

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 28-November 1, 2009, Chicago, IL.

The authors of the first 8 abstracts have been selected as finalists in the AJCP Resident Research Award competition. The papers of these abstracts will be presented Saturday, October 31, 2009, from 8:30 am to 11:00 am at the ASCP Annual Meeting in Chicago, IL.

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

1

Detection of Metastases in Sentinel Lymph Nodes of Breast Cancer Patients: An Evaluation of Molecular Markers Using Intraoperative Real-Time Reverse Transcriptase–Polymerase Chain Reaction Assay (RT-PCR)

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The GeneSearch Breast Lymph Node (BLN) Assay is a molecular diagnostic test for the intraoperative detection of metastases in sentinel lymph nodes (SLNs) of breast cancer patients. This RT-PCR-based assay uses mammaglobin and cytokeratin 19 markers with cutoff values set to detect metastases larger than 0.2 mm. We conducted a prospective clinical validation study to compare the results of intraoperative BLN assay with intraoperative smears and subsequent permanent sections of SLN.

Between April and July 2008, 36 SLNs from 11 patients (range, 2-5 per patient; average, 3.2; median, 3) were studied. Each SLN was cut along the long axis in 2-mm slices. The cut surface of each slice was smeared, Diff-Quik-stained, and examined by 1 pathologist (M.K.S.). The BLN assay was conducted per the manufacturer's recommendations; 50% of each SLN was submitted fresh for RNA extraction, and alternating sections of each node were submitted for permanent H&E sections. Results of BLN assays were compared with intraoperative smears and permanent sections.

Of 36 SLNs, 2 were positive by BLN assay and smears. Both nodes correlated with the presence of metastases (>2 mm) in permanent sections. In addition, the BLN assay was positive in 1 SLN that was negative by cytologic evaluation. Subsequent H&E sections and cytokeratin immunostain of that SLN revealed isolated tumor cells (<0.2 mm). No further axillary nodal evaluation was performed. The remaining 33 of 36 SLNs were negative. No SLNs were involved by micrometastases (0.2-2 mm) in our study population.

The accuracy of intraoperative cytology and BLN assay are comparable in detecting metastases larger than 2 mm in SLNs.

Although the BLN assay was more sensitive in detecting isolated tumor cells, this finding has no clinical significance in terms of patient management.

2

The Extrahepatic Bile Duct Lesion in End-Stage Primary Sclerosing Cholangitis

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Primary sclerosing cholangitis (PSC) is characterized by nonspecific chronic inflammation and fibrosis of the extrahepatic and intrahepatic bile ducts for unknown etiology. Because of lack of specific histology, diagnostic biopsies of extrahepatic bile ducts (EHBDs) are not performed. Therefore, histologic studies on EHBDs have not been studied well. The objective is to know epithelial and stromal changes in EHBDs in PSC and the incidence of bile duct dysplasia.

We evaluated 21 EHBDs of PSC liver explants. The following histologic features were evaluated: inflammation, periductal fibrosis, ulceration, xanthogranulomatous cholangiopathy, metaplasia (pyloric, intestinal, and squamous), hyperplasia, and biliary intraepithelial neoplasia 1-3 (BilIN 1-3) in surface epithelium and intramural glands; 26 EHBDs from non-bile duct disease patients were used as the control. The Fisher exact test was used.

Xanthogranulomatous cholangiopathy was identified in 7 cases (33%); all were associated with bile duct ulceration and most with inspissated bile. BilIN 1 and 2 were present in 3 cases (14%) each. In the control group, BilIN 1 was present in 4 cases (15%). There was no statistical difference in the prevalence of BilIN in the PSC and control groups. Most cases showed mild to severe periductal inflammation (19 cases; 91%), fibrosis (20 cases; 95%), and ulceration (18 cases; 86%). Hyperplasia of surface epithelium was seen in 11 cases (52%), and hyperplasia and ulceration often coexist. Surface epithelium showed squamous metaplasia in 1 case (5%), intestinal metaplasia in 4 cases (19%), and pyloric metaplasia

in 1 case (5%). Intraluminal hyperplasia was a common finding (16 cases; 76%) and always associated with pyloric metaplasia.

Epithelial and intraluminal hyperplasia are common findings (52% and 76%, respectively), although it has not been emphasized previously. Although cholangiocarcinoma is well-known complication, there was no statistical difference in the prevalence of BillIN in the PSC and control groups.

3

Coagulation Factor Level Most Important Variable in Triaging Hepatitis B–Induced Acute Liver Failure Patients for Liver Transplantation

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In acute liver failure, variables predicting survival of patients are based on age, interval between onset of jaundice and encephalopathy, coagulation factor level, metabolic parameters like arterial blood pH, blood gas level, and lactic acid level, but all these variables were concluded from the studies in which the etiology most commonly is paracetamol poisoning and Wilson disease in which metabolic derangement is much higher, whereas in the Indian subcontinent, the major causative factor is viral hepatitis B. Thus, we exploited the database collected by retrospective cohort study to investigate major predictors of survival in viral hepatitis induced–acute liver failure to know factors that can help in triaging patients for liver transplantation.

Subjects were 40 patients admitted to the gastroenterology ICU following a diagnosis of acute liver failure who were followed up for 1 month. Coagulation factors V and VIII, arterial blood gas, and lactic acid levels were measured on day 1 and day 3 of admission.

The analysis showed that patients who survived had a factor V level on day 3 of diagnosis in the range of 10% to 25% and who died had a factor V level below 10% ($P < .004$), with other predictors of survival being nonsignificant.

Thus, the factor V level on day 3 of diagnosis is the most important variable in predicting survival in patients with viral hepatitis B–induced acute liver failure, thus helping in triage for the need for orthotopic liver transplantation, which is the only definite curative therapy in patients who have poor survival rates.

4

Validation of a Novel Rapid Automation Technology for Immunohistochemical Testing of Estrogen Receptor in Breast Cancer

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Automated immunohistochemistry (IHC) platforms have been used for in vitro diagnostic applications of cancer markers. Each of these platforms exhibits unique technical advantages and disadvantages. The common denominator is a prolonged automated run time of 1.5 to 3.5 hours. A recently developed novel automated platform based on capillary gap technology and vacuum/motion (Celerus Diagnostics, Santa Barbara, CA) claims significantly shorter run

time. We wanted to test this hypothesis and validate our in vitro testing of estrogen receptors (ERs) in breast cancers on this platform.

Three institutions with high-volume breast cancers participated in this study, and 165 previously tested invasive breast cancers were included (whole sections, $n = 34$; tissue microarray, $n = 131$). The previously validated method consists of 2 similar robotic reagent-dispensing systems (Autostainer, DAKO, Carpinteria, CA; and Autostainer-360, Thermo, Fremont, CA). A rabbit monoclonal antibody (SP3, Thermo) was used. Tumor sections were pretreated in pressure cooker (citrate pH 6) before incubating with the primary antibody, followed by a 2-step polymer detection (DAKO), followed by enzyme chromogenic localization. Parallel testing on the new technology was performed using similar polymer detection. Slides were scored using a combined method of signal intensity/fraction of positive cells.

Significant concordance of ER scores between the 2 systems was achieved, with 99% agreement on the positive and negative subgroups. Within the positive cores, few ($n = 6$) showed slight, 1-step differences in the proportion of positive cells, with 2 cores showing difference in status. The average turnaround time for ER testing on the preexisting platforms is 2.5 hours, compared with 20 minutes on the new platform. IHC evaluation for ER performed on breast biopsy specimens using Celerus wave technology showed identical results compared with the more established, automated IHC platform.

Celerus Wave technology is a valid method for evaluating ERs on breast cancer. It offers the advantage of significantly shorter turnaround time.

5

Target Organ Damage in Hypertension: Is Malondialdehyde a Sensitive Marker?

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Alteration of free radical generation is an essential event for the pathogenesis of hypertensive target organ injury. Various clinical studies have demonstrated increased reactive oxygen species production in patients with primary hypertension and with associated target organ damage. In mild to moderate hypertension, the oxidative stress and consecutive lipid peroxidation may not be significant, but an increased oxidative injury is noted in severe hypertension associated with target organ damage. During the chain reaction of lipid peroxidation, many intermediate species and end products are formed, and one of them is malondialdehyde (MDA).

The study was conducted with the aim to evaluate the correlation between the level of MDA and severity of hypertension with the number of target organs involved in primary hypertension.

The present prospective, observational, case-control study included 50 subjects; of these 50, 30 (group A) had primary hypertension with some form of target organ damage, 10 subjects (group B) were patients with primary hypertension without any target organ damage, and the remaining 10 (group C) were healthy normal volunteers from the same socioeconomic strata and they constituted the control group. Serum MDA was estimated as a marker of lipid peroxidation by the method of Nadigahr et al using a spectrophotometer.

The mean serum MDA level of subjects in group A was 8.25 ± 1.75 ng/dL, in group B was 5.5 ± 0.37 ng/dL, and in group C (control) was 3.59 ± 0.53 ng/dL. The mean serum MDA level in group A

was significantly higher than both groups ($P < .001$); furthermore, the mean level in group B was significantly higher than in group C.

Serum MDA was found to be a surrogate marker for target organ damage in people with primary hypertension.

6

Fluorescence In Situ Hybridization (FISH) Testing for $-5/5q$, $-7/7q$, $+8$, and $\text{del}(20q)$ in Primary Myelodysplastic Syndrome (MDS) Is Unnecessary in the Setting of an Adequate Conventional Cytogenetic Study

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Multiple studies have compared the sensitivity of FISH with conventional cytogenetics in detecting clonal genetic abnormalities in MDS with differing results. Based on our experience, we hypothesized that FISH provides no additional information about large, macroscopic genetic abnormalities involving chromosomes 5, 7, 8, and 20 in MDS in the setting of an adequate conventional cytogenetic study.

We identified 101 MDS cases diagnosed by a hematopathologist with normal adequate conventional cytogenetics (≥ 19 metaphases) and performed FISH using Abbott Molecular Probes (Abbott Park, IL) for $-5/5q$, $-7/7q$, $+8$, and $\text{del}(20q)$ in all cases on fixed bone marrow (BM) cell pellets (86) or BM aspirate smears (15). For controls, we performed the same FISH panel on 35 MDS cases with abnormal conventional karyotypes. The analysis was performed blinded by an experienced, qualified cytotechnologist.

Of the 101 MDS cases with normal karyotypes, only 1 had a discrepancy between FISH and cytogenetics ($< 1\%$). In this case, FISH revealed $+8$ in 20% of cells, while rereview of metaphase spreads confirmed a normal karyotype. Interestingly, concurrent flow cytometry showed 19% monoclonal B cells. Given that MDS specimens are not routinely mitogen-stimulated for karyotyping, we think the $+8$ is present in the neoplastic lymphocytes, which would not have routinely divided in culture. Of the 35 control cases, 1 showed a minor discrepancy with a complex karyotype including a monosomy 5, but FISH demonstrated $\text{del}(5q)$. Review of this case with a complex karyotype revealed that 5p may be present as additional chromosomal material on chromosome 12.

FISH for common, recurring MDS abnormalities ($-5/5q$, $-7/7q$, $+8$, and $\text{del}(20q)$) is not indicated in cases with adequate conventional cytogenetics and should be limited to cases with suboptimal conventional cytogenetics. Furthermore, conventional cytogenetics should be considered the "gold standard" for detecting chromosomal abnormalities in MDS.

7

Less Is More: Repeat HER-2/neu Testing and Universal Implementation of Fluorescence In Situ Hybridization

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There is currently no consensus in HER-2/neu testing regarding specimen type, multiple specimen testing, and the utility of universal fluorescence in situ hybridization (FISH). Recent reports have suggested that HER-2/neu testing is "missing" patients that may respond

to trastuzumab, prompting institutions to perform excessive testing in an attempt to identify all candidates. This study evaluates the sensitivity and cost-effectiveness of performing HER-2/neu testing on biopsy and excision specimens and the use of universal FISH to increase identification of trastuzumab-responsive tumors.

We identified 48 cases of infiltrating breast carcinoma in which both biopsy and excision were performed at our institution in 2008. Evaluation included type and grade of carcinoma, immunohistochemistry (IHC) results, FISH results, and treatment received. Correlation between FISH and IHC results on biopsy and excision specimens and costs of testing and reimbursement were analyzed.

Of 48 patients, 45 (94%) had IHC for HER-2/neu performed on both the biopsy and excision specimens. Of the IHC results, 43 (96%) were concordant. Of the 48 cases, 47 had FISH performed; 14 (30%) of 47 had FISH performed twice (biopsy and excision specimens); all had identical results. There was a concordance rate of 87% (41/47) when comparing IHC with FISH with 1 case negative by IHC but positive by FISH; the remaining 5 discrepant cases were negative by FISH.

Performance of IHC or FISH on both the biopsy and excision specimens did not yield any increase in sensitivity and resulted in \$5,226.75 unreimbursed costs to the laboratory. Most discrepancies between FISH and IHC resulted in a negative FISH result, and, therefore, no significant increase in sensitivity was appreciated by universal testing either. In addition, all patients at our institution were treated solely on the FISH result, raising the question of the utility of performing IHC in this setting.

8

Expression of AIF and HtrA2/Omi in CLL/SLL and DLBCL

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The pathogenesis of non-Hodgkin lymphoma may involve apoptosis. In response to apoptotic stimuli, several proapoptotic proteins are released into the cytoplasm from the mitochondria, including second mitochondria-derived activator (Smac/DIABLO), apoptosis-inducing factor (AIF), and high temperature requirement protein A2 (HtrA2/Omi). AIF promotes apoptosis through a caspase-independent pathway, while Smac/DIABLO and HtrA2/Omi do through caspase-dependent and caspase-independent pathways. Smac/DIABLO was reported to be strongly positive in diffuse large-B cell lymphoma (DLBCL) and virtually absent in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Little is known about AIF and HtrA2/Omi expression in lymphomas. Here we assessed their expression in SLL/CLL and DLBCL by immunohistochemistry using formalin-fixed, paraffin-embedded tissue sections.

