

abstracts of papers

AJCP Resident Research Symposium Finalists

Selected abstracts from the AJCP Resident Research Symposium and Poster Sessions, Annual Meeting of the American Society for Clinical Pathology (ASCP), October 27-October 31, 2010, San Francisco, CA.

The authors of the first 10 abstracts have been selected as finalists in the AJCP Resident Research Award competition.

The papers of these abstracts will be presented Saturday, October 30, 2010, from 9:30 AM to 12:00 PM at the ASCP Annual Meeting in San Francisco, CA.

Content, typographical errors, and inconsistencies in these abstracts are the responsibility of the abstract authors.

1 Cytomorphological Features of Pancreatic Mucinous Cystic Neoplasms: Comparison of Two Pancreatic Cystic Lesions With Endoscopic Ultrasound-Guided Fine-Needle Aspiration (EUS-FNA) and Histological Correlation

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Pancreatic cystic lesions are diagnostically challenging in EUS-FNA practice. Combining serological, radiological, and cytological findings can be helpful. However, carcinoembryonic antigen (CEA) and amylase can be complicated by comorbid conditions, and radiological findings may not be definitive for cyst type. Also, limited literature is available regarding cytomorphological features of pancreatic cysts. As such, definitive cytomorphological criteria for the variety of pancreatic cystic lesions are essential in establishing the correct diagnosis on EUS-FNA. We studied 2 cases in which different cystic neoplasms were suspected on EUS-FNA but confirmed as mucinous cystic neoplasms (MCN) by surgical histology.

Two women, 47 and 50 years old, underwent EUS-FNA and follow-up partial pancreatectomies. One patient presented with only slightly elevated CEA levels. The other patient had elevated amylase and lipase and normal CEA levels. On-site cytologic evaluation was conducted for only one of the patients. On cytology, macrophages and tall columnar cells were identified. One patient had additional findings of muciphages, multinucleated giant cells, and intracellular and extracellular mucin. Special stains and immunohistochemical studies were conducted on this patient. Additional studies were not available on the other patient owing to scant material submitted. As such, the cytopathologic impression differed for both cases, with only one case "suspicious" for MCN. On histology, both patients had areas of low-grade tall columnar cells with intracytoplasmic mucin vacuoles. The final diagnostic impression for both patients was MCN.

Cytological criteria such as muciphages, multinucleated giant cells, and mucin in addition with ancillary studies such as immunohistochemistry can further differentiate MCNs from other entities prior to surgical resection and, thus, better guide patient care and prognosis. However without specific criteria and adequate cytologic material, pancreatic cystic lesions still pose diagnostic difficulties on FNA regardless of serological

and radiological findings, requiring further cytomorphological studies.

2 Prediction of Final Grade in Adenocarcinoma of Endometrium Using p53, Ki-67, and Zeb1 Immunostains in Preoperative Biopsies

Marier Hernandez-Perez, MD, and Antonio De Las Morenas, MD. Pathology and Laboratory Medicine, Boston Medical Center, Boston, MA.

Endometrial biopsies can underestimate the final grade of differentiation of endometrioid adenocarcinomas. We have noted that the final grade of some cases of endometrioid adenocarcinomas may change from low grade in diagnostic biopsies to high grade in hysterectomy specimens.

We selected 10 cases with grade I endometrioid adenocarcinoma in endometrial biopsies. In 5 of these cases, the final grade remained unchanged after hysterectomy. In the other group of 5 cases, the grade changed from grade I to grade III in the final hysterectomy. Cases were immunostained with antibodies to p53, a transcription factor staining tumor cell nuclei; Ki-67, a protein associated with cell proliferation staining tumor cell nuclei; and the transcription factor ZEB1, staining stromal cell nuclei in aggressive endometrioid carcinomas. All cases were graded on a scale from 0 to 3. A score of 0 corresponded to lack of staining; 1 to weak staining of less than 50% of cells; 2 to strong staining of fewer than 50% or weak staining of more than 50% of cells; and 3 to strong staining of more than 50% of cells.

The scores for p53 for the cases that remained as grade I were 1, 1, 1, 1, and 1; for Ki-67 were 2, 1, 3, 1, and 1; and for ZEB1 were 2, 2, 2, 1, and 2. The scores for the cases that changed to high grade for p53 were 3, 3, 2, 3, and 3; for Ki-67 were 3, 3, 2, 3, and 3; and for ZEB1 were 3, 2, 2, 3, and 2. The 2 groups were tabulated and compared for each antibody using the *t* test; *P* values were .0004 for p53, .05 for Ki-67 and .1 for ZEB1. p53 and Ki-67 appear to be sensitive immunomarkers to predict the presence of a higher grade epithelial tumor in endometrial biopsies with grade I endometrioid adenocarcinoma. ZEB1 did not show a significant difference between cases that remained as grade I and cases that changed to grade III endometrioid adenocarcinoma.

3

Quantifying the Extent of Invasive Carcinoma and Margin Status in Partial Mastectomy Cases Having a Gross Lesion: Is a Defined Tissue Processing Protocol Needed?

Lisa D. Duncan, MD, and George Sneed, DO. Department of Pathology, University of Tennessee Graduate School of Medicine, Knoxville.

Accurate estimation of disease extent and margin status are critical when evaluating partial mastectomies because both are predictors of recurrence. The College of American Pathologists has published a protocol describing sampling recommendations for ductal carcinoma in situ to maximize precise determination of disease extent and margin status because most of these cases have no gross lesion. No published standards exist for processing specimens involved by invasive carcinoma, presumably because such cases have a grossly identifiable lesion.

To demonstrate the need for a standardized processing protocol for partial mastectomies having a gross lesion containing invasive carcinoma, we retrospectively studied 100 cases. In our laboratory, partial mastectomies are subjected to a mapping process in which the specimen is differentially inked, measured in 3 dimensions, and sequentially sectioned into slices, and slides are prepared from gross lesions and all fibrous stroma to facilitate microscopic reconstruction of the breast tissue. Disease extent is determined by reapproximation of involved slides and slices using calculation of average slice thickness. Using each case's slide key and slide diagram, disease extent and margin status of slides taken from lesional and surrounding marginal and fibrous tissue only (7-20 blocks) were compared with findings from the entirely mapped specimen (10-76 blocks).

In mapped vs nonmapped tissue, tumor size was larger in 17 cases (17%), pT was upstaged in 12 cases (12%), and positive margins were discovered in 8 cases (8%). Mapping revealed 6 cases (6%) of ductal carcinoma having an extensive intraductal component and 4 cases (4%) have additional foci of invasive carcinoma. Our study confirms that restricting histologic sampling to grossly abnormal tissue results in inaccurate pathologic staging and margin assessment in a significant number of cases. A standardized tissue mapping protocol for partial mastectomy specimens is needed to ensure adequate pathologic examination, even in cases having a gross lesion.

4

Is There a Role for Fatty Acid Synthase in the Diagnosis of Prostatic Adenocarcinoma? A Comparison With AMACR

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Although AMACR is a useful marker for prostatic adenocarcinoma (PC), it is less sensitive in certain PC variants and can be expressed in benign PC mimics. Fatty acid synthase (FASN) is up-regulated in PC and has been found to have high sensitivity (99%) and specificity (100%) in distinguishing PC from benign prostatic glands (BPGs). The aim of this study was to compare the utility of FASN to that of AMACR in the diagnosis of PC.

A tissue microarray containing foci of BPG (n = 54) and PC (n = 24) was immunostained with AMACR and FASN (BD Bioscience; 1:1,600). The intensity of expression (0-3+), percentage of positive cells (0%-100%), and the H score (0-300) were evaluated. In addition, the discriminatory powers of the 2 markers were compared by ROC analysis and by studying cases with coexistent BPG and PC on the same core.

FASN expression in PC was not significantly different from that of AMACR; however, there was significantly more FASN

expression in BPGs compared with AMACR, evidenced by higher averages of intensity (1.6 vs 0.6; $P < .05$), percentage of positive cells (66.8% vs 10.5%; $P < .01$), and H score (94.1 vs 11.6; $P < .0001$). ROC analysis showed that FASN had significantly less discriminatory power than AMACR (AUC, 0.848 vs 0.956; $P = .04$). Moreover, in 16 cases with coexistent PC and BPGs on the same core, FASN could distinguish PC in only 6 cases, compared with 13 cases by AMACR ($P = .03$).

Although FASN can be used to distinguish PC from BPGs, significant expression in BPGs reduces its discriminatory power and makes it less useful than AMACR in this capacity. Testing with further dilutions of the FASN antibody is being performed to assess whether its diagnostic utility can be improved and to confirm or refute its reportedly high sensitivity and specificity for the diagnosis of PC.

5

Is There a Role for Routine Evaluation of Prophylactic Sentinel Lymph Node Biopsy in Breast Carcinoma?

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Contralateral or bilateral prophylactic mastectomies are performed to reduce the risk of developing breast cancer. Sometimes an occult carcinoma is found in the "prophylactic breast," which prompts the surgeon to sample the lymph nodes for staging. Given the high morbidity of complete axillary lymph node dissection, some surgeons perform prophylactic sentinel lymph node biopsy (SLNB).

We undertook the study to examine if routine prophylactic SLNB is indicated in all patients undergoing prophylactic mastectomy. A retrospective review of all prophylactic mastectomies between January 2004 and April 2010 was performed. The stage of tumor on the disease side and the pathologic findings in the prophylactic breast were analyzed. The number of SLNs and the frequency of metastases were noted. The data for patients who received preoperative chemotherapy were analyzed separately.

A total of 142 prophylactic mastectomies on 135 patients were performed for breast carcinoma, of which 14 were bilateral prophylactic mastectomies performed in high-risk patients (for LCIS, presence of *BRC A* mutation, or strong family history). Before mastectomy, 19 patients received chemotherapy. Of the 142 prophylactic breasts, 15 had occult carcinomas (10.6%): 9 noninvasive (4 DCIS, 3 LCIS, 2 both DCIS and LCIS) and 6 invasive (all were T1; 4 were well-differentiated and 2 were moderately differentiated ductal carcinomas). The median number of prophylactic SLNs removed was 2 (range, 1-11). Only 1 of 135 patients had a positive prophylactic SLN, which originated from the disease side of the breast.

Although it is not uncommon to find occult carcinomas in the prophylactic breast, it is rare for the occult carcinoma to spread to the lymph nodes. Rarely, locally advanced carcinoma crosses over to the contralateral lymph nodes. Therefore, routine prophylactic SLNB is unnecessary and should be reserved for patients with advanced tumor in the disease side.

6

Follow-up Findings in Patients With a Negative ThinPrep Pap Test

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The significance of endocervical cells (ECs) and transformation zone (TZ) component in preventing false-negative cervical Pap smears remains controversial, while the revolutionary HPV DNA testing has increased the detection of cervical premalignant lesions.

We searched our archival file for a 1-year period and identified 600 cases of ThinPrep Pap tests with a diagnosis of negative for intraepithelial lesion or malignancy, including 295 lacking ECs/TZ component and 305 having ECs/TZ component. Results of concurrent high risk (HR)-HPV DNA testing by the HC2 System were obtained. Follow-ups are composed of Pap test(s) and/or biopsies within 1 year. The χ^2 test was used for statistical analysis.

The epithelial abnormality on follow-up was noted in 31/295 (10.5%) cases lacking ECs/TZ component and 29/305 (9.5%) cases with ECs/TZ component. The detection rates in these 2 groups were not significantly different ($P = .59$). A concurrent HR-HPV test was performed in 83 cases lacking ECs/TZ component and 57 cases with ECs/TZ component. The HR-HPV DNA was detected in 10/83 (12.0%) cases without ECs/TZ component and in 9/57 (15.8%) cases with ECs/TZ component. The positive rates in these 2 groups were not significantly different ($P = .53$). However, the epithelial abnormality on follow-up was identified in 7/19 (36.8%) cases in the HR-HPV DNA-positive group, which was significantly higher than 11/119 (9.2%) in the HR-HPV DNA-negative group ($P < .05$).

This study shows no significant difference in the follow-up results between the groups with and without ECs/TZ component. The absence of ECs/TZ component does not affect the detection of HR-HPV DNA. The cases with HR-HPV DNA more likely have epithelial abnormality on follow-up than cases without HR-HPV DNA detected. Therefore, HR-HPV DNA results may provide valuable information for risk assessment of patients with or without ECs/TZ component.

7

Detection of Myeloproliferative Leukemia W515L/K Mutation in JAK2^{V617F}-Negative Myeloproliferative Neoplasms

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A mutation in the myeloproliferative leukemia (*MPL*) gene, *MPL* W515L/K, was discovered in JAK2^{V617F}-negative patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF), with a prevalence of 2% to 8.5% and 10%, respectively. The World Health Organization in 2008 reclassified the myeloproliferative neoplasms (MPNs), incorporating this mutation as one of the major criteria in the diagnosis of these entities. Our study's objectives were to evaluate the prevalence of the *MPL* W515L/K mutation in patients diagnosed with or highly "suspicious" for MPNs and to evaluate the clinical utility of *MPL* genotyping findings.

Laboratory data were reviewed to identify patients who had *JAK2* mutation analysis done on their peripheral blood and/or bone marrow. Chart reviews were performed to select patients diagnosed with or suggestive of harboring an MPN. Archived DNA, originally used to assess *JAK2* status, was tested for the *MPL* W515L/K mutation by real-time PCR.

The test arm (JAK2^{V617F}-negative) had 44 patients, 26 women and 18 men, with ages ranging from 32 to 91 years (mean, 66 years). The *MPL* W515L/K mutation was observed in 3 cases in the test arm (6.8%); 2 for *MPL* W515L and 1 for *MPL* W515K. Of the 3 positives, one was diagnosed with ET, and a second was diagnosed with PMF. The third case had a clinical note indicating a history of ET, but no bone marrow biopsy was on record at our institution. None

of the patients in the control arm (JAK2^{V617F}-positive) were positive for the *MPL* mutation.

This clinical study detected the presence of the *MPL* W515L/K mutation in 3 of 44 patients (6.8%) diagnosed with or suspicious of harboring an MPN, consistent with what has been reported in the literature. *MPL* W515L/K screening in patients negative for the *JAK2* mutation can provide an additional criterion to aid in the diagnosis of MPN.

8

Gastrointestinal Stromal Tumor Markers in Cutaneous Melanomas: Relationship to Prognostic Factors and Outcome

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Melanoma is known to express c-kit, the prototypical marker for gastrointestinal stromal tumors (GISTs). Two new stains for GISTs, DOG1 and protein kinase C- θ (PKC), have not been evaluated for their staining patterns in primary and metastatic cutaneous melanomas. Furthermore, none of these stains has been correlated with prognostic factors or outcome.

Tissue microarrays were constructed with two 1-mm, formalin-fixed, paraffin-embedded cores from each of 62 primary cutaneous melanomas and 15 metastatic melanomas. Five-micrometer tissue sections were immunostained for polyclonal c-kit (pc-kit), monoclonal c-kit (mc-kit), PKC, and DOG1. Negative controls had primary antibody replaced with buffer. Slides were assessed for the presence of staining, percentage of cells staining, and lesional distribution of stain (in situ, radial, vertical). Results were correlated with prognostic parameters (Clark level, Breslow depth, lymph node status, local recurrence, and distant metastases) and outcome (overall and recurrence-free survival).

Thirty-four primary cutaneous melanomas (55.7%) stained for pc-kit, and 30 (48.4%) stained for mc-kit. PKC expression was seen in 10 cases (16.4%), and DOG1 expression in 2 of 59 cases (3.4%). Of the cutaneous melanomas that expressed pc-kit, 21 (67.7%) had a Breslow depth less than 1 mm, compared with 7 of 26 (26.9%) negative for pc-kit ($P = .002$). Also associated with a less than 1-mm Breslow depth was 3+ staining intensity for pc-kit (16/20 [80%]; $P = .003$). Melanomas positive for pc-kit had less nodal disease (1/31 [3.2%]; $P = .001$) and less chance of local recurrence (1/33 [3.0%]; $P = .021$), but no significant correlation with distant metastatic disease (19/32 [54.2%]; $P = .388$). This applied to in situ, radial, and vertical growth phase melanomas.

While c-kit is neither a sensitive nor a specific marker for cutaneous melanoma, its expression is correlated with a lower Breslow depth, less nodal disease, and less local recurrence. DOG1 and PKC have no diagnostic or prognostic value in cutaneous melanomas.

9

Expression of Thyroid Transcription Factor 1 (TTF-1) in Male Breast Cancer: A Case Report and Review of Literature

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TTF-1 has long been used as a highly specific marker for carcinomas of lung and thyroid origins. Recently, we identified a primary male breast cancer with strong, nuclear TTF-1 staining in invasive

and in situ components. We did a retrospective study of TTF-1 expression in male breast cancers in the archives of our department.

We identified 33 cases of primary invasive ductal carcinomas and 4 cases of in situ carcinoma in the archives from 1991 to 2009. In addition, 44 cases of male gynecomastia specimens were identified. Immunohistochemical stains for TTF-1 were performed with adequate controls on all 81 cases by automated methods (Leica Bond III, Bannockburn, IL) using TTF-1 mouse monoclonal antibody (M3575; DAKO, Carpinteria, CA).

Of the 33 cases of invasive cancer, 67% had associated in situ carcinoma. The mean age of the patients was 63.9 years, and the mean tumor size was 2.0 cm. The majority of cases were moderately to poorly differentiated (17/33 and 14/33, respectively), and 2 were well differentiated. Of the 33 cases, 31 were estrogen receptor-positive, and only 9 were HER-2/neu-positive. Only the index case revealed unequivocal nuclear staining for TTF-1, in the invasive and in situ components. In addition, strong, diffuse, cytoplasmic staining was seen in 1 case in the region of mucinous differentiation, but not in the typical ductal carcinoma component. All gynecomastia cases were negative.

Lung cancer is about 60 times more common in men compared with primary breast carcinoma. In the absence of a distinct in situ component, a diagnosis of primary male breast cancer includes a differential diagnosis of metastasis from an undiagnosed lung primary. Our study reveals that TTF-1, to date considered a very specific marker of lung and thyroid origins, can rarely be positive in male breast cancer. This is of particular importance when analyzing a carcinoma of unknown primary.

10

The Added Value of Molecular Testing in Small Pancreatic Cysts

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Pancreatic cysts occur in more than 1% of medical patients and range from nonneoplastic pseudocysts to malignant mucinous neoplasms. The most recent international guidelines recommend resection of mucinous cysts larger than 3 cm or smaller cysts with positive cytology, mural nodules, or symptoms. Cytology plays a central role in diagnosis in conjunction with imaging, cyst fluid analysis, and, more recently, molecular testing. While an elevated level of CEA supports mucinous differentiation, it cannot distinguish benign from malignant. Recent studies have shown high-amplitude *k-ras* gene mutation and allelic imbalance are predictive of malignancy. Our aim was to determine the added benefit of molecular testing in diagnosing small (≤ 3 cm) pancreatic cysts.

We retrospectively obtained 59 pancreatic cysts 3 cm or smaller with fine-needle aspiration cytology, cyst fluid CEA levels, and molecular analysis (PathFinder TG; RedPath Integrated Pathology, Pittsburgh, PA). Diagnoses were classified as unsatisfactory, benign nonmucinous, benign mucinous, and suspicious/malignant. RedPath criteria for mucinous lesions include *k-ras-2* gene point mutation, high DNA quantity (optical density ratio >10)/DNA quality, or loss of heterozygosity (LOH) in 2 or more genomic loci; criteria for malignancy include *k-ras-2* gene mutation, high amplitude ($>75\%$), or 2 or more genomic loci with LOH, high amplitude ($>75\%$).

Concordant diagnoses were seen in 53% (31/59) of cases. In 9 cases (15%), there was disagreement between cytology and molecular (benign mucinous vs benign nonmucinous). Molecular testing provided a diagnosis in 19 cases (32%) when cytology was unsatisfactory or CEA not elevated (<192 ng/mL). Elevated CEA levels were seen in 25% of cases, each diagnosed as a mucinous lesion with molecular analysis.

Molecular analysis of pancreatic cyst fluid demonstrates added diagnostic value in scant specimens when cytology may be unsatisfactory and CEA unreliable.

11

Utility of CD44s and CD44v6 Expression in the Differentiation Between Non-Small Cell and Small Cell Carcinomas of the Lung

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CD44, a transmembrane glycoprotein receptor, plays a major role in tumor progression, invasion, and metastasis. The purposes of this study were to evaluate the expression of CD44 standard (CD44s) and one of its variants, CD44v6, in normal lung tissue and in common lung tumors and to determine their prognostic significance in these tumors.

This retrospective study consisted of 5 cases of normal lung, 52 cases of non-small cell carcinomas (NSCLC; 21 squamous cell carcinomas and 31 adenocarcinomas), and 23 cases of neuroendocrine tumors (15 small cell lung carcinomas [SCLCs] and 8 carcinoid tumors). We evaluated the expression of CD44s and CD44v6 receptors by immunohistochemical staining.

CD44s and CD44v6 expression was correlated with lymph node metastasis, tumor size ($>$ or $<$ 3 cm), and pleural invasion. In normal lung tissue, positive staining for CD44s and CD44v6 was restricted to the epithelial lining of the lower third of the major bronchus. Pneumocytes and bronchial gland epithelium expressed only CD44s. CD44s and CD44v6 were expressed in all squamous cell carcinomas and variably expressed in adenocarcinomas (39% and 45%, respectively). Carcinoid tumor expressed only CD44s in 88%. All cases of SCLC were negative for CD44s and CD44v6. A restricted panel that consists of CD44s and CD44v6 could differentiate NSCLC from SCLC with a sensitivity of 67%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 53%. In adenocarcinomas, CD44s was expressed in 64% (9/14) of tumors <3 cm and in 33% (4/17) of tumors larger than 3 cm. No correlation was found between CD44s or CD44v6 and lymph node metastasis or with pleural invasion.

We conclude that in normal lung tissue, CD44s is expressed in a regional distribution. A limited immunohistochemical panel that consists of CD44s and CD44v6 could potentially be used to discriminate NSCLC from SCLC. CD44s expression correlates with tumor size in adenocarcinomas.

12

The Utility of GLUT-1 Staining in the Immunohistochemical Evaluation of Peripheral Nerve Sheath Tumors

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Peripheral nerve tissue consists of different types of cells, including Schwann cells, perineurial cells, fibroblasts, and nerve axons. Perineurial cells play an important role in maintaining a diffusion barrier for the peripheral nerve, creating a blood-nerve barrier. The goal of this study was to determine the presence of perineurial cells in a subset of common peripheral nerve sheath tumors (PNSTs).

This retrospective study consisted of 91 cases of PNSTs, including 20 neuromas, 32 neurofibromas, 2 perineuriomas, 17 schwannomas, and 20 malignant PNSTs (MPNSTs). Perineurial cells were evaluated using GLUT-1 and EMA immunohistochemical staining. Staining was observed in 19 cases (95%) of neuroma, 16 cases (94%) of schwannoma, and 7 (22%) cases of neurofibroma. In these cases, GLUT-1 and EMA staining were confined within 1 to 3 cell layers in the capsule around the lesion. All perineuriomas showed diffuse staining for both markers. MPNSTs exhibited a unique staining pattern for GLUT-1 in 16 cases (80%) in which tumor cells around the blood vessels were negative but those away from the blood vessels were positive. All MPNSTs were negative for EMA. Based on histologic review and GLUT-1 and EMA results, a case of neuroma and of neurofibroma were reclassified as perineurioma.

We conclude that the capsules of neuromas, schwannomas, and some cases of neurofibromas contain perineurial cells. These cells may have originated from the perineurium and were pushed to the periphery by the proliferating tumor cells. GLUT-1 staining in malignant tumors could be attributed to the cellular response to hypoxia and might be a survival adaptation of tumor cells away from the blood supply. A trend for GLUT-1 staining emerged in benign and malignant nerve sheath tumors. However, owing to the limited samples of this study, we cannot be certain that these staining patterns could be used reliably to differentiate benign from malignant PNSTs.

13

The Relative Performance of ProEx C for Detection of Women at Risk of Cervical Cancer

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Minichromosome maintenance protein 2 (MCM2) and topoisomerase 2 α (TOP2 α) are markers of aberrant S-phase cell cycle induction triggered by HPV E6/E7 oncogenes. Therefore, MCM2/TOP2 α has the potential to serve as a specific marker of persistent transforming infection with HPV. ProEx C (BD Tripath) is an immunocytochemical assay designed to detect MCM2/TOP2 α . As part of a multicenter study in Canada to assess the application of HPV DNA, HPV mRNA, and biomarker testing in cervical cancer screening, the ProEx C test was evaluated in comparison with the Hybrid Capture 2 HPV DNA (HC2; Qiagen) and PreTect HPV-Proofer (Proofer; Norchip) and APTIMA (Gen-Probe) HPV mRNA assays.

Patients referred to colposcopy in 5 of the 10 Canadian provinces were enrolled. Cervical specimens obtained at enrollment in SurePath LBC were utilized for ProEx C testing with separate parallel specimens collected in PreservCyt LBC used for HPV DNA and mRNA testing. Histology-confirmed CIN 2+ served as the disease end point to assess the relative performance of ProEx C in comparison with the HPV DNA and mRNA tests.

The interim analysis was based on 796 patients having all 4 tests with histologic diagnosis available for baseline. This included 232 histology-confirmed CIN 2+, 198 CIN 1, and 366 negative cases. ProEx C showed a sensitivity of 73.7% for the detection of CIN 2+ with a specificity of 76.2%. The corresponding figures for the HPV tests were as follows: HC2, 95.3% and 38.3%; Proofer, 78.9% and 75.2%; and APTIMA, 94.4% and 46.8%.

ProEx C showed a lower sensitivity and higher specificity than both HC2 and APTIMA and strikingly similar performance to that of Proofer. Owing to its significantly higher specificity, ProEx C has the potential to serve as a more specific test for the triage of borderline cytologic abnormalities as an adjunct to cytology. The ongoing longitudinal portion of the study will further assess the higher clinical specificity of ProEx C and explore its potential utilization in cervical cancer screening.

14

"Wrong Blood in Tube": Solutions for a Persistent Problem

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Over the past 5 years (2005-2009), 59,373 type and screens were performed at our institution, and a total of 26 major errors ("wrong blood in tube" [WBIT]) were identified, posing potential risk for our patients. Indeed, in 2006, 1 patient was given ABO-incompatible blood owing to a WBIT-type error. Of the errors, 8 (30%) were detected by discrepant typing results (comparison with historic blood type), 6 were discovered by the clinical service, and 12 were identified in the blood bank by other means (eg, second label found under the first). Our estimated "raw" WBIT rate (1 in 2,283 samples) is comparable to that (1:2,262) in the published literature.

Since 2006, our nursing policy mandates that "all type, screen and cross will have two witnesses to the correct ID of the patient and labeling is done at the bedside at the time of the draw." This has reduced (from 11 in 2006 to 5 in 2007) but did not eliminate our WBIT problem that persisted into 2008 and 2009 (3 and 7 incidents, respectively). Since February 27, 2009, as an additional safety measure, we also require a second, independently drawn sample in previously untyped patients (that is, no historic record) who are likely to receive transfusions. So far, all of the requested second draws (258 specimens) have confirmed the results of the first draw.

We conclude that miscollected specimens (WBITs) continue to represent a leading cause of potential mistransfusions at our institution. Changes in nursing (2 witnesses to correct ID) and/or blood bank policy (check-type with a second specimen) may reduce but not eliminate this persistent problem. Clearly, additional safety measures (eg, blood recipient verification systems such as Typenex, scannable bar-codes, or radio frequency identification) are required to prevent WBIT-type errors.

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Ephrin B2 Receptor Expression Is Decreased in Stage III Colon Cancers With a High Level of Microsatellite Instability

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Ephrin B2 receptor (EPHB2) is involved in colonic epithelial cell adhesion. Loss of EPHB2 expression has been associated with invasive colorectal adenocarcinoma (CRC). It is unclear if inactivation of DNA mismatch repair genes is associated with decreased EPHB2 expression.

We evaluated EPHB2 expression and its prognostic significance in stage III CRC patients stratified by microsatellite status.

We identified all stage III CRC patients from 1996 to 2006 who received adjuvant chemotherapy at our institution. Tissue microarrays were constructed to evaluate EPHB2 expression (high, 2+/3+; low, 0/1+) and microsatellite status by immunohistochemistry (IHC). Microsatellite stable (MSS) tumors were defined as MLH1/MSH2 (+/+) by IHC; tumors staining otherwise were classified as having a high level of microsatellite instability (MSI-H). Chart review was conducted to determine disease-free survival (DFS) and overall survival.

We identified 149 cases (median age, 63 years; 49% male), of which 123 had adequate tissue for microarray analysis. EPHB2 stained high in 78 (70%) and low in 45 (30%). Of the cases, 107 (87%) were MSS and 16 (13%) were MSI-H. MSS tumors were more likely to have high EPHB2 expression (73/107 [68%]) than MSI-H tumors (5/16 [31%]; $P < .01$). DFS at 3 years was 60% for EPHB2-low and 38% for EPHB2-high ($P = .08$). By 4 years, DFS curves converged and no difference was observed during complete follow-up ($P = .13$). No difference in DFS was observed among 4 subsets of MSS and MSI-H tumors staining EPHB2 high or low ($P = .09$).

As MSI-H tumors are closely associated with CIMP phenotype, the finding of decreased expression of EPHB2 in MSI-H tumors supports the possibility of *EPHB2* gene inactivation in MSI-H tumors through the mechanisms of *EPHB2* gene mutation and/or DNA hypermethylation. EPHB2 did not significantly discriminate as a prognostic factor but showed a trend toward identifying earlier relapse.

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Lyme Disease Testing by Multiplexed Bead Analysis Using VlsE1 and pepC10 Antigens Compared With 2-Tiered Testing

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Currently, the diagnosis of Lyme disease is based on clinical manifestations and history of exposure to vector ticks in an area where Lyme disease is endemic. Laboratory tests with high diagnostic accuracy are important because appropriate treatment of Lyme disease is highly effective and a missed diagnosis can lead to adverse consequences due to sequelae of untreated *Borrelia burgdorferi* (Bb) infection.

Since 1995, the Association of Public Health Laboratories and the CDC have recommended a 2-tiered approach to serologic testing for Lyme disease in the United States. In our laboratory, serum is first tested by a sensitive method using a fluorescent-based serologic test (ELFA). Positive or equivocal samples are "reflexed" to Western blot procedure. This two-tiered testing is relatively insensitive for patients with early-stage disease.

Recently, new serologic tests based on recombinant antigens and synthetic peptides from Bb have been developed. We obtained and validated 2 such peptides of Bb (VlsE1 and pepC10) from ZEUS Scientific, packaged as a multiplexed bead assay, and compared these tests with the results of the standardized 2-tiered testing as a retrospective study. The sample size was 75.

The likelihood ratios were as follows: positive likelihood ratios: PepC10, 2.40; Western blot, 2.38; VlsE1, 2.14; and ELFA, 1.34; negative likelihood ratios: VlsE1, 0.76; ELFA, 0.59; PepC10, 0.55; and Western blot, 0.38. A simple correlation of the number of positive tests to the available clinical diagnosis using the Pearson correlation coefficient showed significant correlation (0.456; $P < .001$).

PepC10 and VlsE1 have high positive likelihood ratios with low negative likelihood ratios, comparable to the current 2-tiered method.

VlsE1 showed the highest specificity for the clinical diagnosis of Lyme disease. When taken in aggregate, the more positive tests there are, the more likely it is that the independent clinical diagnosis will be positive for Lyme disease.

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VCS Parameters Are Additional Methods for Diagnosing Chronic Lymphocytic Leukemia (CLL)

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Our purpose was to investigate the value of VCS parameters generated by VCS technology of the Coulter LH750 hematology analyzer as a sensitive indicator for the diagnosis of CLL. VCS parameters such as neutrophil volume (NV), neutrophil volume distribution width (NVDW), neutrophil conductivity, neutrophil conductivity distribution width (NCDW), neutrophil scatter, neutrophil scatter distribution width (NSDW), lymphocyte volume (LV), lymphocyte volume distribution width (LVDW), lymphocyte conductivity, lymphocyte conductivity distribution width (LCDW), lymphocyte scatter, and lymphocyte scatter distribution width (LSDW) from 17 patients with CLL proven by flow cytometry/immunohistochemistry and from 40 age-matched control subjects were analyzed by using the Student *t* test.

Patients with CLL in comparison with a control group demonstrated a statistically significant increase in the following parameters: NV (154 vs 142; $P < .0001$), NVDW (33.5 vs 20.4; $P < .0001$), NCDW (8.8 vs 5.9; $P < .0001$), NSDW (16 vs 11; $P < .0001$), LVDW (21.4 vs 13.9; $P < .0001$), LCDW (14.3 vs 10.6; $P < .0001$), and LSDW (19.6 vs 17; $P < .0001$) and a decrease of LV (73 vs 82.3; $P < .0005$).

VCS quantitative parameters are additional tools for diagnosing CLL.

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Liver Histopathology in Patients With Nonalcoholic Fatty Liver Disease and Obstructive Sleep Apnea

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Obstructive sleep apnea (OSA) and obesity are common in patients with nonalcoholic fatty liver disease (NAFLD). Whether intermittent nocturnal hypoxemia associated with OSA influences the severity of NAFLD has not been established.

This case-control study included 134 morbidly obese Caucasians with OSA and race, age, and body mass index (BMI)-matched control subjects (CTLs). All subjects underwent intraoperative liver biopsy during bariatric surgery. Subjects with more than 20 g of daily alcohol use or those with evidence of other liver disease were excluded. OSA was defined by having a documented diagnosis in the patient's medical record and/or diagnostic sleep study in our records. Liver histology was assessed for steatosis, fibrosis, lobular and portal inflammation, hepatocyte necrosis, ballooning, and Mallory hyaline. Liver biopsies were diagnosed as normal, simple steatosis, borderline nonalcoholic steatohepatitis (NASH), and NASH. Frequency and severity of different NAFLD histological features were compared between the 2 groups.

The study included 67 subjects with OSA and 67 CTLs. The mean BMI was 49.2 for OSA and 46.3 for CTLs. Subjects with OSA had a higher frequency of dyslipidemia (43% vs 27%; $P < .07$) and advanced fibrosis (\geq stage 2, 11% vs 1%; $P < .06$). Liver biopsies from the OSA and CTL groups showed normal histology (34% vs 48%), simple steatosis (34% vs 31%), borderline NASH

(24% vs 13%), or NASH (7% vs 7%). Different clinical variables between the 2 groups not statistically significant included diabetes, hypertension, and metabolic syndrome. Differences in steatosis, lobular and portal inflammation, hepatocyte necrosis, ballooning, and Mallory hyaline were also not statistically significant.

Morbidly obese Caucasian subjects with NAFLD and OSA tend to have more advanced fibrosis. Early evaluation of NAFLD severity in these subjects may be warranted.

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***Campylobacter jejuni*-Induced Apoptosis of THP-1 Macrophages Correlates With Virulence Genes and In Vitro Invasiveness**

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Induction of host cell apoptosis has emerged as a common virulence mechanism among bacterial pathogens. Toxin production, secreted proteins, and invasion are the 3 virulence mechanisms that have been identified in the pathogenesis of *Campylobacter*. In this study, we investigated the relation between invasiveness of isolates of *Campylobacter jejuni* with different invasive capacities and their extent of apoptosis induction in the differentiated human macrophage cell line THP-1. We further assessed the impact of possession of 3 different virulence genes (*cia*, *iam*, and *cdtB*) by these isolates on this process.

Eight *C jejuni* isolates from patients in the kingdom of Bahrain with established invasive capabilities and expressing all, only 2, or none of the virulence genes were assessed for extent of apoptosis induction of THP-1 cells. Apoptosis was assessed using 3 different methods. Gel electrophoresis was used to detect DNA laddering. Phosphatidylserine translocation and DNA strand breaks were detected by flow cytometry following annexin-V labeling or fluorescein-dUTP end-labeling (TUNEL assay), respectively.

Gel electrophoresis revealed clear DNA laddering following 36 hours of in vitro invasion of THP-1 cells (suggesting extensive apoptosis) with the 2 most invasive isolates, which possess all 3 virulence genes, while faint laddering was evident following invasion by the 2 isolates of intermediate invasiveness, which possess *ciaB* and *cdtB*. Isolates of lower invasiveness, including 2 possessing only *iam* and *cdtB* and 2 that lack all 3 virulence genes, did not show any laddering.

Flow cytometry following labeling with the 2 methods (annexin-V and fluorescein-dUTP) similarly showed the highest levels of apoptosis following invasion by the 2 most invasive isolates that possessed all 3 virulence genes, while the 2 isolates with lowest invasiveness and that did not possess any of the 3 genes showed the lowest levels of apoptosis. Isolates possessing only 2 virulence genes and that were intermediate in their invasiveness showed intermediate levels of apoptosis induction. Although the 2 isolates possessing *cdtB* and *cia* were more invasive than those possessing only *cdtB* and *iam*, this was not sharply reflected in their extent of apoptosis induction.

These data suggest a direct correlation between possession of virulence genes, invasiveness, and extent of apoptosis induction. Correlation between apoptosis induction and the clinical picture of patients from whom isolates were obtained showed an association between the higher degree of invasiveness, extent of apoptosis, and severity of clinical symptoms. The importance of apoptosis in the pathogenesis of *Campylobacter* is, however, still undefined.

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Generating Evidence-Based Interpretation of Hematology Screens via Anomaly Characterization

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We have developed a simple, workable, computer-generated second opinion for any set of data from a hemogram with high accuracy with an electronic medical record. This has been made possible as a result of advances in mathematics, low computational costs, and rapid transmission of the necessary data.

The database used for this study is a reasonable file of 22,000 laboratory hemograms generated by 2 Beckman-Coulter analyzers. All control samples, patient identifiers, and data for patients younger than 23 years were stripped from the data set. A reviewer experienced in medicine reviewed the data and labeled all diagnoses identifiable. We propose a novel method for anomaly detection and classification via anomaly characterization. For a given sample in the system, we characterize its anomalous behavior via nonlinear differential diagnosis processes. Then, once its characterization profile, which is its unique differential metric, is constructed, we identify other anomalies that match this differential profile.

For 99.84% of the patients, the algorithm identified the diagnoses that were given by the reviewer. The algorithm detection rate is as follows: microcytic anemia, 99.63%; normocytic anemia, 98.03%; moderate/severe SIRS, 96.69%; thrombocytopenia, 99.52%; leukocytopenia, 84.83%; and normal, 93.18%.

This limited analysis of only automated hematological information gives promising results for extending the method to the case of more complicated conditions than presented and for extension to a combination of chemistry, hematology, immunology, and other data.

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Classical Hodgkin Lymphoma in Elderly Patients: A Single Institutional Experience

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Classical Hodgkin lymphoma (cHL) has a bimodal age incidence curve with most patients presenting either in adolescence/young adulthood or late in life. While the clinicopathologic features of cHL in young adults are well described, less is known about elderly patients. Recent studies have shown that elderly cHL has a more aggressive course if Epstein-Barr virus (EBV) is detected.

Archived histologic specimens were retrieved from the University of Michigan files for cases in which the diagnosis of cHL was made on or after age 60 years. The pathologic and clinical features of 65 patients were examined.

Cases included 51% men and 49% women aged 60-90 years (median 68 years). 39 (60%) cases were subclassified as nodular sclerosis, 13 (20%) mixed cellularity, 5 (8%) lymphocyte-rich, and 8 (12%) unclassifiable. In 33 cases, in situ hybridization for EBV was performed. Forty-five percent had EBV-positive tumor cells; more than half of these were of the nodular sclerosis (NSHL) type. Fifty-one cases had bone marrow staging biopsies evaluated at our institution and 16 (31%) were positive or suspicious for cHL. An additional 4 patients (8%) showed bone marrow involvement at the time of relapse. There was no correlation between EBV status and bone marrow involvement. Ten patients (15%) had evidence of non-Hodgkin lymphoma and 13 (20%) had a history of carcinoma.

In this study, cHL in the elderly affected males and females equally and many patients had comorbid malignancies. Elderly cHL often presented with stage IV disease or EBV positivity, features that have been associated with immunodeficiency in other patient populations. In this study, the presence of EBV positivity did not correlate with bone marrow involvement. The frequency of bone marrow disease was nearly 40%, supporting the utility of staging bone marrow biopsies at diagnosis and recurrence, regardless of EBV status.

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A Comparison of Three Different dsDNA Assays

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The clinical symptoms of connective tissue diseases are not specific for autoimmune disease and occur in other diseases, making a diagnosis based on clinical symptoms alone difficult. Laboratory testing of autoantibodies is an important adjunct to diagnosing connective tissue diseases. Double-stranded DNA (dsDNA) is a traditional biomarker for screening connective tissue diseases. Antibodies to dsDNA are heterogeneous with respect to class, avidity, and cross-reactivity. Several different methodologies for dsDNA testing are commercially available.

In an effort to improve accuracy for dsDNA testing, we conducted a small study to compare 3 different dsDNA assays. Thirty-eight patient samples were tested using the following methods: Farr radiobinding assay, multiplex flow immunoassay, and ELISA. A single specimen from each patient was used for each assay. Clinical presentation and results from serological testing established diagnosis of SLE.

