Young Investigator Awards Program Abstracts

The Academy of Clinical Laboratory Physicians and Scientists (ACLPS) established the Paul E. Strandjord Young Investigator Awards Program in 1979 to encourage students and trainees in laboratory medicine to consider academic careers. Each year a call for abstracts is sent to each member, inviting submission of scientific papers.

All submitted abstracts are peer reviewed by a committee of ACLPS members selected confidentially by the director of the Young Investigator Program, Eric D. Spitzer, MD, PhD, FASCP. Reviewers are blinded to authors and institutions. Young Investigator Award recipients are granted free registration to the annual meeting, reimbursement for a portion of travel expenses, and the opportunity to present their scientific work before an audience of peers and mentors.

The following abstracts were presented at the 42nd Annual Meeting of the Academy of Clinical Laboratory Physicians and Scientists, June 7-9, 2007, in San Diego, CA. Authors receiving a 2007 Young Investigator Award are marked with an asterisk (*).

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A 2-Year Study of Patient Safety Competency Assessment in 29 Clinical Laboratories.
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Competency assessment is a critical component of laboratory operations and is mandated by the Clinical Laboratory Improvement Amendments. However, there have been no previous reports on methods for assessing competency in patient safety. In partnership with an online publishing company (Medical Training Solutions, Seattle, WA), we developed and implemented a computer-based tool to assess objectively the performance of 875 laboratory staff from 29 laboratories in patient safety. Web-based tests were administered semiannually for 2 years; the 4 tests contained a total of 40 questions. Of these, 5 questions were primarily about workplace culture, 10 about types of error (eg, cognitive vs noncognitive), 8 about prioritization of projects for patient safety interventions, 13 about specific interventions, and 4 about general patient safety concepts.

Overall, the mean score was 85% (range, 56%-100%). The questions were reliable (KR-20 coefficient, 0.79). When categorized by question type, the mean for workplace culture was 84%; types of error, 86%; prioritization of projects for intervention, 84%; specific interventions, 81%; and general concepts, 88%. Of the questions in the highest quartile (>98% correct), 3 were about types of error, 3 about prioritization of projects for intervention, 3 about specific interventions, 1 about workplace culture, and 1 about general concepts. For example, when asked about an event in which a nurse submitted a specimen with mismatched labels and laboratory staff failed to detect the error and analyzed the specimen, 100% of technologists correctly indicated that an intervention should be directed to the nursing and laboratory staff. Of the questions in the lowest quartile of scores (<72% correct), 6 were about specific interventions, 3 about types of error, 1 about workplace culture, and 1 about prioritization of projects for intervention. For example, 1 question asked, “A ‘blame-free’ approach is reasonable to apply to which of the following data entry errors?” Only 56% of technologists correctly chose “an error made by an employee when the lab was busy and understaffed”; 1% chose “an error made by an employee who was drunk”; and 39% chose “neither of the above.” Of 13 questions about specific interventions, 6 (46%) were in the lowest quartile, suggesting that this may be a relatively difficult topic for laboratory technologists.

Computer-based competency assessments help laboratories identify topics for continuing education in patient safety. This method allows supervisors to monitor individual performance of laboratory staff, to compare the group’s performance with that of other laboratories, and to set and monitor quantitative goals for performance improvement.

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Role of Cardiac Marker Screening Panels in the Detection of Acute Myocardial Infarction.
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Although cardiac-specific troponin appears to be the marker of choice for the diagnosis for acute myocardial infarction (AMI), many institutions continue to offer routine clinical screening with creatine kinase (CK) and troponin. At the Veterans Affairs Puget Sound Healthcare System Seattle Division Hospital, the screening protocol to rule out an AMI includes measurement of total CK, CK-MB fraction, and troponin T within 2 hours of presentation with repeated screening within 10 hours. To determine whether routinely providing total CK and CK-MB provides any additional benefit, all screening panels done between August 2005 and March 2006 at the Veterans

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Tissue inhibitor of metalloproteinase 1 (TIMP-1) is a glycoprotein that has an important role in the growth and spread of cancer. Past studies have demonstrated clinical utility for plasma TIMP-1 as a marker for colorectal cancer. Other studies have found associations between plasma TIMP-1 concentrations and other medical conditions, including other cancers, cardiovascular disease, diabetes mellitus, hepatic fibrosis, renal disease, and increasing age. The purpose of our research was to examine the clinical sensitivity and specificity of TIMP-1 for colorectal cancer in subjects undergoing colonoscopy for any indication.

EDTA plasma samples were collected from 2,003 subjects before colonoscopy, 11 of whom had colorectal cancer. Twelve additional plasma samples were collected from subjects before undergoing colorectal cancer resection. Of 23 subjects with cancer, 15 had stage I or II A and 8 had stage III or IV disease. TIMP-1 was measured using a 2-step sandwich microplate immunoassay or an enzyme-linked immunosorbent assay. Of the 23 subjects with cancer, 15 had elevated TIMP-1 plasma concentrations. Further studies on the use of TIMP-1 combined with CEA for colorectal cancer screening are warranted.
9 False-Positive Clonal Restriction of Lymphocytes in a Patient With Refractory T-Cell Prolymphocytic Leukemia Receiving Anti-CD52 Therapy: A Case Study.
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Our objective was to measure the interference, if any, of the drug alemtuzumab on fluorescence-activated cell sorting (FACS) analysis in the setting of a clinical immunohematology laboratory. Alemtuzumab, a monoclonal antibody that binds to surface CD52 on lymphocytes, is used to treat several forms of non-Hodgkin lymphoma, including refractory T-cell prolymphocytic leukemia (T-PLL). Based on the biologic properties of alemtuzumab, we speculated that this drug may influence FACS analysis of peripheral blood lymphocytes, particularly in the assessment of lymphocyte clonality and the uptake of anti-CD52 antibodies.

Peripheral blood specimens were obtained from 2 subjects, 1 receiving alemtuzumab for refractory T-PLL (of known immunophenotype) and 1 from a healthy volunteer. T and B lymphocytes from the specimens were analyzed by standard FACS protocol for the expression of several surface markers, including CD3, CD4, CD5, CD52, and \( \kappa \) and \( \lambda \) light chains. In addition, an aliquot of blood from the healthy donor was treated with alemtuzumab ex vivo and interrogated by FACS techniques.

In the patient with refractory T-PLL, a subset of malignant T cells expressing CD52 was identified. This malignant subset of T cells also demonstrated bright surface expression of \( \kappa \) light chain. In the healthy volunteer, no malignant cells were identified, and there was no expression of surface \( \kappa \) light chain within the untreated aliquot; CD52 was demonstrable. However, T cells from the volunteer sample treated with alemtuzumab demonstrated bright expression of surface \( \kappa \) light chain; expression of CD52 was similarly unaffected.

The administration of the anti-CD52 agent alemtuzumab causes binding of anti-\( \kappa \) to T cells, resulting in apparent \( \kappa \) restriction by FACS analysis. Therefore, treatment with this drug should be recognized as a potential interference for FACS-based diagnosis of a monoclonal lymphocyte population in peripheral blood. However, alemtuzumab does not diminish recognition of the CD52 receptor, nor does it hinder the ability to detect residual neoplastic T-cell disease by FACS analysis.

10 Refining von Willebrand Disease Reference Ranges by Blood Type to Decrease Equivocal Results.
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Diagnosing von Willebrand disease (vWD) can be difficult and must include clinical history and laboratory tests. Routine testing should include von Willebrand factor antigen (vWF:Ag), von Willebrand factor activity (vWF:RCo), and factor VIII activity (FVIII) levels. Caution should be observed when interpreting results of these assays as vWD. It has been shown that normal subjects with type O blood have lower levels of these vWD factors than people with non-type O blood. The purpose of this investigation was to establish reference ranges for vWF:Ag, vWF:RCo, and FVIII for blood type O and non-O individuals to help better detect the presence of vWD.

We tested 137 normal control subjects with no history of bleeding and 27 subjects with known or suspected vWD for vWF:Ag, vWF:RCo, and FVIII. Of the normal subjects, 61 had type O blood and 76 had non-type O blood. Mean results and 2 SD ranges for non-type O, type O, and vWD subjects were as follows, respectively: vWF:Ag, 128 (67-189), 92 (31-152), and 40 (3-76); vWF:RCo, 112 (42-181), 86 (27-145), and 28 (0-65); FVIII, 142 (61-224), 112 (43-181), and 58 (12-103); and vWF:RCo/vWF:Ag ratios, 0.88 (0.53-1.22), 0.94 (0.67-1.21), and 0.65 (0-1.3). For vWD patients with historic blood types available, 4 were non-type O and 4 were type O. Results were as follows: vWF:Ag, 31 (0-81) and 47 (26-68); vWF:RCo, 18 (0-51) and 45 (15-75); mean FVIII, 42 (0-93) and 66 (24-107); and mean vWF:RCo/vWF:Ag ratio, 0.53 (0.13-0.93) and 0.94 (0.7-1.2), respectively.

By determining the vWD reference ranges for O and non-O blood types, we can reduce the number of patients in the equivocal range, which we define as the overlap between the upper limit of the 2SD range for patients with known vWD and the lower limit of the 2SD range for normal non-type O patients. When a patient’s blood type is known to be non-O, the chance of an equivocal result is diminished because only 1 of the 76 non-type O normal subjects’ vWF:Ag result was within the equivocal range.

12 Effect of Hawthorn on Calcium Influx in Rat Cardiomyocytes: Differences Between Two Products.
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Hawthorn extracts (Crataegus species) are among the most popular herbal products in the United States and Europe (Pittler et al. Am J Med. 2003;114:665-674). These extracts are readily available and used by the general population to treat many cardiac diseases, including heart failure and angina. There are limited data on beneficial or detrimental effects of Hawthorn at the cellular level.

We studied potential cardiac effects of commercially available Hawthorn using rat cardiomyocytes. Two liquid extracts, a blend of Hawthorn flowers, leaves, and berries manufactured by Herb Pharm, Williams, OR (product 1), and an extract of Hawthorn berry manufactured by Gaia Herbs, Brevard, NC (product 2), were used to determine their effects on calcium influx in adult rat cardiomyocytes isolated using the Langerdorff procedure. Cells were incubated with Fluo4 (3 µmol/L), and calcium transients were recorded by real-time fluorescence spectrophotometry. Concurrent acquisitions of fluorescent images were also made after addition of the individual extracts, which mimicked in vivo serum concentrations after recommended dosages.

Addition of product 1 (1 µL/mL) resulted in initiation of robust calcium transients and eventual calcium overload. Addition of product 2 (1 µL/mL) resulted in increased sparking, eventual initiation of calcium transients, and, ultimately, an increased rate of beating, but no calcium overload was observed.