AIF was strongly and diffusely expressed in 9 (82%) of 11 cases of DLBCL, weakly positive in 10 (83%) of 12 cases of CLL/SLL, with increased intensity in pseudofollicles, and moderately to strongly positive in follicular centers of 10 benign lymph nodes with reactive follicular hyperplasia. In contrast, HtrA2/Omi was weakly expressed in CLL/SLL (12/12 [100%]), DLBCL (18/23 [78%]), and the follicular center and mantle zone of 10 benign lymph nodes.

The different expression levels and patterns of AIF and HtrA2/Omi in CLL/SLL and DLBCL suggest different apoptotic mechanisms involved in the pathogenesis of these diseases. High frequency and level of AIF and Smac/DIABLO (previous studies) in DLBCL indicate that caspase-dependent and caspase-independent apoptotic

pathways are functionally active in this lymphoma. Weak expression of AIF and lack of Smac/DIABLO (previous studies) in CLL/SLL support the notion that this neoplasm is characterized by defective apoptosis. Weak expression of HtrA2/Omi in CLL/SLL, DLBCL, and benign lymph nodes suggests that HtrA2/Omi may not be a major proapoptotic protein in these disease entities. Additional studies are required to further elucidate the role of these proapoptotic proteins in the pathogenesis of these lymphomas.

9

Correlation of T-Cell Immunophenotypes Between Skin Biopsy and Peripheral Blood Findings in Mycosis Fungoides

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Flow cytometry (FC) is useful to evaluate peripheral blood involvement in mycosis fungoides (MF); however, correlation between blood and skin T-cell immunophenotypes has not been studied. Our study aims to compare T-cell immunophenotypes detected by immunohistochemistry (IHC) in skin with FC findings in peripheral blood in MF.

For the study, 37 MF patients had skin biopsy and concomitant peripheral blood analysis. Morphologic, immunophenotypic, laboratory, and molecular findings of 47 skin and 56 blood samples were reviewed. T-cell immunophenotypes detected in skin by IHC were compared with FC findings in peripheral blood.

Of 37 MF patients, abnormal peripheral blood findings were present in 16 (43%), represented by 29 blood samples. Diagnostic findings in 29 blood samples included absolute lymphocytosis in 12, abnormal FC immunophenotype in 29, CD4/CD8 ratio more than 10 in 20, and clonal T-cell gene rearrangement in 19. Of 16 patients, 10 (63%) had discrepant T-cell immunophenotypes between blood and skin, including the following: (1) additional antigen aberrancies detected in blood such as dimCD3 (12%), dimCD2 (18%), and dimCD5 (6%); (2) identification of CD8+ T-cell immunophenotype in 12%, when skin interpreted CD4+; (3) different combination of antigen aberrancies in blood than in skin (6%); and (4) no antigen aberrancies in blood while CD7 loss was reported in skin (12%).

Absolute lymphocytosis is rare in MF, yet 43% of MF patients showed evidence of blood involvement. T-cell immunophenotype commonly differs between skin and blood, mostly due to limitations of IHC in skin to detect partial antigen loss of CD3 (12%), CD2 (18%), and CD5 (6%) and to identify CD8+ immunophenotype (12% of patients). Meanwhile, loss of CD7 is overcalled in skin in 12% of patients. These findings suggest that CD8+ MF might be more common than assumed. Practicing pathologists should be aware of limitations of IHC performed on skin biopsy specimens in the diagnosis of MF.

10

Pitfalls of Human Papillomavirus Diagnostic Tests for Oral and Pharyngeal Epithelial Dysplasia and Squamous Cell Carcinoma

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Human papillomavirus (HPV) has a role in certain oral and pharyngeal epithelial dysplasia and invasive squamous cell carcinoma (SSC). The preferred methods for detecting HPV are in

situ hybridization (ISH) and polymerase chain reaction (PCR). HPV infection correlates with immunohistochemical expression of p16. However, there are conflicting reports regarding the reliability of p16 in oral dysplasia and carcinoma. Immunohistochemical detection of HPV is available, labeling HPV types 6, 11, 16, 18, 31, 33, 42, 51, 52, 56, and 58. This study evaluated combining IHC for HPV and p16 as a less expensive alternative to ISH or PCR for detecting HPV.

Immunohistochemical expression of p16 (MTM Laboratories, Westborough, MA) and HPV (DAKO, Denmark) was analyzed in tissue blocks from 14 patients recently diagnosed with oral and pharyngeal epithelial dysplasia (n = 5) or invasive SCC (n = 9), with confirmed HPV status by ISH or PCR, by outside reference laboratory. Cases were evaluated for distribution, extent, and degree of intensity of p16 and HPV.

Tonsillar (5/5) and oropharyngeal SCC (1/1) were high-risk HPV+ by PCR or ISH and strongly diffusely positive for p16. Cases of squamous papilloma and dysplasia (4/4) were low-risk HPV+ by PCR and showed focal p16 staining. The degree of p16 expression correlated with an increase in the severity of dysplasia. The remaining cases included 3 SCC HPV- by PCR and negative for p16; 1 oral SCC HPV- by PCR and strongly diffusely positive for p16. All cases (14/14) were HPV- by IHC. Positive and negative controls were adequate.

These data indicate that immunohistochemical expression of p16 is a reliable marker for HPV infection in oral and pharyngeal epithelial dysplasia and carcinoma, while HPV IHC is not dependable. High-risk HPV infection shows strong correlation with tonsillar carcinoma.

11

NF-κB Proteins Expression in High-Grade B-Cell Lymphomas With MYC Rearrangement: Analysis of 37 Cases

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B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (IDB/BL) as defined in the World Health Organization (WHO) classification are aggressive lymphomas that often share characteristics, including *MYC* rearrangement and Epstein-Barr virus (EBV) infection, with Burkitt lymphoma (BL). Nuclear factor κB (NF-κB) is a family of transcription factors with a crucial role in cellular development, proliferation, and survival. Current data suggest that the NF-κB pathway is a promising therapeutic target. Little is known about NF-κB in BL and IDB/BL. Our aims were to study and compare the expression of members of the NF-κB transcription factors in IDB/BL with *MYC* rearrangement and BL and search for possible differences caused by EBV coinfection and relationship with mortality.

Paraffin blocks of 37 cases (24 BL and 13 IDB/BL) were obtained. NF-κB transcription factors p65, p50, c-Rel, and p52 were evaluated by immunohistochemistry. The presence of EBV was evaluated by in situ hybridization. *MYC* rearrangement was evaluated by FISH, CISH, and cytogenetics. BL and IDB/BL cases were classified according to WHO criteria. Only cases with *MYC* rearrangement were included.

Increased expression of c-Rel was found in BL (54%) and IDB/BL (76%). Expressions of the rest of NF-κB transcription factors

were negligible. EBV was 37.5% for BL and 30% for IDB/BL. BL has a mortality of 33% and IDB/BL, 50%. A trend for increased c-Rel expression and greater index of mortality was identified in BL (40% vs 60%). EBV coinfection was associated with decreased mortality in BL (60% vs 20%). Males with BL and EBV had a better survival (83%) than males without EBV (20%).

Our results suggest no differences in NF- κ B expression in BL and IDB/BL. Increased c-Rel expression and greater mortality in BL is suggested. Males with BL and EBV showed better survival, suggesting EBV is a good prognostic factor in males.

12

Histologic Autoimmune Gastritis Is Frequently Unrecognized and Twice as Common as the Clinical Prevalence of the Disease

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Autoimmune gastritis (AG) is an immune-mediated, destructive, chronic inflammation of the oxyntic mucosa with a risk of pernicious anemia, carcinoid tumors, and adenocarcinoma. Prevalence estimates (0.1%-2%, United States) are based primarily on clinical features and may not reflect the prevalence of histologic AG. Guidelines for follow up of AG lack specificity. The goals of this study were to determine the histologic prevalence of AG and to examine the clinical response to the biopsy diagnosis.

For the study, 802 randomly selected body biopsies from 2007 were reviewed for the histologic features of AG, defined as follows: (1) deep, chronic lymphoplasmacytic inflammation; (2) atrophy of oxyntic glands, with or without intestinal and/or mucous gland metaplasia; and (3) ECL cell hyperplasia and/or a normal antral biopsy. The clinical records of 43 patients whose biopsies had been reported as AG were reviewed for the clinical response.

In the 802 biopsies, 13 cases of AG were found that had not been recognized by the pathologist diagnosing the case. Based on this sample, and adding those that were appropriately diagnosed, the histologic prevalence of AG was 4%, twice the clinical estimates of prevalence. The median age of AG patients was 62 years (range, 9-84 years). Of the patients, 74% were female, and 85% were Caucasian. Of the AG patients, 14 had a history of B₁₂ deficiency, 5 of whom had no previous diagnosis of AG. Twenty-one patients had no history relevant to AG. Pain was the most common presentation (29%). Endoscopically, 43% had erythema, 38% polyps/nodules, 31% atrophy, 12% erosions, and 10% a mass. Proton pump inhibitors were being taken by 44%. The clinical responses to a histologic diagnosis of AG were inconsistent and varied. Thirty-seven percent had at least 1 repeated EGD. Among the 26 patients followed up by gastroenterologists, serum B₁₂ was ordered for 11, parietal cell antibody for 7, intrinsic factor antibody for 4, and serum gastrin for 6.

The prevalence of AG as determined by histologic features is at least twice that based on clinical features, suggesting that there is significant subclinical AG. Patients with AG present with symptoms such as abdominal pain and endoscopic findings such as erythema, polyps, atrophy, and erosions, common to many patients having EGD. In addition, histologic AG is underrecognized by pathologists. Gastroenterologists' responses to the histologic diagnosis are variable, possibly owing to the subjectivity of the published guidelines for AG. However, follow-up studies are initiated in many patients, suggesting the diagnosis is considered significant.

13

Investigation of a Possible Association Between Refractory Iron Deficiency Anemia With an Underlying Remote *Helicobacter pylori* Infection

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Helicobacter pylori is a bacterial infection that accounts as the prevalent gastric pathogen. *Helicobacter* has been associated with many extradigestive disorders, such as refractory iron deficiency anemia (IDA; sideropenic). The aim of this case-control study was to investigate the role of remote *H pylori* infection in refractory IDA by comparing 2 different methods for diagnosis of *H pylori* infection.

The study was conducted on 30 patients proved to have refractory IDA by therapeutic trial. Thirty normal nonanemic subjects were included as controls. *H pylori* testing included stool antigen and *H pylori* PCR.

The *H pylori* stool antigen test revealed 12 positive cases out of 30 IDA cases. Of the cases, 5 were stool PCR cagA-positive, 4 were stool PCR ureC-positive. There was 100% agreement between PCR cagA and the stool antigen test in the detection of *H pylori* infection ($P = .003$). Stool PCR cagA had a diagnostic accuracy of 76.67 and a likelihood ratio of 3.57. There was 100% agreement between PCR ureC and the stool antigen test in the detection of *H pylori* infection ($P = .009$). Stool PCR ureC had a diagnostic accuracy of 73.33 and a likelihood ratio of 3.25. There was a very highly significant difference between the means of serum ferritin, serum iron, TIBC, and transferrin saturation of *H pylori* stool antigen positive and negative subjects ($P < .001$).

There was a very highly significant association between *H pylori* infection and refractory IDA ($P < .0001$). Serum ferritin levels were significantly lower in *H pylori* stool antigen-positive cases than in negative cases ($P < .001$). *H pylori*-positive cases are 2.7 times more likely to develop anemia than negative cases (confidence interval, 1.9-3.8). *H pylori* stool antigen testing by ELISA proved to be easier compared with the stool PCR, which is tedious and time-consuming. *H pylori* infection raised the risk of IDA by 2.6-fold.

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A Survey of the Ordering Rate and Distribution of "Solo" Hemoglobin A_{1c} (HbA_{1c}) Measurements During a Three-Year Interval at a University Hospital

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Monitoring of HbA_{1c} in diabetes is recommended to occur at least twice per year. Although no formal recommendations as yet exist for use of HbA_{1c} in diabetes screening, there is literature advocating such use, with a suggested cutoff for a diabetes diagnosis of an HbA_{1c} of 7% or more (reference range, 4%-6%). The rate at which HbA_{1c} is used for diabetes screening is unknown, however, and the overall fraction of HbA_{1c} measurements that are not part of serial monitoring is unknown.

In relation to these questions, we examined the rate and results distribution of "solo" HbA_{1c} measurements during a 3-year interval in which a solo measurement was that from any patient represented only once in the 3-year interval database. Primary data were 48,681 HbA_{1c} measurements made at the University of Nebraska Medical

Center during the 3-year interval of 2006-2008. These measurements were from a total of 18,448 patients, of whom 9,422 (51.1%) were from solo patients; 9,026 patients (48.9%) had 2 or more measurements during this interval (average, 4.4 measurements per patient). The accumulation of solo patients was essentially linear with the accumulation of HbA_{1c} orders during the interval: No. of Solo Patients = 18.7% × No. of Orders ($r^2 = 0.998$). Among solo orders, the average HbA_{1c} was 6.5% ± 1.7% (median, 6.0%); this differed ($P < .001$) from the average among all measurements from multiple-order patients of 7.3% ± 1.7% (median, 6.9%). Of the solo orders, 45.2% had elevated HbA_{1c} (≥6.1%). By this analysis, the rate of solo orders may reflect a combination of factors such as patient population turnover, patient noncompliance, and screening with loss to follow-up.

By whatever means solo orders are derived, however, we conclude that currently approximately 19% of HbA_{1c} measurements at our institution represent solo orders and that approximately 45% of such orders have elevated HbA_{1c} for which there is no evidence of follow-up within our hospital system.

15

Applying the Bethesda Terminology for Thyroid Cytology: A Comparative Study With a Preexisting Three-Tiered Classification

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The terminology for reporting fine-needle aspiration biopsy (FNA) of the thyroid, a critical component in management, has not been fully standardized, but the recent 7-tiered Bethesda classification for thyroid FNA cytology proposes to resolve this. We compared classifying thyroid FNA specimens by the new Bethesda criteria with our in-house 3-tiered system in all cases with histologic follow-up.

We retrospectively retrieved 55 consecutive thyroid FNAs with surgical resections from 2006-2008. Four insufficient cases were excluded. The remaining 51 cases were blindly reclassified using the new Bethesda criteria and by our preexisting system (benign, indeterminate, neoplastic).