When using the appropriate cutoff for the kit, the multiplex assay detected fewer known SLE cases (9/38) than the Farr (21/38) or ELISA (14/38) method. The Farr assay results were consistent with the clinical diagnosis of SLE than the ELISA and multiplex methods.

These assays differ in the source of DNA used as antigen and specificity and sensitivity to antibodies. The Farr assay detects a high-avidity subset of antibodies, while ELISAs detect lower avidity antibodies. Owing to the variability in antibody reactivity, it may be useful to combine high-avidity antibody methods with low-avidity antibody methods to improve specificity and sensitivity. This study also demonstrates the need for some type of standardization or disclosure of differences that might be expected among these assays. Until test procedures are standardized, clinicians should be aware that analytical differences can exist among methods that may not reflect clinical severity of the disease. Monitoring patients with one method for dsDNA is recommended.

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Utility of UroVysion FISH in Distinguishing Nested Urothelial Carcinoma From Proliferative von Brunn Nests

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Nested urothelial carcinoma is a rare and clinically aggressive histologic variant of urothelial carcinoma, characterized by deceptively bland histologic features resembling benign von Brunn nests, which can pose significant diagnostic problems, particularly in limited superficial biopsy material. No unique immunoprofile has reliably differentiated von Brunn nests from nested urothelial carcinoma. The aim of this study was to determine if the UroVysion fluorescence in

situ hybridization (FISH) probe set could reliably distinguish benign proliferative von Brunn nests from nested urothelial carcinoma.

UroVysion FISH was performed on paraffin-embedded tissue sections from 10 negative and 5 positive controls, 13 proliferative von Brunn nests, and 17 pure nested urothelial carcinomas. The number of cells exhibiting polysomy, single chromosomal gains, and homozygous 9p21 deletion were recorded in 50 cells. Normal cutoffs for each chromosomal abnormality were determined from negative controls using beta inverse function with 95% confidence level.

All nested urothelial carcinomas were positive by FISH (100%). Single gains, polysomy, and homozygous 9p21 deletion were present in 77%, 59%, and 35% of cases, respectively. Exclusive homozygous 9p21 deletion was common in the nested urothelial carcinoma (24%), and polysomy and/or single gain with concurrent homozygous 9p21 deletion was observed in a smaller number of cases (12%). No chromosomal alterations were identified in any of the proliferative von Brunn nests.

UroVysion FISH can be performed on paraffin sections and accurately distinguish nested urothelial carcinoma from its benign mimicker, proliferative von Brunn nests. Nested urothelial carcinomas have complex chromosomal alterations composed of polysomy, single gains, and/or homozygous 9p21 deletion, each of which are absent in benign von Brunn nests. UroVysion may have clinical laboratory application to help differentiate nested urothelial carcinoma from proliferative von Brunn nests in minute biopsies.

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Leptin Expression in Estrogen Receptor–Positive Breast Carcinoma Compared With Commonly Utilized Prognostic Markers

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The adipocyte-derived peptide leptin plays a role in the development of breast cancer by inducing growth of breast cancer cells through activation of several pathways. Human breast cancers have been found to have increased leptin and receptor isoform expression. Evidence suggests that breast tumorigenesis is associated with an increase in leptin expression (LE). Our goal was to compare the level of LE with breast cancer prognostic markers, including Oncotype DX recurrence score (RS) (Genomic Health, Redwood City, CA).

Estrogen receptor–positive breast carcinomas from patients with Oncotype DX RS testing over a 2-year period were included. Tissue microarrays were created from representative tumor samples and stained with rabbit polyclonal antibody to leptin (Santa Cruz Biotechnology, Santa Cruz, CA). Staining was scored using the following scale: less than 10%, 0; 10% to 50% weak staining, 1+; more than 50% weak staining, 2+; and more than 50% strong staining, 3+. LE was compared with tumor grade, tumor size, lymph node status, MIB-1 proliferation index, and Oncotype DX RS.

Of the 133 cases, the majority (52.6%) showed little or no LE (70/133), 33.8% (44/133) stained 1+, and 10.5% (14/133) scored 2+, while only 3.0% (4/133) demonstrated strong staining. Strong LE was significantly associated with high RS (2/4, 50%) compared with tumors with no/weak expression, the majority of which (71/129) had a low RS ($P = .02$). Results trend toward association of strong LE with higher grade, but this was not statistically significant ($P = .08$). No association was noted between strong LE and tumor size, lymph node metastasis, or MIB-1. While the majority of breast tumors demonstrated focal and/or weak LE, only a small percentage exhibited strong diffuse staining. Tumors with strong LE demonstrated a

significant association with higher RS compared with absent/weakly stained tumors ($P = .02$). Our findings are limited by small sample, and analysis of a larger cohort may prove useful.

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High-Grade Cervical Lesions Following High-Risk Human Papillomavirus–Positive Atypical Squamous Cells SurePath Specimens: A Quality Assurance Study

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Diagnosis of atypical squamous cell of undetermined significance (ASC-US) represents 5% to 15% of Papanicolaou (Pap) tests. Testing for high-risk human papillomavirus (HR-HPV) with Hybrid Capture 2 (HC2) is a well-established and highly sensitive method for triaging patients for further management. Based on the ASCUS/LSIL Triage Study (ALTS), the rate of HR-HPV positivity in ASC-US ranges from 31% to 59.7%, and overall detection of CIN 3 is 25%. In this retrospective study, we evaluated the rate of HC2 HR-HPV positivity on ASC-US SurePath Pap smears preceding the histological diagnosis of high grade (CIN 2-3) lesions.

From 2006 to 2008, liquid-based SurePath (TriPath Imaging, Burlington, NC) Pap smears interpreted as ASC-US with reflex HC2 (Digene, Gaithersburg, MD) HPV testing and histological follow-up (cervical/cone biopsy, endocervical curettage, hysterectomy) were analyzed ($n = 348$). Rates of high-grade histology in HR-HPV+ and HR-HPV– groups were compared. The ASC-US rate in our laboratory is 11.5%. Among patients with ASC-US, the HPV+ rate was 63%. All cases of CIN 3 and 96% of CIN 2 tested positive for HR-HPV. Among HPV+ patients, 54% had high-grade dysplasia on their histological follow-up. The rate of severe dysplasia/carcinoma in situ was 10.4% for the HR-HPV+ and 0% for the HR-HPV– group. Only 4 of 128 patients with HR-HPV ASC-US had moderate dysplasia (CIN 2) on histological follow-up (3.1%). Of 220 HR-HPV+ samples, 60 (27.3%) had negative histological follow-up.

By performing HPV testing on ASC-US samples, the number of unnecessary colposcopy referrals can be significantly reduced. Our results confirm the excellent sensitivity of the HC2 HPV assay for detection of cervical cancer precursor lesions on SurePath specimens by identifying all patients with CIN 3 and virtually all (96%) with CIN 2 lesions. HR-HPV+ samples with negative histological findings (27.3%) are possible indicators of regression or limited sampling and suggest further need for follow-up.

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Cytopathologic Interpretative Variation of Hashimoto Thyroiditis: Synopsis of 1,886 Responses From the ASCP NonGYN Assessment Program

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Hashimoto thyroiditis is a relatively common disease seen in thyroid cytopathology samples; therefore, specimens from FNA and touch-prep samples of this entity were incorporated into the ASCP NonGYN Assessment Program. The ASCP NonGYN Assessment Program is a glass-slide program developed with oversight from the ASCP NonGYN Assessment Committee. Each annual program is composed of FNA and NonGYN samples for a total of 20 patient cases, divided into 4 quarterly shipments of 5 cases. Laboratory and individual peer-comparison statistics for each case reviewed are provided to all participants after event participation.

Performance data from 1,886 total responses from 7 cases of Hashimoto thyroiditis, circulating in the NonGYN Assessment program since 2007, were extracted and reviewed. Of the participants, 82.1% chose the correct response of a negative or inflammatory process consistent with Hashimoto thyroiditis, while 12.1% classified the cases as positive for malignancy, the most common choice (6.4%) responding with malignant lymphoma. Less common, yet interesting, were malignant responses of 3.0% anaplastic carcinoma and 2.3% lesion of uncertain biologic potential, follicular neoplasm. The remainder of responses included 0.3% nonneoplastic goiter, 1.4% granulomatous thyroiditis, 0.7% metastatic melanoma, and 1.1% papillary carcinoma. Of the participants, 2.7% did not provide an interpretation response.

In light of the 12.1% response rate for positive for malignancy, further microscopic evaluation of these cases was performed. The somewhat pleomorphic Hürthle cells with prominent nucleoli and the lack of colloid may have contributed to the anaplastic carcinoma responses and to the follicular neoplasm responses. Responses of malignant lymphoma are self-evident, in light of the prominent lymphoid component in Hashimoto cases. Generally lymphomas have a more monotonous population with minimal, if any, epithelial component. However, such a distinction may not always be possible based on the morphology alone, and immunostaining or flow cytometry might be necessary.

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A Rare Case of Hemoglobin San Bruno: Could This Hemoglobin Cause Thalassemia Trait–Like Hematologic Changes?

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Hemoglobin San Bruno (HSB) was relatively recently described in a 37-year-old African American woman from San Bruno, CA, and named according to this city (Hoyer et al, 2002). It was established that HSB is a β -chain hemoglobin variant caused by a mutation involving the codon 39, CAG \rightarrow CAC; [β 39 (C5) Gln \rightarrow His]. The mutation involves the α 1/ β 2 contact region. In this initial report, it was documented that the presence of the HSB trait is not associated with any clinical and hematological manifestations.

We present here a case of HSB that shows thalassemia trait–like changes in the peripheral blood. This is, to the best of our knowledge, a second case of HSB in the literature and the first pediatric case.

A blood specimen was received from a 4-year-old African American boy who presented to his pediatrician with mild microcytic hypochromic anemia (hemoglobin, 9.5 g/dL; hematocrit, 32.3%; RBC count, 4.44/mm³; MCV, 72.8 fL; MCH, 21.4 pg; and RDW, 13.9%). Hemoglobin studies, which included alkaline and acidic electrophoresis and high-performance liquid chromatography

(HPLC), were conducted. Abnormal hemoglobin with the following characteristics was identified. First, this hemoglobin did not separate from hemoglobin A on both alkaline (pH 8.6) and acidic (pH 6.3) electrophoresis. Second, it coeluted with hemoglobin A₂ on HPLC. And third, it constituted about 36% of total hemoglobin. These characteristics exactly match the initial description of HSB.

Since the patient's anemia could not be explained by iron deficiency (serum Fe, 63 µg/dL; ferritin, 236 ng/mL; Fe saturation, 23%; TIBC, 275 µg/dL), this raises the possibility of the causal role of HSB in the thalassemia trait-like peripheral blood changes in this patient.

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Apoptotic Markers in Bilharzial-Related Invasive Bladder Cancer

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Cleaved caspase and Bcl-2 are markers of apoptosis. We assessed the expression pattern of these apoptotic markers in *Schistosoma* infection-related bilharzial bladder cancer (BBC) and the association with advanced pathological stage.

Immunohistochemical staining for cleaved caspase and Bcl-2 was performed on archival bladder specimens from 205 patients treated with radical cystectomy for invasive BBC between 1997 and 2003. Altered immunohistochemical expression of cleaved caspase and Bcl-2 were defined as cleaved caspase less than 10% and Bcl-2 more than 20%. Expression was correlated with clinicopathological characteristics with a mean follow-up of 3 years (range, 0-8 years).

The study included 205 patients (158 [77%] men and 47 [23%] women) with a mean age of 52.5 years (range, 35-74 years). Squamous cell carcinoma (SCC), transitional cell carcinoma (TCC), and adenocarcinoma (AC) represented 60%, 33%, and 7% of cases, respectively. There was extravesical extension in 53% and lymph node (LN) invasion in 29% of cases. Cleaved caspase was altered in 180 (88%) patients (108 [88%] of SCC, 60 [88%] of TCC, and 12 [86%] of AC), while Bcl-2 was altered in only 6 (3%) patients (all TCC). There was significant association between advanced pathological stage and cleaved caspase expression (extravesical extension, $P = .004$; LN invasion, $P = .04$) but not with Bcl-2 (extravesical extension, $P = .49$; LN invasion, $P = .26$). We found an association between extravesical extension and cleaved caspase expression in SCC ($P = .01$) and LN invasion and cleaved caspase expression in TCC ($P = .026$).

Cleaved caspase expression is associated with advanced pathological stage at radical cystectomy for invasive BBC. Cleaved caspase expression may play a significant role in BBC progression in the different histology of BBC. Our findings support the need for further evaluation of apoptotic pathways in locally advanced bilharzial-related SCC and metastatic bilharzial-related TCC.

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CD5 Immunoreactivity in Urothelial Carcinomas

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CD5 is a 67-kDa glycoprotein present in the membranes of lymphocytes and their neoplastic counterparts, thymic carcinomas, and adenocarcinomas. Immunohistochemical (IHC) studies reported granular cytoplasmic staining in malignant mesothelioma. Two alternative exons for the CD5 gene account for production of full-length (membranous) protein (exon 1A) or truncated (cytoplasmic) protein (exon 1B). The purpose of this IHC study was to determine the sensitivity of CD5 for urothelial carcinoma.

IHC stains for CD5 (clone 4C7) were done on high-grade (HG) and low-grade (LG) urothelial carcinoma: metastatic HG urothelial carcinoma (n = 1), invasive HG (n = 23) and LG (n = 3) urothelial carcinoma, noninvasive HG (n = 9) and LG (n = 11) papillary urothelial carcinoma, and carcinoma in situ (n = 4). Stains were scored as focal (5%-90%) or diffuse (>90%). Intensity of staining was graded as weak, moderate, or strong.

Staining localized to the cytoplasm and appeared granular. The sensitivity of CD5 for LG and HG urothelial carcinoma was 93% (13/14) and 58% (21/36), respectively. The overall sensitivity was 68% (34/50). Focal staining predominated in LG (8/13 [62%]) and HG (13/21 [62%]) urothelial carcinoma. Staining intensity was moderate to strong in LG (11/13 [85%]) and HG (20/21 [95%]) urothelial carcinoma.

CD5 is a sensitive but nonspecific urothelial marker. Nevertheless, granular cytoplasmic immunoreactivity for CD5 in metastatic carcinoma of unknown primary supports a urothelial origin, especially if other urothelial markers are expressed in an IHC panel. The granular cytoplasmic staining for CD5 in urothelial carcinoma indicates preferential selection of exon 1B over exon 1A.

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BCL6 Expression in B-Cell Lymphoblastic Leukemia Correlates Strongly With the t(1;19) Translocation

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Study to date suggests that the distribution of BCL6 protein expression in B-cell neoplasia predominates in tumors displaying a germinal center or post-germinal center phenotype. This knowledge is frequently used in diagnosis of B-cell leukemia/lymphoma when morphology and other immunophenotypic markers are inconclusive. We, however, found 1 patient with B-cell lymphoblastic leukemia (B-ALL), a pre-germinal center cell, whose blasts aberrantly expressed BCL6. This finding was in conflict with the scant literature addressing the issue. Therefore, for use in diagnosis, we performed an extensive study that included 52 B-ALL specimens from a wide spectrum of patients (in age and immunophenotype) to conclusively document the proportion of B-cell lymphoblastic leukemias that express BCL6.

In our screening study, we found that 6 of 52 (12%) B-ALL specimens demonstrated positive BCL6 staining. More interesting, we discovered that all 3 B-ALL cases with the t(1;19)(q23;p13) karyotypic translocation demonstrated strong staining for BCL6. In addition, FISH analysis for a cryptic t(1;19) in the remaining 3 BCL6+ screen cases revealed 1 more screen case to be t(1;19)+, as well as the index case. Expansion beyond the screening study showed BCL6 protein expression in 4 of 5 additional cases of t(1;19) B-ALL. These BCL6 protein expression data are supported by a microarray expression database demonstrating that BCL6 mRNA expression is significantly higher in B-ALLs containing t(1;19) when compared with the various other B-ALL subtypes. Finally, to investigate whether other protein markers of germinal center phenotype are expressed in the t(1;19) BCL6+ B-ALL cases, we stained for LMO2 and HGAL proteins

and found them to be expressed in 67% (6/9) and 89% (8/9) of cases, respectively. Overall, BCL6 expression is consistent with a diagnosis of B-ALL, especially in cases containing a t(1;19) translocation.

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Comparison Between Dual- and Single-Probe Chromogenic In Situ Hybridization (CISH) in Assessing *HER2* Gene Status of Chinese Breast Cancer Patients

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Dual-probe CISH is a recently launched commercial kit for determining the *HER2* status of breast cancer, in which *HER2* and chromosome 17 are simultaneously detected. This study was to evaluate the performance of dual-probe CISH by comparing with the results of single-probe CISH. *HER2* status was analyzed using dual- and single-probe CISH in 129 paraffin-embedded tissue sections of Chinese breast cancer patients, respectively. The results were scored by *HER2* gene copy number or the ratio of *HER2*/chromosome 17 according to the American Society of Clinical Oncology/College of American Pathologists criteria.

Of 129 sections, 7 cases (5%) were scored as equivocal based on the results of single-probe CISH, but none of these cases was scored as equivocal based on the results of dual-probe CISH. Of 122 cases, 50 were scored as negative and 72 were scored as positive by both single- and dual-probe CISH, in which the overall agreement between the 2 methods was substantial ($\kappa = 0.80$). The distribution frequencies of chromosome 17 aneusomy in concordant and discordant cases of the 2 methods were 38% (42/110) and 53% (10/19), respectively. This study shows that dual-probe CISH can reduce the frequency of equivocal results of single-probe CISH, indicating aneusomy 17 may have an impact on the interpretation of *HER2* status results in breast cancer tissue.

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Molecular Classification Method for Discriminating Metastatic vs New Primary Cancer

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Distinguishing metastatic from a second, independent cancer has dramatic implications for treatment, yet in challenging cases (eg, 2 squamous cell carcinomas), morphologic similarity and immunostain pattern do not provide sufficient information to distinguish these possibilities. We report further development of a classification algorithm to discriminate metastasis from new primary cancer based on a panel of microsatellite markers.

Archival specimen pairs from 3 cohorts were used to create a reference set of 155 patients consisting of 63 patients with breast, colorectal, and lung cancer with corresponding known metastasis; 21 patients with known independent primary cancer, 1 tumor involving breast, large bowel, or lung metastasis; and 71 patients (lung, head and neck, gynecologic tract, and other sites) with metastases

or new primaries confirmed by follow-up. These routine pathology specimens were microdissected to isolate tumor cells and then tested for loss of heterozygosity (LOH) in 16 markers for tumor suppressor genes. We created a measure of the dissimilarity of the pattern of mutations between specimen pairs using allele ratios (representing the degree of LOH clonality). Dissimilarity values are low for similar mutations, indicating tumors of common origin (metastasis), and high for dissimilar mutations, indicating tumors of distinct origin (new primary). The dissimilarity measure was applied to the reference set and cutoff values were determined that best separated metastatic from new primary cases; the accuracy of the cutoff was estimated by cross-validation.

By using the cutoffs, specimen pairs were classified as concordant (metastasis) or discordant (independent) with a cross-validated accuracy of 98% (95% CI, 95.8%-100%).

A classification method for discriminating metastatic from new primary tumors, focused on representative microdissections selected according to histopathologic features, demonstrated high accuracy with cross-validation. External validation is planned to demonstrate the utility of this method for resolving indeterminate diagnoses needed to guide effective treatment.

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Oral Bioavailability of Nattokinase (NSK-SD)

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We believe this to be the first study to detect and measure nattokinase in human serum following the ingestion of a single daily dose (2,000 FU) of nattokinase. Softgel capsules, each containing 100 mg of purified nattokinase (NSK-SO) from Japan Bio Science Laboratory were used in this IRB-approved study. Eleven healthy subjects (5 men, 6 women; aged 21-65 years) who met eligibility criteria were enrolled. Baseline blood samples were collected, and subjects were observed swallowing a single capsule of the NSK-SO supplement before returning at 2, 4, 8, 12, 24, and 48 hours postingestion for subsequent blood draws. The presence of nattokinase in serum was measured by ELISA, using a rabbit polyclonal antinattokinase capture antibody.

A pharmacokinetic pattern was observed for NSK-SO between baseline and 48 hours postdose, peaking at approximately 13.3 ± 2.5 hours postdose. Statistically significant increases in detectable serum nattokinase from baseline were seen at time points t = 2 hours through t = 24 hours.

Nattokinase, a serine protease derived from the traditional Japanese fermented soybean food natto, has significant profibrinolytic and antihypertensive effects and is increasingly used as a supplement for the benefit of cardiovascular health. Little is known regarding the pharmacokinetic and pharmacodynamic properties of this enzyme, and while this study provides important information regarding the bioavailability of nattokinase, further studies are warranted.

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Is HPV-16 a Marker for Progression of Cervical Intraepithelial Neoplasia 1 (CIN 1)?

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Although approximately 25% of CIN 1 progress to CIN 2/3 (high-grade [HG] CIN), currently there are no reliable predictors for CIN 1 progression. A biomarker that identifies women at greatest risk for CIN 1 progression would facilitate triage for LEEP or cone biopsy. HPV-16 has been shown to cause about 60% of cervical squamous carcinomas. Testing cervical biopsies with CIN 1 for HPV-16 may identify women at greatest risk for progression.

We retrieved 62 cervical biopsies with a diagnosis of CIN 1 and a minimum follow-up of 6 months (range, 6-85 months; mean, 16.7 months; median, 11 months) from our files. Based on histological and/or cytological diagnoses at follow-up, these cases were classified as 16 progressed, 30 regressed, and 16 persistent CIN 1. The persistent CIN 1 were excluded from further study. After confirming that the lesion was still present, each of the remaining 46 biopsies was tested for high-risk (HR) HPV and subtyped for HPV 16/18 (Third Wave Technologies, Hologic, Madison, WI). Results of HR-HPV testing were correlated with follow-up.

HR HPV was detected in 28 (61%) of the CIN 1 cases (7 HPV-16+, 0 HPV-18+, 21 non-16/18 HR HPV+). Of the cases, 18 (39%) were HR HPV-. Of the HPV-16+ CIN 1 cases, 71% progressed compared with 29% of the non-16/18 HR HPV+ and 28% of the HR HPV- CIN 1 cases.

HPV-16 positivity in cervical biopsies with CIN 1 is a useful biomarker for risk of progression to HG CIN ($P = .04$; Fisher exact test). Prospective studies are warranted to confirm our findings.

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Significant Amounts of Free Light Chains May Not Be Demonstrated or Be Considerably Underestimated by Serum Protein Electrophoresis

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The clinical utility of free light chain (FLC) measurement has been established for lymphoproliferative patients with low tumor burden. This study, focused on patients with a diagnosis of multiple myeloma and significantly elevated FLC measurements, was to evaluate the measurement of paraproteins by serum protein electrophoresis (SPE) and compare it with total light chain (LC) and FLC assessment.

Patients' data were extracted from the LIS, and those with FLCs of more than 1 g/L were reviewed. Patients' SPEs were performed on a Sebia hydragel system with amidoblack staining (Norcross, GA); LCs were measured on Beckman Immage (Brea, CA); and FLCs were measured by Freelite reagents (San Diego, CA).

In a 6-month interval, 22 patients were identified with FLC values of more than 1 g/L in at least 1 measurement. Eight patients' paraprotein concentrations on SPE were significantly lower than FLCs, with differences from 2.5 to 78.0 g/L. Among those, 3 patients with a diagnosis of multiple myeloma had no visible paraprotein in SPE. Case 1 had 2 nonvisible paraproteins on SPE, the presence of which was in the middle gamma region, that were confirmed by immunofixation as non-G, M, A, D, E free light chains (FLCs at 3.05 g/L). It is interesting that in the same patient, 2 paraproteins were clearly visible and measured at 1.0 and 1.4 g/L in urine protein electrophoresis with same staining method. Case 2 had two paraproteins at 3.3 and 1.3 g/L on SPE; LC for κ was at 26.0 g/L, while FLC for free κ was at 82.4 g/L. Case 3 had 2 separate SPEs with no visible paraprotein, and FLCs showed free κ at 4.2 g/L.

Significant amounts of FLCs may not be demonstrated or may be considerably underestimated by SPE, which emphasizes that when the clinical indication is high, FLC measurements should be pursued, regardless of SPE findings.

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Breast Cancer After Irradiation for Hodgkin Lymphoma: Molecular Subtyping Based on Immunohistochemistry

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Breast cancer is a well-documented complication of irradiation treatment for Hodgkin lymphoma. Several clinicopathologic characteristics of these radiation-associated cancers have been examined to enable their early detection and appropriate management. In this study, we attempted to perform the molecular subtyping of this group of cancers using an immunohistochemical approach.

We retrieved 22 cases of invasive breast cancer arising after irradiation for Hodgkin lymphoma from our database from 1991 to 2009. Immunohistochemistry was performed on all cases for CK8/18, CK19, CK5/6, CK14, and EGFR. The results of ER, PR, and HER2 expression were known in all cases. The mean age of the patients at diagnosis of breast cancer was 44.4 years (range, 34-62 years). All tumors were of the ductal type, except for 1 case of lobular carcinoma. In 64% cases, only the luminal cytokeratins, CK8/18 and CK19, were expressed; 36% of cases expressed luminal and basal-type cytokeratins (CK5/6 alone in 4 cases, CK5/6 and EGFR in 3 cases, and CK5/6 and CK14 in 1 case). None of the cancers expressed the basal-type cytokeratins exclusively. Based on the expression of hormone receptors, HER2, cytokeratins, and EGFR, the carcinomas were categorized as luminal A (54%, ER+/HER2-), luminal B (9%, ER+/HER2+), HER2 (23%, ER-/HER2+), and basal-like (14%, ER-/HER2- and CK5/6+ or EGFR+) subtypes. All triple-negative cancers were associated with a high nuclear grade, and all were categorized as basal-like subtype.

Our study demonstrates that in comparison with sporadic breast cancer, this group of radiation-associated breast cancers shows a similar distribution of basal-like subtype while exhibiting a greater prevalence of the HER2 type, suggesting a more aggressive behavior. A much larger group of cases needs to be evaluated to determine if these cancers really differ from sporadic breast cancer.

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Examination of an Association Between Clinically Significant Outcome Measures and the Quality Indicator Turnaround Time

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Turnaround time (TAT) primarily is accepted as an indicator of laboratory efficiency. Anecdotal information implies that clinical outcomes also are significantly impacted by TAT. The purpose of this study was to obtain evidence for testing the hypothesis that TAT impacts clinically significant outcome measures outside of the laboratory.

A retrospective review at 4 different hospitals examined at least 100 consecutive cases of 6 routine surgical pathology specimen types. Independent variables were specimen type and TAT. Potential outcome measures were time to next diagnostic procedure, definitive or ancillary treatment (time to surgery, radiation, and/or chemotherapy), or next office visit. For inpatients, LOS also was recorded. Specimen covariates potentially impacting TAT also were recorded and included in the analysis.

An anonymous, self-administered written questionnaire measuring a possible association between TAT for increasingly "serious" specimen types and patient level of anxiety was administered to 150 adult volunteers waiting for appointments in the University of Pittsburgh Cancer and Breast centers.

No statistically significant associations were found at any institution between TATs for all specimen types and measures of time to treatment or LOS. The exception was breast core biopsies given immediate interpretations; the availability of immediate diagnostic information was associated with decreased time to treatment. Some level of anxiety was reported by volunteers that correlated with specimen type and TAT; all specimen types and TATs were associated with at least mild levels of anxiety.

Time to treatment and LOS do not appear to be valid outcome measures for the quality indicator TAT. Only when the TAT is extremely fast, as with the provision of an immediate interpretation, is patient time to treatment impacted. Further studies should be performed to confirm our patient anxiety findings and to potentially develop TAT into a patient-centered quality indicator with anxiety as the outcome measure.

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Ovarian Malignant Mixed Müllerian Tumor (MMMT): A Clinicopathologic Review of 28 Cases

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Ovarian MMMTs are reportedly rare, and the literature is limited to case reports and a few clinical series. Our aim was to review clinicopathological features of a contemporary series of ovarian MMMT.

A retrospective search was performed to identify cases of ovarian MMMT. Clinical and follow-up information was obtained from medical records. Histological sections were reviewed to determine the epithelial and sarcomatous elements.

Between 2002 and 2010, 28 cases were identified. The patients ranged in age from 51 to 81 years (mean, 63 years). The most common presenting symptom was abdominal pain (50%). In 16 (57%) cases, presentation was as a unilateral ovarian mass, while the remainder were synchronous bilateral masses. Extraovarian extension was noted in 21 (75%) cases. In 16 (57%) tumors, there was a single epithelial component including endometrioid (8 [29%]), papillary serous (5 [18%]), or poorly differentiated carcinoma (3 [11%]), whereas 12 (42%) tumors were mixed, with the most common being papillary serous and endometrioid. Of the tumors, 5 (18%) had a clear cell component. In 11 (39%) tumors, there was a homologous sarcoma type showing endometrioid stromal sarcoma or undifferentiated sarcoma. Of the tumors, 17 (61%) were heterologous showing chondrosarcoma, rhabdomyosarcoma, and/or myxoid sarcoma. After a median follow-up of 12 months for 27 patients (range, 1-91 months), 11 patients developed local recurrences after a median of 10 months (range, 6-23 months). Of the patients, 13 died with disease after a median of 9 months (range, 1-48 months). The 13 patients included 8 (72%) with local recurrence, 2 (40%) with clear cell component, and 4 (66%) with rhabdomyosarcoma component.

In this study, local recurrence was associated with a worse outcome. However, in this study, a clear cell or a rhabdomyosarcoma component, which have been reported to be associated with a worse outcome, were not.

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Higher Expression of Serine-213 Phosphorylated Androgen Receptor Level Is Associated With Prostate Cancer Recurrence

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The majority of prostate cancers (PCa) are indolent and do not affect survival, yet patients are subjected to overtreatment that affects quality of life. Thus, it is important to identify biomarkers to distinguish aggressive (recurrent and metastatic) from less aggressive tumors. Androgen receptor (AR) plays a critical role in PCa progression. Phosphorylation of AR at various serine residues may affect AR activity. In this study, we examined the expression of phosphorylated AR at serine residues 213 and 650 in PCa in relation to clinicopathological features.

Immunohistochemistry was performed using antibody against AR, ARSer(P)-213 and AR Ser(P)-650, to characterize the expression pattern on tissue microarray (n = 134) of PCa. The percentage and intensity levels were scored semiquantitatively: 0, negative; 1+, weak; 2+, moderate; and 3+, strong for nuclear and cytoplasmic expression. The final histoscore (H score) was calculated as the sum of the percentage of staining multiplied by the corresponding intensity level (range, 0-300). Statistical analyses were performed by using the *t* test.

An H score of more than 100 for nuclear phospho-AR 213 staining was found in 11 of 35 (31.4%) of nonrecurrent and 20 of 32 (62.5%) of recurrent PCa. It is interesting that the H score of cytoplasmic ARSer(P)-213 expression was significantly higher in PCa with recurrence (mean H score, 9.8 ± 4.9) compared with nonrecurrent cases (0.7 ± 0.5 ; $P < .05$). In addition, the intensity and the percentage of cytoplasmic staining were significantly higher in the recurrent group ($P < .05$); however, the difference in nuclear staining was not significant.

There was no difference in the H score of ARSer(P)-213 with respect to other clinicopathological parameters, eg, PSA level, Gleason score, and stage. There was no statistical difference in the H score of ARSer(P)-650 in any subgroups.

This study demonstrates significant up-regulation of ARSer(P)-213 in PCa epithelial cells with recurrence in comparison with nonrecurrent cancer. This suggests a role of ARSer(P)-213 in high-risk PCa.

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Relationship Between ADAMTS13 Antibody Property and Thrombotic Thrombocytopenic Purpura's Clinical Outcomes

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Primarily acquired thrombotic thrombocytopenic purpura (TTP) is an autoimmune disease caused by production of an autoantibody that impairs ADAMTS13 function and, in turn, leads to platelet thrombosis. Biochemical characteristics of ADAMTS13 autoantibody are known to be diverse and heterogeneous among TTP patients. This study was designed to characterize ADAMTS13 antibody properties in the context of clinical outcomes using longitudinal samples from a large TTP cohort.

In this study, we collected, from 38 patients with idiopathic TTP, more than 450 longitudinal samples throughout the clinical course of acute presentation, hospitalization, and follow-up during remission. We assayed for ADAMTS13 activity (reportable ranges from 0.5% to 100%), ADAMTS13 antibody (IgG) concentration, and quantitative ADAMTS13 inhibitory titers. All laboratory data were then analyzed in the context of clinical data.

Based on correlative analyses, we can group TTP patients into 3 categories. In the first group, patients display good agreement between antibody concentration and antibody inhibitory titer. ADAMTS13 antibody amount and titer are high during acute TTP.

When clinical remission is obtained, the ADAMTS13 antibody amount and titer are significantly reduced, although the antibody amount remains above normal reference ranges throughout the period of clinical remission. In the second group, there is a significant discordance between ADAMTS13 antibody amount and antibody titer. When compared with clinical outcomes, ADAMTS13 antibody inhibitory titers appear to be more associated with the clinical course of disease. There may be a third group with fewer patients. This group lacks association of clinical outcomes with either antibody amount or antibody inhibitory titer.

Our study is the first to report ADAMTS13 antibody characteristics in relation to clinical outcomes using longitudinal samples from a large cohort of TTP patients. This novel information provides new insights and offers the basis to further investigate molecular details of autoimmune responses in TTP.

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Silver In Situ Hybridization and p57 Immunohistochemistry Are Useful Adjunctive Studies in the Diagnosis and Differentiation of Molar Pregnancy and Hydropic Abortion

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The distinction between complete hydatidiform mole (CHM), partial hydatidiform mole (PHM), and hydropic abortion (HA) can be difficult owing to overlap of morphologic features. Cytogenetic analysis to establish ploidy can identify most PHMs, but it is labor-intensive and does not separate CHMs from HAs. The purpose of this study was to evaluate the combination of p57 immunohistochemistry (IHC) and in situ hybridization for a common chromosomal marker as a reliable method to establish a diagnosis.

We identified 15 cases diagnosed morphologically as PHM (6 cases), "suspicious" for PHM (3 cases), CHM (2 cases), molar NOS (1 case), or HA (3 cases) in our institutional database from January 2004 to August 2009. Only cases with accompanying cytogenetic or other ploidy analysis were selected and included for study. Six additional cases of nonhydropic products of conception with cytogenetic data were included as control cases. Immunohistochemical staining for p57 in the villous mesenchyme and cytotrophoblast was scored as negative, limited (<10%), focal (10%-50%), or diffuse (>50%). For in situ hybridization, a chromogenic probe for chromosome 17 was used, and the average number of signals/cell from 50 cells was recorded.

Of 15 cases of mole or suspected mole diagnosed morphologically, only 4 correlated with p57/SISH/cytogenetic data (3 PHMs, 1 CHM), highlighting the need for ancillary studies in such cases. Ploidy as assessed by SISH correlated with cytogenetics in 20 of 21 cases, and the addition of p57 staining allowed for accurate separation of CHM (diploid/p57-negative), PHM (triploid/p57-positive), and HA (diploid/p57-positive) in a relatively straightforward manner.

Although molecular genotyping remains the "gold standard" for assessment of molar pregnancy, the combination of p57 IHC and SISH appears to be a simple and reliable substitute for this highly complex procedure.

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Comparing Histopathologic Features of Uterine Tumors Associated With Abnormal Glandular Cells in Cervical Cytology

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Cervical cytology is primarily a screening test for SILs; the role for screening glandular lesions is limited. Abnormal glandular cells (AGCs) remain challenging for cytopathologists. Pap smear (PS) sensitivity for glandular lesions is low and limited by sampling error and interpretation. The aim of this study was to determine if a correlation between AGCs and specific tumor characteristics exists on PS.

A retrospective 10-year study of 189 hysterectomy specimens was performed that included 184 endometrial adenocarcinomas (EMAs) and 5 endocervical adenocarcinomas (ECAs). Cases were divided into AGC and negative based on PS results obtained 6 months prior to hysterectomy. Histopathologic information (age, lymph node status, invasion depth, histologic subtype, grade, stage, size, volume, and cervical involvement) was retrieved. Statistical analysis was performed.

Of 184 EMA, 97 (53.7%) had cervical cytology specimens. Of these, 48 (49.5%) were abnormal, including 18 (18.6%) adenocarcinomas (ACs) and 30 (30.9%) AGCs. Of the 5 ECAs, 2 had cervical cytology specimens and were positive for AC. Using the Fisher exact test, tumor histologic grade had statistical significance for abnormal PS ($P = .02$, G1 vs G2; $P = .02$, G1 vs G3; $P = .012$ G1 vs G3/serous carcinomas [SC]/MMMT). SC/MMMT had a higher abnormal rate compared with EMA (75% vs 52.7%) but was not statistically significant. Similarly, tumors with cervical involvement had a higher abnormal PS rate (76.9% vs 45.8%) but were not statistically significant.

Tumor grade is a significant histopathologic feature affecting detection of AGC on cytology. Grade 2 or higher AC is likely to have abnormal PS. Grade of uterine AC is determined by architectural features and nuclear atypia. Our results indicate that significant cytologic nuclear atypia aids cytopathologists in recognizing the atypical cells. Also, solid tumor growth may promote tumor cell shedding, resulting in enhanced sampling. Histologic subtypes of tumors along with cervical involvement may show statistical significance when additional cases are evaluated.

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Assessment of Platelet Large Cell Ratio (P-LCR): A New Platelet Volume Parameter in Patients With Ischemic Events

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Platelet function can be conveniently estimated by measuring the mean platelet volume (MPV). The MPV looks at the volume of the entire platelet pool; a new measurement, the P-LCR, is able to quantify the percentage of large platelets that contribute to the total platelet count.

Our objective was to correlate larger, hence, more reactive, platelets with ischemic events by using the measurements of P-LCR. For the study, 239 patients were evaluated at Danbury Hospital Laboratory with a troponin I test. The Sysmex X2100 analyzer was used to calculate the P-LCR in EDTA-anticoagulated whole blood. The patients were stratified into 3 groups: group 1 ($n = 50$), negative troponin I (control); group 2 ($n = 100$), negative troponin I, and patients were evaluated with a lipid profile; and group 3 ($n = 89$), elevated troponin. The groups were then compared.

Groups 1, 2, and 3 had mean P-LCRs of 27.13 (SD = 5.12), 31.39 (SD = 5.89), and 36.14 (SD = 10.71), respectively. The difference between the means was calculated using a 2-tailed t test rejecting the null hypothesis at a P value of .05. There was a statistically significant difference between groups 1 and 2, groups 1 and 3, and

groups 2 and 3 with a P value of $< .001$. Group 2 was further statistically evaluated to determine the relationship of P-LCR with the lipid profile using Pearson correlation coefficient. Our data show a moderate correlation between P-LCR and cholesterol levels with an r value of 0.23 and a P value of .026.

The P-LCR appears to increase as the traditional risk factors for MI (lipid profile) increase, may be a useful marker to detect patients with high risk for ischemic events due to the increase in platelet activity, may identify patients with hyperlipidemia who have increased platelet activity, and may be a useful parameter for the consideration of antiplatelet therapy.

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The Cytomorphological Spectrum of Small Cell Carcinomas and Large Cell Neuroendocrine Carcinomas in Serous Effusion: Our Experience With 68 Cases

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Small cell and neuroendocrine carcinomas are uncommon in serous body cavity effusions. The purpose of this study was to examine the cytomorphological spectrum of small cell carcinoma and large cell neuroendocrine carcinoma in body cavity serous fluid.

We have examined 5,171 serous body effusion specimens during 5 years in which 723 (14%) were positive for malignancy. All cytology slides and the available clinical data, histological follow-up confirmation, and ancillary studies were reviewed.

A total of 68 cases (60 pleural, 5 peritoneal, and 3 pericardial effusions) from 50 patients with an average age of 73 years were reported as diagnostic or suggestive of small cell carcinoma (52 cases) or large cell neuroendocrine carcinoma (16 cases). The primary site was lung in 56 cases, pancreatic in 6 cases, and cervical, colonic, and head and neck region (2 cases each). Of the 68 cases, 48 (71%) had no history of malignancy of the same type. Of the 20 cases with a history of malignancy, the average period from primary to positive effusion was 36 months. Ancillary studies were utilized in 46 cases (68%), including flow cytometric studies in 5 cases. Three cytomorphological predominance patterns were seen: predominance of small clusters, 33 cases; predominance of large clusters mimicking non-small cell carcinoma, 18 cases; and predominance of a single cell pattern mimicking lymphoma, 17 cases. Significant apoptosis was seen in 22 cases, and marked cannibalism was seen in 11 cases. Prominent nucleoli were noted in 16 cases (24%).

