional protocols that call for rapid freezing of more tissue samples. At the Mayo Clinic, for example, the protocol for freezing solid tumors will likely be similar to that already employed for lymphoid tissues. Currently, when a diagnosis of lymphoma is clinically suspected, one portion of the tissue is frozen in the cryostat at −20°C using an embedding medium for frozen tissue as the mounting medium, and another portion of the tissue is kept as fresh as possible. If lymphoma is still a possible diagnosis after frozen section evaluation, part of the fresh tissue is snap frozen in liquid nitrogen and stored at −80°C. This frozen tissue is then retrieved if gene rearrangement studies are warranted to confirm the diagnosis. If only small amounts of tissue are available, tissue is transferred from the cryostat to the −80°C freezer. The other portion of the fresh tissue is then placed in formalin or other fixatives, such as B5 in the case of lymphoid neoplasms, for later processing and morphologic and IHC evaluations.

Based on experience in this type of workup for lymphomas, many pathology laboratories affiliated with larger cancer centers appear to have adequate training and facilities to implement a similar tissue handling protocol for solid tumors. The indication to freeze tissue from solid tumors will need to be discussed with the clinician but will surely evolve with experience. Cases where a biopsy is performed in an office setting may present an initial challenge. In some of these cases, where typically the entire tissue specimen is placed immediately in formalin, discussion and planning will be necessary to ensure that a frozen section is requested by the clinician. These will be carefully selected cases in which gene expression analysis or any molecular technique requiring high quality nucleic acids is anticipated. As mentioned above, iced transport to an available freezer location may be one practical option for handling the office biopsy.

Alternative Methods for RNA-Friendly Tissue Preservation

What about alternative “RNA-friendly” preservative methods, such as RNAlater and the organic solvents and alcoholic fixatives? As reviewed in the previous section, several of these alternative fixatives deliver better results than formalin in terms of RNA quality or detection of specific expression markers. In some cases, these alternative fixatives may even allow pathologists to conserve a single tissue sample for combined morphologic, histologic, and molecular analysis. This may be an attractive alternative...
to saving separate samples, as discussed above, especially when access to appropriate freezer facilities is limited.

In particular, RNAlater has undergone scrutiny as a possible alternative to freezing or formalin fixation in expression studies. This solution precipitates out RNases into an aqueous sulfate salt solution and thereby preserves the intact RNA. Although RNA yields and specific gene RNA abundance with RNAlater are generally comparable with those seen with frozen tissue, some studies indicate that freezing still may be preferred over RNAlater for RNA preservation. Also, because of the critical importance of RNA quality to quantification of less abundant transcripts, more studies are needed that compare snap-frozen tumors with tumors in RNAlater in genome-wide expression microarrays. In terms of preserving tissue for histology and IHC, results with RNAlater have been mixed.

Other alternative fixatives that avoid the cross-linking associated with formalin include ethanol, methanol, Carnoy fixative (60% ethanol, 30% chloroform, 10% glacial acetic acid), and methacarn solution (substituting 60% methanol for the ethanol in Carnoy). While all these non-formalin methods may eventually prove to be useful RNA-friendly alternatives to freezing, validation in microarray-based genome-wide expression studies will be required before they can be put into routine use.

Other Issues to Consider

In addition to considering the preservation method, pathologists interested in genomics-based testing will need to monitor several other tissue-handling issues that will impinge, either directly or indirectly, on sample quality and hence on the clinical reliability of the expression result. Examples of such issues include the potential value of frozen specimens in traditional IHC analysis (eg, when traditionally processed tissue is nondiagnostic); the cost effectiveness of collecting and storing frozen specimens in selected clinical situations; the impacts of variations in tissue handling techniques (eg, timing of collection and preservation, use of various buffers in fixatives, method of RNA extraction, quantity of tissue); the best methods for checking RNA quantity and quality (which may differ for RT-PCR vs microarray assays); and the implications of using fine-needle aspiration to obtain the specimen.

These issues are beyond the scope of this review but deserve future study. Fine-needle aspiration, for example, has been used with success in some gene expression profiling studies, but acquiring enough fine-needle aspiration tissue to perform both the standard pathologic tests and the new expression tests may remain a challenge for certain tumors. Similarly, adequate portions of other biopsies (eg, breast, pancreatic, renal, adrenal) may be unavailable for frozen storage and eventual expression testing because of the understandable priority in using unfrozen tissue to make the initial histologic diagnosis. It may also be challenging to identify areas of tumor within prostatic tissue using gross examination of the biopsy, a key issue for microarray analysis since a high percentage of normal cells can prejudice the analysis. Minimum standards for volume and percentage of tumor tissue will need to be developed and validated for each sample collection method used in expression analysis. At the institutional level, decisions on how or when to best capture a specimen with RNA quality suitable for full-genome expression testing will need to be made by a team of specifically pathologists, surgeons, and internists who understand the demands and limitations of biopsy in each organ system. The long-term stability of mRNA signatures in frozen or fixed specimens is another issue worthy of additional study. In particular, studies need to address the potential value of using RNAlater in frozen specimens and of freezing isolated RNA. Factors such as fixative buffers and storage temperatures (eg, −80°C vs liquid nitrogen) must also be investigated. In some studies, even storage at −80°C has been inadequate in completely preventing RNA degradation, and thus any evaluations of expression tests based on frozen samples should also verify the reproducibility of results with samples of different vintage. In one recent analysis performed as part of the clinical validation studies for a microarray-based test for tumor tissue of origin, 64 frozen specimens stored at −80°C for 2 years or less demonstrated accuracy of results equivalent to that seen with 34 frozen specimens stored under similar conditions for 5 to 8 years (C.T.R., oral communication, April 2007). Although such findings are encouraging, especially given the documented decline in mRNA quality in stored FFPE specimens (eg, Figure 4), clinicians need to remain acutely aware that the performance of any expression test will depend critically on the specific gene markers included in the test panel. If the markers chosen are inherently unstable, either during the brief period of ischemia/warmth before freezing or a long period of frozen storage after preservation, then test performance will suffer. This reinforces the need for carefully designed studies to validate test performance under a variety of demanding clinical conditions and with specimens of varying quality.

Major Opportunities for Improved Patient Care

While many questions remain about the practicality and logistical details of collecting a frozen specimen for expression analysis, the potential benefits to patients of what, for many laboratories, is a simple tissue-handling step are clear. As discussed earlier, genome-wide expression analysis will probably provide the highest clinical value in those settings in which a clinician needs to weigh multiple variables in order to answer an inherently complex question involving diagnosis, classification, or outcome. It is in these complex situations where a gene-by-gene or IHC approach leading to a simple binary assay result will often fall short. Based on preliminary studies, the settings where genome-wide expression testing will be preferred will likely include: the classification of certain lymphomas, leukemias, and tumors of unknown origin; the identification of previously unrecognized subsets of cutaneous melanomas, breast carcinomas, colorectal cancers, and adult soft tissue sarcomas; and the stratification of patients with cancer into fine-level subgroups with distinct clinical manifestations and specific prognoses or predicted responses to therapies.

For example, based on seminal studies using microarrays and frozen tissues, researchers recently reported on a microarray-based tissue of origin assay that quantifies the similarity of tumor biopsy specimens across 15 known sites of origin. If the preliminary results can be confirmed in clinical studies, then many more patients with poorly differentiated tumors or cancers of unknown origin, which represent anywhere from 2% to 5% of all diagnosed cancers, depending on the definition and popu-
lation considered,125,144–147 will be more likely to receive disease-specific treatment.

Another setting in which genome-wide expression analysis is likely to be adopted involves prediction of response to chemotherapy. Because each patient’s oncogenic state typically involves deregulation of multiple cell signaling pathways, full-genome expression analysis may provide the clearest window into the inherently complex nature of cancer.143,148 The high degree of tumor complexity and heterogeneity explains why a particular chemotherapy can be so effective in one patient but spectacularly ineffective or toxic in another. While progress has been made in selecting appropriate targeted therapies for individual patients based on biomarkers or single-gene analyses, only recently have researchers begun analyzing the simultaneous expression of thousands of genomic markers to predict a patient’s response to the older agents and, importantly, to commonly used combination regimens. This new pharmacogenic approach has now been tested, with promising results in several oncology settings,16–24,149,150 and it may eventually lead to improved prediction of clinical response to various chemotherapy regimens. But, as emphasized throughout this review, achieving this next level of sensitivity and accuracy in expression analysis results may require procedural changes at the time of specimen collection and handling. The first evidence of this evolving requirement can be seen in the recent approval—in fresh-frozen or RNA-preserved breast tissue—of a microarray-based expression test to assess risk of distant metastasis.11

More diagnostic tests of this nature are in development. As recently suggested in a commentary on attempts to use formalin-fixed tissues for expression-based prognosis in breast cancer, “It is unrealistic to expect that optimal molecular measurements should always be obtained from formalin-fixed paraffin-embedded blocks . . . we are obliged to reconsider our methods of handling clinical tissue samples.”78(p995) The author’s conclusion parallels our own: “It is long accepted that to characterize lymphoma, one must obtain extra samples specifically for molecular testing. We should learn to do likewise to accurately assess prognosis and select treatment for . . . cancer.”

**SUMMARY**

RNA quality is a critical determinant in genome-wide analysis of gene expression. Many studies have confirmed that frozen tissues yield more intact RNA than FFPE tissues and hence allow a far more comprehensive and clinically insightful interrogation of tissue expression. As commercially produced high-density microarrays become a common platform for expression profiling in the clinical laboratory, pathologists will increasingly need to collect frozen tissues or other RNA-stabilized samples along with the standard tissues taken for morphologic and histopathologic study. Ideally, when expression analysis is contemplated, a tumor specimen would be frozen in liquid nitrogen within half an hour (preferably within minutes) of surgical resection and stored at −80°C or below. While more studies are needed to determine the ideal method and timing of tissue extraction and processing for genome-wide expression profiling, it is clear that the pathologist’s current tissue handling strategy of choice (i.e., FFPE) is often incapable of tapping the full potential of genomics-based expression tests. In many clinical situations, the minimal amount of upfront time and cost required to collect and freeze tissue for expression analysis will pay dividends in terms of improved diagnosis and prognosis.