By using the prior system, 28 nonneoplastic cases were diagnosed as benign (54%), indeterminate (43%), and neoplastic (4%); 15 adenomas were diagnosed as indeterminate (60%) and neoplastic (40%); 8 carcinomas were diagnosed as indeterminate (36%) and neoplastic (64%). By using the Bethesda criteria, 28 nonneoplastic cases were diagnosed as benign (86%) and atypical cells of undetermined significance (ACUS; 14%); 15 adenomas were diagnosed as ACUS (33%) and suspicious (67%); 8 carcinomas were diagnosed as ACUS (12.5%), suspicious (50%), and malignant (37.5%). Both systems were 100% sensitive in diagnosing the neoplasms. However, the indeterminate and neoplastic categories of the prior system included a significant proportion of nonneoplastic lesions (13 cases [36%]) that, using the Bethesda system, were reassigned to the benign (9 cases) or ACUS (4 cases) categories. By using the Bethesda criteria in these cases, we would have prevented 9 unnecessary repeated FNAs and/or surgical resections.

Our data confirm the clinical utility of the Bethesda classification system and show that it provides better guidance for clinical workup and treatment of thyroid nodules than the prior system.

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α-Methylacyl-Coenzyme A Racemase (AMACR) and ProEx C Expression in Serrated Colonic Adenomas

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AMACR is a cytoplasmic enzyme involved in bile acid biosynthesis and fatty acid oxidation. AMACR is expressed in colon adenomas and adenocarcinomas, but not in normal colonic epithelium. AMACR expression has been investigated in inflammatory bowel disease, Barrett esophagus, and gastric dysplasia. ProEx C targets the expression of topoisomerase II-α (TOP2A) and minichromosome maintenance protein-2 (MCM2) and is overexpressed in cervical dysplasia. To date, AMACR expression has never been investigated in sessile serrated (SSA) and traditional serrated adenomas (TSA). ProEx C expression has never been investigated in colon polyps. We evaluated the expression of AMACR and ProEx C in colon polyps, with emphasis on serrated adenomas.

We studied AMACR and ProEx C immunohistochemical expression in 10 SSAs, 10 TSAs, 10 tubular adenomas (TAs), 6 TAs with high-grade dysplasia (HGD), 5 intramucosal carcinomas (IMCs) arising in TA, and 5 cases of normal colonic mucosa.

AMACR immunoreactivity was scored as negative, weak (faint cytoplasmic stain or granular apical staining), moderate (diffuse granular cytoplasmic stain), and strong (diffuse intense cytoplasmic stain). Only moderate and strong staining were considered positive. ProEx C expression was scored as negative if no or less than 5% nuclear stain was present and positive if more than 5% nuclear staining was present. The surface epithelium and the crypts were scored separately with both antibodies.

AMACR and ProEx C are consistently expressed in SSA, TSA, HP, TA, TA with HGD, and TA with IMC. ProEx C is expressed in the crypts of normal colon. The surface-positive/crypt-negative AMACR and surface-negative/crypt-positive ProEx C staining patterns can help discriminate between SSA and TSA. HPs show similar expression to SSA for AMACR and ProEx C. AMACR and ProEx C are overexpressed in the different compartments of all colon adenomas and IMC.

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Are Mixing Studies Useful in Pediatric Patients?

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A mixing study is a screening test used to investigate the prolongation of prothrombin time (PT) and/or activated partial thromboplastin time (PTT) and to differentiate between factor deficiency and circulating inhibitor. Few studies have shown the utility of mixing studies in pediatric patients.

Data for 300 patients, 5 days to 24 years old (mean, 7 years), M/F ratio 1:1, with prolonged PT, PTT, or both were retrospectively reviewed. After incubation at 37°C, patient plasma mixed (1:1) with normal plasma was measured for PT at 0 minutes and PTT at 0 and 90 minutes. Correction was defined within 2.0 seconds above the upper limit of normal for PT and within normal range for PTT, which suggested factor deficiency. No correction at 0 minutes and/or 90 minutes suggested presence of a circulating inhibitor. After

evaluation of mixing studies, individual factor assays and lupus anticoagulant (LAC) assays were performed. A total of 121 with corrected PT and 104 with corrected PTT were evaluated. Of these with PT and PTT correction, factor deficiencies were found in 82 (68%) and 55 (52%), respectively, and LAC in 29 (24%) and 15 (14%), respectively. In 182 cases with no PTT correction, 50% were positive for LAC, but also revealed 20% with factor deficiency alone. Forty-four with corrected PT and PTT revealed neither factor deficiency nor LAC and 48 had factor deficiency and LAC.

A mixing study may not be used as a screening test for factor deficiency or circulating inhibitor among pediatric patients because the analysis shows the test by itself is not very sensitive or specific.

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B-Cell Lymphoma Involving Bone Marrow Without Clinical Evidence of Concurrent Extramarrow Lymphoma

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Bone marrow biopsy is needed for lymphoma staging evaluation before therapy. However, bone marrow biopsy performed for other medical reasons can occasionally unveil lymphoma involvement without concurrent extramarrow lymphoma. This study was done to evaluate the clinical and pathologic features of these lymphomas.

By reviewing bone marrow biopsy pathology reports, 28 patients positive for B-cell lymphoma, without clinical evidence of concurrent extramarrow involvement, were identified. Bone marrow biopsy is performed for evaluation of abnormal CBC. Diagnosis of B-cell lymphoma is based on combined histologic, immunophenotypic, and gene rearrangement studies. Hospital charts were retrospectively reviewed, and the follow-up period was more than 6 months.

Patients included 19 males and 9 females with a mean age of 72.5 years. On follow-up evaluation, 3 patients showed lymphadenopathy, and subsequent lymph node biopsy confirmed B-cell lymphoma. In another 3 patients, imaging studies revealed suspicious lymphadenopathy, without further tissue examination. Serum protein electrophoresis identified monoclonal proteins in 14 patients, and 8 of them have lymphoplasmacytic lymphoma (LPL). Six patients have CLL, and 1 has mantle cell lymphoma. One CLL and one LPL show minimal morphologic evidence of lymphoma, and the diagnosis is based primarily on flow cytometry findings. In 10 patients, however, the lymphomas (including low grade B-cell lymphoma and large cell lymphoma by morphologic criteria) could not be further classified.

Patients with abnormal CBC undergoing bone marrow biopsy may occasionally show B-cell lymphoma without concurrent extramarrow involvement. LPL is a common disease in these patients. In some patients, however, the B-cell lymphomas could not be further classified. With minimal histologic involvement, flow cytometry may be the only method to detect lymphoma in rare cases. Since these patients present with an abnormal CBC, clinical treatment is needed and may be challenging.

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High- and Intermediate-Grade Ductal Carcinoma In Situ of the Breast: A Comparison of Pathologic Features in Core Biopsies and Excisions and an Evaluation of Core Biopsy Features That May Predict a Close or Positive Margin in the Excision

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Low- and high-grade ductal carcinoma in situ (DCIS) are known to be highly disparate by a multitude of parameters, including progression potential, immunophenotype, gene expression profile, and DNA ploidy. In this study, we analyzed a group of intermediate- and high-grade DCIS cases to determine how well the core biopsy predicts the maximal pathology in the associated excisions and to determine if there are any core biopsy morphologic features that may predict a close (≤ 0.2 cm) or positive margin in the subsequent excision. We evaluated in detail 49 consecutive paired specimens (core biopsies with a maximal diagnosis of DCIS and their corresponding excisions, which included 20 and 29 specimens from mastectomies and breast-conserving surgeries, respectively).

In 5 (10%) of 49 cases, no residual carcinoma was found in the excision. In another 4 cases, the changes were diagnostic only of atypical ductal hyperplasia. There were 4 and 3 respective cases of invasive and microinvasive carcinoma of the 49 excision specimens, for an overall invasion frequency of 14%. In 28 cases in which a sentinel lymph node evaluation was performed, only 1 was found to be positive. Among the 40 cases with at least residual DCIS in the excision, there were 5 cases in which comedo-pattern DCIS was present in the excision but not in the core biopsy, attributed to the lower maximal nuclear grade in the biopsy proliferation in 4 cases and the absence of central necrosis in the fifth case. For the other main histologic patterns, in 8 (20%) of 40 cases, there were more patterns identified in the core biopsy than in the corresponding excision. For the other 32 cases, 100%, 66%, 50%, 33%, and 25% of the number of histologic patterns in the excisions were captured in 35%, 5%, 17.5%, 15%, and 7.5% of the preceding core biopsies, respectively. Therefore, the core biopsy reflected at least half of the noncomedo histologic patterns in 77.5% of cases. In 6 (15%) of the 40 cases, the maximum nuclear grade of the excision (grade 3) was higher than that seen in the core biopsy (grade 2). Overall, however, the maximum nuclear grade in the excision was significantly predicted by maximum nuclear grade in the core biopsy ($P = .028$), with a ϕ of 0.347, indicating a moderately strong association. At a size threshold of 2.7 cm, there was no significant association between lesional size and core biopsy features. Furthermore, the clear margin width of the cases with lesional size 2.7 cm or less (mean, 0.69 cm) was not significantly different ($P = .4$) from the cases with lesional size more than 2.7 cm (mean, 0.56 cm). Finally, among a variety of core biopsy features that were evaluated, including maximum nuclear grade, necrosis, cancerization of lobules, number of tissue cores with DCIS, number of DCIS ducts per tissue core, total DCIS ducts, and comedo pattern, only necrosis was significantly associated with a positive or close (≤ 0.2 cm) margin on multivariate analysis (ϕ of 0.350).

It is concluded that a significant change (to invasive disease [14%] or to no residual disease [10%]) is seen in approximately 24% of excisions that follow a core biopsy diagnosis of intermediate- or high-grade DCIS. Core biopsy features are of limited value in predicting a close or positive margin in this group.

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A Biochemical Index for Prediction of Cirrhosis and Bridging Fibrosis in Egyptian Chronic Hepatitis C Virus-Infected Patients

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Hepatitis C virus (HCV) is a major health problem globally and is associated with chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Egypt has the highest HCV prevalence in the world, with an average of approximately 13.8% in the general population. Assessment of liver histology is pivotal in prognostication and decision making regarding therapeutic intervention in patients with HCV. The liver biopsy is an invasive procedure with complications, and a noninvasive alternative would be preferable. The beginning of this century has witnessed a new era in noninvasive tools in the diagnosis of stage of liver fibrosis. In this study, an index depending on standard biochemical serum markers (the Goteborg University Cirrhosis Index [GUCI]) was calculated.

Serum samples from Egyptian chronic HCV patients and living liver donors serving as controls collected at the time of liver biopsy were analyzed using routinely available biochemical markers of liver disease. Liver histology was evaluated using the Ishak protocol, and the relationship between the serum biochemical markers and cirrhosis (Ishak stage ≥ 5) and bridging fibrosis (Ishak ≥ 3) was examined.

The GUCI index was calculated for each chronic HCV patient and compared with the histopathologic stage of fibrosis. Three cutoff points of the GUCI score were chosen. With a GUCI cutoff value of 0.5, the sensitivity was 100% and specificity 0% for excluding bridging fibrosis. The negative predictive value (NPV) and positive predictive value (PPV) were 100% and 53%, respectively. Using a cutoff value of 2.1, the sensitivity was 96% and specificity 100% for diagnosis of bridging fibrosis, and the NPV and PPV were 88% and 100%, respectively. Using a cutoff point of 4.1, the sensitivity was 100% and specificity 100% for diagnosing liver cirrhosis, and the NPV and PPV were both 100%.

A GUCI score less than 0.5 or more than 4.1 in HCV patients can reduce the need for liver biopsy and has been suggested as a potential alternative new-era tool with a high degree of accuracy. Ultimately, liver biopsy still has a "gold" role in accurate staging of liver fibrosis in HCV patients with a GUCI score between 0.5 and 2.1.

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Discriminating Hepatocellular Carcinoma From Metastatic Adenocarcinoma on Fine-Needle Aspiration Biopsy of the Liver: The Utility of Immunocytochemical Panels

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The cytomorphologic features of hepatocellular carcinoma (HCC) in fine-needle aspiration (FNA) biopsy are well described. However, correctly diagnosing HCC on cytologic features alone and differentiating it from metastatic adenocarcinoma (MAC) remains a challenge. Studies have recommended the use of various immunocytochemical (ICC) stains to aid in the diagnosis and distinction of these tumors, with variable success rates. The aim was to identify the most sensitive and specific markers and the best panel for accurate diagnosis.

In this study, we performed a panel of 7 ICC stains, HepPar1, glypican-3, polyclonal and monoclonal carcinoembryonic antigen (pCEA, mCEA), MOC-31, cytokeratin (CK)7, and CK20, on cell block sections of 42 FNA cases of HCC and 48 FNA cases of MAC. The staining was performed according to standard avidin-biotin-complex method using the Ventana System.

Overall, 38 of 42 HCC and 44 of 48 MAC tumors were correctly identified by a panel of 4 markers, CK7, MOC-31, HepPar1, and glypican-3 with accuracy rates of 90.5% and 91.7%, respectively. In the HCC group, glypican-3 was most sensitive and detected in 34 (81%) of 42, while HepPar1 and pCEA were less sensitive and detected in 30 (71%) and 21 (50%) of 42, respectively. In the MAC group, MOC-31 was most sensitive and detected in 38 (79%) of 48, followed by CK7 in 20 (42%) of 48. Stepwise logistic regression analysis showed that a panel of glypican-3, HepPar1, MOC-31, and CK7 is most helpful in diagnosing and accurately differentiating HCC from MAC on FNA biopsies of the liver.

We conclude that a panel of HepPar1, glypican-3, MOC-31, and CK7 can be used in a practical and cost-effective approach to maximize the accuracy of malignant diagnosis on liver FNA biopsies. Judiciously used in combination with cytomorphology, this panel can accurately distinguish HCC from MAC on liver FNA samples ($P < .05$).

