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 41st Annual Meeting of ACLPS, June 1-3, 2006, in Chicago, IL. Authors receiving a 2006 Young Investigator Award are marked with an asterisk (*).

ACLPS abstracts are published in the *American Journal of Clinical Pathology* (*AJCP*) as received by ACLPS without *AJCP* editorial involvement. Content and typographical errors and inconsistencies in these abstracts are the responsibility of the abstract authors.

1

Anatomic and Clinical Pathology of Chronic Pulmonary Thromboembolism Treated by Pulmonary Endarterectomy.

Mingyi Chen, MD, PhD,[1] Michael Madani, MD,[2] and Paul L. Wolf, MD.[1] Departments of [1]Pathology and Laboratory Medicine and [2]Surgery, Division of Cardiothoracic Surgery, University of California, San Diego, VA Medical Center, San Diego.

The objective of this investigation is to study the anatomic and clinical pathologic changes of chronic pulmonary thromboembolism treated by pulmonary endarterectomy. Pulmonary endarterectomy for the treatment of chronic, thromboembolic pulmonary hypertension is an important surgical procedure. It provides immediate and permanent relief of the pulmonary hypertension associated with the sequelae of unresolved pulmonary thromboembolic disease. This investigation describes the anatomic and clinical pathology studies performed at our institution in hundreds of patients who are referred to the cardiothoracic surgery division of the department of surgery, which was initiated by the chairmen of the division, Stuart W. Jamieson, MB, FRCS.

Patients with chronic pulmonary hypertension resulting from chronic occlusion of the pulmonary vessels by thromboemboli were selected. Neither anticoagulant nor vasodilators were effective in the treatment. The initial steps in the diagnosis hinge on a suspicion of pulmonary hypertension. The calculation of the degree of pulmonary hypertension is made by measurement of the reverse gradient across the tricuspid valve. A perfusion lung scan differentiates other causes of pulmonary hypertension. The definitive test is the pulmonary angiogram. The common cause resulting in chronic thromboembolic disease is deep vein thrombosis, which may be caused by prolonged immobilization or fracture of the pelvis or lower extremities. In addition, the clinical pathology workup includes the following coagulation tests: antithrombin III deficiency, deficiency of protein C and protein S, mutation of factor V Leiden, and the presence of lupus anticoagulant.

We have studied numerous pathologic pulmonary artery endarterectomy specimens removed after surgery and autopsy specimens. The new Jamieson classification of pulmonary occlusive disease due to thromboembolus proposed by the cardiothoracic surgeons and our pathology group is as follows: type I disease (30% of cases), major vessel thrombus present and readily visible on the opening of the pulmonary arteries; type II disease (60% of cases), no major vessel thrombus can be appreciated with only thickened intima, occasionally with webs; type III disease (10% of cases), very distal and confined to the segmental and subsegmental branches; type IV disease, inoperable with only intrinsic small-vessel disease, although secondary thrombus may occur as a result of stasis.

Pulmonary artery endarterectomy is a curative operation for patients with chronic thromboembolic pulmonary hypertension. Clinical coagulation tests are important in the assessment of the cause of the chronic thromboembolic pulmonary hypertension. Clinical and anatomic pathologists cooperate with cardiothoracic surgeons to benefit the patients.

3

Effect of Asian, Siberian, and Indian Ginseng on Calcium Efflux in Adult and Neonatal Cardiomyocytes: Cardiotonic in Adult Myocytes but Cardiotoxic in Neonatal Cells.

Ashley W. Allison, Brian J. Poindexter, Roger J. Bick, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston.*

Ginseng is used widely by the general population to improve cardiac health and circulation. Although loosely termed as ginseng, Asian (Panax), Siberian, and Indian ginseng are prepared from different plants.

We tested the popular belief of cardiotonic effects of various ginseng (liquid extract of Siberian and Asian ginseng manufactured in China and distributed by Z.T. Universal, Glen Head, NY, and Indian ginseng manufactured by Herb Pharmacy, William, OR) using rat cardiomyocytes. Neonatal rat cardiomyocytes were isolated by enzymatic digestion and cultured on laminin-coated

coverslips. Adult cardiomyocytes were isolated by enzymatic digestion using a Langendorff procedure. Cells were incubated with the calcium indicator fluroprobe Fluo4 (3 μmol/L final concentration), and calcium fluctuations (transients) were recorded with a real-time fluorescence spectrophotometer (Wallace/Perkin Elmer). Concurrent acquisitions of fluorescent images also were made following the addition of microliter quantities of extract mimicking in vivo serum concentration after recommended doses and an overdose. Extracts of Panax, Indian, and Siberian ginseng were compared with both cardiomyocyte types.

Addition of 10 μL of extract (per milliliter of culture medium) from each of 3 extracts tested on neonatal cardiomyocytes resulted in a rapid cessation of beating (<10 seconds) due to calcium overload. Sequential dilutions of the extracts revealed that addition of 0.1 μL of the extract (per milliliter of the medium) resulted in constant, regular beats (transients) and a slightly elevated diastolic concentration without calcium overload. Addition of extracts to isolated adult cardiomyocytes revealed that calcium transients were initiated in sparking, calcium-tolerant myocytes, and adult cells were able to tolerate high concentrations of ginseng extract. The magnitude of cardiotonic effects in adult cells (or cardiotoxicity in neonatal cells) was highest with Asian ginseng (2.6 times that of Siberian ginseng; 1.6 times that of Indian ginseng) because the active ingredients (ginsenosides in Asian, eleutherosides in Siberian, and withanolides in Indian ginseng) are structurally different. Whereas fully developed cardiomyocytes were able to accommodate moderate to high doses of ginseng, neonatal cells could not, and the effects on newly formed, developing myocytes, such as are found in the fetus, could be extremely deleterious. However, for adults, it might well be a "tonic" to myocytes in its ability to increase beating and intramyocytic calcium levels.

4

Reflexive Coagulation Testing.

Geoffrey S. Baird and Wayne L. Chandler. University of Washington Medical Center, Seattle.

At our institution, we offer a panel of coagulation tests to aid in the diagnosis of hemostatic disorders consisting of a prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), and fibrinogen. This panel was designed to evaluate and monitor patients with ongoing hemostatic defects, but we noted a large number of panels ordered for patients with no clinically apparent hemostatic abnormality. The panel was not intended for use in routine heparin or warfarin monitoring. TT testing was primarily done to detect heparin contamination falsely elevating PTT results. We hypothesized that if the PT and PTT were normal, it was unlikely that the fibrinogen level was critically low (<100 mg/dL) or that the TT was diagnostically useful.

Of 28,737 total panels collected during 6 months from 2 hospitals, 9,184 were initial tests and 19,553 were repeated tests; the average PT was 16.8 ± 6.0 seconds, the average PTT was 42 ± 24 seconds, and the average fibrinogen level was 471 ± 193 mg/dL. Of all panels, 11,103 (38.6% of the total) had PT and PTT values within the reference range, and none of this subset had fibrinogen levels less than 100 mg/dL, the institutionally recommended cutoff for cryoprecipitate use for a low fibrinogen level. In 26 panels with normal PT and PTT, the fibrinogen level was less than the lower limit of the reference range (150 mg/dL) but more than 100 mg/dL, and chart review showed that none of the 26 cases with borderline low fibrinogen levels were treated with plasma or cryoprecipitate. Two patients being monitored for hemostatic factor levels after undergoing plasmapheresis had normal PT and PTT values with borderline low fibrinogen levels of 138, 142, and 133 mg/dL. One patient had disseminated intravascular coagulation, initially with a prolonged PT and a critical fibrinogen level. After therapy, PT and PTT normalized, with 1 fibrinogen level of 141 mg/dL that was not treated. In patients with normal PT and PTT, the TT was normal or slightly prolonged at most, and there was no evidence the TT was used to change treatment or diagnosis.

A reflexive panel that performed fibrinogen and TT testing only in patients with an abnormal PT or PTT would not have missed any critical fibrinogen levels less than 100 mg/dL but would have missed 26 fibrinogen levels between 100 and 150 mg/dL that were not associated with changes in therapy. The number of missed borderline low fibrinogen levels could be reduced by using more conservative cutoffs for normal PT and PTT or by use of panels specific to individual practice, like a nonreflexive PT and fibrinogen level for monitoring plasmapheresis or a nonreflexive disseminated intravascular coagulation panel. Reflexive coagulation panels may be a viable method for reducing unnecessary clinical testing.

5

Heparin-Induced Thrombocytopenia Antibody Testing.

Geoffrey S. Baird and Wayne L. Chandler. University of Washington Medical Center, Seattle.*

Heparin-induced thrombocytopenia (HIT) develops in up to 1% to 3% of patients who are administered heparin, is thought to be due to the formation of antibodies to heparin-platelet factor 4 complexes, and can result in platelet destruction and activation, leading to thrombosis. The responsible antibodies (HIT Abs) can be identified with the Asserachrom kit (Stago), which uses an enzyme-linked immunosorbent assay (ELISA) method on citrated plasma. The ELISA generates a colored product that is measured by optical density (OD), and each kit provides a cutoff value for the OD, above which a positive result is reported. Published reports, however, indicate that the OD value itself can be used to stratify patients into at least 4 categories, such as negative (no antibodies present), borderline negative (a repeated test may be positive), positive (antibodies present), and strong positive (antibodies present with elevated risk of thrombosis).

During the first 4 months of testing after test introduction at 2 large hospital laboratories in the western United States, 206 HIT Ab tests were performed, of which 25 were reported as positive and 34 were repeated tests done after an initial negative test result. To investigate the possibility of stratified reporting, the OD values of patients who underwent repeated testing were analyzed to assess the likelihood of a subsequent positive test result given a prior negative test.

Patients with negative results and with "low" OD values, defined as less than 66% of the OD cutoff, were 10 times less likely to have a subsequent positive test result than those who had "borderline" OD values, defined as 66% to 99% of the OD cutoff (10% vs 100%; $P < .01$). Next, the medical records of the patients with positive test results were evaluated for 2 outcomes, death or thrombotic event, with the latter defined as a clinical diagnosis of thrombosis based on radiologic or laboratory evidence of a new thrombotic episode since receiving heparin. Patients with positive test results and OD values greater than 1.0 (strong positive) were approximately 4 times more likely to have a thrombotic episode than those with OD values between the cutoff and 1.0 (77% vs

20%; $P < .025$). Patients with "strong positive" results also were 3 times more likely to die during the 4-month period studied, but the result was not statistically significant (30% vs 10%; $P > .2$).

Because these results are consistent with those of several studies done at other institutions, we now report the results of the HIT Ab test as "negative," "borderline, may be positive on repeated testing" for tests with OD values between 66% and 99% of the cutoff, "positive," and "strong positive, elevated risk of thrombosis" for tests with OD values of more than 1.0. This hopefully will lead to larger studies prospectively evaluating this reporting strategy and its effect on patient outcomes.

6

ZAP70 Expression Assessed by Immunohistochemistry on Peripheral Blood: A Simple, Robust, and Prognostically Important Assay for Patients With Chronic Lymphocytic Leukemia.

Michele Roullet,[1] Rachel Sargent,[1] Terry Pasha,[1] Isabela Cajiao,[2,3] Stephen Liebhaber,[2,3] Paul Zhang,[1] and Adam Bagg.[1] Sponsor: Donald Young. Departments of [1]Pathology and Laboratory Medicine, [2]Medicine, and [3]Genetics, University of Pennsylvania School of Medicine, Philadelphia.*

ZAP70 expression is a potential surrogate marker for mutational status (SHM) of the immunoglobulin heavy chain variable (IgV_H) gene, which is currently one of the most powerful prognostic factors in patients with chronic lymphocytic leukemia (CLL). Given its complexity and cost, however, IgV_H SHM analysis is not well-suited to routine use in the clinical diagnostic laboratory. ZAP70 expression can be assessed by flow cytometry (FCM), immunohistochemical analysis, and other newer methods. However, technical constraints and standardization issues currently complicate FCM assessment. The usefulness of immunohistochemical analysis is restricted largely to tissue samples, precluding its routine application to most patients with CLL, in whom the diagnosis typically is made on peripheral blood (PB) sample analysis. Thus, we developed a simple, reliable, and robust immunohistochemical assay that can be performed on PB.

Whole blood samples from 29 patients with a confirmed diagnosis of CLL were collected. PB mononuclear cells (PBMCs) were purified, and a paraffin-embedded cell block was prepared. Immunohistochemical analysis for ZAP70 was carried out using a standard StreptAvidin Biotinylated Horseradish peroxidase immunohistochemical approach. Samples were scored as ZAP70+ if unequivocal staining was observed in more than 20% of the B cells. In all cases, IgV_H SHM analysis was performed by comparing complementary DNA sequences with a database and was scored as positive when there was a more than 2% deviation from germline sequences.

Of the 29 specimens, 17 (59%) were positive for ZAP70 expression and 12 (41%) were negative for ZAP70 expression. SHM was evident in 20 specimens (69%) and absent in 9 (31%). Of the specimens, 22 (76%) displayed "concordant" ZAP70 and SHM results, in that 15 (52%) were SHM+/ZAP70–, and 7 (24%) were SHM–/ZAP70+. Of the 7 "discordant" specimens, 5 (17%) were SHM+ and ZAP70+, and 2 (7%) were SHM– and ZAP70–. Whereas our 76% concordance rate is less than the approximately 90% initially reported, it is comparable to several subsequent studies. Indeed, ZAP70 expression may be a more powerful—and independent—prognosticator than SHM. It is important to note that immunohistochemical analysis of PB is a simple assay that circumvents the current standardization issues that confound the FCM assessment of ZAP70 expression, suggesting that this novel approach may have an important role in the routine clinical laboratory assessment of patients with CLL.

7

The Use of Mean Corpuscular Volume (MCV) With ABO/Rh and Select Antigen Phenotyping for Determination of Incorrectly Labeled Specimens.

Rachel Beddard[*] and Chantal Harrison. Department of Pathology, University of Texas Health Science Center at San Antonio.

The ramifications of specimens labeled with the wrong patient name are far-reaching and can lead to improper treatment decisions, delayed therapy, prolonged hospital stay, and even death. The discovery of these improperly labeled specimens before release of laboratory values would be of great value. An initial study of parameters, including hemoglobin, MCV, platelet count, and blood urea nitrogen, showed that a patient's MCV was stable across time, age, and type of diagnosis. The only exception was in the newborn period when MCV is known to drop quickly from birth during the subsequent weeks.