To further identify the mechanisms of increased calcium influx, adult rat cardiomyocytes were challenged with 10 µmol/L of ouabain, an Na+,K+-ATPase inhibitor, and, despite this addition, increased calcium transients were still observed, resulting in myocyte calcium overload. Adult myocytes were also challenged with nifedipine, a dihydropyridine calcium channel blocker, but the myocytes continued to proceed to increase Ca\(_2+\) (ionized calcium) and eventual calcium overload.

Our findings reveal that 2 readily available Hawthorn preparations demonstrate markedly different effects on an isolated adult rat myocyte model, suggesting important implications for patients who are using these preparations to supplement or even replace their prescribed
cardiac activity of Hawthorn, at least to some extent, is independent of the Na+,K+-ATPase and the l-type calcium channel.

14 Surrogate Markers of Epidermal Growth Factor Receptor Inhibition in Skin and Buccal Mucosa.
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Tyrosine kinase inhibitors targeting epidermal growth factor receptor (EGFR) have shown promise for treating some malignancies, but methods for selecting patients and determining optimal dosing are not yet defined. Demonstration of the desired effect on the targeted pathway during treatment could help guide therapy. Repeated sampling of the tumor is not always possible; therefore, surrogate markers are needed. Because EGFR has a role in renewal of the epidermis and oral mucosa, these are possible sites for measuring EGFR inhibition.

In a clinical trial of patients with advanced malignancies treated with the tyrosine kinase inhibitor gefitinib using 2 different dosing regimens, skin biopsy specimens were obtained before and on day 28 of therapy. These were stained immunohistochemically for the proliferation marker Ki-67 and for phospho-extracellular signal-related kinase (pERK), a molecule downstream from EGFR. Buccal swabs were collected before therapy and weekly during therapy, as were blood samples for measurement of serum gefitinib levels by immunoassay. Expression of c-fos mRNA, shown to be correlated with anti-EGFR response in preclinical experiments, was measured by RT-PCR in the buccal swabs.

Skin biopsies showed decreased nuclear staining for Ki-67 in the day 28 biopsy specimens relative to before therapy in 11 (79%) of 14 patients with available samples. Also, 11 (79%) of 14 cases showed the expected decrease in staining for pERK. However, neither the proportion of cells staining nor the magnitude of decrease correlated with steady-state serum gefitinib concentration measured on day 28. There was no correlation between buccal c-fos expression and serum drug levels. Patients displayed 2 distinct patterns of variation in buccal c-fos expression, with 13 (72%) of 18 showing initial suppression followed by rebound increase. The remaining 5 showed wide variation, some with more than 100-fold changes in c-fos expression, mostly above the pretreatment baseline.

Proliferation index and pERK expression in the epidermis decreased as expected during gefitinib therapy, but changes were not proportional to serum drug levels, suggesting a more complex relationship between dose and EGFR inhibition. Expression of c-fos in the buccal mucosa did not correlate with serum gefitinib levels. The finding of 2 distinct patterns of c-fos expression during treatment is intriguing and suggests distinct functional response to EGFR blockade by gefitinib.

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The prevalence of RBC alloantibodies has been previously reported to range from 0.6% to 2.0% in general patient populations. The frequency and properties of RBC alloantibodies have never been studied in male military veterans in a hospital setting.

Transfusion records of 18,750 military veterans at a VA medical center were retrospectively reviewed. For patients with RBC alloantibodies, the following data were collected: sex, decade of birth, results of antibody screens, alloantibody specificity, reaction phase, and whether alloantibodies were detected at the time of initial testing or after at least 1 prior negative antibody screen.

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RBC alloimmunization occurred in 2.36% (443 patients with 577 antibodies) of patients from a military veteran population that was 95% male. Alloimmunization rate varied with decade of birth, ranging from 1.23% (1941-1950) to 3.28% (1911-1920). The 10 most frequently identified alloantibodies in men, as a percentage of total antibodies, were as follows: K, 21.8%; E, 18.2%; D, 9.06%; Le_a, 7.39%; Fy_a, 5.36%; c, 4.81%; C, 4.62%; P_1, 3.88%; Jka, 3.70%; and Le_b, 3.51%. The stimulus for D alloimmunization in most men could not be identified because 80% developed this antibody before treatment at the VA. In the other 20% of cases, anti-D was most commonly associated with the infusion of Rh+ platelets. The percentage of patients making anti-D was highest in veterans born between 1911 and 1920 (0.44%). The majority of alloantibodies reacted at antiglobulin (AG) phase (93.9%). Even antibodies known typically to react at room temperature instead reacted mostly at AG and/or 37°C: anti-M, 18 of 18 (100%); anti-Jk_a, 10 of 11 (91%); and anti-P_1, 18 of 21 (86%). Of 405 alloantibodies for which at least 2 antibody screens were performed, 35.6% became undetectable over time. Anti-Jk_a had the highest rate of evanescence (11/14 [79%]) among alloantibodies occurring at least 10 times, whereas anti-D had the lowest rate (2/36 [6%]).

Overall alloantibody prevalence was as high as, or higher than, that reported in most mixed-gender populations. Anti-D was common despite the lack of pregnancy-related alloimmunization. Total and D alloimmunization varied by decade of birth and may reflect differences in medical and transfusion practice across combat eras. Compared with reports on other populations, alloantibodies made by male veterans tended to react more in AG phase and less at room temperature. Evasion was found to vary with alloantibody specificity.

A Rapid HPLC Method Used to Establish Pediatric Reference Intervals for Vitamins A and E.
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Retinol (vitamin A) and tocopherol (vitamin E) are fat-soluble vitamins that are measured in the clinical laboratory to assess deficiency or toxicity. Premature infants and children with diseases such as cystic fibrosis are often at risk of developing fat-soluble vitamin deficiencies; thus, establishing a pediatric reference interval is important. The purpose of this study was to evaluate reference intervals for vitamins A and E using an HPLC method that had been modified to decrease sample volume, run time, and mobile phase consumption.

More than 400 samples from healthy subjects were analyzed using this rapid HPLC method to establish pediatric reference intervals. Serum specimens were collected from healthy boys and girls, 7 to 17 years old. Samples were precipitated with ethanol, and retinol and α-tocopherol were extracted into hexane, evaporated under nitrogen, dissolved into ethanol, and injected onto an Agilent 1100 HPLC. The rapid HPLC method uses an Agilent Eclipse Plus C-18, 4.6 × 50 mm, 1.8-µm particle analytic column; 0.12-mm internal diameter tubing; and a diode array detector with a 10-mm path-length flow cell. Analytes were detected by their UV absorbance at 325 nm for retinol and 295 nm for α-tocopherol. The limit of quantification is 0.06 mg/L for retinol and is 0.6 mg/L for α-tocopherol.

Total imprecision values (% coefficient of variation) for retinol were 9.0%, 7.8%, and 8.0% at concentrations of 0.3, 0.59, and 2.04 mg/L, respectively. Total imprecision values for α-tocopherol were 6.3%, 5.0%, and 5.5% at concentrations of 5.6, 10.8, and 28.3 mg/L, respectively. For retinol, the Deming regression analysis gave a slope of 0.951 ± 0.023, a y-intercept of 0.004 ± 0.012, and an S_e of 0.019. For α-tocopherol, the Deming regression analysis gave a slope of 1.030 ± 0.044, a y-intercept of –0.35 ± 0.69, and an S_e of 1.17. The partitioning test for sex showed no statistical difference for either analyte. The nonparametric reference interval for retinol is 0.23 to 0.55 mg/L for ages 7 to 9; 0.28 to 0.68 mg/L for ages 10 to 12; 0.33 to 0.67 mg/L for ages 13 to 15; and 0.27 to 0.83 mg/L for ages 16 to 17. Partitioning test for age showed no statistical difference for α-tocopherol; the nonparametric reference interval is 5.0 to 12.9 mg/L. The modified HPLC method reduced sample volume by 50%, decreased run time by 60%, and decreased mobile phase consumption by 38%. The method showed comparable results to the previous method and is employed for clinical use. Reference intervals determined using this rapid HPLC method are comparable to previously published intervals (Soldin et al, 2005).

Pediatric Reference Intervals for Urine Calcium, Phosphorus, and Total Protein.
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Lack of pediatric reference intervals is an important issue in laboratory medicine. The aim of this study was to determine pediatric reference intervals for calcium, phosphorus, and total protein in urine. Determining pediatric reference intervals for these analytes in urine has important clinical implications. For example, urine total protein is helpful in monitoring children with renal disease. Urine phosphorus and calcium reference intervals can aid in predicting kidney stone formation and detecting disorders of calcium metabolism in children.

In this study, urine specimens were collected from 940 healthy children 7 to 17 years of age (469 boys and 471 girls). All analytes were measured using a MODULAR P analyzer (Roche Diagnostics, Indianapolis, IN). Results were normalized for creatinine and were reported as analyte/creatinine ratios. Reference intervals were partitioned by age and sex for each analyte. The 4 age groups were 7 to 9, 10 to 12, 13 to 15, and 16 to 17 years for boys and girls (8 groups total), with at least 83 samples in each group. Nonparametric analysis using EP Evaluator Release 5 software (David G. Rhoads Associates, Kennett Square, PA) was conducted to nonparametrically determine the central 95% reference intervals.

Results (in mg/g creatinine for all) were as follows: calcium: boys, 7 to 9 years, 7 to 434; 10 to 12 years, 7 to 300; 13 to 15 years, 7 to 304; and 16 to 17 years, 10 to 277; girls, 7 to 9 years, 17 to 589; 10 to 12 years, 11 to 355; 13 to 15 years, 5 to 334; and 16 to 17 years, 21 to 291; phosphorus: boys, 7 to 9 years, 164 to 1,707; 10 to 12 years, 121 to 1,309; 13 to 15 years, 102 to 1,269; 16 to 17 years, 67 to 913; girls, 7 to 9 years, 118.5 to 1,347; 10 to 12 years, 152 to 1,365; 13 to 15 years, 80 to 996; and 16 to 17 years, 56 to 905; urine total protein: boys, 7 to 9 years, 63 to 233; 10 to 12 years, 60 to 243; 13 to 15 years, 40 to 510; and 16 to 17 years, 30 to 179; girls, 7 to 9 years, 71 to 545; 10 to 12 years, 60 to 429; 13 to 15 years, 33 to 287; and 16 to 17 years, 27 to 321.