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**CAP ‘08 ABSTRACT PROGRAM**

Abstract and case study submissions for the upcoming CAP ‘08 meeting will be accepted beginning on February 1, 2008 through March 28, 2008. Accepted submissions will be published in the September 2008 issue of the Archives.
Thrombotic Microangiopathy in Cocaine Abuse–Associated Malignant Hypertension

Report of 2 Cases With Review of the Literature

Xin Gu, MD; Guillermo A. Herrera, MD

Acute renal failure is well described in cocaine-induced acute intoxication. The cause is multifactorial. In most cases reported, acute renal failure was associated with rhabdomyolysis, direct vasoconstriction, and alteration of systemic hemodynamics. In recent in vitro studies, cocaine-induced direct or indirect diffuse injury to endothelial cells has also been described. Clinically, cardiovascular symptoms with elevated blood pressure are common findings in patients with acute cocaine intoxication. However, significant elevation of blood pressure reaching malignant hypertension is infrequent. We report 2 cases of cocaine abuse–associated malignant hypertension with renal failure. Kidney biopsies revealed thrombotic microangiopathy with fibrinoid necrosis of arterioles and glomerular tufts. Cocaine-mediated endothelial injury and platelet activation may play important pathogenetic roles in cocaine abusers who develop acute renal failure and malignant hypertension. (Arch Pathol Lab Med. 2007;131:1817–1820)

REPORT OF CASES

Case 1

A 48-year-old man came to the emergency department for bleeding from the left ear because of trauma. He had no chest pain, shortness of breath, or other complaints. During physical examination, he was found to have an elevated blood pressure (210/110 mm Hg). Laboratory tests revealed a serum creatinine of 11 mg/dL and blood urea nitrogen of 90 mg/dL. Platelet counts and lactate dehydrogenase levels were within normal limits, and schistocytes were less than 1% in the peripheral blood smear. The patient had a history of hypertension for 1 year and was taking antihypertensive medications. His last known serum creatinine (1 year ago) was 1.2 mg/dL. Serology tests for hepatitis, antineutrophil cytoplasmic antibodies, antinuclear antibodies, rapid plasma reagin, and rheumatoid factor were all negative. Twenty-four-hour urine protein was measured at 2 g. A kidney biopsy was done for worsening renal function with uncertain etiology. Prebiopsy, the patient denied using illegal drugs. When the nephrologist discussed the biopsy report with the patient, he admitted that he had used cocaine a few days before his admission. The patient's renal function was improved after his hypertension was controlled using multiple antihypertensive medications. However, he remained in chronic renal insufficiency with elevated creatinine (3–4 mg/dL) after 1 year.

Kidney biopsy revealed small arteries with hyperplastic change (onion-skinning) and myxoid intimal change. Segmental fibrinoid necrosis of vascular walls with fragmented red blood cells was also present. The glomeruli revealed segmental fibrinoid necrosis of capillary tufts. Moderate interstitial fibrosis with tubular atrophy and dropout was also present (Figures 1 through 3). Immunostain for cocaine metabolites was positive in the cytoplasm of proximal tubular cells (Figure 4) proving that indeed cocaine use prior to the renal biopsy had occurred.

Case 2

A 39-year-old man visited the hospital and complained of a 2-day history of headache with blurred vision. The patient had no significant medical history. He appeared agitated but not confused. Physical examination revealed a normal heart rate and regular rhythm with no signs of congestive heart failure. The blood pressure was elevated (200/110 mm Hg). He denied illicit drug use. Laboratory tests revealed an elevated serum creatinine (9 mg/dL). He was given dialysis for acute renal failure. A kidney biopsy was performed for acute renal failure with unknown etiology. Drug abuse was suspected. Urine chemical analysis revealed cocaine metabolites. The patient was lost to follow-up.

Kidney biopsy revealed segmental glomerular fibrinoid necrosis and prominent fibrinoid necrosis of afferent and efferent arterioles. Cocaine toxicity not only induces vasoconstriction but may also precipitate diffuse endothelial injury as a result of malignant hypertension and, eventually, acute renal failure.

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Figure 1. Case 1. Fibrinoid necrosis of arterioles (hematoxylin-eosin, original magnification ×500).

Figure 2. Case 1. Fibrinoid necrosis of glomerular capillary tufts (hematoxylin-eosin, original magnification ×150).

Figure 3. Case 1. Fibrinoid necrosis of arteriole with collapse of capillary loops (hematoxylin-eosin, original magnification ×350).

Figure 4. Case 1. Immunohistochemical stain for cocaine metabolites; peroxidase–anticocaine–cocaine amphetamine regulatory transcripts as marker. Proximal tubular damage with vacuolization, apical blebs, desquamation and fragmentation of tubular cells, and staining in their cytoplasm for cocaine metabolites (original magnification ×500).

Figure 5. Case 2. Fibrinoid necrosis of glomerular arteriole and capillary tufts with fragmented red blood cells (hematoxylin-eosin, original magnification ×500).

Figure 6. Case 2. Segmental fibrinoid necrosis of glomerulus with fragmented red blood cells (hematoxylin-eosin, original magnification ×500).
Cocaine is a stimulant extracted from the leaf of the Erythroxylon coca plant and is available as a hydrochloride salt for intravenous or intranasal administration or as “freebase” for smoking. The major pharmacologic effects of cocaine include blockage of norepinephrine reuptake and release of catecholamines from adrenal glands.8,9 The pathogenesis of cocaine-induced acute renal failure is multifactorial and includes rhabdomyolysis, acute ischemia because of vascular constriction, and direct toxicity.10–13 Most cocaine is metabolized by the plasma and liver cholesterol esterases to water-soluble metabolites. This enzyme is responsible for the detoxification of cocaine in the body, and it has been shown that patients with life-threatening cocaine toxicity have lower plasma cholinesterase activity than those who have non-life-threatening toxicity.14 Most cocaine metabolites are eliminated through the urine.15 Cocaine and its metabolites often combine with cocaine amphetamine regulatory transcripts and can be detected in the urine or blood and can also be found in renal tubular epithelial cells using an immunohistochemical stain, as seen in case 1. Because the transcripts are quickly absorbed and metabolized, presence of strong positive staining in the tubular epithelial cells indicates recent use of cocaine.

The first reports of cocaine abuse–related acute renal failure were associated with rhabdomyolysis.16,17 It is still the most common renal complication of cocaine abuse. Cocaine-related rhabdomyolysis has a high mortality. In 1 report,18 13 of 39 patients with cocaine-related rhabdomyolysis developed acute renal failure. Among those patients with acute renal failure, 6 died. The mechanism of cocaine-induced rhabdomyolysis is unclear. Increased muscle activity resulting from sustained and repetitive sympathetic stimulation, ischemia because of vasoconstriction, and direct toxicity are potential causes of cocaine-related rhabdomyolysis.

Myocardial and cerebral ischemia because of cocaine-induced vasoconstriction has been well documented in many clinical and experimental studies. The vasoconstriction effects are mediated via competitive antagonism of norepinephrine reuptake.8 In animal studies, the cocaine effects on various blood vessels and vascular beds are different, suggesting variable distribution of catecholamine loci and their receptors.9 Although there is no direct evidence of cocaine inducing renal vascular constriction in humans, clinical reports of acute tubular necrosis and kidney infarcts following cocaine abuse strongly suggest that vasoconstriction most likely occurs in the renal circulation.11 The result of renal vasoconstriction is commonly acute tubular necrosis, but there may be a direct toxic effect of cocaine metabolites on the proximal epithelial cells.12,19

Acute renal failure with accelerated or malignant hypertension is occasionally seen in cocaine abusers. Thakur and colleagues20 reported 2 chronic hypertensive patients who developed accelerated hypertension with renal failure after consuming cocaine. Histologic examination of kidney biopsies from these patients revealed typical hyperplastic vasculopathy with onion-skinning of the interlobular arteries and ischemic changes of glomeruli with cerebiform waviness of glomerular basement membranes. Segmental fibrinoid necrosis of an interlobular artery was also noted.

Reported cocaine-associated TMA is rare, with only 2 previously reported cases in the literature.21,22 In those reported cases, patients presented with hemolysis, thrombocytopenia, renal failure, and other systemic manifestations mimicking thrombotic thrombocytopenic purpura.

Herein we have reported 2 cases of cocaine abuse-associated acute renal failure in patients who denied illicit drug use. The kidney biopsies revealed typical features of TMA. Clinically, these 2 patients presented with headache and significantly elevated blood pressure in the malignant hypertension range. Symptoms and signs of cardiac or cerebral ischemia were not present. Laboratory tests ruled out rhabdomyolysis. There were also no clinical indications and laboratory results to support thrombotic thrombocytopenic purpura or scleroderma. The renal biopsy from patient 1 revealed not only fibrinoid necrosis of arterioles and glomeruli but also evidence of vascular sclerosis and glomerulosclerosis. In case 2, the patient had no significant medical history, and the renal biopsy revealed prominent fibrinoid necrosis of glomerular arterioles and capillary tufts. The chronic changes of interstitium and vasculature were rather mild. Abuse of cocaine was confirmed by positive cocaine metabolites in urine by chemical analysis and by the finding of cocaine metabolites in proximal tubular cells.

Thrombotic microangiopathy is not a specific entity but a lesion seen in many conditions, including thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, scleroderma, malignant hypertension, and toxicity related to various drugs and radiation nephritis. In our last 5-year renal biopsy archives, 24 (0.9%) of 2750 kidney biopsies were diagnosed as TMA. In these 22 cases, 6 presented clinically as malignant hypertension, and 2 of these 6 cases, as we have reported here, were cocaine related with clear laboratory evidence of abuse cocaine. The other 4 cases include 2 young females with preeclampsia and 2 chronic hypertensive patients with accelerated malignant hypertension. In these 4 patients, the blood pressures reached as high as 220 to 240 over 120 to 140 mm Hg, slightly higher than what we found in patients with cocaine abuse.

The pathogenesis of TMA is unclear. Proposed mechanisms suggest direct damage of vascular endothelium such as occurring in malignant hypertension, preeclampsia, chemotherapy-associated toxicity, and burns.23,24 In animal studies, cocaine abuse has been shown to induce a marked pressor response, which is mediated by sympathetic activation and α,β-adrenoceptor stimulation.25 However, this hypertensive effect was short lasting because of the rapid recruitment of compensatory mechanisms that quickly normalize blood pressure, such as β-adrenoceptor-mediated relaxation of vascular smooth muscle cells. Clinically, most cocaine-intoxicated patients have short-lasting hypertension with no significant renal impairment. The hypertension is generally controlled by administration of medications that directly target the central effects of cocaine, such as the benzodiazepines.26,27 Therefore, hypertension alone appears to be insufficient for inducing TMA in cocaine-associated acute renal failure.

As indicated in several in vitro studies, cocaine is toxic to endothelial cells and can directly or indirectly injure the endothelium.5,6 Enhancement of catecholamines has...
been demonstrated to be an effect of cocaine. Exposure to catecholamines has been suggested to induce endothelial injury and affect the permeability of small blood vessels in animal models. Clinically, disseminated intravascular coagulopathy is not an uncommon finding in patients who have died from cocaine abuse.11 These findings imply that either direct or indirect cocaine toxicity may damage endothelial cells and induce a vasculopathy with TMA features.