The most common cytomorphological pattern was predominance of small clustering with nuclear molding. However, in 51% of the cases, the predominant cytomorphological pattern was a single cell pattern mimicking lymphoma or a large clustering pattern mimicking non-small cell carcinoma. Knowing the cytomorphological spectrum of small cell and neuroendocrine carcinoma in fluid cytology may help in establishing timely and accurate diagnoses.

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Cytomorphological Spectrum and Ancillary Studies in Liver Fine-Needle Aspiration (FNA) Biopsies of Nonepithelial Neoplasms: A Study of 57 Cases

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Nonepithelial lesions in liver FNAs are rare entities and encompass a wide variety of benign and malignant conditions. The current

review emphasizes the cytomorphologic features, differential diagnosis, and potential pitfalls associated with these lesions.

We retrospectively reviewed ultrasound-guided liver FNAs with positive diagnosis for malignancy or neoplasm in a 4-year period. For each case, we reviewed the cytomorphology, the final cytologic diagnoses, ancillary studies, and available corresponding histological material and clinical follow-up.

We retrieved 576 ultrasound-guided liver FNAs with positive diagnosis of malignancy or neoplasm from our records for a period of 48 months. Of the cases, 57 (1%) were diagnosed as nonepithelial lesions. The average age of patients was 48 years (range, 30-86 years). Of these 57 FNAs, 26 were metastatic melanoma, 15 were mesenchymal tumors (7 metastatic sarcomas, 5 hemangiomas, and 3 metastatic gastrointestinal stromal tumors [GISTs]), and 13 (23%) were lymphomas, and there was 1 case of each of metastatic meningioma, metastatic ovarian granulosa cell tumor, and bile duct hamartoma. A history of the neoplasm was present in 42 cases (74%). The cases with no history included 5 hemangiomas, 5 lymphomas, 2 GISTs, 1 melanoma, 1 leiomyosarcoma, and 1 bile duct hamartoma. Ancillary studies were utilized in 52 cases, including immunohistochemical stains in 42 cases, flow cytometry in 9 cases, and molecular testing in 5 cases (fluorescence in situ hybridization studies in 4 cases and PCR in 1 case). Histological correlations were available in 52 cases with no discrepancies seen.

Nonepithelial neoplasms of the liver are rare entities, and 26% of these cases occurred in patients with no history of malignancies. By correlating clinical and radiological data, cytologic findings, and ancillary studies, a definitive specific diagnosis can be achieved with FNA cytology.

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Phosphorylated CXCR4 Is Associated With Poor Survival in Adult Precursor-B Lymphoblastic Leukemia

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CXC chemokine receptor 4 (CXCR4) is involved in the trafficking of normal and malignant hematopoietic cells. CXCR4 is activated by phosphorylation. While CXCR4 overexpression was reported to predict extramedullary organ infiltration in childhood acute lymphoblastic leukemia (ALL), its role in ALL remains unclear. We analyzed the prognostic impact of total and phosphorylated CXCR4 in adult patients with precursor B-ALL.

Expression of total and phosphorylated CXCR4 was analyzed in bone marrow samples from patients newly diagnosed with pre-B-ALL who were treated at M.D. Anderson Cancer Center between January 2006 and June 2007. Total CXCR4 was measured by flow cytometry (FC) using anti-CXCR4 antibody (RD Systems). We also used formalin-fixed, paraffin-embedded bone marrow biopsy specimens to detect total and phosphorylated CXCR4 by immunohistochemistry (IHC) using anti-CXCR4 antibody (Abcam) and anti-phosphoCXCR4 antibody (*Cancer Res.* 2007;67:651), respectively. Clinical data were obtained from the institutional database with the last follow-up in July 2008. The Kaplan-Meier product-limit method and log-rank test were utilized to assess the difference in overall survival (OS) and progression-free survival (PFS) between groups. Multivariate Cox proportional hazards models were used to assess the hazard ratios.

There were 30 men and 24 women with a median age of 42 years (range, 17-84 years). Of the patients, 49 had a complete response and 12 had a relapse; 15 patients died. In 36 patients, there

was overexpression of total CXCR4; 18 of them had pCXCR4. There was a strong correlation between total CXCR4 expression detected by FC and IHC ($P < .001$). There was no association between total CXCR4 and other laboratory data. pCXCR4 was associated with a higher WBC count ($P = .006$) and a higher bilirubin level ($P = .03$). In the multivariate analysis, a high creatinine level ($P < .01$), Philadelphia chromosome ($P = .017$), late response ($P < .001$), and presence of pCXCR4 ($P = .027$) were associated with worse OS. No association between OS and total CXCR4 was found. Total CXCR4 and pCXCR4 were not associated with worse PFS.

While IHC and FC techniques provide comparable data assessing total CXCR4 expression, there was no correlation of total CXCR4 expression and clinical outcome in our study group. In contrast, activation of CXCR4 via phosphorylation is associated with worse survival of patients with precursor-B lymphoblastic leukemia.

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Screening of Males for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in an Emergency Room Setting

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Despite effective treatment, sexually transmitted infection (STI) is a persistent problem in our society, and screening is of high priority according to the Centers for Disease Control and Prevention (CDC). Particularly, screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) is emphasized because infections may be asymptomatic, thereby permitting inadvertent transmission. This study presents data to support the screening of males for CT and NG in an emergency room (ER) setting.

Polymerase chain reaction results from the LSUHSC-Shreveport Diagnostic Virology Laboratory were reviewed to investigate CT and NG positivity rates. We further subclassified positive results according to the triage center (ie, fast track, pediatric, or traditional ER) and compared the number of positive specimens from each setting.

In 2002, 153 of 1,793 (8.5%) of screened males were positive for CT or NG. Of the 153 positive specimens, 52% (80/153) were obtained during an ER visit. In 2008, there was a statistically significant increase in CT/NG detection, with 200 of 1,821 (11%) of screened males testing positive ($P = .014$). Of these, 62% were from the ER.

These data suggest that screening males in an ER setting may provide a unique opportunity to proactively identify infected patients who may otherwise go untreated. As advocated by the CDC, screening is of particular importance not only because infections may be asymptomatic, but also because ongoing efforts to combat CT/NG transmission have been minimally effective. Further investigation into cost-effectiveness of ER screening and its overall effectiveness in reducing STI transmission may be warranted.

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Sulfatase 2 (SULF2) Protects Hepatocellular Carcinoma Cells Against Apoptosis Induced by PI3K Inhibitor LY294002

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide. Because of frequent de novo and acquired

resistance of HCCs to chemotherapy, there are limited options for therapy of HCC. There is, therefore, an urgent need for improved therapy of HCC. Consequently, there is strong interest in identifying novel molecular targets for therapy of advanced HCC. SULF2, a heparan-degrading sulfatase, has an oncogenic effect in HCC. The oncogenic activity of SULF2 in HCC is partially mediated through up-regulation of glypican 3, which promotes heparin-binding growth factor signaling and HCC cell growth.

By using oligonucleotide microarray analysis, real-time quantitative PCR, gene transfection, MTT and apoptosis assay, Western blotting, immunocytochemistry, and confocal microscopy, we investigated the role of SULF2 in drug-induced apoptosis.

SULF2 expression is associated with a decreased apoptosis index in human HCCs ($P = .00001$). Forced expression of SULF2 in the SULF2-negative Hep3B cell line significantly induces resistance to drug-induced apoptosis. Forced expression of SULF2 activates the Akt pathway and down-regulates activity of the apoptotic caspases 3 and 7, leading to enhanced cell cycle progression. Conversely, knockdown of SULF2 using an shRNA construct targeting the SULF2 mRNA induces profound cell growth arrest, decreases Akt phosphorylation, down-regulates cyclin D1 and the antiapoptotic molecule bcl-2, and sensitizes the SULF2-expressing HCC cell lines Huh7 and SNU182 to chemotherapy-induced apoptosis.

Our results confirm the prosurvival, antiapoptotic effect of SULF2 in HCC is mediated through activation of the PI3K/AKT pathway.

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Electronic Pathology Reporting: A Practical Public Domain Solution

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Pathologists are under increasing pressure because of rising workload and more stringent requirements for pathology reports. Synoptic checklists have been developed to ensure that the pathology reports on cancers include all essential data elements, and commercial electronic anatomic pathology information systems are being offered to meet this requirement. However, these software packages are expensive, and interfacing them with the hospital information system can be a major hurdle. We are presenting a pathology reporting system that is designed to integrate with the existing pathology workflow and can be easily implemented in most laboratories.

We developed InfoPath forms with fields for all required data elements for cancer reporting. Drop-down lists and automatic calculators for grade and stage facilitate data entry. Synoptic reports are generated from these InfoPath forms. The data are automatically captured into a fully searchable Microsoft Access database.

The staff and residents find the user interface intuitive and easy to use. The reports have a consistent, easy-to-read format and include standardized language. When necessary, a pathologist may type in additions or comments to the report. The electronic template can be edited to reflect changes in terminology or staging parameters, eg, synoptic reports utilizing the latest AJCC Cancer Staging system. The system is practical, flexible, easily updated, and compatible with other informatics systems.

This software application, which will be placed in the public domain, provides an alternative to the commercial systems. Since it is run on a widely installed MS Office suite, it can be readily utilized in most laboratories. It can provide pathologists with a relatively simple way to deal with the increasing complexity of pathology reporting.

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A Cocktail of E-Cadherin and p120 Catenin Reliably Distinguishes DCIS From LCIS

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Treatment of ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) can be dramatically different. Immunohistochemical (IHC) staining for E-cadherin is routinely used to differentiate DCIS from LCIS in difficult cases. Recent studies utilizing p120 catenin have shown diffuse membranous staining in DCIS and diffuse cytoplasmic staining in LCIS. For small lesions, the number of sections available for IHC studies may be limited. We developed a cocktail composed of E-cadherin and p120 catenin primary antibodies so that only 1 slide is needed for the double stain.

We retrieved 27 blocks of formalin-fixed, paraffin-embedded tissue from 26 cases previously diagnosed as DCIS or LCIS. The E-cadherin antibody was a rabbit polyclonal antibody, and the p120 catenin antibody was a mouse monoclonal antibody. The E-cadherin primary antibody was detected using a secondary antibody raised against rabbit antibody and visualized with a brown color. The p120 catenin primary antibody was detected using a secondary antibody raised against mouse antibody and visualized with a red color.

With the single antibodies, 15 of 15 DCIS samples had diffuse membranous staining with E-cadherin or p120 catenin; all 12 LCIS samples showed cytoplasmic red staining with p120 catenin. When stained with the antibody cocktail, all 15 DCIS samples showed red and brown membranous staining without cytoplasmic stain; all 12 LCIS samples showed diffuse cytoplasmic red staining for p120 catenin and no staining for E-cadherin.

IHC staining with the antibody cocktail showed 100% concordance compared with the traditional single antibody staining for E-cadherin or p120 catenin. Our antibody cocktail includes E-cadherin as a positive membranous stain for DCIS and p120 catenin as a positive cytoplasmic stain for LCIS, which may enhance our accuracy in diagnoses. This antibody cocktail may be especially useful in small samples and small foci of CIS.

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Prostate-Specific Antigen Screening Test: Is It Overused in Prostate Cancer Practice? Retrospective Study of the Data From Brooklyn VA Medical Center From 1997 to 2007

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Prostate-specific antigen (PSA) has been used as a serodiagnostic screening tool for prostate cancer worldwide for many years. Recent studies suggest that about 20% of prostate cancers diagnosed by biopsy occur in patients whose serum PSA levels are <4.0 ng/L, the cutoff for diagnosis of prostate cancer. We have performed the inverse study, ie, what percentage of patients with elevated PSA levels have initial and even follow-up biopsies that are negative but who are subsequently found to have prostate cancer.

We retrospectively studied more than 1,000 total cases at the Brooklyn Campus of the New York Harbor VA Medical Center from 1997 to 2007. Of these, the serum samples of 203 cases were found to be positive for PSA but negative for prostate cancer on initial biopsy. Each of these patients was followed up for PSA and biopsy results for a maximum of 3 years. Of the 203 patients, 46 (22.6%)

were subsequently found to have prostate adenocarcinoma in the follow-up biopsies at a mean age of 65 years within 3 years. Of these patients, 35% were younger than 60 years when they were diagnosed with prostatic adenocarcinoma.

Without the PSA test, around 20% of patients with prostate cancer would be missed by prostate biopsy alone, at least the first time, assuming that each cancer did not develop subsequent to the first biopsy. Since more than half of the Gleason scores were 7 or greater, this seems to be a reasonable assumption. The opportunity for early effective treatment would, therefore, be lost. We conclude that the PSA screening test is still the most useful test and should continue to be used in our practice.

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Decreased WWOX and FHIT Protein Expression Correlates With Tumor Grade in Cholangiocarcinoma

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Common fragile sites are large regions of genomic instability that are found in all people and are hot spots for chromosomal rearrangements and deletions. Two of these genes, *WWOX* and *FHIT*, which are tumor suppressors, have been found to show loss of expression in several carcinomas. We compared the expression of *WWOX* and *FHIT* in cholangiocarcinoma (CC) with benign common bile ductal epithelium (CBD) and correlated protein expression with tumor differentiation.

Archival files were searched for CC resections, and 69 were reviewed and arrayed to create a tissue microarray of cores each 1.5 mm in diameter. Sections were stained with *WWOX* and *FHIT* and controls stained appropriately. Stains were graded as negative (0), weakly positive (1+), or strongly positive (2+). A Cox regression survival analysis was performed.

In CC, *WWOX* and *FHIT* protein expression was absent or reduced as compared with benign CBD. Negative and weak positive staining for *WWOX* were seen in 41% (28/69) and 52% (36/69) of CCs, respectively; negative and weak positive staining for *FHIT* were seen in 77% (53/69) and 23% (16/69) of CC, respectively. *WWOX* and *FHIT* expression was greater in CBD, with weak or strong positive staining in 100% (13/13) and 93% (13/14) of cases, respectively. Decreased immunoreactivity was observed in CC as the tumor grade increased. There was no statistically significant correlation between *WWOX* and *FHIT* expression and clinical outcome.

WWOX and *FHIT* were absent or decreased in CCs, particularly those of high histological tumor grade, when compared with benign CBD. Down-regulation or absence of *WWOX* and *FHIT* expression indicates that these tumor suppressors may play a key role in carcinogenesis of CC. Additional studies with larger numbers of cases are warranted to evaluate the association of *WWOX* and *FHIT* with clinicopathological factors.

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Immunohistochemical Studies of Kidney Tumors on Tissue Microarrays

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Kidney tumors of various types behave differently and have different prognoses. Owing to some overlapping morphological

features and immunohistochemical staining pattern, they may pose diagnostic challenge. Therefore, it is necessary to explore additional immunohistochemical stains to help subclassify these epithelial neoplasms.

Tissue microarrays of 20 cases each of renal cell carcinomas (RCC) of clear cell, chromophobe, and papillary variants and oncocytoma were constructed and used to test a panel of immunohistochemical markers including carbonic anhydrase (CA) IX, galectin 3, kidney injury molecule 1 (Kim1), p63, and heterochromatin-associated protein (HP) 1. Additional tumors (including 6 collecting duct carcinomas, 4 mucinous tubular and spindle cell carcinomas, and 1 metanephric adenoma) were stained for Kim1 and p63 on conventional paraffin block sections. Tissue microarrays of 221 cases of urothelial carcinoma were stained for p63 and Kim1.

Nuclear staining for p63 was positive in 78% of urothelial carcinomas and negative in all renal epithelial tumors except for 2 oncocytomas and 1 collecting duct carcinoma. CA IX is highly sensitive for clear cell RCC (90% positivity) and is negative in all other renal epithelial tumors except for 1 chromophobe RCC. Expression of galectin 3 was found mostly in renal tumors with oncocytic features, including oncocytomas (100%) and chromophobe RCCs (85%). Expression of HP1 was found mostly in papillary RCC (79%) and oncocytoma (75%) but less in chromophobe (30%) and clear cell RCCs (35%). Immunostain for Kim1 is less specific and positive in the majority of tumors of both urothelial and renal origins.

p63 is a sensitive and specific marker for tumors of urothelial origin. The panel of CA IX, galectin 3, and HP1 is useful in differentiating different types of renal epithelial neoplasms.

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Adjudication of Local vs Central Histology Interpretation of Cervical Intraepithelial Neoplasia End Points From the CERVISTA Clinical Trial

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Interpretation of cervical intraepithelial neoplasia (CIN) end points across clinical trials is often inconsistent as a result of different methodologies. This analysis evaluated the histologic agreement among pathologists in diagnosing CIN in women infected with high-risk (HR) human papillomavirus (HPV) from the CERVISTA (Hologic, Marlborough, MA) clinical trial. As part of a multicenter, prospective clinical study, women with atypical squamous cells of undetermined significance (ASC-US) or worse cytology underwent colposcopy and cervical biopsy according to local standard of care guidelines. Clinical management was based on diagnoses from local colposcopists and pathologists. However, a central histology review panel provided the histological diagnoses that were used for final case definitions. The pathology results provided by the central review panel were reported as a consensus report from 2 expert pathologists blinded to each other's interpretation. If the pathologists disagreed, a third pathologist provided an independent review. If that pathologist disagreed with both initial reviews, a final adjudicated review was performed by all 3 pathologists to achieve a consensus diagnosis.

Among women with ASC-US cytology, 1,003 were included in the final analysis irrespective of CERVISTA HR HPV status. The following diagnoses were made by the central review panel: 73.8% (740/1,003) had no CIN, 18.8% (189/1,003) had CIN 1, 5.0% (50/1,003) had CIN 2, and 2.2% (22/1,003) were diagnosed with CIN 3. Approximately 14% (137/1,003) of all cervical biopsies

required review by a third pathologist. Of the CIN 2 cases, 60% (30/50) required a third reviewer for diagnosis, the most of any grade CIN. Fewer than 1% (3/1,003) of cases required adjudication. These data demonstrated that the highest disagreement with histologic review in women with ASC-US cytology occurred among CIN 2 cases, consistent with other studies. The variable nature of CIN 2, in addition to differing methodologies, makes comparison of similar data across clinical trials challenging.

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Utility of Immunohistochemistry for Tissue Transglutaminase in the Diagnosis of Celiac Disease on Formalin-Fixed, Paraffin-Embedded Tissue

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The diagnosis of celiac disease (CD) rests on a combination of clinical, biochemical, and histologic findings. In serum samples, most useful are antibodies to tissue transglutaminase-2 (TTG); however, its utility in the tissue biopsy has been controversial. The objective of this study was to clarify the utility of TTG in duodenal biopsies in the diagnosis of CD.

We selected 106 formalin-fixed, paraffin-embedded duodenal biopsies from the anatomic pathology archives at The Ottawa Hospital. Immunohistochemical stains for CD3, CD4, CD8, and TTG were performed on all cases, and the number of lymphocytes per 100 enterocytes was recorded. The intensity of TTG staining was graded semiquantitatively (0, negative; 1, weak; 2, moderate; and 3, intense) and the location of the staining recorded. Results were subjected to statistical analysis.

The 106 cases were divided into 4 groups: group 1, normal duodenum, 33 cases; group 2, duodenitis not attributed to CD, 25 cases; group 3, untreated CD, 49 cases; and group 4, treated CD, 9 cases. The intraepithelial lymphocytes expressed CD3 and CD4 and were negative for CD8, and their count overlapped between groups 1 and 2 but was statistically less than group 3. TTG was expressed in surface and crypt epithelium, the superficial lamina propria, and muscularis mucosa in group 3 (intensity 2-3) and was less in groups 2 and 4 (intensity 1-2) and absent in normal mucosa (group 1). The presence of this difference in staining correlated with a diagnosis of CD ($P = .02$).

TTG overexpression in intestinal epithelium and lamina propria is characteristic but not specific to CD. Further validation of this technique at different institutions in a prospective study would assist in the assessment of the clinical utility of this test. TTG expression alone is not useful in the diagnosis of early CD.

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Study of Surgical Margins in Breast Pathology With Imprint Cytology

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The combination of breast conservative surgery and radiotherapy has become standard treatment for most malignant breast tumors. The status of surgical margins is an important prognostic factor for local recurrence. We need a reliable method of intraoperative assessment to resolve the lesions in a single surgical procedure. Gross examination is usually used with or without X-ray study, frozen biopsies, cytology, or a combination of these.

We prospectively studied 218 cases with imprint cytology, including 145 partial mastectomies and 73 total mastectomies. Samples were categorized into negative, positive, and atypical-suspicious. Of the cases, 189 were malignant, most commonly infiltrating ductal carcinoma (120), intraductal carcinoma (36), and lobular invasive carcinoma (21), and 30 cases were benign, the most common were papilloma (8), low-grade phyllodes tumor (6), and fibroadenoma (5). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 88%, 97.2%, 89.8%, 96.7%, and 95.2% respectively.

The cytologic examination of surgical margins in breast disease is a very good method. It allows examination of practically 100% of the marginal surface quickly, to have good quality of material and efficiency. The operators need good expertise in surgical breast pathology and cytopathology.

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Comparative Assessment of Zinc-Based Fixatives in Hematopathology Practice

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Mercury-based fixatives (such as B-5) have been traditionally used for hematopathology specimens. Alternative zinc-formalin fixatives (ZFFs) are available, but few studies to date assessed their performance. The morphologic quality and antigen, DNA, mRNA, and microRNA preservation in ZFF-fixed lymphoid tissues was assessed.

Five fixatives were evaluated: 10% normal buffered formalin (NBF; Pharmco, Brookfield, CT), B-5 (American Mastertech, Lodi, CA), zinc-formalin (ZF; Richard Allan, Kalamazoo, MI), and 2 zinc-formalin/acetic acid fixatives (ZFA1, Newcomer, Middleton, WI, and ZFA2, Anatech, Battle Creek, MI).

Seven fresh, deidentified clinical specimens (3 spleen and 4 lymph nodes) were fixed for 3 and 6 hours, processed routinely, and stained for H&E and IHC (CD5, CD15, CD20, CD23, CD30, PAX5, BCL1, and BCL6). Five 20- μ curls were used for DNA, mRNA, and miRNA extraction. DNA/RNA quality was assessed with PCR/RT-PCR (VAMP2 for DNA, TBP for mRNA, and SNORD44 for microRNA). H&E and IHC slides were independently scored (1, suboptimal; 2, limited; 3, good; and 4, excellent) by 3 hematopathologists blinded to the fixative used and fixation time.

The morphologic score was similar in 3-hour-fixed compared with 6-hour-fixed tissue: mean values for NBF, B-5, ZF, AZF1, and AZF2 were 2.9, 3.4, 3.7, 2.7, and 3.3, and 3.1, 3.2, 3.5, 2.7, and 3.0, respectively. ZF provided the best morphologic detail overall.

The mean scores for IHC quality for NBF, ZF, AZF1, and AZF2 were 3.2, 3.3, 3.2, and 3.1, respectively. Similar DNA quality was observed with all ZFFs: average Ct values for ZF, AZF1, AZF2, and NBF were 27.0, 27.3, 27.2, and 26.8, respectively. Similar results were obtained for extracted miRNA: average Ct values for NBF, ZF, AZF1 and AZF2 were 36.8, 38.5, 37.1, and 36.8, respectively. mRNA was undetectable for most samples.

ZFFs provided quick fixation, good to excellent morphology, adequate antigen preservation for IHC, and good quality DNA and miRNA. ZFFs are excellent replacements for B-5 in hematopathology practice and are suitable for clinical and research purposes.

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Gastrointestinal Signet-Ring Cell Carcinoma vs Metastatic Lobular Carcinoma to Gastrointestinal Tract: An Immunohistochemical Differential Diagnostic Strategy

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Metastatic lobular carcinoma (MLC) to the gastrointestinal (GI) tract or peritoneal surface can be extremely difficult to distinguish morphologically from primary GI signet-ring cell carcinoma (SRC), a distinction essential for appropriate therapy. Various markers and their value in the differential diagnosis of these 2 entities were studied.

The study included 18 gastric, 7 colorectal SRC, and 9 MLC samples from 8 patients. We compared MLC and SRC immunoprofiles using commercially available antibodies for CK7, CK20, ER, PR, GCDFP-15, mammaglobin (MAM), CDX2, and MUC1. The percentage of positive cells was recorded, and cases with expression in more than 5% of tumor cells were considered positive. The Fisher exact test was used for statistical analysis.

All MLC patients were women with a median age of 73 years (range, 48-79 years) and had documented breast tumors. The interval from diagnosis to metastasis ranged from 0 to 20 years (mean, 7 years; median, 5 years). Three primary tumors compared with the subsequent metastases. MLC to the stomach (6) and small (1) and large bowel (2) was identified in 7 biopsies and 2 resection specimens.

CK7, CK20, ER, PR, GCDFP-15, MAM, MUC1, and CDX2 were expressed in 84%, 72%, 0%, 0%, 0%, 0%, 8%, and 76% of SRC and in 100%, 0%, 77%, 23%, 67%, 53%, 86%, and 0% of MLC, respectively. MUC1 was significantly more often expressed in MLC than in SRC ($P = .01$). Focal expression of CDX2 was present in 1 MLC. Two MLCs were negative for ER and PR. No ER nuclear expression was present in SRCs, but 2 cases showed weak nuclear PR expression. Three MLCs (33%) were misdiagnosed; 1 patient underwent right hemicolectomy. MLC retained its original phenotype even in late metastases.

MLC to mucosal GI sites can occur late and poses significant diagnostic challenges, and its misdiagnosis can have serious consequences. Several markers have excellent discriminating value: a panel of CK20, ER, CDX2 and GCDFP-15 suffice in most cases, but additional studies (PR, MAM, and MUC1) might be occasionally needed for the correct diagnosis.

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High Levels of *c-myc* Sequences in Early-Stage Tumors From Patients With Bladder Carcinoma

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Bladder carcinoma of the squamous cell type is the most prevalent type in Egypt. It is thought that chronic schistosomiasis infections take part in initiation of the precancerous state. Early detection of these tumors is essential for successful treatment without recurrence. DNA copy number changes detected in schistosomiasis-associated (SA) tumors had more changes than non-SA tumors, whereas the number of changes in SCC and TCC tumors was similar. The cellular *myc* (*c-myc*) proto-oncogene is a homologue of the transforming gene of avian myelocytomatosis virus. It has been linked to cell proliferation in a number of different systems.

We studied *c-myc* oncogenic alterations in DNA from tumors of 18 patients with bladder carcinomas associated with chronic schistosomal infections. Samples from these patients were compared with 10 samples of normal bladder tissue, peripheral blood lymphoblasts, and SCaBER and T24 squamous and transitional cell carcinoma cell lines. High levels of *c-myc* sequences were detected in the DNA from samples taken from early-grade squamous cell carcinoma. Amplification of *c-myc* DNA sequences initiated in the precancerous cells and correlated with the tumor grade.

DNA and RNA extracts were prepared from solid tumor tissue. Spots of 2-fold serial dilutions of the samples were fixed on a nitrocellulose membrane by using a manifold suction device from S&S, USA. We performed Dot-spot hybridization by using a biotinylated *c-myc* probe from Oncogene, USA. Signal was developed by using the streptavidin-HRP/TMB system from R&D.

C-myc was amplified about 150-fold in DNA samples from squamous cell carcinoma as compared with DNA from normal tissue. A linear dose-response relationship was found between the degree of amplification and grade of tumor. This is the first report to present data correlating *c-myc* amplification with the grade of SA-SCC.

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Endoplasmic Reticulum (ER) Stress in Human Nonalcoholic Steatohepatitis (NASH)

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ER stress-induced pathways are implicated in several diseases, including diabetes. Accumulation of misfolded/unfolded proteins induces ER stress, which is sensed by transmembrane proteins, including protein kinase-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). Activation of such proteins results in complex signaling responses through c-Jun, p38 MAPK, and NF- κ B pathways to dampen the stress. Studies have shown that ER stress plays a role in liver injury in animal models and bariatric NAFLD. However, data for nonbariatric human NASH and ER stress are limited.

Liver biopsies diagnosed with NASH between 2007 and 2009 were retrieved from the files. Immunohistochemistry (IHC) for PERK, eukaryotic initiation factor 2 α (eIF2 α), IRE1, and ATF6 was performed on paraffin-embedded tissue. We analyzed 21 cases (16 NASH and 5 control cases). Nontumor liver and hepatitis C biopsies served as control cases. NAS and stage were determined. IHC results were evaluated for cell type and nuclear and cytoplasmic reactivity.

NASH cases showed increased expression of ER stress proteins in hepatocytes with little variation between steatotic and nonsteatotic cells. ATF6 was positive in hepatocyte and biliary nuclei; the remaining markers were positive only in hepatocytes. ATF6 showed strong, diffuse nuclear reactivity, while eIF2 α and IRE1 showed mild, diffuse cytoplasmic staining in hepatocytes. PERK showed mild, patchy nuclear reactivity in hepatocytes. Control cases were negative for IHC markers except ATF6. It is interesting that eIF2 α expression was decreased in a cirrhotic NASH; otherwise, no correlation was observed between immunoreactivity of ER stress proteins and disease severity.

ER stress response proteins are up-regulated in NASH, and their expression is not limited to steatotic/ballooned hepatocytes. This suggests a broader role of ER stress and characterization of a specific pathway may provide insights about the specific stress response involved in human NASH.

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Elevated Plasma Level of Clotting Factor VIII Predicts Venous Thrombosis in Children With End-Stage Kidney Disease (ESRD)

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Clotting factor abnormalities are common in children with ESRD, although their impact on subsequent thrombotic complication is unclear. We investigated the prevalence of factor VIII and other clotting factor abnormalities and their effect on thrombotic events.

Between January 2007 and October 2009, 29 pediatric patients with CKD IV and ESRD on hemodialysis (HD) or peritoneal dialysis (PD) were screened for inherited and acquired thrombophilic disorder: protein C, protein S, factor V Leiden mutation (FV506Q), thrombin III deficiency, prothrombin mutation (G20210A), homocysteine, factor VIII, factor IX, factor XI, DRVVT, PTT-LA, and antiphospholipid antibodies. We collected baseline demographic data, including age, sex, race, duration and type of dialysis, type of access, and cause of ESRD, and analyzed their impact on the development of thrombotic complications.

Of 29 patients, 22 were on dialysis and 7 had stage 4 CKD. Ages ranged from 3 to 21 years. There were 16 males (55.1%), 18 (62%) AA. Renal diseases included FSGS in 14 (48.2%), dysplasia/hypoplasia in 6, obstructive uropathy in 2, and others in 7. Of the patients, 19 were on HD and 3 on PD. Fifteen patients had CVC, and 4 had an AV graft/fistula. Fifteen (51%) had more than one thrombotic risk factor. Hyperhomocysteinemia (48%) and elevated factor VIII (44%) were the most common abnormalities. Venous thromboembolism occurred in 7 of 22 (31.8%) dialysis patients. We analyzed the data with separate logistic regressions of thrombotic events on homocysteine, factor VIII, age, and type of disease. None of these were statistically significant owing to the small sample. However, there was a trend toward higher levels of homocysteine associated with less risk (95% CI, 0.68 to 1.02; OR, 0.83; $P = .08$) and higher levels for high factor VIII associated with greater risk (OR, 1.01; 95% CI, 0.996 to 1.02; $P = .15$).

There is a linear relation between factor VIII level and risk of thrombosis. We recommend that prophylactic anticoagulation should be considered in these patients.

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Frequency and Clinical Meaning of Fibroelastotic Changes in the Gastrointestinal Tract

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Fibroelastotic changes (FECs) in the gastrointestinal tract (GIT) are rarely described and defined with abnormal accumulation of elastic fibers within the mucosal, submucosal, or muscular layer. Their frequency, origin, and clinical meaning are unclear. Therefore, a prospective study was performed to address these issues.

Within a 2-year period, 1 pathologist (B.M.) screened all incoming GIT specimens for the occurrence of amorphous accumulations that are "suspicious" for elastotic lesions on H&E-stained slides. In all suspicious cases, elastic van-Gieson staining was performed to verify the elastotic origin. The corresponding endoscopic findings were correlated by an experienced gastroenterologist (S.G.).

FECs were found in 5 esophageal, 47 gastric, and 15 colonic biopsies, representing about 2% of all investigated cases. Another 4 lesions were found in surgical specimens of the colon. One lesion occurred in an appendectomy specimen.

Two cases showed elastofibroma-like fibers. In 3 specimens, lesions were found similar to changes we defined as angioelastosis, recently. Polyps or mucosal elevations were the most common endoscopic findings (15 cases). In 1 of these cases, a right hemicolectomy

was performed because of severe stenosis caused by angioelastosis. In 3 polypoid lesions, the elastotic change was associated with neoplasia ranging from low-grade dysplasia to invasive cancer. One patient received intensive endoscopic surveillance in very short intervals because of a clinical highly suspicious polypoid lesion in the stomach. The second most associated lesion was ulcerous disease (11 cases). Other related clinical findings were postradiation, postlymphoma, gastrectomy, and postintervention.

FECs in the GIT are not as exceedingly rare as thought before. Our data suggest a mainly reactive origin. Although rarely associated with a neoplastic disease, it is the only histomorphological finding in a certain number of polypoid lesions that are partially highly suspicious for malignancy.

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Tumor Marker Multiconstituent Controls for Use in Quality Assurance of Assay Performance: The Importance of Clinically Relevant Analyte Levels and Appropriate Matrix Composition

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Quality assurance of tumor marker assays requires access to control materials that closely resemble patient samples. Several multiconstituent tumor marker control kits are marketed for this purpose. The objective of this study was to compare 6 commercially available products in terms of analyte levels and control matrix.

The analyte levels for AFP, CA15-3, CA19-9, CA125, CEA, ferritin, free PSA, and total PSA were determined for 6 multiconstituent tumor marker control kits: Fujirebio Diagnostics Control (Gothenburg, Sweden), Liquichek (Bio-Rad Laboratories, Irvine, CA), Lyphocheck (Bio-Rad), Liquid QCTM (Cliniqa, San Marcos, CA), MAS T-marker (Microgenics, Fremont, CA), and Quality Control Sera (Randox Laboratories, Oceanside, CA). To evaluate matrix composition, the different control materials and normal serum samples were analyzed with a Serum Protein Electrophoresis (SPE) kit. All kits contained 1 control where the levels for each analyte corresponded to the normal range and at least 1 control with analyte levels in the pathological range.

Clinically relevant percentages of free PSA to total PSA were observed only in the controls from Fujirebio and Cliniqa, with 30% and 10% free PSA, respectively. The remaining control materials tested contained 90% or more free PSA. The Fujirebio and Lyphocheck controls showed similar patterns on SPE compared with normal serum samples, including bands in the albumin and α -, β -, and γ -globulin regions. The α - and β -globulin bands were merged for the Cliniqa control. The Liquichek, MAS, and Randox controls showed a distinct albumin band only.

Clinically relevant analyte levels and appropriate sample matrix are the most important parameters for any quality control. The Fujirebio and Cliniqa controls showed a clinically relevant ratio of free PSA to total PSA and were identified as suitable for quality assurance of total PSA assays. The controls with a matrix most similar to patient samples were Fujirebio and Lyphocheck.

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CD117 (c-kit) and Mast Cell Tryptase Are Equivalent for Enumerating Mast Cells in "Mastocytic Enterocolitis"

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Mastocytic enterocolitis (ME) is a proposed morphologic disorder that occurs in patients with chronic intractable diarrhea and is characterized by increased gastrointestinal mucosal mast cells.

As originally described, the increased mast cells are demonstrated by immunohistochemical staining for mast cell tryptase (MCT) and predict a favorable therapeutic response to drugs affecting mast cell mediator function. Because antibodies to MCT may not be available in all laboratories, we sought to compare the performance of CD117 vs MCT for the enumeration of mast cells in colonic biopsies.

Colonic biopsies from 43 patients were selected (4 with ME and 39 randomly selected biopsies with histories ranging from no symptoms to chronic intractable diarrhea). The biopsies were immunohistochemically stained for MCT and c-kit, and the immunopositive cells for each stain were enumerated in 10 high-power fields (HPF) and averaged. There was a 96% concordance in the mast cell counts between the MCT and c-kit stained slides, calculated by Pearson product-moment correlation. In addition, mast cell counts for both stains were statistically higher in patients with known ME compared with the randomly selected biopsies. The mast cells per HPF in the randomly selected biopsies ranged from 6.4 to 39.2. When stratified according to the clinical history, the number of mast cells was statistically higher in patients with diarrhea than in asymptomatic patients. However, several patients with no diarrhea symptoms exhibited greater than 20 mast cells per HPF.

Immunohistochemistry for c-kit is equivalent to MCT for the enumeration of mast cells in patients with suspected ME. This supports the use of this alternative stain for laboratories that may not have access to MCT. An unanticipated finding was that several biopsies in patients with no symptoms exhibited mast cell counts exceeding 20/HPF, raising the question of whether a threshold of 20 mast cells per HPF reliably separates normal from pathological numbers of mast cells.

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Utility of UroVysion Fluorescence In Situ Hybridization Assay for Characterization of Renal Cell Carcinoma in Paraffin-Embedded Fixed Tissue: A Study of the Genetic Composition of Renal Cell Carcinoma With Clear Cell Features

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The most common forms of renal cell carcinoma (RCC) are clear cell (CC) and papillary (PC), but tumors often demonstrate a mixed phenotype. Immunohistochemical (IHC) positivity for CK7 and Amacar (P504S; Ventana, Tucson, AZ) supports PC, but exceptions exist. We tested the ability of the UroVysion fluorescence in situ hybridization (UV FISH) assay (Abbott Molecular, Des Plaines, IL) to characterize these lesions.

RCCs were classified morphologically as CC (48) or PC (17), and the extent of clear cell and papillary features was noted. Tissue microarray sections of 0.6- μ m core samples constructed from paraffin-embedded tumor were processed for UV FISH, CK7, and Amacar. We analyzed 15 to 30 cells from each tumor for copy number of chromosomes 3, 7, and 17. A tumor was deemed CC when it was disomic for chromosomes 3, 7, and 17; PC when at least 2 of 3 chromosomes were trisomic; and aneuploid when 3 or more copies for any of the 3 chromosomes was present. A correlation of histology, IHC studies, and cytogenetic composition was performed.

In 32 of 48 CCs, the aggregate of histology and IHC and cytogenetic composition agreed and 4 were aneuploid. In all 9 CC cases in which histology and IHC disagreed, cytogenetic composition agreed with histology. Seven CC cases had concurrent histology and IHC but discordant cytogenetics. Of 17 PCs, 8 were concordant. Of the 10 PC cases with CC cytologic features, 4 had CC cytogenetics. Overall, cytogenetics concurred with final diagnosis in 86% of CC and 64% of PC cases.

In cases of discrepant histology and IHC, UV FISH is useful in the final characterization of RCC. UV FISH was also key in tumors demonstrating papillary architecture with CC cytologic features.

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Laboratory Performance Improvement by Automated Reporting of Immature Granulocytes on the Sysmex XE-2100

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Leukocyte differentials are performed automatically using the Sysmex XE-2100 and then “reflexed” to manual differentials based on a set of flags. The flag for immature granulocytes (IGs) is triggered by a default setting of IG more than 0.9%, but the setting can be customized by the user. Although the Sysmex XE-2100 can be validated to automatically report IGs, a manual differential will still be performed if any additional flags are present in a sample. We sought to specifically determine the effect of using automated IG counting on the number of manual differentials we perform in a major academic institution.

We retrospectively evaluated data on 3,177 automated and manual differentials performed in a 7-day period to determine the number of manual differentials that were performed secondary to isolated IG flags. We found that 3.0% of all automated differentials in that period reflexed to a manual differential for a pure IG flag and, therefore, decided to pursue automated IG counting. We then prospectively validated automated IG counting with duplicate manual counting and determined a linear correlation up to 7%. We reset the flag for IGs to 7% or more and measured the number of manual differentials that we were able to avoid by the new criteria. We evaluated the IG percentage from 10,334 automated differentials and found that we prevented a total of 1,028 manual differentials in the first month after implementing the new criteria.

We were able to prevent a significant number of manual differentials by resetting the Sysmex XE-2100 to reflex to manual differentials only if the IGs are 7% or more. The benefit of automated IG counting in a given institution likely depends on the patient population. Our measured outcome was even better than predicted, reducing our percentage of manual differentials from 32.5% of total differentials to 25%.

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Helicobacter pylori: To Stain or Not to Stain?

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Debate continues regarding the necessity of performing routine special stains for the identification of *Helicobacter pylori* (HP) in gastric biopsies. We performed a retrospective study in order to investigate the utility of special stains for the diagnosis of HP.

We retrieved 200 consecutive cases from our files using the key words “gastritis” and “*Helicobacter pylori*.” H&E-stained slides and special stains for HP were reviewed independently by 2 pathologists and examined for the presence of HP and the degree and types of inflammation present. The minimum number of high-power fields (HPF) required to see the organisms on H&E was recorded. Our criteria were matched by 196 biopsies obtained from 181 patients. We found that 32 biopsies were positive for HP by special stains; of those, HP was seen on H&E in 29 cases (91%). Four biopsies (2%) were considered “suspicious” or equivocal on H&E but negative by stain. In 2 biopsies (1%), HP was initially missed on H&E but sub-

sequently seen on review. There were no cases in which the special stain was negative where definitive HP was identified on H&E. The number of fields required to detect HP on H&E ranged from 1 to 25 (mean, 5.8; median, 4). Significant (2+ or 3+) amounts of plasma cells showed 94% sensitivity for HP. Combined significant acute and chronic inflammation had a specificity of 98% and a negative predictive value of 97%.