In general, reference intervals decreased with age for all analytes, and boys had higher analyte/creatinine ratios than did girls in the comparable age group, although there were exceptions to both trends. The establishment of these pediatric reference intervals for urine will aid in the diagnosis and management of a variety of pediatric disorders.
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Thrombin Generation Alterations in Patients With Trauma and Disseminated Intravascular Coagulation.
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Severe trauma can induce coagulation factor consumption and disseminated intravascular coagulation (DIC), presumably due to release of tissue factor (TF) and phospholipids by the injury. We evaluated thrombin generation in 28 trauma patients, of whom 14 showed evidence of DIC. Diagnosis of DIC was based on the International Society on Thrombosis and Haemostasis (ISTH) DIC score, which incorporates changes in platelet count, fibrinogen, prothrombin time (PT), and D dimer.

In trauma patients without DIC, median values were as follows: platelets, 183,000/μL; fibrinogen, 199 mg/dL; PT, 18.8 seconds; and D dimer, 3.7 μg/mL; in trauma patients with DIC, median values were as follows: platelets, 60,000/μL; fibrinogen, 101 mg/dL; PT, 24 seconds; and D dimer, 18.7 μg/mL. Citrate-anticoagulated plateletpoor plasma was recalcified in the presence of corn trypsin inhibitor (which blocks contact activation) and a thrombin-sensitive fluorogenic substrate. Thrombin generation was initiated by in vivo TF present in the plasma. In normal plasma, the lag time (from recalcification to the onset of thrombin generation) was found to be inversely proportional to the TF concentration, whereas the peak thrombin generation was a complex function of the coagulation factor, phospholipid, and TF levels. To simulate hemodilution in severe trauma patients during resuscitation, we diluted normal plasma with physiologic buffer to 60% of the normal coagulation factor concentration, which had no effect on the lag time or peak thrombin generation. In 15 healthy subjects, the lag time was 38.3 ± 11.1 minutes, and peak thrombin generation was 101 ± 56 nmol/L. Trauma patients without DIC (ISTH DIC score, 0-4; mean, 2.7) had the shortest lag times (19.9 ± 7.4 minutes; P < .001 vs normal subjects), and trauma patients with DIC (ISTH DIC score, 5-8; mean, 6.0) had lag times shorter than normal subjects but higher than trauma patients without DIC (28.6 ± 9.4; P = .009 vs normal subjects; P < .02 vs trauma patients without DIC; analysis of variance [ANOVA]). Peak thrombin generation had a similar distribution, with the most elevated levels in trauma patients without DIC (320 ± 117 nmol/L; P < .0001 vs normal subjects), whereas trauma patients with DIC had thrombin generation levels higher than normal subjects but lower than trauma patients without DIC (169 ± 70 nmol/L; P < .04 vs normal subjects; P < .0001 vs trauma patients without DIC, ANOVA).

These data suggest that moderate trauma and hemostatic insults but not overt DIC lead to increased thrombin generation, possibly due in part to TF in the plasma released during the trauma. In more severe trauma leading to overt DIC, thrombin generation is still elevated, even though coagulation factor levels are severely reduced.

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Measurement of the Active Leflunomide Metabolite A77 1726 in Human Plasma by High-Performance Liquid Chromatography.
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Leflunomide is a disease-modifying antirheumatic drug that not only alleviates symptoms of active rheumatoid arthritis, but also inhibits structural damage in affected joints. In vivo, essentially all leflunomide present is converted to its active metabolite, A77 1726. Therefore, most methodologies developed for pharmacokinetic studies monitor A77 1726 concentrations and not leflunomide concentrations.

A simple method for the measurement of A77 1726 in human plasma by high-performance liquid chromatography analysis has been developed. The sample preparation protocol is short and straightforward, the main step consisting of protein precipitation with methanol. Chromatographic separation of A77 1726 and the internal standard, 4-aminopyridine, was achieved using a strong cation exchange polySULFOETHYL Aspartamide column with UV detection at 280 nm. Isocratic elution was performed using a pH 3 buffer containing 25% acetonitrile, 10 mM of KH2PO4, and 150 mM of KCl. Each run was 7 minutes long. The reproducibility (β coefficient of variation) for intraday and interday assays of spiked controls was less than 3%. The limit of quantification was 1 μg/mL. The average absolute recovery was approximately 100%. Based on these results, this assay is highly suited to the determination of A77 1726 concentrations in patients taking leflunomide owing to its simplicity, reproducibility, and rapid analysis time.

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Differentiation of Bone Marrow Mesenchymal Stem Cells Toward the Smooth Muscle Cell Lineage by Blocking the ERK-MAPK Pathway.
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Smooth muscle cells (SMCs) are major components of blood vessels, required for the engineering of large and medium caliber vessels. Adult bone marrow–derived mesenchymal stem cells (BM MSCs) are multipotent cells that may be used in vessel regeneration. This study aimed to evaluate whether adult BM MSCs can be converted into SMCs. We examined the ERK-MAP kinase pathway because in vitro differentiation of SMCs is blocked by the MEK inhibitor, PD98059.

We used PD98059, an inhibitor of MEK to block ERK-MAPK pathway activation in BM MSCs. After 7-day treatment with PD98059, we evaluated SMC-specific marker gene expression in BM MSCs by RT-PCR and immunodetection. To elucidate the molecular target responsible for PD98059-induced BM MSC differentiation into SMC lineage, we used siRNA to knock down Elk-1, an antimyogenic transcription factor and a downstream target molecule of ERK-MAPK. We also evaluated ligand-induced cell contractility by measuring contraction of cell-embedded collagen gel (gel contraction).

Undifferentiated BM MSCs express most SMC marker genes, albeit mainly at low levels, except smooth muscle myosin heavy chain (SMMHC), the most definitive marker of differentiated SMCs. PD98059 treatment of BM MSCs up-regulated the expression of α-smooth muscle actin (ASMA), h-caldesmon, and SMMHC expression in BM MSCs in low serum conditions. PD98059-treated BM MSCs also acquired ligand-induced contractility to endothelin and carbachol. Interestingly, Elk-1 knockdown by siRNA induced SMMHC expression but decreased h-caldesmon expression and did not change ASMA expression.

ERK-MAPK pathway inhibition by PD98059 induces SMC marker genes, including SMMHC in BM MSCs. These cells gain ligand-induced contractility to endothelin and carbachol. Elk-1 might be partially responsible for PD98059-induced SMMHC expression; however, signaling molecules for up-regulation of ASMA and h-caldesmon expression remain uncertain. These findings provide impetus for rationale bioengineering of blood vessels.

Study supported by the CAP Foundation (K.T.) and NIGMS (A.W.).
**24 Pediatric Reference Intervals for Three Markers of Bone Metabolism.**

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The interpretation of pediatric bone marker results can be difficult because children have elevated bone marker concentrations compared with adults and bone marker values are known to vary with age. Presently, there is limited information on pediatric reference intervals for bone markers. The purpose of this study was to determine the reference intervals for 2 serum markers of bone formation, bone-specific alkaline phosphatase (BSAP) and osteocalcin (OSTEO) and 1 urine marker for bone resorption, cross-linked N-telopeptide (NTXc).

BSAP and OSTEO are molecules synthesized by osteoblasts that can be assayed in serum, and NTXc is generated by osteoclasts by cleaving the N-terminal domains of collagen and is present in serum and urine. Serum for BSAP and OSTEO and urine for NTXc was collected from 940 healthy children (470 boys and 470 girls) 7 to 17 years of age and assayed by chemiluminescent immunoassays according to manufacturer instructions. BSAP measurements were made using a Beckman Coulter DxI analyzer, OSTEO measurements were made using a Roche Elicys E170 analyzer, and NTXc measurements were made using an Ortho Clinical Diagnostics Vitros ECI analyzer and reported in nM bone collagen equivalent (BCE)/mM of creatinine. In groups with 120 or more subjects, the reference interval was determined nonparametrically by the central 95% range, whereas in groups with fewer than 120 subjects, the reference interval was determined as a nonparametric index.

Results were as follows: BSAP (µg/L): boys, 7 to 9 years, 49 to 140; 10 to 12 years, 49 to 156; 13 to 15 years, 28 to 211; and 16 to 17 years, 15 to 127; girls, 7 to 9 years, 36 to 159; 10 to 12 years, 44 to 163; 13 to 15 years, 15 to 136; and 16 to 17 years, 11 to 45; NTXc (nM BCE): boys, 7 to 9 years, 167 to 578; 10 to 12 years, 152 to 505; 13 to 15 years, 103 to 776; and 16 to 17 years, 34 to 313; girls, 7 to 9 years, 201 to 626; 10 to 12 years, 173 to 728; 13 to 15 years, 38 to 515; and 16 to 17 years, 20 to 144; OSTEO (ng/mL): boys, 7 to 9 years, 66 to 182; 10 to 12 years, 85 to 232; 13 to 15 years, 70 to 336; and 16 to 17 years, 43 to 237; girls, 7 to 9 years, 73 to 206; 10 to 12 years, 77 to 262; 13 to 15 years, 33 to 222; and 16 to 17 years, 24 to 99.

The results of this study show that the reference intervals for each of these 3 analytes change rapidly with pubertal development and should be partitioned by age and sex. It is our hope that with these new reference intervals, clinicians will better be able to evaluate diseases and conditions that affect bone growth and to monitor the effects of therapy in pediatric patients.

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**26 Cytokine Production by Macrophage Cultures to Goldenseal and Astragalus Extract.**

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Goldenseal is a native American medicinal plant used as an immune stimulant. Astragalus is a widely used herbal product in China, other Asian countries, and the United States as an immune stimulant. The interpretation of pediatric bone marker results can be difficult because children have elevated bone marker concentrations compared with adults and bone marker values are known to vary with age. Presently, there is limited information on pediatric reference intervals for bone markers. The purpose of this study was to determine the reference intervals for 2 serum markers of bone formation, bone-specific alkaline phosphatase (BSAP) and osteocalcin (OSTEO) and 1 urine marker for bone resorption, cross-linked N-telopeptide (NTXc).

BSAP and OSTEO are molecules synthesized by osteoblasts that can be assayed in serum, and NTXc is generated by osteoclasts by cleaving the N-terminal domains of collagen and is present in serum and urine. Serum for BSAP and OSTEO and urine for NTXc was collected from 940 healthy children (470 boys and 470 girls) 7 to 17 years of age and assayed by chemiluminescent immunoassays according to manufacturer instructions. BSAP measurements were made using a Beckman Coulter DxI analyzer, OSTEO measurements were made using a Roche Elicys E170 analyzer, and NTXc measurements were made using an Ortho Clinical Diagnostics Vitros ECI analyzer and reported in nM bone collagen equivalent (BCE)/mM of creatinine. In groups with 120 or more subjects, the reference interval was determined nonparametrically by the central 95% range, whereas in groups with fewer than 120 subjects, the reference interval was determined as a nonparametric index.