In addition, platelet activation and thrombus formation also occur secondary to cocaine. Platelets can be directly activated by cocaine27 and indirectly activated through an α-adrenergic–mediated increase in platelet aggregation.28 In the presence of cocaine, adenosine diphosphate–induced platelet aggregation is enhanced,29 and tissue plasminogen activator inhibitor is increased.30

It is likely that cocaine-induced toxicity and the hypertensive effect resulted in injury of endothelial cells in the 2 cases presented. Damage to the endothelium and activation of platelets further triggered thrombosis and fibrinoid necrosis in small vessels and capillaries and resulted in the morphologic changes characteristic of TMA. Other factors, such as chronic hypertension, arteriolosclerosis, and low cholinesterase activity, may also be involved in selected cases.

In most cases, a history of cocaine abuse is not readily available. A high index of clinical suspicion is necessary when patients present with acute renal failure associated with significantly elevated blood pressure and poor response to conventional treatment. Serum and urine testing for cocaine and cocaine metabolites are indicated in this clinical situation. Ancillary diagnostic techniques are often helpful in working out acute renal failure in suspicious cocaine abuse cases if a renal biopsy is performed. In some cases associated with cocaine use, the main renal lesion is infarct or necrosis,31,32 in others myoglobinuria-related, and in another group associated with acute tubular necrosis resulting from vasconstriction and possibly as a direct toxic effect on proximal tubular cells by cocaine metabolites.19

In conclusion, 2 cases of cocaine-induced acute renal failure with no rhabdomyolysis or ischemic or toxic-induced acute tubular necrosis are reported. Renal insufficiency with malignant hypertension was the major clinical presentation. The histologic features are those of TMA with conspicuous fibrinoid necrosis of glomerular capillary tufts and arterioles. The mechanisms responsible for these pathologic changes are unclear but most likely multifactorial. Diffuse vascular endothelial injury because of direct toxicity or cocaine-enhanced catecholamine release is likely a major contributor to the renal pathology noted in the 2 reported cases. It remains imperative to recognize that acute renal failure following cocaine abuse can occur in the absence of concomitant rhabdomyolysis.

References

Filiform Polyposis Mimicking Familial Adenomatous Polyposis in a Patient Without Inflammatory Bowel Disease

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● Filiform polyposis is an uncommon entity that is most often encountered in the colon of patients with a history of inflammatory bowel disease (IBD). Filiform polyposis is characterized by a large number of “wormlike” polyps lined by histologically normal colonic mucosa. These polyps can mimic adenomatous polyps. Only rare cases without a history or evidence of IBD have been reported. Neuromuscular and vascular hamartoma of the small bowel is a rare, focal disorder characterized by disorganized smooth muscle fascicles throughout the submucosa accompanied by fibrosis, nerve fibers, ganglion cells, and vessels. To our knowledge, there is only one report of this lesion in the large bowel (cecum), where it presented as a mass. Here we report the case of a 50-year-old man with no known history or symptoms of IBD presenting with filiform polyposis involving the entire colon, clinically mimicking familial adenomatous polyposis, and showing histologic features similar to neuromuscular and vascular hamartoma of the small bowel.

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REPORT OF A CASE

A 50-year-old white man presented for a routine checkup with a medical history significant for obesity, body habitus, hypercholesterolemia, and sleep apnea. His surgical history was significant for appendectomy at age 8 years and arthroscopic repair of a torn meniscus a year prior. There was a questionable family history of inflammatory bowel disease (IBD). His appetite had not changed; indeed, he had recently gained a few pounds. He reported no nausea, vomiting, abdominal pain, changes in bowel habits, or alterations in stool size, shape, or consistency. He denied melena and hematochezia. His social history was significant for drinking 12 beers on the weekend; he quit smoking tobacco 25 years prior. It was decided that he should undergo a routine screening colonoscopy, which discovered diffuse involvement of the entire length of his colon with numerous polyps of an adenomatous, filiform appearance. Biopsies were not obtained. The patient was diagnosed clinically with an adenomatous polyposis syndrome and was referred to our institution for genetic counseling. Genetic screening for APC gene mutations (for familial adenomatous polyposis) and MYH gene mutations (for MYH-related adenomatous polyposis, a condition with a phenotype similar to familial adenomatous polyposis that inherited in an autosomal recessive manner) revealed no evidence of the mutations most commonly associated with these disorders. Unable to completely exclude malignant transformation of these polyps, the decision was made to undergo a total colectomy. During the operation, it was noted that the entire colon was redundant. The postoperative course was uneventful, and the patient was discharged on the sixth postoperative day.

A 143-cm length of colon, including the anus and a 2.6-cm segment of terminal ileum, was submitted for pathologic examination. Macroscopically, the entire length of the large intestine was involved with more than 100 filiform polyps ranging in size from 3.5 × 1.0 cm to 0.2 × 0.1 cm (Figure, A). The mucosa was otherwise tan, with normal folds and without apparent defects or diverticula; the maximum wall thickness was 0.5 cm. The appendix was surgically absent, and the serosa, adventitia, terminal ileum, and mesentery appeared grossly unremarkable. The polyps were liberally sampled for histologic evaluation, along with representative sections of grossly normal-appearing large intestine. Microscopically, the filiform polyps were lined by normal mucosa, including normal lamina propria and epithelium. The submucosal cores of some of the polyps were composed of fibrovascular tissue (Figure, B), but there were also several cores where the fibrovascular tissue was admixed with disorganized smooth muscle fascicles, nerves, and ganglion cells (Figure, C and D). No epithelial dysplasia was identified (there was no evidence of tubular adenomas or villous adenomas in any of the submitted sections). In addition, there was no evidence of arborizing smooth muscle or hyperplastic mucosa to suggest Peutz-Jeghers polyps (nor was there clinical evidence of mucocutaneous pigmentation), and there was no evidence of cystically dilated glands or expansion of the lamina propria to suggest juvenile polyps. The uninvolved (nonpolypoid and grossly normal) colon also showed submucosal abnormalities histologically. In random areas throughout the entire colon, a disordered and focally thickened muscularis mucosae merged with a submucosa that was fibrotic and had an increase in disorganized smooth muscle bundles and dilated vessels (Figure, E). Little to none of the loose connective tissue comprising normal submucosa was observed, and the muscularis mucosae often appeared in close approximation to the muscularis propria, with regions of possible direct continuity between the two smooth muscle layers (Figure, F).

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A. Gross appearance of the patient's large intestine, showing numerous slender, wormlike filiform polyps. B through F, Microscopic features of the filiform polyps and nonpolypoid colon (all stained with hematoxylin-eosin). B, Tip of a filiform polyp showing normal colonic epithelium overlying a fibrovascular core without significant inflammation (original magnification ×40). C, The tip of another filiform polyp showing submucosal fibrovascular tissue with dilated vessels admixed with disordered smooth muscle fascicles, nerves, and a lymphoid aggregate (original magnification ×40). D, At higher magnification, the disordered smooth muscle bundles (*), nerves (arrowheads), and ganglion cells (arrows) are more evident and are interspersed with fibrous tissue and dilated vessels (original magnification ×100). E, In areas, even the grossly normal, nonpolypoid colon showed abnormal submucosal tissue with a lack of a defined muscularis mucosae and increased fibrosis with interspersed dilated vessels and disorganized smooth muscle bundles (original magnification ×40). MP indicates muscularis propria. F, There were areas where there was marked fibrosis of the submucosa with smooth muscle disarray, making it difficult to determine the location of the normal muscularis mucosae (original magnification ×100). MP indicates muscularis propria.
There was no evidence of acute inflammation, granulomatous inflammation, or chronic mucosal injury, features that would suggest active or inactive IBD. All of the submitted lymph nodes were benign, without granulomatous inflammation or lymphoid hyperplasia.

**COMMENT**

This is a case of so-called filiform polyposis that endoscopically mimicked an adenomatous polyposis syndrome, since it occurred in a patient with no history or evidence of IBD. In addition, the submucosal cores of these polyps, as well as the submucosa in nonpolyoid areas of the colon, demonstrated an interesting finding of neuromuscular and fibrovascular hyperplasia and disarray. Filiform polyps of the colon are typically considered to be variants of inflammatory pseudopolyps occurring in the setting of IBD. Filiform polyps are long, slender, worm-like or finglike projections that can extend up to 9 cm in length and can have occasional “bridging” between adjacent polyps. The polyps can be localized, or they can diffuse involve the colon, and they have been associated with benign strictures. Filiform polyposis has a slight preponderance in men, affecting a range of ages from 6 to 77 years, with an average of 38 years. In some cases, the polyps are difficult to distinguish from villous adenomas, and biopsies are needed to make the diagnosis. Microscopically, the mucosa overlying the filiform polyps ranges from normal to containing nonspecific acute or chronic inflammation, but always without dysplasia. The pathogenesis of filiform polyposis is uncertain, but since the vast majority of cases occur in the setting of IBD, the polyps are generally believed to form as a result of mucosal repair.

Since filiform polyposis typically occurs in the setting of IBD (ulcerative colitis and Crohn disease), it is important to realize that rare cases, including ours, have occurred with no prior history or evidence of IBD. There are 2 reports of filiform polyposis occurring in the setting of unusual inflammatory conditions (colonic tuberculosis and histiocytosis X) but, to our knowledge, only 3 other reports have noted filiform polyposis without evidence of an underlying inflammatory condition. One case, reported in 1989, was that of a 69-year-old woman with no reported history of IBD or colitis who presented with filiform polyps 1 to 2 cm in length within the transverse colon, histologically consisting of normal colon mucosa with “nonspecific inflammatory changes.” The second case, reported as a letter to the editor in 1989, was that of a 47-year-old Chinese woman who was in good health and presented with rectal bleeding; she was found to have multiple filiform polyps within the cecum and proximal ascending colon. The third case, reported in 1996, was that of a 74-year-old man who was found to have multiple filiform polyps in the distal transverse colon that, histologically, were polyoid ganglioneuromas. There was 1 additional case, in which a single filiform polyp located in the distal colon was described in a 77-year-old man without a previous or subsequent diagnosis of IBD. Our case differs from these reported cases because the filiform polyposis involves the entire length of the colon. As demonstrated by our case, the occurrence of diffuse colonic filiform polyposis in a patient without IBD and with a family history of colon polyps/cancer led the clinicians to determine that this was a case of adenomatous polyposis. Since adenomatous transformation could not be excluded, the patient underwent a total colectomy.