Our results show that HP can be seen relatively easily on H&E in the majority of cases; however, a small number of cases can be missed if special stains are not employed. Immunostaining is very sensitive and useful in cases morphologically suspicious for the presence of HP (ie, significant inflammation). We do not advocate the routine use of special stains on all gastric biopsies.

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A 15-Year Perspective on Pathological Factors and Outcomes for Oral Tongue Invasive Squamous Cell Carcinoma (OTISCC)

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The purpose of this study was to find useful factors for predicting local recurrence, later cervical metastasis, and disease-free and overall survival in patients with OTISCC. We investigated clinicopathologic factors and TNM stages for all OTISCC patients from 1994 to 2009. Pathologic factors included depth of invasion (DOI), tumor differentiation (TD), angiolymphatic invasion, perineural invasion (PI), mode of invasion, and lymphocytic infiltrate. Follow-up data were collected for all patients. A log-rank test was selected for *P* values.

A total of 66 patients were identified with mean age of 61.7 years (range, 23-91 years); 42 (64%) were men. The distribution of T stage was as follows: T1, 37%; T2, 31%; T3, 20%; and T4, 12%; N-stage distribution was as follows: N0, 66.7%; N1, 9.1%; N2, 21.2%; and N3, 3%. The median follow-up time was 2.2 years. There were 9 local recurrences with a median time of 0.63 years. There were 6 later cervical metastases with a median time of 1.38 years. Of the patients, 16 (24%) died of disease-specific causes. The overall 1- and 3-year survival rates were 0.83 (95% CI, 0.71-0.91) and 0.67 (95% CI, 0.52-0.78), respectively. The pathological factors correlating with local recurrence included a DOI of more than 2 mm (*P* = .0032), TD (*P* = .025), PI (*P* < .001), and T stage (*P* = .003). The factors associated with worse disease-specific survival at 3 years included DOI (87%, CI, 0.65-0.96, DOI ≤ 2 mm vs 57%, CI, 0.36-0.73, DOI > 2 mm), PI (76%, CI, 0.60-0.87 without PI vs 35%, CI, 0.5-0.69 with PI), TD (90%, CI, 0.66-0.97, well; 60%, CI, 0.37-0.77, moderate; and 55.6%, CI, 0.20-0.80, poor), and advanced T stage (90%, T1; 70%, T2; 47%, T3; and 20%, T4).

Besides TNM stage, DOI, PI, and TD were significantly associated with local recurrence and disease-specific survival. No statistical significance was found among pathologic factors with later cervical metastasis. Future biologic markers and molecular studies are necessary to guide surgeons in clinical management.

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Association of Wild-Type p53-Induced Phosphatase 1 (Wip1) and BCR-ABL Gene Expression in Chronic Myeloid Leukemia

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The *BCR-ABL* fusion oncogene is an activated tyrosine kinase that activates several signaling pathways leading to chronic myeloid leukemia (CML). In addition, the Wip1 has been shown to be amplified and overexpressed in multiple human cancer types. This cross-sectional study aimed to determine the relationship between Wip1 and *BCR-ABL* gene expression in patients with CML.

We studied 30 patients in the chronic phase of CML with various molecular responses, including complete, major, partial, and no molecular responses (informed consent obtained). Total RNA was extracted from peripheral blood, and cDNA was synthesized accordingly. *BCR-ABL* gene expression was evaluated by TaqMan-based real-time polymerase chain reaction, and Wip1 gene expression was assessed by SYBR Green-based real-time polymerase chain reaction. Final data were analyzed statistically.

In this study, we found a significant correlation between Wip1 and *BCR-ABL* gene expression in CML patients ($P = .042$).

Our data may suggest a *BCR-ABL*-dependent or *BCR-ABL*-independent role for Wip1 in the pathogenesis of CML.

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SIRT1 Overexpression in Tubular Adenomas, Tubular Adenomas With High-Grade Dysplasia, and Invasive Adenocarcinomas

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Silent mating-type information regulation 2 homologue 1 (SIRT1) is an NAD-dependent, class III histone deacetylase. SIRT1 plays an important role in cell survival by deacetylation of key cell cycle molecules, such as p53. SIRT1 is involved in the development of many malignant tumors. SIRT1 can function as an oncogene (inhibiting p53 activity) and a tumor suppressor (inhibiting β -catenin activity). The role of SIRT1 in colon cancer is not clear.

Tubular adenomas (TAs; 21 cases), tubular adenomas with high-grade dysplasia (TAHGs; 20 cases) and invasive adenocarcinomas (CAs; 34 cases) were stained for SIRT1 expression. SIRT1 expression in normal colon was used as the basal line. The IHC intensity was scored as 0 to 4, and the percentage of positive tissue was scored as 0 to 100. The total raw scores were the multiplication of intensity and percentage. SIRT1 expression was defined as no overexpression (score <100), low (score 100-200), or high (score >200).

The SIRT1 expression scores were 263.3 ± 55.7 for TA ($n = 21$), 228.7 ± 103.5 for TAHG ($n = 20$), and 132.3 ± 104.4 for CA ($n = 34$). There was statistical significance between the premalignant lesions and carcinomas ($P = 2.37E-06$ for TA vs CA; $P = .0018$ for TAHG vs CA). TAHG had lower SIRT1 scores than TA, but it was not statistically significant ($P = .188$). Most TAs (95.2% [20/21]) had high SIRT1 overexpression, except 1 case. The TAHGs had 70% (14/20), 10% (2/20) and 20% (4/20) high, low, and no SIRT1 overexpression, respectively. The invasive adenocarcinomas had 29.4% (10/34), 32.4% (11/34) and 38.2% (13/34) high, low, and no SIRT1 overexpression, respectively.

SIRT1 overexpression was present in TAs, TAHGs, and CAs. The premalignant lesions (TA and TAHG) had significantly higher SIRT1 expression scores and higher percentages of high SIRT1 overexpression than the CAs. The CAs were heterogeneous lesions (about one third each for no, low, and high SIRT1 overexpression).

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Correlation of Grade and Stage With Risk-Stratification Gene Expression Profiling for Invasive Breast Carcinoma

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Mammaprint (Agendia, Huntington Beach, CA) is a 70-gene, FDA-approved gene expression profile (GEP) performed on fresh tissue used to stratify invasive breast cancer patients into recurrence risk categories. The purpose of this study was to correlate pathological grading and staging to GEP risk stratification in women with invasive breast carcinoma.

Fresh tissue was sent for Mammaprint analysis on 47 patients treated at the University of South Florida Breast Health Center with a suspected or biopsy-proven diagnosis of invasive breast cancer from June 2009 through December 2009. The specimens were obtained by core biopsy or at the time of surgical excision. A GEP was included in the analysis when tumor volume was sufficient ($\geq 30\%$ tumor/connective tissue ratio). The remaining tissue was processed for routine pathologic analysis, including Nottingham grade and AJCC stage assessment.

Of the tumors, 2 grade 1, 14 grade 2, and all grade 3 (17) were high-risk. There were 2 low-risk tumors, 1 grade 1 and 1 grade 2. The low-risk cancers were stage IA and IIA, respectively. The high-risk cancers consisted of 12 stage IA, 2 stage IIA, 3 stage IIB, 1 stage IIIB, and 6 stage IIIC. Eleven cases were reported as insufficient tumor cellularity; of these, 6 were obtained by core biopsy, 4 had at least a focal mucinous component, 1 had extensive DCIS, and 2 were small (≤ 3 mm).

Mammaprint analysis provides good correlation between higher grade tumors and high-risk status. Twelve high-risk stage I tumors were identified, 3 of which were T1b (0.6-1.0 cm). Identifying the patients who would benefit from chemotherapy is critical to the care of women with breast cancer. These findings may challenge the treatment now being offered for these early-stage tumors. Sampling issues related to method of procurement, low cellularity of low-grade and mucinous carcinomas, extensive DCIS, and small lesions need to be resolved by future studies utilizing larger cohorts.

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Statistical Analysis of Frequencies of Mutations in the Cystic Fibrosis Transmembrane Regulator Gene and Their Associations With IVS8-polIT and Y Chromosomal Microdeletions

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An association between cystic fibrosis transmembrane regulator (*CFTR*) gene mutations and male and female infertility has been hypothesized. This study investigated the frequency of the *CFTR* gene mutation in a group of consecutive patients who were candidates for assisted reproductive techniques and the relation between *CFTR* mutation, IVS8-polIT, and Y chromosomal microdeletions in azoospermic males.

We screened 7,851 patients (4,234 females and 3,617 males) for 53 *CFTR* gene mutations and IVS8-polIT polymorphism by multiplex PCR; 223/3,617 (6.2%) were azoospermic men investigated for Y chromosomal deletion by multiplex PCR. Frequencies of mutation were separately calculated in all samples for men and women; the χ^2 test was used for comparisons; P values $\leq .01$ were considered significant.

CFTR mutations were detected in 4.6% of the subjects, a percentage similar to that reported in the general population. The most common mutation was $\Delta F508/N$, observed in 1.71% of patients. No difference in sex distribution was evidenced; 52.8% of all *CFTR* mutations included in the test used were never detected; this percentage increased to 64% when considering the sex distribution of each single mutation.

The IVS8-poly-T showed a frequency of 70.9% for 7T/7T alleles, 19.8% for 7T/9T alleles, and only 0.20% for 5T/5T alleles. In 2 of 223 azoospermic patients, there was a Y chromosomal microdeletion and negativity for the *CFTR* mutation; 6 of 223 azoospermic patients had a *CFTR* mutation. The following distribution of IVS8-poly-T polymorphism in azoospermic patients was detected: 5T/5T, 0.86%; and 9T/9T, 0.86%.

Our data show no evidence of associations between azoospermia, *CFTR* mutations, IVS8-poly-T, and Y chromosomal microdeletions. A great percentage of the mutations included in the screening test used were absent in the large series of cases analyzed.

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Fast Bedside Measurement of Blood Count and C-Reactive Protein in Newborns: Comparison With Conventional Methods

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Abnormal CBC and high plasma C-reactive protein (CRP) are associated with neonatal infections and could be helpful in the diagnosis of neonatal sepsis and to monitor the antibiotic treatment. This work has the aim of evaluating and comparing the performance of the ABX-MicrosCRP200, a bedside analyzer that requires 18 μ L of whole blood to perform a CBC and CRP dosage in 4.5 minutes, with conventionally measured CBC and CRP in a neonatal population.

We collected 150 capillary or venous blood samples of term and preterm newborns. All samples were processed on an ABX-MicrosCRP200 analyzer and on a Sysmex XE-2100 (conventional hematology analyzer), for CBC, leukocyte differential, reticulocytes, and nucleated RBCs; high-sensitivity CRP (hs-CRP) was performed on a ModularPE. According to the Bland-Altman procedure, the CBC and CRP differences were regressed against their means and assessed also by means of intraclass correlation (ICC).

The ICC for WBCs was 0.98 (95% CI, 0.95, 0.99); for hemoglobin, 0.97 (95% CI, 0.96, 0.98); for hematocrit, 0.96 (95% CI, 0.93, 0.98); for MCV, 0.95 (95% CI, 0.93, 0.96), and for platelets, 0.98 (95% CI, 0.96, 0.98). However, the ABX-MicrosCRP200 overestimated the WBC count (+1.18; 95% CI, +0.94, +1.42; $P < .001$), hematocrit (+1.58; 95% CI, +1.02, +2.15; $P < .001$), and platelets (+12.1; 95% CI, +6.8, +17.5; $P < .001$). No evidence of overestimates or underestimates was found for hemoglobin and MCV. The ICC for CRP was high (0.973; 95% CI, 0.966, 0.973), without any systematic difference between the 2 values ($P = .64$). The SD of the difference was as follows: for WBCs, 1.28; for platelets, 34.17; and for hemoglobin, 0.64.

The agreement between the 2 methods was high for each parameter. The SD of the difference for hemoglobin has no impact on the possible transfusion threshold but could be clinically important for WBCs and platelets in leukopenic or thrombocytopenic newborns.

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A Systematic Review and Meta-analysis of the Diagnostic Accuracy of FNAC for Parotid Gland Lesions

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The value of FNAC for diagnosis of salivary gland lesions is controversial. Numerous accuracy studies have been published with variable results; however, the literature has not been summarized. We performed a meta-analysis to summarize the diagnostic accuracy of FNAC in parotid gland lesions.

MEDLINE, EMBASE, and the bibliographies of retrieved articles were searched for studies published between January 1, 1985, and January 1, 2010, evaluating FNA accuracy in parotid gland lesions. SCOPUS was searched to find articles citing the set of retrieved articles. Studies were eligible if they were based on cohorts of consecutive patients with histological verification of all cases. Eligible studies were included if accuracy data (true-positive, false-positive, false-negative, true-negative) could be extracted directly or calculated. Quality assessment was performed using QUADAS. Data extraction and quality assessment of included articles were done independently by 2 researchers. Meta-analytic methods based on a bivariate random effects model were used to construct summary receiver-operating characteristic (SROC) curves. Sources of heterogeneity were studied using meta-regression.

We screened 3,848 titles and abstracts to obtain a set of 75 articles that met our inclusion criteria. The area under the SROC (AUSROC), pooled sensitivity, and specificity for determination of malignancy were 0.96 (95% CI, 0.94-0.97), 79% (95% CI, 75%-83%), and 97% (95% CI, 96%-98%), respectively. The AUSROC, sensitivity, and specificity for the discrimination of neoplastic lesions from nonneoplastic lesions were 0.98 (95% CI, 0.98-1.00), 96% (95% CI, 83%-98%), and 98% (95% CI, 68%-100%), respectively.

FNAC has greater power to discriminate neoplastic from nonneoplastic lesions than to discriminate malignant from benign parotid gland lesions. FNAC is highly specific but moderately sensitive for the detection of malignancy. FNAC is highly sensitive and specific for the detection of neoplasia.

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Sensitivity for Quality Control (QC) Detection of Random-Error 2-Standard-Deviation (2s) and 3-Standard-Deviation (3s) Departures From True Mean, When QC Mean and Standard Deviation Are Estimated by Only 20 Prior Measurements

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Replicate QC material measurements are almost always characterized by a normal distribution. For such normally distributed data, random-error 2s and 3s deviations of the measurement (x) from the mean (x_m) occur at fixed rates (0.045 and 0.0027, respectively). If x_m and standard deviation (s) are known exactly, then sensitivity for detection of such deviations will be 100%. In practice, however, the mean and standard deviation are known only as estimates based on a finite number of measurements, such that sensitivity of the detection of true 2s and 3s random-error deviations from the true mean is necessarily less than 100%. The Clinical and Laboratory Standards Institute (CLSI) recommends estimation of means and standard deviations for quality control materials via 20 measurements. We know of no source that documents the associated sensitivity for true 2s and 3s detection for this specific circumstance, and as the answer is not directly calculable analytically, we

determined 2s and 3s sensitivity by simulation of a QC procedure using $n = 20$ measurements for estimation of $xm(n)$ and $s(n)$.

In 10 runs of 10,000 trials each, $xm(20)$ and $s(20)$ were determined by random-number-generated sampling of 20 replicates of x , according to probabilities of a standard normal distribution ($x = 0 \pm 1s$). The next (21st) sampling for each trial represented a QC measurement, x . For 2s deviations, true-positives (TP) occurred if $|x| > 2s$ and $|x - xm(20)| > 2s(20)$; false-negatives (FN) occurred if $|x| > 2s$ but $|x - xm(20)| < 2s(20)$. Sensitivity was calculated as $TP / (TP + FN)$. The analogous calculations were made for 3s deviations. For 2s, sensitivity for detection was $78.4\% \pm 1.8\%$. For 3s, sensitivity for detection was $67.0\% \pm 6.4\%$. These results document the extent to which correct QC classifications of random-error deviations from the true mean are limited, even when QC parameters are determined by 20 measurements per CLSI recommendations.

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Utilization of a Molecular Method for the Identification of *Scopulariopsis brevicaulis* as a Cause of Disseminated Hyalohyphomycosis

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Scopulariopsis brevicaulis, although highly resistant to antifungals, is rarely identified as a cause of invasive disease owing to difficulty in species identification. We describe a case of disseminated infection caused by *S brevicaulis* in an immunocompromised patient using histology along with a molecular approach for identification.

A 26-year-old man with a history of diffuse large B-cell lymphoma underwent high-dose chemotherapy followed by a matched unrelated donor stem cell transplant. The patient was discharged 3 weeks after transplantation but was readmitted 1 month later with graft-vs-host disease of the skin and gastrointestinal tract. Five weeks after readmission, black skin lesions developed on the chest and gluteal areas. A punch biopsy of each area was submitted for culture and histopathological examination. Hyaline septate hyphae with acute-angle branching, consistent with *Aspergillus*, were identified in the deep tissues. Cultures after 9 days grew a mold identified as *Scopulariopsis* species. The patient, who had been receiving prophylactic voriconazole, was subsequently treated with micafungin and amphotericin B. The skin lesions continued to spread, and the patient died 7 days after diagnosis, with the cause of death listed as an invasive fungal infection.

Following DNA extraction from culture, a PCR-based assay using universal fungal primers was done to amplify the internal transcribed spacer 2 region of the rDNA complex. The amplicon was sequenced (272 bp) and compared with sequences in GenBank using a BLAST search. The sequence showed a more than 99% homology with multiple strains of *S brevicaulis*. The isolate identification was verified by the production of characteristic morphological structures on microscopic examination.

This case highlights the utility of using molecular methods in combination with histology to identify emerging fungal pathogens as causes of invasive disease. Additional studies are needed to refine this methodology as a valid process for optimal patient management.

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Evaluation and Comparison of Histopathologic Features in Liver Wedge Biopsies of Older and Younger Donors

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Owing to a shortage of liver donors, allografts from older donors (>65 years) have been accepted for liver transplantation. The effects of older liver donors on allograft survival have been variable. The aim of our study was to evaluate and compare histological changes in liver allograft wedge biopsies between older and younger liver donors.

A retrospective review of liver donor wedge biopsies was conducted on older donors and a control group of younger donors for patients who underwent liver transplantation from 2005 to 2009. A H&E stain was evaluated for steatosis, inflammation, glycogenated nuclei, lipogranulomas, sinusoidal dilatation, zone 3 necrosis, bile ductular proliferation, and arterial sclerosis in all cases. Additional stains for trichrome, PAS after diastase digestion, and iron stain were performed to evaluate for fibrosis, cytoplasmic globules consistent with α_1 -antitrypsin, iron accumulation, and ceroid-laden histiocytes.

Older allograft donors (>65 years; $n = 60$) showed increased portal inflammation (17% vs 7%; $P = .02$), glycogenated nuclei (48% vs 29%; $P = .007$), and lipogranulomas (12% vs 3%; $P = .002$) and decreased macrovesicular steatosis (10% vs 29%; $P = .002$) when compared with younger donors (≤ 65 years; $n = 199$). Fibrosis was more common in older donors (17%) than in younger donors (7%; $P = .03$); however, there was no difference when evaluated for periportal or bridging fibrosis (7% vs 3%; $P = .24$). There was no statistical difference between the 2 groups for microvesicular steatosis ($P = .61$), lobular inflammation ($P = .41$), bile ductular proliferation ($P = .4$), sinusoidal dilatation ($P = .32$), zone 3 necrosis ($P = 1.0$), arterial sclerosis ($P = .05$), presence of PASD-positive globules ($P = 1.0$), ceroid-laden histiocytes ($P = .9$), and iron accumulation ($P = .07$).

Older liver allograft donors had increased portal inflammation, mild portal fibrosis, glycogenated nuclei, and lipogranulomas as compared with younger donors. Younger donors had an increased incidence of macrovesicular steatosis. There was no statistically significant difference in the extent of advanced fibrosis between the 2 groups.

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Quality Control Planning of a Hepatitis B Viral Load Assay

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HBV DNA measurement has an important role in management of patients with chronic viral hepatitis B. The aim of the study was to determine the performance characteristics and to plan a statistical quality control system of a laboratory-developed, real-time quantitative PCR assay for HBV DNA quantification.

Values of systematic and random error at 4.2 log IU/mL (20,000 IU/mL) and 3.2 log IU/mL (2,000 IU/mL) were determined. Candidate quality control procedures were selected, and performance of the method was determined employing a normalized operational process specifications (OPSpecs) chart.

The performance results of the assay at levels of 4.2 log IU/mL and 3.2 log IU/mL were excellent and good, respectively. Moreover, a 13.5S rule with 2 measurements offered 90% probability of error detection at a level of 4.2 log IU/mL, while no rule offered 90% probability of error detection at a level of 3.2 log IU/mL. Minimizing the formation of primer-dimer and nonspecific products and concentrating the target DNA during the purification process are proposed for accurate quantitative PCR, particularly when CT values are high.

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Electronic Entry of Point-of-Care Testing Results in Remote Alaska Villages

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The 12 native villages of the Maniilaq Health System (MHS) are the first Alaska Native rural sites to begin entry of all point-of-care testing (POCT) into the resource patient management system (RPMS) by community health aid practitioners (CHAPs) and nursing staff who perform testing. The goal is to fully implement use of the RPMS electronic health record (EHR) throughout the MHS by 2011.

In March 2009, the Maniilaq Health Center Laboratory began training all CHAPs and nursing staff, approximately 85 people, in the entry of POCT results into the RPMS EHR. Between March 2009 and September 2009, laboratory personnel trained staff at the main clinic facility in each village. MHS has seen increasing compliance with the entry of POCT results entered into the RPMS. Approximately 1,000 POC tests are performed throughout the MHS every month. Each month, a sampling of POC tests representing approximately 15% of tests is reviewed for compliance with entry of POCT results into the RPMS. In August 2009, only 34% of POCT results were entered electronically into the RPMS. As of April 2010, all CHAPs and nursing staff are trained in electronic entry of POCT results into RPMS, and approximately 90% of all POCT results are entered into the RPMS EHR.

The MHS strives to achieve 100% compliance with entry of POCT results into the RPMS EHR and has made great progress toward achieving this goal. This effort will help the MHC achieve its goal of full implementation of the RPMS EHR across the organization by 2011, in compliance with requirements of Meaningful Use for CMS.

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Suppression of Polyclonal γ -Globulin Background in the Presence of an M Protein on Serum Protein Electrophoresis Correlates With the Number of Plasma Cells in the Bone Marrow

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Multiple myeloma (MM) is characterized by clonal expansion of malignant plasma cells (PCs) in the bone marrow. Serum protein electrophoresis (SPEP) is usually used as an initial diagnostic approach, and the detection of a monoclonal band (M protein) on SPEP raises the suspicion of possible MM. The concurrent suppression of the polyclonal γ -globulin background (SPB) on the SPEP gel has not been investigated as a possible predictor of MM vs MGUS or other monoclonal gammopathies.

We examined 61 patients with an M protein on SPEP who had a bone marrow biopsy performed and correlated the SPEP findings of SPB with the number of PCs in the bone marrow. We found that 100% of patients who had an M protein of more than 3.0 g/dL exhibited SPB. About three fourths of these patients had more than 20% PCs in the bone marrow. In the other one fourth of the patients, the PC level was less than 10% (half of them were in the range of 5%-10% and the other half <5%). On the other hand, 88% of patients who had more than 20% of PCs in the bone marrow showed almost completely suppressed polyclonal background, whereas only 44% of patients with fewer than 5% of PCs in the bone marrow showed mostly partial suppression of the polyclonal γ -globulin background.

These results indicate that suppression of polyclonal γ -globulin background in patients with a strong M protein (>3.0 g/dL) could be a predictor of PC expansion in the bone marrow and serve as an additional indicator of MM. Additional studies are necessary to confirm the results of this pilot study.

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p16, WT1, and Fli-1 Proteins and Growth-Phase Assignment in Cutaneous Melanomas

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The ability to distinguish between radial growth phase (RGP) and vertical growth phase (VGP) melanoma is a significant prognostic aid. Despite established morphological criteria for these growth phases, use of specific molecular markers to differentiate between them has not been well-established. Our goal was to investigate the roles of p16, WT1, and Fli-1 in RGP-to-VGP progression.

In melanoma, p16 is thought to play a tumor suppressor role, while WT1 and Fli-1 act as transcriptional activators. Hence, we hypothesized that as tumors enter the VGP, p16 labeling should decrease, while WT1 and Fli-1 labeling should increase. We immunostained 18 RGP and 15 VGP melanoma cases (45% male; 55% female; mean age, 54 years) with well-characterized antibodies to p16, WT1, and Fli-1, separating VGP cases into superficial/lateral and deep components based on morphologic appearances.

In the RGP melanomas, p16 was expressed at least weakly in 15 of 18 (83%), weak-to-strong WT1 reactivity was seen in 17/17 (100%), and Fli-1 was expressed focally in 6 of 18 (33%). The deep component of VGP melanomas showed strong WT1 staining in 10 of 15 (67%) cases, Fli-1 staining in 9 of 14 (64%) cases, but strong p16 expression in only 2 of 15 (13%) cases in that tumor compartment. The majority of RGP cases stained for p16 and WT1, while fewer stained for Fli-1.

Positivity for WT1 is inexplicable in the context of the paradigm given above, but it indicates that it is not likely to be a good indicator of transition from RGP to VGP. On the other hand, one may suspect the presence of the VGP when lesions label moderately for Fli-1 or weakly/not at all for p16 in their deep dermal components. Histological criteria remain the best method for assignment of growth phase in melanomas. However, p16 and Fli-1 may prove to be useful adjuncts in morphologically indeterminate cases.

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Comparison of the Analytical Performance of Cyclosporine and Tacrolimus With Abbott ARCHITECT i2000SR and Siemens Dimension Xpand Plus

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Routine monitoring of cyclosporine (CSA) and tacrolimus levels is of great importance to ensure effective immunosuppression and minimize adverse toxic effects. Currently, Abbott TDx/FLx and Abbott IMx batch analyzers are used by our laboratory to assay CSA and tacrolimus, respectively. The ARCHITECT, with improved functional sensitivity using chemiluminescent microparticle immunoassay, and the Xpand Plus, with automated pretreatment and antibody-conjugated magnetic immunoassay, can automate the process and possibly provide improved analytical

performance. The purpose of this study was to evaluate the analytical performance of the ARCHITECT and Xpand Plus.

Linearity, precision, correlation, and functional sensitivity were examined using patient whole blood specimens. Data were analyzed using EP Evaluator. For the ARCHITECT immunoassays of CSA and tacrolimus, the linearity, precision, and functional sensitivity findings validated the manufacturer's claims. Values obtained with the ARCHITECT immunoassay correlated well with the current assays for CSA ($r = 0.9596$) and tacrolimus ($r = 0.9935$). For Xpand Plus, the findings validated manufacturer's claims for linearity, precision, and functional sensitivity for CSA and tacrolimus (2.4 ng/mL) immunoassays. Values obtained with the Xpand Plus immunoassays correlated well with the current assays for CSA ($r = 0.985$) and for tacrolimus ($r = 0.990$). However, using the CLSI EP10 protocol with patient samples, the precision and bias, respectively, of the tacrolimus Xpand Plus immunoassay are 21.5% and -0.01 ng/mL for the low-range pool (mean, 2.29 ng/mL), 4.9% and -4.26 ng/mL for the middle-range pool (mean, 8.84 ng/mL), and 4.2% and -5.25 ng/mL for the high-range pool (mean, 18.65 ng/mL).

The assays of CSA with the ARCHITECT and Xpand Plus and the assay of tacrolimus with ARCHITECT are sensitive and precise and provide clinically relevant drug levels. However, the Siemens Xpand Plus tacrolimus assay with a functional sensitivity of 2.4 ng/mL is not suitable for measuring at the 2- to 4-ng/mL proposed target trough levels, and negative bias observed with middle- and high-range patient pool samples may confound routine monitoring.

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Evaluation of Shifting Patterns of On-Call Consultations in Clinical Laboratory

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On-call consultations received by clinical pathology trainees provide insight into the most current problems encountered by the clinical laboratory and the health care personnel relying on the results released. This study aimed to identify changes in the pattern of consultations received in the clinical laboratory in terms of the total number of referrals, subjects, and type of callers between 2 periods spanning 7 years.

A review of all documented consultations among on-call clinical pathology trainees for two 12-month periods (July 2001-June 2002 and July 2008-June 2009) was performed. The consultations were classified according to subject and type of caller. The findings between the 2 periods are subsequently compared.

In the 2001-2002 period, a total of 54 documented consultations were received and acted upon by the on-call clinical pathology trainees. The 3 most common on-call consultations were request for blood smear review (29.6%), release of blood products and supply issues (18.5%), and panic values and interpretation of clinical chemistry results (11%). The calls came mainly from medical technologists (91%), with few coming from physicians (5%) and nurses (4%).

In the 2008-2009 period, after 6 intervening years, the total number of documented calls increased to 82 (up by 51.8%). The 3 most common on-call consultations were requests for blood smear review (35.4%), panic values and interpretations of clinical chemistry results (21.9%), and release of blood products and supply issues (20.7%). The calls still came mainly from medical technologists (83%), with increasing proportions from physicians (10%) and nurses (7%).

The shifting patterns of on-call consultations over time signify the broadening and increasingly relevant role of the clinical laboratory and its personnel in acute care settings in hospitals. Continuous monitoring

and analysis of these changes could be a valuable learning opportunity for clinical pathology trainees, a potential quality assurance tool, and an important customer satisfaction assessment exercise.

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Discrepancies Between Molecular Genotyping and Serologic Phenotyping in Multitransfused Patients

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Blood incompatibility continues to be a significant problem, despite advances in immunohematology. Hemagglutination is the classic method used for testing blood group antigens and antibodies; however, hemagglutination-based assays can be labor-intensive, time-consuming, and unreliable in the multitransfused patient. Recently, blood group genotyping has been commercially available for use as an adjunct to serologic phenotyping. With the development of any new technology, it is important to test for correlation. The following study was conducted to examine the value of molecular genotyping, identify discrepancies to serologic phenotyping, and discuss possible causes.

Between May 2008 and April 2010, our institution performed molecular genotyping with Bioarray on 40 patients who had warm autoantibodies and/or had recently been multitransfused as an aid in providing phenotypically matched blood. We retrospectively reviewed all of these cases to evaluate the correlation of genotype and phenotype and also to identify the antigens for cases with discordance.

Of the 40 patients who received Bioarray testing, 5 were excluded because phenotyping had not been performed or no definitive alloantibody had been identified to speculate the possible antigen phenotype. We found 10 of 35 (28%) cases to be discrepant, and, of these, 4 were Fy^b and 2 were Jk^a or Jk^c . A single case of LW^a , C , Fy^a , and Jk^b discrepancy was also identified. Among the 10 cases, 2 had discrepancy of 2 antigens.

The genotype and phenotype were discrepant in 28% of cases, and, of these, Fy^b was the most common. Possible reasons include variations caused by polymorphisms, antibody reactivity of non-epitope region(s), and interference caused by warm autoantibodies. Molecular genotyping can be a useful adjunct in providing compatible blood to multitransfused patients; however, caution should be taken until further investigation can identify the source of these discrepancies.

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Diagnostic and Histogenetic Significance of PAX2 and PAX8 Expression in Three Uncommon Tumors of the Male Lower Urogenital Tract

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Adnexal tumor of probable Wolffian origin (ATWO), endometrioid carcinoma of the seminal vesicle (EMCSV), and clear cell adenocarcinoma (CCA) of the lower urinary tract are rare tumors of the male genital tract. Their diagnosis is often challenging owing to their rarity. The histogenesis of these tumors is elusive, and

Wolffian and müllerian origins have been suggested. In this study, we investigated the expression of PAX2 and PAX8, novel markers for Wolffian and müllerian ducts, in these tumors utilizing immunohistochemistry (IHC).

We retrieved 1 case of each ATWO, EMCSV, and CCA of the urinary bladder from our case files. IHC for PAX2 (Zymed) and PAX8 (Proteintech) was performed on formalin-fixed, paraffin-embedded tissue sections with the avidin-biotin peroxidase method following antigen retrieval. IHC was also performed on 100 prostatic adenocarcinomas (PAs), 44 urothelial carcinomas (UCs), 50 colonic adenocarcinomas (CAs), and 37 malignant peritoneal mesotheliomas (MPMs) from male patients, which were assembled separately on 6 tissue microarrays. We also stained 3 adenomatoid tumors (ATs), and normal male genital tract tissues.

PAX2 and PAX8 were detected in the epithelial cells of the male genital tract, including epididymis, vas deferens, seminal vesicles, and ejaculatory ducts. The ATWO, EMCSV, and CCA showed diffuse, strong, nuclear staining for PAX2 and PAX8. No PAX2 or PAX8 expression was detected in PAs, UCs, CAs, MPMs, or ATs.

PAX2 and PAX8 are cell lineage-specific transcription factors and are expressed in the Wolffian duct-derived male genital tract, as reported for the müllerian duct and its derivatives. Their detection in ATWO, EMCSV, and CCA supports the assertion that they are derived from the remnants of the genital tract. PAX2 and PAX8 IHC may help to differentiate these uncommon tumors from other entities in the male genital tract.

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Verification of the Roche Real-Time PCR COBAS AmpliPrep/COBAS TaqMan 48 HCV Test With the Roche COBAS AMPLICOR HCV Monitor, Version 2.0 Assay

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With the likely increase of patients diagnosed with hepatitis C virus (HCV), quantitation of plasma HCV RNA levels for the proper clinical management of the patients will become a larger function of the laboratory. Traditional methods can be time-consuming and technically difficult. Our laboratory recently evaluated the COBAS AmpliPrep-TaqMan automated nucleic acid isolation plus real-time PCR system and compared it with our current methodology, the Roche COBAS AMPLICOR HCV Monitor, version 2.0 assay, for measuring HCV viral loads.

In the present study, viral load quantification using the Roche COBAS AmpliPrep/COBAS TaqMan 48 HCV Test (Roche; Pleasanton, CA) was compared with the Roche COBAS AMPLICOR HCV Monitor, version 2.0 assay. Comparison data were obtained using 82 archived patient specimens previously quantitated for HCV RNA by the COBAS Monitor test, as well as OptiQuant-S HCV RNA Quantification Panels from AcroMetrix (Benicia, CA).

Two specimens with viral loads of 369,000 and 426,000 IU/mL were invalid by TaqMan, meaning that the internal standard did not quantitate. Thirty specimens were "target not detected" by TaqMan and less than 600 IU/mL by the Monitor. An additional 6 specimens were below and 12 specimens were above the linear range for the Monitor but were quantitated by the TaqMan. (Only 1 of these 12 quantitated as <700,000 by TaqMan, and none were above the TaqMan range.) The remaining 33 sample pair values were compared using the Wilcoxon signed ranks test and found to be not significantly different ($P = .39$). Kendall correlation had a tau statistic of 0.69; the regression coefficient was 0.76. A significant difference

was obtained when comparing the measured TaqMan values with the AcroMetrix concentrations, but the latter values are obtained using a different PCR assay. Nevertheless, the AcroMetrix controls along with several series of diluted patient specimens showed the TaqMan to be linear.

These comparison data demonstrate equivalence in the capabilities of the 2 methodologies to quantify HCV viral load. Significant differences were obtained when TaqMan viral loads were compared with the AcroMetrix panels but not when compared with the COBAS Monitor assay previously used in our laboratory.

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Does Total Submission of Neck Contents Impact Pathologic Staging in Head and Neck Carcinoma? A Study of 30 Cases at the University of Pittsburgh Medical Center

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Despite node negativity by pathologic examination of neck dissection specimens for head and neck squamous cell carcinoma (HNSCC), up to 10% of patients will have recurrence in the neck, suggesting inadequate assessment by conventional techniques. We sought to determine if total submission of neck dissection specimens for histologic evaluation would improve the pathologic staging of the neck.

We prospectively accrued 30 consecutive neck dissection cases for HNSCC. Each case was prosected by a pathology assistant or resident using the standard technique of submitting all grossly identifiable possible lymph nodes. Subsequently, the remaining tissue was entirely submitted for histologic examination. Data collected included number and size of lymph nodes identified by an initial gross examination and histologic review, number of additional blocks required to submit the remainder of specimen, number and size of additional lymph nodes identified, number of additional lymph nodes involved by metastatic carcinoma identified, and final pathologic stage. Initial examination identified an average of 45 lymph nodes per case. Of 30 cases, 17 (57%) were pathologically node-positive. After total submission, additional lymph nodes were identified in 27 of 30 cases (average, 20 additional lymph nodes per case). Additional lymph node metastases were noted in 4 of 30 cases (13%), but only 1 case (3%) was upstaged: pN2b to pN2c (owing to identification of contralateral metastasis). However, total fat submission did not yield metastases in any initially node-negative cases. None of the additional positive lymph nodes demonstrated extracapsular spread.

Total submission identifies more lymph nodes and more positive lymph nodes in a small portion of cases, although upstaging as a result of these additional positive nodes appears negligible. Thus, in our opinion, the standard gross technique for lymph node dissection is adequate.

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Peripheral CD19+ B-Cell Populations in HIV- and Hepatitis C Virus-Infected Veteran Patients: A Retrospective Study

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HIV+ and HCV+ coinfection, as high as 35% in Western populations, is associated with liver fibrosis/decompensation, hepatoma, faster AIDS progression, and HAART liver toxicity. Few data are available on the peripheral blood CD19+ B-cell population in HIV+ and HCV+ coinfection.

A retrospective review of 50 HIV+/HCV+ patients for peripheral blood percentage and absolute number of CD19+ B cells and HCV viral load (VL) by PCR was performed on a veteran population and compared with 47 normal control cases.

In 26 coinfecting patients, there was a high HCV-VL (>500,000 IU/mL); 10 had negative HCV-VL (<45 IU/mL); 1 had an intermediate HCV-VL; 13 had no quantitative data. In coinfecting patients, the mean B-cell percentage (15.65%; SD, 9.63%) with high HCV-VL was higher than the mean B-cell percentage (8.34%; SD, 6.84%) with negative HCV-VL ($P = .018$, t test; $P = .01$, Wilcoxon rank sum test). Also, the mean absolute B-cell number (mean, 284.38; SD, 246.42) with the high HCV-VL was higher than the mean absolute B-cell number (mean, 143.4; SD, 137.89) with the negative HCV-VL ($P = .049$, t test; $P = .027$, Wilcoxon rank sum test). When the mean B-cell percentage and absolute B-cell count were compared between the coinfecting group (14.57%; SD, 9.25%; and 260.98; SD, 217.5) and control group (13.6%; SD, 4.76%; and 280.62, SD, 126.8) there was no difference ($P = .78$ and $P = .062$; Wilcoxon rank sum test).

The mean percentage and absolute number of peripheral CD19+ B cells in coinfecting HIV+/HCV+ patients with a high HCV-VL is higher than in patients with a negative HCV-VL, suggesting B-cell chronic antigenic stimulation by HCV.

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Longitudinal Management With Crossmatched Platelets, 2002-2010: Alloimmunization, Corrected Count Increments, and Patient Outcomes

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The use of crossmatch-compatible platelets has been shown to improve posttransfusion corrected count increments (CCIs) in patients refractory to transfusion with random platelet units. This method is widely used at many institutions, yet little has been reported as to the laboratory results and clinical outcomes in patients requiring continuing management with crossmatched platelets.

This retrospective cohort study involved patients identified at UCSF Medical Center, from 2002 to 2010, for whom 2 or more platelet crossmatch assays were performed. All patients were refractory to random platelet units as defined by 2 consecutive 1-hour post-transfusion CCIs less than 7.5 prior to first crossmatch. Available medical records and laboratory results were reviewed.

Of 71 patients included in the study, a median of 4 cross-match assays was performed per patient (range, 2-17). The average percentage reactivity in initial (58.6%) vs last (55.3%) crossmatch assay for each patient showed no trend toward progressive alloimmunization. Of the 71 patients, 66 (93%) were transfused with a total of 729 crossmatched units. The overall CCI for all units showed wide variation at 6.97 ± 7.87 ($n = 419$ units with adequate 1-hour postcounts). The mean CCI \pm standard deviation for the 1st unit transfused, averaged across all patients, was 6.64 ± 8.01 ($n = 43$); for the 5th unit, 8.91 ± 8.22 ($n = 21$); for the 10th unit, 8.15 ± 6.88 ($n = 12$); and for the 15th unit 8.32 ± 8.95 ($n = 11$), showing no trend toward decreased responsiveness with continuing transfusion. Of the patients, 9 (13%) were transfused with HLA-matched platelets owing to nonresponsiveness to or lack of crossmatch-compatible units. No patients developed spontaneous intracranial

hemorrhage, nor was there any mortality from bleeding-related complications during management with crossmatched platelets.

There was no trend toward increasing alloimmunization to platelets despite the use of non-HLA-matched, crossmatch-compatible platelets during longitudinal patient management. Clinical outcomes demonstrate that this approach is a safe and viable medium- to long-term management strategy for thrombocytopenic patients refractory to random platelet units.