During a 4-month period, all specimens with a change in MCV of more than 5% within 24 hours were sequestered along with the prior specimen. Those associated with transfusion of RBCs between the 2 MCV values were excluded from further testing. ABO and Rh testing was performed on the remaining specimens. If one or both of these were discordant, no further testing was performed. If they were concordant, all Rh+ specimens were phenotyped for the C, c, and E antigens and all Rh– specimens were phenotyped for the M, N, and S antigens.

During this period, 280 specimens showed more than a 5% MCV change in 24 hours. Of these, 61 had no prior sample available for further testing and 154 were transfused RBCs between the 2 MCV determinations. Of the remaining 65, 9 had ABO inconsistencies (1 also showed Rh inconsistency). Additional phenotyping for selected antigens did not detect additional inconsistencies. Of the 9 with ABO inconsistencies, the percentage of MCV change ranged from 6.6% to 26%. Testing of duplicate CBC samples selected for a change in MCV of more than 5% showed that at least 13.8% were drawn from the wrong patient. The sensitivity of ABO testing for detecting a mismatch between samples of 2 different individuals is about 65%; thus, we estimate that 21.2% of samples that show an MCV change of more than 5% may have been drawn from the wrong patient. The extended antigen phenotyping did not seem to identify any additional inconsistencies.

These results show that a simple ABO/Rh test on samples suspected of being labeled improperly based on a significant change in MCV can be used to prevent the release of laboratory values that may otherwise compromise patient care. This approach also can be used to verify the effectiveness of processes put into place to improve positive patient identification.

8

Effect of the Indian Ayurvedic Medicine Ashwagandha on Measurement of Serum Digoxin Using Immunoassay: Study of Protein Binding and Interaction With Digibind.

Amanda Peterson,[*] Jeffery K. Actor, Margaret Olsen, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston.

Ashwagandha, a popular ayurvedic medicine for general well-being, has been in use in India for more than 3,000 years. Now, it is commercially available in the United States and gaining popularity. Alkaloids found in this herb have structural similarity with digoxin. We studied the effects of ashwagandha on measurement of serum digoxin concentrations and on 12 other commonly monitored drugs in vitro and in vivo (in-mouse model) using immunoassays.

Two liquid extracts of ashwagandha (product 1, Herbs Pharmacy, Williams, OR; and product 2, Herbs etc, Santa Fe, NM) and a dry powder (product 3) were purchased from a local herbal store. Aliquots of drug-free serum were supplemented with 10, 25, or 50 μL of each product (expected in vivo concentrations after recommended doses) per milliliter of serum (products 1 and 2 were used directly; product 3 was extracted with ethanol/water 60:40 by volume); and the apparent digoxin was measured using the fluorescence polarization immunoassay (FPIA), microparticle enzyme immunoassay (MEIA), and Beckman assay.

Significant apparent digoxin concentrations (range, 0.28-0.74 ng/mL) were observed using only the FPIA (MEIA and Beckman assay showed no apparent concentration). The magnitude of interference was highest with product 1 and lowest with product 3. Blood samples collected from mice after feeding herbs demonstrated similar in vivo apparent digoxin concentrations using only the FPIA. Apparent digoxin concentrations were stable in serum samples at room temperature for 1 week. Digoxin pools prepared from patients receiving digoxin were supplemented further with herbs, and falsely elevated digoxin concentrations were observed using the FPIA. Conversely, values were falsely lower (negative interference) with the MEIA. The Beckman assay was free of any interference. The immunoreactive components of ashwagandha were 46% to 54% bound to protein (mostly albumin). Although free apparent digoxin activity could be completely neutralized by using Digibind (as measured by free digoxin using the FPIA) in products 1 and 3, even a high concentration of Digibind (20 μg/mL) only partially neutralized the free components of product 2. Ashwagandha affected only serum digoxin measurement by the FPIA and MEIA but showed no interference with the measurement of concentrations of salicylate, theophylline, tobramycin, valproic acid, acetaminophen, amikacin, gentamicin, phenobarbital (Roche assays), carbamazepine, phenytoin, procainamide, and *N*-acetyl procainamide (FPIA assays; Abbott) in serum samples.

10

Anatomic and Clinical Pathology of Pseudomembranous Colitis Associated With a Virulent *Clostridium difficile.*

Armen Kasyan and Paul L. Wolf. Departments of Pathology and Laboratory Medicine, University of California, San Diego, VA Medical Center, San Diego.

The objective of this investigation was to study the causes and anatomic and clinical pathology of recent cases of pseudomembranous colitis. A marked increase in incidence and severity of pseudomembranous colitis has occurred at our medical centers. Nationwide reports and international cohort studies in Europe suggest similar worrisome evolution of *Clostridium difficile*–associated disease caused by a previously uncommon strain of *C difficile* with variations in toxin genes.

Patients with a clinical diagnosis of *C difficile* pseudomembranous colitis were selected. Medical records were reviewed to ascertain the presence of chosen criteria, including recent antibiotic therapy, comorbidities, etc. The collected data were correlated with the *C difficile* toxin level, WBC counts, and severity of clinical manifestations. One of our typical patients studied had diabetes mellitus and diabetic gangrene of a leg; he was treated with multiple antibiotics; and *C difficile* pseudomembranous colitis developed with fever, diarrhea, and a WBC count of 48,000. Despite treatment with metronidazole and vancomycin, the patient died with toxic megacolon. An autopsy revealed a classic *C difficile* pseudomembranous colitis with yellow pseudomembranes throughout the colon.

C difficile infection results in a broad spectrum of disease ranging from mild diarrhea to severe life-threatening conditions. Colonic injury results from the production of protein toxins, A and B. New binary CDT toxin has been mapped to a different gene locus. Exotoxin A causes an outpouring of fluid resulting in watery diarrhea, whereas exotoxin B causes damage to the colonic mucosa leading to pseudomembrane formation. The *cdtA* gene of a binary toxin causes cell death by disrupting actin filament assembly through ribosylation of adenosine diphosphate, whereas the *cdtB* gene mediates cell-surface binding and intracellular translocation. The recent increase in virulence is thought to be due to up-regulation of toxins A and B and coexpression of CDT binary toxin.

We found that the new virulent *C difficile* isolates were resistant to conventional treatment with metronidazole and vancomycin. Prognosis depended on the presence of comorbidities, including diabetes mellitus, cancer, and deficient immune system status. Aggressive antibiotic use is responsible for the emergence of a more virulent strain of *C difficile* refractory to usual treatment, resulting frequently in fatal pseudomembranous colitis.

11

Development and Characterization of a Real-Time PCR Assay for the Detection of a Marker of Carbapenem Resistance in *Klebsiella pneumoniae.*

Kar Fai Chow, Fann Wu, Richard C. Huard, Susan Whittier, and Phyllis Della-Latta. Sponsor: Steven Spitalnik. Department of Pathology, Columbia University Medical Center, New York, NY.*

The pervasive use of wide-spectrum antibiotics has led to a rapid increase in the incidence of multidrug resistant strains of *Klebsiella pneumoniae*. Carbapenems, once reserved as the antimicrobial drugs of last resort, now are used frequently to treat these infections. As a result, *K pneumoniae* strains resistant to carbapenems are commonplace in major medical centers, severely limiting therapeutic choices. A plasmid-transmitted class A β-lactamase, bla_{KPC}, has been reported as a marker for the detection of resistance to carbapenems. We developed a real-time polymerase chain reaction (PCR) assay that can rapidly and accurately detect this gene from *K pneumoniae*.

We studied 102 clinical isolates of *K pneumoniae* resistant to imipenem or meropenem (MIC, ≥ 8 μg/mL) by culture-based methods collected from November 2004 to November 2005. PCR primers were designed to amplify 203-base-pair and 183-base-pair regions from positions 506 to 708 and positions 853 to 1035 of the purported bla_{KPC} coding region, respectively. Fluorophore beacons were constructed to identify a single nucleotide difference between bla_{KPC-1} and $bla_{KPC-2\ \&\ 3}$ (position 520 A → G) and between bla_{KPC-2} and bla_{KPC-3} (position 814 C → T). Complementary DNA sequences of bla_{KPC-1} spanning the aforementioned regions were used as positive controls. Reference strains for bla_{KPC-2} and bla_{KPC-3} were identified by DNA sequencing. Strain relatedness was determined by PFGE.

Real-time PCR was 100% sensitive compared with the Etest for resistance to carbapenems, whereas automated systems were 89% sensitive. PFGE showed that 78% of bla_{KPC}+ isolates were indistinguishable or closely related, suggesting that the majority of the carbapenem-resistant *K pneumoniae* strains in our collection are composed of 1 dominant hospital-associated clone. In addition, because bla_{KPC} was identified in 11 additional clones, resistance to carbapenems was not clone-dependent. Our positive isolates carried bla_{KPC-2} or bla_{KPC-3}. This is consistent with published data that have shown that $bla_{KPC-2\ \&\ 3}$ are the prevalent strains in New York City. Our attempts at distinguishing bla_{KPC-2} from bla_{KPC-3} by real-time PCR were unsuccessful, possibly because position 814 is in a GC-rich region. Although other mechanisms of resistance to carbapenems are known, resistance among strains in our study is reliably determined by detection of bla_{KPC}.

Our real-time PCR assay can quickly and accurately detect bla_{KPC}, expediting appropriate therapy and infection control precautions. Clinical implementation is underway to identify the presence of bla_{KPC} directly from patient specimens.

12

Analysis of Zinc Protoporphyrin (ZPP) Testing in a Low-Income Primary Care and Lead Clinic.

Jonathan R. Genzen and Henry M. Rinder. Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT.

Whole blood ZPP levels often are elevated after moderate to severe lead exposure and frequently are requested along with measurements of serum lead in childhood screening. ZPP levels also are elevated in iron deficiency, often before the onset of anemia. To determine whether ZPP testing in our laboratory was providing valuable clinical information, a retrospective analysis of ZPP, lead, iron, and hemoglobin measurements was performed. All ZPP assays in the 12 months preceding February 2005 were included in this analysis, and the laboratory database was searched for additional information in cases in which the ZPP measurement was elevated (≥ 29 μg/dL). Clinical follow-up was documented through chart review.

In the year of analysis, 289 ZPP tests were performed for a total of 209 patients, almost exclusively children (98%; mean age, 3.9 ± 2.1 years). For comparison, 3,530 lead assays were ordered in our laboratory during this same interval. Of our 209 patients, 75 (36%) had at least 1 ZPP level of 29 μg/dL or more; 71 (95%) of these patients also had corresponding venous lead studies ordered. Of the 71 patients, 49 (69%) had at least 1 venous lead measurement of 10 μg/dL or more. Iron studies were ordered in only 15 (20%) of the 75 patients with elevated ZPP levels, and only 11 had documented iron deficiency anemia or iron supplementation. Thus, only slightly more than 50% of at-risk patients (15/26) were evaluated for iron deficiency.

Of our patients, 37 were identified as having 2 or more pairs of corresponding ZPP and lead measurements, permitting an analysis of these values over time. A poor statistical correlation (inverse or scattergram) between lead and ZPP measurements was detected in 19 cases (51%). The present analysis confirms that although the whole blood ZPP level poorly correlates with an elevated serum lead level (at initial evaluation and during follow-up), appropriate investigation into iron deficiency occurred infrequently, often only when anemia was apparent.

We propose that the substitution of iron studies for ZPP testing will improve patient care in this population by identifying and correcting an underrecognized nutritional deficiency with an equally important developmental impact. Results of this study were shared with the clinical service, and an initiative to emphasize screening for iron deficiency in this population is underway. Once iron deficiency screening is implemented, the laboratory plans to discontinue the ZPP assay.

14

Investigation of Simultaneous Identifications of Morphine and Hydromorphone in Urine of Patients on Morphine Alone.

Ping Wang,[1] Judith A. Stone,[1] Christine A. Haller,[1,2] Katherine H.Chen,[1] and Alan H.B. Wu.[1] Departments of [1]Laboratory Medicine and [2]Medicine, University of California at San Francisco.*

Accurate identification of opiates in urine samples is important for clinical and forensic purposes. In our practice of opiate confirmation using gas chromatography–mass spectrometry (GCMS), we have frequently observed morphine and hydromorphone for patients whose medications only listed morphine. Hydromorphone was detected at levels approximately 1% to 3% of morphine levels for a majority of urine samples with morphine concentrations more than 35,000 ng/mL. To study the source of hydromorphone in these patients, we examined the following possibilities: (1) generation of hydromorphone from morphine during the analytic process; (2) hydromorphone contamination of the morphine formulation administered; and (3) hydromorphone as a minor metabolite of morphine in humans. The metabolism of morphine to hydromorphone has been reported in nonhuman mammalian species.

All GCMS analyses were performed using the same procedure as that for patient urine samples. First, analysis of morphine and morphine-6-glucuronide standards at concentrations 3 times greater than the highest total morphine concentration observed or morphine-6-glucuronide expected in our patient samples showed no detectable hydromorphone, indicating that it was not created from morphine during the testing process. We further analyzed the specific morphine preparation administered to 7 patients. No hydromorphone was detected at the limit of detection (<10 ng/mL), suggesting that the drug itself was not contaminated with hydromorphone. The detection of hydromorphone in clinical urine samples is highly dependent on the hydrolysis method. We found that hydrolysis of hydromorphone glucuronide using β-glucuronidase, which is a common practice in many clinical laboratories, resulted in only 53% recovery of hydromorphone, greatly limiting its detection. We used an acid hydrolysis procedure to reliably detect hydromorphone.

Collectively, our data suggest that hydromorphone is a minor metabolite of morphine in humans. The inability to identify this potentially could be due to incomplete hydrolysis. A clinical study in postoperative patients under controlled dosing conditions is underway to further test this hypothesis. The detection of low levels of hydromorphone in urine samples containing high concentrations of morphine should be interpreted with caution because it may not be evidence of hydromorphone exposure.

16

Drug-Herb Interaction: Effect of St John's Wort on Absorption and Metabolism of Procainamide in Mice.

Melissa Hovanetz,[*] Jeffrey K. Actor, Margaret Olsen, Alice Wells, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston.

St John's wort, a popular herbal antidepressant, induces CYP3A4, causing treatment failure owing to increased metabolism of many drugs. Procainamide is metabolized by a different pathway to *N*-acetyl procainamide (NAPA). However, interaction between procainamide and St John's wort has never been studied.

We studied this drug-herb interaction using a mouse (Swiss Webster) model. St John's wort (liquid extract, Gaia Herbs, Brevard, NC) was purchased from a local health food store; the presence of active ingredients, hypericin and hyperforin, was confirmed by TLC analysis. We used the fluorescence polarization immunoassay for measuring procainamide and NAPA in serum samples of mice. Components of St John's wort did not interfere with these assays. One group of mice (group A, 4 mice in each group) was fed 75 μL of St John's wort a day for 2 weeks (last dose 1 day before feeding procainamide); another group (group B) received a same single dose of St John's wort for 1 week; the third group (group C) received only a single dose 1 hour before feeding with procainamide (to study acute effects); and the control group (group D) received no St John's wort. All groups received procainamide. Blood was drawn by retro-orbital bleeding, 1, 4, and 24 hours after feeding with a single dose of procainamide.