The clinical management of patients with filiform polyposis is variable and should be assessed on a case-by-case basis. Filiform polyps themselves are not considered precancerous. However, tight collections of filiform polyps or “giant inflammatory polyps” can mimic adenomatous polyps and even malignancy. While some authors believe that filiform polyposis in and of itself is not an indication to operate, others, such as Bauknecht et al, have argued that “where there are broad findings, a malignant degeneration cannot be definitely ruled out by means of clinical methods” and “spontaneous involution of the finding cannot be expected.” Given the nondysplastic nature of the disorder, the majority of authors have advocated surgical intervention only in those cases in which symptomatic relief is desired, frequently for abdominal pain or severe hemorrhage and anemia.

In those cases in which filiform polyposis arises in association with active IBD, preemptive surgical resection would seem reasonable, particularly given the increased risk of colon cancer in ulcerative colitis and Crohn disease. Less certain, however, is the management of an asymptomatic patient without this history who is found to have filiform polyposis. The preponderance of cases with associated IBD suggests that these patients may, in fact, have occult IBD. Thus, if a biopsy of the polyps demonstrates a lack of dysplasia, additional biopsies from intervening nonpolyoid mucosa may reveal inactive or minimally active chronic colitis and establish an underlying diagnosis of IBD. However, some cases of filiform polyposis may arise instead from a separate etiology without a known risk for colon cancer; in these cases, filiform polyposis itself may not warrant a preemptive colectomy. Given the dearth of data on the actual rate of malignant transformation, if any, of the filiform polyp, it is difficult to say for certain. However, monitoring for concurrent or subsequent development of adenomatous polyps in a colon afflicted by filiform polyposis would be nearly impossible, and it might put the patient at risk for occult colon cancer arising in an adenoma “camouflaged” in the background polyposis. Ultimately, the onus may be upon the physician and the patient to weigh the risks of preventative colectomy versus the difficulty in predicting malignant transformation based on the available data and/or monitoring for a concurrent or subsequent adenomatous neoplasm if the segment of colon involved by filiform polyposis is not removed.

Since rare cases of filiform polyposis without evidence of IBD or other inflammatory conditions do exist, the pathogenesis of the filiform polyps in this setting (and possibly even filiform polyps occurring in IBD) may not be related to a postinflammatory reparative process. The neuromuscular and fibrovascular hyperplasia/disarray observed in our case is strongly reminiscent of the changes first described by Fernando and McGovern in a disorder they termed *neuromuscular and vascular hamartoma of the small bowel*. Since that time, there have been additional descriptions of small intestines affected with the appearance of bundled smooth muscle seemingly derived from the muscularis mucosae admixed with unmyelinated nerve fibers and ectatic blood vessels in the submucosa, often with some degree of fibrosis. Some have since challenged the hamartomatous nature of these changes, as several cases have been reported with clear or suggestive changes...
polyps with submucosal neuromuscular and fibrovascular hyperplasia/disarray that mimicked an adenomatous polyposis syndrome in an asymptomatic 50-year-old man. The neuromuscular and fibrovascular disarray noted within the cores of the polyps as well as the submucosa in grossly “normal” colon is reminiscent of neuromuscular and vascular hamartoma of the small bowel. We hypothesize that neuromuscular and fibrovascular hamartomas can form within the submucosa of the colon in some patients, possibly as a response to chronic mucosal prolapse, and can then lead to the formation of filiform polyps.

References
Sclerosing Epithelioid Fibrosarcoma of the Cecum

A Radiation-Associated Tumor in a Previously Unreported Site

Jared C. Frattini, MD; Julie Ann Sosa, MD, FACS; Susanne Carmack, MD; Marie E. Robert, MD

Data from the nuclear reactor explosion in Chernobyl and the atomic bomb detonations in Hiroshima and Nagasaki demonstrated an association between ionizing radiation and radiation-induced tumors. Sclerosing epithelioid fibrosarcoma is a rare form of malignant fibrosarcoma that is low grade and indolent with distinct immunohistopathologic characteristics that usually occurs in the soft tissues of the extremities. A 62-year-old man from Kiev who aided in the cleanup at Chernobyl presented with crampy abdominal pain, nausea, and vomiting. His workup revealed a cecal mass, and the final pathology from his laparotomy confirmed sclerosing epithelioid fibrosarcoma with metastasis to the liver. In addition to a review of the literature, we report the first case of sclerosing epithelioid fibrosarcoma arising from the large bowel. Exposure to ionizing radiation from Chernobyl could have played a role in the development of his tumor.

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The world’s worst nuclear power accident occurred April 25 to 26, 1986, at Chernobyl in the Ukraine. The subsequent release of more than a hundred different radioisotopes caused the evacuation of 135,000 people and had severe environmental and medical effects on Western Russia. Most studies have focused on the incidence of solid tumors in the workers involved in the cleanup effort.

An association between ionizing radiation and cancer was made more than a century ago; the earliest reports of radiation-induced sarcoma were made more than 80 years ago. The introduction of external beam radiation in the treatment of cancer has led to a better understanding of the relationship between radiation and radiation-induced sarcomas. Additional data were collected in Japan after World War II on the subsequent development of solid tumors in atomic bomb survivors.

Sclerosing epithelioid fibrosarcoma (SEF) is a rare variant of fibrosarcoma first described by Meis-Kindblom in 1995. It is a low-grade neoplasm found in deep muscle tissue, bone, periosseum, fascia, or neural tissue.

We describe a case of SEF of the cecum in a man who aided in the cleanup after the Chernobyl nuclear accident and provide a review of the literature to date. To our knowledge, this is the first report of SEF involving the gastrointestinal tract.

REPORT OF A CASE

A 62-year-old man with no medical or surgical history presented with several days of crampy abdominal pain, nausea, and vomiting. He denied melena, bright red blood per rectum, or weight loss. He had never undergone colonoscopy. He was a nuclear engineer in Kiev and aided in the cleanup of Chernobyl 19 years prior to presentation. He appeared well upon physical examination. His abdomen was slightly distended, with right lower quadrant tenderness to deep palpation. Laboratory values were within normal limits. His carcinoembryonic antigen (CEA) was 1.3. Chest x-ray was normal, but abdominal computerized tomography (CT) showed a 5 × 7-cm mass in the right lower quadrant. A gentle bowel preparation was administered, colonoscopy confirmed a cecal mass, and biopsy revealed only inflammatory tissue.

At laparotomy, purulent fluid was seen associated with an ileal interloop abscess. A cecal mass with perforation was identified, and a right hemicolectomy was performed. A 1-cm mass in the liver was sent for frozen section, which returned as a “sclerotic nodule, final diagnosis deferred to permanent section.” Pathologic examination revealed a 7.5 × 5.5 × 4.4-cm fungating, friable mass in the cecum that obstructed the appendix orifice. A draining sinus extended from the mucosa to the serosal surface. Large areas of necrosis extended to the mucosa and obliterated the muscularis propria (Figure 1). Small tumor nodules were noted in the mesenteric fat. Microscopic examination revealed a sclerotic neoplasm (Figure 2, A through C). The submucosal and muscularis propria were completely replaced by round cells with clear cytoplasm and pleomorphic nuclei. These “epithelioid” cells were arranged in single file, as well as in sheets, entrapped in a dense fibrous stroma. Other areas of tumor were composed of spindle-shaped cells arranged in fascicles. Large areas of necrosis were noted. Immunohistochemical staining was positive for vimentin (V9, Dako, Carpinteria, Calif) and Bcl-2 (124, 1:160, high pH steam antigen retrieval [AR], Dako). Focal smooth muscle actin (1A4, Dako) and CAM 5.2 (1:140, protein kinase AR, Becton Dickinson, San Jose, Calif) staining of tumor cells was also present. Stains for S100 (1:8, Dako), c-Kit (1:200, low pH steam AR, Dako), CD34 (QBEN10, 1:4, Dako), factor VIII (1:4000, protein kinase AR, Dako), myeloperoxidase (1:10,000, low pH steam AR, Dako), desmin (D33, 1:250, low pH steam AR, Dako), and HMB-45 (1:50; Dako) were negative. The overlying colonic mucosa was unremarkable. These histologic and immunohistochemical pat-
Sclerosing Epithelioid Fibrosarcoma of the Cecum—Frattini et al

Figure 1. Cut surface of the cecum and pericolic fat showing a sclerotic tumor with necrosis extending from the mucosa to the pericolic fat. The muscularis propria is obliterated. Arrow points to mucosa.

Figure 2. A, Low-power view showing extension of the neoplasm from mucosa to serosa. Increased cellularity near the mucosa can be appreciated (short arrows). Less cellular, more sclerotic areas (long arrow) are present deeper in the bowel wall (hematoxylin-eosin, original magnification ×25). B, This medium-power view reveals a spindle cell component that was found in portions of the lesion (hematoxylin-eosin, original magnification ×100). C, High-power view of sclerosing epithelioid fibrosarcoma. Cords of infiltrating epithelioid cells in a dense fibrous stroma were noted in other regions of the tumor (hematoxylin-eosin, original magnification ×200).

teams were diagnostic of SEF. Final pathologic diagnosis of the liver lesion was consistent with metastatic disease.

The patient’s postoperative course was uneventful. Thyroid ultrasound was unremarkable, and he is undergoing evaluation for an elevated prostate-specific antigen.

COMMENT

Most data on the medical effects of radioactive fallout from Chernobyl were collected from the 120,000 citizens of Belarus who served as liquidators. There has been a national cancer registry in Belarus since 1973. All malignant tumors are registered. They were mobilized to decontaminate the nuclear power plant and a 30-km zone around the facility. The isotope responsible for exposure early on was 131iodine (50–300 Ci/km²), but more than 43,500 square miles were contaminated by other long-acting isotopes, such as cesium, strontium, and transuranians. On average, liquidators were exposed to 0.1 Gy of radiation. By comparison, 80% of Hiroshima and Nagasaki survivors were exposed to <0.1 Gy. Patients undergoing external beam radiation who develop radiation-induced sarcomas receive, on average, 10 to 50 Gy.

There is compelling evidence from the nuclear disasters at Chernobyl, Hiroshima, and Nagasaki that residents exposed to radioactive fallout are susceptible to gastrointestinal malignancies. Approximately 25% of cancers diagnosed in Chernobyl liquidators were of the digestive system, whereas 56% of cancers from Hiroshima and Nagasaki residents were of the digestive system. The excess relative risk per 1 Gy for digestive malignancies is fairly high (relative risk, 1.21–2.41). In contrast, the excess relative risk per 1 Sievert (Sv) for digestive system malignancies after Hiroshima and Nagasaki was 0.38 (1 Gy = 0.7 Sv).