Results were as follows: BSAP (µg/L): boys, 7 to 9 years, 49 to 140; 10 to 12 years, 49 to 156; 13 to 15 years, 28 to 211; and 16 to 17 years, 15 to 127; girls, 7 to 9 years, 36 to 159; 10 to 12 years, 44 to 163; 13 to 15 years, 15 to 136; and 16 to 17 years, 11 to 45; NTXc (nM BCE): boys, 7 to 9 years, 167 to 578; 10 to 12 years, 152 to 505; 13 to 15 years, 103 to 776; and 16 to 17 years, 34 to 313; girls, 7 to 9 years, 201 to 626; 10 to 12 years, 173 to 728; 13 to 15 years, 38 to 515; and 16 to 17 years, 20 to 144; OSTEO (ng/mL): boys, 7 to 9 years, 66 to 182; 10 to 12 years, 85 to 232; 13 to 15 years, 70 to 336; and 16 to 17 years, 43 to 237; girls, 7 to 9 years, 73 to 206; 10 to 12 years, 77 to 262; 13 to 15 years, 33 to 222; and 16 to 17 years, 24 to 99.

The results of this study show that the reference intervals for each of these 3 analytes change rapidly with pubertal development and should be partitioned by age and sex. It is our hope that with these new reference intervals, clinicians will better be able to evaluate diseases and conditions that affect bone growth and to monitor the effects of therapy in pediatric patients.
immune stimulatory patterns, which may offer insight into differential macrophage responses. However, both products differ in their observed after 72 hours of incubation. MCP-1 production compared with controls. Similar results were also 3.2). In addition, both products of astragalus also demonstrated significant fold suppression of IL-10 at 24 and 72 hours for all 3 dilutions tested and significant fold suppression of IL-12 at 72 hours at the lowest dilution tested (1:50). AS-2 demonstrated MCP-1 production at 1:50 dilution, whereas AS-1 demonstrated no significant MCP-1 production compared with controls. Similar results were also observed after 72 hours of incubation.

Goldenseal and astragalus exhibit the ability to stimulate macrophage responses. However, both products differ in their immune stimulatory patterns, which may offer insight into differential use and the therapeutic potential of these products to regulate macrophage immune responses and activation events.

Goldenseal and astragalus exhibit the ability to stimulate macrophage responses. However, both products differ in their immune stimulatory patterns, which may offer insight into differential use and the therapeutic potential of these products to regulate macrophage immune responses and activation events.

Interaction of Grapefruit Juice With Acetaminophen in Mice. Meredith A. Reyes,* Semyon A. Risin, Jeffrey K. Actor, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School.

Grapefruit juice increases the bioavailability of a number of drugs due to inhibition of the P-glycoprotein pump by furanocoumarins contained within the juice. Moreover, intestinal CYP3A4 enzyme is also inhibited by these molecules. Acetaminophen is a popular over-the-counter pain medication that is toxic to the liver in high concentrations. The interaction of grapefruit juice with acetaminophen was examined in an in vivo mouse model. This drug-food interaction has never been previously reported.

Swiss Webster mice (Harlan, Houston, TX) were fed with 200 µL of white grapefruit juice (Ocean Spray, Lakeville, MA) or pink grapefruit juice (Tescum; Lake Wells, FL) by oral gavage (3 mice in each group) followed by orally delivered, 100 or 15 mg/kg, acetaminophen 1 hour later. Blood was withdrawn by retro-orbital bleeding 1 and 2 hours after feeding with acetaminophen. The concentrations of acetaminophen in serum samples of mice were determined by the fluorescence polarization immunoassay (FPIA) using an AxSYM analyzer (Abbott Laboratories, Abbott Park, IL). Our initial study with mice serum samples indicated that the FPIA designed for determination of acetaminophen in human serum samples can also accurately determine acetaminophen concentrations in serum samples of mice.

White grapefruit juice increased the bioavailability of acetaminophen in mice and also significantly prolonged its half-life. In contrast, pink grapefruit juice only prolonged the half-life of acetaminophen without significantly increasing bioavailability compared with the controls. Specifically, in mice fed with white grapefruit juice followed by acetaminophen, the mean acetaminophen concentrations were 107.8 µg/mL (SD, 18.3) and 40.27 µg/mL (SD, 16.1 µg/mL) 1 and 2 hours after feeding with acetaminophen, respectively. In contrast, the mean acetaminophen concentration in control mice was 62.2 µg/mL (19.3 µg/mL) 1 hour after administration of acetaminophen and 7.0 µg/mL (SD, 4.0 µg/mL) 2 hours after feeding. These differences were statistically significant by independent t test and Mann-Whitney U test (P < .05). When mice were fed with pink grapefruit juice followed by acetaminophen, the concentrations of acetaminophen were 76.9 (SD, 20.5) and 34.5 µg/mL (SD, 17.2 µg/mL) 1 and 2 hours after administration. Similar trends were observed when mice received a much lower dose of acetaminophen (15 mg/kg). In separate experiments, the duration of the effect of white grapefruit juice (up to 24 hours) was studied. The most significant effects lasted for 4 hours. We conclude that white grapefruit juice increased the bioavailability and prolonged the half-life of acetaminophen, whereas pink grapefruit juice only prolonged its half-life.

HPC-A Collection Efficiency and Engraftment Between HIV and Non-HIV Patients With Lymphoma. Andrea Chakrapani,* Carlo Palesi, Kala Mohandas, Joan Uhlinger, and Liljiana Vasovic. Department of Pathology, Montefiore Medical Center; Albert Einstein College of Medicine, Bronx, NY.

This retrospective study evaluated hematopoietic progenitor cell-apheresis (HPC-A) collection efficiency and engraftment in HIV and non-HIV patients with lymphoma. We reviewed the files of patients who had HPC-A collection/ transplantation at Montefiore Medical Center between 2003 and 2007. We identified 8 HIV lymphoma patients (HIV lymph pts) and 35 non-HIV lymphoma patients (non-HIV lymph pts). The HPC-A collections were performed after mobilization with G-CSF and/or chemotherapy.

Mobilization of peripheral blood (PB) CD34+ cells was significantly lower in the HIV lymph pts with an average number of 27.7×10^6 (range, 7.9-87.6 µL) compared with 85.1×10^6 (range, 8.9-746.7 µL) in non-HIV lymph pts (P < .01). Also, the total CD34 cell collection average was significantly lower for the HIV lymph pts, 1.71×10^9/kg (range, 0.26-4.2×10^9/kg) compared with 5.46×10^9/kg (range, 0.04-6.2×10^9/kg) in non-HIV lymph pts (P < .01). Collection efficiency in both populations correlated with the PB CD34+ cell count. For HIV lymph pts (n = 20 collections), the correlation coefficient was r = 0.82; for non-HIV lymph pts (n = 78 collections), it was r = 0.87. The infused CD34+ HPC dose was lower in the HIV lymph pts (4.22 vs 7.28 CD34+ cells×10^9/kg) in non-HIV lymph pts; P < .05). Posttransplant engraftment data for the HIV lymph pts were similar to those for non-HIV lymph pts. The median times until engraftment for absolute neutrophil count (ANC) more than 500µL, WBC count of 1,000µL, and platelet count more than 20,000µL were 11.80 days (range, 10-13 days), 11.80 days (range, 10-13 days), and 18.50 days (range, 12-35 days) in the HIV lymph pts and 10.89 days (range, 8-33 days), 11.33 days (range, 8-25 days), and 19.50 days (range, 7-118 days) in the non-HIV lymph pts, respectively.

HIV lymph pts have significantly lower PB CD34+ HPC mobilization and HPC-A collection average than non-HIV lymph pts, but there is a similar collection efficiency in both groups. Although the infused CD34+ cell dose was lower in the HIV lymph pts, engraftment was similar in both groups.

Blood Detection in Urine: Lower Threshold for the Clinitek Status Compared With the Yellow IRIS. William Nicholas Rose, Eric D. Spitzer, and Jay L. Bock. Department of Pathology, Stony Brook University Medical Center, Stony Brook, NY.

Presence of even a trace of blood in urine can reflect a serious condition such as bladder cancer. However, a tiny amount of blood can also be normal, and commercial systems using peroxidase dipstick methodology may differ in their threshold of detection. Because
we recently adopted the Clinitek Status (Siemens Medical Systems) dipstick reader for use in a point-of-care (POC) setting, we compared its blood detection with our main laboratory system, the Yellow Iris (IRIS International), which provides a dipstick analysis and RBC count. We retrospectively examined results during two 7-day periods, 2 months apart. The Status reports urine blood in 1 of 6 categories: negative (NEG), trace intact (TI), trace lysed (TL), small (S), moderate (M), and large (L). The Iris dipstick reader does not distinguish intact and lysed, reporting only trace (T). The quantitative cell counts from the IRIS were categorized as POS if more than 2 RBCs per high-power field were reported, and otherwise as NEG. A total of 501 urine samples were examined in the POC laboratory during the study. The POC laboratory serves only the Emergency Department (ED), so the study population included patients who presented to the ED for any reason and had a stat urinalysis ordered. The patients were 48% male and ranged in age from 1 month to 94 years (median, 44 years).

The results of the Status blood dipstick test were as follows: 241 (48%) NEG, 76 (15%) TL, 15 (3%) TL, 42 (8.4%) S, 49 (9.8%) M, and 78 (16%) L. The Status NEG specimens were not, by our clinical protocol, retested on the Iris. However, 10 Status NEG specimens were NEG on the Iris by dipstick and cell count. Trace and negative results were reproducible on the Status. Of the 76 specimens that were TI on the Status, 73 (96%) were NEG on the Iris by dipstick and cell count. Of the 15 specimens that were TL, 14 (93%) were NEG on the Iris by dipstick and cell count. Of specimens that were S, M, or L on the Status, NEG results by dipstick and cell count on the Iris occurred, respectively, in 10 (24%) of 42, 1 (2%) of 49, and 3 (4%) of 78 cases. None of the 260 specimens in any positive category on the Status were in a higher “more positive” category on the Iris dipstick.

In a diverse population of patients presenting to an ED, 52% tested at least trace positive for urine blood using the Status, and 87 (96%) of 91 of the Status trace positives were not confirmed as positive by dipstick or cell count using the Iris. By contrast, only 14 (8%) of 169 Status results in a higher positive category (S, M, or L) were not confirmed as positive by dipstick or cell count using the Iris. Using the Clinitek Status method for urine screening could result in considerably more follow-up testing than using the Yellow Iris, but further studies will be needed to determine if such follow-up is clinically appropriate.