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Differential Expression of Galectin-3, CK19, HBME1, and Ret Oncoprotein in the Diagnosis of Follicular-Patterned Thyroid Lesions by Fine-Needle Aspiration Biopsy

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Fine-needle aspiration (FNA) is a commonly used procedure for the evaluation of thyroid nodules and is recognized as an excellent method for identifying lesions that require surgical resection. However, cytodiagnosis can be difficult, particularly for follicular-patterned lesions, based solely on cytologic features. Previous studies on surgical or FNA specimens have shown that some immunohistochemical (IHC) markers may enhance the diagnostic accuracy. Our goal was to evaluate 4 recently investigated markers for their utility in differentiating benign from malignant thyroid nodules on FNA biopsies.

We performed IHC staining of galectin-3, Ret oncoprotein (Ret), HBME-1, and cytokeratin (CK)19, on cell-block sections of surgically confirmed thyroid FNA cases, including 44 benign lesions (37 hyperplastic/cellular nodules and 7 follicular adenomas) and 27 malignant tumors (6 follicular carcinomas, 19 classic papillary carcinomas, and 2 follicular variants of papillary carcinoma).

Statistical analysis showed significantly different immunoreexpression between the 2 groups for all markers. Sensitivity of expression for all benign lesions vs malignant tumors was as follows: 10/44 (23%) vs 25/27 (93%) for galectin-3; 14/44 (32%) vs 23/27 (85%) for Ret; 12/44 (27%) vs 24/27 (89%) for HBME-1; and 13/44 (30%) vs 23/27 (85%) for CK19. The sensitivity and specificity were highest for galectin-3 (92.6% and 77.3%, respectively). HBME-1 had the second best sensitivity (88.9%). The panel of galectin-3 + HBME-1 showed the highest sensitivity (90.7%) and specificity (75%), but this was, surprisingly, lower than galectin-3 alone (92.3% and 77.3%, respectively).

Galectin-3 is the best single marker for differentiating benign from malignant thyroid lesions. Different combinations of these markers had lower sensitivity and specificity than did galectin-3 alone, although galectin-3 + HBME-1 demonstrated the best combination. Because galectin-3 and HBME-1 were the best 2 independent markers, together they may be the best panel when one desires to use more than one marker.

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BRAF Mutational Analysis: Limited Utility in the Preoperative Evaluation of Thyroid Nodules

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Fine-needle aspiration (FNA) is a valuable method for preoperative diagnosis of thyroid cancer. It can be challenging, however, to definitively recognize malignancy on cytology alone, specifically the follicular variant of papillary carcinoma (FVPTC). Nuclear changes readily identified in conventional papillary carcinoma (PTC) are not reliably detected in the follicular variant. The *BRAF* V600E mutation is specific for PTC and is an accepted marker in the assessment of thyroid nodules. We studied its potential role in the preoperative evaluation of thyroid nodules. It is at this stage that knowledge of *BRAF* V600E mutational status would be most useful in routine practice, specifically cases cytologically suspicious for FVPTC.

All thyroid FNA designated indeterminate or malignant with cell block preparations (CBPs) and corresponding postoperative malignant thyroidectomy specimens during a 4-year period at our institution were obtained. DNA was purified from the specimens and analyzed for the *BRAF* V600E mutation using a homogeneous polymerase chain reaction melting curve assay. Of 16 CBPs examined, none were positive for the *BRAF* V600E mutation. This included 1 case each of FVPTC and conventional PTC. Both corresponding excisions showed FVPTC and were *BRAF*⁻. Four other cases designated indeterminate on aspiration had malignant final diagnoses. One showed FVPTC on excision and was *BRAF*⁻. Three showed conventional PTC on excision, with 1 *BRAF*⁺ and 2 *BRAF*⁻. Our study suggests that the *BRAF* V600E mutation cannot be reliably demonstrated in FNA CBPs of PTC and, more important, FVPTC.

These findings reflect the low frequency of the mutation in FVPTC, a low fraction of malignant cells in some FNA CBPs, and heterogeneity in mutational status of malignant cells even for malignancies that harbor the mutation. Thus, we propose that *BRAF* V600E mutational analysis has limited utility in the preoperative evaluation of thyroid nodules.

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Use of Free-Thyroxine (FT4)-Dependent Thyrotropin (TSH) Reference Ranges in Diagnosis of Subclinical Hypothyroidism: Argument in Favor Based on a Simulation Study of How a TSH Reference Range Is Obtained

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Subclinical hypothyroidism is diagnosed solely on laboratory measurements of TSH and FT4. Whereas median values for log TSH vary linearly with FT4 ($\log \text{TSH [mIU/L]} = -0.478 \text{ FT4 [ng/mL]} + 0.846$), an FT4-independent TSH reference range (typically 0.4-5.0 mIU/L) is used, even when associated with FT4 measurement. We examined the fraction of TSH measurements at low-normal FT4 likely to be misclassified by use of a non-FT4-dependent TSH reference range.

Given that the distribution of TSH values at any given FT4 is approximately log-normal, we posited a constant log-width distribution of TSH for all FT4, for which distribution was then centered on the median log TSH value as a function of FT4. We then determined the log-width that would result in the conventional log-normal TSH reference range (log-width = 1.1) when the TSH reference range was defined as the central 95% of results obtained from sampling from a

population with a normal FT4 distribution (reference range, 0.8-2.0 ng/mL). Computer simulation of random sampling of this population ($n = 10,000$) reproduced the TSH reference range when the log-width of the FT4-dependent TSH reference range was 1.0. Thus, conventional sampling for determination of the FT4-independent TSH reference range overestimated the specified TSH log-width by +10%. More important, this FT4-independent TSH reference range significantly misclassified statistically normal (central 95%) TSH values at low-normal FT4. At FT4 = 0.8 ng/mL (posited TSH reference range, TSH = 0.94-9.0 mIU/L), high TSH designations (>5 mIU/L) occur for results above just 1 SD from the mean of the FT4-dependent TSH reference range, whereas low TSH designations (<0.4 mIU/L) occur only for results more than 3.5 SD below the mean.

Laboratory practitioners should be aware that non-FT4-dependent TSH reference ranges likely significantly misclassify statistically normal TSH values at low-normal FT4, at which subclinical hypothyroidism is diagnosed.

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Mycophenolate-Induced Chronic Colitis Resembling Ulcerative Colitis

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Mycophenolate mofetil (MMF) is an immunosuppressive agent used in solid organ transplant patients that is known to cause diarrhea, sometimes with endoscopic and histologic features of an erosive enterocolitis, and an acute graft-vs-host disease (GVHD)-like histologic pattern. MMF colitis has not been previously described as having significant lamina propria plasmacytosis or other features of a chronic colitis. We have observed patients taking MMF who present with diarrhea and a chronic colitis histologically resembling inflammatory bowel disease. The purpose of this study was to characterize the morphologic findings in patients taking mycophenolate who develop a chronic colitis that resembles ulcerative colitis or Crohn colitis.

The study consisted of 13 patients (M/F, 8/5; mean age, 50 years; range, 13-70 years) who were solid organ transplant recipients (7 heart, 5 kidney, and 1 liver), were taking MMF, developed diarrhea, and had histologic findings of chronic colitis, including lamina propria plasmacytosis and crypt injury, along with apoptoses of crypt epithelium.

Colonic biopsies from all 13 of these patients have significant lamina propria plasmacytosis and eosinophilia that varies from patchy to diffuse and is, in some cases, basally predominant. In addition, crypt architectural distortion is present in all biopsies and varies from slight to marked. Of 13 patients, 9 had dilated crypts lined by flattened regenerative epithelium, containing neutrophils and/or eosinophils. All had crypt apoptoses, similar to acute MMF colitis; 5 biopsies from 3 patients had been diagnosed as ulcerative colitis on initial evaluation.

In addition to an apoptotic dominant colitis (resembling GVHD), MMF is capable of causing a colitis, resembling ulcerative colitis, but sharing the dilated damaged crypts and apoptoses of acute MMF colitis.

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The Significance of Marked Nuclear Atypia in Grade 1 Cervical Intraepithelial Neoplasia

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Approximately 10% to 15% of cases of grade 1 cervical intraepithelial neoplasia (CIN 1) are found to have progressed to a high-grade squamous intraepithelial lesion (HSIL) or higher at follow-up, and there are presently no reliable morphologic predictors of this subset. It has recently been reported that cases of CIN 1 that display marked nuclear atypia (defined as cases with at least 5 epithelial cells with nuclear enlargement of at least 5 times the size of an intermediate cell and/or multinucleation of at least 5 nuclei) have a substantially higher rate of HSIL on short-term follow-up and may, therefore, require more aggressive initial management. We report herein our experience with a cohort of such cases.

Following a review of consecutive cervical biopsies, 352 cases with CIN 1 were classified into group 1 (CIN 1 with marked atypia, n = 31) and group 2 (CIN 1 without marked atypia, n = 321). The average follow-up rates for groups 1 and 2 were 94% (29/31) and 90.7% (291/321), respectively. Average follow-up durations were 14.3 and 17.9 months, respectively. The follow-up HSIL rate of the cases with marked atypia was 10.34% compared with 11.68% for cases without marked atypia. The follow-up interpretive frequency (in cytologic samples) of "low-grade squamous intraepithelial lesion" was significantly higher in group 1 (19/29 vs 114/291; $P = .009$). However, no significant differences were identified between groups 1 and 2 regarding the interpretive frequencies of HSIL (3/29 vs 34/291; $P = 1$) or "negative for intraepithelial lesion or malignancy" (6/29 vs 56/291; $P = .8$) in follow-up cytologic samples. In subsets of both groups in which high-risk human papillomavirus testing was performed in the Papanicolaou test sample that immediately preceded the index cervical biopsies, no significant differences in viral load were found.

CIN 1 with marked atypia does not have a higher follow-up HSIL rate than CIN 1 without marked atypia in our patient population. Further studies are required to address the significance of marked atypia in CIN 1 and whether patients with this finding should be managed differently.

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Dendritic Cell Density and Chemokine CXCL14 Expression in Prostate Cancer

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To perform immunosurveillance and initiate antitumor immune response, dendritic cells (DCs) must be physically present in malignant tissue. Attraction of DCs into tissue is regulated by chemokines. One of them, breast- and kidney-expressed chemokine (BRACK; CXCL14), is known to be selective for monocytes and DCs and is expressed ubiquitously in normal tissues but absent in a variety of cancer tissues and tumor cell lines. However, the mechanisms responsible for CXCL14 loss in malignant tissues and cells are unknown. The goals of this study were to evaluate DC density in prostate carcinoma tissue and cell lines in correlation with CXCL14 expression at the transcriptional and translational levels and to elucidate possible mechanisms of CXCL14 gene regulation.

Detection of DCs in formalin-fixed and paraffin-embedded prostate tissue and cancer cell lines was performed by immunohistochemistry with monoclonal antibodies recognizing CD83, CD1a,

and S-100 proteins. Expression of CXCL14 protein in tumor tissue sections, BPH, normal prostatic epithelium, and prostate carcinoma (PCa) cell lines was determined by immunohistochemistry with anti-CXCL14 monoclonal antibodies (R&D Systems). CXCL14 gene expression was evaluated by reverse transcription-polymerase chain reaction (PCR), using specific primers for CXCL14 (forward, 5'-tccggctcagcatgaggctcc; and reverse, 5'-cacctattctctgaagacc; 313 base pairs). Primers for β -actin transcript were used as an internal control. The state of CXCL14 gene promoter methylation was evaluated by bisulfate-specific polymerase chain reaction (MS-PCR), followed by DNA sequencing. Cultured LNCaP, PC-3, and DU145 cells were treated with demethylating agent 5-aza-2'-deoxycytidine (5-aza-dC, 10 and 25 μ mol/L), and expression of CXCL14 protein and mRNA was determined by immunohistochemistry and RT-PCR, respectively.

We demonstrated that DC density in prostate carcinoma tissue is significantly lower than in benign prostate. CXCL14 protein expression, evaluated by immunohistochemistry in human prostate cancer tissues and prostate cancer cell lines LNCaP, PC3, and DU145, is significantly decreased. Likewise, expression of CXCL14 messenger RNA (mRNA), determined by RT-PCR, is decreased. Treatment of CXCL14+ prostate tumor cells with demethylating agent 5-aza-2'-deoxycytidine resulted in recovery of CXCL14 mRNA and protein expression in these cells, indicating CXCL14 gene promoter methylation as a possible mechanism of gene silencing. Finally, using bisulfate-specific PCR, we found that DNA from prostate cancer cells contains hypermethylated CpG island sequences encompassing the transcriptional regulatory region of the *CXCL14* gene. Thus, our results are the first evidence for the epigenetic regulation of CXCL14 expression in prostate cancer cells.

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Reevaluation of CD20 Expression in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

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Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a monoclonal B-cell neoplasm characterized by frequent coexpression of CD5 and CD23. While the expression of CD20 is usually described as dim positive, our experience indicates that CD20 is more often moderately positive.

In this study, we collected 40 CLL/SLL cases from the last 2 years from our department file (21 from peripheral blood, 13 from bone marrow, and 6 from lymph node). These cases were analyzed by using 4-color flow cytometry, including CD20, CD19, CD22, CD5, CD10, CD23, FMC7, CD38, and Zap70. We found CD20 was moderately expressed in 65% of cases (26/40), brightly expressed in 5% (2/40), dimly expressed in 23% (9/40), and negative in 8% (3/40). All cases in which CD20 expression was negative were from bone marrow. The CD20- cases displayed morphology consistent with CLL/SLL, and CD23 was moderately expressed in those cases. In contrast with the variability observed in the intensity of CD20 expression, other B-cell markers, including CD22 and CD19, were expressed more consistently. CD22 was dimly expressed in 85% of cases (34/40), and CD19 was moderately expressed in 88% (35/40). In addition, CD5 was moderately expressed in 38% of cases (15/40), and CD23 was moderately expressed in 55% (22/40) and brightly expressed in 13% (5/40).

Contrary to the frequently described immunophenotype of CLL/SLL, our results indicate that the expression of CD20 is more

variable, with moderate expression of CD20 in more than half of the cases. Other B-cell markers have a more consistent pattern, with dimly positive CD22 and moderately positive CD19. We believe these results would be helpful to avoid confusion in the differential diagnosis of small B-cell lymphomas.