The procainamide concentration after 1 hour of feeding was highest in group C (mean, 11.5 μg/mL; SD, 2.3 μg/mL) followed by group A (mean, 9.9 μg/mL; SD, 1.3 μg/mL), whereas group B (mean, 7.4 μg/mL; SD, 0.9 μg/mL) and group D (mean, 7.3 μg/mL; SD, 2.0 μg/mL) showed comparable values. The concentration in group C was significantly greater than in the control group by independent *t* test ($P = .048$). We observed no statistically significant difference in procainamide concentrations between any groups after 4 hours, and values were not detected after 24 hours, indicating complete clearance of procainamide. It is interesting that no difference was observed between groups in NAPA concentrations or in the calculated half-life of procainamide (group A, 1.59 hours; group B, 1.82 hours; group C, 1.58 hours; and group D, 1.89 hours), indicating that St John's wort has no significant effect on metabolism of procainamide, but acute ingestion increases bioavailability. We hypothesized that the change is due to short-term inhibition of P-glycoprotein in the gut. In a different experiment in which mice were fed purified hypericin, the active component of St John's wort that inhibits P-glycoprotein, a significant increase in bioavailability (53%) of procainamide was observed compared with the control group, confirming our hypothesis.

17

Neutrophil CD64 Expression Is a Marker for Serious Infection.

Margret Oethinger, Christopher Tormey, George Fedoriw, and Henry Rinder. Department of Laboratory Medicine, Yale University, New Haven, CT.*

Prompt diagnosis of serious community-acquired infections is essential to improve prognosis. We prospectively studied neutrophil CD64 expression as an initial marker of serious infection in adults and children who underwent blood cultures within 24 hours of admission. Newly admitted, consecutive adult and pediatric patients (n = 600) were eligible if a CBC count was also obtained on admission; patients younger than 7 days old and those with neutrophil counts less than 2,000/μL were excluded. Infection was documented by positive cultures (blood and/or tissue) and chart review. Blood from the first available CBC count was used to quantify neutrophil CD64 expression as an index using a bead-based (Trillium Diagnostics) flow cytometric assay. Follow-up samples were studied when drawn within 12 hours of the initial sample.

An interim analysis of samples from 296 patients showed that the 124 patients with no infection had a mean ± SD CD64 index of 1.39 ± 1.70, significantly lower than the 2.05 ± 1.82 index in all 159 patients with bacterial infection and the index of 2.58 ± 2.30 in the 42 bacteremic patients ($P < .007$ for both). Receiver operating characteristic (ROC) analysis demonstrated area under the curve values for "all infection" and "bacteremia" to be 0.65 and 0.67, respectively. An index value of 1.5 was determined as the inflection point for all infection; indices of 1.5 or more yielded respective positive and negative predictive values of 69% and 51%. It is interesting that the mean ± SD neutrophil CD64 index in the 13 children with documented viral infection was 3.35 ± 2.17 ($P < .0001$ compared with patients without infection), and 12 of these 13 had an index of 1.5 or more (92% sensitivity). By contrast, the identified inflection for bacteremia was 2.0, which resulted in respective positive and negative predictive values of 53% and 83%. Among 92 patients with and 50 patients without infection who had follow-up CD64 indices, only the 27 bacteremic patients demonstrated a continuing rise in index; ROC analysis identified an inflection value of 0.5 for this increase. A rise in the subsequent neutrophil CD64 index by 0.5 or more had a 50% positive predictive value; more important, an increase of less than 0.5 was 90% predictive that bacteremia was *not* present.

This preliminary study of neutrophil CD64 expression suggests that it may be a useful index for identifying patients with serious infections, especially pediatric viral infection, and for excluding bacteremia in patients at risk by clinical criteria. Analysis of samples from the remaining 304 patients is underway to confirm and enlarge these findings.

18

Quantifying Microalbuminuria by Chip Microfluidic Electrophoresis.

Owen T.M. Chan and David A. Herold. Departments of Pathology, Veterans Affairs San Diego Medical Center and University of California at San Diego.

Microalbuminuria is an important prognostic marker in diabetic nephropathy and cardiovascular disease. Most commercial assays use immunoreactivity to quantify microalbuminuria; however, nonimmunoreactive forms exist that may not be detected by these tests. The aim of this study was to develop an assay that would measure total albumin (immunoreactive and nonimmunoreactive) without loss in sensitivity and accuracy.

We adapted the Experion Pro260 Analysis Kit developed by Bio-Rad Laboratories to quantify urine albumin. The Experion automated electrophoresis system uses Caliper Life Sciences' LabChip microfluidic separation technology and fluorescent sample detection to perform automated analysis of 10 protein samples per chip. Each albumin sample (66 kd) was mixed with the manufacturer's sample buffer and a chicken albumin (44 kd) internal standard. The samples then were electrophoresed under nondenaturing conditions and compared with the supplied sizing ladder.

With variable concentrations of bovine serum albumin normalized to the internal chicken albumin standard, the electrophoresis system was best fit with a polynomial ($R^2 = 0.99$) in the concentration range of 5 to 300 mg/L. The lower limit of detection was determined to be 5 mg/L. To analyze precision, patient urine samples were measured. For intrachip variation (1 chip = 10 wells), an 11-mg/L urine albumin specimen ("low concentration") yielded urine albumin/internal standard ratios having coefficients of variation

(CVs) ranging from 3% to 11%. A 123-mg/L urine albumin specimen ("high concentration") demonstrated CVs ranging from 6% to 7%. For interchip variation, the CV was 9% for the 11-mg/L urine albumin specimen and 8% for the 123-mg/L urine albumin specimen (n = 3 chips per albumin concentration). Compared with the Beckman Synchron LX20 immunoassay method, the chip electrophoresis system identified higher albumin concentrations in all patient urine samples with positive microalbuminuria (n = 14; y = 0.5212x + 10.819; R^2 = 0.8553). In addition, the chip was able to quantify albumin concentrations below the 20-mg/L threshold of the compared immunoassay (n = 28 cases).

The Experion chip automated electrophoresis system is able to detect immunoreactive and nonimmunoreactive forms of albumin, as evidenced by the consistently higher albumin concentrations compared with immunoassay. The chip electrophoresis system is a simple method to quantify microalbuminuria with good sensitivity, precision, and accuracy.

19

The Other Group G *Streptococcus*: Increased Detection of *Streptococcus canis* Ulcer Infections in Dog Owners.

Maggie M. Lam and Jill E. Clarridge III. Sponsor: Petrie M. Rainey. Veterans Affairs Puget Sound Health Care System, Seattle, WA.*

Large colony-forming, β-hemolytic Lancefield group G streptococci traditionally have been divided into human strains, which include *Streptococcus dysgalactiae*, and animal strains, such as *Streptococcus canis*. Although rare cases of *S canis* infections in humans have been reported in the literature, most clinical laboratories cannot distinguish between *S dysgalactiae* and *S canis*, because only Lancefield typing is performed; biochemical identification is not done and has been unreliable. The objectives were to determine the true incidence and pathogenic spectrum of *S canis* infection in humans and to further identify the species within the group G streptococci.

All clinically significant isolates of β-hemolytic streptococci were identified by a combination of Lancefield grouping, biochemical testing (Vitek instrument, API 20 Strep identification system), and 16s ribosomal RNA gene sequencing (when necessary). *S canis* isolates were selected for further study. Biochemical profiles found in the literature for *S canis* isolates infecting animals and humans were compared, and antibiotic susceptibility testing was performed.

We identified 3 isolates of *S canis* (confirmed with 16s RNA gene sequencing); all were associated with ulcer infections in dog owners. Identification with the Vitek 1 database was incorrect (99% probability for *Streptococcus equi* subspecies *equi*) but correct with Vitek 2 and API 20 Strep test systems. Literature values for biochemical phenotypes of *S canis* isolates infecting animals and humans varied. Key differences between our biochemical profiles and those reported in the literature were the CAMP reaction, esculin hydrolysis, β-glucuronidase, α-galactosidase, pyrrolidonylarylamidase, and acid production from lactose and trehalose. The organisms were susceptible to tested antibiotics, except for gentamicin and bacitracin.

Some detection methods have incorrectly identified strains of *S canis*. Thus, there may be an underestimation of the true number of infections due to *S canis.* However, with better identification methods, more infections due to *S canis* may be identified. Clinicians and laboratorians should be aware that this does not represent an emerging epidemic or an increase in the virulence of *S canis.* In addition, it may be important to identify group G streptococci to the species level because this could have epidemiologic and clinical implications. Finally, patients with wounds, especially ulcers, might be counseled to avoid or minimize contact with dogs.

21

Cutaneous Lymphoproliferative Disorders: A Retrospective Study at the University of Alabama at Birmingham.

Nicholaus J. Hilliard and Darshana Jhala. Department of Pathology and Laboratory Medicine, University of Alabama at Birmingham.

Cutaneous T-cell lymphoproliferative disorders, including primary cutaneous anaplastic large cell lymphoma (cALCL), cutaneous peripheral T-cell lymphoma (cPTCL), lymphomatoid papulosis (LyP), and other lesions are rare entities that may be difficult to differentiate. These cutaneous T-cell lymphomas can be related clonally, and they often show overlapping clinical and/or histologic features. Accordingly, a correct diagnosis always requires assessment of clinical, histologic, and immunophenotypic features. In addition to these T-cell disorders, classical Hodgkin disease (HD) also can involve the skin and can mimic some of the cutaneous T-cell lymphoproliferative disorders. The aim of this study was to explore cutaneous T-cell lymphoproliferative disorders and cutaneous HD at the University of Alabama at Birmingham.

A retrospective search was performed for the 1980-2005 period for cutaneous lymphoma and lymphomatoid papulosis. All patients with a diagnosis of a cutaneous lymphoma and lymphomatoid papulosis were included in the study and their biopsy specimens reviewed. The H&E-stained sections and immunohistochemical stains were reevaluated independently by 2 pathologists. Flow cytometry and molecular studies also were included whenever available.

We found 15 cases, including 13 men and 2 women (mean age, 54.4 years; range, 34-71 years) in our records with the diagnosis of cutaneous lymphoma (12 cases) and LyP (3 cases). Two patients had secondary cutaneous involvement, and 10 had primary cutaneous lymphoma. The histologic subtypes included the following: primary cALCL, 8; systemic ALCL involving skin, 1; LyP, 3; HD, 2 (1 primary cutaneous HD and 1 cutaneous involvement by systemic HD); and cPTCL, 1. Flow cytometric analysis (2/15 cases) and molecular studies (1/15 cases) supported the diagnosis in 3 cases.

Cutaneous lymphoproliferative disorders are more common in men. Two women had a diagnosis of ALCL (1 cALCL and 1 systemic ALCL). The 3 LyP cases and the 2 HD cases occurred in men. Because these entities overlap in clinical and/or histologic features, immunohistochemical panels with clusterin, survivin, and MAP-kinase expression may help delineate the biology of these different entities and aid in their differential diagnosis. Studies of these markers are underway.

22

SDHB Mutations in Malignant Extra-adrenal Pheochromocytomas.

Roger D. Klein, Long Jin, Kandelaria Rumilla, and Ricardo V. Lloyd. Sponsor: John F. O'Brien. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Germline mutations in the genes encoding the B (SDHB) and D (SDHD) subunits of the heterotetrameric protein succinate dehydrogenase (mitochondrial complex II) are important causes of inherited and apparently sporadic paragangliomas. Mutations in

SDHB seem to be found more frequently in association with extra-adrenal and malignant pheochromocytomas, whereas mutations in SDHD are identified more often in benign head and neck paragangliomas. Malignant and benign paragangliomas are difficult to distinguish by tumor morphologic features. In an effort to further study the role of SDHB and SDHD in apparently sporadic malignant extra-adrenal pheochromocytomas, we screened a series of such tumors for mutations in the SDHD and SDHB genes.

Mutation testing was performed on DNA extracted from paraffinized tumor and adjacent normal tissue by polymerase chain reaction amplification and direct sequencing of the coding regions and intron-exon junctions of the SDHB and SDHD genes. Among 10 extra-adrenal pheochromocytomas with proven metastatic disease, 3 nonsense, 1 insertion causing a frameshift, and 2 probable missense mutations were found in SDHB. One previously reported nonsense mutation, Arg90X, was found in tumors from 2 presumably unrelated patients. The same mutation was detected in DNA extracted from accompanying normal tissue for each of the 5 cases in which this analysis was performed.

Germline mutations in SDHB may be common in patients with malignant extra-adrenal pheochromocytomas. Our results support recommendations for genetic testing for SDHB in patients with pheochromocytomas as a potential risk factor for malignancy.

23

The Influence of Migration on the Abilities of Naive and Memory T Cells to Induce GVHD.

Britt E. Anderson,[1] Warren D. Shlomchik,[2] and Mark J. Shlomchik.[1] Sponsor: Brian R. Smith.[1] Departments of [1]Laboratory Medicine and [2]Medical Oncology, Yale University School of Medicine, New Haven, CT.*

Allogeneic stem cell transplantation (alloSCT) can cure many hematologic malignancies and hematopoietic stem cell disorders but frequently is complicated by graft-vs-host disease (GVHD). We and others have published that memory phenotype ($CD62L^{lo}CD44^{hi}$) T cells do not cause GVHD but can engraft and mount immune responses, including graft-vs-tumor effects. These findings apply to GVHD induced by CD4 or CD8 T cells and across major MHC differences. Thus, the inability to induce GVHD seems a fundamental property of memory phenotype (M) cells. One hypothesis to explain why naive cells (N) cause potent GVHD but M cells do not is that most M cells lack CD62L (termed effector memory [EM]) and fail to traffic to LN and PP, 2 sites that may be essential for initial priming to allogeneic antigens. However, a subset of memory cells, termed central memory (CM) cells *do* express CD62L, and it is of clinical interest for the design of M cell transfusion to determine the contributions of CM vs EM cells in GVHD. We therefore compared GVHD initiated by N, EM, and CM CD4 cells in the B6 ($H\text{-}2^b$) $\rightarrow$ BALB/c ($H\text{-}2^d$) model.