### 32

**The Role of Flow Cytometry in Routine CSF Specimens.**

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In patients with central nervous system involvement of hematologic malignancies, conventional cytomorphic analysis of cerebrospinal fluid (CSF) has limited sensitivity in detecting malignant cells (15%–25%). Flow cytometric immunophenotyping (FCI) has been found to have at least 25% higher sensitivity than conventional cytomorphic analysis in detecting malignant cells. At our institution, the clinician directs CSF specimens to hematology for a cell count or to cytology for conventional morphologic analysis (cytopath). After cytopath review, the specimen can then be redirected for FCI, usually with a delay of up to 24 hours. We wanted to investigate whether this is the best use of a sample that is difficult to collect and easily degraded by time and improper collection media.

Cytopath results were examined on all CSF specimens collected in 2005 at a large academic medical center. Cytopath and FCI reports were correlated for all specimens available. Results were collected and catalogued according to the age, sex, submitting specialty, stated clinical history, cytopath diagnosis, and FCI diagnosis. Cytopath diagnoses were classified as negative or abnormal, which consisted of atypical, “suspicious,” or positive. We reviewed 211 CSF cytopath specimens. Of the 211 specimens reviewed, 85 (40%) were classified as abnormal. Of the cytopath abnormal cases, 55 (65%) were sent for FCI, in which 38 (69%) were negative, 8 (15%) were insufficient, and 9 (16%) were positive. Of the 85 abnormal cytopath cases, 30 did not have FCI performed, although 9 were submitted with a suspicion of hematologic malignancy. Of 211 specimens, 126 (60%) were classified as negative by cytopath. Of the cytopath-negative cases, 56 (44%) were sent for FCI, in which 27 (48%) were negative, 27 (48%) were insufficient, and 2 (4%) were positive.

Based on the data, proper handling of CSF includes a laboratory capable of immediate triage of specimens to the appropriate diagnostic modality and proper preservation media. Use of FCI with its greater diagnostic sensitivity might help the diagnosis in the 48% of cytopath-negative/FCI-insufficient specimens and in the 15% of cytopath-abnormal/FCI-insufficient cases. Routing of specimens in this way would eliminate the 35% of cytopath-abnormal specimens with no FCI analyses performed, especially when 11% of these cytopath-abnormal cases had a diagnosis suspicious for a hematologic malignancy. Proper routing of limited specimens such as CSF is paramount to institutional quality assurance.

### 33

**M Proteins as Potential Early Markers of Posttransplant Lymphoproliferative Disorder.**

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Posttransplant lymphoproliferative disorder (PTLD) is a potentially life-threatening complication occurring in up to 30% of graft recipients. Early diagnosis and intervention are important prognostic factors. Currently available screening assays have significant limitations; Epstein Barr viral (EBV) load, EBV-specific T-cell response, and serum interleukin-10 concentrations have been studied with varying results but are not routinely available. An increased incidence of monoclonal gammopathy associated with an elevated risk of developing PTLD was reported in liver and kidney allograft recipients. We investigated the prevalence of monoclonal gammopathy in serial serum samples from heart transplant recipients by 2 widely available tests: serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE).

For the study, 61 samples from 20 heart transplant recipients with PTLD were analyzed by SPE and IFE and interpreted by 3 blinded interpreters. Samples were interpreted as positive or negative for monoclonal gammopathy based on unambiguous analysis. Discordant results were reviewed, and resolution was attempted in a blinded manner. Tests were scored as indeterminate if no unanimous interpretation was achieved.

The median time from transplantation to the diagnosis of PTLD was 5.6 years (SD, 4.4 years), and the median time from the first sample to diagnosis of PTLD was 5.1 months (SD, 0.4 months). The interobserver variability was 14.8%. All 5 available pretransplant samples were negative. M proteins were detected in 11 of the remaining 56 samples; 2 were interpreted as indeterminate. These 13 samples came from 7 PTLD patients (35%). It is important to note that IFE was positive in all 7 patients, whereas SPE detected the M protein in only 3 patients. Of the 7 patients, 6 (86%) had an abnormal SPE/IFE before the diagnosis of PTLD (4 positive, 2 indeterminate). The M proteins were IgGκ (n = 3), IgGλ (n = 1), IgMκ (n = 1), IgAκ (n = 1), and λ light chain (n = 1). It is interesting that 1 EBV-negative patient and 1 patient with T-cell PTLD had monoclonal gammopathy.
SPE and IFE are widely available, noninvasive, and affordable tests that could complement current investigational screening assays for PTLD. With 1 exception, M proteins were always detected before diagnosis of PTLD in our series. IFE detected more than twice as many cases of M protein–positive PTLD than did SPE. This emphasizes the need for clinical laboratories to consider using both tests rather than using reflex IFE testing only for positive SPE samples.

**34**

Comparison of Hemagglutination and a Solid Phase ELISA for Determination of ABO Blood Group Antibody Titors.

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One solution to the increased demand for solid-organ transplants is the use of ABO-incompatible (ABO) organs. The ABO blood group anti-A/B titer, as measured by classical hemagglutination, is widely used to make clinical decisions on the timing of transplant patients and on the diagnosis of acute humoral rejection in ABOI cases. However, studies have shown poor interinstitutional reproducibility of this assay, and we sought to develop and validate an enzyme-linked immunosorbent assay (ELISA) using red cell ghosts as the solid phase antigen to produce a more precise and accurate quantitative measure of anti-A/B antibody concentrations in this growing patient population.

As proof-of-principle, we used 3 anonymized plasma samples from group B individuals representing high, medium, and low anti-A titers, 2 serial dilutions of each of these 3 samples, each in replicates of 5; and 3 undiluted, anonymized samples from an AB individual; to prepare a total of 48 blinded samples for testing. We then compared results obtained by a single trained blood bank technologist performing standard hemagglutination IgM titters in saline with group A red cells with the optical density results detecting IgM anti-A obtained by ELISA. These results showed a good correlation between ELISA and standard hemagglutination ($R^2 = 0.912$). The ELISA showed an interassay coefficient of variation ranging from 1.3% to 11.5% for low and high antibody concentrations, respectively. In contrast, the difference between maximum and minimum titer obtained by hemagglutination for replicates of the same sample was at least 2-fold, resulting in serial 2-fold dilutions that differed from each other by 0- to 4-fold.

We developed a solid-phase ELISA method for quantifying IgM anti-A concentrations that is more precise and accurate than the standard hemagglutination titer. In addition, this approach has the capability of quantifying IgG anti-A concentrations and those for individual IgG subclasses (ie, IgG1, IgG2, IgG3, and IgG4), which may prove to be more clinically relevant in this setting.

**35**

Determination of 8-Isoprostanes in Human Serum Using LC/MS/MS.

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The isoprostanes, which are prostaglandins produced via free radical–induced peroxidation of arachidonic acid, are gaining acceptance as reliable indicators of lipid peroxidation in vivo. However, owing to their labile nature and complicated formation pathway (different products and isomers are formed), the methods available for their measurement remain a challenge. Current methods for measuring isoprostanes in serum and urine include immunoassays or mass spectrometry. However, most of these methods suffer from poor reproducibility or long sample preparation times. The aim of this project was to develop a method to measure 8-iso-prostaglandin F2α (8-iso-PGF2α) in human serum using liquid chromatography–tandem mass spectrometry (LC/MS/MS).

To accomplish this, hormone-free human serum was spiked with 8-iso-PGF2α and its deuterated internal standard and extracted using a solid-phase extraction technique. We injected 50 µL of the sample into a Waters 2795 HPLC system using a Waters XTerra MS C18 3.5-µm column. The initial gradient consisted of 5% acetonitrile in ddH2O with a flow rate of 0.3 mL/min, ending at 33% acetonitrile in ddH2O at 20 minutes. 8-iso-PGF2α and 8-iso-PGF2α-d4 were detected using a Waters Micromass Quattro Micro Micro tandem MS operated in the negative ion, multiple reaction mode with the following transitions: 8-iso-PGF2α, m/z 353.20>193.25; 8-iso-PGF2α-d4, m/z 357.20>197.25 (retention time [Rt], 16.16 minutes for both). Our results showed clear separation of the 8-iso-15(R)-PGF2α (Rt, 15.71 minutes), 15(R)-PGF2α (Rt, 17.22 minutes), PGF2α (Rt, 17.8 minutes), and 15β-PGF2α (Rt, 17.82 minutes) isomers. The assay was linear from 20 pg/mL to 25 ng/mL (correlation coefficient = 0.9999). Recovery of 8-iso-PGF2α ranged from 81% to 106%. Precision studies showed an intra-assay coefficient of variation (CV) of 5.3% at 1.4 ng/mL.

We introduced a simple, sensitive, and reproducible LC/MS/MS assay to quantify 8-iso-PGF2α in human serum. Determination of the interassay CV and reference range is underway.

**36**

The Utility of Fetal Fibronectin in Preterm Labor Decision Making.

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Approximately 2% of pregnancies are complicated by threatened preterm labor (tPTL), treated with ultrasound (US), steroids, and tocolysis although 80% of these pregnancies will carry to term. Fetal fibronectin (fFN) has been used to predict outcome for these pregnancies. We evaluated fFN testing, with turnaround time (TAT) of 24 to 96 hours, to determine if fFN results predicted PTL or affected treatment and if fFN testing through a reference laboratory provided adequate care.

The fFN test was provided to 240 women with tPTL during 12 months. Retrospective chart reviews determined symptoms, treatment, fFN result, and gestational age (GA) at presentation, testing, and delivery. The 2-tailed, unpaired t test and the Fisher exact test (significance $P < .05$) were used for analysis. Demographics were similar in the test positive (fFN+) and negative (fFN-) groups. During the study, 236 of 240 women met GA requirements. Forty-nine had not delivered by the conclusion of the study or were lost to follow-up. An fFN+ result correlated with a relative risk of 4.3 (95% confidence interval, 2.2–8.6; $P = .001$), sensitivity was 43.8%; specificity, 89.9%; positive predictive value, 25.9%; and negative predictive value, 95.2% for delivery within 7 days of testing. The fFN result was available before delivery in 94.7% of patients (177/187). The median times from testing to delivery were 23 and 47.2 days for fFN+ and fFN– results, respectively. One patient delivered before...
fFN+ and 9 before fFN− results were available. Twenty-six patients had fFN+ results before delivery, and 17 (65.4%) received treatment. In the fFN− group, 44.0% (71/151) received treatment. Tocolytic use was similar between fFN+ and fFN− groups (P = 0.071). Steroids (P = .0008), US (P = 0.018), and antibiotics (P = .02) were associated with fFN+ results and increased length of hospital stay (LOS; P = .0078). Seven patients with an fFN+ result and no pTTL-related treatment had utraneous tract infection, closed cervix, and no signs or symptoms of pTTL. In the fFN− group, 85.4% (129/151) were discharged before a result was issued. The fastest TAT of our results (0.73 days) was used for comparing patients with an fFN− result and hospital stay longer than 0.73 days (n = 34). Treatment was different in patients discharged before or after a result (P = .03), but treatment was similar whether the patient was discharged the day of or multiple days after a negative result (P = .0). The LOS was the same if the patient was discharged before or after a negative result (P = .4).