Before the advent of nuclear power, other forms of ionizing radiation were associated with malignancy. The first cases were skin cancers in radiation workers in the 1900s. Soon thereafter, sarcomas were reported in tuberculosis patients treated with radiation and in workers painting radium watch dials. Radiation-induced sarcomas include...
osteosarcomas, angiosarcomas, fibrosarcomas, leiomysarcomas, and spindle cell sarcomas found in bone, muscle, soft tissue, and nerves. The risk of radiation-induced sarcoma is 0.03% to 0.8%.2

In 1995, Meis-Kindblom7 described a neoplasm composed of epithelioid cells arranged in strands, nests, and sheets set in fibrotic and extensively hyalinized stroma. There have been 57 reported cases of SEF, confirming it as a distinct clinicopathologic entity.7 This rare tumor has been reported in bone, muscle tissue, fascia, and/or periosteum of the extremities, trunk, and head and neck.4 We were unable to find reports of this tumor in the gastrointestinal tract. The average age of patients is 45 years, with no sex predilection.4 Most reports describe the tumor as a low-grade, indolent malignancy, with a local recurrence rate of 48%, a metastatic rate of 60%, and a mortality rate of 35%.7 In 2001, Antonescu et al8 described a series of 16 cases, with a recurrence rate of 50%, a metastatic rate of 86%, and a mortality rate of 57%, suggesting that this neoplasm is more aggressive than originally reported. They later reported on bony invasion and necrosis, which had not been previously described.9

According to the literature to date, SEF has a set of characteristic pathologic features. On gross examination, SEF averages 9 cm in diameter (range, 3.7–22 cm) and has a lobulated, firm texture with a tan, homogeneous appearance on cut section. Some cases have necrotic areas on gross examination. Sclerosing epithelioid fibrosarcoma is defined primarily by histologic criteria. Microscopically, the tumor is characterized by small, round epithelioid cells with sparse cytoplasm arranged in nests and cords, associated with a densely fibrotic, hyalinized stroma. There is minimal nuclear pleomorphism, and mitotic figures are rare. The chromatin pattern is usually fine, with a small nucleolus. The cytoplasm is clear, probably representing an artifact due to shrinkage during processing. The cellularity varies throughout the tumor, with some cases having patchy necrosis.

Immunohistologically, tumors stain consistently and strongly for vimentin with varying degrees of staining for epithelial membrane antigen (EMA), S100 protein, HMB-45, and cytokeratin (CAM 5.2). Nearly all reported cases have been positive for vimentin, with the exception of the Meis-Kindblom study, in which 1 of 14 cases was negative for vimentin.4,8–14 Cytoplasmic staining of CD99 has been noted.10,11,13 Focal EMA staining is noted in nearly half of reported cases (15/34), with rare noted positivity for CAM 5.2, AE1/AE3, S100, and neuron-specific enolase. All previously reported cases are negative for leukocyte common antigen, α-smooth muscle actin (2 cases with equivocal staining), desmin, HMB-45, and CD68.4,8–15 In our case, focal smooth muscle actin positivity was noted within tumor cells, an exception to the previously published literature.

Ultrastructurally, the tumor consists of spindle cells surrounded by tight bundles of collagen fibers. There is abundant, well-developed rough endoplasmic reticulum, which is distended with granular material. Cells are not surrounded by a basal membrane.4,7,8,14 Some reports have identified SEF with ultrastructural nerve sheath11 or myofibroblastic differentiation.4 These disparate ultrastructural findings call into question whether SEF represents a single entity or a heterogenous group of neoplasms with similar histologic features.

The histologic differential diagnosis of SEF includes a wide variety of benign and malignant tumors with significant sclerotic or epithelioid components. Immunohistochemical analysis is an important adjunct to the diagnosis in cases of SEF, largely by revealing pertinent negatives in the immunohistochemical profile that allows the exclusion of epithelial tumors and other mesenchymal lesions. Differentiating between carcinomas and SEF can be exceptionally difficult, as up to half of reported SEFs have EMA or CAM 5.2 immunostaining.9,10 The single strands of epithelioid cells can mimic lobular or signet ring carcinomas in particular. Electron microscopy can easily differentiate these entities, and it should be used for a definitive diagnosis in difficult situations.

Benign fibrous entities to be considered include fibromatosis, fibrous histiocytoma, myositis ossificans, and nodular fascitis. Other variants of fibrosarcoma, such as myxofibrosarcoma, can be differentiated from SEF by the presence of myxoid zones, a whirling growth pattern, and curvilinear blood vessels in the latter tumor. Low-grade fibromyxoid sarcoma/hyalinizing spindle cell tumor contains poorly formed collagen rosettes, consisting of a hyalinized collagenous core cuffed by epithelioid fibroblasts, a feature not seen in SEF. Interestingly, areas suggestive of typical adult fibrosarcoma, with a herringbone pattern and prominent atypia, can be noted in SEF. Additionally, poorly differentiated areas in SEF can feature a hemangiopericytoma-like pattern, which can be confused with synovial sarcoma. Cytogenetic identification of t(X;18), found in synovial sarcoma, can differentiate these two entities. Gastrointestinal stromal tumors can be epithelioid but are rarely sclerotic. c-Kit immunohistochemical staining would differentiate the 2 lesions. Likewise, smooth muscle neoplasms, such as hyalinized leiomyoma or leiomyosarcoma, could superficially resemble SEF but would be characterized by diffuse smooth muscle actin and desmin positivity. Clear cell sarcoma may be difficult to differentiate due to positive S100 immunostaining in both entities; however, SEF is negative for HMB-45. Sclerosing lymphoma can be ruled out by negative leukocyte common antigen (CD45) immunostaining. In several of these entities, electron microscopy can be very helpful in differentiating these lesions, as SEF reveals purely fibroblastic origin as a rule, with only a single reported case having myofibroblastic differentiation.4

The treatment for this tumor is wide local excision. There is no evidence to support the use of adjuvant chemoradiation.7,12

We believe this to be the first reported case of the rare tumor SEF arising in the large bowel. The patient’s exposure to ionizing radiation in the remote past likely played a role in tumorigenesis.

References


**CAP ’08 ABSTRACT PROGRAM**

Abstract and case study submissions for the upcoming CAP ’08 meeting will be accepted beginning on February 1, 2008 through March 28, 2008. Accepted submissions will be published in the September 2008 issue of the ARCHIVES.
Amnion Nodosum Revisited
Clinicopathologic and Placental Correlations

Adebowale J. Adeniran, MD; Jerzy Stanek, MD, PhD

Amnion nodosum (AN) is a placental lesion consisting of numerous small nodules on the amnion of the chorionic disc, placental membranes, or the umbilical cord. Prior to 1950 when Landing1 first used the term amnion nodosum, the characteristic plaques containing squamous cells on the fetal surface of the amnion were referred to as annicotic nodules. Grossly, the lesion consists of multiple, firm, circumscribed, round to ovoid, raised, shiny, yellow nodules, ranging from 1 to 5 mm in diameter visible on the amniotic surface1-3 (Figure 1). Microscopically, they are composed of varying proportions of squamous cells (occasionally keratinized) embedded in degenerative amorphous acidophilic debris1,4,5 (Figure 2). These lesions either are found on the amniotic surface or occasionally embedded in amniotic mesoderm or project through the amnion into the spongy layer cleft between it and the chorion.5 The amniotic epithelium may be present either as a complete or as an incomplete cell layer between the basal side of the nodule and the basement membrane. The epithelium is identical to that of the adjacent uninvolved amnion between nodules and occasionally appears continuous with it, often showing hyperplastic changes.6

Ultrastructural analysis shows that the nodules are composed mostly of closely packed bundles of fibillary material of high electron density and cellular elements of various kinds, irregularly dispersed within the fibillary mass. The basement membrane of the amnion epithelium is usually present under the nodules and sometimes appears multilaminated.6 This indicates that the process is largely superficial and the underlying stroma does not participate in the formation of the lesion. The microscopic and ultrastructural features lend credence to the belief that AN represents deposits of cellular elements from the fetal skin accumulating and organizing on the surface of the amniotic epithelium and undergoing secondary degenerative changes, with subsequent invasion of the squamous cell mass by connective tissue.

Two gross lesions commonly confused with AN are subchorionic fibrin deposits and squamous metaplasia of the amnion (Figure 4, A and B).7

The pathogenesis and mechanism of formation of the nodules in AN are largely unknown and have been the subject of controversy in the past, but several hypotheses have been formulated. Simpson2 suggested that AN was caused by hemorrhagic effusion from which the blood pigment goes in time, leaving sacs of serum with contracted coagula. That was in part because the clinical pictures preceding the findings were consistent with abortion or antepartum hemorrhage. The nodules were also previously suggested to be the result of amniotic metaplasia or transplantation of fetal epithelial cells. It is possible that some of the cells accumulated in the nodules are of amniotic origin, but most of the cellular elements embedded in the nodular masses are fetal epidermal cells in various stages of preservation and maturation.5,8 Another possible pathogenetic mechanism that has been suggested is inflammation; however, there is no associated inflammatory or other tissue reaction.10

Amnion nodosum does not represent degenerate areas of squamous metaplasia, as it consists of amorphous acidophilic material intermingled with squamous elements of varying age under the amniotic epithelium.4 It has been observed in the membranes of 9- and 16-week-old fetuses, thereby supporting the hypotheses that the amniotic lesions seen in late pregnancy are the end result of abnor-

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Figure 1. Gross appearance of the amniotic surface of the fetal membranes showing multiple, firm, circumscribed, round to ovoid, yellow nodules, typical of amnion nodosum.

Figure 2. Microscopic appearance of amnion nodosum nodules showing varying proportions of squamous cells embedded in degenerative amorphous acidophilic debris, attached to the amniotic epithelium (hematoxylin-eosin, original magnification ×100).

Figure 3. Firm white subchorionic nodules of subchorionic fibrin deposition.

Figure 4. Squamous metaplasia of amnion. A, Irregular white hydrophobic plaques. B, Keratinizing squamous epithelium on the chorionic plate of squamous metaplasia (hematoxylin-eosin, original magnification ×100).