CM cells caused severe GVHD, similar to that induced by N cells. This suggests that LN homing enables M cells to initiate GVHD and/or that CM cells have unique functional properties required to initiate GVHD that are lacking in EM cells. We next asked whether LN entry was required for donor cells to initiate GVHD. N cells induced GVHD, albeit less severe than in WT recipients, in recipients lacking all secondary lymphoid organs (LN, PP, and spleen), suggesting that priming in tissues is sufficient. However, we also found that N cells from CD62L-deficient donors caused GVHD. Thus, because CD62L function and even secondary lymphoid tissue are dispensable for GVHD induction, we conclude that homing differences alone do not control GVHD and that critical functional properties other than homing must limit the ability of EM cells to induce GVHD.

24

Non-Hodgkin Lymphoma Involving Ovaries: A Retrospective Study at the University of Alabama at Birmingham.

John C. Lavelle and Darshana Jhala. Department of Pathology and Laboratory Medicine, University of Alabama at Birmingham.

Occurrence of lymphoma in the ovary is extremely rare. The most common initial signs and symptoms of malignant lymphoma involving ovaries are abdominal or pelvic pain or mass. Rarely, non-Hodgkin lymphoma (NHL) of ovary also can cause marked elevation of CA-125. Thus, these NHLs involving the ovaries can cause confusion for clinicians because the manifestations might resemble other, much more frequent ovarian tumors. Because the treatment and management is different for NHL involving the ovaries and for other more frequent ovarian tumors, it is important to diagnose NHL of the ovary correctly. The literature is sparse regarding NHL of the ovary. The aim of this study was to explore NHL of the ovary, including cytology and histology, at the University of Alabama at Birmingham.

A retrospective search was performed for the 1994-2004 period for ovarian lymphoma. All female patients with a diagnosis of lymphoma involving ovary were included in the study. The H&E-stained sections and immunohistochemical stains were reevaluated independently by 2 pathologists. Flow cytometry data also were included when available.

We found 6 women (mean age, 51.2 years; range, 22-80 years) with the diagnosis of a NHL involving the ovary. Of the 6 cases, 4 were secondary and 2 were primary. Cytology (touch prep) showed findings consistent with lymphoma in 3 cases. Histologic examination showed lymphoma in all 6 cases (diffuse large B-cell lymphoma, 2 cases; follicular center cell lymphoma, 3 cases; and Burkitt lymphoma, 1 case). Flow cytometry (3/6 cases) supported the diagnosis of lymphoma.

Although it is extremely rare, NHL involving the ovaries may cause confusion for clinicians with other much more frequent ovarian tumors. It should always be considered in the differential diagnosis of an ovarian tumor so it can be diagnosed correctly, leading to a proper treatment and management. Expression of clusterin, survivin, and p53 may help delineate the biology of the various subtypes of ovarian lymphoma. Studies of these markers are underway.

25

Epstein-Barr Virus (EBV) Shedding Patterns in Acute and Chronic Infection.

C.J. Holman,[1] C.-H. Webb,[1] K.M. Hokanson,[1] D.O. Schmeling,[1] M.E. Cook,[2] C.A. Romain,[3] and H.H. Balfour Jr.[1] Departments of [1]Laboratory Medicine and Pathology and [2]Pediatrics, and [3]Clinical Virology Laboratory, University of Minnesota Medical Center, Fairview, Minneapolis.

EBV is responsible for a variety of diseases in immunocompetent and immunocompromised patients. We postulated that the pattern and quantity of EBV shedding in body fluids could distinguish specific EBV-associated illnesses and predict their outcome. Because there is no Food and Drug Administration–approved method, we developed our own real-time TaqMan quantitative EBV polymerase chain reaction (qEBV). The target is a highly

conserved 71-base-pair portion of the EBNA-1 gene, and our assay can reliably detect 4 copies per reaction. Results are expressed as EBV copies per milliliter of whole blood (WB), CSF, bone marrow, bronchial lavage, or oral wash fluid.

From December 2002 to February 2006, 3,012 qEBV assays were requested on 831 clinic patients, and 1,102 qEBV tests were done on 51 young adults (median age, 20 years) participating in clinical trials of acute infectious mononucleosis (IM). The median age of the 831 clinic patients was 22 years (range, 3 months to 82 years); 469 (56%) were male, and the majority (58%) were recipients of solid organ or bone marrow transplants.

Of 831 patients, 162 (19%) had at least 1 positive qEBV (range, 10^2 to $10^{7.3}$ copies/mL). WB levels of more than 10^4 that persisted for more than 3 months were indicative of serious EBV disease; patients with PTLD had WB levels of more than 10^4 for a median of 12 months. In contrast, a single high qEBV WB level or steadily decreasing WB levels were associated with asymptomatic or self-limited illness. IM due to primary EBV infection was distinguished readily by its qEBV shedding pattern. In 40 IM subjects with primary EBV infection, the median WB level on enrollment was $10^{4.2}$ but became undetectable within 3 weeks, whereas median oral viral loads remained $10^{4.4}$ for at least 3 months. Oral viral shedding was ablated rapidly in 10 IM subjects with primary EBV who were treated with valacyclovir. Median viral loads in WB and oral washes were always less than 10^2 in the 11 subjects whose IM syndrome was not due to primary EBV. Patterns of EBV shedding can be useful in sorting out EBV infection from EBV disease, and the qEBV assay has the potential to evaluate efficacy of candidate anti-EBV drugs.

26

Using a Secure Internet-Accessible Database to Document Calls to Clinical Pathology Residents and to Improve Laboratory Medicine Education.

Andrew N. Hoofnagle and David Chou. Department of Laboratory Medicine, University of Washington, Seattle.

Clinical pathology residents and fellows serve as the liaisons between the laboratory and clinicians. Consultations to our department take place around the clock and come from a variety of patient care locations. To streamline the documentation of resident calls for subsequent discussion and to permit searchable access to similar calls previously addressed by their peers, we set out to establish an online database. To provide access from any computer on the Internet, we used an active server page-driven html front-end with a Microsoft Access back-end using secure sockets layer and authentication provided through IIS on a Windows 2000 server. Visualization of data was provided in html format and rich text format for easy import into word processors.

In the first 17 full months, the database has accrued 3,430 calls, an average of 7.1 calls per day for the first 10 months and 5.9 for the next 7 months. At the end of the first 10 months, the department tried to respond to resident concerns and reduce the burden of calls received during business hours, 8:00 AM to 5:00 PM Monday through Friday, when residents are busy learning the fundamentals of laboratory medicine. Two interventions were used, both involving changes in technologist behavior. The first placed the responsibility of interpreting prothrombin time and partial thromboplastin time mixing studies on the coagulation resident rather than the resident on call. The second intervention changed the first call from the chemistry and microbiology laboratories during business hours to the chemistry and microbiology fellows on call. These interventions resulted in a reduction in the average number of calls per day, as mentioned; however, the difference was not statistically significant ($P < .06$; Student t test). Although the number of business-hours mixing study interpretations decreased significantly from 0.43/d to 0.13/d ($P < .001$; Student t test), the overall number of calls during business hours did not decrease significantly (5.0/d vs 4.0/d; $P < .06$; Student t test).

The database permits access to a wealth of information obtained by other residents and reduces the amount of research needed to address questions from our clinical colleagues. In addition, the database can be used to monitor interventions aimed at improving resident education and to provide permanent secure electronic documentation of resident involvement in patient care, and it could be used to assess interventions aimed at improving client satisfaction with laboratory services.

28

Evaluation of Sure-Vue Serum/Urine hCG Test Kit Sensitivity and Result Reading Time in the Serum Pregnancy Test.

Shiwen Song, Surekha Gandhi, S. Farhat Quadri, Douglas Kimball, Alan Chakrin, Yi-Hua Chen, and Leonard Boral, MBA. Clinical Chemistry Laboratory, Department of Pathology, John H. Stroger Jr. Hospital of Cook County, Chicago, IL.

The serum human chorionic gonadotropin (hCG) test has become popular as a screening test to evaluate the pregnancy status of female patients who are simultaneously having serum testing for other analytes. Otherwise, a urine pregnancy test can be performed more easily and with less discomfort to the patient. A proper result reading time and the sensitivity of the test are important for physicians to appropriately manage their patients. Because the Sure-Vue serum/urine hCG kit recently won the bid for hCG screening at our facility, we tested the kit's sensitivity and result reading time using patients' serum samples.

In this study, we tested the sensitivity of the Sure-Vue serum/urine hCG kit (Fisher Scientific) on 20 positive serum samples from clinical patients, and the results were compared with those from the ICON serum/urine hCG test kit (Beckman-Coulter). Serial dilutions were made on all samples using hCG diluents from DPC or normal male serum to levels between 25 and 30 mIU/mL. The quantification of hCG was performed on the DPC Immulite 1000. Serum hCG tests were performed on the Sure-Vue and ICON kits according to the manufacturers' instructions. The results were recorded for at least 7 minutes, by which time all samples showed positive results.

The original concentrations of the serum hCG from the 20 patients ranged from 28 to 600 mIU/mL, and the final concentrations of the diluted samples were between 24.3 and 30.3 mIU/mL. Serum samples diluted with hCG diluent from DPC or male serum gave similar hCG concentrations when quantified by the DPC Immulite 1000. With the Sure-Vue kit, none of the samples showed positive results at 4 minutes (the claimed test time to detect 25 mIU/mL by Sure-Vue) regardless of the diluents used. However, positive results were observed between 5 and 7 minutes on all 20 samples. When tested with the ICON kit, all samples showed positive results within 4 minutes (claimed test time, 5 minutes to detect 25 mIU/mL).

Our results indicate that the Sure-Vue serum/urine hCG kit from Fisher Scientific is not able to detect hCG levels near 25 mIU/mL in 4 minutes in serum samples from patients. It appears that at least 7 minutes are required to reach the claimed sensitivity.

Therefore, all serum samples using this kit should be read up to 7 minutes to reduce false-negative results. This is longer than the 4 minutes indicated in the package insert for this test. On the other hand, the ICON test met our expectations and performed as described in the package insert.

29

Is Reduction of Additive Solution From Packed Red Blood Cells as Effective as Washing in Reducing Extracellular Potassium Concentration?

I. Bansal, B. Calhoun, C. Joseph, M. Pothiawala, and B. Baron. Department of Pathology, University of Chicago, Chicago, IL.

The extracellular potassium concentration (K^+) increases in the supernatant of whole blood and packed RBCs (pRBCs) with duration of blood product storage at 2°C to 6°C in anticoagulant-preservative solution (citrate-phosphate-dextrose-adenine [CPDA-1]) and additive solution (AS), which contains varying concentrations of adenine, dextrose, sodium chloride, and other substances. Previous studies have shown that to avoid hyperkalemia, washed pRBCs are the preferred alternative if fresh pRBCs (≤3 days old) are not available. To determine whether a simpler procedure, AS reduction, results in reduction of K^+ in pRBCs comparable to that achieved by washing, we compared the K^+ levels in blood units subjected to both methods.

Before and after K^+ levels were measured in 6 washed and 11 AS-reduced pRBC units. Each unit was weighed, hematocrit was determined, K^+ was measured on the Roche Modular System by the ion selective electrode method, and total K^+ was calculated using the following formula: Total K^+ (mEq) = (Unit Weight/1.05) × (1 – Hematocrit)/100) × extracellular K^+ (Weiskopf et al. *Transfusion.* 2005;45:1295-1301). Washed units were 3 to 21 days old, and AS-reduced units were 4 to 30 days old. Statistical analysis was performed using the Student *t* test.

Although there was no significant difference ($P > .35$) in the initial K^+ between the 2 groups (mean ± SD = 36.95 ± 13.16 mEq prewashing and 39.78 ± 19.94 mEq pre-AS reduction), washing and AS reduction both led to significant lowering of K^+ levels (mean ± SD = 2.15 ± 0.10 mEq postwashing and 4.41 ± 3.04 mEq post-AS reduction; $P < .0005$ in both groups). However, washing was significantly better than AS reduction in reducing K^+ in stored pRBCs ($P < .05$).

K^+ increases in pRBCs stored for extended periods (days to weeks) in CPDA-1 and AS. Washing pRBCs results in very low levels of residual K^+. AS reduction also significantly reduces K^+ levels. Selection of the method of K^+ reduction depends on several factors, including the stringency of K^+ reduction needed and the availability of facilities and staff for washing.

30

Disseminated Fungal Infection After Human Heart Valve Allograft Implantation: Probable Transmission by Contaminated Tissue Allograft.

L.J. Ryan[1] and T. Eastlund.[1,2] [1]Department of Laboratory Medicine and Pathology, University of Minnesota Medical School and [2]Pathology and Laboratory Medicine Service, Veterans Affairs Medical Center, Minneapolis.

Human heart valve allografts are advantageous because anticoagulant is not needed and they are relatively resistant to calcification and bacterial infection. The ability of contaminated human heart valve allografts to transmit fungal infections was reported in 1998, yet the incidence of transmitting infections from a tissue allograft to a recipient is unknown. We report a similar case involving valves provided by the same tissue bank.

A 50-year-old man with atrial fibrillation and aortic insufficiency received a human aortic valve allograft. Six months later, hemiparalysis developed secondary to intracerebral bleeding. Blood cultures were positive for *Candida albicans,* and a septic embolus from an infected heart valve was suspected. One month later, the patient had cervical spine osteomyelitis due to *C albicans.* An infected aortic valve allograft was removed and replaced 1 month later. Five months after valve replacement, the osteomyelitis of the lumbar spine developed.

Inspection of tissue bank records revealed yeast contamination of the pericardium and meniscus obtained from the same cadaveric donor; heart and valve tissue was not cultured before processing. An antifungal soak of the heart valve was used by the tissue bank during valve processing, but the actual heart valve was not cultured before cryopreservation and packaging. Postprocessing culture of contiguous heart tissue was negative, but antifungal residues used during processing may have led to false-negative findings, as shown in the previously reported case.

Although nucleic acid sequencing of the *C albicans* identified in cadaver donor tissue and in the patient was not performed, the disseminated *C albicans* infection in this patient probably was acquired from the use of a contaminated aortic valve allograft. Discarding tissues from cadaver donors that yield positive yeast cultures should prevent similar cases in the future.

31

Host Immunomodulation of *Schistosoma mansoni* Worm Development.

Steve Miller,[1] Emmitt Jolly,[2] and Jim McKerrow.[2] Sponsor: Enrique Terrzazas.[1] Departments of [1]Laboratory Medicine and [2]Pathology, University of California, San Francisco.*

Schistosoma mansoni is the second-most prevalent parasitic infection in humans and a significant cause of morbidity and mortality in endemic regions. The developing trematodes are relatively large, multicellular parasites that have evolved mechanisms to evade and exploit the host immune system. Although in most infectious disease, impairment of host immunity leads to an increased and sometimes overwhelming disease burden, in schistosome infection, the opposite is true. Since the 1940s, studies have shown impairment of parasite growth and egg production in immunocompromised hosts and people treated with corticosteroids. More recently, our laboratory has shown that parasites grown in recombination activating gene (RAG-1) knockout mice develop more slowly and do not pair or produce eggs effectively. These mice lack functional T and B cells, and the worm phenotype can be rescued by the addition of $CD4^+$ T cells.