Although a +fFN result correlates with a higher likelihood of delivering within 7 days and additional therapy with tocolytic, our data suggest that clinical decisions may be independent of a negative result.

37 The Functional Polymorphism of the Hemoglobin-Binding Protein Haptoglobin Influences Susceptibility to Idiopathic Parkinson Disease.
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Oxidative stress and iron have been widely implicated in the etiology of Parkinson disease (PD). Hemoglobin is the richest source of iron in the body and is capable of inducing oxidative stress damage. Human haptoglobin (Hp) is a plasma α2-glycoprotein that removes free hemoglobin from circulation and is important to protect kidneys and other tissues from hemoglobin-induced damage. Hp also functions in immune system regulation and angiogenesis; thus, it is potentially involved in protection from the development of PD. A common genetic polymorphism of Hp exists in the population of 312 idiopathic PD patients and 420 normal control subjects.

Genotyping was performed by polymerase chain reaction (PCR) on the subjects’ genomic DNA using Hp allele–specific primers, followed by agarose gel electrophoresis of the PCR products. A significant increase in the number of subjects carrying the Hp allele was determined in a population of 312 idiopathic PD patients and 420 normal control subjects.

Our results show that subjects with different Hp phenotypes have significantly different risks for idiopathic PD. To our knowledge, this is the first study that investigated the association of Hp phenotypes with idiopathic PD risk.
Clinical Chemistry Education of Pathology Residents. Jonathan R. Genzen¹ and Matthew D. Krasowski.² Department of Laboratory Medicine, Yale University, New Haven, CT; and Department of Pathology, University of Pittsburgh, Pittsburgh, PA.

The Academy of Clinical Laboratory Physicians and Scientists recently published a detailed proposed curriculum for training of pathology residents in clinical pathology. Our main objective was to focus on the current state of clinical chemistry education and identify areas of success and difficulty in teaching this subject to pathology residents.

We first attempted to identify the primary person responsible for clinical chemistry education at all accredited pathology residency programs in the United States. An anonymous, open-ended survey was sent to these faculty that inquired about the following: (1) aspects and methods of the clinical chemistry rotation that work particularly well, (2) major challenges in teaching residents clinical chemistry, and (3) impact of the shortening in length of pathology residency on chemistry education. Faculty members from 49 institutions returned surveys or agreed to telephone interviews.

Many respondents described in detail their efforts in engaging residents, including the challenges of having residents serve effectively on call. The major challenge in teaching clinical chemistry to pathology residents was identified by 59% of respondents as a lack of interest by residents. This was especially evident for residents seeking combined training in anatomic and clinical pathology, most of whom plan a future career with little or no responsibility for clinical chemistry. Nearly 40% of respondents indicated that the shortening of pathology residencies in 2002 negatively impacted clinical chemistry by shortening rotation time and/or forcing more anatomic pathology responsibilities during the chemistry rotation. The respondents also highlighted poor background knowledge and skills exhibited by many residents, especially quantitative, statistical, and abstract problem-solving skills. This hampers understanding of chemistry instrumentation and quality control methods. Last, some faculty members were positive about the future of clinical chemistry education in that areas such as genomics, proteomics, and molecular techniques are developing rapidly and are likely to be of more interest to pathology residents than “classical” clinical chemistry.

Clinical chemistry education of pathology residents faces a number of challenges, including lack of resident interest in the subject, compressed rotation schedules, and deficiencies in background knowledge and skills by residents. Opportunities to address these challenges include ongoing initiatives to reform medical school curricula and the incorporation of genomic and proteomic applications into clinical chemistry.
43 Molecular-Based Identification of Nocardia Species in the Clinical Microbiology Laboratory.

Nocardiae are ubiquitous, saprophytic bacteria that can also cause human disease (>30 species are known to infect humans). Correct identification of Nocardia to the species level is important because mortality rates and antimicrobial resistance patterns differ among species. However, the microbiologic culture and chemotaxonomic methods for species assignment are time-consuming, inaccurate, and not commonly practiced in many clinical laboratories. Molecular methods, such as 16S rRNA gene sequencing, have the potential to improve species identification. We evaluated a multigene sequencing approach to study the relative prevalence of Nocardia species at our institution.

We subcultured 34 clinical isolates, initially identified as Nocardia species by routine microbiologic methods, from frozen stocks or collected prospectively. Direct polymerase chain reaction and sequencing analysis of the 16S rRNA (1,512 base pairs [bp]), hsp65 (441 bp), and rpoB (559 bp) genes were performed from cultures of single colonies. The resulting sequences were compared with entries in GenBank by BLAST analysis. The 16S rRNA gene was considered the “gold standard” for species assignment. Selected chemotaxonomic tests were used to validate identity.

Since 2002, 34 clinical strains have been isolated at our New York City institution. By the 16S rRNA, 13 (38%) were identified as Nocardia nova, 9 (26%) as Nocardia cyriacigeorgica, 6 (18%) as Nocardia farcinica, 2 (6%) as Nocardia niigatensis, and 1 (3%) each as Nocardia abscessus, Nocardia otitidiscaviarum, and Nocardia transvalensis. The sequence of 1 isolate did not significantly match any GenBank entry and is most likely a novel species. Species identities based on hsp65 gene sequences were identical to those obtained by 16S rRNA analysis for 33 of 34 isolates; the hsp65 sequence of the N abscessus isolate was most similar to a Nocardia arthritidis GenBank sequence entry. Owing to the current paucity of entries, reliable identification based on rpoB sequences was not possible by BLAST analysis in GenBank. However, hsp65 and rpoB consistently showed higher intraspecies variability than the 16S rRNA gene.

Overall, 82% of our New York City clinical isolates belonged to 3 distinct species: N nova, N cyriacigeorgica, and N farcinica. This distribution differs from reports in the southwestern United States and central Europe. Identification of Nocardia species at our institution requires multigene sequencing, and 16S rRNA sequencing provides rapid and accurate identification that can be used to determine the number of species. However, the higher ratio of polymorphisms to length in hsp65 and rpoB potentially makes these individual targets more suitable for Nocardia strain discrimination than 16S rRNA.

44 Histologic and Microbiologic Diagnosis of Infective Endocarditis.
Sophie Arbefeville, Philip Kane, and Eric D. Spitzer. Department of Pathology, Stony Brook University Medical Center, Stony Brook, NY.

Treatment of infective endocarditis (IE) often involves replacement of the damaged valve. Histologic examination and culture of the excised valve confirm the diagnosis and can provide information on the adequacy of previous antibiotic treatment and/or guide future treatment. The objective of this study was to correlate histologic and bacteriologic results in patients with acute endocarditis.

We identified patients at our institution who had a histologic diagnosis of IE based on visualization of microorganism and/or changes consistent with IE during the 2002-2006 period and correlated this with valve tissue Gram stain (GS), culture (vcult), and blood culture results. The most frequent etiologic agents were viridans streptococci (n = 9), Staphylococcus aureus (n = 8), and Enterococcus faecalis (n = 5). In 29 specimens, organisms were seen in the histologic section; 15 were GS–/vcult–, 5 were GS+/vcult–, 4 were GS–/vcult+, and 5 were GS+/vcult+. In 3 specimens, organisms were not seen in the histologic section but were seen in the tissue GS (1 was also vcult+). The low incidence of positive valve cultures (10/32) was probably due to antibiotic treatment before valve replacement. Of the 10 positive valve cultures, 5 grew S aureus (3 were methicillin resistant). This probably reflects the difficulty of treating and/or the rapidly progressive nature of S aureus endocarditis. Of 29 specimens, 19 had a negative GS despite the observation of organisms in the paraffin section. This discrepancy is not readily explained by previous antibiotic treatment. Examination of the histologic slides revealed that only small numbers of microorganisms were present in many of the sections, even when there were large vegetations. In addition, organisms were often localized in microcolonies rather than uniformly distributed.

At our institution, the surgeon sends part of the surgical specimen to the anatomic pathology laboratory and part to the microbiology laboratory for GS/culture. We suspect that the low yield of the tissue GS in the microbiology laboratory is due to inadequate sampling. Having the surgical pathologist assess the entire specimen and decide which part of the specimen should be sent to the microbiology laboratory may improve the yield of valve GS/culture.

46 Sepsis-Induced T- and B-Lymphocyte Apoptosis Is MyD88-Dependent.
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Sepsis induces global lymphocyte apoptosis, resulting in impaired immune defenses and subsequent increased morbidity and mortality. There are multiple potential triggers or signaling molecules in mediating death signals. Elucidating the specific signaling pathways in sepsis-induced lymphocyte apoptosis may lead to improved therapies of this highly lethal disorder.

Presently, our studies were performed using the cecal ligation and puncture (CLP) model of clinical sepsis using specific gene-targeted deletions of the following receptors and signaling molecules: toll-like receptors 2 and 4 (TLR2/4), interleukin 1 receptor (IL-1R), myeloid differentiation factor 88 (MyD88), toll/IL-1 receptor domain–containing adapter-inducing interferon-β (TRIF), signal transducer and activator of transcription 1 and 4 (STAT1 and STAT4), and c-Jun N-terminal kinase (JNK). CLP-induced lymphocyte apoptosis was evaluated 24 hours postoperation by active caspase-3 and TUNEL staining.

Surprisingly, the only genetic construct that ameliorated sepsis-induced apoptosis occurred in the MyD88–/– mice. T- and B-lymphocyte apoptosis was significantly decreased in MyD88–/– mice by 80% and 85%, respectively, as evidenced by decreased active caspase-3
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Comparison of Roche’s New Prolactin II Assay With Roche’s Initial Method Based on Three Months of Patient Samples Before and After the Change.
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Our objective was to determine if the new, reformulated Roche Elecsys Prolactin II assay has less cross-reactivity with macroprolactin than the initial Roche Elecsys Prolactin assay.

We reviewed data from the last 3 months of use of the initial Roche method (August 2006-October 2006; total number of patients, 356; number of hyperprolactinemic patients, 88 [females, 53; males, 35]). The presence of macroprolactin was reported when the prolactin level fell to within the normal range after treatment with polyethylene glycol.

The data revealed an increase in the number of patients reported to have hyperprolactinemia with macroprolactin than the initial Roche Elecsys Prolactin assay.