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Minimally Enhanced Detection of *Mycobacterium tuberculosis* Complex Using Polymerase Chain Reaction on Resected Culture-Negative Necrotic Pulmonary Granulomas

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Patients with necrotic pulmonary granulomas and negative mycobacterial cultures can present a diagnostic challenge. Molecular testing of surgical pathology specimens is not routinely performed. To determine if polymerase chain reaction (PCR) amplification of *Mycobacterium tuberculosis* complex (Mtb) DNA would enhance diagnostic yield in such cases, we performed a retrospective review of formalin-fixed, paraffin-embedded (FFPE) pulmonary specimens with necrotic granulomas for the 5-year period 2002-2007.

DNA from archived FFPE tissue was extracted, and nested PCR was performed to detect the insertion sequence 6110 found only in Mtb. In 89 patients, necrotic pulmonary granulomas were resected (68 patients) or biopsied (21 patients), were without associated pulmonary neoplasms during the study interval, and had accompanying mycobacterial culture results available. Of these, 40 (45%) had negative mycobacterial cultures and negative fungal stains, 12 (13%) had Mtb+ cultures, 29 (33%) had cultures positive for mycobacteria other than tuberculosis (MOTT) and had negative Mtb cultures and fungal stains, and 8 (9%) had positive fungal stains. *Mycobacterium avium* complex was cultured in 27 of 29 MOTT+ patients. Using FFPE tissue from 26 of 33 culture-negative, necrotic granuloma resections, PCR detected Mtb DNA in 1 patient who was asymptomatic and had presented due to a positive workplace tuberculin skin-test screen. PCR was not performed for the patients for whom adequate clinical follow-up could not be ensured in the event of a positive result. Mtb DNA was detected in 4 of 4 Mtb culture-positive control cases.

In our patient population, these results suggest that Mtb PCR on pulmonary resection specimens will result in minimally enhanced detection of tuberculosis.

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Persistent Localized Bone Marrow Aplasia Following Radiotherapy With Preserved Peripheral Counts: A Study of Seven Cases

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Prolonged bone marrow (BM) suppression is a known effect of radiation therapy (XRT). Localized, XRT-induced sternal BM aplasia was described in early studies in the radiation oncology literature; however, no pathologic studies have examined in detail the phenomenon of XRT-induced, localized BM aplasia in random iliac crest biopsies and its relationship to overall hematopoiesis. We retrospectively reviewed aplastic iliac crest BM specimens with discrepant peripheral blood (PB) counts following XRT to the pelvic region.

BM aplasia was defined as 5% or less cellularity in an adequate biopsy and/or similarly hypocellular particles on aspirate smears. Discrepant PB counts were defined as within or more than normal limits or mild cytopenias (WBC count, 2.5-3.9 10e3/ μ L; hemoglobin, 10.0-12 g/dL; and platelet count, 120-149 10e3/ μ L). Seven patients with BM aplasia and discrepant PB counts were identified; each had received localized XRT to the sacrum, lumbar spine, or pelvis for myeloma, rectal cancer, or metastatic tumor.

Aplastic BMs showed core biopsies with complete replacement by mature fat, lacking fibrosis and radiation atypia, and/or virtually acellular spicules. Mild cytopenias were seen in 5 cases and normal or increased counts in 1 case each. Aplastic BMs were observed as early as 5 months following XRT and as late as 43 months. A myeloproliferative disorder was diagnosed in 1 case based on PB findings and JAK-2 mutation, despite BM aplasia. In 1 case, a right-sided aplastic BM, diagnosed 8 months after XRT, was followed 14 months later by a normocellular right aspirate and aplastic left BM biopsy.

Prolonged, localized BM sterilization may be seen as a result of XRT to the lumbar and sacral spine and pelvis for several years. In the setting of preserved PB counts, this is not likely representative of overall hematopoiesis and serves as a potential diagnostic pitfall. Regeneration of hematopoietic activity at exposed sites may be possible.

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Angiolipoma of the Breast

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Angiolipoma is a well-demarcated benign neoplasm containing mature adipose tissue and variable amount of capillaries. It may present a diagnostic challenge, particularly when it presents as a mass in the breast. Our target was to study characteristic clinical and morphologic aspects of angiolipoma of the breast.

We retrospectively studied 39 cases of breast angiolipoma (13 biopsies and 26 excisions) during a 19-year period (1990-2009). We also analyzed clinical data to determine age and sex distribution and clinical presentation of the tumor.

Angiolipoma was detected in 24 women (age range, 36-76 years) and 15 men (age range, 28-70 years). All men and 20 women had a palpable subcutaneous mass. Four cases were detected radiologically. Two cases presented as a tender mass. Age distribution for women was 36 to 76 years and for men, 28 to 70 years. Diagnosis was made by needle core biopsy in 13 cases (2 men and 11 women). Lumpectomy was performed to confirm the diagnosis in 4 cases. All angiolipomas were superficial, and the largest tumor was 4 cm. The vascularity varied from 10% to 50% with the exception of 1 case with 95% vascularity (cellular angiolipoma). All cases demonstrated proliferating capillaries. The endothelium was flat with uniform hyperchromatic nuclei. Neither nucleoli nor mitoses were found.

It is difficult to diagnose lipomatous tumor of the breast on a needle core biopsy specimen. However, the presence of benign

capillary-sized vascular proliferation within mature adipose tissue in a biopsy performed in the context of a breast mass can lead to the correct diagnosis and may avoid unnecessary lumpectomy. Subcutaneous fat is highly vascular, but tortuosity of capillaries with at least 3 interconnecting capillary channels in 1 spot constitutes minimal criteria for diagnosis of angiolipoma on a breast biopsy.

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The Distinction of Clear Cell Carcinoma of the Female Genital Tract, Clear Cell Renal Cell Carcinoma, and Translocation-Associated Renal Cell Carcinoma: An Immunohistochemical Study

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Clear cell carcinoma of the female genital tract (CCCa) shares histologic features with clear cell renal cell carcinoma (CCRCC) and translocation-associated renal cell carcinoma (TA-RCC), the latter in particular. It is critical to distinguish CCCa, CCRCC, and TA-RCC when CCRCC or TA-RCC is metastatic to the female genital tract. Such distinction is not always possible based on the morphology alone. To our knowledge, this was the first study to directly compare the immunophenotypes of these 3 tumors.

A tissue microarray (TMA) was constructed from 23 CCRCC, 5 TA-RCC, and 13 CCCa cases. TA-RCC was confirmed by the immunostain for TFE3 protein. Each case was represented by triplicate cores. This TMA was stained with cytokeratin (CK)7 and CK20, CD10, AMACR, carbonic anhydrase 9 (CA9), and WT-1. Each stain was graded as positive or negative, and the percentage of positive cells was also recorded for each positive case.

Of 13 CCCa cases, 6 originated from the endometrium and 7 from the ovary. Histologically, CCCa, CCRCC, and TA-RCC all consisted of clear cells. CCCa and TA-RCC displayed papillae lined with clear cells and hyalinized fibrovascular cores. CK7 was positive in all CCCa cases (12/12) but in only 20% of the TA-RCC (1/5) and 4% of the CCRCC cases (1/23). In contrast, CD10 was positive in all TA-RCC cases (5/5) and 91% of the CCRCC cases (21/23) but in only 8% of the CCCa cases (1/12). TFE3 was positive in all TA-RCC cases but negative in all CCCa and CCRCC cases. CA9 was positive in 87% of the CCRCC cases but in only 20% of the TA-RCC cases and was negative in all CCCa cases. CK20, AMACR, and WT-1 were positive in only a small percentage of the tumors, and the results were not contributory.

Although morphologically similar, CCCa can be reliably distinguished from TA-RCC and CCRCC. CCCa is mostly CK7+/CD10-/CA9-/TFE3-, TA-RCC is usually CK7-/CD10+/CA9-/TFE3+, while CCRCC is mostly CK7-/CD10+/CA9+/TFE3-.

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T-Cell Large Granular Lymphocytic Leukemia Mimicking Idiopathic Myelofibrosis: A Report of Two Cases

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T-cell large granular lymphocytic leukemia (T-LGL) is a rare, indolent lymphoproliferative disorder of clonal cytotoxic T cells that is difficult to diagnose because it mimics reactive and neoplastic

processes. T-LGL presents with anemia, neutropenia, marrow hypercellularity, T-lymphocyte-predominant aggregates, and, occasionally, increased reticulin fibrosis. In contrast, primary idiopathic myelofibrosis (PIMF) is a chronic myeloproliferative disorder that also presents with cytopenias, hypercellular marrow, diffuse reticulin fibrosis, and, occasionally with reactive T-lymphocyte-predominant aggregates. We report 2 unusual presentations of T-LGL in which the most striking finding was diffuse marked increase in marrow fibrosis, leading to an initial impression of PIMF.

Two women, 96 and 68 years old, presented with anemia, neutropenia, and slight splenomegaly. Both underwent bone marrow aspiration with biopsy to exclude myelodysplasia or PIMF. The bone marrow aspirate was dry in one patient and unremarkable in the other. Both cases displayed marrow hypercellularity, marked diffuse fibrosis, and interstitial lymphoid aggregates predominated by CD3+ T cells. Although the initial impression was PIMF, given the T-cell-predominant lymphocytic aggregates, additional studies were obtained, including immunohistochemistry (IHC) for natural killer (NK) cytotoxic T cells and peripheral blood polymerase chain reaction studies for T- γ receptor gene rearrangements. One case showed NK-cell marker positivity (CD8, CD56, CD57, and granzyme) by IHC in numerous scattered interstitial cells and in the aggregates. In the other case, the aggregates were lost on the levels used for IHC, but NK-cell marker positivity was obvious in scattered interstitial cells. Both cases were clonal by T- γ receptor gene rearrangement studies and negative for the *JAK-2* mutation.

Although T-lymphocyte-predominant aggregates are frequently interpreted as reactive, these cases highlight the necessity to investigate these aggregates further for T-LGL in the setting of anemia and neutropenia, even in presence of marked fibrosis. Molecular studies for T- γ receptor gene rearrangements and *JAK-2* mutation may prove helpful.

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Comparison of Immunostaining for Maspin, Fascin, and VHL as Diagnostic Markers for Ductal Adenocarcinoma of Pancreas

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Ductal adenocarcinoma of the pancreas (DAP) is the most frequent neoplasm of the pancreas. Recent studies have shown the presence of new possible markers for DAP. Maspin is a serine protein inhibitor that has been identified as a potential prognostic tumor marker. Fascin, actin-bundling protein involved in cellular motility, is up-regulated in neoplasms. *VHL* is a tumor suppressor gene. Positive staining of *VHL* has been demonstrated in normal exocrine pancreatic tissue. We investigated and compared the diagnostic utility of maspin, fascin, and *VHL* for differentiating between DAP and benign reactive lesions of the pancreas (BRL).

A total of 23 pancreatic specimens were retrieved and reviewed. Of the 23 cases, 10 (43%) were DAP. The remaining 13 cases were diagnosed as BRL and served as controls. Immunohistochemical (IHC) staining was performed on formalin-fixed, paraffin-embedded sections using a HIER technique. Intensity, pattern, and distribution of staining were recorded. Cases that showed weak or less than 5% staining were considered negative.

DAP was positive for maspin in 9 (90%), fascin in 9 (90%), and *VHL* in 2 (20%) cases. BRL was positive in 0 (0%), 1 (8%), and 12 (92%) cases, respectively. Fisher exact tests indicated that maspin, fascin, and *VHL* are all significant markers for differentiation of

the diagnosis. The *P* values of the 3 markers were .0001, .0001, and .007, and the relative risks were 14.000, 11.700, and 0.2167, respectively. The correlation coefficients and the *P* values between maspin and fascin, maspin and VHL, and fascin and VHL were 0.91417 (<.0001), -0.63492 (.0011), and -0.55476 (.0060), respectively.

Our results indicate that the staining results for maspin, fascin, and VHL are statistically different between DAP and BRL. The staining of the 3 IHC markers is strongly correlated, and all 3 antibodies can be helpful in distinguishing DAP from BRL.

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Positive Predictive Value of the Expression of HPV Oncogenic Proteins E6 and E7 in Cervical Cancer Screening

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In almost all cervical cancers, human papillomavirus (HPV) DNA has been identified. Persistent infection with high-risk HPV types is the first step in carcinogenesis. Women with active HPV infection will express E6/E7 oncogenes, which are required for malignant transformation. As only a small proportion of infections progress toward cancer, it is important to distinguish transient HPV infection from infection that is likely to persist or progress.

We tested 196 samples by the following: (1) conventional Papanicolaou (Pap) smear; (2) HPV-DNA test and typing (Innogenetics NV Belgium); (3) E6/E7 messenger RNA (mRNA) expression from the carcinogenic HPV types 16, 18, 31, 33, and 45 by the PreTect HPV-Proofer assay (NorChip, Italy), which uses a nucleic acid sequence-based amplification technique. The following cytologic disease categories at Pap smear were represented: negative (*n* = 83), atypical cells of undetermined significance (ASCUS; *n* = 72), low-grade squamous intraepithelial lesion (LSIL; *n* = 31), and high-grade squamous intraepithelial lesion (HSIL; *n* = 10).

Of the samples, 124 were positive by HPV DNA testing and 50 by the HPV-Proofer. HPV DNA testing was positive in 56.6% of negative, 70.8% of ASCUS, 58.1% of LSIL, and 80% of HSIL cases (*K* and *P*, not significant). E6/E7 mRNA positivity rates were 12% of negative, 29.2% of ASCUS, 38.7% of LSIL, and 70% of HSIL cases (*K* = 0.212; *P* < .001).

HPV-Proofer may be more useful in predicting high-grade disease as it has a higher specificity and positive predictive value than DNA testing and might offer an improvement for the triage of women with an ASCUS or LSIL Pap smear.