Developmental *S mansoni* gene expression was assayed by high-density microarray and quantitative polymerase chain reaction techniques. Poly-A RNA samples were compared for juvenile worms grown in RAG-1 knockout mice and wild-type controls. Several genes are up-regulated in worms exposed to a functional host immune system including ribosomal proteins, structural proteins, proteases involved in blood digestion, and TGF-β signaling components. Interaction with host T cells is key to the initiation of worm growth and maturation, and this work has identified several

genetic pathways induced by this host-parasite interplay. These will serve as probes for further studies of worm development and parasite responses to host signals.

32

Decreased Apoptosis in Lung Cancer Cells Is Associated With an Increase in the Expression of Inducible Nitric Oxide Synthase.

George G. Chen, Tak W. Lee, Hu Xu, Johnson H.Y. Yip, Ernest C.W. Chak, and Anthony P.C. Yim. Sponsor: David E. Bruns. Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, N.T. Hong Kong.

Inducible nitric oxide synthase (iNOS) is expressed in response to several stresses, including inflammatory cytokines, bacterial endotoxin, and cigarette smoking. The link between cigarette smoking and the alteration of iNOS is particularly relevant to the pathogenesis of lung cancer because cigarette smoking is one of primary risk factors that cause lung cancer.

The present study was set to analyze the level of iNOS in non–small cell lung carcinoma (NSCLC) tissues and in lung cell lines. The results showed that the expression of iNOS was significantly higher in tumorous tissues than in nontumorous tissues. A similar result also was obtained in vitro by using human lung cancer cell lines and normal lung cells. However, the intensity of iNOS was stronger in the nucleus than in the cytoplasm of tumorous tissues. More than 50% of the cases tested did not express iNOS protein in nontumorous tissues. Statistical analysis indicated a negative correlation between iNOS expression and apoptosis in NSCLC tissues.

This study demonstrated a discrepant expression pattern of the increased iNOS and the decreased apoptosis in NSCLC tissues. Because the generation of iNOS is associated particularly with inflammatory and environmental factors of lung cancer risk, the increase in iNOS expression is believed to be associated with the pathogenic process in the development of human lung cancer.

34

***Nocardia cyriacigeorgica* as a Cause of Serious Pulmonary Infection: Diagnostic Approaches for This Emerging Pathogen.**

Robert Schlaberg,[*] Richard C. Huard, and Phyllis Della-Latta. Sponsor: Steven Spitalnik. Department of Pathology, Columbia University Medical Center, New York, NY.

Life-threatening infections caused by *Nocardia* species are increasing among immunocompromised patients and are associated with up to 50% mortality. Laboratory identification of *Nocardia* and its differentiation from rapidly growing mycobacteria can be challenging and time-consuming using routine culture and biochemical methods. *Nocardia cyriacigeorgica* is an emerging pathogen outside the United States, has a characteristic antimicrobial susceptibility pattern, and is misidentified often as *Nocardia asteroides*.

We recently encountered a *Nocardia*-like organism causing serious pulmonary disease in a heart transplant recipient. The strain eventually was revealed to be *N cyriacigeorgica* by 16S ribosomal RNA (rRNA) sequencing and secondary targeted biochemical testing. These methods then were applied to stocked *Nocardia* strains to characterize the prevalence of *N cyriacigeorgica* isolated at our institution.

Routine microbiologic methods for identification of gram-positive, filamentous, branching bacteria were originally applied. Antimicrobial susceptibility testing was done by disk diffusion and broth microdilution methods. Polymerase chain reaction (PCR) of the 16S rRNA gene base pair was done from single colonies grown on blood agar plates, followed by sequence analysis of the resulting PCR products. The data were compared with sequences in GenBank by BLAST analysis. Once characterized to the species level by molecular methods, secondary biochemical tests were used to confirm identity.

A total of 7 sequential pulmonary isolates from the index case were acquired, but routine microbiologic testing failed to identify the organisms beyond the genus level. 16S rRNA sequences (n = 7) matched 100% to GenBank entries for *N cyriacigeorgica*. The antimicrobial susceptibility pattern of 1 strain also was consistent with *N cyriacigeorgica*. In a retrospective analysis of 15 *Nocardia* strains isolated at our institution, we identified 2 as *N cyriacigeorgica* by 16S rRNA sequencing and targeted biochemical testing.

We describe the first diagnosed *N cyriacigeorgica* infections in the United States. For the purpose of generating quality data, microbiology laboratories need to recognize that *N cyriacigeorgica* can cause serious disease in immunocompromised patients, and they need to adapt laboratory protocols for its specific differentiation from *N asteroides* and other *Actinomycetes*. Molecular approaches for the identification of *Nocardia*, such as 16S rRNA sequencing, permit rapid, accurate diagnosis and appropriate therapy.

35

First-Time Platelet Apheresis Donors in a Hospital-Based Program: Demographics, Donation Outcome, and Return Behavior.

Brian T. Edelson,[1,2] Barbara A. Clutts,[2] Marian S. Dynis,[2] and Douglas M. Lublin.[1,2] [1]Department of Pathology and Immunology, Washington University and [2]Barnes-Jewish Hospital, St. Louis, MO.

Single donor platelets collected by apheresis represent a valuable and increasingly used blood component. As the demand for apheresis platelets increases, it is imperative that apheresis programs understand the demographics of new donors and the outcomes of their donations to most effectively ensure a continued platelet supply. Although several studies have examined the characteristics of first-time whole blood donors, few studies have focused on platelet apheresis donors. Our specific objectives were to assess the demographics, the deferral and miscollection rates, and the return behavior of first-time platelet apheresis donors.

We retrospectively identified all first-time platelet donors scheduled for donation at our hospital-based apheresis program (5,200 apheresis platelet donations per year) during the 1-year period from August 2003 to July 2004. Available donor demographics were analyzed, and all attempted donations by these donors during their first year in our program were assessed for outcome. To determine whether the time to second attempted donation predicted donor return behavior, donation attempts during the 365-day period following the second attempted donation also were tracked.

Of 437 first-time platelet donors scheduled for donation, 384 (88%) attended their scheduled donation attempt. First donation attempts by these 384 donors ended in successful product collection for only 65% of donors. Of the first-time donors, 27% were deferred on the first attempt; low hemoglobin values and poor venous access were the most common reasons for deferral. Apheresis was initiated on 282 first-time donors, with 11% of

these procedures ending in miscollection owing to aborted procedures. Women made up a disproportionate number of the deferred donors, and their first donation attempts were more likely to end in aborted procedures. Overall, a platelet product was obtained successfully from 49% of female and 71% of male first-time donors scheduling appointments.

Donors successful on their first attempt were more likely to return and to be successful on repeated donation than those whose first attempt ended in deferral or miscollection. A shorter interval between first and second attempted donations was correlated with increased repeated donations. Platelet collection centers and donor recruiters can make use of this information when attempting to recruit and retain new apheresis donors.

36

Rapid Quantitative Determination of Porphobilinogen in Urine Using Microspin Columns.

Mikhail Roshal and Petrie M. Rainey. Department of Laboratory Medicine, University of Washington Medical Center, Seattle.*

Large increases of urinary porphobilinogen (PBG) are indicative of an acute porphyria attack due to acute intermittent porphyria, variegate porphyria, or hereditary coproporphyria. Although these conditions are relatively rare, they share symptoms with a number of other more common conditions, such as acute surgical abdomen, and often need to be ruled out rapidly. Most common laboratory methods for PBG are modifications of the Mauzerall and Granick method using Dowex resin ion exchange followed by acid elution and reaction with *p*-dimethylaminobenzaldehyde in acid solution. The published quantitative methods are time-consuming and inconvenient. Reports of 2 rapid methods using bulk extraction have been published recently, but both methods seem to be qualitative.

We developed a 15-minute quantitative method using resin-packed microspin columns and 3-wavelength spectrophotometry to compensate for nonspecific interference. Dowex resin is added to a microcolumn and packed by a rapid spin. Urine is applied to the packed column and rapidly spun; the resin containing the PBG then is washed twice with water. PBG is eluted in 1 mol/L of acetic acid, and Ehrlich reagent is added to the eluate. After 5 minutes, absorbance is measured at 520, 553, and 585 nm. The concentration of PBG is calculated as $66*(A_{553} - ½(A_{520} + A_{585}))$. Subtraction of the background lowers variability in replicate measurements and allows more accurate determination of PBG concentrations. The reportable range is 0.1 to 15 mg/L (reference range, <2.3 mg/L). Between-day imprecision was 8.4% at 1.2 mg/L and 6.0% at 4.4 mg/L. Deming regression comparison with our established method yielded $y = 0.889x + 0.08$ ($r = 0.97$). No interference was noted from highly colored urine specimens. Implementation of this method should result in substantial reduction of turnaround time, improved accuracy and precision, and lower costs.

37

Automated BLAST Searching and Sequence Analysis for the Molecular Microbiology Laboratory.

Noah G. Hoffman, Dhruba J. SenGupta, and Brad T. Cookson. Department of Laboratory Medicine, University of Washington Medical Center, Seattle.*

Sequencing of polymerase chain reaction–amplified genes is the "gold standard" for species identification of certain slow-growing, unculturable, or phenotypically variable bacterial and fungal isolates and is being used increasingly to identify more routine organisms. Identification of an organism is achieved by comparison of sequences to a reference database and also may require phylogenetic analysis that describes evolutionary relationships between the clinical isolate and a set of related organisms. The National Center for Biotechnology Information provides fast searching of a vast collection of nucleotide sequences in the form of the BLAST Web interface. Unfortunately, the public consortium of databases that make up ENTREZ and the Web interface to BLAST prove difficult to use in a clinical setting. One obstacle is the large proportion of sequences that are not helpful (or indeed prove a hindrance) to making an identification; for example, of the nearly 240,000 ENTREZ entries identified as bacterial 16S ribosomal RNA, about 80% are too short or are from strains that are uncultured or incompletely identified. Of the remaining entries, only a subset are well-described in the literature and of high sequence quality. A further difficulty is the absence of nonproprietary software that unifies the multistep process comprised of a BLAST search, selection of a set of reliably identified records, and assembly of the corresponding sequence data. To address the aforementioned difficulties, we have written software that partially automates these components.

After a set of related sequences are identified via a BLAST search, additional information about each entry (including full-length sequence data) is retrieved from GenBank. Data from both queries, and some calculated parameters, are aggregated into a spreadsheet, which can be used to select a subset of sequences to include in a multiple sequence alignment and phylogenetic tree. Sequences are selected based on title (for example, by excluding uncultured isolates), number of ambiguities, association with a peer-reviewed publication, length, BLAST alignment score, percent identity, and number of gaps. The software is written in the Python programming language, is platform-neutral, and is installed on 2 computers in the laboratory running Windows XP. It is intended for the nontechnical user and is now being applied routinely to bacterial and fungal identification. The software accomplishes in a few minutes what previously took up to several hours of manual data retrieval and reformatting and enables a more systematic and comprehensive approach to sequence identification.

38

Performance of Sebia CAPILLARYS-II for Detection and Immunotyping of Monoclonal Paraproteins.

Zhaohai Yang, D. Keith Harrison, Yara A. Park, Carolyn H. Chaffin, Beatrice Thigpen, Pattye L. Easley, C. Andrew Robinson Jr, Robinna G. Lorenz, and Robert W. Hardy. Department of Pathology, University of Alabama at Birmingham.

Capillary electrophoresis systems for the analysis of monoclonal paraproteins offer improved turnaround time and potential cost savings. We evaluated the performance of the Sebia CAPILLARYS-II capillary electrophoresis (CE) system for the detection and identification of monoclonal proteins in serum samples. We randomly selected 104 serum specimens and analyzed them by Sebia Hydragel, agarose gel electrophoresis (AGE)/immunofixation electrophoresis (IFE), and CE/immunosubtraction (IS). AGE and IFE were used as the "gold standard," and results were interpreted by 8 readers blinded from the IFE results when interpreting the CE results.

We found that CE correctly identified 84% of cases that were positive for monoclonal band on AGE (sensitivity) and 97% cases that showed no monoclonal band on AGE (specificity), with an

overall agreement of 91%. The positive predictive value of CE was 95% and negative predictive value, 89%. AGE and CE missed IgA cases. IS and IFE agreed on the immunotype in 86% of cases that were positive for a monoclonal band. The percentages of each immunotype identified with IFE and IS were similar except for IgM (which IS failed to detect) and free light chains.

To further study the apparent discrepancy with IgM detection, we assessed 5 more samples that were quantitated for IgM by immunoassay. These samples had IgM concentrations ranging from 545 to 4,310 mg/dL, and all samples were detected equally using both methods and showed strong IgM bands on IFE. The failure to detect IgM initially was likely due to the low concentrations, as judged by weaker but clear bands on IFE.

We found that both systems had similar performance with respect to paraprotein detection and the failure to detect IgA in some cases; however, IFE seems to be more sensitive than IS for the detection of IgM paraproteins.

39

Diagnostic Yield in Posttreatment Lymphoma Biopsies.

Irfan Warraich, Nichlaus Hilliard, and Vishnu Reddy. University of Alabama at Birmingham.

Posttreatment residual or recurrent masses often are biopsied to evaluate neoplastic or nonneoplastic reactive changes. These biopsies include true cut needle biopsies, fine-needle aspirations (FNAs), and excisional biopsies. Morphologic evaluations and emerging molecular studies (eg, gene expression arrays) of these posttreatment biopsy specimens have a key role in the assessment of therapeutic response. However, posttreatment changes of these biopsy specimens can be a hampering factor in complete evaluation of these specimens.

The purpose of this study was to compare the diagnostic yield of pretreatment and posttreatment lymph node biopsies. Moreover, the incidence of various posttreatment changes and their effect on diagnostic yield is described. All posttreatment biopsy specimens (n = 79) obtained during the past 5 years were compared with the pretreatment biopsy specimens for the quality of the sample, morphologic features, flow cytometric yield/viability, immunohistochemical quality, and polymerase chain reaction (PCR) yield.