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Aberrant T-Cell Antigen Expression in B-Cell Lymphoma.
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B-cell lymphomas often aberrantly coexpress T-cell antigens other than CD5 and CD43. We studied the incidence and spectrum of aberrant T-cell antigen expression in a large series of B-cell non-Hodgkin lymphomas analyzed in our laboratory between 2003 and 2006 using 4- or 8-color flow cytometry.

We searched our database for B-cell lymphomas in blood, body fluid, lymph node, tissue, or bone marrow specimens that were analyzed for expression of B- and T-cell antigens. We identified 1,042 cases of B-cell lymphoma in which neoplastic B cells represented more than 10% of the sampled WBCs, and we analyzed these cases for expression of CD2, CD3, CD4, CD7, and CD8. A T-cell antigen was considered aberrantly expressed if more than 25% of the abnormal B cells expressed this antigen and if the staining was confirmed not to represent nonspecific antibody binding, autofluorescence, or other artifact.

Overall, 3.5% (36/1,042) of the B-cell lymphomas aberrantly expressed 1 or more of these T-cell antigens. The most commonly expressed antigen was CD7 (17 cases) followed by CD8 (14 cases), CD2 (9 cases), and CD4 (1 case). No cases of CD3+ B-cell lymphoma were identified. Aberrant T-cell antigen expression was identified in 2.6% of cases having a CD5+ small cell immunophenotype (72/273), 1.6% of cases having a CD5–/CD10– small cell immunophenotype (3/186), 20% of hairy cell leukemias (3/15), 8.0% of cases having a CD10– large cell immunophenotype (9/113), and 3.1% of cases having a CD10+ large cell immunophenotype (14/455). Of the 14 CD10+ cases with T-cell antigen expression, 8 had a strikingly similar immunophenotype characterized by coexpression of CD7, CD5 (5 of 8 cases), CD8 (5 of 8 cases), and λ light chain restriction (all 8 cases). These represent the only cases in our series with convincing aberrant coexpression of multiple T-cell antigens. Histologic review revealed that 6 of these 8 cases were diffuse large B-cell lymphomas and that 2 were low-grade follicular lymphomas. Further characterization of these cases will determine whether this unique immunophenotype represents a novel subtype of follicle center lymphoma.

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Impact of Critical Value Cutoffs on Laboratory Operations.
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Immediate reporting of critical laboratory values is an integral part of quality patient care; however, no clear consensus exists in the literature as to what values should be considered critical for each laboratory test. As a result, defining critical values varies widely among hospitals. Our objectives were to determine the distribution of critical value results in our institution, compare our cutoffs for critical values with those for a peer group of 8 academic medical centers, and assess the impact of altering those values on call volume and allocation of laboratory personnel time.

We assessed our weekly critical result reporting data and determined the most frequent laboratory tests for which critical result calls were necessary. We then compared our critical value cutoffs for these tests with those used at 8 peer institutions. By using the average critical value for each test, we determined the potential impact on the number of critical result calls per week. In addition, we assessed the average time necessary to document notification of the critical result. By using
Based on this review, the median fluorescent intensity of CD38 is significantly decreased in follicular lymphoma compared with the germinal center B cells in follicular hyperplasia as measured by flow cytometry and does not vary significantly between background bystander T- and B-cell populations (serving as internal controls). These findings suggest CD38 is a useful marker for differentiating follicular lymphoma from follicular hyperplasia and may be helpful in identifying low-level involvement by follicular lymphoma in bone marrow aspirates.

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Use of a Resident On-Call Database to Characterize Communication Failures in Critical Value Reporting.

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The Clinical Laboratory Improvement Amendments require that laboratories have a procedure for reporting “imminent life-threatening results” to caregivers. Although this system has improved timely intervention, communication failures undermine its impact. When laboratory personnel at our institution are unable to reach care providers with critical laboratory results (CLRs), the laboratory medicine resident on-call ensures that the value is communicated and/or that appropriate action is taken on the patient’s behalf. We evaluated all resident-involved CLR calls to determine reasons for the communication failure and to define potential interventions.

The University of Washington Clinical Pathology program maintains an online database of all calls received by residents and fellows on call. From August 2004 through January 2006, the database contained 6,700 calls to residents and fellows on call. For the study, 592 calls regarding critical values were accrued by searching the “Call Classification,” “Specific Request,” and “Relevant Information” fields for the word critical.

In 318 (61%) of the 524 proven CLR calls, there was a documented reason for a communication failure. Of these 318 cases, 117 calls (37%) were because the ordering or covering providers did not return pages or calls; 37 calls (12%) were for patients from closed outpatient clinics; 26 calls (8%) were for discharged inpatients; 35 calls (11%) were related to confusing requisitions; 16 calls (5%) were related to confusing and/or unusual results; 7 calls (2%) were because the provider refused to accept the CLR; and in 6 calls (2%), there was an insufficient attempt to contact the provider. Of interest, 43% of the unreturned pages or calls were calls to outpatient providers, which was in keeping with the fraction of the total number of calls to outpatient providers (38%). Microbiology and chemistry initiated 44% and 36% of all calls, respectively.

Unreturned pages and inability to reach care providers for outpatients or discharged patients constitute the largest sources of communication failures. We are devising interventions to address the causes of communication failures identified in this analysis.

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Prognostic Potentials of Haptoglobin Phenotypes in Identifying Diabetics at Risk for Developing Diabetic Retinopathy.

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Information on 83% of patients (1,309) of these cases: hematocrit, 318 (24%); hemoglobin, 201 (15%); potassium, 230 (18%); troponin, 167 (13%); platelet count, 134 (10%); WBC count, 117 (9%); glucose, 78 (6%); and calcium, 64 (5%). Wide variability was found in the critical value cutoffs used among peer institutions. By using the average critical values for our 8 most common critical results, we estimate a 59% reduction in our critical result call volume, eliminating 933 total calls per week. This significant decrease in total calls, with an average time to documentation of 1 minute, would save an estimated 16 hours per week of laboratory personnel time, or the equivalent of 0.4 full-time equivalents per year.

In this study, we showed that critical value cutoffs vary widely among peer institutions. Although critical values must be chosen to accurately represent the severity of the clinical situation, appropriate choice can significantly impact call volume and, therefore, utilization of laboratory personnel time. Recently, the Joint Commission on Accreditation of Healthcare Organizations established a requirement that clinicians document the action taken on receiving a critical result. Review of this documentation could lead to the establishment of institution-appropriate critical values that maintain the highest degree of patient safety while improving laboratory personnel time management.

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Use of CD38 to Differentiate Follicular Lymphoma From Follicular Hyperplasia by Flow Cytometry.

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Follicular lymphoma can be difficult to differentiate from follicular hyperplasia in some cases by flow cytometry. In addition, low-level involvement of bone marrow by follicular lymphoma can be difficult to detect by flow cytometry given the normal CD10+ immature B-cell population typically present. It has been our observation that follicular lymphoma demonstrates CD38 expression at decreased levels compared with that present in follicular hyperplasia. This study was aimed at documenting this observation in an effort to help differentiate follicular lymphoma from follicular hyperplasia.

CD38 expression was assessed by flow cytometry in 11 lymph nodes with immunophenotypic changes characteristic of follicular hyperplasia and 18 lymph nodes selected based on the presence of a mature B-cell neoplasm with CD10 coexpression. Each of the 18 lymph nodes demonstrating neoplastic immunophenotypic changes by flow cytometry had a confirmatory morphologic diagnosis of follicular lymphoma. The level of CD38 was measured as the median fluorescence intensity of the atypical CD10+ cells in cases of follicular lymphoma and compared with the median fluorescent intensities of the CD10+ polyclonal germinal center population in cases of follicular hyperplasia.

The median fluorescent intensity in the neoplastic population was significantly different compared with normal germinal center B-cell cases of follicular hyperplasia (P < .005). Levels of CD38 did not differ significantly between the nonneoplastic B cells in cases of follicular lymphoma and non–germinal center B cells in follicular hyperplasia (P < .2), between T cells and nonneoplastic B cells in cases of follicular lymphoma (P < .3), between T cells and non–germinal center B cells in cases of follicular hyperplasia (P < .3), or between T cells in cases of follicular lymphoma and follicular hyperplasia (P < .3).
Diabetic retinopathy (DR), a retinal microangiopathy, is the leading cause of blindness in persons aged 20 to 74 years in the United States. Retinopathy has 2 stages: nonproliferative (NPDR), which consists of intraretinal microaneurysms, hemorrhages, and soft and hard exudates, and proliferative (PDR), a more serious condition characterized by neovascularization and fibrovascular growth from the retina. Retinal neovascularization has a tendency to be fragile and prone to hemorrhage and leakage. It has been suggested that hemorrhagic events, inflammation, and angiogenesis can have a major role in the etiology of DR. Free hemoglobin (Hb) released from RBCs following a hemorrhagic event can cause oxidative damage in different tissues. Haptoglobin (Hp), an α₁-glycoprotein, can bind free Hb and remove it from the circulation to prevent Hb and/or iron-mediated tissue damage. Also, Hp can modulate immune response and affect angiogenesis. Hp has 3 major phenotypes, Hp 1-1, Hp 2-1, and Hp 2-2. The overall biologic activity of Hp 2-2 is considerably lower than that of Hp 1-1. The focus of this project was to study the possible role and frequency of Hp phenotypes and oxidative stress (OS) in diabetics with various stages of DR.

So far, 20 diabetics with at least 20 years of type 1 diabetes have been involved in this project. Hp phenotyping was performed by gel electrophoresis. OS was assessed by measuring 8-isoprostan (LC/MS/MS), conjugated dienes, serum free iron, and total antioxidant capacity (photometry).

Our results showed that 63.2% of diabetics with DR had Hp 2-2, 26.3% had Hp 2-1, and 10.5% had Hp 1-1 phenotypes compared with 36%, 48%, and 16% in the general population, respectively. Also, the Hp concentration was lowest in Hp 2-2 (85 ± 36 mg/dL) and highest in Hp 1-1 (190 mg/dL), with Hp 2-1 (95 ± 26 mg/dL) in between. At this point, we have not seen significant differences in levels of serum markers of OS among our subjects. This study will be continued in our laboratory for the next few years.

Our preliminary results suggest that Hp 2-2 is highly associated with the presence of DR in type 1 diabetics and may be used to identify diabetics at higher risk for developing DR.
Methylmalonic acid (MMA), an intermediate in the metabolic transformation of propionic acid to succinic acid, is used as an indicator of vitamin B12 deficiency. Vitamin B12 (cobalamin) is an essential cofactor for the enzymatic carbon rearrangement of MMA to succinic acid. Vitamin B12 deficiency leads to increased concentrations of MMA. MMA reference intervals found in the literature were developed from studies of relatively small samples using gas chromatography–mass spectrometry, and they vary considerably. The objective of our study was to evaluate reference intervals for MMA in serum and urine, measured by a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method used in our laboratory, to provide interpretive guidance to clinicians.