Figure 5. Microscopic appearance of chorion nodosum showing flat nodules containing squamous debris buried in the chorionic mesenchyme (hematoxylin-eosin, original magnification ×100).
mal processes that begin early in development rather than recent events. One must be careful with diagnosis of AN in cases of prolonged intrauterine fetal demise as amniotic debris attached to the amnion as part of the maceration and autolysis process may simulate it. It is possible however to distinguish between keratin from fetal skin (cytokeratin 14 positive and cytokeratin 13 negative) and amnion (cytokeratin 13 positive and cytokeratin 14 negative). It is generally believed that AN is associated with conditions that lead to significant prolonged oligohydramnios. The association with marked oligohydramnios was first reported in 1912, when it was thought that perforation of the amnion by fetal hair had resulted in proliferation of the epithelium forming the nodules. It is found in placentas from fetuses with renal agenesis, following premature rupture of membranes, in the donor twin of the twin transfusion syndrome, in diamniotic acardiac twins, and in sirenomelia. Relationship between AN and oligohydramnios have been explained in 2 ways: (1) squames from the hyperconcentrated amniotic fluid might adhere to the surface of the amnion, producing secondary degeneration of the amniotic epithelium, and (2) fetal movement could erode the amniotic epithelium, leading to the incorporation of fetal squames and further proliferation of the exposed amniotic mesoderm. Available evidence suggests that reduced liquor in oligohydramnios allows the fetus to come into direct contact with the amnion, thereby leading to transfer of fetal squames to the amnion by a detritic mechanism.

As our preliminary results suggested that AN is an important lesion associated with extremely high perinatal mortality and morbidity, the review of existing literature showed that the most recent original reports on AN date back decades ago. At the same time, even recent textbooks on placental pathology quote only well-known data on morphology and associations with oligohydramnios and predisposing conditions therto. No data on incidence of AN or its association with other placental features are quoted, however. Therefore, we decided to devote more attention to this lesion by analyzing our 10-year experience in this case-controlled study.

### MATERIALS AND METHODS

Forty-five cases of AN were retrieved (study group [SG]) from our placenta and autopsy database at the University of Cincinnati Medical Center during a period of 10 years (1994–2003). Cases of chorion nodosum were excluded. Patients' medical records and placental reports were reviewed for clinicopathologic correlation. The hematoxylin-eosin slides for all the cases were also reviewed. In a similar way, 45 cases of a control group (CG) without AN were matched for gestational age but otherwise randomly selected from the placental database. The clinical and placental (gross and microscopic) features were statistically compared using the 1-tail Fisher probability test or analysis of variance when appropriate. The difference was considered significant when \( P < .05 \). Amnion nodosum was diagnosed grossly by finding multiple, firm, circumscribed, round to ovoid, raised, shiny, yellow nodules, ranging from 1 to 5 mm in diameter on the amniotic surface of the membranes (Figure 1). Microscopically, these nodules were located on the surface of the amnion and occasionally embedded in the amniotic mesoderm and were made up of varying proportions of squamous cells embedded in degenerative amorphous acidophilic debris (Figure 2).

### RESULTS

Forty-five cases of AN were found among 4342 placentas analyzed (1%). The clinicopathologic and placental features are summarized in Tables 1 and 2, respectively. The mean gestational age in both groups was 28.6 weeks. The mean fetal weight was 1349 g in the SG, whereas it was 1534 g in the CG. In the SG, oligohydramnios was diagnosed clinically in 10 (22.2%) cases, whereas it was diagnosed in 2 (4.4%) cases in the CG. Congenital malformations were found in 12 (26.7%) cases in the SG, and none in the CG. In the SG, associated multiple gestations, perinatal mortality, prolonged preterm premature rupture of membranes, and chronic twin-twin transfusion syndrome (chronic TTTS) were reported in 13 (28.9%), 16 (35.6%), 11 (24.4%), and 4 (8.9%) cases, respectively. In the CG, multiple gestations, perinatal mortality, and prolonged preterm premature rupture of membranes were present in 12 (26.7%), 10 (22.2%), and 8 (17.8%) cases, respectively. There was no reported case of chronic TTTS. The mean placental weight was 314 g in the SG, whereas it was 325 g in the CG. In the SG, the most common placental findings included acute chorioamnionitis (14 cases, 31.1%), fi-

<table>
<thead>
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<th>Clinical Parameters</th>
<th>Study Group n = 45</th>
<th>Control Group n = 45</th>
<th>( P ) Value*</th>
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<tr>
<td>Gestational age, mean ± SD, wk</td>
<td>28.6 ± 6.6</td>
<td>28.6 ± 6.5</td>
<td>.30</td>
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<td>Premature rupture of membranes, No. (%)</td>
<td>11 (24.4)</td>
<td>8 (17.8)</td>
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<td>Preecclampsia, No. (%)</td>
<td>4 (8.9)</td>
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<td>Substance abuse, No. (%)</td>
<td>4 (8.9)</td>
<td>2 (4.4)</td>
<td>.34</td>
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<tr>
<td>Abruption, No. (%)</td>
<td>2 (4.4)</td>
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<td>.34</td>
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<td>0 (0)</td>
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<td>Oligohydramnios, No. (%)</td>
<td>10 (22.2)</td>
<td>2 (4.4)</td>
<td>.01</td>
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<td>Multiple pregnancy, No. (%)</td>
<td>13 (28.9)</td>
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<td>Monochorionic, No. (%)</td>
<td>5 (11.1)</td>
<td>4 (8.9)</td>
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<td>Dicchorionic, No. (%)</td>
<td>8 (17.8)</td>
<td>8 (17.8)</td>
<td>.60</td>
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<td>Caesarean section, No. (%)</td>
<td>12 (26.7)</td>
<td>12 (26.7)</td>
<td>.60</td>
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<td>Malformations, No. (%)</td>
<td>12 (26.7)</td>
<td>0 (0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Perinatal mortality, No. (%)</td>
<td>16 (35.6)</td>
<td>10 (22.2)</td>
<td>.12</td>
</tr>
<tr>
<td>Macerated stillbirths, No. (%)</td>
<td>7 (15.6)</td>
<td>1 (2.2)</td>
<td>.03</td>
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<td>Chronic twin transfusion syndrome, No.</td>
<td>4 (8.9)</td>
<td>0 (0)</td>
<td>.06</td>
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*Boldface values are significant.
brosis of chorionic villi (9 cases, 20%), chorangiosis (6 cases, 13.3%), meconium staining (5 cases, 11.1%), villous edema (5 cases, 11.1%), decidual arteriolopathy (4 cases, 8.9%), and chronic villitis (3 cases, 6.7%). The most common placental findings in the CG included acute chorioamnionitis (22 cases, 48.9%), chorangiosis (10 cases, 22.2%), decidual arteriolopathy (7 cases, 15.6%), chronic villitis (4 cases, 8.9%), meconium staining (4 cases, 8.9%), and fibrosis of chorionic villi (2 cases, 4.4%). There was no associated villous edema (Table 2).

Four cases of chorion nodosum associated with the severe early amnion rupture sequence were identified. In these cases, the mean gestational age was 22 weeks, the mean fetal weight was 499 g, and the mean placental weight was 165 g.15 These were not included in the study because they did not meet the diagnostic criteria for AN.

**COMMENT**

Amnion nodosum is a lesion that merits more attention and reevaluation as our analysis has shown that, probably unlike any other single placental feature, it is associated with an extremely high risk of fetal and perinatal mortality (35%). This outcome is mainly because of lethal congenital malformations, predominantly of the genitourinary system and oligohydramnios-associated fetal pulmonary hypoplasia. The perinatal mortality in the CG was also very high, hence absence of statistically significant differences, but this was because of other causes, predominantly the amniotic sac infection syndrome and complications of prematurity (average gestational age was 28.6 weeks in both the SG and the CG). It is probably impossible to select a true gestational age–matched CG, because (1) only placentas from abnormal pregnancies are sent for examination in our department at the discretion of referring obstetricians and (2) the SG is composed predominantly of premature deliveries (average gestational age 28.6 weeks). It is theoretically possible that because of significantly higher proportion of macerated stillbirths in the SG (Table 1), AN may be the result of oligohydramnios secondary to amniotic fluid resorption after fetal death. However, this mechanism is unlikely and is not raised in the literature.

Half of our AN cases occurred in the second trimester, hence this is in variance with the statement that “AN develops only late in fetal life because earlier in gestation there is insufficient vernix and that when oligohydramnios is present in the second trimester, AN does not develop.”13(p7) An opinion that placentas from gestations of 26 weeks or less rarely show AN despite an accompanying history of marked oligohydramnios16 is more acceptable. Although AN is generally regarded as a hallmark of prolonged oligohydramnios, surprisingly, this clinical condition was diagnosed in only 10 (22%) cases of AN. This may mean that AN may not be a reliable sign of oligohydramnios, and mechanisms other than a simple increased concentration of vernix in the amniotic fluid such as adhesion of squames to traumatic erosion of the amnion because of fetal movements48 might be operational. Another explanation could be that clinically significant oligohydramnios is much more prevalent than that diagnosed antenatally by sonography or that antenatal sonography was not performed in most cases. However, the patient population of our hospital is composed predominantly of poor socioeconomic groups that do not have benefits of the whole spectrum of antenatal care.17

Although the typical model association of AN are anomalies of the fetal urinary system, our analysis has shown that AN secondary to prolonged premature rupture of membranes is almost as common as AN associated with congenital malformations and more common than AN secondary to genitourinary malformations alone. This, however, may be because prolonged premature rupture of membranes is a very common condition, much more common than fetal genitourinary malformations.

Also, in our material, AN in multiple pregnancy was 3 times more common than the TTTS diagnosed clinically or at autopsy. Amnion nodosum may therefore be the first clinical indicator of the TTTS before other features thereof appear, or, alternatively, the oligohydramnios in monochorionic pregnancy may occur independently and not as a part of chronic TTTS or may occur in dichorionic twin pregnancy. The diagnosis of TTTS is usually made based on the findings of polyhydramnios-oligohydramnios sequence with weight discordance judged by obstetrical sonogram in monochorionic twins.18 Surprisingly, our CG

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**Table 2. Placental Correlations of Amnion Nodosum**