Most significant interval changes in posttreatment biopsy specimens were noted in morphologic evaluation of H&E-stains, including artifacts and marked increase in mixed inflammatory cells. Flow cytometry cellular yield/viability was low (25%) in posttreatment biopsy specimens compared with pretreatment biopsy specimens (85%). The most useful immunohistochemical markers were CD3, CD20, CD79a, CD15, CD30, EBV, and ALK-1 because their expression was seen in pretreatment and posttreatment biopsy specimens. Malignancy was found in 53% of the posttreatment biopsy specimens by morphologic evaluation, but PCR results were positive in 18% of the cases, and 75% of the pretreatment neoplastic cases had positive PCR results.

Posttreatment biopsy specimens pose new diagnostic challenges. A significant degree of fibrosis, mixed inflammatory infiltrate, and necrosis were noted in this study, which limits morphologic (architecture and cytologic) evaluation and cellular yield/viability for ancillary tests, thus limiting diagnostic yield. Although minimally invasive procedures decrease morbidity and mortality in patients, decreasing biopsy specimen size and FNA procedures further compound the problem because the small sample may provide only a small glimpse of the architecture. However, FNA preparations provide better cytomorphologic features. In addition, mixed inflammatory cell infiltrates and fibrosis also influence gene array test results.

40

A Profile of Rh Antibodies and Phenotypes Among Ethnic Groups in the Southwest.

Mary Kay Vaske and Mary Berg. University of Arizona Health Sciences Center, Tucson.

Traditionally, Latinos have been grouped with whites in Rh phenotyping studies. During a previous study examining the incidence of antibodies in the local population, several uncommon Rh phenotypes were found. We hypothesize that these uncommon phenotypes come from the minority populations within our community, specifically Latinos and Native Americans, and, thus, they should not be grouped with whites in phenotyping studies. To test this hypothesis, we studied the ethnic background of patients whose Rh phenotype had been recorded.

We compared the Rh phenotypes found among different ethnic groups by doing a retrospective review of records maintained in the transfusion medicine laboratory. These records contained Rh phenotyping results from patients who had made an Rh antibody (D, C, c, E, e). Rh phenotypes of people found to have anti-D due to RhIg therapy were not included. The records used included computer records, paper records, and microfiche when available.

From 1979 through 2005, 653 Rh phenotypes were performed. Of these, 620 patients had a designated ethnicity (white, 437; Latino, 145; Native American, 14; African American, 10; other, 9; Asian, 4; and Pacific Islander, 1). The designation "other" refers to a background that is multiracial and/or cannot be defined within the aforementioned ethnic designations. No indication of ethnicity could be found for 33 patients. In looking at antibody formation, the number of Latinos who made anti-c was almost twice that of whites (19% vs 11%). In addition, although the number of Native Americans in the review is small (just >2% of the known ethnicity group), they comprised 15% of the anti-e antibodies. Similarly, the incidence of the R2R2 phenotype reflected this difference. The most common Rh phenotypes among whites and Latinos were rr, R1R1, and R1r. The r"r phenotype was found among 2% of whites but not at all in Latinos. Likewise, the R1Rz and r'r" phenotypes seen in 2% and 1%, respectively, of the Latino population were not found in any white patients.

Because all of these patients were exposed to the same donor population, it would be expected that the frequency of their antibody production also would be similar if their phenotypes were similar. Although we appreciate that these results are biased because they reflect only patients who made Rh antibodies and not the entire patient population, they support our hypothesis that Latinos (and likely Native Americans) should not be grouped with whites for phenotyping purposes.

42

Differentiation of *Candida albicans* Directly From Blood Culture Using Gram Stain Morphology.

Amanda T. Harrington, David Nowowiejski, and Ajit P. Limaye. Department of Laboratory Medicine, University of Washington and Harborview Medical Centers, Seattle.

Because the rate of azole resistance has remained low for *Candida albicans*, rapid differentiation of *C albicans* from other

yeasts provides timely information useful for guiding therapy in patients with fungemia. The aim of this study was to evaluate whether the Gram stain morphologic characteristics of yeast directly from blood culture could be used to accurately differentiate *C albicans* from other yeasts and *Candida* species, an observation that previously has been supported only by anecdotal evidence.

In a prospective single-center study, Gram stain morphologic characteristics in the first positive blood culture bottle were evaluated from samples of 60 consecutive patients with fungemia detected in the BACTEC Blood Culture System. Categorization of Gram stain morphologic features as compatible with *C albicans* vs other yeast was performed using systematic, predetermined definitions by a reviewer who was blinded to the final organism identification. Final yeast identification was determined subsequently by using standard biochemical techniques (YBC VITEK card, API20C, CHROMagar). The morphologic categorization of *C albicans* vs other yeast was evaluated for sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values. As a secondary analysis, usefulness of this method was evaluated as a function of the type of blood culture bottle.

From August 2004 to June 2005, yeast was identified from blood cultures of 60 patients (*C albicans,* 43.3%; *Candida glabrata,* 28.3%; *Candida parapsilosis,* 8.3%; *Candida krusei,* 6.7%; *Candida tropicalis,* 5.0%; *Cryptococcus* species, 3.3%; *Candida dubliniensis,* 1.7%; *Candida lusitaniae,* 1.7%; and *Rhodotorula mucilanginosa,* 1.7%). Differentiation of *C albicans* from other yeasts based on Gram stain morphologic characteristics had a sensitivity of 85.2%, a specificity of 100%, a PPV of 100%, and an NPV of 89.2%. Differentiation of *C albicans* by Gram stain morphologic characteristics from different bottle types revealed 95.8% sensitivity and 100% specificity for the aerobic bottle and 25.0% sensitivity and 100% specificity for the MycoF bottle ($P = .0002$).

Specific Gram stain morphologic characteristics can be used to accurately differentiate *C albicans* from other yeasts in aerobic blood culture bottles. Additional studies are required to determine the clinical impact on antifungal therapy choice and to evaluate the validity for blood cultures from other instrumented blood culture systems.

43

Growth Characteristics of Psychrophilic and Nonpsychrophilic Bacteria in Cold-Stored Platelets.

Margret Oethinger,[1,2] Sheldon Campbell,[1,2] and Gary Stack.[1,2] Sponsor: Brian Smith.[2] [1]VA Connecticut Healthcare System, West Haven, and [2]Department of Laboratory Medicine, Yale University, New Haven.*

Storage of platelet products in the cold, as opposed to the typical 20°C to 24°C, has been studied as a means to better preserve platelets and reduce bacterial contamination. However, the extent to which cold storage reduces bacterial contamination in platelet products is not well-studied. Therefore, the goal of the present study was to characterize the growth kinetics of psychrophilic ("cold-loving") and nonpsychrophilic bacteria in cold-stored platelets.

We studied nonpsychrophilic bacteria commonly associated with septic reactions to platelet products (*Escherichia coli*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*) and psychrophilic bacteria commonly associated with septic reactions to RBCs (*Yersinia enterocolitica*, *Pseudomonas fluorescens*, and *Serratia liquefaciens*). Leukocyte-reduced, whole blood–derived platelet concentrates (PCs) obtained from a regional blood center were combined in a sterile manner, split into approximately 50-mL portions in platelet storage bags, and inoculated to a final concentration of 10^3 colony-forming units (CFU)/mL. PCs then were stored at 4°C without agitation or at 22°C with gentle agitation (4-6 bags per microorganism and temperature). Viable cell counts were determined initially and on days 1, 2, 3, 5 or 7, and 14 days after inoculation.

The results showed that all 3 nonpsychrophilic organisms survived at 4°C but did not proliferate. *Y enterocolitica* did not grow by 14 days at 4°C, and in 4 (80%) of 5 bags did not survive. *P fluorescens* grew to 10^4 CFU/mL by day 5 and 10^8 by day 14. *S liquefaciens* showed no detectable growth at day 7 but reached 10^5 CFU/mL by day 14. By comparison, all organisms, except *Y enterocolitica*, grew well at 22°C and reached stationary growth after 2 days. *Y enterocolitica* did not grow at 22°C, as at 4°C, and did not survive in any bag. In a separate experiment, *Y enterocolitica* grew in PCs in tube cultures at 22°C only if the PC suspension (n = 12 donors) was first heat treated at 56°C for 30 minutes.

The growth of nonpsychrophilic organisms studied was inhibited by cold storage. Growth of the psychrophilic bacteria *P fluorescens* and *S liquefaciens* was delayed by cold storage but not eliminated. *Y enterocolitica*, which is a clinically significant contaminant of RBCs, unexpectedly did not grow in most PCs at 4°C and 22°C, apparently due to a heat-labile plasma factor. Our results indicate that cold storage should reduce but not eliminate the risk of bacterial contamination of PCs.

44

Combined Use of Fetal Gestational Age and TDx FLM-II Fetal Lung Maturity Assay Results to Predict Risk of Neonatal RDS.

Anders H. Berg,[1] Stacy Melanson,[1] Kimberly Springer,[1] Milenko Tanasijevic,[1] Thomas McElrath,[2] and Petr Jarolim.[1] Departments of [1]Pathology and [2]Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA.*

Assays for amniotic fluid surfactant levels as an indicator of fetal lung maturity are used to assess risk of respiratory distress syndrome (RDS). Manufacturer's guidelines for the widely used Abbott TDx FLM-II (FLM) assay dictate that assay values be interpreted as mature, immature, or intermediate. Our obstetricians often request better-defined clinical significance for these results, however. In 2004 McElrath et al published a logistic regression model estimating the probability of RDS based on FLM and gestational age (GA); since then, 2 similar models have been published. These formulas' predictive values have never been validated using independent data, and their predictions are often significantly different. We sought to test the performance of the McElrath formula over time using a new data set and compare its performance to use of manufacturer's guidelines and to another published model. The FLM assay results and associated GA, RDS status, and clinical histories were assembled for the 2001-2005 period. Included were singleton births, FLM sampling within 72 hours of delivery, and no fetal anomalies.

The criteria were met by 332 cases; there were 22 cases of RDS (6.6%). Hosmer-Lemeshow analysis demonstrated that the McElrath formula produced an excellent overall fit ($P = .66$, indicating we cannot reject the hypothesis that it correctly predicts observed risks), whereas the alternative formula was a poor fit ($P = .0025$). Using 5% RDS risk as a threshold for defining positive vs negative results, the McElrath formula demonstrated a sensitivity of 86%, and a negative predictive value (NPV) of 98.5%, whereas the manufacturer's guidelines were only 68% sensitive and had an NPV of 97.5%. The model performed particularly well for FLM intermediate (40-55 mg/g) values. Finally, the performance of model in diabetic mothers is presented.

The McElrath formula for RDS risk when applied to an independent data set seemed to be an accurate predictor of risk in our patient population and outperformed FLM testing alone in some contexts. The predictive value was demonstrated to remain stable over time. This formula provides accurate estimates of RDS risk, enabling clinicians to make more informed and confident peripartum decisions. These results represent a step toward implementation of this model into interpretive FLM reports at our hospital.

45

A Rapid Method for Quantification of Urinary Isoprostanes by LC/MS/MS.

Amy K. Saenger, Thomas J. Laha, and S.M. Hossein Sadrzadeh. University of Washington Medical Center and Harborview Medical Center, Seattle.*

The role of oxidative stress (OS) in the pathobiology of numerous clinical conditions is well established. The main challenge is to find an accurate marker to detect OS in vivo. F_2-isoprostanes are a class of prostaglandin-like compounds formed nonenzymatically by free radical–induced peroxidation of arachidonic acid. They are produced in abundance in vivo in quantities far exceeding cyclooxygenase-derived prostaglandins. One of the F_2-isoprostanes (8-*iso*-$PGF_{2\alpha}$) has proven to have potent biologic activity. 8-*iso*-$PGF_{2\alpha}$ has been demonstrated to be a reliable marker of oxidative stress in humans, and levels have shown to correlate with the severity of some disease states. Current methods for measurement of isoprostanes have relied on gas chromatography/mass spectrometric (GC/MS) methods or immunoassays. Many of these methods have poor precision and long analysis times, and they require extensive sample preparation. The aim of this project was to develop a liquid chromatographic/tandem mass spectrometric (LC/MS/MS) assay for quantitative analysis of 8-*iso*-$PGF_{2\alpha}$ and its metabolite 2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$-M) in human urine.

After addition of the internal standard 8-*iso*-$PGF_{2\alpha}$-d_4, 500 µL of urine was centrifuged, and 30 µL of the sample was injected directly onto the HPLC column. Analytic chromatography was performed using a Restek C_8 3.5 µM column with an isocratic mobile phase consisting of 88% methanol/12% acetonitrile with 0.1% ammonium hydroxide. 8-*iso*-$PGF_{2\alpha}$, 8-*iso*-$PGF_{2\alpha}$-M, and 8-*iso*-$PGF_{2\alpha}$-d_4 were detected using tandem mass spectrometry operated in the negative ion, multiple reaction mode with the following transitions: 8-*iso*-$PGF_{2\alpha}$-d_4, *m/z* 357.25>197.1; 8-*iso*-$PGF_{2\alpha}$, *m/z* 353.25>193.1; and 8-*iso*-$PGF_{2\alpha}$-M, *m/z* 325.25>237.1. Elution of 8-*iso*-$PGF_{2\alpha}$, 8-*iso*-$PGF_{2\alpha}$-M, and 8-*iso*-$PGF_{2\alpha}$-d_4 was achieved after 2.5 minutes, with a total run time of 5.0 minutes. Our results demonstrate consistent linearity in the range of 250 pg/mL to 100 ng/mL. Precision studies showed interassay and intra-assay coefficients of variation of less than 8.0% for 8-*iso*-$PGF_{2\alpha}$ and 8-*iso*-$PGF_{2\alpha}$-M. The lower limits of detection were 110 and 50 pg/mL for 8-*iso*-$PGF_{2\alpha}$ and 8-*iso*-$PGF_{2\alpha}$-M, respectively. Recovery of 8-*iso*-$PGF_{2\alpha}$ ranged from 93% to 101%, whereas 8-*iso*-$PGF_{2\alpha}$-M recovery from spiked urine samples was 91% to 98%. To establish a normal range, samples were obtained from 24 apparently healthy people, and levels of 8-*iso*-$PGF_{2\alpha}$-M were quantified as 45.6 ± 10.9 ng/mg creatinine (mean ± SD). We have developed a sensitive, precise, and rapid assay to detect 8-*iso*-$PGF_{2\alpha}$ and its metabolites in urine using a direct-inject LC/MS/MS method.

46

Rapid Molecular Classification of Leukemias by Liquid Bead Array Using Signature LTx.

Joanna Steere, Crystal R. Weatherill, Adam Bagg, Debbie Nielsen, and Vivianna Van Deerlin. Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia.