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Incidental von Meyenburg Complexes Detected at Hepatic Core Biopsy

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von Meyenburg complexes are small, periportal, segmental biliary dilations usually encountered on the Glisson capsule during frozen sections or autopsies. Multiple von Meyenburg complexes have been linked to polycystic liver disease, polycystic kidney disease, and cholangiocarcinoma. von Meyenburg complexes might be confused with ductular reaction. Incidental von Meyenburg complexes detected via hepatic core biopsies performed for unrelated medical diseases have yet to be studied.

We reviewed a large series of specimens from medical hepatic core biopsies to establish the existence of incidental von Meyenburg complexes, alert pathologists to the diagnostic pitfall of mistaking von Meyenburg complexes for ductular reaction, and determine whether incidental von Meyenburg complexes are surrogate markers for polycystic disease. We retrospectively studied specimens from adult medical hepatic core biopsies and excluded specimens from frozen sections or autopsies.

Specimens accessioned from 2003 to 2009 with incidental von Meyenburg complexes diagnosed were reviewed. We then reviewed 100 consecutive specimens accessioned in 2009. We calculated the likelihood of multiple von Meyenburg complexes, and hence, polycystic disease, using dimensions of a specimen, von Meyenburg complex, and liver. Four specimens harbored von Meyenburg complexes, all but 1 of which were misinterpreted as ductular reaction by senior general pathologists and correctly diagnosed during quality control by a hepatopathologist. One specimen had 2 von Meyenburg complexes. Review of 100 consecutive specimens revealed a prevalence of 1%. We let the volume of 1 specimen equal 1/50,000 of a 1,750-cm³ liver and the diameter of a von Meyenburg complex equal 5 mm. The probability of detecting 1 solitary incidental von Meyenburg complex by random biopsy was 0.0037%, strongly suggesting multiplicity if detected incidentally. Incidental von Meyenburg complexes detected at hepatic core biopsy are rare, easily overlooked, and likely are surrogate markers for polycystic disease.

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Evaluation of an Automated Digital Image System, Nextslide Digital Review Network, for Examination of Peripheral Blood Smears

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Examination of peripheral blood smears by light microscopy remains one of the major labor-intensive procedures in the hematology laboratory. Several automated digital image systems have

been introduced in recent years to improve turnaround time and proficiency. The goal of this study was to investigate a new automated digital image system, Nextslide Digital Review Network, to examine peripheral blood smears.

The Nextslide Digital Review Network consists of a high-resolution scanner, a computer containing acquisition software, and a central data center that performs processing, classification, and review. We evaluated 207 peripheral blood smears (prepared manually or from automated slidemaker), including 50 normal blood samples and 157 abnormal blood samples of various clinical conditions such as infection, leukopenia, acute leukemia, chronic leukemia, lymphoma, myelodysplastic syndrome, and plasma cell leukemia. Each blood smear was examined by an experienced technologist using standard microscopy and the Nextslide system. Smears were examined for WBC morphology and classification and RBC and platelet morphology. Statistical analysis was carried out using Microsoft Excel for evaluation of accuracy.

Comparison of Nextslide results and manual differential counts for 207 samples showed excellent correlation for all major WBC classes (segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, atypical lymphocytes, blasts, and immature granulocytes) with regression coefficients ranging from 0.78 to 0.97. Evaluation of RBC and platelet morphology also showed good correlation between microscopy and Nextslide. Manually prepared smears and abnormal smears, such as leukopenic samples, are particularly challenging for digital imaging systems and, therefore, were a focus of this study. Leukopenic samples demonstrated markedly decreased review time. In addition, the Web-based review application provides easy accessibility from any location with Internet access.

The Nextslide system shows excellent correlations when compared with conventional manual differentials for evaluation of peripheral blood smears.

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Relationship Between Apolipoprotein AII Gene Polymorphisms and Type 2 Diabetes Mellitus With Coronary Heart Disease

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Apolipoprotein AII (apoAII) is a major apolipoprotein in the human HDL particle. The physiological and biochemical role of apoAII is still in dispute. Lacking a large population epidemiological study, apoAII has long been regarded as a less important component of apolipoprotein. Recently, with the exploration of gene expression regulation and new polymorphisms of the apoAII encoding gene (*APOAII*), as well as the results of clinical trials and studies of transgenic mice, the role of *APOAII* in the development, prevention, and treatment of atherosclerosis and hyperlipidemia has gained more concern from researchers. However, many contradictions exist in different studies of *APOAII* polymorphisms and risk of cardiovascular diseases. Numerous genetic studies in different populations revealed the relationship between *APOAII* polymorphism and type 2 diabetes. Now, *APOAII* is a hot genetic target for type 2 diabetes and its complications all over the world. Our research was to explore the effect of the interaction between *APOAII* -256T/C on the presence of polymorphism and type 2 diabetes mellitus with coronary heart disease in the Han nationality from northern China.

Genotypes of *APOAII* -256T/C polymorphisms of the *APOAII* gene were analyzed by using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) in 155 unrelated healthy people, 101 patients diagnosed with type 2 diabetes mellitus (DM), and 78 patients with type 2 DM combined with coronary heart disease (CHD). The relationship of gene polymorphisms of *APOAII* -256T/C and levels of HbA_{1c}, serum lipids, and glucose was also analyzed.

Neither the frequencies of genotypes nor frequencies of alleles of *APOAII* gene -256T/C polymorphisms were statistically different among DM patients, DM + CHD patients, and control subjects ($P > .05$). Lipid levels, HbA_{1c}, and glucose were presented differently in the genotype subgroups. Regardless of C allele gene carriers or TT genotype, in the DM + CHD group, TC was lower than that of the control group and DM group, and TG, GLU, and HbA_{1c} were higher than in the control group, while LDL-C, GLU, and HbA_{1c} were lower than in the DM group. No relationship between sex, family history of hypertension, BMI, and *APOAII* gene -256T/C polymorphisms was found. Logistic regression analysis showed that age and HbA_{1c} were the risk factors, but HDL-C was a protective factor ($\beta = -0.649$; $\text{Exp}(\beta) = 0.785$; 95% CI, 0.287~2.148; $P = .02$) for type 2 diabetes mellitus with coronary heart disease.

In our study, we developed the methods of genome DNA extraction by NaI and *APOAII* -256C polymorphism detection by PCR-RFLP, which are rapid and convenient for large-scale epidemiological screening, human biology research, and clinical study in the laboratory. No significant differences within the genotype or allele distributions were observed among different groups. Age, BMI, GLU, and HbA_{1c} are the independent risk factors for type 2 diabetes. BMI, GLU, and TG were the independent risk factors for coronary heart disease, while HDL-C was a preventive factor for CHD. Age and HbA_{1c} were the independent risk factors for type 2 diabetes with CHD, while HDL-C also was an independent preventive factor. Our study unveiled no clue of *APOAII* -256C as a risk factor or preventive factor in patients with type 2 diabetes, CHD, and type 2 diabetes with CHD. The *APOAII* -256T/C polymorphism shows no obvious correlation with serum HDL-C, TG, and HbA_{1c} levels. The -256T/C polymorphism of the *APOAII* gene may not be the major genetic risk factors for type 2 DM with CHD in the Han nationality.

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A Case of Primary Pulmonary Intravascular Large Cell Lymphoma With Plasmablastic Morphology and Unusual Phenotype Closely Mimics Non-Small Cell Carcinoma

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Plasmablastic lymphoma and intravascular large cell lymphoma are recognized as 2 distinctive clinical pathologic entities in the 2008 WHO classification. Herein we report a case of large cell lymphoma that initially presented as a lung mass and showed overlapping features of these 2 entities and an unusual immunophenotype and closely resembled non-small cell carcinoma.

We investigated clinical, morphologic, immunohistochemical, and molecular features of an unusual case of large cell lymphoma with clinical and morphologic features of plasmablastic lymphoma and intravascular lymphoma. IHC, CISH using an anti-EBER-1 probe and κ and λ light chain probes, and PCR for *IgH* and *TCR γ* gene rearrangement were performed on a paraffin block.

This 57-year-old HIV+ and heavy smoker (1.5 packs/d) patient was found to have a lung mass on the left lower lobe. No lymphadenopathy or other masses were evident. The CBC showed pancytopenia with an absolute CD4 count of 278/ μ L. Frozen section was requested on the initial wedge biopsy of the lung mass, and a diagnosis of “non-small cell carcinoma” was made. Lobectomy was performed, revealing a poorly defined, peripherally located, 2.5-cm tan-gray mass. Sections of the mass showed numerous round, various sized nests of monotonous atypical large cells in the lumens of dilated, small, angiolymphatic spaces around the peribroncho- or bronchiolar wall and lobular and alveolar septa. The lung architecture in the tumor area was largely preserved. The tumor cells showed typical plasmablastic or immunoblastic

morphology with round nuclei, 1 prominent nucleolus, moderately clumped chromatin, and a moderate amount of basophilic cytoplasm. Many tumor cells had slightly eccentrically located nuclei. The tumor cells were strongly and diffusely positive for CD45Rb, CD30, BCL-2, EBER, and Ki-67 (MIB-1 index, >90%) and were negative for CD138, CD38, CD20, CD79a, PAX5, ALK-1, EMA HHV-8, CD15, c-κ and c-λ, CD2, CD3, CD4, CD8, CD5, CD7, panCK, CK1/3, CK7, CK20, CK5/6, CD56, synaptophysin, chromogranin, p63, and TTF-1. The PCR study for *IgH* and *TCRγ* gene rearrangement was negative.

This case illustrated a primary lung presentation of an aggressive lymphoma with overlapping features of EBV+ plasmablastic lymphoma in an HIV+ patient with intravascular lymphoma and an unusual immunophenotype. Owing to the close resemblance to non-small cell carcinoma, caution should be made during frozen section consultation.

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Bacterial Vaginosis as a Risk Factor for Acquiring Sexually Transmitted Diseases

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Few studies have demonstrated that bacterial vaginosis (BV) is associated with sexual behavior risk factors similar to those for other sexually transmitted diseases. In the present study, the prevalence of these in a multivariate analysis of data from sexually active women infected with BV and *Chlamydia trachomatis* (CT), *Treponema pallidum* (syphilis), *Neisseria gonorrhoeae* (NG), or HIV was observed. Non-BV-infected women were used as control subjects.

Data from 788 women screened in the SAVVY HIV gel phase 3 clinical trial in Accra (West Legon Study Site) from 2004 to 2006 were analyzed. Participants were evaluated for the presence of BV, CT, *T pallidum*, NG, *Trichomonas vaginalis* (TV), and HIV and were interviewed in detail with respect to sexual behaviors. Statistical comparisons were made using the *t* test, χ^2 test (Pearson), and logistic regression multivariate analysis.

This study showed a high association between BV and HIV ($P < .01$) with a risk factor of 0.4, which does not occur in the other sexually transmitted diseases like NG, syphilis, and *Chlamydia* with insignificant association ($P < 1$) and risk factors of 0.6, 0.7, and 0.9, respectively. HIV was found to be the most prevalent sexually transmitted disease with 11.2%; *Chlamydia* was 9.2%; TV, 2.3%; syphilis, 1.7%; and NG was the least, with 1.5%. Also, BV and candidiasis were found to be the most common causes of vaginitis in the women studied. We also observed mixed infection with the organisms that cause vaginitis in the women studied.

Bacteria associated with BV increase female genital-tract infection with HIV, but the mechanism by which this happens is not clear. BV is not a sexually transmitted disease but predisposes one to HIV infection. It is strongly suggested that all cases of BV, symptomatic and asymptomatic, that are seen in sexual-health clinics should be treated to reduce the risk of PID, preterm delivery, and/or HIV transmission. Also, sexually active and pregnant women should be encouraged to frequently visit sexual-health clinics for BV screening and treatment.

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Bcl-2 Overexpression and Prognostic Factors of Colorectal Carcinoma: Our Experience in Iraq

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Colorectal cancer is one of the first 10 cancers among the Iraqi population. The relative frequency of colon and rectal cancers and the sex frequency rate of colorectal carcinoma in Iraq have been investigated to date, but none included bcl-2 expression. Bcl-2 expression in colorectal carcinoma and its association with prognostic factors in different populations has been controversial. Our aim was to investigate bcl-2 expression in colorectal carcinomas among Iraqis and associate its expression with other prognostic factors.

We stained 35 colorectal carcinoma cases (33 resections and 2 biopsies) with bcl-2 immune stain. Staining patterns were assessed by combining qualitative and quantitative data and related with age and sex of the patient, tumor grade, tumor stage, tumor size, mucinous differentiation, anatomic location, and lymph node status. Statistical analysis was performed using Microsoft Office Excel, and the results were analyzed using the χ^2 test. *P* values were calculated, and the significance was assessed at an α level of .05.

A statistically significant association was seen with regard to tumor grade ($P = .013$), tumor stage comparing stage A vs D and stage B vs D ($P = .035$ and $P = .043$, respectively), nonmucinous type of colorectal carcinoma ($P = .031$), small tumor ($P = .036$), and negative lymph node status ($P = .036$). There was no association between bcl-2 expression and age ($P = .127$) and the sex ($P = .127$) of the patient and the anatomic location of the tumor ($P = .0651$).

A significant association was observed between bcl-2 overexpression and favorable prognostic parameters of colorectal carcinoma in this group of Iraqi patients. This agrees with previously published studies among Caucasian, African American, and Egyptian patients.

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Non-EBV-Related Low-Grade Small B-Cell Lymphomas in Posttransplant Patients

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Posttransplant lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphoplasmacytic proliferation, mostly Epstein-Barr virus (EBV)-related, with a spectrum of morphologic, phenotypic, and molecular features. Low-grade (indolent) small cell lymphomas have not been considered as a part of PTLT under the WHO classification of tumors of hematopoietic and lymphoid tissues. In recent literature, extranodal marginal zone lymphoma, mantle cell lymphoma, and hairy cell leukemia have been reported in posttransplant patients. To our knowledge, lymphoplasmacytic lymphoma has not been described in the posttransplant setting.

We report 2 cases of low-grade small B-cell lymphoma with bone marrow involvement and IgM monoclonal gammopathy, one involving a 53-year-old man occurring 10 years after renal transplantation and another involving a 71-year-old man 3 years after heart transplantation. Neither patient showed evidence of lymphoma before the transplant procedure. The posttransplant course remained stable until the development of clinically indolent, histologically low-grade

lymphoproliferative disorders that were classified as non-EBV-related low-grade small B-cell lymphoplasmacytic lymphomas using immunohistochemistry, flow cytometry, and molecular studies.

Low-grade small cell lymphomas in transplant patients are often excluded from the PTLD category owing to the low incidence of these cases. Without well-established epidemiological data, it remains difficult to ascertain the overall rate of development of low-grade small cell lymphomas in transplant patients. Although the exact etiology of low-grade small cell lymphomas in transplant patients is unclear, these cases should be studied in depth to determine whether these lymphomas in transplant patients are secondary to the posttransplant immunosuppressive therapy or merely represent a coincidental event in these patients. By closely monitoring the clinical course of these patients, we may be able to shed light on the proper clinical management of these unusual cases. The accumulation of these unusual cases may also contribute to the future classification of PTLD and a better understanding of the oncogenesis of low-grade lymphomas.

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Blood Management by Transfusion Triggers: When Less Is More

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We have reviewed the annual blood utilization data at our institution for the past 6 years. The number of packed RBC units for allogeneous transfusions gradually increased from 3,989 (in 2004) to 4,762 (in 2008), a 19% increase. This had exceeded the 7% increase in annual patient admissions during the same period (from 20,470 in 2004 to 21,908 in 2005).

In 2009, we introduced new transfusion guidelines ("triggers"), essentially adopting the recommendations of the Society for the Advancement of Blood Management. Most important, we reduced the trigger of blood transfusions in normovolemic, symptomatic chronic anemia patients from 8 to 7 g/dL of hemoglobin. At the same time, we created a new trigger of 9 g/dL of hemoglobin for high-risk (eg, cardiovascular and/or chronic pulmonary disease as well as chemotherapy) patients. We monitored the indications for blood transfusions during 2009 (2,717 consecutive orders) and sent out letters of reminder of the new guidelines to our clinicians if criteria were not met (a total of 102 letters, representing 4% of the reviewed orders). Our annual blood utilization in 2009 showed some improvement (4,648 units) compared with the previous years (4,762 units), despite the increase in patient admissions (from 21,908 to 22,734): this represents a 6% decrease in blood utilization when corrected for patient admissions. If this trend holds up, the predicted blood utilization for 2010 based on the January to March data (4,280) promises to show further improvement (an 11% decrease compared with 2008).

We conclude that blood utilization may be improved in a community hospital setting by combining new, evidence-based transfusion triggers with physician education.

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Bioactive Assay and Antimicrobial Screening of Selected Philippine Seaweeds

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Oceans constitute the greatest source of biological diversity on the planet. The Philippines is blessed with a diverse marine environment that offers vast opportunities for the discovery of a vast array of chemical compounds that can be developed into products with beneficial medical and industrial uses. This study was undertaken to identify bioactive components and antibacterial properties of 7 seaweeds from Oriental Mindoro and Pangasinan, Philippines.

Bioactive components were determined using thin-layer chromatography and spray reagents, while antibacterial activity was screened using a disk-diffusion technique. Results of the phytochemical analysis showed the seaweeds contain alkaloids, coumarins, anthranoids, phenols, higher alcohols, and sugars. Among the seaweeds tested, only the *Amphiron* species contained anthraquinones. Results of the antibacterial screening showed that *Ceratodictyon* species, *Padina* species, *Sargasum* species, and *Amphiron* species were able to inhibit the growth of *Staphylococcus aureus*. On the other hand, only *Halimeda opuntia* inhibited the growth of *Pseudomonas aeruginosa*. However, no significant activity was shown against *Escherichia coli*. These results show that the seaweeds tested contain different phytochemicals and have antimicrobial properties.

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Bioactive Assay and Antimicrobial Screening of the Fruits of Mangosteen (*Garcinia mangostana*), Leaves of Durian (*Durio zibethinus*), and the Rinds of Tabon-Tabon (*Hydrophytune orbiculatum*)

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The use of medicinal plants with proven efficacy is beginning to be widely accepted because aside from being inexpensive, they have minimal side effects. It is in this endeavor that we have studied the chemical components of the crude methanolic extracts prepared from the fruit of mangosteen, rind of tabon-tabon, and leaves of durian.

The plant samples were air dried for 2 weeks, soaked in 95% methanol for 24 hours, and concentrated to a syrupy consistency. These were then subsequently chromatographed in thin-layer silica gel 60 F254 using the appropriate solvent systems. The solvent system that gave good resolution spots was the chloroform-methanol in 9:1 and 7:3. The chromatograms were subjected to different testing by spraying them with different reagents. The antibacterial screening was done using the Kirby-Bauer diffusion technique. The plant samples were tested against *Staphylococcus aureus* and *Escherichia coli*.

Results showed that the methanolic extract of the fruits of mangosteen contained important bioactive constituents, namely, flavonoids, steroids, alkaloids, and essential oils. Only steroids and essential oils were present in tabon-tabon rind. Durian was found to contain alkaloids, coumarins, anthranoids, and anthraquinones. Mangosteen and durian give a positive zone of inhibition against *S aureus*. This means that mangosteen and durian possess antibacterial properties against *S aureus* organisms.

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Uterine Lymphangioma: A Case Report and Review of the Literature

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We report a case of a 55-year-old woman with a pelvic mass who underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. The uterus contained several intramural and subserosal leiomyomas, the largest measuring 12.5 cm in diameter. An incidental finding was a multicystic lesion within the myometrium at the lower uterine segment, measuring 2.5 cm in greatest dimension. On histologic sections, this cystic lesion was composed of small and large lymphatics with a flattened lining epithelium, consistent with a lymphangioma.

Most lymphangiomas represent malformations rather than true neoplasms and are thought to result from failure of the lymphatic system to communicate with the venous system. Vascular tumors are rare in the female genital tract and include hemangioma, lymphangioma, lymphangioma circumscriptum, angiomas, and arteriovenous malformation. These lesions can present with symptoms (abdominal pain/mass, postcoital bleeding, and vaginal and vulval mass) similar to epithelial malignancies and may lead to unwarranted radical surgery. Clinically, they have been misdiagnosed as cystadenoma in ovarian tumors and endocervical polyp in cervical tumors. The vascular tumors occur most commonly in the ovary, followed by the vulva, cervix, and vagina.

To our knowledge, this is the first reported case of lymphangioma in the uterus. The risk of Kasabach-Merritt coagulopathy (thrombocytopenia and other bleeding problems related to vascular tumors) has to be considered in larger tumors. Most of these cases can be treated by surgery.

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Concurrent Loss of CD34 and CD10 With Diminished TdT Expression in Relapsed Adult Precursor B Lymphoblastic Leukemia Bringing Significant Challenge in the Evaluation of Minimal Residual Disease: A Case Study With Literature Review

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Examination of bone marrow biopsy specimens for minimal residual disease (MRD) in patients with acute lymphoblastic leukemia (ALL) after chemotherapy can provide clinicians with valuable information in disease relapse, therapy efficiency, and survival. The most important laboratory techniques currently used for the detection of MRD are flow cytometry and polymerase chain reaction (PCR). We describe a case of relapsed precursor B-cell ALL with loss of CD34, CD10, and diminished TdT expression and review the related literature.

Flow cytometric evaluation of the initial bone marrow demonstrated a typical B-ALL phenotype. The leukemic cells were positive for CD10, CD19, CD34, and TdT. Cytogenetic studies demonstrated a hypodiploid karyotype with 37 to 38 chromosomes. The bone marrow biopsies 1 month later after induction chemotherapy and 4 months later after consolidation chemotherapy showed no evidence of residual disease by flow cytometric, cytogenetic, and morphologic studies. However, the disease relapsed 6 months later after the initial diagnosis, and the leukemic cells become negative for CD34 and CD10 with diminished TdT expression by flow cytometry and demonstrated a complex hypertriploid clone with 72 to 76 chromosomes. PCR gene rearrangement for the immunoglobulin heavy (*IGH*) chain and T-cell receptor (*TCR*) γ , performed on all bone marrow biopsy samples, was negative.

Relapsed B-ALL with concurrent loss of CD34 and CD10, diminished TdT expression, and negative *IGH* and *TCR* γ gene

rearrangements associated with cytogenetic progression has not been previously reported in adult patients. Although flow cytometry and PCR have been shown to detect MRD in nearly 100% of patients with B-ALL, occasionally owing to phenotypic shift and unavailable specific molecular markers, these tests may fail the task. Patient-specific molecular testing may be the solution in these cases. These changes also highlight significant challenges in the evaluation of MRD in acute leukemia after chemotherapy.

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Sudden Fetal Demise Due to Umbilical Cord Ulceration in Cases of Proximal Small Bowel Atresia: An Underappreciated Association: Report of a Series of Three Cases and Review of Literature

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Proximal small bowel atresia (SBA) has been associated with trisomy 21 and polyhydramnios. Umbilical cord ulceration has been linked to meconium exposure; however, the association of SBA with cord ulceration and potentially fatal cord hemorrhage is not well known. Three sudden third-trimester fetal deaths, each with SBA, were identified in an 18-month period. Literature was reviewed for prior reported cases.

Case 1 involved a 21-year-old diagnosed antenatally with fetal SBA who presented at 32 weeks with loss of fetal movements. A stillborn fetus was delivered, with "bloody" amniotic fluid. An autopsy confirmed proximal SBA. The cord showed linear ulcerations with focal umbilical artery erosion. In case 2, a 39-year-old with a suboptimal second trimester ultrasound examination presented at 35 weeks' gestation for loss of fetal movements. A C-section delivered a stillborn fetus with "port-wine" amniotic fluid. An autopsy revealed jejunal atresia with duodenal dilatation and cord ulcerations with umbilical artery erosion. In case 3, a 23-year-old diagnosed with fetal duodenal atresia and polyhydramnios on antenatal ultrasonogram presented at 31 weeks' gestation for loss of fetal movements. A C-section delivered a stillborn fetus. No autopsy was performed. The proximal cord showed an ulcer with a necrotic umbilical cord artery.

The first report documenting SBA with cord ulceration and hemorrhage was published in 1991 (Brendon et al). Since then, 21 additional cases have been reported in 12 papers, with a mortality rate of 48%. SBA with cord ulceration may be due to reflux of bile-rich duodenal contents into the amniotic fluid. The factors predisposing fetuses with SBA to catastrophic cord hemorrhage, typically in the third trimester, have not been ascertained. Our case series has crucial implications for gynecologists and pathologists. Fetal SBA should categorize the pregnancy as "high risk," with appropriate prenatal counseling and vigilant monitoring. Careful umbilical cord examination is crucial to avoid missing this diagnosis.

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Unsatisfactory Pap Smears With HR-HPV–Negative Status Should Not Be Neglected

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The Bethesda System criteria for a satisfactory liquid-based preparation is at least 5,000 well-visualized/well-preserved squamous

cells with 10 endocervical or squamous metaplastic cells. A sample may also be unsatisfactory for a number of reasons, including obscuring factors and unlabeled or broken slides. The recommendation for unsatisfactory Pap smears endorsed by the American Society for Colposcopy and Cervical Pathology (ASCCP) is to repeat the Pap test within 2 to 4 months. In many cases, high-risk HPV DNA (hr-HPV) testing can still be performed on unsatisfactory samples. There are no standard guidelines for the follow-up of women with unsatisfactory Pap smears and concurrent hr-HPV status. The follow-up care in such cases mainly relies on the clinician. The goal of this study is to analyze the follow-up results of women with unsatisfactory Pap smears with concurrent hr-HPV testing.

From January 1, 2008, to December 31, 2009, 1,192 of all Pap tests (1%) were interpreted as unsatisfactory smears. Of these, 340 (29%) had concurrent hr-HPV. Follow-up cytology and histology reports of this cohort were reviewed.

Of 340 unsatisfactory Pap smears with concurrent hr-HPV testing, 259 were HPV-, 66 were quantity not sufficient (QNS), and 15 were HPV+. Only 90 HPV- women had follow-up, 6 of whom became HPV+. Of the 6 cases, 3 showed dysplasia, including 1 high-grade squamous intraepithelial lesion. Of the 66 QNS cases, 21 women had follow-up and 2 became HPV+. Of the 2 cases, 1 showed dysplasia. A total of 7% of the 325 HPV-/QNS cases became HPV+. In addition, of the 15 HPV+ cases 5 of 9 cases with follow-up showed dysplasia.

Women with unsatisfactory Pap smears and hr-HPV-/QNS are not at low risk and should not be neglected. This study strongly supports the ASCCP guidelines for repeated Pap smears.

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Incidental Bilateral Ectopic Adrenal Rests

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A 50-year-old Caucasian woman presented with a pelvic mass found on routine physical examination. A pelvic ultrasound revealed a uterine mass, measuring 6.7 cm in greatest dimension. A hysterectomy and bilateral salpingo-oophorectomy were performed and revealed the mass as a leiomyoma. The ovaries were unremarkable. The right and left fallopian tubes were sectioned to reveal a 0.5-cm and a 0.6-cm tan-yellow nodule, respectively, which were identified as adrenal rests. Adrenal rests, or ectopic adrenal tissue, can be found in many organs and structures within the abdomen and pelvis.

Reports of adrenal rests have been well documented in testicular tissue, hernia sacs, broad ligaments, kidneys, liver, appendiceal mesentery, and fallopian tubes. It is rare to have bilateral adrenal rests in adnexal structures. The majority of adrenal rests are discovered incidentally during the performance of other surgical procedures and autopsies and may mimic a neoplasm, either primary or metastatic. True adrenal heterotopia is rare. This case report presents a rather rare finding of bilateral adrenal rests, and a succinct review of the literature is performed.

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Immature Teratoma of the Maxillary Sinus

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Teratomas are neoplasms composed of tissue elements from at least 1 of the 3 embryonic germ layers, ie, ectoderm, endoderm, and mesoderm. The tissue elements are foreign to the organ or anatomic

site of origin. The word *teratoma* is derived from the Greek word "teras," which literally means monsters. Teratomas can happen at any age in life; however, they mostly occur in infancy and childhood. Teratomas are classified as benign (mature) or immature depending on their histological composition and degree of maturation/differentiation. Immature teratomas contain varying amounts of neuroectodermal or blastemal tissue. Immature teratomas in the sinuses or paranasal regions are extremely rare.

We report a case of a 10-year-old boy who presented with right maxillary sinus mass. A CT scan showed an erosive mass that measured 8.9 × 4.6 × 4.4 cm in the right maxillary sinus and extended into the orbit and right side nasal cavity. The biopsy of the lesion revealed an immature teratoma, with the tumor composed of immature neuroectodermal, epithelial, and stromal elements. Areas of overt malignancy, such as teratocarcinoma, were absent in the sample. Immunohistochemical stains were negative for myogenin, excluding any area of rhabdomyosarcoma, and focally positive for GFAP and neurofilament protein, confirming neuroectodermal differentiation. The patient subsequently underwent resection of right maxillary sinus mass via midface degloving, and reconstruction with split calvarial bone graft was performed. The tumor recurred 4 months later, and the patient is now being treated with chemotherapy after endoscopic debridement of the maxillary sinus.

The 2 most important prognostic factors of immature teratomas are age at the time of diagnosis and anatomic site and/or resectability of the tumor. Immature teratomas usually behave in a benign fashion in infants and small children; however, they can be more aggressive in older children, as seen in this case.

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Composite Adenoma and Microcarcinoid in Sigmoid Colon: A Study of Two Cases and Review of Literature

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Microcarcinoid has been a well-recognized lesion in the lung, stomach, and colon. In the colon, microcarcinoids are often found to be associated with inflammatory bowel disorders, eg, ulcerative colitis, and, rarely, with adenomatous polyp. The prevalence and significance of the latter association is not clear.

We present 2 cases of colonic adenoma containing microcarcinoid in a 62-year-old woman and a 52-year-old man who underwent routine endoscopic screening for colon cancer. Endoscopy revealed an 11-mm sessile polyp in the sigmoid colon of the woman and a 15-mm pedunculated polyp in the sigmoid colon of the man. The remaining colonic mucosa was normal. Histologic features were similar in both polyps, and they showed tubular adenoma with a small aggregate of epithelial cells with round central nuclei with stippled chromatin and eosinophilic, granular cytoplasm situated within the lamina propria near the base of the polyp. Endocrine differentiation of these cells was confirmed by synaptophysin and chromogranin.

Our cases demonstrate the subtlety of microcarcinoid in the colonic polyp that may frequently be overlooked by pathologists. Awareness of composite adenoma and microcarcinoid in the colon will lead to improved diagnosis and provide a bigger study series to characterize this group of lesions.

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Overexpression of the *adeB* Gene in Clinical Isolates of Tigecycline-Nonsusceptible *Acinetobacter bowmannii* Without Insertion Mutations in the *adeRS* Gene

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Tigecycline-nonsusceptible *Acinetobacter baumannii* is emerging after the usage of tigecycline for eradicating infections caused by the multidrug-resistant *A baumannii* resistant to carbapenems (MRAB-C). We collected 13 tigecycline nonsusceptible MRAB-C clinical isolates since March 2008. The isolates were mostly obtained from patients with severe underlying diseases, comorbidity, long hospital stay, and previous exposure to multiple antibiotics. Only 7 of the 13 isolates lost their susceptibility to tigecycline related to clinical prescription chronologically. None of the 13 isolates shared the same strain characteristics while analyzed by RFLP, OXA typing, and integron detection. All had increased adeB transcription demonstrated by TaqMan RT-PCR, and they converted to become tigecycline susceptible while exposed to a nonspecific efflux pump inhibitor, 1-(1-naphthylmethyl)-piperazine. These results indicated that active adeABC efflux pump may reduce tigecycline susceptibility of those MRAB-C isolates. However, none of these tigecycline-nonsusceptible MRAB-C isolates acquired previously known adeRS mutations. Up-regulation of adeB expression may result from cross-stimulation by another mechanism.

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Lung Involvement by Nodular Lymphocyte Predominant Hodgkin Lymphoma With Unfavorable Histology and Concurrent *Mycobacterium avium* Complex Infection

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Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is typically an early-stage, indolent lymphoma with an excellent prognosis. A careful workup is necessary to exclude classical Hodgkin lymphoma and more aggressive entities such as T-cell/histiocyte-rich large B-cell lymphoma. We report a case of a 5-year-old Hispanic/African American boy with persistent cervical lymphadenopathy in whom progressive pulmonary opacities developed, originally interpreted as an infectious process. After referral to our institution, a wedge lung biopsy was obtained, and a staging evaluation showed PET uptake in the lung, hilum, and mediastinum.

Other diagnostic specimens included the original lymph node biopsy from the referring institution and a repeated biopsy of the cervical lymph nodes. The histopathology in the lung was similar to the lymph node biopsies and showed a nodular lymphoid infiltrate including lymphocyte predominant (LP) cells that were CD45+, CD20+, CD3-, CD30-, and CD15- and present in a CD20+ lymphocyte-rich background with expanded CD21+ follicular dendritic cell meshworks. Suggesting clinically aggressive disease, prominent extranodal and morphologically atypical LP cells were seen in the lung and lymph nodes. In situ hybridization stains for EBER and bilateral bone marrow aspirates and biopsies were negative.

Noncaseating granulomatous inflammation, not an uncommon finding in Hodgkin lymphoma, was also present in the lung biopsy. GMS and AFB special stains were negative for organisms in the lung and lymph nodes. However, 3 of 5 gastric aspirates submitted for mycobacterial culture grew *Mycobacterium avium* complex (MAC), confirmed by commercial DNA hybridization probes.

NLPHL uncommonly presents at an advanced stage and rarely involves the lung. Besides lung involvement, our unusual case was

also associated with unfavorable histology and a coexistent MAC infection. It is unclear whether these factors are related. The patient is currently being treated with triple-agent antimycobacterial therapy and is doing very well following 4 cycles of chemotherapy.

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Lymphoepithelial Sialadenitis vs Lymphoma in HIV Patients: A Case Report

Ana Lucia Cota, MD, and Andy Nguyen, MD. University of Texas Health Science Center at Houston.

Lymphoepithelial sialadenitis (LESA) is a benign disorder in which there is a lymphoid infiltration throughout the salivary gland with atrophy of parenchyma, ductal hyperplasia, and presence of lymphoepithelial lesions. It mainly affects the parotid and submandibular glands. LESA is a risk factor for the development of extranodal marginal zone B-cell lymphoma, up to 44-fold. Differentiation of LESA from marginal B-cell lymphoma is imperative, and it frequently presents a challenge owing to histological changes in lymphoid tissue associated with HIV infection (persistent generalized lymphadenopathy).

We present a case of a 55-year-old HIV+ man with submandibular enlargement for the past few months. He had several abscesses in the lower extremities and face, which were surgically drained previous to the development of this lesion. Excisional biopsy was performed.

The biopsy specimen consisted of a 3-cm, well-circumscribed soft tissue mass. Microscopic examination demonstrated a lesion with islands of epithelial tissue with squamous differentiation. Basement membrane-like material was identified within the islands. Intraepithelial lymphocytes are also seen. A dense lymphoid background surrounded the epithelial islands with an ill-defined follicular pattern, folliculolysis, an attenuated mantle zone, and a marked increase in vascular proliferation. Immunohistochemical stains showed that the follicles are positive for CD20, CD10, and pax5. They were negative for bcl-2. Interfollicular areas were positive for CD3, CD5, and CD43. CD23 showed follicular dendritic cells in the follicles. Together, the immunohistochemical stains showed no evidence of lymphoma. EBER stain was negative. Immunophenotyping by flow cytometry showed B cells and T cells with normal immunophenotypes.

Differentiation of LESA from marginal B-cell lymphoma in HIV+ patients is often challenging and requires correlation of HIV-associated morphological findings, immunohistochemical stains, and immunophenotyping by flow cytometry. LESA in HIV+ patients has also been known as HIV-associated salivary gland disease and salivary diffuse infiltrative lymphocytosis syndrome. Even though LESA is a benign condition, it is associated with a high risk for development of extranodal marginal zone B-cell lymphoma, and patients with LESA would need to be followed up closely.

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A Case Report: Refractory Cytopenia With Multilineage Dysplasia With Features of Myelodysplastic Syndrome With Isolated del(5q)

Ana Lucia Cota, MD, and Andy Nguyen, MD. University of Texas Health Science Center at Houston.

Myelodysplastic syndrome (MDS) associated with isolated del(5q) is an MDS in which the sole cytogenetic abnormality is deletion of 5q. This type of MDS is associated with a much better prognosis than other subtypes. We present a case of a 71-year-old woman with persistent macrocytic anemia. A bone marrow was

performed that showed morphological evidence of myelodysplasia. Cytogenetics showed deletion of 5q and t(4;22). Specific morphological findings and the clinical course in this patient revealed features of MDS with isolated del(5q).

Peripheral blood and bone marrow specimens were received in pathology. Wright and H&E stains were evaluated. Cytogenetics were performed.

Peripheral blood and bone marrow showed features of MDS with isolated del(5q): macrocytic anemia, normal platelet count, hypercellular bone marrow with erythroid dysplasia, marked increase in megakaryocytes with small size and hypolobated nuclei, blast count of 4%, and no ringed sideroblasts. However, chromosome analysis of 20 cells from the bone marrow showed 18 cells with interstitial deletion of 5q as the only abnormality and 2 cells with both del(5q) and t(4;22). According to the WHO criteria, this case is best classified as refractory cytopenia with multilineage dysplasia. Follow-up of this patient 8 months after diagnosis showed unchanged blood counts. The patient has been in stable condition and requires no treatment.

The patient in this report had most of the cells (18 of 20) in cytogenetic study showing isolated del(5q). Even though a diagnosis of refractory cytopenia with multilineage dysplasia was rendered in this case, the clinical course of this patient would likely be similar to that of MDS with isolated del(5q). Given the fact that MDS with isolated del(5q) responds well to thalidomide analogues, a trial of lenalidomide may be considered in this patient if clinically indicated in the future.

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Positive Placental Iron Staining in Neonatal Hemochromatosis

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Neonatal hemochromatosis (NH) is an extremely rare and severe disease. Patients who are premature or small for gestational age present with hepatic and extrahepatic siderosis at birth, frequently with liver or multiorgan failure. Prognosis is poor, with death often imminent within days postpartum, particularly when aggressive medical interventions are delayed.

A 25-year-old G3, P1, A1 delivered at 36 weeks a 1,976-g girl with jaundice, diffuse petechiae, hepatosplenomegaly, increased total bilirubin, and thrombocytopenia. Placentomegaly with villous edema and thrombotic fetal vasculopathy were noted. The patient was diagnosed with coagulopathy, underwent a complete infectious disease workup that was negative, and received multiple transfusions of fresh frozen plasma and platelets. High suspicion for neonatal hemochromatosis was based on high ferritin and low total iron binding capacity. Gomori iron stain of labial salivary gland biopsy revealed abundant iron deposition in the mucous acinar cells. This biopsy was delayed for 2 weeks owing to coagulopathy.

Noninvasive studies of the placenta might render an immediate diagnosis and help rapid initiation of adequate treatment. Six placental sections of this NH case and placental sections of 10 random control cases were stained with Gomori iron stain. Positive chorionic villi were grouped based on the extent of their villous interstitium and Hofbauer cells staining as follows: <25% staining, +; 25% to 50%, ++; 50% to 75%, +++; and more than 75%, ++++. Results reported in number of positively stained chorionic villi per 10 low-power-fields showed a significantly increased number of iron-stained chorionic villi in NH placenta compared to control cases: 46.3 vs 0.9, $P = .0001$, for less than 25% staining; and 54.8 vs 0, $P = .0001$, for more than 25% staining.

This is the first study to show significantly increased iron staining in the placental chorionic villi of an NH case compared

with control cases. Therefore, placental iron staining could be an important noninvasive tool for immediate diagnosis of NH.

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How Useful Is the Existent Uterine Cervical Mucous, Comparison With a Second Sample, to Detect Adequate Samples and CIN?

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Our objective was to compare 2 cytology samples: the existent mucous in the uterine cervix "first mucous" (FM) and a second sample (SS) obtained after removing all the mucus to determine the adequacy of each sample according to the Bethesda System to detect CIN.

In 495 patients scheduled for a colposcopy from January 2005 to August 2006, the existent FM was sampled and conventional cytology (CC) was done. In a clean cervix, an SS was obtained and processed in liquid-based cytology (LBC), Liqui-PREP. All positive cases by any method were confirmed by biopsy, and immunohistochemistry with p16 was done in the biopsies and in all positive cases in LBC for FM and SS. Also, the CC-positive cases and the corresponding "false-negative" cases of SS were restained with p16.

CC detected 50% fewer cases of CIN compared with the SS. All of these lesions had a positive colposcopy and biopsy with p16+ in biopsy and LBC. One case was detected in the FM but not in the SS, confirmed also by colposcopy and biopsy. Endocervical cells were present in 98% in the SS and only in 41% in the FM ($P = .002$). The cells obtained in the SS are more viable cells attached to the surface with the cytological characteristics well preserved. The inflammatory material was present in both, but more epithelial cells were present in the SS compared with the FM.

The FM contains 50% less diagnostic material than an SS.