<table>
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<tr>
<th>Placental Parameters</th>
<th>Study Group n = 45</th>
<th>Control Group n = 45</th>
<th>P Value*</th>
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<tr>
<td>Weight, mean ± SD, g</td>
<td>314 ± 21.7</td>
<td>325 ± 19.0</td>
<td>.82</td>
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<td>Two-vessel umbilical cord, No. (%)</td>
<td>3 (6.7)</td>
<td>0 (0)</td>
<td>.12</td>
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<tr>
<td>Acute chorioamnionitis, No. (%)</td>
<td>14 (31.1)</td>
<td>22 (48.9)</td>
<td>.07</td>
</tr>
<tr>
<td>Chronic villitis, No. (%)</td>
<td>3 (6.7)</td>
<td>4 (8.9)</td>
<td>.07</td>
</tr>
<tr>
<td>Luminal vascular abnormalities of chorionic villi, No. (%)†</td>
<td>11 (24.4)</td>
<td>1 (2.2)</td>
<td>.002</td>
</tr>
<tr>
<td>Chorionic villi fibrosis, No. (%)‡</td>
<td>9 (20.0)</td>
<td>2 (4.4)</td>
<td>.02</td>
</tr>
<tr>
<td>Placental edema, No. (%)</td>
<td>5 (11.1)</td>
<td>0 (0)</td>
<td>.03</td>
</tr>
<tr>
<td>Laminar necrosis of membranes, No. (%)</td>
<td>1 (2.2)</td>
<td>6 (13.3)</td>
<td>.05</td>
</tr>
<tr>
<td>Meconium macrophages in membranes, No. (%)§</td>
<td>5 (11.1)</td>
<td>4 (8.9)</td>
<td>.50</td>
</tr>
<tr>
<td>Infarction, No. (%)</td>
<td>1 (2.2)</td>
<td>4 (8.9)</td>
<td>.18</td>
</tr>
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<td>Decidual arteriolopathy, No. (%)</td>
<td>4 (8.9)</td>
<td>7 (15.6)</td>
<td>.26</td>
</tr>
<tr>
<td>Chorangiosis, No. (%)</td>
<td>6 (13.3)</td>
<td>10 (22.2)</td>
<td>.20</td>
</tr>
<tr>
<td>Retrophlacental hematoma, No. (%)</td>
<td>3 (6.7)</td>
<td>3 (6.7)</td>
<td>.60</td>
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<tr>
<td>Marginal placenta, No. (%)</td>
<td>5 (11.1)</td>
<td>3 (6.7)</td>
<td>.36</td>
</tr>
</tbody>
</table>

* Boldface values are significant.
† Vascular luminal abnormalities of stem villi, including fibroblast “septation” and total luminal obliteration.
‡ Fibrosis of terminal villi (secondary to † above).
§ Macrophages, frequently vacuolated, containing a nonrefractile yellow pigment present in the placental membranes.
|| Partial or complete (circummarginate) placenta.
and SG comprised similar numbers of monochorionic versus dichorionic placentas (Table 1).

Among other clinical conditions that were more common, although insignificantly, in the SG than in the CG were maternal preeclampsia and diabetes mellitus, both of which may be associated with oligohydramnios. The mechanism may be a marked uteroplacental underperfusion because of pathologic changes in uterine arteries providing blood to the placenta or decreased fetal glomerular filtration rate. Oligohydramnios in postterm pregnancies is associated with a reduction in renal artery end-diastolic velocity, suggesting that increased arterial impedance is an important factor. Our analysis also revealed a conspicuous paucity of other placental findings associated with AN. The high macerated stillbirth rate in the SG was most likely responsible for the placental regressive changes (stem vessel vascular luminal abnormalities and fibrosis of chorionic villi). Placental edema, more commonly seen in the SG, may be explained by decreased venous return to the heart because of compression of chorionic plate vessels and by congenital malformations—both known to be associated with hydrops, seen earlier in the placenta than in the fetus. Laminar necrosis of placental membranes was more commonly observed in the CG. It is a placental lesion diagnosed microscopically as a bandlike coagulative decidual and/or trophoblastic necrosis in the placental membranes in cases with perinatal acute hypoxia, particularly in cases complicated by hypertensive disorders in pregnancy. The lesion, unlike AN, is a feature of acute hypoxia therefore less commonly diagnosed in the SG. As this is an acute placental lesion, it was less commonly found in the SG. This is most likely the consequence of perinatal hypoxia as a cause of fetal death. Although AN has no relevance to management of pregnancy as it is diagnosed only postnatally, it may be important to identify AN at the time of birth, because this could rapidly suggest previously unrecognized oligohydramnios and its consequent, frequently serious conditions including congenital malformations of the neonate thus leading to rapid initiation of relevant diagnostic procedures or in cases of perinatal mortality to strengthen indications of perinatal autopsy.

Two gross lesions commonly confused with AN are subchorionic fibrin deposits (Figure 3) and squamous metaplasia of the amnion (Figure 4). In the former, the amnion can be made to slide over these deposits, whereas in the latter, there is a more diffuse thickening of the amnion and the plaque cannot be separated from the membrane. The term squamous metaplasia is a misnomer because the amnion is an immature squamous epithelium continuous with the fetal skin. The histologic appearance of squamous metaplasia is obvious, showing stratified squamous epithelium with focal keratinization. Histologically, AN should also be differentiated from chorion nodosum, a rare grossly inapparent lesion occurring in placentas from pregnancies complicated by severe early amnion rupture sequence associated with extra-amniotic pregnancy, particularly in the limb-body wall complex. Microscopically, chorion nodosum features flat nodules containing squamous debris, buried in the chorionic mesenchyme and without overlying amnion (Figure 5).

In conclusion, much remains to be learned about the pathogenesis of AN. Nevertheless, this placental condition portends poorly for pregnancy as it is associated with extremely high perinatal morbidity and mortality.

References

17. Sundarajaran V, Levin LS, Stanek J. High recurrence rates of acute and chronic placental inflammatory conditions. 9th International Federation of Placenta Associations Meeting; Mainz, Germany; September 24–27, 2006; Atlanta, Ga. Abstract 45.
Bernard-Soulier Syndrome
An Inherited Platelet Disorder

Angie Pham, MD; Jun Wang, MD

Bernard-Soulier syndrome is an inherited platelet disorder, which is transmitted in an autosomal recessive manner. This syndrome is characterized by variable thrombocytopenia and large defective platelets. Bernard-Soulier syndrome often presents early with bleeding symptoms, such as epistaxis, ecchymosis, menometrorrhagia, and gingival or gastrointestinal bleeding. Diagnosis can be confirmed by platelet aggregation studies and flow cytometry. The differential diagnosis includes the other inherited giant platelet disorders, as well as von Willebrand disease and immune thrombocytopenia purpura. Treatment is generally supportive with platelet transfusions when absolutely necessary and avoidance of antiplatelet medications. Recombinant activated factor VII and desmopressin have been used in attempts to shorten bleeding times; however, no definitive studies regarding their effectiveness have been reported.

(Arch Pathol Lab Med. 2007;131:1834–1836)

Bernard-Soulier syndrome (BSS) was first recognized in 1948 by two French hematologists—Jean Bernard and Jean Pierre Soulier. They described a patient from a consanguineous family afflicted with severe bleeding episodes, thrombocytopenia, and very large platelets. Bernard-Soulier syndrome is a very rare quantitative and qualitative platelet disorder with an autosomal recessive mode of inheritance. Due to frequent misdiagnosis and underreporting, the true prevalence is unknown. However, based on reported cases from North America, Europe, and Japan, the estimated prevalence is less than 1 in 1,000,000. Heterozygous carriers are usually asymptomatic, although they may have mild bleeding tendencies. There have also been reports of a mild form of BSS with an autosomal dominant inheritance trait.

PATHOPHYSIOLOGY

Platelets play a critical role in normal primary hemostasis and clot formation. The platelet membrane contains specific glycoprotein (GP) receptors, which function in platelet adhesion, activation, and aggregation. The GP Ib-IX-V receptor complex, which is responsible for platelet adhesion through its interaction with von Willebrand factor on the exposed subendothelium, is composed of 4 transmembrane polypeptide subunits—disulfide-linked alpha and beta subunits of GP Ib, and noncovalently bound subunits GP IX and GP V. The platelets of BSS lack or have a dysfunctional GP Ib-IX-V receptor resulting in defective adhesion to the subendothelium. The dysfunctional platelets found in BSS can result from one of several different glycoprotein mutations such as missense, nonsense, or deletion mutations of the GP Ib-α, GP Ib-β, or GP IX genes. This variety of mutations is most likely responsible for the heterogeneity of BSS.

CLINICAL MANIFESTATIONS

Bernard-Soulier syndrome presents early with bleeding symptoms, most commonly epistaxis, ecchymosis, and cutaneous and gingival bleeding, as well as menometrorrhagia and gastrointestinal bleeding. Rarely, patients will have severe hemorrhage at times of injury or surgery. The severity of these bleeding symptoms is variable among patients and may range from mild to life-threatening and may even become more or less severe during puberty and adulthood. Heterozygous patients may have mild to moderate bleeding tendencies.

LABORATORY FINDINGS

Thrombocytopenia is variable in BSS, and the platelet count typically ranges from less than 30 to 200 x 10⁹/µL. Bleeding times may range from marginal to markedly prolonged. Evaluation of the peripheral blood smear will reveal large platelets; typically more than one third of the platelets are about half of the size of a red blood cell (3.5 µm), and some platelets are as large or larger than a lymphocyte (Figure 1). Bone marrow biopsy specimen will show normal numbers of megakaryocytes without significant morphologic abnormalities. Modern platelet function tests, such as the PFA-100, may be useful for identifying qualitative platelet disorders such as BSS, but with variable sensitivity, depending on the severity of the defect. Currently, their use is limited to screening for platelet dysfunction, and further testing, such as aggregometry or flow cytometry, is necessary for confirmation.

In vitro platelet aggregation studies characteristically show a failure to aggregate with ristocetin and slow response with low doses of thrombin. This failure to aggregate cannot be corrected with the addition of normal plas-
ma, which distinguishes BSS from von Willebrand disease. The platelets show normal aggregation with epinephrine, adenosine diphosphate, collagen, and arachidonic acid. Flow cytometry can be used to confirm defects in the GPIb-IX-V complex by antibodies directed against platelet surface antigen CD42b, revealing a severe reduction or deficiency of GPIbα (Figure 2, A through H). Other platelet antigens, CD41 (GPIIb) and CD61 (GPIIIa), are normal.

The cause of thrombocytopenia in BSS is unknown, and early studies suggested decreased platelet survival. However, a later study using 111indium-oxine labeled platelets shows little or no decrease in platelet survival time, suggesting ineffective or decreased thrombopoiesis.

**DIFFERENTIAL DIAGNOSIS**

Included in the differential diagnosis for BSS are other inherited giant platelet disorders such as May-Hegglin anomaly and the other MYH9-related thrombocytopenia syndromes (Fechtner syndrome, Sebastian syndrome, and Epstein syndrome), which are characterized by giant platelets, autosomal dominant inheritance trait, and mutations of the MYH9 gene on chromosome 22q12-13, which is the gene encoding for the heavy chain of nonmuscle myosin IIA (NMMHC-IIA). May-Hegglin anomaly is the most common inherited giant platelet disorder and has a clinical manifestation much like that of BSS with mild bleeding tendencies. These patients will often have other clinical findings including nephritis, familial spastic paraplegia, and pituitary growth hormone deficiency. In vitro platelet aggregation tests show normal response to adenosine diphosphate, collagen, epinephrine, and ristocetin; however, impaired response to epinephrine has been described. Peripheral smear evaluation shows large platelets and Dohle bodies, a blue spindle-shaped inclusion, within the cytoplasm of neutrophils. Gray platelet syndrome is an extremely rare giant platelet disorder that appears to have an autosomal dominant

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**Figure 1.** Peripheral blood smear from a patient with Bernard-Soulier syndrome showing presence of large and giant platelets (modified Wright-Giemsa stain, original magnification ×1000).