Genetic testing has assumed a central role in the diagnostic and prognostic evaluation of leukemias, with rapid and accurate classification being crucial to appropriately guide therapy, particularly in the case of acute promyelocytic leukemia. We currently offer a 2-step multiplexed reverse transcriptase–polymerase chain reaction (RT-PCR) assay (HemaVision-7, DNA Technologies A/S) to aid in the diagnosis of acute leukemias; however, this assay has a minimum 2-day turnaround time for positive results. We evaluated the use of a novel liquid bead array technique (Signature LTx) for rapid detection of the same 7 common translocations/inversion associated with acute leukemias and can produce a result in 1 day.

Samples previously tested by HemaVision (57) or single t(15;17) or t(9;22) RT-PCR (50) and 8 translocation- or inversion-positive cell lines were tested using Signature LTx, multiplex RT-PCR followed by PCR amplification and direct hybridization to allele-specific capture probes with signal detection on a Luminex IS2.2 platform. Samples submitted for diagnostic testing proved to be completely concordant with results available within 1 day; however, samples with a lower proportion of neoplastic cells (eg, minimal residual disease specimens) fell below the level of detection for this assay. Further sensitivity assessment using RNA from translocation- or inversion-positive cell lines sequentially diluted with RNA from the HL60 translocation/inversion–negative cell line revealed the assay to be sensitive to 1% for t(8;21)+ (KASUMI) RNA. Results with other cell lines were variable.

Signature LTx provides accurate and rapid detection of the 7 common translocations and inversions in diagnostic samples of acute leukemias, whereas detection of minimal residual disease requires an alternative more sensitive method. In this assessment, the liquid bead array format is highly compatible with the workflow of a clinical laboratory and provides clinically relevant data in a timelier manner.

47

Transfusion Protocol for Implantation and Explantation of Cardiac Devices in an Academic Medical Center.

Angela N. Bartley and Mary P. Berg. University of Arizona Health Sciences Center, Tucson.

There are few reports in the literature describing the amount of transfusion support needed for implantation and explantation of cardiac-assist devices. Because such devices are used frequently at this institution, we established a protocol to support patients needing such devices.

We retrospectively reviewed blood bank records to document the use of blood components for all 215 patients on cardiac-assist devices in the 1989-2005 period. The data were separated into components given at the time of implantation and those given at the time of explantation of the cardiac device. Data were compiled and analyzed by using the Statview statistical program.

Implantation data for 214 patients showed the mean use as follows: RBCs, 5.7 U; platelets, 2.4 U; fresh frozen plasma (FFP), 7.5 U; and cryoprecipitate, 0 U. The median values were as follows: RBCs, 4.5 U; platelets, 1.0 U; FFP, 6.0 U; and cryoprecipitate, 0 U.

Of 215 patients, 1 had no transfusion record and died before explantation. Explantation data for 134 patients showed mean use as follows: RBCs, 7.7 U; platelets, 2.4 U; FFP, 9.9 U; and cryoprecipitate, 3.7 U. The median values were as follows: RBCs, 8.0 U; platelets, 2.0 U; FFP, 9.0 U; and cryoprecipitate, 0 U. Of 215 patients, 81 died before explantation and did not receive transfusion.

A suitable protocol for the preparation of blood components for implantation and explantation of a cardiac device is as follows: implantation, RBCs, 4 U; platelets, 1 U; and FFP, 6 U; and explantation, RBCs, 8 U; platelets, 2 U; and FFP, 8 U. Our data do not support the need for preparation of cryoprecipitate before implantation or explantation. The process of thawing and pooling cryoprecipitate is labor-intensive. Excess production may lead to unnecessary waste of a product that has a short life span (4 hours).

48

Positive and Negative Heterophil Antibody Interference in a Chromogranin A Immunoassay.

Joshua A. Bornhorst,[1,3] Jeffrey J. Smith,[2] J. Alan Erickson,[2] and Edward R. Ashwood.[1,2] [1]Department of Pathology, University of Utah, and [2]ARUP Laboratories and the Institute for Clinical and Experimental Pathology, Salt Lake City; and [3]Department of Pathology, University of Arkansas for Medical Sciences, Little Rock.

Interference caused by heterophil antibodies can adversely affect the immunoassay of patient samples. Heterophil antibodies interact with antibody components used in sandwich immunoassays and can elevate or, in some cases, reduce measured analyte concentration values. Estimates of the prevalence of heterophil antibodies in the clinical population vary widely and range from less than 1% to 80%.

An immunoassay for the neuroendocrine tumor marker chromogranin A (CgA) was examined to determine the frequency of heterophil antibody interference. This sandwich assay uses a polyclonal anti-CgA rabbit capture antibody and a monoclonal mouse anti-CgA detection antibody. The coefficient of variation (CV) for a control serum sample with a mean measured CgA concentration of 82 ng/mL was 13.6%, and a 10.8% CV was observed for a control serum sample with a mean concentration of 588 ng/mL. Immunoglobulin inhibiting reagent (Bioreclamation) was incorporated selectively into the CgA assay sample diluent at a final concentration demonstrated to block as much as 3 mg/mL of heterophil antibody. Patient samples that yielded a ratio of less than 0.4 or more than 2.5 for the unblocked immunoassay–measured CgA concentration vs the blocked immunoassay–measured CgA concentration were considered to exhibit significant heterophil antibody interference.

Significant numbers of clinical samples displayed positive or negative heterophil antibody interference. Of the 101 randomly selected clinical samples assayed in the presence and absence of heterophil blocking agent, 11 samples exhibited positive heterophil antibody interference and 16 exhibited negative antibody interference. The median measured CgA concentration was 113 ng/mL for unblocked samples and 121 ng/mL for blocked samples. Measured concentration differences of greater than 250 ng/mL between the blocked and unblocked measured concentrations were found in 5 samples with positive heterophil interference and 6 samples with negative interference. For all 11 samples displaying strong heterophil interference, the measured analyte concentrations were falsely elevated above or falsely depressed below the upper limit of the assay reference interval (0-375 ng/mL) in the absence of heterophil blocking agent. These results demonstrate that clinically significant heterophil interference can occur in at least 11% of clinical samples and that negative and positive heterophil interference can occur at comparable frequencies in a single immunoassay.

49

A Real-Time PCR Assay for the Detection of T-Cell Receptor Excision Circles Based on FRET Probes: A Screening Test for Severe Combined Immunodeficiency.

Patricia R. Slev,[1] Carl T. Wittwer,[1,4] Edward W. Taggart,[4] Christine M. Litwin,[1,4] Gudrun H. Reed,[1] and Harry R. Hill.[1-4] Departments of [1]Pathology, [2]Pediatrics, and [3]Medicine, University of Utah; and [4]ARUP Laboratories, Salt Lake City.*

Severe combined immunodeficiency (SCID) is a group of inherited, treatable immune system disorders that result in impaired humoral and cellular immunity and are characterized by reduced or absent T cells. The incidence of SCID has been estimated to be 1/100,000 live births. Patients with SCID are asymptomatic at birth but are quickly ravaged by infections and die unless treated with immune reconstitution. Prompt treatment with bone marrow transplantation in the first month of life has been associated with 95% survival rate vs 70% survival for patients in whom treatment was delayed for 3 months or longer. However, prompt treatment cannot be provided without a timely diagnosis. For these reasons, it would be beneficial to develop a screening test for newborns that could detect the many forms of SCID. Although SCID is genetically characterized by a variety of mutations in more than 8 separate genes, all genotypes exhibit lymphopenia. A simple, inexpensive but reliable test that would detect lymphopenia could be used to screen for all SCID genotypes.

Recently, the quantitative measurement of T-cell receptor excision circles (TRECs) has been proposed as a screening test for SCID. TRECs are episomal by-products of T-cell rearrangements and occur in recently formed T cells. We originally developed a quantitative real-time polymerase chain reaction (PCR) assay to screen for SCID based on the detection of TRECs using SYBR Green I, but we have now developed a more specific assay based on HybProbe (FRET) technology. Although FRET technology has been previously used to detect TRECs in HIV applications, to our knowledge, it has never been applied in an SCID assay.

Cord (n = 8) and SCID (n = 5) DNA samples were quantified for DNA by spectrophotometry and standardized to equal concentrations. FRET probes were used to quantify the TREC target and β-globin (reference gene) for all samples. The relative β-globin copy numbers were consistent with DNA concentrations and similar for all samples. The relative TREC copy number for the cord samples was approximately 1/80 of the β-globin control samples, which is consistent with previous reports. Because 4 of the 5 SCID samples were below the assay's level of detection for TREC, relative quantification was impossible. However, qualitatively the assay was 100% accurate because all 8 normal control samples had lower crossing thresholds than any SCID sample (P = .0008). These results suggest this real-time PCR approach could be useful as a SCID screening strategy.

50

Women With Both $p16^{INK4a}$ and High-Risk HPV Positivity Are Candidates for Intensified Triage.

Swati Mehrotra,[1] Ron McGlennen,[2] Stefan E. Pambuccian,[2] and

Suman Setty.[1] Departments of Pathology, [1]University of Illinois at Chicago, and [2]University of Minnesota, Minneapolis.

The advent of liquid-based cytology has revolutionized Papanicolaou (Pap) test screening by increasing the test specificity and providing liquid-based Pap (LBP) residue for ancillary tests. The emphasis in recent times is on identifying women at risk for high-grade dysplasia (cervical intraepithelial neoplasia 2-3). Women with abnormal Pap test results of atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) undergo reflex human papillomavirus (HPV) DNA testing. Because the presence of high-risk HPV infection does not necessarily signify the presence of disease, surrogate markers of dysplasia would minimize the use of colposcopy. We tested for a cell cycle–related molecule ($p16^{INK4a}$), which is a marker for the genomic chaos induced by HPV when the viral DNA (E7 gene of the oncogenic HPV types) integrates into the host genome. We assessed the conjoint value of $p16^{INK4a}$ on cellblock preparations and high-risk HPV by DNA polymerase chain reaction (PCR) for isolating a subset of women with negative, equivocal, or low-grade cytologic findings who need further workup.

We tested 65 consecutive cases of LBP, which had HPV identification performed by PCR. The cytologic diagnoses made on these Sure Path LBPT were as follows: LSIL, 18; ASCUS, 19; and within normal limits (WNL), 28. HPV DNA testing was performed using PCR with Myo9/11 consensus primers and restriction digestion to obtain the HPV type. Cell-block sections were stained with H&E and immunostained with an antibody against $p16^{INK4a}$ (DAKO, Carpinteria, CA). The sections were analyzed for adequate cellularity and for positive nuclear and/or cytoplasmic staining for $p16^{INK4a}$. The results of immunostaining were correlated with HPV subtype.

All cases included in this analysis were positive for HPV. Our results show that of the 18 cases of LSIL, 11 were positive for high-risk HPV types, of which 6 cases demonstrated $p16^{INK4a}$ immunostaining. In the ASCUS category of 19 cases, 10 cases were high-risk HPV+, of which 9 stained with $p16^{INK4a}$. The WNL category had 28 cases, with 17 high-risk HPV+, of which 8 demonstrated $p16^{INK4a}$ positivity. In a subset of the cases, repeated HPV testing showed high-risk HPV type-specific persistence for 3 to 24 months with negative $p16^{INK4a}$ staining, indicating nonprogression to dysplasia.

We demonstrated that $p16^{INK4a}$ is positive in a subset of LSIL/ASCUS cases that were positive for high-risk HPV DNA. We propose that the addition of this test identifies a subset of patients at risk for high-grade dysplasia. We suggest a further refinement of the current algorithm whereby high-risk HPV+ and $p16^{INK4a}$+ cases of ASCUS/LSIL should be subjected to further clinical follow-up. The addition of this test would reduce colposcopy for women at risk.

51

Increased Tissue Factor–Bearing Microparticles Derived From Endothelial Cells in Hemolytic Uremic Syndrome.

Morayma Reyes and Wayne Chandler. Department of Laboratory Medicine, University of Washington, Seattle.*

Microparticles (MPs) are circulating cellular fragments that are increased in thrombotic conditions, including thrombotic thrombocytopenic purpura, antiphospholipid syndrome, and heparin-induced thrombocytopenia. Tissue factor (TF)-bearing MPs are thought to be procoagulant in these conditions. Hemolytic uremic syndrome (HUS) is a thrombotic disease characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure secondary to glomerular thrombotic microangiopathy. In children, HUS most often occurs after infection with *Escherichia coli* O157H7. It is hypothesized that *E coli* verotoxin injures renal and other endothelium leading to activation of hemostasis, thrombotic microangiopathy, and HUS.

We studied 42 children at the time of admission with bloody diarrhea associated with *E coli* O157H7 infection (typically day 4 of illness) before HUS developed, and 5 healthy adult control subjects (samples from age-matched control subjects is ongoing). In 13 children (pre-HUS cases), HUS subsequently developed, whereas in 29 (uncomplicated cases) the illness resolved without the development of HUS. We analyzed the total number of MPs, percentage of TF-bearing microparticles, and their cellular derivation using a 5-color flow cytometry assay. MPs were collected from plasma by ultracentrifugation (100,000*g*). MPs were defined based on size (0.5-1 μm) and strong annexin V binding.

The MP distribution in the adult control subjects was similar to that reported by others: $70 \pm 24 \times 10^3$ MPs/mL of plasma (28% platelet-, 1.8% endothelial-, and 6.0% monocyte-derived). The MP distribution in uncomplicated cases was $647 \pm 83 \times 10^3$ MPs/mL (24% platelet-, 7.6% endothelial-, and 10% monocyte-derived), whereas pre-HUS cases showed $1{,}127 \pm 451 \times 10^3$ MPs/mL (9.0% platelet-, 9.6% endothelial-, and 9.2% monocyte-derived). Compared with adult control subjects, children infected with *E coli* O157H7 showed more endothelial MPs ($P = .004$, unpaired t test). Compared with the uncomplicated cases, the pre-HUS cases showed increased MPs that were TF+ (3.0% ± 0.2% vs 4.8% ± 1.1%; $P = .02$) and increased MPs from endothelium that were TF+ (1.0% ± 0.1% vs 1.8% ± 0.5%; $P = .02$) but fewer platelet MPs ($P = .02$). In a previous study, the pre-HUS cases showed increased hemostatic activation as indicated by higher levels of F1.2 and D dimer.

E coli infection injures endothelium as indicated by increased release of endothelial MPs. In contrast with patients infected with *E coli* and an uncomplicated outcome, patients in whom HUS developed were more likely to show TF-bearing MPs that often were derived from endothelial cells and increased hemostatic activation, suggesting a pathologic role for endothelial injury and TF expression in HUS.

52

Factors Affecting Survival, Dedifferentiation, and Redifferentiation of Adult Rat Cardiomyocytes in Culture.