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The Role of Bone Marrow Biopsy Immunohistochemistry in Myelodysplastic Syndromes: Staining Pattern and Patient Survival Analysis

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Myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic stem cell (HPC) disorders. Currently, prognosis of MDS is defined by the IPSS (International Prognostic Scoring System), which is based on objective measurements of blast counts, morphologic examination of dysplasia, and cytogenetic findings. However, the IPSS is limited by accuracy of blast counts and a high number of cases with no detectable cytogenetic abnormalities. Immunohistochemistry (IHC) of bone marrow biopsy specimens is not routinely performed in MDS disease stratification.

We chose 20 patients diagnosed with MDS during 2001 to 2008, who had more than 1 bone marrow biopsy to document disease progression in our hospital. We performed IHC on the initial diagnostic bone marrow biopsy specimen of all 20 cases with anti-CD34, Ki-67, and anti-glycophorin antibody on selected cases. The percentage of CD34+ cells ranged from 2 to 65 (25%-75% quartiles, 4-20) and that of Ki-67+ cells ranged from 2 to 65 (25%-75% quartiles, 24-35). Length of follow-up ranged from 3 to 78 months (25%-75% quartiles, 13-32 months). We assigned scores according to the percentage of CD34+ and Ki-67+ cells (1, < 20%; 2, 20-39%; and 3, >40%) and cellularity (1, hypocellular; 2, normal cellular; 3, hypercellular) and used a Cox regression model to identify prognostic variables.

Increased CD34+ cells were associated with shorter survival (risk ratio, 5.6; 95% CI, 1.43-21.78). Ki-67 positivity (risk ratio, 1.53; 95% CI, 0.46-5.05) and cellularity (risk ratio, 1.04; 95% CI, 0.34-3.22) were not associated with prognosis. In 2 cases with high cellularity and low CD34+ cells, IHC with glycophorin confirmed that more than 50% of these cells were erythroid precursors, differentiating MDS from AML.

IHC with CD34 may provide prognostic information in newly diagnosed MDS. In cases in which the HPC shows ambiguous differentiation, glycophorin staining will differentiate between MDS and emerging AML. More studies are needed to confirm the findings.

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Incidental Prostate Carcinomas in Radical Cystoprostatectomies: A Comparison With Clinically Detected Tumors

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Cystoprostatectomy specimens offer a unique chance to evaluate incidental prostatic carcinoma (IPC) for comparison with clinically detected prostate carcinoma (CPC). The clinical and pathological features of cases of IPC diagnosed over 6 years (2004-2010) were compared with a cohort of 100 sequential well-characterized cases of CPCs diagnosed in radical prostatectomy specimens within the same period.

IPC was identified in 48 (34.5%) of 139 cystoprostatectomy specimens. The mean age of the patients with IPC was greater than that in patients with CPC (69.6 vs 62.6 years; $P < .0001$), whereas their mean serum PSA, when known, was significantly lower (3.59 vs 7.22 ng/mL; $P < .0001$). The mean Gleason score in IPC was significantly lower than that in CPC (6.25 vs 6.74; $P = .001$), with 19% of cases having a score of 7 or above, vs 52% of cases with CPC ($P = .0002$). A tertiary Gleason grade 5 component was limited to cases of CPC (seen in 9 cases). The percentage extent of carcinoma in IPC ranged from 1% to 50% (mean, 7.5%) while that in CPC ranged from 1% to 74% (mean, 9.8%; $P = .338$). Compared with CPC, IPC was more likely to be organ-confined (94% vs 70%; $P = .002$), limited to a single lobe (60% vs 11%; $P < .0001$), and lack positive margins (13% vs 21%; $P = .305$).

The incidence of IPC in cystoprostatectomy specimens in this series is similar to previous studies. Although IPC tended to have less adverse features than CPC, a certain proportion of cases can have aggressive features such as high Gleason score, extraprostatic extension, and positive margins. These findings confirm the need for adequate prostate sampling in cystoprostatectomy specimens.

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Body Cavity Lymphoma With TCR Gene Rearrangement in an HIV-, Epstein-Barr-, and Human Herpes Virus-8- Patient

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An 81-year-old man with a complicated medical history, including atrial fibrillation, type 2 diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease, hypertension, monoclonal gammopathy of unknown significance, tobacco dependence, history of alcohol abuse, and small cell carcinoma of the left lung (s/p treatment with chemotherapy and radiation) presented to the emergency room complaining of shortness of breath. Radiologic studies showed a large left-sided pleural effusion. Thoracentesis yielded 1 L of yellow serous fluid; cytology demonstrated large atypical monoclonal cells positive for CD45, CD20, CD79a, BCL-6, and MUM-1. Flow cytometry studies of the pleural fluid were consistent

with B-cell-type malignant lymphoma. This case of lymphomatous pleural effusion is unusual in that no evidence of primary tumor or lymphadenopathy could be found elsewhere (CT, PET scans). Test results for HIV, human herpesvirus 8, and Epstein-Barr virus were negative. Given the HHV-8- status of the patient, the diagnosis would be "body cavity lymphoma" in lieu of the more common HHV-8+ "primary effusion lymphoma."

Body cavity lymphomas are a heterogeneous group of non-Hodgkin lymphomas, first recognized in 1989. In our patient, cellular morphology, flow cytometry, and immunohistochemistry were consistent with diffuse large B-cell lymphoma. HIV serology and PCR assays for EBV and HHV-8 in paraffin-embedded tissue were negative. Given the lack of evidence for primary disease outside the pleural cavity, the final clinical diagnosis was nonprimary effusion lymphoma body cavity lymphoma.

A review of the literature shows that HIV-, HHV-8-, non-Burkitt-type body cavity lymphomas are extremely rare, with fewer than a dozen cases reported. The prognosis for body cavity lymphoma is generally poor; however, given the rarity of this entity, optimal therapy remains unknown. Currently, treatment is geared toward the underlying tumor type, and, in this case, the patient will be treated with the standard B-cell lymphoma chemotherapy protocol.

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Mucoepidermoid Carcinoma With Cilia of the Hard Palate and Maxillary Sinus

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Cilia have rarely been described in mucoepidermoid carcinoma (MEC). We report 2 examples of low-grade MEC with cilia. This study highlights a potential pitfall in the diagnosis of low-grade MEC, as it is quite possible to misdiagnose a low-grade MEC as a benign lesion because of the presence of cilia.

After identifying a case of MEC of the hard palate with cilia, we reviewed existing archived MEC cases to reassess for cilia. Histology slides and pathology records from 21 low-grade MEC cases at our institution were retrieved. Patient ages ranged from 27 to 83 years (average, 57 years) with 8 men and 13 women. Tumor sites included the hard palate, soft palate, unspecified palate, nasal cavity, lateral maxillary wall, lower lip, lung upper lobe, and metastatic MEC in a lymph node and vertebral body.

Unexpectedly, 1 additional case of MEC in the maxillary wall had ciliated cells, for a total of 2 of 21 cases. A search of the literature showed reports of MEC with cilia in the thyroid gland, lacrimal sac, stomach, and thymus. To our knowledge, cilia have never before been reported in MEC of the hard palate or of the maxillary sinus.

Frozen section diagnosis of malignant salivary gland tumors is known to be difficult, and this problem is compounded by the potential of finding cilia in frozen sections, as the presence of cilia is often seen as a marker of benignity. MEC, in particular, is associated with false-negative diagnosis at frozen section. Although apparently only rarely seen in MEC, cilia should not be considered as diagnostic for a benign tumor. In fact, in this case series, cilia were found in 9.5% of low-grade MECs.

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Multiple Granular Cell Tumors of the Cecum

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A granular cell tumor is a benign, neural neoplasm of Schwann cell origin that usually occurs in the fourth to sixth decades as a solitary, painless mass involving the tongue, oral cavity, skin, or soft tissue. However, it can occur anywhere in the body. In the gastrointestinal tract, the most common site is the esophagus, followed by the duodenum, anus, and stomach. It is uncommon in the colon and rectum. When discovered in this location, the tumor is usually a solitary, submucosal nodule and measures less than 2 cm.

We discuss a case of a 23-year-old woman who presented with abdominal pain with multiple pain episodes over the past year. She was diagnosed by CT scan to have an ileocolic intussusception. She underwent a right hemicolectomy, and a mass was found in the cecum. Gross pathologic evaluation of the surgical specimen revealed a 4.0 × 3.5 × 2.0-cm submucosal mass with a white-tan, cut appearance. In addition, another submucosal nodule with a pale, yellow-tan, cut surface was seen. Histopathologic evaluation of these nodules showed nests of large, polygonal cells with granular, eosinophilic cytoplasm and centrally located oval-to-slightly irregular nuclei with granular chromatin and inconspicuous nucleoli. The tumor was strongly positive for S-100 immunohistochemical staining with negative staining by CD117, CD34, and smooth muscle actin. The pathologic findings were consistent with multiple granular cell tumors.

Review of the world literature has only demonstrated a few reported cases of multiple granular cell tumors within the cecum. None have been reported to be as large as 4.0 cm or in a patient in the third decade. Our patient was 23 years old with 2 granular cell tumors, the largest measuring 4.0 cm, located within the cecum. This is a very uncommon case.

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Cell Phone Contamination With Nosocomial Pathogens in Intensive Care Unit

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Cell phones are widely used as portable electronic devices for communication and are in close contact with the body of health care workers (HCWs). Cell phones may serve as vectors for the nosocomial transmission of microorganisms. The aim of this work was to evaluate the role of cell phones in relation to the transmission of bacteria from cell phones to the hands of HCW in intensive care units.

This study was conducted in a 40-bed intensive care unit (ICU). A total of 136 staff, including 20 senior staff, 8 intensivists, 30 ward physicians (assistant doctors), 48 nurses and nurse aides, and 30 supportive services staff and housekeepers were included in this study.

Samples and cultures were obtained from the dominant hand of the participants and their cell phone at the same time. Isolated microorganisms were identified and allocated to appropriate genera. Results revealed contamination of cell phones by bacteria and other microorganisms representing a rate of 96.5%. Microorganisms from cell phones and hands were similar, and some of them are known causes of nosocomial infections. *Staphylococcus aureus* (SA) strains isolated from cell phones constituted 48.0%, and 31% of these isolated from the hands were methicillin-resistant (MRSA+). The gram-negative strains isolated from 30.0% of the cell phones and 32.0% from the hands were ceftazidime-resistant strains.

The nosocomial isolates in the ICU were as follows: 33% staphylococcus, 20% nonfermentative gram-negatives, 24% coliforms, 11% enterococci, and 12.0% yeasts. The mean colony count was higher in ring using staff's phones ($P > .05$). The rate of routine cleaning of HCW's cell phones was 8.0%, and 92% of the participants never cleaned their cell phones. Although the assistant doctor's phones had higher colony counts, there was no significant difference

in the rates of specific types of bacterial growth and colony counts isolated on all different groups' cell phones.

This study confirmed that cell phones were contaminated with nosocomial pathogens. The use of cell phones in the ICU may have serious hygienic consequences. Work is needed at various levels to minimize the risk of cell phones as vectors for pathogen transmission. Multidisciplinary hospital infection control teams should develop active preventive policies and strategies to reduce cross-infection caused by cell phones in ICUs.

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Novel High-Yield Tissue Microarray Method Performance Characteristics Compared With a Conventional Tissue Microarray Method With Regard to Immunohistochemical Control Adequacy

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Tissue microarray (TMA) technology has aided in pathologic investigation, allowing the analysis of multiple examples of an entity from the same paraffin block (PB). TMAs have also been implemented as controls, providing multiple tissue types in the same PB control. However, conventional (Beecher) TMA methods yield only about 150 slides per TMA PB, sometimes necessitating frequent TMA PB construction.

Donor PBs were sampled (punched) in the X/Y axis in this study rather than the Z axis used in the conventional TMA sampling method. Tissue is transferred into deeper recipient wells (15 vs 2-3 mm) using a novel injection process employing a 2-phase solid-liquid mixture of tissue and liquid wax in order to fill the deeper well with a continuous column of tissue. These TMAs were compared with conventional TMAs using multiple immunohistochemical antibodies: calponin; calretinin; CD20 (L26); CD3; CD34; CD45 (LCA); CDX2; chromogranin; cytokeratins 5, 7, 20, and AE1/3; CAM5.2; HMB45; melan A; S-100 protein; smooth muscle actin; synaptophysin; and TTF-1.

The novel TMA method produced nearly 1,000 to 1,500 slides from the same TMA PB, 7 to 10 times the conventional method. Both methods served as excellent positive and negative controls for all antibodies tested, with essentially 100% staining of tissues when adequate controls were included in the TMA.

Since this novel method for constructing TMAs provides more slides than the conventional method, it decreases the frequency of control construction, contains positive and negative controls on the same slide, and provides a greater amount of tissue from certain lesions for research studies and for multiple laboratories to compare their immunohistochemical staining patterns. The TMA uses tissues fixed and embedded similarly to patient specimens and can serve as a control repository for histochemistry, immunohistochemistry, and in situ hybridization. Once established in a laboratory, it has several advantages over current methods.

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Mitochondrial Myopathy With Inclusion Bodies in a 3-Month-Old Female Infant

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We present a case report of a 3-month-old African American girl who presented with difficulty breathing, pneumonia, failure to thrive (less than the third percentile in height and weight), dysphagia, and decreased truncal muscular tone. Worsening dysphagia and difficulty breathing resulted in gastrostomy tube placement with Nissen fundoplication and tracheostomy. The patient was delivered by cesarean section at 37 weeks' gestation owing to maternal preeclampsia.

Genetic studies demonstrated normal karyotype and *SMN1* (survivor motor neuron 1 gene). A right thigh muscle biopsy was obtained and examined by light microscopy, immunohistochemistry, and electron microscopy. H&E-stained sections revealed small, immature, round myocytes with rhabdoid morphology. No angular fibers, inflammation, or vasculitis were identified. A trichrome stain demonstrated eosinophilic cytoplasmic inclusions and ragged red fibers. Nonspecific esterase showed focal cytoplasmic staining in scattered myocytes. CD68 immunohistochemical staining highlighted focal macrophages within myocytes. Scanning electron microscopy performed on the tissue demonstrated small, abnormally shaped mitochondria with irregularly shaped cristae and no "parking lot" structures. Inclusion bodies, most consistent with lipid droplets, were also noted within scattered muscle fibers. Representative tissue was submitted for molecular analysis.

Literature review for documented cases of a mitochondrial myopathy with inclusion bodies in pediatric patients was negative. We believe this is the first documented case of this type to date.

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Two Foci of Breast MALT Lymphoma in a 68-Year-Old Woman

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Breast low-grade MALT lymphoma is rare. We report a case of MALT lymphoma with plasmacytic differentiation in a 68-year-old woman.

She presented with 2 lesions at the 1 and 9 o'clock positions of her right breast. Needle core biopsies both showed sheet-like lymphocytic infiltrate intermixed with scattered plasma cells. The lymphocytes were small to medium sized, with slightly irregular nuclei, moderately dispersed chromatin, inconspicuous nucleoli, and relatively abundant, pale cytoplasm. Occasional larger lymphoid cells were present. No lymphoid follicles were seen. These lymphocytes surrounded some residual normal-appearing ducts. Immunohistochemical stains for CD20 and CD45 were strongly and diffusely positive. Reactive CD3+ T cells were interspersed. A diagnosis of extranodal marginal zone B-lymphoma of mucosa-associated lymphoid tissue type with plasmacytic differentiation was rendered. The patient underwent chemotherapy and had been free of lymphoma since then. On rebiopsy 8 years later, fibrocystic changes with foci of mild periductal chronic inflammation were present.

Foci of breast MALT lymphoma may rarely occur, and patients may survive well after appropriate treatment.

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Triplet Gestation With Complete Hydatidiform Mole and Subsequent Gestational Trophoblastic Neoplasm: A Case Report

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We report the rare case of a 26-year-old woman with a triplet gestation including a live fetus in its own gestational sac and a separate sac containing a complete mole (β -hCG of 600,000 mIU/mL) and a separate population of normal villi consistent with a conceptus.

To our knowledge, there have been 13 reported cases of triplet pregnancy with coexistent complete hydatidiform mole. A living fetus can coexist with a complete or partial hydatidiform mole. A complete mole has a greater propensity (20% risk) to develop into persistent trophoblastic disease. In multiple gestations, the incidence of persistent trophoblastic disease requiring further management with chemotherapy is 3 to 4 times higher with a multiple pregnancy compared with a singleton. Of the 13 reported cases of women with triplet pregnancies including a mole, 5 women developed GTN.

Our patient was also found to have GTN with a postevacuation rising β -hCG that never fell below 1,300 mIU/mL. A hysterectomy and bilateral salpingectomy were performed, and 2 courses of post-surgical prophylactic actinomycin D were given. Gestational trophoblastic disease was confined to the uterus. Soon after, the patient's β -hCG became undetectable.

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CD20+ T-Cell Large Granular Lymphocytic Leukemia Associated With Concurrent Hairy Cell Leukemia and Plasma Cell Myeloma

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We report the case of a 69-year-old man with 3 concurrent distinct hematolymphoid malignancies, including a CD20+ T-cell large granular lymphocytic leukemia, a hairy cell leukemia, and a plasma cell myeloma. It is the first case report, to the best of our knowledge, in the English literature with these 3 malignancies in 1 patient.

This patient had a history of T-cell large granular lymphocytic leukemia with dim CD20 expression before he was referred to our institution owing to anemia, monocytopenia, thrombocytopenia, and absolute lymphocytosis; however, he has received no treatment for T-cell large granular lymphocytic leukemia. On admission, he was also diagnosed with hairy cell leukemia, which went into complete clinical remission after 1 cycle of 2-CdA chemotherapy. Five years later, he was diagnosed with a third hematolymphoid malignancy—a plasma cell myeloma. The hairy cell leukemia and plasma cell myeloma are λ immunoglobulin light chain restricted, suggesting that these 2 B-cell neoplasms might have arisen from a same population of neoplastic B cells. Complex cytogenetic aberrancies were present at the time when plasma cell myeloma was diagnosed. Despite intensive chemotherapy, the patient died 10 months after the diagnosis of plasma cell myeloma.

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An Unusual Case Report: Acute Lymphoblastic Leukemia From a Patient With Previously Treated Multiple Myeloma

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We report a case of a 72-year-old Caucasian man in remission from previously diagnosed multiple myeloma in 2000, who presented to our hospital with pancytopenia. The patient had been receiving

maintenance therapy with Revlimid and in remission for the last 4 years prior to this admission. A bone marrow biopsy was performed and revealed a precursor B lymphoblastic lymphoma/leukemia arising in an extensively fibrotic marrow.

B lymphoblastic leukemia is a hematologic disorder involving lymphoblasts of the B-cell lineage that most commonly affects young children. Multiple myeloma is a plasma cell neoplasm typically affecting adults older than 50 years. The association between these 2 entities remains unknown.

Peripheral blood and bone marrow specimens were received in pathology to rule out a possible relapsed myeloma. H&E, reticulin, trichrome, and immunohistochemical stains were evaluated. In addition, flow cytometry was performed.

Histological sections from the bone marrow core biopsy revealed a diffuse infiltrate by B lymphoblasts in an extensively fibrotic marrow. The blast population was consistent with a B-lymphoblast phenotype, demonstrating positivity for CD10, CD19, CD20, and TdT and negativity for CD2, CD3, CD4, CD7, CD8, CD34, CD38, CD56, CD138, CD117, cyclin D1, and EBV-LMP. Stromal fibrosis was confirmed by reticulin and trichrome stains. Recurrent multiple myeloma was ruled out with normal results of serum and urine protein electrophoresis and polyclonal plasma cells. A hyper-CVAD chemotherapy regimen was subsequently started.

In this report, we document an unusual presentation of acute B-lymphoblastic lymphoma/leukemia arising from a previously treated multiple myeloma. To the best of our knowledge, there have been no previously documented reports. The precise association between these 2 hematologic neoplasms in this patient remains unknown but is very intriguing.

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Comparison of WBC Differential Manual Count vs Sysmex-2100 Autoanalyzer Research W: Danbury Hospital's Experience

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The Sysmex-2100 is a blood autoanalyzer that can analyze 30 different hematological parameters from a single blood sample. The following parameters were analyzed in our study: neutrophils, lymphocytes, basophils, eosinophils, and monocytes. Research W of the Sysmex-2100 is a unique feature of the Sysmex-2100 that gives more accurate information on count and granularity of the WBC, as compared with the standard Sysmex-2100, which sometimes fail to give the report.

We randomly selected 90 WBC differential reports in which the standard Sysmex-2100 failed to give the final report. On these 90 cases, peripheral smears were made, manual differentiation was done by laboratory technologists, and these were reviewed by an experienced pathologist. Finally, the results were compared between manual differentiation done by laboratory technologists, pathologist, and Research W of the Sysmex-2100.

Of 90 sample reports, 97.77% reviewed by a pathologist were in agreement with the differential report submitted by Research W of the Sysmex-2100. On further comparing of the results between a pathologist and the laboratory technologist, there was only 70% concordance. In only 1 sample report, the pathologist did not agree with the Research W report but agreed with the manual differentiation count done by the laboratory technologist. Of 90 samples, 1 sample was recognized as autoanalyzer failure and the Research W of the Sysmex-2100 also failed to generate a report.

Research W of the Sysmex-2100 can be used as a powerful tool by laboratory technologists to compare their results after doing

manual differentiation counts. This would greatly help clinicians who depend solely on the manual differential count report for managing their patients.

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Metastases to the Gastrointestinal Tract: A Retrospective 5-Year Experience

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Metastases to the gastrointestinal (GI) tract come from a wide variety of organs. We review our experience for a consecutive 5-year period in a tertiary care center.

A retrospective search was performed from January 2004 to January 2008, using the key words "malignancy," "gastrointestinal tract" (including each anatomic subsite), and "metastatic." Reports were reviewed for demographics, diagnosis, primary site of malignancy, nature of diagnostic specimen, and specific GI location. Follow-up data were found for 88% of cases. Overall survival up to September 1, 2009, was calculated. Kaplan-Meier curves were used for comparison.

There were 376 malignancies metastatic to the GI tract (0.33% of the total GI volume). Of these, 20% were diagnosed on mucosal biopsies obtained at endoscopy; the rest were from staging laparotomies or en-bloc resections. Sites involved on mucosal biopsies were stomach (22%), duodenum (21%), small bowel (3%), colon (33%), and rectum (21%). The most common primary sites were female genital tract (49%), urinary tract (10%), and malignant melanoma (8%). Fewer than 12% were from elsewhere in the luminal GI tract, breast, lung, peritoneum, and adrenal; 20% were of unknown primary. The most common site was the ovary, and the most common histologic type was carcinoma.

The median survival for all patients was 255.5 days. Survival was highest for breast (501.5 days), female genital tract (446 days), urinary tract (339 days), and malignant melanoma (309 days). The mean survival was 80 days for hepatopancreaticobiliary, 46 days for lung, 30 days for unknown primary, and 21 days for GI primary tumors.

The overall prevalence of metastatic disease to the GI tract is low, and the range of possible primaries is broad. The female genital tract is the most common source of metastases to the GI tract. There are differences in survival depending on the site of primary tumor.

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Exsanguination Due to Inferior Vena Cava Filter Migration and Vessel Perforation

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A 37-year-old man with a history significant for morbid obesity, dilated cardiomyopathy, deep vein thrombosis, and atrial fibrillation treated with warfarin therapy died suddenly following uncomplicated laparoscopic gastric sleeve surgery with preoperative inferior vena cava filter placement. The decedent was discharged from the hospital in good condition 3 days prior to his death. On the day of his death, he complained of back pain and dyspnea. He was admitted to the hospital and found to have a hemoglobin level of 6 g/dL. The decedent received 5.5 units of packed RBCs and 2 U of fresh frozen plasma, but died despite resuscitative efforts.

At autopsy, 10 L of blood was found in the abdominal cavity, in addition to congealed blood in the right retroperitoneal space. Examination of the inferior vena cava revealed migration of the filter above the level of the renal arteries with perforation of the IVC wall. The filter was intact and nondeformed.

This case represents a rare but life-threatening complication of vena cava filter placement. Reports in the literature highlight the varied presentation of vena cava filter migration, which may include massive hemorrhage, gastrointestinal bleeding, cardiac failure, cardiac tamponade, and abdominal pain.

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Placental Findings in Pregnancies Complicated by Intrahepatic Cholestasis

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Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific complication associated with a high incidence of preterm birth and stillbirth. The pathogenesis is poorly understood. There are rare data on histopathological findings of placentas in ICP in the literature. This study aimed at describing common placental histopathological patterns in ICP to provide guidance for future studies.

This was a retrospective study searching the archives for placental examinations performed here (October 2007-October 2009) for the key word "cholestasis." Clinical information, including gestational age, obstetric and prenatal history, and laboratory test results, was extracted from the hospital health information system.

There were 53 placentas (50 singleton and 3 twin placentas) returned from 51 patients with a history of ICP. All were treated with ursodiol. The maternal peak bile acid level ranged from 4 to 175 $\mu\text{mol/L}$ (normal, 0-10). Other common maternal complications included gestational diabetes mellitus (6/53 [11.3%]) and hypertensive disorders (4/53 [7.5%]). Most (37/53 [69.8%]) deliveries occurred at 36 to about 37 weeks' gestational age by induction or cesarean section to avoid the unpredictable stillbirth. Fetal outcomes included stillbirths (4/53 [7.5%]) and IUGR (2/53 [3.8%]). Commonly seen histopathology included perivillous fibrin deposition with extravillous trophoblastic (EVT) proliferation (24/53 [45.3%]), evidence of meconium exposure (23/53 [43.4%]), villous dysmaturity (17/53 [32.1%]), villous edema (15/53 [28.3%]), erythroblastosis (12/53 [22.6%]), decidual vasculopathy (9/53 [17.0%]), acute chorioamnionitis (6/53 [11.3%]), and villitis of unknown etiology (3/53 [5.7%]). Placental parenchyma vascular lesions or fetal circulation abnormalities are rarely seen. No consistent histopathological findings were observed in placentas from 2 patients with recurrent ICP.

This is the largest reported study of placental histopathology in ICP. The main pathological findings consist of increased perivillous fibrin deposition with EVT proliferation and evidence of intrauterine distress. No obvious patterns of inflammation or circulation problems were observed. The above statistics also point out the need for a controlled prospective study of placental pathology, placental-fetal correlation, and clinical-pathological correlation of laboratory test results such as peak bile acid levels and duration.

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Use of Toluidine Blue Stain of Umbilical Cord Mast Cells to Distinguish Stillborn From Liveborn Infants

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Distinction between stillborn and liveborn infants is an important issue in pediatric pathology and legal medicine. At many situations, proof of life is hard to find or establish. Mast cells have been used as vitality marker in cutaneous sections of gunshot wounds. One previous study suggested a significant difference of expression of the mast cell marker tryptase in the umbilical cord. The current study aimed to investigate the usefulness of toluidine blue stain for mast cells in the umbilical cord to distinguish stillborn from liveborn infants.

This was a prospective study. Placentas associated with stillbirth and control (gestational-age-matched liveborn) infants were identified from clinical practice during January to August 2009. Paraffin-embedded umbilical cord sections were stained with toluidine blue and reviewed by 2 pathologists independently and blinded to clinical information. Maternal age, gestational age at delivery, fetal sex, degree of maceration, morphology, and number of toluidine blue-stained cells were recorded.

We collected 40 umbilical cord samples (34 singleton and 3 twin placentas). The maternal age ranged from 17 to 37 years and gestational age at delivery, 19 to 39 weeks. Of 20 umbilical cord samples from placentas of liveborn infants, 18 (90%) demonstrated the presence of toluidine-stained cells (cytoplasmic granules) in the umbilical cord. Of 20 stillborn samples, 7 (35%) had positively stained cells. The difference in the qualitative staining result was significant (positive vs negative staining, $P < .001$; Fisher exact test). Among the stillborn cases with positive staining, the 3 cases with the highest number of positive cells were all intrapartum death. In both groups, the number of positively stained cells was not dependent on gestational age. In the group of stillborns, there was no significant association with degree of maceration or the sex of the infants.

This simple test using a toluidine blue stain to identify mast cells in the umbilical cord may be a useful adjunct in determining livebirth or stillbirth.

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Unusual Cutaneous CD4+ T-Cell Lymphoma in a 59-Year-Old Male: A Report and Literature Review

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Several cutaneous T-cell lymphomas (CTCLs) exist in the literature, of which mycosis fungoides is the classic type. Diagnosis of non-mycosis fungoides CTCLs is challenging, especially when the clinical presentation is atypical. With the assistance of molecular studies, flow cytometry, and immunohistochemistry, more definitive criteria are available for diagnosticians to classify these rare forms of lymphoma and differentiate unusual presentations from mycosis fungoides. However, not all histological variants can be grouped into the current classification systems. We report the case of a man who had an unusual clinical presentation and whose disease could not be definitively classified based on histology and ancillary studies.

A 59-year-old man presented in stage IV disease with erythroderma, bilateral inguinal adenopathy, and no history of mycosis fungoides-like lesions. Flow cytometry of his peripheral blood showed only a high CD4/CD8 T-cell ratio. He underwent skin punch biopsies that were sent to our institution. On histology, small atypical lymphocytic infiltrates with folliculotropism and minimal epidermotropism were seen. More specifically, fewer than 30% of the atypical cells

constituted a large cell component. Immunohistochemistry demonstrated peripheral neoplastic T cells with nonatypical central B cells within ill-formed follicles. The atypical T-cell phenotype was CD4+/CD8- cells with positivity for CD3, CD5, CD7, and BCL2. In addition, CD30, BCL1, and BCL6 were negative. T-cell receptor gene rearrangement was present without B-cell rearrangement.

These findings supported a peripheral T-cell lymphoma over T-cell hyperplasia but did not offer any specific diagnostic grouping. Based on histology, immunophenotype, and clonality of the small neoplastic T cells, one possible grouping is under CD4+ small/medium-sized pleomorphic T-cell lymphoma. However, the initial systemic presentation and the folliculotropism are inconsistent with the above provisional grouping.

As such, this unique case poses difficulty in CTCL classification and possibly represents a histological variant to cutaneous CD4+ T-cell lymphomas, of which further study and correlation are recommended.

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Mesothelioma in Egypt: an Emerging Health Concern and a Diagnostic Challenge

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Malignant pleural mesothelioma (MPM) is an aggressive and deadly malignancy that is on the rise worldwide. Despite its increasing incidence, no treatment modality is accepted as the standard of care.

The aim of this article is to review the literature on world values for MPM and compare them with the Egyptian series, with special emphasis on the data collected from the Department of Pathology, Cairo University hospitals, during 5 years. The article also focuses on some important risk factors involved in the pathogenesis of such tumors in Egypt and outlines the challenges in the diagnosis of MPM.

A review of literature was carried out, together with collection of all available archival data covering the 2004-2008 period from the Pathology Department in Kasr El Aini Medical School and New Kasr El Aini Teaching Hospital.

From the findings of the present study and the results of similar studies carried out in other Egyptian centers, it can be concluded that mesothelioma in Egypt is also on the rise and is mainly environment related with a high incidence in females nearing those of males.

The increasing incidence and the earlier age of presentation of such cases in Egyptians stress the importance of developing a means of protection for the high-risk populations with frequent medical surveillance, particularly in individuals involved in the asbestos and asbestos-related industries.

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Impact of Proliferative Indices and Tumor Suppressor Genes on Treatment Response in Marginal Zone Lymphomas

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First, increased proliferation as assessed by Ki-67 and MUM-1 staining has been associated with worse prognosis in marginal zone B-cell lymphomas (MZL). However, ours is the first study to assess the impact of proliferative indices on response to therapy.

Second, loss of p53 and p16 tumor suppressor genes has traditionally been associated with cancer progression. The significance

of these markers in MZL remains to be elucidated and was investigated in our study.

Third, we explored the role of proliferative indices and tumor suppressor genes in identifying MZLs with lymphoplasmacytic differentiation (LPD). Consecutive MZL cases (2006-2008) in patients 18 to 99 years old were identified by searching the Copath database at WU/BJC. Cases with insufficient diagnostic material were excluded. Slides were reviewed by 2 pathologists to confirm the diagnosis; immunohistochemical stains for Ki-67, MUM-1, WT1, p53, and p16 were subsequently performed. Slides were manually graded for percentage staining. Less than 1% tumor staining was considered negative. Statistical analyses were performed using the Wilcoxon 2-sample test and Pearson correlation coefficient.

We reviewed 29 cases. Some degree of lymphoplasmacytic differentiation was seen in 34% of cases. Tumor staining for WT1 was not seen. Mean percentages of tumor staining for Ki-67, MUM-1, p53, and p16 were 19.1%, 14.7%, 4.1%, and 6.1%, respectively. Of the assessable patients, 78% responded to treatment with no evidence of progressive disease. None of the assayed markers were correlated with treatment response. Ki-67 and MUM-1 staining, however, correlated with LPD ($P = .0136$ and $P = .0042$, respectively). A correlation between MUM-1 and p16 staining was also seen ($P = .0006$).

Assaying for tumor suppressor gene status (p53 and p16) and proliferative indices (Ki-67, MUM-1, WT1) is unlikely to predict treatment response in low grade MZL. Ki-67 and MUM-1 staining, however, may serve as a useful adjunct in ascribing lymphoplasmacytic differentiation to these tumors.

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Prognostic Factors in Endometrioid Adenocarcinoma and Serous Carcinoma of the Endometrium: A Cancer Institute Experience

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Endometrial cancer is the most common gynecologic malignancy in the developed countries. There are numerous recent articles exploring the risk factors for early recurrence and survival in early-stage type I and type II endometrial carcinoma. However, the difference of tumor outcome in pure endometrial adenocarcinoma (EAC) and uterine serous carcinoma (USC) in disease extending beyond the endometrial cavity is yet to be explored. The aim of this study was to explore and compare the outcome of patients with II stage or higher EAC with that of patients with USC.

A total of 107 patients (73 with EAC and 34 with USC) were studied. For statistical analysis, the following baseline variables were considered for their prognostic value: age at presentation, tumor size, depth of myometrial invasion (MI), lymphovascular involvement (LVI), and USC and EAC subtypes (considered as binary variables). Disease-free survival, death of disease (DOD), and overall survival were assessed by using univariate and multiple Cox proportional hazards models. Patients with USC tend to be older than patients with EAC ($P < .001$). In univariate analysis, USC tends to recur more frequently than EAC ($P = .004$). However, this significance disappeared in multivariate analysis. Among all prognostic factors and after adjusting for the aforementioned factors, EAC with high-grade FIGO and MI of 50% or more were the only independent factors in predicting DOD ($P = .009$) (HR, 2.64; 95% CI, 1.27-5.47) in stage II

or higher and in stages II + IV. Similarly, LVI was the only independent factor in predicting recurrences ($P = .004$) (HR, 5.36; 95% CI, 1.72-16.72) in stage II or higher but not in stages III + IV.

Based on our study, there was no significant difference in outcome between USC and EAC in stage II or higher disease after adjustment. However, MI and LVI were the only independent predictors of bad outcomes in these tumors.

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CD44v6 Expressed Exclusively in the Epithelial Component of Synovial Sarcoma

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Synovial sarcomas are aggressive soft tissue neoplasms that may consist solely of spindle cells (monophasic type) or spindle and epithelioid cells (biphasic type). Despite the recent advances in the therapy for local disease, distant metastasis remains the predominant cause of death. Therefore, there is a great need for understanding this tumor at a molecular level. CD44 is an 85- to 90-kDa integral transmembrane glycoprotein belonging to a distinct family of adhesion molecule receptors. There has been recent intense interest in the association of the CD44 variant, CD44v6, expression and tumor progression and metastasis. The purpose of this study was to determine the expression of CD44v6 in monophasic and biphasic synovial sarcomas.

A total of 16 synovial sarcoma cases (10 monophasic and 6 biphasic) were retrieved from the archival surgical pathology files. The ages of patients ranged from 12 to 55 years (mean, 58 years). Representative tissue sections from each case were selected, and the corresponding paraffin-embedded tissue block was used to prepare tissue microarrays. Immunohistochemistry was performed using a monoclonal antibody against CD44v6. Reactivity for CD44v6 was defined as uniform membrane staining in at least 10% of cells. In addition, its intensity was graded as follows: 0+, negative; 1+, weak; 2+, moderate; and 3+, strong.

Strong CD44v6 expression was noted only in the epithelial component in all cases of biphasic synovial sarcoma (6/6). In contrast, the spindle cell component was negative in all cases. None of the monophasic synovial sarcoma cases (0/10) expressed CD44.

CD44v6 is expressed exclusively in the epithelial component in biphasic synovial sarcomas. The spindle cells did not express CD44v6 in monophasic or biphasic synovial sarcomas. CD44v6 expression appears to be associated with the degree of epithelial differentiation.

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Immature Platelet Fraction Suggests a Role for Peripheral Destruction of Platelets in Jacobsen Syndrome

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Paris-Trousseau/Jacobsen thrombocytopenia is a rare genetic syndrome associated with a deletion of the long arm of chromosome 11 at 11q23.3-24. This deletion includes 2 ETS transcription factors encoding genes *FLI1* and *ETS1*. The complete lack of *FLI1* expression in a portion of MK progenitors leads to small, immature MK that undergoes massive intra-bone marrow lysis, the proposed mechanism for the severe thrombocytopenia in the patients.

A 69-year-old man with a medical history of Jacobsen syndrome was admitted with respiratory distress secondary to aspiration of tube feedings. Serial CBC examinations were performed and, using the Sysmex XE-2100, demonstrated anemia and severe thrombocytopenia with an increased immature platelet fraction (IPF). A peripheral blood smear showed macrocytic anemia and thrombocytopenia with large platelets containing giant granules.

The patient had a cytogenetics study performed 9 years earlier that showed 46,XY,del(11)(q23), and the bone marrow demonstrated normocellular bone marrow with prominent dysmegakaryopoiesis and an adequate number of megakaryocytes with hypolobated forms and micromegakaryocytes. During 30 days, his peripheral blood was collected and his IPF measured 6 times. His IPF ranged from 21.0% to 29.5% with a mean of 24.7%.

Measurement of the platelet index IPF has proved to be an important diagnostic tool in the determination of the etiology of thrombocytopenias. Determination of reticulated platelets helps differentiate thrombocytopenia due to decreased production vs peripheral destruction of platelets. A high IPF has been reported in hyperproductive thrombocytopenia due to peripheral destruction of platelets. The IPF levels in our patient were consistently elevated. Therefore, it suggests that for patients with Jacobsen syndrome, peripheral destruction leads to decreased life span of platelets with release of immature platelets from the affected bone marrow. This could be a concurrent mechanism, accounting for the severe thrombocytopenia seen in patients with Paris-Trousseau/Jacobsen.

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Down-Regulation of CD28 Receptor in Lyme-Positive Patients: A Test for Chronic Lyme Disease

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Lyme disease is caused by the tick-borne spirochete *Borrelia burgdorferi* (Bb). In infected people, disease can progress to chronic joint inflammation. The CD28/B7 pathway plays a central role in immune responses against pathogens, autoimmune disease, and graft rejection. Our objective was to study the expression of CD28 in the T cells of patients with Lyme disease performed in the off-peak season (December 2009-February 2010).

Multiparametric analysis consisting of cellular light characteristics and the simultaneous staining for CD45 and CD28 was performed on available EDTA-anticoagulated blood. We selected 62 patients for evaluation. In 32, there was a positive Lyme 2-tiered test, and 30 were used as random patient controls with no history of Lyme disease or Lyme testing.

The range of CD28 counts in Lyme-positive patients was 176 to 2,772 cells per μL (mean, 1,234/ μL). Normal control subjects showed a range of 1,080 to 3,138 cells per μL (mean, 1,756.85/ μL). A 2-tailed *t* test was performed that showed statistical significance between the means, with a *P* value of .005. Owing to the low number of patients and our not wanting to assume a parametric distribution, a Mann-Whitney test was performed, which also showed a statistically significant difference with a *P* value of .0060.

A significant down-regulation of the CD28 receptor was observed in patients with a positive Lyme 2-tiered test compared with control subjects, suggesting a causative association of decreased CD28 expression and Bb infection. The cohort of patients tested belonged to the off-peak Lyme season and may be more representative of persistent cases of Lyme disease. The findings need to be validated in a cohort of clinically defined or self-defined patients with chronic Lyme disease to better understand the relationship in

humans. The findings would have therapeutic implications since CD28 blocking antibodies have been developed for therapeutic use in other inflammatory diseases.

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Granular Cell Astrocytoma: Case Report and Review of Literature

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Granular cell astrocytoma (GCA) is a rare type of infiltrating malignant brain tumor, first described by Markesbery et al in 1973, with only a small number of cases in North America. We present a case of a 75 year-old man with no significant medical history who presented with progressive expressive aphasia. An MRI revealed a 3.6-cm, irregular, heterogeneous, centrally enhancing periventricular mass with peritumoral edema in the right parietal white matter and an evolving cerebral infarct.