**Figure 2.** Flow cytometric analysis of the expression of platelet surface antigens CD41a (GPIIb), CD42b (GPIIbα), and CD61 (GPIIIa) is used to compare peripheral blood samples from a normal control (A through D) and a patient with Bernard-Soulier syndrome (E through H). The peripheral blood from the normal control shows presence of a distinct platelet population (gated) and a distinct erythrocyte population (not gated) (A). There is adequate expression of surface antigens CD41a (B), CD42b (C), and CD61 (D) on the platelets. Peripheral blood from a patient with Bernard-Soulier syndrome shows a merging zone between platelets (gated) and erythrocytes (not gated) (E) due to the presence of numerous large and giant platelets, as well as a complete loss of surface CD42b antigen expression on the patient’s platelets (G). However, CD41a (F) and CD61 (H) show adequate expression of GPIIb and GPIIIa on the patient’s platelets.
mode of inheritance as well as some seemingly sporadic cases. Patients tend to present early with epistaxis, ecchymosis, and other bleeding symptoms. Thrombocytopenia is common; however, bleeding time is prolonged even in patients with normal platelet counts, suggesting a qualitative platelet disorder. Platelet aggregometry shows reduced response to collagen and thrombin, but normal responses to adenosine diphosphate and arachidonic acid. Ristocetin may have normal or reduced, but not absent, response. The peripheral blood smear reveals large agranular platelets that appear gray-blue on Wright-Giemsa stain. The bone marrow biopsy specimen usually shows normal megakaryocytes and reticulin fibrosis.

Patients with von Willebrand disease, the most common inherited bleeding disorder, may present with symptoms similar to those of BSS such as mucocutaneous bleeding, epistaxis, and ecchymosis. However, von Willebrand disease is not typically associated with thrombocytopenia or significant peripheral smear findings. Platelet aggregation tests show failure to aggregate in the presence of ristocetin, much like BSS. However, a ristocetin cofactor activity test, using the patient's plasma and freshly washed platelets to measure the von Willebrand factor activity in the plasma, will be normal in patients with BSS and reduced in patients with von Willebrand disease.

Patients with BSS are often mistakenly diagnosed with immune thrombocytopenic purpura, an immune-mediated thrombocytopenia caused by antiplatelet antibodies, leading to the accelerated destruction of platelets. Peripheral smear evaluation will show decreased platelets, and bone marrow evaluation will show normal or increased numbers of megakaryocytes. The diagnosis of immune thrombocytopenic purpura requires the exclusion of other causes of thrombocytopenia. Possible causes of immune thrombocytopenia include infections, autoimmune diseases, lymphoproliferative diseases and drug therapy. Immune thrombocytopenic purpura can be separated into childhood and adult types. The childhood immune thrombocytopenic purpura is typically acute onset, often develops after viral infection or vaccination, and is frequently self-limited with resolution in weeks to months, while the adult type is usually a chronic disease with insidious onset, more often involves women, and rarely resolves spontaneously.

TREATMENT

Platelet and/or blood transfusions remain the best therapeutic measure for uncontrolled bleeding and prophylaxis to control bleeding during surgery. The benefits of receiving the transfusions must be weighed against the risks of exposure. Repeated exposure to blood products raises concern for alloimmunization and platelet refractoriness. The use of leukoreduced blood components has been shown to decrease alloimmune platelet refractoriness. Although some authors have suggested that patients should receive platelets from human leukocyte antigen–matched donors in order to avoid alloimmunization, currently this is not a widely accepted strategy. Activated factor VIIa (FVIIa) has been reported to reduce bleeding times in patients with BSS. However, FVIIa is an experimental drug in treatment of inherited thrombocytopenia, and adverse reactions have been reported. Desmopressin, a synthetic analog of antidiuretic hormone, may transiently increase factor VIII and von Willebrand factor by causing their release into blood. It is used for treatment of mild hemophilia A and von Willebrand disease. Desmopressin has been reported to shorten bleeding episodes for some patients, but a test dose is recommended to determine those patients who will benefit. Stem cell transplantation has been successfully used to treat 2 children with BSS who had severe, life-threatening bleeding episodes; however, based on the study results, the use of transplantation should only be considered in severe disorders and after patients have developed antiplatelet antibodies. Splenectomy, often performed when immune thrombocytopenia is mistakenly diagnosed, does not improve the platelet count or function in BSS. Patients with BSS should be counseled about the importance of preventing even minor trauma as well as avoiding aspirin-containing medications and other platelet antagonists.

CONCLUSION

Bernard-Soulier syndrome is one of several inherited giant platelet disorders distinguished by a functional abnormality of the GPIb-IX-V platelet GP receptor complex. The disease is highly variable with bleeding tendencies that can range from mild to severe and life-threatening. Platelet aggregation studies and, more definitively, flow cytometry can provide an accurate diagnosis of this rare disease and allow for adequate therapeutic management.

References

Biopsy Pathology in Colorectal Disease


In the late 1980s, I was fortunate enough to purchase the first edition of Biopsy Pathology in Colorectal Disease, then authored by I. C. Talbot and A. B. Price and published as part of the Biopsy Pathology Series. I remember as an apprentice gastrointestinal pathologist cherishing the clear style and diagnostic pearls provided by the authors. During the years, I have frequently boasted to colleagues and trainees that I had in my possession the best biopsy book of colorectal pathology ever written!

This was until I received the second edition of this great title, now published by Hodder Arnold, with the addition of a third coauthor, Dr. Manuel Salto-Tellez.

Under a slightly larger format and a handful more pages, this second edition replicates and improves on the quality of the 1987 edition. The same basic structure is preserved, with great opening chapters: “Normal Features” and “Assessment of Abnormalities,” in which the authors systematically review each mucosal component and critically evaluate their utility in reaching a diagnosis in the setting of pathology.

A total of 20 chapters exhaustively cover the entire biopsy pathology of the colorectal mucosa and anus. Although numerous chapters/sections are similar to the previous edition, several welcome additions include, for example, chapter 7, “Ileoanal Punch Pathology”; chapter 9, “Microscopic Colitis”; and chapter 11, “Iatrogenic Diseases.”

In addition to extended coverage, keeping with advances in the field, the ouvrage offers a completely new iconography, with color pictures cover to cover. Most illustrations are of good quality, with the exception of a few low-power views. A slight deception, though, is the limited number of endoscopic pictures. Another minor criticism is that the font size has been slightly decreased. The eyesight of this reviewer has not improved since the 1987 edition; the font size may be strenuous to the reader.

A cardinal element of this masterpiece is the presentation of diagnostic pearls, allowing the authors to share with us the angst and limitations sometimes associated with the evaluation of biopsy material, even if one is well versed in the diagnostic criteria.

In conclusion, this book is a must-keep and a worthy successor to the first edition, which sits proudly next to it on my shelves. I wish only that each segment of the gastrointestinal tract could get such a devoted textbook. One would hope that all surgical pathologists, whether practitioner or academic, residents or fellows, would read this superb publication.

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Test Your Memory

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 edition of the ARCHIVES. Registered continuing medical education participants should answer these questions either online or use the December 2007 answer sheet.

1. A retrospective review of 136 extranodal marginal zone B-cell lymphomas (EMZLs) determined which of the following statements to be false?
   a. lymphoepithelial lesions (LELs) were present in 100% of all lung and thyroid gland EMZL cases
   b. 94% of all salivary gland EMZLs contained LELs
   c. the frequency of LELs was lowest in the breast and skin and soft tissue cases
   d. the frequency of destructive-type LELs tended to be higher in EMZLs with a larger number of total LELs, regardless of anatomic site
   e. monoclonal plasma cells were most commonly found in cases involving the lung and stomach
   (from Site-Specific Morphologic Differences in Extranodal Marginal Zone B-Cell Lymphomas—Rawal et al)

2. A recent study found that the number of Epstein-Barr virus (EBV)-positive Hodgkin lymphoma (HL) cases with the genotype GG at position −1082 was significantly higher compared with EBV-negative HL cases with the same genotype.
   True or False?
   (from Epstein-Barr Virus Infection and Single Nucleotide Polymorphisms in the Promoter Region of Interleukin 10 Gene in Patients With Hodgkin Lymphoma—da Silva et al)

3. When discussing cytologic features of nonneoplastic diseases of the lung and pleura, which of the following statements is true?
   a. Charcot-Leyden crystals have been frequently reported in serous fluids from patients with eosinophilic pleural effusions
   b. once inside the alveolar parenchyma, the mechanisms by which inhaled inorganic particles or fibers reach the pleural surface are not well understood
   c. reserve cell hyperplasia is similar to small cell carcinoma with regard to high nuclear-cytoplasmic ratio, extreme hyperchromasia, and nuclear molding
   d. smoker's pigment has a darker and coarser appearance in bronchoalveolar lavage (BAL) specimens compared with hemosiderin pigment
   e. eosinophils' characteristic red granules are not easily visualized in Diff-Quik preparations
   (from Cytology of Nonneoplastic Occupational and Environmental Diseases of the Lung and Pleura—Laucirica & Ostrowski)

4. Hep Par 1 (hepatocyte antigen) is positive in nearly all adenocarcinomas originating in the pancreas, biliary tree, breast, and colorectum.
   True or False?
   (from Best Practices in Diagnostic Immunohistochemistry—Kakar et al)

5. Upon review of 12 primary cutaneous carcinomas, the authors determined that which of the following findings is false?
   a. all tumors involved superficial to deep reticular dermis, with 4 lesions extending into subcutaneous fat
   b. evidence of intracytoplasmic lipid deposition was identified in 5 cases
   c. all cases showed the presence of tumor necrosis forming variably sized “comedones” in the center of large tumor aggregates
   d. neither epithelial membrane antigen nor carcinoembryonic antigen staining identified ductal differentiation in any cases
   e. expression of cytokeratin 7 was identified in 2 cases
   (from Adnexal Clear Cell Carcinoma With Comedonecrosis—Chaudhry & Zembowicz)

6. The JAK2<sup>V617F</sup> mutation is seen in only 10% to 15% of cases of polycythemia vera and therefore its detection is not viewed as being useful in a diagnostic workup of patients with erythrocytosis.
   True or False?
   (from Transformation of Polycythemia Vera to Chronic Myelogenous Leukemia—Mirza et al)