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Screening for Mutations in the Cystic Fibrosis Transmembrane Regulator Gene (*CFTR*) in 3,578 Infertile Candidates for Assisted Reproductive Techniques and Relation Between *CFTR* Mutation, IVS8-poliT, and Y Chromosomal Microdeletions in Azoospermic Males

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An association between *CFTR* gene mutations and male and female infertility has been hypothesized in the literature. The aim of our study was to investigate the frequency of the *CFTR* gene mutation in an unselected group of 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 3,578 patients (2,139 female and 1,439 male) for 53 *CFTR* gene mutations and the IVS8-poliT polymorphism by multiplex polymerase chain reaction (PCR; INNO-LiPA *CFTR*); 117 azoospermic men were investigated for Y chromosomal deletion by multiplex PCR (ABanalitica). Frequencies of mutation were separately calculated in all samples for men and women; the χ^2 test was used for comparisons. Only *P* values of .01 or less were considered significant.

CFTR mutations were detected in 3.8% of subjects, a percentage that overlaps with the one reported in the general population. The most common mutation was Δ F508 (del/N), observed in 1.19% of patients; only mutation N1303k/N (0.34%) showed a different distribution between sexes (0.63% in men and 0.14% in women). The IVS8-poly-T showed a frequency of 71.5% for 7T/7T alleles, 19.1% for 7T/9T alleles, and only 0.31% for 5T/5T alleles. Of 117 azoospermic patients, 1 had a Y chromosomal microdeletion and was negative for the *CFTR* mutation; 4 of 117 azoospermic patients had a mutation for *CFTR*. 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* mutation, IVS8-poly-T, and Y-chromosomal microdeletions.

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Determining the Utility of the Lymphosum in CD4 Evaluations

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The CD4+ T lymphocyte count is used to evaluate HIV-infected people to stage HIV infection, monitor immune system functions, and initiate prophylaxis for opportunistic infections. Assessing CD4+ T lymphocytes by flow cytometry requires accurately determining the lymphocyte population and the coexpression of CD4 with CD3. Lymphocytes exhibit low side scatter and bright CD45 in the CD45/side-scatter histogram. This gating is assumed to provide high lymphocyte purity and recovery; however, the Centers for Disease Control and Prevention recommends the use of a lymphosum tube that contains CD3, CD19, and a natural killer cell marker if lymphocyte recovery is questionable. A lymphosum of 90% to 110% indicates high purity and recovery of the lymphoid population. In this study, we evaluated the need to use the lymphosum tube when the CD3 percentage is less than 60%.

From December 1, 2006, to January 15, 2009, 1,433 whole blood samples (sodium heparin or EDTA) from immunocompromised patients were evaluated for percentage and absolute count of CD3, CD4, and CD8 by using a 4-color monoclonal panel (CD3, CD4, CD8, and CD45). If the CD3 percentage was less than 60% and if the patient did not have a previous similar low result, a second reflex tube for lymphosum evaluation containing CD3, CD16,

CD19, and CD45 was additionally set up. The lymphosum (CD3% + CD16% + CD19%) was computed.

Of 1,433 specimens, 106 had a CD3 percentage from 19% to 59%. The lymphosum of these samples ranged from 90% to 105%, with an average of 96%.

All lymphosum results were greater than 90%, which indicated that initial gating for the lymphoid population was pure and correct. The use of the lymphosum tube was not necessary in determining the CD4+ T lymphocyte count.

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Are We Uniformly and Accurately Classifying Isolated Tumor Cells, Micrometastases, and Macrometastases in the Sentinel Lymph Nodes of Invasive Lobular Carcinoma?

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Among pathologists, there is low reproducibility in classifying small-volume metastases in sentinel lymph nodes (SLNs) using the current American Joint Commission on Cancer (AJCC) staging criteria, particularly in cases of invasive lobular carcinoma (ILC). Reliable pathologic stage classification of SLNs is essential for patient management.

We reviewed patients treated at our institution who had a diagnosis of ILC and a positive SLN biopsy between 1998 and 2008. All SLNs were reassessed using strict adherence to the 2003 AJCC criteria. Specifically, the single largest tumor cell cluster was measured, and diffuse single cells or small clusters throughout the lymph node were classified as isolated tumor cells (ITCs). Further surgery, including full axillary lymph node dissection (ALND), and other clinical follow-up were reviewed.

Our inclusion criteria were met by 51 cases and were originally classified by the primary pathologist as follows: 10 ITC, 8 micrometastases, and 33 macrometastases. Cases were reclassified as follows: 21 ITC, 2 micrometastases, and 28 macrometastases. Twelve ITC cases underwent full ALND, and 3 (25%) of these patients had additional macrometastases. All micrometastatic cases underwent ALND and had no additional disease. Of the macrometastatic cases, 23 underwent full ALND, and 17 (74%) had additional metastatic disease.

We conclude that it is not appropriate in cases of ILC to classify diffuse single or small clusters of metastatic cells in the SLN as ITC. With strict adherence to the 2003 AJCC criteria, 11 patients' SLN metastases would have been classified ITC, and a full ALND would have not been done. In doing so, additional significant lymph node metastases would not have been discovered in 3 of these patients. The criteria for assessing small-volume metastases in the SLNs of patients with ILC need to be more clearly defined.

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The Diagnostic Role of Multiparameter Immunophenotyping by Flow Cytometry in Multiple Myeloma: A New Model

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Immunophenotypic studies on multiple myeloma (MM) have been performed for more than 15 years. Multiparameter flow cytometry (FC) represents an attractive approach in the detection of aPC due to its capacity to combine an examination of an aberrant immunophenotype and clonality studies. Owing to the large numbers of cells amenable to analysis by FC, it may be additionally useful in the detection of minimal residual disease. Problems with such evaluation of plasma cells (PCs) include those related to the frequent hemodilution of bone marrow aspirates with peripheral blood and the lability of PCs stored outside of the body. At this time, the histologic examination of bone marrow remains the "gold standard" in the diagnosis of MM. We have developed an objective and reproducible new statistical diagnostic model that examines what correlation exists between the immunophenotype and clonality detected by FC and histology that defines the diagnostic role of FC in MM.

Fifty-five patients were enrolled in a pilot study for routine diagnostic analysis of MM; a minimum of 100 PCs were analyzed for each patient sample.

A direct 8-color method was applied to study the immunophenotype of PC using a BD FACSCanto II. Samples were labeled with the following monoclonal antibody combinations (PacificBlue-FITC-PE-PerCP-APC-PeCy7-PacificBlue-APC-Cy7): CD45/CD38/CD221/CD19/CD27/CD138/CD56; CD45/CD38/CD200/CD19/CD81/CD138/CD10; CD45/CD38/CD28/CD19/CD117/CD138/CD33; and CD45/cytoL/cytoK/CD19/CD38/CD138/CD56/CD20.

Analysis of CD38, CD19, and CD10 expression, when applied to our model, resulted in optimal concordance with histology.

This statistical model showed a correlation between FC and histology. This statistical model represents a new objective and reproducible way to interpret the immunophenotype of PC and aPC and correlates this analysis with histologic results. Our goal is to use this information to consolidate this model and test its applicability on a larger scale.

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Evaluation of Lysosomal Storage Disorder Quality Control and Application in Newborn Blood Spot for Screening Program

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Lysosomal storage disorders (LSDs) represent a group of more than 40 genetic diseases caused by the deficiency of enzymes, resulting in the accumulation of undegraded substrates within lysosomes and early death. We have developed a tandem mass spectrometry method using dried blood spots for the identification of the most common lysosomal diseases: Niemann-Pick, Gaucher, Pompe, and Fabry related to the deficiency of acid sphingomyelinase (ASM), acid β -glucocerebrosidase (ABG), acid α -glucosidase (GAA), and acid α -galactosidase (GLA), respectively.

Using dried blood spots (DBS), 3-level quality controls were evaluated by the multiplex enzyme assay developed by Centers for Disease Control and Prevention. DBS extracted enzymes were incubated with related substrate/internal standard followed by liquid-liquid extraction and then solid phase extraction, preparing the sample for injection into a tandem mass spectrometer.

The standard calibration curves showed good linearity within the range 0 to 5 of pure compound/internal standard, and the determined correlation coefficients were $R^2 > 0.992$. The mean \pm STD for low, medium, and high controls were 0.45 ± 0.07 , 1.52 ± 0.12 , and 2.23 ± 0.10 , respectively, for ASM; 2.10 ± 0.40 , 10.31 ± 1.45 , and 13.72 ± 1.10 , respectively, for ABG; 3.16 ± 0.27 , 14.46 ± 2.01 , and

22.91 ± 1.21, respectively, for GAA; and 1.42 ± 0.34, 4.46 ± 0.84, and 6.42 ± 1.58, respectively, μmol/L/h for GLA.

We were able to run successfully the multiplex assay with an acceptable recovery from blood spot enzyme activity. We are currently seeking to reduce the assay time and to include the fifth disorder in a single analytic run to get a method more suitable for a newborn screening program.

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The Detection of *Toxoplasma gondii* in Acquired Toxoplasmic Lymphadenitis and Mimicking Lesions Using Immunohistochemical and Polymerase Chain Reaction Methods

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The histopathologic triad of florid reactive follicular hyperplasia, clusters of epithelioid cells, and focal sinusoidal distention by monocytoïd B cells has been considered to be the pathologic diagnostic criterion of toxoplasmic lymphadenitis. It has a satisfactory specificity, but because of its low sensitivity, some cases could be missed.

To evaluate the use of polymerase chain reaction (PCR) and immunohistochemical (IHC) methods in detecting the microorganism *Toxoplasma gondii*, 3 groups of patients were selected. Groups were the toxoplasmic lymphadenitis (TL) group (n = 18) with 3 of 3 histopathologic changes of the triad, the TL-mimicking group (n = 5) with 2 of 3 of the triad and dog or cat contact history, and a control group (n = 17) with 2 of 3 of the triad but without the dog or cat contact history.

The results showed positive rates for PCR of 94% (17/18), 20% (1/5), and 12% (2/17) and for IHC of 61% (11/18), 60% (3/5), 0% (0/17), respectively. The statistical analysis showed a significant difference between the TL group and the control group by PCR ($P < .01$) or by IHC ($P < .01$). The difference between the TL-mimicking group and the control group was significant by IHC ($P < .01$) but not by PCR ($P > .05$).

Our results suggest that the histopathologic triad is a good morphologic criterion for diagnosis of TL, and it would show doubt about toxoplasmosis for a lymphadenopathy with 2 of 3 of the triad, especially for a patient with a positive medical history. An added auxiliary examination by PCR and/or IHC at this time would improve the diagnosis.

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Prevalence of *Helicobacter pylori* Infection, the Virulence Genotypes of the Infecting Strain, and Associated Disease Outcomes in the Kenyan Population

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Epidemiologic studies attempting to relate *Helicobacter pylori* genotypes and host factors to disease outcome in Africa are scarce. This has led to some concluding that data on *H pylori* infection in Africa are at odds in several aspects with those published in the West. Kenya lies in the cultural crossroads between Africa and the

rest of the world. It is a port of entry to the continent, a regional business hub, and one of the leading tourist destinations in Africa. It can, therefore, be assumed that this is also the region where the predominant *H pylori* genotype changes can be found and, therefore, an ideal place for studies on the molecular epidemiology of *H pylori*.

This study investigated the prevalence of *H pylori* infection, the virulence genotypes of the infecting strain and associated disease outcomes in the Kenyan population. Interleukin (IL)-1b-511, IL-1RN, and TNF-α polymorphisms were genotyped by oligonucleotide allele-specific polymerase chain reaction (PCR) in a sample of 290 patients with dyspepsia undergoing upper gastrointestinal endoscopy at the Aga Khan University Hospital, Nairobi, Kenya. The presence of the *cagA* gene and genotype variations in the *vacA* gene was also determined by PCR. Association of these genetic polymorphisms with the development of gastritis and peptic ulcer disease (PUD) was tested.

The *H pylori* status was 52.2%. Infection was highest in patients with peptic ulcer disease (74.2%). *H pylori cagA* and *vacA* genotypes showed no inclination toward any particular pathology. The IL-1b-511 allele T/T was more common (48.4%) in PUD patients as compared with other pathologies, as was allele 2 of IL-1RN (48.4%), whereas allele A of TNF-α showed no significant association with any pathology. The offending association of the IL-1b-511 allele T/T and 2 of the IL-1RN, which is viewed as more provocative, was not abundantly observed in this study. The IL-1b-511 T/T haplotype was highest in PUD patients (64.5%), as was TNF-α A/A (43.5%). In this study population, *H pylori* infection was associated more with gastritis (73%) as opposed to other pathologies.

In this study, the pathogen virulence factors seem not to have any role in disease outcome, but there seems to be a relationship in host response and disease outcome.

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Relapsing Systemic Polyclonal B-Immunoblastic Proliferation. A Case Report

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The hallmark of lymphoid neoplasia is clonality. However, claims of polyclonal lymphoma surfaced many times in the literature but were mostly discredited. Yet, the literature shows rare, but well-documented cases of systemic polyclonal B-immunoblastic proliferation (SPBIP) that can be life-threatening if not treated. We encountered a case of SPBIP that relapsed despite chemotherapy, documenting the aggressive nature of such polyclonal lymphoproliferations.

A 55-year-old woman with a history of diabetes mellitus and hypertension first presented with lymphadenopathy. Approximately 5 days later, she was admitted to a hospital with weakness, dizziness, dyspnea, lymphadenopathy, hemoptysis, and bleeding gums. Laboratory workup revealed severe thrombocytopenia, anemia, and leukocytosis with large atypical lymphocytes. Serologic studies were negative for autoimmune disease and acute viral infections. Polyclonal hypergammaglobulinemia was detected. The patient underwent bone marrow and lymph node biopsy and flow cytometry evaluation.

Peripheral blood flow cytometry showed B cells with expression of CD45, CD19, CD30, CD38, CD56, CD 71, HLA-DR, and cytoplasmic light chains with a polytypic pattern. An initial lymph node biopsy showed atypical lymphoid hyperplasia with an increase in CD30+ B immunoblasts. A repeated lymph node biopsy showed progression in the disease with numerous immunoblasts effacing the lymph node architecture. A bone marrow biopsy was hypercellular with diffuse immunoblastic infiltration. Flow cytometry was

repeated on lymph node and bone marrow with similar findings. No clonal B-cell populations were detected by molecular testing.

The patient received treatment of dexamethasone, cyclophosphamide, and vincristine with a clinical response. The patient had a subsequent relapse 2 months later. An additional course of treatment resulted in remission, and the patient was alive and free of disease 7 months later.