A right temporal craniotomy was performed, and a needle biopsy of the right temporal mass was obtained. Gross examination showed multiple irregular fragments of tan-pink soft tissue. Microscopically, the lesion consisted of brain parenchyma infiltrated by a moderately cellular neoplasm of glial cell origin. The neoplastic cells were hypercellular with nuclear pleomorphism and increased nuclear anaplasia. Foci of necrosis, capillary endothelial proliferation, and hyperplasia of blood vessels were also noted. Few mitoses were present. The neoplastic cells demonstrated abundant eosinophilic granular cytoplasm with ill-defined cytoplasmic borders. Immunoperoxidase stains demonstrated strong positivity for GFAP, CD 68, and PSA stain. The findings were consistent with GCA.

The patient died approximately 3 months later due to acute panlobar bronchopneumonia. An autopsy confirmed our diagnosis.

GCA is a highly aggressive neoplasm, in contrast with granular cell tumors in other parts of the body, which are benign. Patients range in age from 25 to 79 years (mean, 55 years) with a male/female ratio of 2:1. Patients present with symptoms of increased intracranial pressure. Most occur in the cerebral hemispheres. The differential diagnosis includes progressive multifocal leukoencephalopathy, cerebral infarction, and multiple sclerosis. Electron microscopic studies reveal an increase in intracytoplasmic lysosomes. Cytogenetic analysis shows a loss of 9p or 10q in most cases. Surgical excision with chemotherapy or radiotherapy is the treatment of choice.

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Unusual Presentation of Metastatic Ductal Adenocarcinoma of the Pancreas

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Pancreatic cancer first metastasizes to regional lymph nodes (72%-83%) and then to the liver (64%-80%) and lungs (27%-50%). Pancreatic cancer uncommonly metastasizes to the head and neck. We report a case of an 86-year-old woman with no significant medical history who presented with a right mandible mass. An X-ray and CT scan showed an expansile osteolytic lesion in the right mandible.

The patient underwent a biopsy of the mandibular mass. Gross examination showed multiple fragments of tan-pink soft tissue.

Microscopically, the lesion demonstrated stromal connective soft tissue infiltrated by a moderately differentiated malignant epithelial neoplasm arranged in solid nests and ductal-glandular components. The neoplastic cells demonstrated pleomorphic nuclei with ill-defined cytoplasmic borders. Mitoses and necrosis were present. The intervening stroma demonstrated desmoplastic reaction and mixed inflammatory cell infiltration. Immunoperoxidase staining demonstrated positivity for CK7, CK20, CEA 19-9, and villin and were negative for ER, PR, TTF1, WT1, and CTX2. Histological features were consistent with metastatic, moderately differentiated adenocarcinoma. Primary sites include upper GI (pancreatic and biliary) and head and neck.

A subsequent CT scan showed a mass in the pancreatic body with dilatation of the pancreatic ducts and atrophy of the tail. A PET-CT scan showed a focal increase uptake in the known right mandibular tumor. Other areas of uptake were observed in the T2 vertebral body, liver, and in both lungs that all presumably represented tumor.

Pancreatic cancer uncommonly metastasizes to bone. Metastasis of pancreatic carcinoma to the oral region is extremely rare, with only 5 reported cases, 2 of which reported metastasis to the mandible as the presenting symptom. It is believed that metastasis to this region must occur via a hematogenous route. In this case, it was the first indication of an undiagnosed malignant tumor and prompted a workup for the primary tumor.

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Plasma Cell Extraction: A New Approach to Obtain Prognostic Information From Multiple Myeloma Specimens in the Cytogenetics Laboratory

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Several chromosomal abnormalities are associated with multiple myeloma (MM), including loss of chromosome 13q14, 17q13, and t(4;14)(p16.3;q32). Fluorescence in situ hybridization (FISH) analysis has emerged as the technique of choice for evaluation of cytogenetic abnormalities in plasma cell malignancies. These studies increase the proportion of cases with chromosomal abnormalities from 50% by conventional methods to more than 90%.

The study included 9 confirmed MM patients and 10 non-MM patients (control subjects). Plasma cells were extracted from the bone marrow samples in 4 of 19 cases using MACS magnetic technology. FISH analysis was implemented, with 3 probes from Vysis. Only 1 case showed an abnormal karyotype via conventional cytogenetic analysis. FISH analysis was done using a fluorescence microscope. When possible, 500 nuclei were scored. Only 5 of the expected abnormal group and 9 of the control group had enough material to score 500 cells.

With the LSI IGH SG/LSI FGFR3 SO probe, 3 of 5 cases from the experimental group showed 1 orange/1 green/1 fusion signal, while the control showed two orange/two green signals. With the LSI D13S319 SO/LSI 13q34 SG probe, 2 of 5 cases of the expected abnormal group showed 1 orange/2 green signals (deletion of 13q), while the control showed 2 orange/2 green signals. For the LSI TP53 SO/CEP 17 SG probe, 1 of 5 cases of the expected abnormal group showed 1 orange/2 green signals (deletion of 17p), while the control showed 2 orange/2 green signals.

We attempted to use an antibody-based sorting technique by MACS to purify plasma cells from the bone marrow aspirate before FISH analysis. Although we were unsuccessful in purifying enough

plasma cell material for FISH analysis, a novel technique had been introduced to the Danbury Hospital Cytogenetic Laboratory. From our attempts, the cytogenetics staff members may be able to develop their own techniques to purify plasma cells more successfully in the future.

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The Significance of Intranuclear Inclusions Within Follicular Cells in Papillary Carcinoma of the Thyroid

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Our objective was to identify the significance of intranuclear inclusions and other cytological features on FNA signed out as "suspicious" for papillary carcinoma of the thyroid. All cases of FNA signed out as "suspicious for papillary carcinoma of the thyroid (PCT)" between January 2007 and December 2008 at Danbury Hospital were reviewed, along with 20 random cases from the files of our laboratory.

The following cytological features were recorded for each case: nuclear grooves, overall cellularity, cellular overlap, and total number of intranuclear pseudoinclusions. The follow-up surgical pathology diagnosis was also noted. The slides were independently reviewed by 2 cytotechnologists and two pathologists.

A total of the 58 FNA cases were found using SNOMED, of which 52 were diagnosed as suspicious, 2 as worrisome, 2 as possibility of, 1 as cannot rule out, and 1 as indeterminate for PCT. For 17 cases, there was no surgical follow-up at our institution, and they were excluded from the study. The remaining 34 cases were reviewed. The ages of the patients ranged from 37 to 80 years (mean, 55 years). Of the 34 patients, 27 (79%) were women and 7 (21%) were men. On average, 9 cytology slides were made for each case. Of the 34 patients who had surgical follow-up, 20 (59%) were confirmed to have papillary carcinoma of the thyroid, 6 had multinodular hyperplasia, 5 had nodular hyperplasia, 2 had follicular adenoma, and 1 had a hyalinizing trabecular tumor. Statistically significant features included intranuclear pseudoinclusions, nuclear grooves, and overall cellularity. Cell overlap was not statistically significant.

Our study used a semiquantitative system to assess the likelihood of papillary carcinoma. While no one cytologic characteristic is diagnostic of papillary carcinoma, the presence of intranuclear pseudoinclusions, nuclear grooves, and overall cellularity aid in making this diagnosis with a high level of certainty.

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Classical Hodgkin Lymphoma Presents as Pancytopenia

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Classical Hodgkin lymphoma is the most common subtype of Hodgkin lymphoma, accounting for approximately 95% of cases. Patients classically present with lymphadenopathy of the cervical region. Nonaxial lymph node groups and extranodal involvement of the bone marrow or spleen is rare. We report the case of a 56-year-old Hispanic man who presented with constitutional symptoms and low back pain for several years.

No significant lymphadenopathy was appreciated by physical examination. Pancytopenia was noticed on routine blood work.

Radiological examination demonstrated a 1.1-cm mediastinal lymph node and splenomegaly. Based on the clinical presentation, a myelodysplastic syndrome or malignancy was clinically suspected, and a bone marrow biopsy was performed. The bone marrow biopsy demonstrated extensive granulomatous infiltrate with stromal fibrosis and focal necrosis. There were scattered, large, atypical cells in a mixture of cellular background. These large cells showed abundant cytoplasm and macronucleoli, as Reed-Sternberg cells. The immunophenotype of classical Hodgkin lymphoma was confirmed by immunohistochemical stains on core biopsy: positive for CD15 and CD30, and EBV-LMP+. Special stains for AFB and GMS were negative. No aberrant cell population was demonstrated by flow cytometry.

Primary extranodal classical Hodgkin lymphoma with involvement of the bone marrow is rare. Histopathological features suggestive of bone marrow involvement include necrosis and the presence of Reed-Sternberg cell or its variant in a polymorphic background infiltrate with focal fibrosis and/or myxoid change. In our case, the patient presented with pancytopenia, constitutional symptoms, and low back pain without palpable cervical lymphadenopathy, making the diagnosis an unsuspecting finding on bone marrow biopsy. A high index of suspicion and immunohistochemistry are essential making this diagnosis in the absence of the classical clinical presentation.

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Unusual Presentations of Metastatic Carcinoma of the Breast

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Invasive breast carcinomas preferentially metastasize to lymph nodes, liver, lung, and bone; however, lobular carcinoma metastases have a greater predilection for the peri/retroperitoneal region, internal genital organs, and leptomeninges than ductal carcinoma. We report 3 unusual presentations of metastatic cancer, including 2 invasive lobular carcinomas presenting as diverticulosis and uterine cancer and 1 ductal carcinoma presenting as a pericardial mass.

Three cases of unusual metastatic breast carcinoma, submitted between 2005 and 2009, were retrieved from the surgical pathology archives of Houston-area hospitals. All slides were reviewed and diagnoses confirmed by 2 pathologists. Relevant clinical information was obtained from medical records.

The first patient was a 64-year-old woman with a history of invasive lobular carcinoma (ILC), diagnosed 20 years ago, who underwent sigmoid colectomy for diverticulosis. Incidentally, metastatic carcinoma, involving the full thickness of the colonic wall, was identified, consistent with breast primary. The second patient was a 62-year-old woman with a history of ILC, diagnosed 15 years ago, who underwent hysterectomy for endometrial adenocarcinoma. Lobular carcinoma metastases were identified, infiltrating the myometrium and vaginal and cervical stromas. The last patient was a 46-year-old woman with a history of invasive ductal carcinoma, diagnosed 7 years ago, who presented with pericardial effusion and mass. Biopsy revealed tumor cells with signet-ring features, suggestive of a gastrointestinal primary; however, immunohistochemistry demonstrated the tumor to be consistent with her known breast primary.

The 3 reported cases of metastatic breast carcinoma are remarkable not only in the novelty of their presentations, but also in the interval between initial diagnosis of the patients' primary cancers and the identification of metastases. The cases illustrate that careful survey of these organ systems is essential for identification of sometimes inconspicuous cells. Furthermore, immunohistochemistry

may aid in illuminating a many-times-unexpected diagnosis when metastases are encountered at rare sites.

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High-Risk Human Papillomavirus and p16 Expression Predicts Radiation Response in Oropharyngeal Cancer

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Our objective was to determine if favorable radiation response correlates with high-risk human papillomavirus (HrHPV) and p16 expression in a subset of newly diagnosed oropharyngeal cancers that had not undergone prior radiation or chemotherapy. Approximately 45% of oropharyngeal cancers are shown to harbor HrHPV. Epidemiologic trends during the past 3 decades have demonstrated an increased incidence in a subset of oropharyngeal cancer cases. This patient population tends to be younger and nonsmoking and tends to have a favorable response to radiation therapy. We wanted to determine if a favorable radiation response correlates with HrHPV and p16 expression.

We retrospectively selected 12 cases of oropharyngeal SCC. Eligibility criteria included newly diagnosed patients whose response status to radiation therapy was known and who had no prior treatment. The tumor tissue used was from the original biopsy. HrHPV cocktail in situ hybridization and p16 immunohistochemistry stains were applied to formalin-fixed, paraffin-embedded tumor tissue. The presence of HrHPV by in situ hybridization was determined by a nuclear dot staining pattern. The presence of p16 by immunohistochemistry was determined by dense nuclear and cytoplasmic staining. Results were analyzed and compared with previously known radiation response status.

Of the patients, 8 patients were classified as radiation responders. In responders, 100% (8/8) of tumors were p16+ and 88% (7/8) were HrHPV+. The other 4 patients were classified as radiation nonresponders. In nonresponders, 75% (3/4) of tumors were positive for p16 and 50% (2/4) were HrHPV+. The results showed no correlation with tumor location, TNM stage, or grade.

HrHPV and p16 were positive in 88% to 100% of radiation responders and in 50% to 75% of radiation nonresponders. This finding did not correlate with tumor location, stage, or grade. Our data suggest that expression of HrHPV and p16 predicts a favorable radiation response. HrHPV and p16 molecular studies should be performed on all newly diagnosed cases of oropharyngeal cancer. Further studies with a larger patient population and expanded molecular investigation are warranted.

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Clinical Significance of Anti-U Antibodies

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Because U antigens are nearly ubiquitous, anti-U is rarely formed, and the significance of these antibodies has not been fully elucidated. However, cases of anti-U–induced hemolytic disease of the newborn, hemolytic transfusion reactions, and warm autoimmune hemolytic anemia have been reported. It is, therefore, important to understand the various clinical scenarios in which these antibodies can be encountered and to establish management protocols, particularly for emergency situations. We present lessons learned from the transfusion of several U- patients.

We reviewed clinical records for anti-U–positive patients retrieved from our blood bank database. The transfusion history, management, and clinical outcome of several patients were summarized.

All 3 patients were African American females with anti-U. One patient delivered a neonate that was transferred to NICU 12 hours after delivery with hyperbilirubinemia and a positive direct antiglobulin test (DAT). The newborn was treated with phenobarbital and intense phototherapy as an alternative to double-volume exchange as compatible units were unavailable. Second, a 38-year-old patient with symptomatic anemia required emergency transfusion that was delayed for 2 days while compatible units were located. A third patient was a 23-year-old Rh– patient in sickle cell crisis. Despite a negative monocyte monolayer assay (MMA), she developed acute hemolytic transfusion reaction during an in vivo crossmatch with U+ red cells.

The clinical significance of anti-U is illustrated by the cases presented. Additionally highlighted is the importance of communication between clinicians and the blood bank to facilitate the emergency acquisition of rare units. Patient education as to the potential significance of these antibodies is also important. Furthermore, case 3 underscores the necessity of judicious transfusion in patients with high frequency antibodies, despite a negative MMA.

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Metastatic Prostate Carcinoma to Supraclavicular and Cervical Lymph Nodes as the Presenting Finding

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Rarely is metastatic cancer to cervical lymph nodes the initial presentation of prostate cancer. A fine-needle biopsy from a supraclavicular lymph of this 65-year-old revealed poorly differentiated carcinoma. Immunohistochemistry for PSA was positive and negative for CK7, CK20, TTF-1, chromogranin, and synaptophysin. A subsequent serum PSA was 375 ng/mL (normal <4.0 ng/mL). The physical examination revealed an enlarged prostate. A PET CT scan demonstrated diffuse metastatic disease with adenopathy in the chest, abdomen, and pelvis and osseous metastases, and at least 1 pulmonary nodule was identified in the left lower lobe. Following the oncology treatment with bicalutamide and leuprolide, the serum PSA decreased within 1 month from 375 ng/L to 62.5 ng/L, and the cervical and supraclavicular lymphadenopathy decreased by half.

This case emphasizes the importance of immunohistochemical analysis for PSA in supradiaphragmatic unknown primaries in lymph, even though such initial presentations are rare.

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Metastatic Disease to the Pancreas Documented by Endoscopic Ultrasound–Guided Fine-Needle Aspiration

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Endoscopic ultrasound–guided fine-needle aspiration has become the dominant method for investigation of pancreatic nodules and masses. While the majority of these lesions are primary pancreatic neoplasms, occasional cases represent metastases from primaries arising elsewhere. Metastatic as opposed to primary carcinoma of the

pancreas can have significant prognostic and therapeutic implications. This study was performed to determine the frequency and site of origin for metastatic disease to the pancreas as found in an endoscopic ultrasound-directed fine-needle aspiration series. We also investigated cytologic features helpful in distinguishing metastatic from primary carcinoma.

The records of the departments of pathology at the University of Utah School of Medicine and the David Geffen School of Medicine were electronically searched for all fine-needle aspirates obtained from pancreatic masses during a 7-year period. All cases with a cytologic diagnosis of metastatic disease were reviewed and, whenever possible, correlated with subsequent histologic specimens.

A total of 10 metastatic carcinomas to the pancreas were found in the 7-year period. Eight metastases arose from renal primaries. One case was a squamous cell carcinoma arising in the esophagus, and a second was a squamous cell carcinoma arising in a lung primary.

Metastatic renal cell carcinoma was the most frequent metastasis to the pancreas, representing 80% of the secondary carcinomas in our series of FNAs. The remaining cases were all of squamous morphology arising from the lung or esophagus. The metastatic deposits were recognized as late as 10 years following treatment of the original carcinoma. Separation of metastatic renal cell carcinoma from pancreatic clear cell and foamy gland pattern adenocarcinoma can be difficult. Primary and metastatic squamous cell carcinomas are impossible to separate cytologically.

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The Prevalence of Positive Group B *Streptococcus* in Mothers of Low-Birth-Weight Infants

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Birth weight is a strong indicator for a newborn's chances for survival, growth, long-term health, and psychosocial development. A low birth weight (LBW; <2,500 g) raises grave health risks for children. Infants who survive have impaired immune function and an increased disease risk. They are likely to remain undernourished, have decreased muscle strength, and have a higher incidence of chronic diseases. Children born underweight also tend to have cognitive disabilities. Furthermore, group B *Streptococcus* (GBS) has been identified as the number 1 cause of life-threatening infections in newborns that can cause them severe mental and physical sequelae. Therefore, any correlation between GBS positivity and LBW is critical since the presence of both can compound the health risk in affected infants.

Birth-related clinical information was obtained from September to December 2009, in a local hospital. Twins were regarded as 1 delivery. The data were analyzed and categorized based on the age of pregnancy: 19 years or younger, 20 to 24, 25 to 29, 30 to 34, and 35 years or older. The GBS prevalence in pregnant mothers was compared between the total number of deliveries and the LBW deliveries. There were a total of 1,062 deliveries in this study; 161 (14.7%) were LBW infants. The prevalence of GBS positivity among the mothers of LBW infants was 21.2% (34 deliveries), which was close to the general positive GBS rate, 19.7%. It was observed that GBS positivity in the mothers of LBW infants was higher in the 19 years or younger and 35 years or older age groups than in the other groups.

In general, the mothers of LBW infants may not have a higher incidence of GBS positivity, except within the groups of pregnant women in the 19 years or younger and 35 years or older age groups.

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Uncommon Clinical-Pathologic Correlation in a Case of Acute Myelomonocytic Leukemia

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Acute myelomonocytic leukemia comprises 5% to 10% of all cases of acute myeloid leukemia. It is characterized by the proliferation of neutrophil and monocyte precursors. Common clinical presentations of acute myeloid leukemia include fatigue, fever, anemia, and thrombocytopenia. Leukemia cutis is an uncommon presentation without other symptoms. In acute myelomonocytic leukemia, leukemic infiltration of organs is uncommon in the absence of hyperleukocytosis, with only a few cases published internationally. Clinically significant hyperleukocytosis is classified as a WBC count of 200,000/ μ L in the setting of acute myeloid leukemia.

We report a case of a 63-year-old man who presented to his primary care physician with a nonpruritic, papular skin rash that had been present for several weeks with no other symptoms at that time. Evaluation of the skin biopsy demonstrated leukemia cutis. The patient was subsequently diagnosed with acute myelomonocytic leukemia after an extensive bone marrow evaluation. Soon following this diagnosis, the patient developed progressive shortness of breath, fever, chills, and had a total WBC count of 27,500/ μ L. Chemotherapy was quickly administered, but the patient developed tumor lysis syndrome. His clinical condition rapidly declined, and he died a short time later. An autopsy examination was performed; some of the significant findings include increased weight of both lungs (1,800 g on the right and 1,660 g on the left). Histopathologic evaluation of the lungs revealed diffuse, leukemic infiltration with numerous acute microinfarcts bilaterally. Microscopic evaluation of the liver also demonstrated diffuse, leukemic infiltration.

This is an uncommon presentation and progression of acute myelomonocytic leukemia; furthermore, this case demonstrates that leukemic organ infiltration and subsequent rapid deterioration can occur outside the setting of hyperleukocytosis.

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Transiently Acquired Hemoglobin C Posttransfusion in Sickle Cell Patients

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Sickle cell crisis is a well-described complication of sickle cell anemia. Red cell transfusion, simple or exchange, is the standard therapy. Pretransfusion and posttransfusion hemoglobin S (HbS) levels are measured via high-performance liquid chromatography (HPLC) to assess for adequate response. At times, hemoglobin C (HbC) is detected posttransfusion by HPLC. This is usually attributed to laboratory artifact. However, this does not fully explain the reproducible and transient nature of the result. Currently, donor screening methods do not detect persons with abnormal hemoglobin (Hb) who are heterozygotes. Therefore, the possibility of an acquired HbC from a donor unit should be a consideration when a confirmed sickle cell patient has an unexpected HbC result on HPLC.

HPLC results, pretransfusion and posttransfusion, for transfused sickle cell patients were reviewed from July 2007 to November 2009. Pretransfusion Hb profiles, immediate posttransfusion Hb profiles, and remote posttransfusion profiles were examined.

We found 10 patients with HbS pretransfusion, with subsequent profiles showing both HbS and HbC immediately posttransfusion. In all patients, the HbC percentage was less than 20% of the total Hb.

Also, in all patients, the HbC incrementally decreased in a period of 2 or 3 months until reaching 0.

Sickle cell patients who receive transfusions have a chance of receiving abnormal Hb from donor blood, thereby altering their HPLC results posttransfusion. The altered Hb profiles in our study likely represented a transiently acquired HbC from transfusion since all of the patients showed only HbS profiles pretransfusion, HbS and HbC posttransfusion, and an incremental decrease in HbC to 0 in a 2 to 3 month period. Therefore, in patients with unusual posttransfusion Hb profiles, the possibility of transfusion-acquired abnormal Hb should be considered. Review of pretransfusion records or repeated testing 2 or 3 months later will often clarify the interpretation.

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Image-Guided Core Biopsies in the Breast Clinic Utilizing Large Gauge Needles Are Reliable for Evaluation of Lymphoma

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Traditionally, lymphoma diagnosis is based on incisional/excisional biopsy. In the setting of our breast clinic, core biopsies (CBs) utilizing large-gauge needles are routinely performed in patients with lesions detected by imaging, be it breast or axillary lymph nodes, including those "suspicious" for lymphoma. The aim of the study was to evaluate the performance of image-guided (IG) CBs in evaluation of lesions with suspicion for lymphoma in the breast clinic setting.

From January 2002 to June 2008, we identified and reviewed cases that were biopsied by a breast radiologist using IGCB using the following inclusion criteria: clinical suspicion of lymphoma, history of lymphoma, tissue sent for flow cytometry phenotyping (FC), or lymphoma diagnosis.

We identified 60 cases that fit the inclusion criteria (representing 0.67% of 8,994 IGCB specimens from the breast and axilla). All biopsies were US guided, performed by 14-gauge (80%) or 10- and 12-gauge (20%) needles, with the sample containing 3 to 10 cores (mean, 5.8 cores). The patients ranged in age from 21 to 92 years (mean, 53 years); all were women except 1. Tissue was sent for FC in 53 of 62 cases; 50 had sufficient tissue for FC. Tissue was sufficient for immunohistochemistry in all cases.

Among the 60 cases, 16 were diagnosed as carcinoma or benign breast lesion, 24 as reactive lymph nodes, 18 as lymphoma (10 in breast and 8 in axilla), and 2 as indeterminate cases ("cannot rule out lymphoma"). Definitive subtyping was established in 15/18 (83%) of diagnosed lymphomas based solely on the CB sampling, including primary diagnosis of lymphoma in 7 cases (3 diffuse large B-cell, 2 follicular, and 2 small lymphocytic) and 8 recurrent lymphomas (2 marginal zone, 1 diffuse large B-cell, 1 mantle cell, 1 small lymphocytic, 1 Hodgkin, and 1 anaplastic large cell). Three diagnosed lymphomas (2 Hodgkin and 1 mature B-cell lymphoma) required additional sampling for further subclassification. Additional sampling was required for diagnosis in 2 indeterminate cases (2/60 [3%]).

IGCB sampling of axillary and breast lesions suspicious for lymphoma is increasingly common in our breast clinic. In our experience, the IGCB using large-gauge needles provides ample tissue to study architecture/cytology and sufficient material for ancillary studies leading to a definitive diagnosis in the great majority of cases.

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Distinguishing Phyllodes From Fibroadenoma by Noninvasive SS-OCT Imaging and Invasive Molecular Imaging

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Etiopathologically, phyllodes tumor and fibroadenoma of breast are different, but diagnostic ambiguities prevail in certain situations, especially in stromal hypercellularity. To resolve such diagnostic ambiguity, the current study evaluated these tumors by noninvasive skin swept source optical coherence tomography (SSOCT) and immune histochemical studies. The OCT study tries to find out the differential structural signature in the tumor lesion and immune histochemistry identifies expression levels of proteins like p63 and α -SMA in myoepithelial cells and structural molecules like collagen I, III, and IV.

The OCT demonstrated foaminess of PT skin in contrast with compactness of FA. The IHC findings depicted significant differences between the 2 tumors in terms of p63 ($P < .0001$) and α -SMA expression ($P < .0001$). However, collagen I and III expression was increased in phyllodes compared with fibroadenoma and normal with irregular distribution of collagen IV in some of the blood vessels in PT.

Thus, SSOCT unveils the differential tomographic signature of phyllodes tumor and fibroadenoma, indicating varied optical properties, while significant differences in p63, SMA, and collagen I, III, and IV expression expose the pathological distinctness of these tumors at the molecular level.

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Outside Slide Reviews: Breast Pathology Dominates Workload in a Subspecialty-Aligned Practice

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Tertiary care centers see many referred patients following biopsy at another hospital. Many academic hospitals require that in-house pathologists review outside slides prior to further treatment. Outside slide reviews (OSRs) significantly contribute to workload; some subspecialties are affected more than others. Understanding the volume and subspecialty distribution of OSRs is essential to allocate staff appropriately, distribute work equitably, and accomplish it in a clinically appropriate time frame.

We retrospectively surveyed our first consecutive 400 OSRs in 2009, tabulating the number of cases/subspecialty, the number of slides submitted/case (a validated surrogate for effort/time), and the number of 88321 charges per case, each equivalent to 2.18 relative value units (RVUs). OSRs accounted for 11% of our accessioned surgical pathology cases in 2009. The 400 OSRs were distributed as follows: breast, 86 (22%); GI, 79 (20%); hematopathology, 75 (19%); ENT, 40 (10%); pulmonology, 38 (10%); GU, 33 (8%); dermatology, 24 (6%); gynecology, 11 (3%); and miscellaneous, 6 (2%). The total number of referred slides per subspecialty (% total referred slides) were as follows: breast, 1,836 (42%); GI, 512 (12%); hematopathology, 696 (16%); ENT, 249 (6%); pulmonology, 353 (8%); GU, 303 (7%); dermatology, 171 (4%); gynecology, 127 (3%); and miscellaneous, 82 (2%). Excluding miscellaneous, the average number of referred slides reviewed per 88321 was as follows: breast, 13; GI, 6; hematopathology, 8; ENT, 6; pulmonology, 8; GU, 8; dermatology, 5; and gynecology, 11.

We conclude the following: (1) The OSR caseload at our academic center varies markedly among subspecialties. (2) Breast, GI, and hematopathology services sign out about 60% of all OSR cases. (3) The actual workload (effort/time) varies significantly, even among the 3 highest volume services, with the breast service workload (number of slides reviewed) \gg GI and hematopathology. (4) To accomplish their important clinical mission, breast pathology

subspecialty services must be staffed on the basis of actual workload and not the number of RVUs accrued.

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Sclectrosing Angiomatoid Nodular Transformation of the Spleen: A Case Report and Literature Review

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Sclectrosing angiomatoid nodular transformation (SANT) of the spleen is a vascular lesion composed of multiple confluent angiomatoid nodules surrounded by concentric collagen fibers exhibiting inflammatory and myofibroblastic response accompanied by numerous erythrocytes and siderophages. The nodules are populated by endothelial cells, phenotypically recapitulating normal splenic vasculature such as sinusoids, capillaries, and small veins. Nuclear atypia is minimal, mitotic figures are extremely rare, and necrosis is consistently absent. This lesion has a unique immunohistochemical profile characterized by CD34-/CD31+/CD8+ sinusoids, CD34+/CD31+/CD8- capillaries, and CD34-/CD31+/CD8- small veins. CD68 is positive in macrophages. Occasional cases have shown expression of Epstein-Barr virus RNA. To date, sporadic case reports and occasional series of SANT have been described in the literature associated with various systemic diseases. We report the first case of SANT described in a patient with essential thrombocythemia.

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Clinicopathologic Correlation in Primary FSGS in Pediatric Age Group: A Retrospective Review

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Primary focal segmental glomerulosclerosis (FSGS) is the most common cause of steroid-resistant nephrotic syndrome in children. The progression to end-stage renal disease is fairly common (>60%), although more than a third of patients achieve remission and have normal renal function. The prognostic indicators have not been clearly defined.

We performed a retrospective review of 42 children with primary FSGS diagnoses between 1996 and 2010. Age at time of presentation varied from 2.5 to 19 years with mean follow-up period of 6.3 years. This group of patients included 30 males and 12 females. Of the children, 27 were African American, 9 were Hispanic, 3 were Caucasian, and 3 belonged to other ethnic groups. We examined the clinical, laboratory, histopathologic, and electron microscopic findings on onset in an attempt to determine prognostic indicators.

Univariate analysis revealed that 3 variables at time of clinical presentation have statistically significant impact on prognosis. These factors are absence of hypertension, absence of immune deposits, and normal GFR. Other factors with a favorable impact on the prognosis include low levels of proteinuria, normal serum albumin, and a low percentage of segmentally sclerosed glomeruli, although the impact was not statistically significant. These features may assist in guiding the therapy of FSGS by modulating the type and intensity of immunosuppressive and/or vasoactive therapy.

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Prognostic Value of Cyclooxygenase-2 Expression in Bilharzial-Related Urothelial Cancer

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Cyclooxygenase-2 (COX-2) is an important marker in multiple inflammatory and carcinogenesis pathways. We assessed the expression pattern of COX-2 in bilharzial- and nonbilharzial-related urothelial cancer (BUC and NBUC, respectively) and its association with pathological and clinical outcome after radical cystectomy. The study included 134 patients treated by radical cystectomy for urothelial cancer.

Patients were divided into 2 groups, each containing 68 patients with matching of the patients based on pathologic stage, lymph node involvement, and bilharzial infection. Group 1 comprised patients with BUC, and group 2 comprised patients with NBUC. Immunohistochemical staining for COX-2 was performed on archived bladder specimens. Altered immunohistochemical expression of COX-2 was correlated with pathologic parameters and clinical outcome. The mean follow-up was 3 years (range, 0-8 years).

The study included 119 men and 17 women with a mean age of 56 years (range, 31-79 years). Extravesical extension and lymph node invasion were present in 56% and 35% of patients in both groups. COX-2 expression was altered in 71% of group 1 and 81% of group 2. COX-2 expression was associated with advanced tumor stage ($P = .006$) and recurrence ($P = .02$) in group 1 but not in group 2.

COX-2 expression is a predictive marker for advanced tumor stage and recurrence in BUC. Our findings support further evaluation of the role of inflammatory pathways in BUC.

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Prevalence of Lung and Breast Carcinoma Among HIV+ Women in an Inner City Hospital: A Retrospective Study of Clinicopathological Findings From 2001-2009

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Recent population-based studies have suggested that the aging of the HIV-infected cohort and its improved survival were associated with an increase in non-AIDS-associated malignancies. In women, the 2 most common cancers are lung carcinoma (LC) and breast carcinoma (BC). We determined the prevalence and clinicopathological correlation of LC and BC among the HIV-infected women (HIVW) among our hospital population.

This was a retrospective study. All LC and BC cases in patients diagnosed in our pathology department from 2000 to 2009 were identified, with HIV status determined. Medical records were reviewed, and the following demographic, laboratory, and clinical data were collected: age, duration of HIV, type of cancer with stage, CD4 count, VL closest to cancer diagnosis, clinical course, cancer therapy and outcome.

From 2000 to 2009, 109 LCs and 756 BCs were identified. Of those, 11 (11%) LCs and 19 (2.5%) BCPs were in HIVW. The average age at cancer diagnosis was 56.6 years \pm SD and 48.2 years \pm SD, respectively. The average duration of HIV infection for LCP was 56.57 \pm 53.09 months and for BCP was 92.38 \pm 62.07 months. Of patients with BC, 36.8% received HAART, as did 58.3% of LC patients. The average CD4 count for patients with BC was 348.2 \pm 282 and for patients with LC, 351.45 \pm 200.0 cells/mm³, while the average VL for BC was 99.2 \pm 212.8 and for LC, 164.5 \pm 291.44 * 1,000 copies/mL.

Of the patients with BC, 55.55% were 2B or higher, while half of the patients with LC were diagnosed in stage 4. Of the HIV+

patients with LC, 76.95% were smokers. All patients with BC were diagnosed with infiltrating ductal carcinoma, while the majority of patients with LC had non-small cell carcinoma. The 3-year survival rate for BC was 42.8%, while for LC, it was 15.38% ($P = .00042$).

The prevalence of LC in HIVW is significantly higher than that of BC. HIVW with LC had a shorter duration of HIV infection than patients with BC prior to cancer diagnosis. Therefore, patients with BC have a substantially higher survival rate than patients with LC.

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Brd 4 and Aurora-B Expression in Normal Colon, Adenomatous Colon Polyps, and Colorectal Carcinoma: An Immunohistochemical Study

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Brd 4 is a BET (bromodomains and extraterminal) protein and plays an important role in cellular growth control, cell cycle progression, and tumor suppression. In a recent study, Brd 4 suppression decreased Aurora-B protein levels, and exogenous Brd 4 expression up-regulated endogenous Aurora-B expression in primary keratinocytes and cervical carcinoma cells. Aurora-B is a nuclear chromosomal passenger protein that controls kinetochore attachment to microtubules, allowing proper separation of sister chromatids. Novel Aurora kinase inhibitors are currently in phase 1/2 clinical trials. Our aim was to study Brd 4 and Aurora-B protein expression in adenomatous colon polyps, colorectal adenocarcinomas and normal adjacent colon.

Immunohistochemical staining for Brd 4 and Aurora-B was performed on formalin-fixed, paraffin-embedded tissues from adenomatous colon polyps, colorectal adenocarcinomas, and normal adjacent colon. Control slides reacted appropriately. Brd 4 expression was diffuse, nuclear, and strong in 9 of 9 (100%) colorectal adenocarcinomas, 8 of 8 (100%) adenomatous colon polyps, and 9 of 9 (100%) normal adjacent colon. Aurora-B expression was focal (<25% of the tissue; 8/9 [89%]), nuclear, strong (1/9 [11%]), weak (6/9 [67%]), and negative (2/9 [22%]) in colorectal adenocarcinomas. Aurora-B expression was focal (<25% of tissue), nuclear, strong (1/8 [12.5%]), weak (5/8 [62.5%]), and negative (2/8 [25%]) in adenomatous colon polyps. Aurora-B expression was focal (<25% of the tissue), weak (5/9 [55.6%]), and negative (4/9 [44%]) in normal adjacent colon.

Strong expression of Brd 4 in adenomatous colon polyps, colorectal adenocarcinomas, and normal adjacent colon compared with focal weak expression of Aurora-B supports the earlier findings that Brd 4 may be upstream of Aurora-B and may play a role in controlling Aurora-B expression. Strong expression of Brd 4 in precancerous adenomatous colon polyps and colorectal adenocarcinomas suggests Brd 4 may play a role in the pathogenesis of colorectal adenocarcinoma and be a good target for future antitumor therapy.

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Major and Unique Autopsy Findings of Liver Transplant Patients: A Single Institutional Study of 32 Patients

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Liver transplants are performed for end-stage liver disease due to various causes. Although surgical techniques and postoperative management have improved, patients are still at risk for complications and significant morbidity and mortality. We evaluated 32 autopsy cases of liver transplant patients to determine the major

cause of death and to find possible preventable measures that can be taken to prevent mortality in these patients.

A retrospective analysis of autopsy cases from 1996 to 2010 was performed at our institution, and all cases of liver transplant recipients were selected. Reason for transplantation, posttransplant survival, autopsy-determined cause of death, and selected pre-mortem clinical data were evaluated.

A total of 32 cases (25 males and 7 females) were reviewed. The most common primary indication for liver transplant was cirrhosis, from hepatotropic viruses ($n = 15$), alcohol-related disease ($n = 5$), autoimmune causes ($n = 2$), hemochromatosis ($n = 2$), cryptogenic ($n = 2$), and NASH ($n = 1$). Other indications included amyloidosis ($n = 2$), TPN-related liver injury ($n = 1$), acetaminophen toxicity ($n = 1$), and unknown ($n = 1$). The mean survival was 27 months post-transplant, ranging from 1 day to 15 years. The major causes of death in short-term survivors ($n = 12$), death at ≤ 1 month posttransplant, were operative complications and transplant failure ($n = 6$), cardiac arrest ($n = 4$), intracranial hemorrhage ($n = 1$), and respiratory failure ($n = 1$). The major causes of death in long-term survivors ($n = 20$), death at >1 month, were sepsis ($n = 9$), intracranial or gastrointestinal hemorrhage ($n = 5$), respiratory failure ($n = 3$), cardiac arrest ($n = 2$), and graft failure ($n = 1$).

Hemorrhage is a considerable and unique cause of death in long-term survivors, which suggests that monitoring of graft synthetic function could help prevent this fatal complication. The majority of long-term survivors died of sepsis, most likely due to chronic immunosuppression. Cardiac arrest is seen in both groups, but mainly in short-term survivors. The majority of short-term survivors died of operative complications and graft failure.

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Papillary Thyroid Carcinoma Arising From a Mature Teratoma in a Cryptorchid Testis: Case Report and Molecular Implications

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Struma testis is a rare entity, and malignant transformation of a testicular teratoma to papillary thyroid carcinoma has not been previously described. Furthermore, solid tumor metastases to the testis are rare: a previous autopsy-based study of 738 patients showed a less-than-1% rate of testicular metastases from solid tumors, and there are no reports of primary thyroid carcinomas metastasizing to the testis.

We report the case of a 56-year-old man who was found to have a cryptorchid testis during an emergency appendectomy for acute appendicitis. The testis contained a mature teratoma with malignant somatic component in the form of a 1.6-cm papillary thyroid carcinoma. Multiple lung nodules were subsequently detected on imaging, and resections of this tissue revealed metastatic papillary thyroid carcinoma. The lung metastases were thought to be from the testicular papillary cancer. However, owing to the possibility of an unknown thyroid primary metastasizing to the lung and in order to facilitate radioactive iodine therapy, a total thyroidectomy was performed. Thyroid examination revealed a 0.5-mm papillary microcarcinoma without extrathyroidal extension. This case raises the questions as to which papillary carcinoma (testicular or thyroidal) led to the lung metastases and whether the testicular tumor could be a metastasis from the thyroid primary. Pending molecular studies for BRAF, RAS, RET/PTC and PAX8/PPAR γ will aid in characterizing the testicular, lung, and thyroid carcinomas.

This case highlights that, although malignant transformation to papillary thyroid carcinoma has been described in struma ovarii, it

may also occur in struma testis. Molecular characterization will help delineate the nature of the testicular papillary thyroid carcinoma as it compares with the thyroid and metastatic tumors in this patient and how it compares with papillary thyroid carcinomas in general.

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Epstein-Barr Virus-Associated Lymphoepithelioma-like Gastric Carcinoma in an HIV Patient

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Lymphoepithelioma-like gastric carcinoma of the stomach is a rare type of gastric carcinoma that was found to be related to EBV infection in more than 80% cases. Concurrent HIV and EBV infection results in increased incidence of lymphomas, undifferentiated nasopharyngeal carcinoma, and leiomyosarcoma. We present a rarely reported case of EBV-associated lymphoepithelioma-like gastric carcinoma in a patient with HIV infection.

A 66-year-old woman with a long-term HIV+ history presented to the clinic with abdominal discomfort. Endoscopically, a large, fungating, noncircumferential, extremely friable mass was found on the greater curvature of the stomach. The gastric mucosa showed diffuse moderate erythema. The histological examination revealed the tumor was composed of abortive branching-anastomosing tubular structures occupying the middle of the mucosa with less-differentiated syncytial nests of undifferentiated cells having vesicular nuclei and prominent nucleoli admixed with lymphoid stroma. In situ hybridization for EBV RNAs revealed strong nuclear staining in the tumor cells, while background lymphocytes and normal gastric mucosa were negative. Tumor cells were also positive for epithelial markers. Background lymphoid cells were proven to be a mixture of T and B lymphocytes and plasma cells by immunohistochemical stains. *Helicobacter pylori* infection was not identified.