MMA in serum and urine specimens was identified and quantified by LC-MS/MS. Nonfasting serum samples (n = 40) were collected from self-reported healthy subjects (24 women and 16 men) and random urine samples (n = 130) collected from self-reported healthy subjects (53 women and 77 men) were analyzed. Results were evaluated using a nonparametric method for reference interval determination. We also performed a database study. Results from 4,944 consecutive MMA tests in serum performed between November 1 and November 30, 2006, and results from 5,012 consecutive MMA tests in urine performed between September 2, 2002, and December 30, 2006, were evaluated using the nonparametric method following exclusion of the highest 10% of results. The reference intervals from healthy subjects for MMA in serum and urine had 0.09 to 0.35 µmol/L and 0.43 to 1.88 mmol/mol creatinine, respectively.

Representative ranges obtained from the database study were 0.05 to 0.45 µmol/L for MMA in serum and 0.20 to 3.00 mmol/mol creatinine for MMA in urine. Representative ranges determined by age were less than 0.51 µmol/L for the first decade, less than 0.26 µmol/L for the second decade, less than 0.33 µmol/L for the third decade, less than 0.40 µmol/L for the fourth and fifth decades, less than 0.42 µmol/L for the sixth decade, less than 0.44 µmol/L for the seventh decade, and less than 0.48 µmol/L for older than 70 years. Urine MMA representative ranges determined by age were less than 3.4 mmol/mol creatinine for the first decade and less than 3.0 mmol/mol creatinine for all others. These ranges are consistent with incidence of congenital methylmalonic aciduria in young children and prevalence of vitamin B12 deficiency in elderly people.

We have validated the reference intervals for MMA in serum and urine using an LC-MS/MS method. These results are comparable to those reported in the literature.

An Analysis of the Association Between Cytochrome P-450 2D6 Polymorphism and Incidence of Adverse Effects Caused by Tamoxifen in Women with Breast Cancer:

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Tamoxifen is an important drug for treatment and prevention of estrogen receptor–positive breast cancer. However, not all patients with estrogen-positive tumors can benefit from tamoxifen. No well-established markers are available to predict the efficacy and side effects of tamoxifen. Tamoxifen acts by blocking estrogen receptors and is metabolized in the body by cytochrome P-450 2D6 (CYP2D6) to endoxifen, which is 10-fold more potent in blocking estrogen receptors. Polymorphisms in CYP2D6, such as the *4 allele, can significantly decrease the enzyme activity. The Food and Drug Administration Clinical Pharmacology Subcommittee has recommended that drug manufacturers include information about CYP2D6 genotype and tamoxifen on the package insert label. However, clinical studies that examined the effect of the CYP2D6 *4 polymorphism on efficacy and side effects of tamoxifen have shown contradicting results. Some suggested poor metabolizers had a higher risk of disease relapse and lower incidence of hot flashes; others found no correlation between CYP2D6 *4 allele status and clinical outcomes; 1 study indicated patients with *4 alleles had better outcomes.

We analyzed the effect of CYP2D6 *4 polymorphism on side effects of tamoxifen in an ethnically diverse breast cancer patient population. Sixty patients who received tamoxifen for breast cancer treatment were genotyped for CYP2D6. DNA extracted from whole blood was amplified, followed by allele-specific extension and on which serum free light chain detection was performed. Only 21 of the 28 samples had an abnormal serum free light chain ratio, yielding a sensitivity of 75% for serum free light chain quantitation. Of the patients, 7 had normal serum light chain ratios, and 2 of these had normal serum electrophoresis and immunofixation results. In the group of samples with normal serum free light chain results and monoclonal free light chain proteins in the urine, 4 were from patients with a diagnosis of light chain amyloidosis. The remaining patients had treated multiple myeloma or monoclonal gammopathy of undetermined significance.

We also investigated if urine free light chain measurement by nephelometry can substitute for urine monoclonal protein quantitation by electrophoresis. A total of 133 clinical urine samples were tested. Compared with urine protein electrophoresis and immunofixation, the urine free light chain test had good sensitivity (90%) but poor specificity (55%) in this set. Of note, no samples with monoclonal urine free light chain protein of more than 0.1 g/24 hours had a normal urine light chain ratio. Of the 3 patients with positive immunofixation results but negative urine nephelometric light chain results, 1 had κ and λ monoclonal components. Two others had very faint monoclonal bands. Results of urine free light chain analysis correlated well with Bence-Jones protein quantitation (r = 0.98); however, the slope of the line was 5.9, and at low Bence-Jones protein concentrations, many outliers were encountered.

Urine electrophoresis and immunofixation may still have a role in diagnosis and follow-up of select patients with monoclonal gammapathies, particularly when primary amyloidosis is suspected. Moreover, quantitative urine free light chain analysis may be a reasonable substitute for monoclonal protein urine quantification by electrophoresis.
hybridization to a microarray. Of the 60 patients, 36 (60%) were homozygote wild type, 21 (35%) were heterozygote, and 3 (5%) were homozygote mutant for the *4 allele. Side effect information was available for 58 patients.

In univariate analysis, Caucasians had a significantly higher incidence of *4 polymorphisms compared with Asians (P = .036; 95% confidence interval, 0.01-0.31). No significant association was found between the incidence of adverse effects of tamoxifen (hot flashes, vaginal dryness, and sleeping problems) and *4 allele status (odds ratio, 1.9-8; P = .1-6). However, the number of patients who were homozygote mutant (*4/*4) was very low, making the statistical comparison difficult. Because intake of comedication that are CYP2D6 inhibitors also decreases the enzyme activity, we plan to increase the statistical power of the study by stratifying patients according to their status of CYP2D6 inhibitor intake and also including more study subjects. We will also analyze the association between CYP2D6 polymorphisms and incidence of cancer relapse, as well as serum endoxifen levels, in the same population.

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Development of a Seizure Panel to Detect Common Drugs Involved in Drug-Induced Seizures Using LCMS/MS.
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Many seizures in patients presenting to emergency departments have unexplained causes, and there is currently no method to quickly or reliably determine if a seizure is drug induced. Therefore, our objective was to develop a seizure panel to detect drugs in patient blood samples that may be the cause of a seizure.

A retrospective study of California Poison Control Center cases in 2003 revealed 386 seizures to be drug related. From this study, we chose 12 drugs that accounted for about 75% of all the drugs implicated in seizures to use in a seizure panel. The drugs are bupropion, citalopram, cocaine, diphenhydramine, isoniazid, lamotrigine, methamphetamine, MDMA, quetiapine, tiagabine, tramadol, and venlafaxine. Liquid chromatography–tandem mass spectrometry (LCMS/MS) was chosen as a technique for the seizure panel owing to its sensitivity and ability to detect a diverse range of compounds. Serum and plasma samples were subjected to solid-phase extraction before the 10-minute LCMS/MS run. The assay is semiquantitative, with a cutoff of 50 ng/mL for all of the drugs except isoniazid and lamotrigine, which have a cutoff of 500 ng/mL owing to their higher therapeutic blood levels. Drugs were also qualitatively identified, requiring a 60% or higher purity match between unknown and library product ion spectra. In a preliminary analysis of 33 samples from patients who had seizures, 2 were positive for lamotrigine.

A test that can detect the presence of the common drugs implicated in drug-induced seizures has the potential to help physicians rule in or rule out drug toxicity as the cause of an unexplained seizure. This knowledge can help in triaging patients and improve patient care.

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Evaluation of the Use and Utility of the Coagulation Screen.
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Coagulation tests are typically ordered to evaluate ongoing bleeding episodes, establish a preprocedure baseline, and/or monitor therapy. The number of monthly coagulation screens (prothrombin time [PT], partial thromboplastin time [PTT], thrombin time [TT], and fibrinogen) ordered at our institution almost tripled from just more than 1,200 in May 2000 to more than 3,000 in May 2006, out of proportion to the number of patients seen or admitted. We evaluated coagulation screens for a 6-month period in 2005 to evaluate use and improve utilization of coagulation testing.

We used laboratory criteria to classify the coagulation screens into diagnostic groups and did chart reviews to determine if the designated laboratory criteria were sufficient to accurately identify patients in each group.

A total of 28,737 screens were evaluated; 9,184 were initial screens and 19,553 were repeated screens. Of the repeated screens, 98% had normal initial and subsequent fibrinogen levels. All patients whose charts were reviewed had histories consistent with their assigned cohorts. Of all the coagulation screens, 40% were normal (PT, <15.6; PTT, <35), 36% had mild vitamin K–dependent factor deficiency (international normalized ratio [INR], 1.2-1.9; TT, <30), and another 12% had a probable lupus inhibitor. None of these screens had critical fibrinogen levels (<100 mg/dL). TT and fibrinogen levels are not useful in patients with normal PTs/PTTs, mild vitamin K–dependent factor deficiency, or a probable mild lupus inhibitor. Of the coagulation screens, 4% indicated vitamin K–dependent factor deficiency, most patients were taking warfarin (INR >2), and another 4% of coagulation screens indicated heparin therapy (PT, <20; PTT, >60). TT and fibrinogen levels are not useful in patients being monitored for anticoagulant therapy. Of the screens, 99.5% had fibrinogen levels higher than the treatment threshold. In the 0.5% (160 panels, 70 patients) with critical fibrinogen levels (<100 mg/dL), we recommended the faster emergency hemorrhage panel (PT, fibrinogen, platelets, hematocrit; turnaround time [TAT], 15 minutes) and not a coagulation screen (PT, PTT, TT, and fibrinogen; TAT, 45-90 minutes) to help decide what components (fresh frozen plasma, cryoprecipitate, platelets, RBCs) are needed. These patients all had ongoing bleeding or hemostatic abnormalities, and 73% of them died during or shortly after their hospitalization. Each patient with a critical fibrinogen level had an average of 15.2 panels done (range, 1-55 per patient).

TTs and fibrinogen levels are not routinely needed in patients with normal PT and PTT, mild vitamin K deficiency without bleeding, or lupus inhibitors without bleeding or for monitoring warfarin or heparin therapy. We are now changing the current coagulation screen (PT, PTT, fibrinogen, TT) to one consisting of PT and PTT only with the option to order TT and fibrinogen and recommending an emergency hemorrhage panel for patients who are actively bleeding or in unstable condition. A similar strategy applied to D dimer testing reduced utilization by more than 80%.