The pathogenesis of SPBIP is unclear. However, SPBIP is an aggressive disease that requires chemotherapy to prevent a fatal outcome. The current case is unique by resistance to multidrug chemotherapy.

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HER-2 Gene Analysis by Dual-Probe Chromogenic In Situ Hybridization (DCISH) in 19 Invasive Breast Carcinoma Cases

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HER-2 gene status is essential for the selection of patients with invasive breast carcinoma for treatment with trastuzumab. Several immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) assays are being used routinely for the evaluation of HER-2. The double-staining CISH assay (DAKO DuoCISH) is a new method recently approved by the Food and Drug Administration that targets both the HER-2 gene and chromosome centromere (Cep17).

The purpose of this study was to perform and compare the accuracy of DCISH with FISH and IHC in determining the HER-2 gene amplification status in invasive breast carcinomas. We randomly selected 19 cases from our database that had been tested for HER-2 by IHC and FISH. All cases were processed with the DCISH assay kit, and the slides were reviewed by 2 pathologists, 1 pathology resident, and 2 cytotechnologists, each blinded to the results of the other. A bright-field microscope was used, and 20 tumor cells were counted per case to obtain the HER-2/Cep17 ratio following College of American Pathologists/American Society of Clinical Oncology guidelines.

Normal breast tissue or lymphocytes served as internal positive controls. The IHC results were as follows: 0, 2 cases; 1+, 8 cases; 2+, 4 cases; and 3+, 5 cases. None of the 0 or 1+ cases were amplified by FISH or DCISH. Three of the 2+ cases were nonamplified by FISH; 1 of these was interpreted as amplified by 2 reviewers. One 2+ case and all 3+ cases were amplified by both methods. Correlation was 95% to 100% between the reviewers.

Our results show that DCISH is an effective, reproducible, and convenient method for detecting HER-2 gene amplification. The advantages include HER-2 and Cep17 chromogenic signals on the same cell; convenient evaluation by bright-field microscope, allowing easy detection of areas of invasive carcinoma; and slide storage at room temperature without loss of signal. The disadvantage is that, occasionally, faint blue dots may result in a false-positive interpretation.

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Tumor-Specific CpG Island Methylation in Protocadherin Family Member PCDH- γ -A12

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Protocadherins (PCDHs) are a major subfamily of the cell adhesion molecule cadherin superfamily, but little is known about their biologic functions. The PCDH subfamily consists of more than 70 members sharing the structural repertoire of approximately 110 amino acid repeat sequence motifs and the unique interconnecting loops. Although PCDHs are expressed predominantly in the brain involving synaptogenesis, they may also have other roles in morphogenesis, cell-cell recognition, and cell-matrix and cell-basement membrane interactions. Their roles in tumor suppression, contact inhibition, tumor invasion, and metastasis have only recently been recognized.

Tumor cell line genomic DNAs were purchased from the ATCC (Manassas, VA). Ovarian cancer cell line pallets were provided by Dr Sharon Stack, the University of Missouri-Columbia. The patient peripheral blood samples were obtained at Ellis Fischel Cancer Center (EFCC), University Missouri Healthcare, with institutional review board approval. Genomic DNA was extracted and subjected to digestion with 4 methylation-sensitive restriction enzymes. The undigested hypermethylated CpG island (CGI) region of the PCDH- γ -A12 gene was differentially amplified by conventional polymerase chain reaction.

CGI methylation of PCDH- γ -A12 was detected in major common human carcinoma cell lines including breast cancer (MCF7, HTB-26D), lung cancer (NCI-H69, NCI-H1395), colon cancer (HT-29), ovarian cancer (OVCA433 and DOV13), and prostate cancer (PC-3 and LNCaP), as well as melanoma (SK-MEL-1). A total of 105 random clinical blood samples from EFCC were tested for circulating tumor cells (CTCs) with CGI methylation of PCDH- γ -A12. Methylation bands were observed in 7 samples from clinical cancer cases including metastatic breast cancer (1 case), colon cancer (1 case), rectal squamous carcinoma (1 case), esophageal adenocarcinoma (1 case), and renal cell carcinoma (3 cases) but not in normal blood controls.

Promoter CGI methylation is associated with gene silencing. Loss expression of PCDH may be related to metastasis. This tumor-specific DNA methylation locus could be used as biomarker for CTC detection.

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Comparison of the Serum Free Light Chain Immunoassay With Urine Bence Jones Protein in Patients With Light Chain Multiple Myeloma

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The standard aids for diagnosis and monitoring of treatment response in light chain multiple myeloma (LCMM) patients are serum protein electrophoresis (SPE) and urine (UPE) protein electrophoresis studies along with serum immunofixation (IFE) and urine (UIFE) immunofixation studies. Continuing therapeutic assessment of LCMM patients is complex as they typically have small or no visible peak(s) on the SPE. As such, urine studies, particularly UIFE, are important adjuncts in monitoring the patients.

Increased serum free light chain concentrations (sFLCs) can also be found in the serum samples of patients with LCMM. We compared the clinical utility of the sFLC immunoassay and its calculable κ/λ ratio ($c\kappa/\lambda$) to standard UPE and UIFE in assessing treatment response and detection of residual disease in LCMM patients.

FREELITE kits (The Binding Site, San Diego, CA) are nephelometry-based assays for quantitation of sFLCs. We retrospectively reviewed serial results collected concurrently (within 72 hours) from 53 patients (578 visits) who had both sFLC and UPE testing performed during a 67.5-month period. Light chain clonality was confirmed with prior tissue biopsy reports. Additional results (when

performed) were recorded for SPE, IFE, UIFE, β_2 -microglobulin, and follow-up bone marrow biopsy.

There were 517 comparable visits in which UPE was ordered (with or without UIFE). Of these, 41 (7.9%) showed a quantifiable UPE peak despite a normal κ/λ . There were also 240 visits in which testing for both sFLC and UIFE was performed. Of these, 57 (23.8%) had a positive or "suspicious" UIFE while yielding a normal κ/λ . In addition, there were 7 visits (2.9%) with a positive κ/λ result despite having a negative UIFE.

Our data indicate that while nephelometry-based assays for sFLC are a very important diagnostic adjunct, they do not replace urine studies in this population. Both assays together provide valuable data for monitoring LCMM patients.

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Is There a Utility for Cytokeratin Antibody Cocktails in the Stratification of Atypical Ductal Hyperplasia in Breast Needle Core Biopsy Material?

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Differential cytokeratin expression can help distinguish between different intraductal breast proliferations; however, this has rarely been utilized in needle core biopsy (NBx) material. Moreover, most prior studies only evaluated antibodies directed against various high-molecular-weight cytokeratins and did not investigate the potential for differential luminal cytokeratin (eg, CK8/18) expression in distinguishing between these lesions. The aim of this study was to determine whether such expression is useful in stratifying ADH lesions in NBx material as to the likelihood of finding more significant lesions on excisional biopsy (EBx).

Investigators were blinded to the findings on subsequent EBx, and recut sections of 39 histologically confirmed and previously characterized cases of ADH on breast NBx were immunostained with a CK5/p63/CK8/18 antibody cocktail and evaluated for the percentage of lesional cells expressing CK5 or CK8/18. Results were subsequently correlated with the findings on subsequent follow-up EBx.

Of the cases, 32 (82%) were diffusely CK8/18-positive and CK5-negative, while the remainder variably expressed both markers. On EBx, ductal carcinoma in situ with or without invasive carcinoma was seen in 9 cases (23%). At a cutoff of 80%, CK8/18 expression in ADH on NBx was associated with a sensitivity of 100% and a specificity of 13% for finding a more significant lesion on EBx. The corresponding positive predictive value (PPV) and negative predictive value (NPV) were 26% and 100%, respectively.

Although not specific and with only a marginally better PPV than histology alone (26% vs 23%), CK8/18 expression in more than 80% of cells in ADH on NBx identified all cases with more significant lesions on EBx (100% sensitivity) and was associated with a 100% NPV. This high NPV suggests that patients with ADH on NBx with mosaic CK5/CK8/18 expression in which fewer than 80% of cells express CK8/18, provided that there are no strong indications otherwise, could potentially be spared follow-up surgery.

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Polycythemia Vera with Eosinophilia Associated With FIP1L1-PDGFR α : A Case Report

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Myeloproliferative neoplasms with PDGFA rearrangement generally present as chronic eosinophilic leukemia or acute leukemia. An association between polycythemia and this disease entity, however, has not been documented. We report a case of a 54-year-old Hispanic man with clinically diagnosed polycythemia vera who was found to have FIP1L1-PDGFR α .

Diagnostic specimens included the peripheral blood smear and CBC, and bone marrow aspirate and biopsy. Wright-stained slides for the peripheral blood smear and bone marrow aspirate and H&E-stained slides of the bone marrow biopsy were evaluated. Chromosome analysis, FIP1L1-PDGFR α (fluorescence in situ hybridization [FISH]), and ETV6-PDGFRB (FISH) were also performed.

Peripheral blood showed marked leukocytosis (WBC count, 24,800/ μ L) with marked eosinophilia (51%), erythrocytosis (with hemoglobin of 17.6 g/dL while receiving 500 mg of hydroxyurea daily and therapeutic phlebotomy). Bone marrow showed a marked increase in eosinophils (28%), an increase in mast cells, marked reticulin fibrosis, and absent iron stores.

FISH testing of the bone marrow showed FIP1L1-PDGFR α mutation via loss of CHIC-2. Chromosome analysis and ETV6-PDGFRB were normal.

Induction of 200-mg imatinib mesylate therapy followed by 100-mg daily maintenance improved the patient's leukocytosis (WBC count, 7,400/ μ L), peripheral eosinophilia (6%), and polycythemia (hemoglobin, 12.0 g/dL) without either hydroxyurea or therapeutic phlebotomy. To the best of our knowledge, there has been no case report of myeloproliferative neoplasms with PDGFA rearrangement in the setting of polycythemia vera. Further investigation of the relationship between polycythemia vera and eosinophilia with PDGFA rearrangement may be warranted.

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Evaluating Private-Public Partnership (P3) Models Applicable to Medical Laboratories

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Canadian provincial governments devote as much as 50% of their budget to funding health care, and this percentage is only expected to increase. Private-public partnerships (P3s), cooperative ventures between the public and private sectors, offer a way to reduce the costs of large projects.

We identify and describe 2 specific examples of clinical laboratory P3s in Canada: the alliance between Saskatoon Health Region and Gamma-Dynacare Medical Laboratories and the Calgary Laboratory Services joint venture (1996-2006). With such limited experience, we evaluate different P3 structures for their applicability to the clinical laboratory. We determined that the operations and maintenance (OM) model and the buy own operate (BOO) model are likely to translate best to the medical laboratory setting.

Under the OM model, a private entity under contract operates a publicly owned asset for a specified term. The Saskatoon Health Region partnership falls under this model. In OM arrangements, the public pays for new capital. The OM model is preferred if retaining public control of the assets is important.

Under the BOO model, public assets are transferred to a private or quasi-public entity, usually under contract, and operated for a specified period. This model benefits the public by eliminating all associated liabilities with the operation of the facility from its

balance sheet. The public, in turn, provides fixed payments to its private partner for operating the facility and maintaining capital assets. Cost overruns are the responsibility of the private entity.

New P3 models are still emerging, and it is possible that the best P3 model for a medical laboratory is not one that falls into one of the two identified. For medical laboratories, it may be that a combination of these and other models is the most mutually beneficial for the public and private entities involved.

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A Case of Multiple Myeloma With Unusual Restriction of Surface and Cytoplasmic κ Light Chains

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We report a case of a 56-year-old woman with a left femoral neck fracture. Imaging revealed multiple lytic bone lesions, including the left femoral neck and the right scapula. The patient was diagnosed with multiple myeloma based on the combined laboratory and clinical data. Immunophenotyping of the lytic lesions showed an unusual restriction of surface and cytoplasmic κ light chains.

Diagnostic specimens included the femoral head biopsy received after hemiarthroplasty and biopsy of the right scapula lesion. H&E-stained slides of the biopsies were evaluated. Immunophenotyping of both lesions was also performed.

Histologic sections of both biopsies showed diffuse infiltration of plasma cells with mature cytologic features. Immunophenotyping of both lesions confirmed the presence of a predominant monoclonal plasma cell population. As expected in a typical plasmacytic neoplasm, the plasma cells showed CD38 positivity with cytoplasmic κ light chain restriction in conjunction with CD45 negativity. An unexpected finding in this case was the aberrant restriction of surface κ light chain. In addition, the malignant plasma cells were positive for CD7, CD19, and CD20 and negative for CD56. The lymphocytes in flow cytometric analysis were small in number and showed normal immunophenotypes (polyclonal B and T cells). In addition, serum protein electrophoresis was negative, but immunofixation revealed a monoclonal κ light chain process. A paraprotein was identified on urine protein electrophoresis, and immunofixation confirmed spillage of free monoclonal κ light chains.

The diagnosis of multiple myeloma is based on a combination of laboratory and clinical features. Despite the unusual marker profile of the monoclonal plasma cells in this case, the clinical and histologic evidence firmly established the diagnosis of multiple myeloma. To the best of our knowledge, the unusual restriction of surface and cytoplasmic light chains has not previously been described in multiple myeloma patients.

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Development of an Integrated Point-of-Care Testing Program in Northwest Alaska

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This study describes an innovative program for managing and integrating point-of-care testing (POCT) data collected at multiple rural sites in the Northwest Arctic Borough and Point Hope. Participants included 11 village clinics, Maniilaq Health Center (MHC), WIC, PHN, the Diabetes program, and MedFlight,

performing 10 different POC tests. A team of laboratory personnel was established to design and implement the program. Barriers to implementation were identified regarding procedures, training, quality control (QC), and information management.

The team met regularly with MHC staff to select and design feasible interventions based on project cause analysis and to plan circumventions around known and anticipated barriers. Monthly control logs were developed to monitor the effect(s) of interventions. The team established workshops to provide participants with opportunities for training and interactive networking. The program uses a hub-and-spoke design to address quality barriers. A POC coordinator manages day-to-day operations at each testing site, ensures results are entered into the medical record, and reports monthly QC data to MHC. Community health aides (CHAs) are trained on written procedures and assessed for competency during workshops at MHC. The "train-the-trainer" method is used for CHAs who cannot attend workshops. Program accomplishments include 100% participation, documented training of all CHAs engaged in testing, an 85% documentation rate of QC, and 90% of all results entered into the medical record.