HIV-infected patients have abnormal immune functions at multiple levels, affecting the incidence and pathogenesis of many cancers. Concurrent EBV infection is associated with several malignancies, although the association with gastric carcinoma remains unclear. EBV-associated lymphoepithelioma-like carcinoma of the stomach carries a better prognosis, but HIV may accelerate the development of the disease and, therefore, result in an unsure prognosis.

The incidence of EBV-associated lymphoepithelioma-like gastric carcinoma may be increased in HIV patients. Starting routine gastric cancer screening in such patients, especially in EBV-endemic areas, may be warranted.

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Extramedullary Hematopoiesis in Lymph Nodes Following Neoadjuvant Therapy for Breast Carcinoma

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Extramedullary hematopoiesis (EMH) usually occurs as a compensatory mechanism associated with hematologic disturbance and may arise in association with chemotherapy. We report the findings in misleading cases of EMH arising in axillary lymph nodes following neoadjuvant therapy for breast carcinoma.

The clinicopathologic features of cases demonstrating nodal EMH following neoadjuvant therapy for breast carcinoma were evaluated. Factor VIII and myeloperoxidase (MPO) immunohistochemical stains were performed.

Three cases were identified with EMH involving axillary lymph nodes in women ranging in age from 41 to 47 years. They had unilateral breast masses measuring 0.6 to 4.0 cm in greatest dimension. Infiltrating ductal carcinoma, grade III, was diagnosed in all cases by core needle biopsy. All patients subsequently received neoadjuvant therapy (pegfilgrastim, doxorubicin, cyclophosphamide, paclitaxel). No residual carcinoma was identified in postchemotherapy resection specimens. One patient had metastatic carcinoma in her lymph nodes. Foci of EMH consisting of myeloid, erythroid, and megakaryocytic precursors were present within the nodal parenchyma and/or subcapsular sinuses of all 3 cases. Megakaryocytes were immunoreactive with factor VIII and myeloid precursors with MPO.

With increasing use of neoadjuvant therapy for breast carcinoma, EMH within lymph nodes is more likely to be encountered. Hematopoietic precursors present in lymph nodes may potentially be misdiagnosed as metastatic tumor cells. Therefore, caution should be exercised when evaluating axillary lymph nodes in the clinical setting of neoadjuvant therapy for breast carcinoma.

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The Elusive GI Pathology Fellowship: Digging Through the Book and Hard to Find

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Prospective fellows use the Internet as a main source of program information. Competitive fellowships may be filled as early as 2 years before the anticipated start date. These factors leave prospective applicants in desperate need for information in order to decide where, when, and how to apply for a position. There is no organized match for the specialty fellowship application process in GI pathology. Applicants must search extensively to find a complete list of available program information, application, and interviews. This study surveyed the potential adequacy of gastrointestinal fellowship program Web sites in aiding a fellowship applicant.

Current programs and Web sites were obtained from 3 information sources: the FREIDA list of graduate medical education; the ICPI, which listed current gastrointestinal pathology/hepatic pathology fellowships; and the Rodger C. Haggitt GI Society Web site. Fellowships outside of the United States were excluded.

A total number of 31 total fellowship programs were found. Fellowships were excluded if direct Web site information could not be obtained, leaving 28. An average of 10 minutes was spent per Web site in order to assess 8 content features important to fellowship applicants.

In 50% of GI fellowship programs, there was some type of resident profile; however, only 14% had detailed information about the fellow's previous education, 28% had flexible length for the program, 36% had volume of cases, 89% had a description of the program, 86% had the number of fellows taken yearly; and 93% listed a contact person.

There is no reliable database for GI pathology fellowships. Most lists are up-to-date. No defined distinction was noted between ACGME-accredited and nonaccredited programs. Fellowship programs should update and maintain Web sites with current data and include pertinent program and applicant information to help applicants in their search for the fellowship of their choice.

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Frequent FHIT Tumor Suppressor but Not NIT-1 Expression Is Lost in Barrett Esophagus and Esophageal Adenocarcinomas

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Loss of expression of tumor suppressor fragile histidine triad (FHIT) has been shown in a number of human cancer types. Previous studies showed that the nitrilase family branch 1 (Nit1) deficiency in knockout mice confers a cancer-prone phenotype, as does Fhit deficiency, and that the extent of tumor susceptibility due to Nit1 and Fhit deficiency is additive. Utilizing immunohistochemistry, we analyzed protein expression levels for FHIT and NIT1 in a cohort of Barrett esophagus (BE) and esophageal adenocarcinoma (EAC).

Sections from existing tissue microarrays (TMAs) containing 21 EAC and progression TMAs containing 47 normal glandular and squamous mucosa, BE, and EAC were stained for FHIT and NIT1. Expression intensity was scored as 0 (absent), 1+ (weak), or 2+ (moderate to strong). Normal gastric glands and squamous mucosa served as controls.

FHIT (2+ in 37/39) and NIT1 (2+ in 37/39) were ubiquitously expressed in the normal epithelium of gastric mucosa. Weak expression of FHIT and NIT1 was detected in the basal layer of normal esophageal squamous epithelium. FHIT expression was lost in 34 of 61 EACs. In 9 of 12 cases with the complete progression available for evaluation, BE showed weak or absent expression (1+ in 3; 0 in 6). In contrast, NIT1 expression was lost in 4 of 61, and preserved in 57 of 61 EACs and in 12 of 12 BEs.

While frequent loss of FHIT expression was detected in EAC, loss of NIT1 expression was found in a small number of EACs. Furthermore, in most of the cases in which both the BE and EAC were stained, reduced or loss of FHIT expression but not NIT1 was detected in the precursor lesion and carcinoma. These findings suggest that loss of FHIT tumor suppressor function may contribute to and represent an early event in the pathogenesis of BE and EAC.

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Expression of Basal Cytokeratin and Epidermal Growth Factor Receptor in Triple-Negative Breast Carcinomas and Its Correlation With Response to Neoadjuvant Chemotherapy

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A clinically useful target molecule has not been identified for triple-negative (TN) breast cancers. However, neoadjuvant chemotherapy (NACT) can achieve complete pathologic response in certain TN breast carcinomas. The aim of this study was to assess the expression of basal cytokeratin (CK5/6 and CK14) and epidermal growth factor receptor (EGFR) in pre-NACT TN breast carcinomas and correlate the finding with the follow-up pathologic treatment response.

We retrieved 58 cases (2008-2009) from our files at KU Medical Center as treated by NACT after an initial diagnosis of primary invasive breast carcinoma in core needle biopsy. Among them, 24 were the luminal type, 5 were the ERBB2 type, 7 were the luminal-HER2 hybrid, and 22 were TN breast carcinomas. Lumpectomy or mastectomy was performed after NACT. The pathologic response in the surgical excision specimen was divided into complete response (CR, no residual invasive tumor identified), partial response (PR, small foci of residual tumor and treatment effects identified), and no response (NR, no change in tumor size with no treatment effect identified). The expression of basal cytokeratin and EGFR in the pre-treatment biopsy specimens was correlated with pathologic response in the subsequent surgical excision specimens.

All 22 TN breast carcinomas were invasive ductal carcinomas (21 grade III and 1 grade II); 11 had positive axillary lymph nodes.

The NACT treatment duration was 4.5 to 7 months. In 8 cases, there was a CR (36%), 11 had PR (50%), and 3 had NR (14%). All 8 CR cases had expression of CK5/6 and/or CK14, and 5 of them were also positive for EGFR. Of 11 PR cases, 8 had expression of CK5/6 and/or CK14, and 6 of them were also positive for EGFR. Of 3 NR cases, 1 had expression of CK5/6 and/or CK14, but this case was negative for EGFR.

In our study, 36% of TN breast carcinomas achieved complete pathologic response after NACT. Expression of basal cytokeratin with additional positivity for EGFR appears to be necessary for TN tumors to achieve response to NACT. This finding may help further identify the critical predictive factors in predicting TN breast carcinoma's response to NACT.

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TTF-1-Positive Carcinoma in Pleural Fluid of a Female Patient: A Word of Caution

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Thyroid transcription factor-1 (TTF-1) is a cell-specific transcription protein expressed in normal epithelial cells of the lung and the thyroid. Many diagnosticians rely on the finding of TTF-1 immunoreactive glandular cells in malignant effusion to evaluate for metastatic pulmonary carcinoma. We report a case of a 40-year-old woman, a nonsmoker, with a new onset of massive pleural effusion who has an adenocarcinoma of gynecological tract that demonstrated strong uniform expression with TTF-1 stain.

The patient is a 40-year-old woman who was admitted with massive pleural effusion. Pleural cytology revealed a distinct malignant cell population with features of adenocarcinoma. The neoplastic cells were immunoreactive for CK7 and TTF-1 but were negative for CK20. The findings were initially interpreted as metastatic adenocarcinoma of probable pulmonary origin. The imaging studies did not reveal a primary tumor in the lung or pleura but showed a large mass in uterus/cervix. Histological evaluation of the cervical mass revealed a morphologically similar carcinoma. The carcinoma cells were TTF-1+ with variable ER/PR expression. Based on clinical and pathologic findings, the pleural fluid and cervical mass are most consistent with a poorly differentiated carcinoma of gynecologic origin. The patient is currently receiving chemotherapy (cisplatin and Taxol) for stage IV cervical cancer and malignant pleural effusion.

TTF-1 is considered a relatively specific marker for lung and thyroid neoplasms, but the occasional expression of müllerian origin carcinomas should be kept in mind when evaluating malignancies in body cavity fluids in female patients.

We documented misleading TTF-1 immunoreactivity in malignant cells of müllerian origin in pleural effusion cytology. This case emphasizes the significance of pathologist-clinician intercommunication for the proper interpretation of unexpected/unusual histological and immunohistochemical findings for optimal patient management.

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JAK2 Gene Mutation in Patients With Abdominal Venous Thrombosis

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Abdominal venous thrombosis (AVT) from portal, mesenteric, splenic, and hepatic veins is one of the initial presentations of myeloproliferative neoplasms (MPNs). Patients without overt MPNs at the time of abdominal thrombotic events may later develop MPNs. *JAK2*^{V617F} mutation analysis in such patients can identify latent MPNs. Risk factors for AVT include MPNs, inherited thrombophilia, abdominal surgeries, infection, malignancy, cirrhosis, pregnancy, and estrogen therapy. In our study, we focused on idiopathic AVT to evaluate the prevalence of *JAK2* mutation in such patients and its role in the detection of nonovert MPNs.

This retrospective study screened patients referred for thrombophilia testing at our hospital between January 2000 and December 2009, and identified 68 patients with AVT and no clinical MPN, representing the study group; 58 patients with DVT and/or PE, represent the control group. *JAK2* mutation analysis was performed using allele-specific PCR on DNA isolated from peripheral blood. In 14 patients, there was no evidence of inherited or acquired risk factors, consistent with idiopathic VTE; 14 patients had inherited risk factors (factor V Leiden, prothrombin mutation, *MTHFR* mutation); 32 patients had acquired risk factors, and 8 patients had inherited and acquired risk factors.

JAK2 mutation analysis was negative in all control group patients and in all patients with genetic risk factors and documented acquired risk factors for VTE. In 14 patients with idiopathic abdominal thrombosis, we found 1 *JAK2*+ case (7.1%) with portal and splenic vein thrombosis. *BCR/ABL* gene rearrangement was negative.

The idiopathic AVT cases represented 21% of the study group. The observed prevalence of *JAK2* mutation in our cases with idiopathic AVT (7.1%) is similar to several previous studies (7%-12.7%). All control patients were negative for *JAK2* mutation. Screening for this mutation appears indicated in cases of idiopathic abdominal thrombosis to detect nonovert MPNs.

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Mucinous Carcinoma of Breast: Retrospective Review of 100 Cases Over a 9-Year Period With Emphasis on Axillary Staging

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Although most studies claim pure mucinous carcinoma of breast (PMBC) to have a better prognosis than mixed mucinous carcinoma (MMC), reports of lymph node (LN) and distant metastases are known in PMBC. We evaluated patient demographics, pathologic features, and receptor status of mucinous breast carcinomas to address some of these questions.

Two categories, a PMBC (n = 45), with more than 90% mucinous component, and a mixed type (n = 55), with less than 90% mucinous component, were studied. PMBC was further subclassified as hypocellular/type A (n = 37) and hypercellular/type B (n = 8) based on the amount of mucin and cellularity. A retrospective analysis of these cases diagnosed at the 3 hospital centers between 2000 and 2009 was done. No patient follow-up was done.

Mean ages at diagnosis in PMBC and MMC were 60 and 63 years, respectively, while mean tumor sizes were 1.65 and 2.5 cm, respectively. Mean age in type A was higher (75 years). Surprisingly, we had only 1 case of PMBC with a mucocoele-like lesion. Well-, moderately, and poorly differentiated PMBCs were 58%, 37.5%, and 4.4%, respectively. Sentinel LNs (SLNs) were positive in 18.5% of PMBCs and 16% of MMCs. All PMBCs with LN metastases had micrometastases except 1. ER and PR were positive in a similar percentage of PMBCs and MMCs. Surprisingly, HER2 was positive in 11% of PMBCs.

We emphasize a positive role of axillary staging by SLN biopsy in PMBC. Tumor size does not always correlate with nodal status.

Although limited by the number of cases, type B was less favorable than A, comparing many factors.

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FOXP3 Expression in Benign and Malignant Breast Epithelium: Its Role in Breast Cancer

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FOXP3 immunohistochemistry on breast carcinoma is often used to quantitate Treg cell infiltrate in the tumor and predict a higher recurrence risk. Although the role of FOXP3-high Treg cells in predicting tumor recurrence/progression is well debated and investigated, the use of FOXP3 by immunohistochemistry to identify Treg cells in tissue sections is now open to challenge in view of the recent demonstration of FOXP3 expression in tumor cells and non-malignant epithelial cells using immunohistochemistry, flow cytometry, and PCR. Nevertheless, very few studies have shown FOXP3 expression in actual human tissues.

In this study using real-time PCR, we show FOXP3 expression in pure breast tumor cells and adjacent benign breast lobules obtained by laser microdissection from mastectomy specimens. In addition, FOXP3 expression in tumor cells was found to be lower as compared with benign breast lobules in the same breast, which is consistent with the proposal of FOXP3 being a tumor suppressor gene. We show close association between TGF- β 1 and FOXP3 expression in the breast tumor cells, pointing toward the role of FOXP3 in regulating tumor-infiltrating lymphocytes as also its role in regulating angiogenesis. This is further supported by our finding that higher expression of TGF- β 1 and FOXP3 is associated with lymph node involvement and higher stage.

We have shown FOXP3 expression in breast tumor cells and benign breast epithelial cells. FOXP3 expression is associated with poor prognosis in terms of lymph node involvement and lower density of tumor-infiltrating lymphocytes. On the other hand, FOXP3 expression in tumor was lower as compared with breast epithelial cells, which is consistent with the proposed role of FOXP3 as a tumor suppressor gene. This apparent paradox is possibly explained by the fact that FOXP3 expressed in tumor cells is a different transcript as compared with the wild-type protein.

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HPV mRNA Detection in the Follow-up of Patients With HPV Infections

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HPV DNA has been identified in almost all cervical cancers, and women with active HPV infection express E6/E7 oncogenes. As only a small proportion of infections progress toward cancer, it is important to distinguish transient HPV infections from persistent and progressive ones.

We tested 362 samples by conventional Pap smear; HPV-DNA test and typing (Innogenetics NV Belgium); and E6/E7 mRNA expression from the carcinogenic HPV types 16, 18, 31, 33, and 45 (PreTect HPV-Proofer assay, NorChip, Italy). Statistical tests were carried out using STATA 10.1 software. Data obtained were correlated through K-statistic value with the aim of identifying possible significant associations.

The Pap smear was negative in 248 (68.5%); atypical cells of undetermined significance (ASCUS) were found in 50 (12.4%), low-grade squamous intraepithelial lesion (LSIL) in 36 (9.9%), and high-grade squamous intraepithelial lesion (HSIL) in 28 (7.7%). The HPV-DNA test was positive in 192/362 (53.04%) samples; the HPV-mRNA test was positive in 80/362 (22.10%) samples. In addition, the HPV-DNA test was positive in 110/248 (44.4%) of negative, 37/50 (74.0%) of ASCUS, 22/36 (61.1%) of LSIL, and 23/28 (82.1%) of HSIL; the E6/E7 mRNA test was positive in 25/248 (10.1%) of negative, 17/50 (34.0%) of ASCUS, 15/36 (41.7%) of LSIL, and 23/28 (82.1%) of HSIL.

The detection of HPV mRNA shows greater association with the degree of development of atypical or malignant lesions in comparison with the presence of HPV-DNA. Therefore, the mRNA test might be a potential second-level tool for the appropriate follow-up of ASCUS and LSIL patients with persistent or progressive HPV infections.

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Intravenous Iron for Iron-Deficient Pregnant Females Who Fail or Are Intolerant to Oral Iron Therapy

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The incidence of iron deficiency approaches 50% of pregnant females. Iron deficiency is associated with increased risk of pre-eclampsia and neurologic and cognitive deficits in infancy. Pregnant females are recommended to take 30 to 60 mg of elemental iron daily. With oral iron therapy, hemoglobin is reported to rise 2 g/dL in 3 weeks and normalize by 8 weeks. However, about 10% to 20% of patients fail or are intolerant to oral iron; reasons include patient noncompliance owing to gastrointestinal upset or constipation and limited absorption. Poor compliance and bioavailability often mandate parenteral iron therapy. The newer preparation, iron sucrose, has negligible risks of allergic reactions compared with iron dextran and may provide better bioavailability. We aimed to examine the speed and success of parenteral iron in patients who failed treatment or were intolerant to oral iron.

A hospital database identified 43 pregnant females aged 15 to 45 years with iron deficiency who failed or were intolerant to oral iron and received at least 600 mg of iron sucrose from September 2008 to December 2009. Three patients who received RBC transfusions were excluded. Records were reviewed to record preinfusion and postinfusion hemoglobin and hematocrit (Hb&Hct). Excluded were 12 patients whose Hb&Hcts were not checked monthly.

Preinfusion Hb&Hcts averaged 10 g/dL and 30%, respectively. An average of 5 infusions (1,050 mg iron) were given. Average Hb&Hct levels increased 2 g/dL and 6.1% after therapy. In 64% (18/28), normal Hb&Hct values (12 g/dL and $\geq 36\%$) were reached after treatment for 46 days.

Given the potential complications of iron deficiency for newborns, parenteral iron sucrose is a viable option for pregnant patients who fail or are intolerant to oral iron. Hb&Hct levels appear to improve as quickly, if not more rapidly, than with oral iron. In addition, our low rate of RBC transfusions suggests parenteral iron minimizes the need for transfusions during pregnancy and postpartum.

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Mantle Cell Lymphoma With Uncommon Clinical, Morphologic, and Immunophenotypic Features

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Mantle cell lymphoma (MCL) is a small B-cell neoplasm characterized by constant expression of cyclin D1 and aggressive clinical behavior. We describe 3 cases of MCL with unusual clinical, morphologic, and immunophenotypic features.

The first case was a 71-year-old man with a history of "chronic lymphocytic leukemia" for 16 years. After follow-up for the first 8 years without any clinical intervention and for the second 8 years with medical management, he presented with symptomatic splenomegaly and marked lymphocytosis. His spleen measured 4.76 kg and was diffusely infiltrated by cyclin D1+/CD5+/CD23+ κ -restricted small B cells. FISH analysis confirmed t(11;14) present in more than 80% of cells in the spleen and peripheral blood.

The second case was a 75-year-old man with acute intestinal obstruction. Histologic examination showed a cyclin D1+ small B-cell lymphoma involving his terminal ileum and multiple lymph nodes. While the lymphoma at the ileum rendered the typical polypoid appearance grossly, the morphology from the lymph nodes displayed a very unique pattern: the lymphoma cells presented in a prominent nodular pattern with a distinctive monocytoid appearance at the marginal zones.

The third case was an 81-year-old woman presenting with anemia and thrombocytopenia. Bone marrow biopsy demonstrated a small B-cell lymphoma involving 55% of total cellularity with unremarkable cytogenetics. The lymphoma was λ restricted, CD5+, CD23+, and CD10-. This case was initially interpreted as low-grade B-cell lymphoma, favor CLL. Her second bone marrow biopsy, performed several months later, demonstrated the overexpressed cyclin D1 by both immunohistochemistry and FISH analysis.

Indolent MCL has been sparsely reported in the literature and presents with a rather different clinical process compared with the typical MCL. In addition, MCL could present with atypical morphologic or immunophenotypic features. These cases expand our knowledge on the differential diagnosis of small lymphoid infiltrates with atypical features to include MCL.

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Fine-Needle Aspiration of Retroperitoneal Seminoma/Dysgerminoma: A Correlative Hematoxylin and Eosin Perspective

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Features of germ cell tumors on fine-needle aspiration biopsy (FNA) are well known. However, most descriptions are based on air-dried preparations stained by the rapid Diff-Quik method. We describe cytologic features of seminoma/dysgerminoma using a rapid H&E stain and correlate with corresponding histomorphology.

FNA smears from 3 patients with a clinical diagnosis of a "retroperitoneal mass" were obtained via CT-guided fine-needle aspiration. There was no other clinical history. Slides were fixed in 100% ethanol and rapidly stained with H&E. A cell block was prepared for histologic evaluation. All 3 cases were satisfactory for evaluation, well preserved, and of moderate cellularity.

Two distinct cell populations were evident: scattered mature lymphocytes and single large cells with moderate to marked nuclear pleomorphism, prominent nucleoli, and fine to moderately coarse

granular chromatin and devoid of cytoplasm. Sporadic mitoses were seen. The background was finely granular, lightly eosinophilic, and streaky. This was felt to represent proteinaceous debris from disrupted cytoplasm, likely corresponding to the "tigroid" pattern of air-dried smears. Preliminary diagnosis of the first patient was large cell lymphoma. However, cell-block evaluation initiated further study, and an undescended testis was found. A testicular mass was later identified on the second case, and a clinical suspicion of dysgerminoma was disclosed in the third case. Analysis of cell-block material supported the FNA diagnosis, and positive placental alkaline phosphatase stains confirmed this diagnosis. The first case was diagnosed only through cell block. The 2 subsequent cases were diagnosed on FNA after experience gained from the first case.

Seminoma/dysgerminoma has distinct diagnostic cytological features on H&E-stained FNA apart from those described in the literature, herein described, and correlates well with tissue sections. Rapid H&E affords cytohistologic correlation with known histologic features. The presence of cell blocks also affords immunohistochemical confirmation.

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Oligohydramnios: Sequence of Sirenomelia

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Sirenomelia is a sporadic lethal anomaly whose pathophysiology is unknown. It occurs in 1 of 60,000 live births. Theories range from vascular steal syndrome causing aberrant caudal regression to vitamin A teratogenicity. This study aimed to elucidate the clinico-pathologic findings of this syndrome.

Perinatal autopsies performed at St Barnabas Medical Center from January 2000 to January 2010 were electronically reviewed through its pathology database. Two cases, 21 and 23 weeks age of gestation (AOG), were diagnosed antenatally for congenital abnormalities. Maternal histories were unremarkable. Oligohydramnios was detected during routine prenatal checkup in both cases. In addition, the first case had limb defects on prenatal ultrasound, while the second case had cardiac defects and renal agenesis detected on echocardiogram and magnetic resonance imaging, respectively. Elective terminations were performed. Postmortem, both cases presented with urinary tract agenesis, including bilateral renal, ureteral, and bladder agenesis; symmetrical or single fused extremity having a single femur and tibia; ventricular hypertrophy; absent external genitalia; intra-abdominal testes; and imperforate anus. Lungs were hypoplastic, probably secondary to oligohydramnios caused by the urinary tract agenesis. Anomalous abdominal aorta branching and subtotal sacrococcygeal agenesis were seen in the 21-week AOG fetus. The 23-week AOG fetus presented with Potter facies, upper limb deformities, premature closure of fontanelles, tracheoesophageal fistula, ectopic thymus, and pseudoglandular formation in the adrenals. Acute chorioamnionitis and 2-vessel umbilical cords were seen on placental examination. Cytogenetics studies showed normal XY karyotype in both cases.

Since sirenomelia syndrome carries a poor and lethal prognosis, a thorough and aggressive prenatal diagnosis and surveillance of oligohydramnios is of the essence.

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Serodiagnosis of Some Parasitic Infections in HIV-Positive and HIV-Negative Patients in Lagos, Nigeria

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We carried out this cross-sectional study using a serodiagnostic technique to screen for the presence of serum antibodies to 3 parasitic infections, namely, *Entamoeba histolytica*, *Schistosoma haematobium*, and *Toxoplasma gondii*, that could become opportunistic in HIV-infected and AIDS patients in Nigeria. We randomly selected 1,080 patients who attended 3 health care institutions in Lagos for the study. Venous blood samples of the patients were collected and screened for HIV infection and the presence of serum antibodies to the 3 parasitic infections. Positive serum samples for HIV were confirmed.

Results showed that 65 (6%) patients were seropositive for HIV infection among the sampled patients. *E histolytica* serum antibody was found in 5 (7.7%) HIV-seropositive patients and 24 (2.4%) HIV-seronegative patients. Antibody to *S haematobium* was found in 1 (0.02%) HIV-seropositive and 4 (0.39%) HIV-seronegative patients, while *T gondii* serum antibody was 2 (3.1%) and 6 (0.6%) for HIV-seropositive and HIV-seronegative, respectively. A significant difference ($P < .05$) was recorded between HIV-seropositive and HIV-seronegative patients in infections with *E histolytica* and *T gondii*.

Results thus further exposed the opportunistic potential of *E histolytica* and *T gondii* as part of the opportunistic parasitic infectious agents that affect HIV-infected/AIDS patients in the area.

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Development of an Anatomic Pathology Quality Indicator for the Diagnosis of Prostate Cancer

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Through a cooperative agreement with the Centers for Disease Control and Prevention, we identified a lack of evidence regarding the general impressions that there is large variability in preanalytic periods from patient presentation to the time the patient undergoes the diagnostic procedure and that there is large variability in post-analytic periods from the time a patient undergoes the diagnostic procedure to the time the patient receives definitive treatment. In previous work, we identified that this delay in diagnosis results in significant patient harm.

Through retrospective chart review, we measured the time between each major step ($n = 6$) from initial presentation with signs or symptoms suggestive of prostate cancer to the definitive treatment of prostate cancer for 100 consecutive men (mean age, 60.3 years) at our university hospital. These steps included the following: (1) time from symptom presentation to initial diagnostic testing procedure, (2) time from initial testing procedure to case sign out, and (3) time from sign out to patient provided results. We further evaluated the step intervals for men with different Gleason scores.

We found marked variation in many of the steps of the testing process with the mean and standard deviation (SD) as follows: time from symptom presentation to initial diagnostic testing procedure, 185 days (SD 546 days); time from sign out to patient provided results, 7.3 days (SD 7.1 days); time from symptom presentation to time of treatment, 272 days (SD 571 days). The mean and SD of the entire analytic phase were 2.8 days (SD 1.9 days). Men with higher Gleason scores had higher variability in step times.

We conclude that men who have prostate cancer display a large variability in the time between initial presentation and treatment. The

majority of this variation is secondary to the lack of standardization of preanalytic and postanalytic steps.

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Immunohistochemical Analysis of Pure Squamous Cell Carcinoma of the Breast: A Case Series

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Primary squamous cell carcinoma of the breast (PSqCCB) is a type of metaplastic carcinoma in which the malignant squamous elements appear in pure form without adenocarcinomatous features. Metaplastic carcinomas are considered a subtype of basal-like breast cancers that arise from the myoepithelial cells of the breast ducts and lobules. PSqCCBs have not yet been comprehensively characterized to confirm this classification. In this study, we characterized the histopathologic and immunohistochemical findings in 3 cases of PSqCCB.

We selected immunohistochemical stains based on previous studies that defined luminal-type and basal-like breast cancers. Immunostaining for estrogen receptor, progesterone receptor, HER-2/neu, p63, epidermal growth factor receptor (EGFR), cytokeratin 5/6, cytokeratin 14, and cytokeratin 8/18 was performed.

The patients ranged in age from 56 to 64 years at the time of diagnosis. The tumors ranged in size from 2.0 to 5.0 cm. In all 3 cases, the tumors displayed pure squamous morphology without evidence of continuity with the skin.

By immunohistochemistry performed on a selected section from each tumor, all 3 tumors were strongly positive for p63, cytokeratin 5/6, cytokeratin 8/18, and EGFR, while they were negative for progesterone receptor and HER-2/neu. Two cases were diffusely positive for cytokeratin 14; one case showed focal positivity. Two cases were negative for estrogen receptor; one case showed weak nuclear staining in 1% of tumor cells. As defined by previously published reports using gene microarray studies and immunohistochemical analysis, these immunostaining results are compatible with a basal-like immunophenotype.

Our data support the concept that PSqCCBs, like other forms of metaplastic carcinoma, represent a subtype of basal-like breast cancers. Characterization of additional cases is necessary to confirm these findings.

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A Rare Transformation of Chronic Lymphocytic Leukemia (CLL) to Precursor B-Lymphoblastic Leukemia/Lymphoma of the Breast in a Patient With Concomitant Chronic Myeloid Leukemia and CLL

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The concomitant presentation of CLL and chronic myeloid leukemia (CML) is a rare event, with 6 case reports without follow-up. We present a patient with simultaneous CML and CLL in which the latter transformed to precursor B-lymphoblastic leukemia/lymphoma (PB-LBL) of the breast.

A core biopsy of the presenting breast mass and the initial diagnostic and staging bone marrow studies were examined.

Immunophenotyping of the specimens was performed by flow cytometry and immunohistochemistry. Conventional karyotyping and fluorescence in situ hybridization for deletion (13q14 and 13q34) and *c-myc* translocation was performed on bone marrow and breast specimens. Immunoglobulin heavy chain gene rearrangement studies performed on the presenting CLL and the breast mass were compared.

A 62-year-old woman was diagnosed with concomitant BCR-ABL-1+ CML in chronic phase and CLL in 2001. She was treated for CML with imatinib mesylate. Karyotyping of the presenting CLL elements in the bone marrow revealed interstitial deletion of the long arm of chromosome 13 (del13q14). In 2009, she developed a left breast mass (14 × 8 × 5 cm). A core biopsy revealed dense infiltration of intermediate-sized lymphoid elements with blastic features and a prominent nucleolus. Lymphoid CML blast phase was excluded by negative *BCR-ABL* fusion gene. The neoplastic cells were positive for Tdt, PAX-5, and LCA (weak positive) and negative for CD20, CD3, CD5, CD34, CD33, CD68, MPO, and PAN-CK. The diagnosis of PB-LBL was made. Staging bone marrow was positive for persistent CLL. The CLL and breast PB-LBL neoplasms showed del13q14. In addition, the latter had del13q34 and *c-myc*. Further, both neoplasms possessed the same IgH gene rearrangement product.

This case report illustrates the rare transformation of CLL to PB-LBL of the breast. The evolution of additional genetic lesions might have contributed to the transformation.

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Expression of p63 in Mesenchymal Tumors

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We studied expression of the p63 gene in mesenchymal tumors using immunohistochemistry. This gene was expressed in 10 of 10 low-grade fibromyxoid sarcomas, 7 of 7 synovial sarcomas, 2 of 5 myxoid neurothekeomas, 2 of 6 myofibromas, 1 of 2 ossifying fibromyxoid tumors, 1 of 3 atypical fibroxanthomas, 1 of 4 myofibrosarcomas, 1 of 5 perineuriomas, and 1 of 3 neurofibrosarcomas. In contrast, the following tumors were negative: 12 dermatofibromas, 5 dermatofibrosarcoma protuberans, 4 desmoid tumors, 6 schwannomas, 5 palisaded encapsulated neuromas, 5 neurofibromas, 7 leiomyomas, 4 leiomyosarcomas, 7 superficial angiomyxomas, 1 angiomyofibroblastoma, 1 angiomatoid fibrous histiocytoma, 4 nodular fasciitides, and 1 low-grade myofibroblastic sarcoma. Our data show that expression of the p63 gene in tumors is not per se indicative of their epithelial or myoepithelial histogenesis.

Both low-grade fibromyxoid sarcomas arising in children displayed unique, paranuclear, dot-like immunoreactivity for calponin, while the remaining low-grade fibromyxoid sarcomas were negative for this marker.

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Retrospective Analysis of the Causes of Underdiagnosis of Urothelial Neoplasms in Urine Specimens

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Urine cytology is often the first-line screening test for urothelial neoplasms. Therefore, a high diagnostic sensitivity is important.

To our knowledge, underdiagnosing urothelial neoplasms on urine specimens has not been summarily addressed.

We searched our pathology files for a 7-year period and retrieved 82 cases of urine cytology specimens with concurrent and/or subsequent histologic confirmation. Cytospin and Papanicolaou stain were originally performed on each urine specimen. Cytologic and histologic slides were reviewed for this study.

Of 82 cases, 11 urothelial neoplasms were underdiagnosed by a certain degree on urine specimens. The 11 cases included 7 high-grade urothelial carcinomas (UCs), 3 low-grade papillary UCs, and 1 papillary urothelial neoplasm of low malignant potential (PUNLMP). Among 7 high-grade UCs, cytologic diagnoses were 3 "suspicious," 3 atypical, and 1 negative, while the cytologic diagnoses for the 4 lower grade urothelial neoplasms were 3 negative and 1 atypical. Of the 7 high-grade UCs, 3 had limited tumor cellularity with significant degenerative change, 1 had only mildly atypical cells, and 3 high-grade UCs were called suspicious owing to the pathologist's uncertainty. Three low-grade UCs and the PUNLMP had scant cellularity with no atypical cells or with very mild atypia.

This study shows that the underdiagnosis of low-grade UCs and PUNLMP on urine specimens is due to the absence of tumor cells or tumor cells with insufficient atypia, while the most common causes for the underdiagnosis of high-grade UC are scant cellularity accompanied by cellular degeneration and pathologist's uncertainty, although the latter has no effect on management. Therefore, the negative results cannot exclude lower grade urothelial neoplasms. The awareness of degenerative change in high-grade UCs and utilizing intramural consultation may increase the diagnostic accuracy of urothelial neoplasms on urine specimens.

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Lymphoma-Associated Hemophagocytosis: A Rare Occurrence in the Caucasian Population

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A 29-year-old Caucasian man complained of fever associated with weight loss (30 lb in a month) and generalized fatigue. He noticed a left axillary mass for 8 months. The initial blood work revealed pancytopenia. The patient was referred to our facility. He had a WBC count of 1.61 k/ μ L, an RBC count of 3.65 m/ μ L, and a platelet count of 38 k/ μ L. The LDH was 1,142 U/L, and total bilirubin was 5.2 mg/dL. The patient had deranged proteins, elevated alkaline phosphatase, raised ALT and AST, and elevated D-dimer levels of 8.09 μ g/mL. The CT scan showed hepatosplenomegaly. A bone marrow biopsy and aspirate and axillary lymph node excision biopsy were performed. A diagnosis of diffuse large B-cell lymphoma with hemophagocytosis was made. The patient was treated with chemotherapy and bone-marrow transplantation but died after 8 months.

The left axillary lymph node excision biopsy, bone marrow aspirate, clot section, and core biopsy were submitted for evaluation. The lymph node was submitted for lymphoma protocol, H&E stains, immunohistochemical (IHC) staining with CD3 and CD20 and bug stains. Additional samples were sent for *IgH* gene rearrangement and BCL-6 by FISH and PCR for clonality.

The bone marrow was stained with H&E, iron, reticulin, PAS, and bug stains; IHC stains for CD3/CD20/CD30/CD34/CD163; and for karyotyping and flow cytometry.

There was extensive necrosis and sheeting of large atypical lymphocytes with prominent hemophagocytosis. PCR showed a clonal population, and special stains for organisms were all negative. IHC was positive for CD20, and FISH was negative.

We report a case of LAHS in a young Caucasian man with diffuse large B-cell lymphoma, which is extremely uncommon in Western populations. This coexistence is usually seen in Asian populations, and its recognition is important owing to its extremely poor prognosis.

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Angioimmunoblastic T-Cell Lymphoma With Presentation Mimicking Evans Syndrome

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Angioimmunoblastic T-cell lymphoma (AITL) is an aggressive non-Hodgkin lymphoma that may be complicated by autoimmune hemolytic anemia (AHA; 12%) or thrombocytopenia (AT; 7%). AITL with both AHA and AT is extremely rare. In order to clarify the clinical features, pathological characteristics, and response to treatment of this unusual entity, we describe a case of AITL presenting with AHA and AT mimicking Evans syndrome.

A 64-year-old man was referred to us with Coombs-positive hemolytic anemia and thrombocytopenia and a presumed diagnosis of Evans syndrome. Bone marrow examination revealed erythroid and megakaryocytic hyperplasia consistent with Evans syndrome. However, a CT scan revealed generalized lymphadenopathy. A lymph node biopsy showed histological, immunophenotypic, and molecular features of AITL. Specifically, the node was effaced by a paracortical polymorphous infiltrate of small to medium-sized T cells, histiocytes, plasma cells, eosinophils, and B immunoblasts. The stroma included marked proliferations of arborized vasculature and perivascular follicular dendritic cells. Neoplastic cells were CD3+, CD4+, and CD5+, whereas the immunoblasts were CD20+ and PAX5+, with many positive for EBV RNA by in situ hybridization. PCR for T-cell receptor γ gene rearrangement confirmed clonality. He received CHOP-R followed by cladribine, with very limited response. His clinical condition was complicated by GI bleeding and neutropenic fever. He died of cardiac and renal failure 4 months after diagnosis.

AITL with Evans syndrome is rare with no established standard therapy. Literature suggests CHOP-like regimens are usually used as first-line therapy with a reported complete remission (CR) rate of 60%. AITL with CR induced by alemtuzumab, splenectomy, or HDCT followed by ASCT have been reported. Recent literature suggests novel therapeutic strategies, including immunomodulators, angiogenesis inhibitors, or anti-IL-6 antibody possibly targeting autoimmune phenomena, might be helpful in the future.

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Neuroendocrine and Squamous Composite Carcinoma of the Colon: Case Report With Molecular Analyses and Review of the Literature

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Composite colorectal carcinomas are rare. There are a modest number of cases in the medical literature, with even fewer cases describing composite carcinoma with neuroendocrine and squamous components. There are, to our knowledge, no reports of composite

carcinoma molecular alterations. We present a case of composite carcinoma of the splenic flexure in a 33-year-old Caucasian man.

H&E staining showed admixed neuroendocrine and keratinized squamous cells. There was positive nuclear CDX2 expression, confirming intestinal derivation. Cytokeratins 7 and 20 were negative. Neuroendocrine cells were synaptophysin- and AE1/AE3-positive and chromogranin A-negative and negative for mucin production on mucicarmine stain. Hepatic metastases showed a similar immunohistochemical profile. Molecular analysis revealed *KRAS* mutation G13D. *BRAF* mutational testing was negative, and microsatellite instability was not detected. The patient had rapid disease progression with chemotherapy and died 60 days after presentation. A literature review highlights the unusual tumor location and age demographic in this case.

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Diffuse Large B-Cell Lymphoma With Lack of Surface Immunoglobulin Light Chain Expression by Flow Cytometry: Morphology, Flow Cytometry, and Immunohistochemistry

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The demonstration of surface immunoglobulin (SIg) light chain restriction by flow cytometric immunophenotyping (FCI) is one of the critical diagnostic features of B-cell lymphomas. However, SIg- malignant B-cell lymphomas, most commonly diffuse large B-cell lymphoma (DLBCL), are often encountered, which are not fully understood.

We retrospectively selected 22 SIg- DLBCL cases and 26 SIg+ DLBCL cases from the pathology database at the University of California, Irvine, based on the concurrent availability of immunohistochemistry (IHC), including Bcl-2, Bcl-6, CD10, and MUM-1. The demographic information and histology of all cases from these 2 groups were reviewed, and FCI and IHC results were analyzed.

The SIg- group was composed of 11 females and 11 males (F/M, 1.0), with a median age of 61.5 years, while the SIg+ groups had 9 females and 17 males (F/M, 0.53) with a median age of 57 years. The 2 groups did not differ in morphology, with the vast majority of cases in each group being centroblastic. Although no difference in immunohistochemical subgroups was found in the 2 groups (15 germinal center B-cell-like [GCB] DLBCLs and 7 non-germinal center B-cell-like [non-GCB] DLBCLs in the SIg- group and 20 GCBs and 6 non-GCBs in the SIg+ group), CD10 was expressed in 10 of 22 (45%) cases by FCI and 8 of 19 (42%) by IHC in the SIg- group and in 18 of 26 (69%) by FCI and 15 of 24 (71%) by IHC in the SIg+ group. The average percentages of CD3 cells appeared to be higher (44% vs 26%) in the SIg- group. Compared with SIg+ DLBCLs, which appeared to show a male predominance, SIg- DLBCLs demonstrated no sex predilection. In addition, SIg- DLBCLs revealed less CD10 expression immunophenotypically than SIg+ DLBCLs. Immunoglobulin heavy chain gene rearrangement analysis may help to better understand SIg- DLBCLs.