Access to community-based health care is essential for people living in rural villages, but geographic isolation creates challenges for tribal organizations attempting to establish integrated health care delivery systems and coordination of care. This study describes a unique model of POCT that allows quality test performance and integration of results across a large regional system. This model can be readily adapted to other Alaska Native health settings and other POC tests.

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Report of a Mysterious Chronic Illness in a Family of Six and the Importance of the Pathologist in the Diagnostic Process

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A 44-year-old man with a 4- to 6-month history of debilitating illness with photophobia, confusion, dyspnea, intermittent fevers, nausea, abdominal pain, headache, and rash was evaluated for zoonotic and tick-borne illness. The patient's wife and 3 sons, (9, 12, and 15 years) had similar symptoms. One son, 17 years old, was symptom-free. Per report, symptoms began following delivery of puppies, assisted by the patient and wife with mouth-to-mouth resuscitation to 1 stillborn pup. The family was also active outdoors and routinely picked ticks from their dogs. The patient was treated with doxycycline before our evaluation.

A canine brucellosis workup was begun. Tests for Q fever (phase I/II); leptospirosis; hepatitis A, B, and C; Epstein-Barr virus; tuberculosis; malaria; bacterial/fungal isolator cultures; urine *Histoplasma*; cytomegalovirus; *Ehrlichia*; herpes simplex virus; Rocky Mountain spotted fever; and Lyme disease were negative for acute infection. Thyroid function, rheumatologic and coagulation tests, urinalysis, and CBC were all normal. A metabolic panel showed a normal glomerular filtration rate with an anion gap of 4 and a bicarbonate level of 32.

Further investigation revealed that the patient was employed as an auto mechanic and body worker who also owned a salvage yard of more than 1,500 crashed cars near his home. On pathology recommendation, heavy metal testing was performed. The urinary heavy metals 24-hour urine total volume was 5,735 mL, arsenic was 57 $\mu\text{g/d}$ (normal, <25 $\mu\text{g/d}$), and Hg was less than 30 $\mu\text{g/d}$ (normal, <20 $\mu\text{g/d}$). Serum chromium measured 2.3 $\mu\text{g/L}$ (normal, <1.4 $\mu\text{g/L}$).

Although toxicologic testing revealed mild to moderate elevations in heavy metals, levels were elevated for multiple toxic metals. However, the constellation of symptoms can be easily explained by

chronic heavy metal poisoning. These findings, along with the failure of extensive ID workup to reveal an infectious etiology, support the diagnosis of chronic toxic exposure. The health department, patient, and pediatrician were notified. The active involvement and guidance of the pathologist in this case led to the most likely diagnosis.

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(8;21)(q22;q22) Translocation in Blastic Phase of Chronic Myelogenous Leukemia: A Unique Entity With Heterogeneous Morphology

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The blastic phase of chronic myelogenous leukemia (CML) is frequently associated with cytogenetic evidence of clonal evolution, defined as chromosomal aberration in addition to the t(9;22)(q34;q11.2). The presence of t(8;21)(q22;q22), which is typically associated with specific type of de novo acute myeloid leukemia (AML), is very rare in blastic crisis of CML. We hereby report a case of CML treated with imatinib and subsequently developing blastic phase associated with t(8;21), along with review of literature.

This 59-year-old man with a history of CML (since August 2007) and receiving imatinib therapy, presented to our hospital in May 2008, with an upper respiratory tract infection associated with loss of appetite, fever, chills, and night sweats. Workup included complete hematologic evaluation with all ancillary studies, including flow cytometry and cytogenetic and molecular studies. Peripheral blood studies revealed leukocytosis with 82% blasts. The bone marrow also had 84% blasts, and flow cytometry showed they were of myeloid origin, expressing CD13, CD33, CD117, and CD34 with aberrant expression of CD7 and CD19. Cytogenetics showed complex karyotypic abnormalities, including t(8;21)(q22;q22) and t(9;22)(q34;q11.2) in all 15 bone marrow cells examined. Fluorescence in situ hybridization (FISH) showed AML1/ETO and ABL/BCR fusion product in all interphase nuclei.

The presence of t(8;21) in CML is rare, and we found only 4 cases reported in the English literature. All of them developed t(8;21) in blastic phase; 2 had M2 and 1 had M1 morphology (French-American-British classification), while 1 presented as extramedullary myeloid sarcoma. The case with M1 morphology was Ph⁻ at blastic crisis, whereas the other had a concurrent Ph⁺ abnormality. Our case had M0 morphology with Ph⁺. These finding clearly suggest that presence of t(8;21) in CML has critical role in the development of blast phase but can have heterogeneous morphology as compared with de novo AML with t(8;21).

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Congo Red Fluorescence Microscopy: A Better Screening Test for Amyloid Deposits in Trepine Bone Marrow Biopsies

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Detection of amyloid deposits by a reliable screening test that leaves ample residual tissue for accurate classification from amid an ever-increasing array of causative proteins is vital in practice. The "gold standard" screening test is polarized microscopy of Congo red-stained sections. However, Congo red stain fluorescence microscopy has been described by some as a more sensitive method for detection. We compare the sensitivity of these 2 methods for the evaluation of trephine bone marrow biopsy specimens. The prospect of subsequent

organ damage makes detection of concomitant amyloid crucial.

We retrospectively reviewed 33 bone marrow biopsy specimens from our archives that had previously been examined with Congo red stain polarized microscopy. A plasma cell dyscrasia was clinically suspected or previously diagnosed in each of the cases. Each Congo red-stained slide was examined using a fluorescence microscope, and results were compared with the results reported using polarized microscopy. Confirmation was done with an amyloid P immunohistochemical stain.

Of 33 cases, 3 that were initially classified as negative were reclassified as positive for the presence of amyloid in the paracortical tissue, bone marrow space, or both. Also 3 cases first classified as only focally positive in the paracortical tissue were reclassified as also positive within the bone marrow. These findings were verified by an amyloid P stain.

Our results reveal that the fluorescence technique is superior to the polarization technique. The fluorescence method demonstrated higher sensitivity and was positive in 100% of the cases that were positive for amyloid P staining. The polarization technique was positive in only 72% of the amyloid P-positive cases. Widespread use of the fluorescence method is warranted to help avoid subsequent unnecessary biopsies, to preserve involved tissues for added studies, and to accurately prognosticate cases of plasma cell dyscrasia.

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Assessing the Utility of Repeating Hematologic Critical and Delta Check Results

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Despite the demonstrated accuracy of modern hematology analyzers, many laboratories repeat critical and delta results. These policies can result in a delay in reporting of clinically relevant results, potentially delaying appropriate treatment. Although this is not a universal policy across clinical institutions, no studies have evaluated the efficacy of repeating these results. In this study, we evaluated the analytic yield and the efficiency and usefulness of repeating testing of these samples.

A retrospective analysis of approximately 2,000 consecutive, repeated results were compared graphically and statistically using data derived from patient samples submitted to the University of Utah Health Science Center or the Huntsman Cancer Hospital clinical laboratories. All samples were analyzed on Advia 120 hematology analyzers (Siemens Diagnostics, Rochester). A survey tool assessing current review and repeat practices was sent to a sample of US laboratories.

Deming regression analysis of data demonstrated a highly significant relationship for WBCs ($R^2 = 0.997$; $P < .01$), platelets ($R^2 = 0.995$; $P < .01$), RBCs ($R^2 = 0.988$; $P < .01$), mean corpuscular volume ($R^2 = 0.997$; $P < .01$), hematocrit ($R^2 = 0.989$; $P < .01$), and hemoglobin ($R^2 = 0.994$; $P < 0.01$). The mean corpuscular hemoglobin concentration ($R^2 = 0.815$; $P < .01$) and mean corpuscular hemoglobin ($R^2 = 0.959$; $P < .01$) demonstrated a highly significant relationship with a correlation coefficient lower than those for other parameters.

Modern hematology analyzers are extremely sophisticated instruments with built-in safeguards to alert the technologist when instrument and/or sample problems arise that preclude result reporting. Consequently, we conclude that there is no clinical value in routinely repeating critical and/or delta results and that a considerable potential savings in resources can be achieved if such repeat policies are eliminated or restricted.

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The Expression of Mcm7 and CES2 in Detection of Dysplasia in Barrett Esophagus

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Barrett esophagus (BE) is a strong risk factor for esophageal adenocarcinoma, with 30 to 125 times higher risk than the general population. Early detection of esophageal dysplasia is essential to benefit patient care and improve survival rates. Minichromosome maintenance (Mcm) proteins are critical for DNA replication, and their expression implies potential for cell proliferation. Carboxylesterase 2 (CES2) was previously found overexpressed in cancer arisen from BE. Our aim was to study the expression of Mcm7 and CES2 in normal esophageal mucosa (NE), nondysplastic BE (NBE), and dysplastic BE (DBE) mucosa.

Paraffin-embedded specimens (15 NE, 31 NBE, and 25 DBE; 10 patients with 3-7 years of follow-up with serial biopsies who developed dysplasia) were stained for Ki-67, Mcm7, and CES2. The staining was scored on a percentage basis, as follows: 0, less than 10%; 1, 10% to 25%; 2, 26% to 50%; 3, 51% to 75%; and 4, 75% or more.

Mcm7 was not expressed in the NE. Mcm7 was up-regulated, together with the proliferation marker Ki-67, in NBE and DBE. In NBE and DBE, the level of Mcm7 expression increased compared with that in NE ($P < .005$). In patients who developed dysplasia, biopsies before dysplasia had higher Mcm7 and CES2 expression than in the matched control samples ($P < .001$). Immunostaining also showed increased expression of CES2 in the progression of BE to DBE.

The enhanced expression of Mcm7 and CES2 in BE is associated with dysplasia, and patients are at risk for subsequent development of dysplasia as well.

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Analysis of Cases of HPV Infection With Discrepancies Between Cytological and Histological Diagnosis Using an In Situ Hybridization Method and HPV Genotyping

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A persistent human papillomavirus (HPV) infection is considered to be a decisive risk factor in the development of anal and perianal squamous cell carcinoma. Invasive anal carcinoma develops from precancerous anal intraepithelial neoplasia (AIN). Polymerase chain reaction (PCR) and in situ hybridization (ISH) have increasingly been used for detection of HPV in cervicovaginal samples. However, there are limited studies in the literature regarding detection of HPV in anal lesions using ISH.

Anal biopsies from 31 male patients were studied in which presence of the high- and low-risk subtypes of HPV was determined by ISH using commercial HPV probes.

The diagnoses were as follows: 4 negative biopsies (13%), 12 condylomas without overt dysplasia (39%), 9 AIN I (29%), 3 AIN II (10%), 1 AIN III (3%), and 2 carcinomas (6%). Low-risk HPV was detected in 2 (50%) of 4 negative cases, 9 (75%) of 12 condylomas, 6 (67%) of 9 AIN I cases, and 2 (67%) of 3 AIN II cases. High-risk HPV was detected in 1 (8%) of 12 condylomas, 2 (22%) of 9 cases of

AIN I, in the 1 case of AIN III, and in the 2 cases of rectal carcinoma. Both high- and low-risk HPV were detected in 2 (17%) of 12 condylomas, 1 (11%) of 9 cases of AIN I, and 1 (33%) of 3 cases of AIN II. HPV cytopathic effect was noticed in 10 cases (32%) and, more specifically, in 2 (50%) of 4 negative cases (squamous papillomas), 2 (17%) of 12 condylomas without overt dysplasia, 3 (33%) of 9 AIN I cases, 3 (100%) of 3 AIN II cases.

We conclude that when used together and evaluated in conjunction with histology sections, ISH is a useful tool for ancillary molecular testing of HPV infection in AIN. When AIN, condylomas, or histologic markers of HPV infection in anal lesions are detected, ISH can be used for detection of HPV in these tissues.

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Significance of CMYC, t(14;18), BCL2 Protein Expression, and Ki-67 Index in Diffuse Large B-Cell Lymphoma

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The diffuse large B-cell lymphoma (DLBCL) prognosis is generally based on the International Prognostic Index. Adverse outcome has been associated with a high proliferative rate, p53 overexpression, and immunoblastic variants and improved survival with BCL2 protein expression and t(BCL6). A germinal center B-cell (GCB) immunophenotype (IP) has shown better survival than those with an activated GCB (AGCB) or activated B-cell (ABC) IP.

We investigated the correlation between chemotherapeutic response (CTXR) and survival status (SS) with the presence of t(14;18) and/or CMYC abnormalities, BCL2 protein expression, and IP, as well as the correlation of the Ki-67 index with these cytogenetic abnormalities.

We stained 115 DLBCLs for CD10, BCL6, CD138, MUM1, BCL2, and Ki-67. Fluorescence in situ hybridization studies (CMYC and t(14;18)) were performed. Cases were subclassified into GCB, AGCB, and ABC IPs, grouped into those with and without CMYC and/or (14;18) abnormalities, and available SS and CTXR were tabulated. The Fisher exact test compared proportions/percentages for covariate data of interest, producing various contingency tables. Exact 95% confidence intervals were calculated for each proportion/percentage of interest.

A greater than expected percentage of patients (66%) without any cytogenetic abnormalities showed complete CTXR, as compared with 34% of patients with one or both abnormalities ($P = .03$), but there was no significant difference in SS. When classified by IP, a higher than expected percentage of ABC and AGCB cases showed BCL2 staining (100% and 79%, respectively), and a lower than expected percentage of GCB cases showed BCL2 staining (58%; $P = .02$). There was no association between the Ki-67 index and the presence or absence of cytogenetic abnormalities. Significance was noted between SS, CTXR, and BCL2 staining; BCL2 negativity indicated better SS ($P = .02$) and CTXR ($P = .02$).

The Ki-67 index did not correlate with cytogenetic abnormalities. BCL2 expression was associated with poorer SS and worse CTXR. The absence of any cytogenetic abnormalities was associated with better CTXR.