Kenneth H. Clark and Jorge L. Sepulveda. Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

A difficulty in studying adult cardiomyocytes in cell culture stems from limited cell survival and the phenomenon of sarcomeric disassembly or dedifferentiation. Although dedifferentiation is well described, little is known about the molecular pathways involved in this process. Our goal was to investigate the factors that promote cardiomyocyte survival and those that may positively or negatively affect the process of dedifferentiation and sarcomeric reassembly, or "redifferentiation." Adult rat cardiomyocytes were isolated via a perfusion technique and plated on laminin under a variety of culture media and growth factors. Cells were monitored for survival and dedifferentiation for a course of up to 7 days.

Culture in the presence of fetal calf serum (FCS) promotes survival in culture and also promotes dedifferentiation. In the first 2

to 3 days in FCS, the cells begin to flatten and spread out, undergo sarcomere disassembly, and induce atrial natriuretic peptide (ANP) expression. At 4 to 5 days, they begin to reassemble sarcomeres, initiate vigorous beating, and form attachments with neighboring cells. Treatment of myocytes with FCS plus G protein–coupled receptor (GPCR) agonists phenylephrine (20 μmol/L) or endothelin-1 (30 nmol/L) accelerates this process and, ultimately, results in a greater than 2-fold increase in cell size and ANP expression when compared with FCS-cultured control samples at days 6 and 7. Insulin-like growth factor 1 (IGF1), at 100 nmol/L, partially substitutes for FCS, whereas treatment with phosphatidylinositol 3 kinase (PI3K) and MEK1/2 inhibitors inhibits dedifferentiation.

Cardiomyocyte de-differentiation is a complex process involving GPCR, IGF1, PI3K, and MAP kinase activation. Elucidation of the signaling pathways regulating adult cardiac myocyte dedifferentiation and hypertrophic redifferentiation is potentially important to better study these cells in culture and to understand the pathologic process of cardiac hypertrophy and regeneration.

53

Not All Microangiopathic Hemolytic Anemias Are Thrombotic Thrombocytopenic Purpura: A Clinicopathologic Correlation.

Yara A. Park, Christina J. Tatum, John E. Reardon, C. Bruce Alexander, Kang-Jey Ho, and Marisa B. Marques. Department of Pathology, University of Alabama at Birmingham.

Microangiopathic hemolytic anemias (MAHAs) include thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome, disseminated intravascular coagulation, and malignant hypertension (MH). We recently encountered 2 patients with MAHA whose clinical and laboratory features overlapped; both died of the disease but had different pathologic features at autopsy.

Case 1 was a previously healthy 33-year-old black woman with abdominal pain, hematemesis, scleral icterus, and pleuritic chest pain. Her blood pressure was 119/96 mm Hg, and the laboratory evaluation was consistent with MAHA (hemoglobin, 8.0 g/dL; platelet count, 19,000/μL; schistocytosis; and lactate dehydrogenase [LDH], 1,951 U/L). In less than 12 hours, she developed respiratory distress and required intubation. Because TTP was suspected, plasmapheresis was planned, but she died immediately before the procedure was started. Postmortem examination revealed platelet microthrombi in multiple organs, especially the brain and heart. Subsequently, complete ADAMTS-13 deficiency due to the presence of an inhibitor (0.7 Bethesda units) was determined from an admission sample, consistent with TTP.

Case 2 was a 43-year-old black woman with a history of hypertension who had slurred speech, headache, and confusion. Her blood pressure was 249/168 mm Hg, and her laboratory studies showed the following: hemoglobin, 9.5 g/dL; platelet count, 114,000/μL; schistocytosis; LDH, 454 U/L; and renal failure. With the presumed diagnosis of TTP, plasmapheresis was started on an emergency basis on arrival. However, immediately after starting the second procedure, she was noted to have fixed and dilated pupils. Subsequently, brain imaging demonstrated diffuse cerebral edema and uncal and tonsillar herniation, and she died on the following day. Autopsy revealed arteriolosclerosis in the kidneys and fibrinoid necrosis in the brain, consistent with MH. Premortem ADAMTS-13 activity was mildly decreased (32%), and there was no detectable inhibitor.

TTP and MH can be difficult to differentiate because MH can exhibit signs and symptoms of TTP as seen in case 2. On the other hand, TTP can be associated with severe hypertension, particularly when renal failure is present. Rapid diagnosis of TTP is essential given the high mortality without immediate plasmapheresis. As exemplified herein, we suggest that severe hypertension and mild thrombocytopenia can be distinguishing features separating MH from TTP, as confirmed by the autopsy findings. Although an ADAMTS-13 activity level of less than 5% is specific for TTP, this result is not readily available when the decision to start plasma exchange needs to be made. Ultimately, the differentiation remains a clinical diagnosis between the patient's primary physician and the transfusion medicine consultant.

54

Prolonged Partial Thromboplastin Times Without Bleeding in Two Patients With Metastatic Melanoma and Diffuse Melanosis Cutis.

Holly N. Burford,[1] Patti Tichenor,[1] John E. Reardon,[1] Vishnu V.B. Reddy,[1] Scott A. McDaniel,[2] and Marisa B. Marques.[1] Departments of [1]Pathology and [2]Medicine, University of Alabama at Birmingham.

A prolonged partial thromboplastin time (PTT) does not necessarily indicate an increased risk of bleeding. We describe 2 asymptomatic patients with very abnormal PTTs who subsequently were diagnosed with metastatic melanoma.

Case 1 was a 56-year-old white man with a history of stage IB melanoma 3 years before admission who now had a 2-month history of gray skin, nausea, vomiting, and significant weight loss. On admission, he had a PTT of more than 200 seconds (reference range, 25-35 seconds), a prothrombin time (PT) of 17.5 seconds (reference range, 12.6-14.6 seconds), and a D-dimer level of 1,559 ng/mL (reference range, 110-240 ng/mL). Further testing revealed a normal thrombin time and a 1:1 PTT mixing study that showed complete correction (36 seconds). A bone marrow biopsy was diagnostic for metastatic melanoma, and the patient's skin color was attributed to diffuse melanosis cutis. Despite lack of bleeding, levels of factors VIII, IX, XI, and XII were assayed, and all were within the reference ranges. Repeated PTT with a 10-minute preincubation with silica decreased to 123 seconds, suggestive of a component of prekallikrein (PK) deficiency. Subsequently, PK and high-molecular-weight kininogen (HMWK) activities were found to be decreased at 32% and 16%, respectively (reference ranges, 65%-135%).

Case 2 was a 58-year-old white man with a complicated medical history that included squamous cell carcinoma removed from his ear 1 year earlier and who was admitted for workup of gray discoloration of skin and constitutional symptoms. Coagulation testing on admission also revealed a PTT of more than 200 seconds, a PT of 34 seconds (international normalized ratio, 2.9), and a D-dimer level of 722 ng/mL. Although the thrombin time was 33 seconds (reference range, <20 seconds), no heparin was detected. A 1:1 PTT mixing study had a result of 53 seconds, 1:2 (1 part patient/2 parts normal plasma) PTT was 40 seconds, and 1:3 PTT was 33 seconds. Based on these results, which suggested an inhibitor, lupus testing was performed, but results were negative. Because various factor assays (XII, 42%; VIII, 387%; X, 58%; V, 22%; and II, 41%) could not explain the very prolonged PTT, we tested for PK and HMWK activities. PK was 20% and HMWK was 3%. Whereas multiple deficiencies of factors necessary to initiate activation in the PTT assay may be why there was no correction in the mixing studies, an unknown inhibitor cannot be excluded.

The literature has evidence of coagulation activation by metastatic melanoma without the involvement of tissue factor and

factor VII. Because both patients had elevated D-dimer levels consistent with ongoing fibrin generation, we postulate that they may have had activation of the contact pathway. However, activation alone could not account for the degree of deficiency observed, which we postulate resulted from adsorption of HMWK, PK, and factor XII onto circulating malignant cells.

55

Acquired Hemophilia and Autoimmune Cholangitis: Are They Related?

Jeremy W. Henderson,[1] Audrey J. Lazenby,[1] Debra K. Horton,[1] Kevin W. Harris,[2] Martin J. Heslin,[3] and Marisa B. Marques.[1] Departments of [1]Pathology, [2]Medicine, and [3]Surgery, University of Alabama at Birmingham.

Acquired hemophilia (AH) is a rare condition caused by an autoantibody to factor VIII. AH manifests with bleeding into soft tissue and/or from the gastrointestinal and urinary tracts and may be idiopathic or secondary to a variety of conditions, such as pregnancy, rheumatoid arthritis, or malignancy. We encountered a patient with AH in the setting of autoimmune cholangitis and investigated a possible relationship between the 2 conditions.

A 71-year-old man with a history of sudden onset of abdominal pain, nausea, vomiting, and chills was noted to have a dilated common bile duct and was diagnosed with cholangitis. Jaundice and clay-colored stools subsequently developed, and endoscopic ultrasonography revealed a distal common bile duct mass. Despite a cytologic diagnosis negative for malignancy, a pancreaticoduodenectomy was performed. His preoperative CBC count and prothrombin time (PT) were normal. However, during surgery, he lost 1.4 L of blood. His PT and partial thromboplastin time (PTT) later on the same day were only slightly prolonged at 16 and 46 seconds, respectively. Within the next 24 hours, he continued to bleed; hemodynamic instability developed, and he had decreased urine output and a rapidly falling hematocrit.

During reexploration on postoperative day (POD) 1, he required massive transfusion, and almost 3 L of serosanguineous fluid and organized clot were removed from the abdominal cavity. Yet, no obvious source of bleeding was found. In the following days, there was persistent bleeding from the gastrointestinal and urinary tracts, concurrent with increasing PTTs which peaked at 98 seconds on POD 6, despite administration of multiple units of fresh frozen plasma. On POD 8, a PTT mixing study showed no correction, and a factor VIII inhibitor was suspected. Factor VIII activity was 2%, and a factor VIII inhibitor was 25 Bethesda units. After 5 to 6 days of administration of an activated prothrombin complex concentrate (FEIBA), bleeding was controlled and hematocrit stabilized. At 4 and 7 days after initiation of corticosteroids, factor VIII levels were 49% and 73%, respectively.

Pathologic examination of the mass revealed a fibroinflammatory process with IgG4+ plasma cells, consistent with autoimmune cholangitis. Of note, autoantibodies to factor VIII have been described to be predominantly of the IgG4 subclass.

The patient was discharged on POD 26 with a steroid-taper regimen. Seventeen days later, he had finished the steroid-tapering regimen, was asymptomatic, and has a factor VIII level of 178%.

We are unaware of another case of AH and autoimmune cholangitis. Immunoperoxidase stains of the lesion did not demonstrate reactivity of plasma cells with anti–factor VIII following incubation with normal plasma. Although a definite cause-effect relationship cannot be proven, the diagnosis of a factor VIII inhibitor concurrent with manipulation of the lesion during resection and the subsequent rapid disappearance of the antibody strongly suggest an association between the 2 rare entities.

56

Improved Assessment of Coagulation Factor Activities in the Presence of Strong Lupus Anticoagulants Achieved by Immunoglobulin Depletion of Patient Plasma.

Patricia V. Adem, Rebecca L. Wilcox, and Jonathan L. Miller. Department of Pathology, University of Chicago, Chicago, IL.

In patients with a history of clinically significant bleeding, together with a demonstrable lupus anticoagulant (LA), determining whether there are true deficiencies of specific coagulation factors can be quite difficult. In some cases, simply performing sequential dilutions of the patient plasma with buffer is adequate to dilute the effects of an LA. But in patients with sufficiently strong LAs, the dilution approach may prove inadequate to allow discrimination between normal and abnormal for individual factors. The present study addressed whether this problem can be overcome through depletion of the LA before coagulation testing.

A 55-year-old woman with a history of metastatic breast cancer who was admitted to our institution with portal hypertension and gastrointestinal bleeding had a prolonged prothrombin time (PT) of 16.5 seconds (normal, 12.5-15.3 seconds) and a partial thromboplastin time of 57.7 seconds (normal, 24-34 seconds). The PTT-LA was markedly prolonged, without significant shortening in the mixing phase, but with marked shortening in the correction-neutralization phase (platelet neutralization procedure). The DRVVT also was markedly prolonged, but showed dramatic shortening in the correction phase. Both results were accordingly consistent with the presence of an LA. Anticardiolipin IgM was markedly increased, at 118.6 MPL, whereas IgG and IgA were normal. Levels of coagulation factors in all PTT-based assays seemed at least moderately decreased and in PT-based assays, typically mildly decreased. Moreover, sequential dilutions of patient plasma showed no apparent increase in factor activity through a dilution of 1:40; for factor VIIIa, 1:80 dilution also could be performed, which now began to show a rise in apparent factor activity. Based on the high level of anticardiolipin IgM, we sought to determine whether removal of IgM would result in an improvement in the apparent activity of coagulation factors.

Patient plasma (25 μL) diluted with Owren-Koller buffer (475 μL) was incubated for 30 minutes at 20°C with goat antihuman IgM conjugated to agarose beads. The beads were removed by centrifugation, and the plasma was tested again in coagulation factor assays. Incubation with the beads reduced the IgM level of the diluted patient plasma from 47.4 to 1.1 mg/dL (98% reduction). Bead treatment of pooled normal plasma produced only minimal reduction in the assayed level for factor VIII, 12% for factor IX, and 25% for factor XI. In contrast, the apparent factor levels in patient plasma before and after IgM removal increased from 7% to 90% for factor VIII, from 18% to 80% for factor IX, and from 7% to 40% for factor XI. Accordingly, this study demonstrates that, at least in the case of IgM, plasma may be depleted of interfering LA inhibitors in such a manner that allows a more accurate determination of coagulation factor activity levels.

57

Differentiation of Bone Marrow Mesenchymal Stem Cells Into Smooth Muscle Lineage.

Kenichi Tamama and Alan Wells, Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Smooth muscle cells (SMCs) are the major components of hollow visceral organs, including blood vessels, urinary bladder, uterus, and gastrointestinal organs. For tissue engineering and regeneration of these organs, an accessible autologous cell source for SMCs would be ideal, and this study is aimed to evaluate whether adult bone marrow mesenchymal stem cells (BMMSCs) could be used for that purpose.

BMMSCs, pluripotential cells, are easily obtainable and highly expandable ex vivo. We have shown that addition of the EGF growth factor will promote proliferation and migration of cells without affecting concomitant or subsequent differentiation into at least 3 different lineages. However, there is no protocol established for differentiating BMMSCs into SMC lineage.

We are taking 2 approaches to generating SMCs from BMMSCs. Molecularly, we are introducing myocardin, a potent transcriptional coactivator that functions as a master regulator of SMC-selective genes. We hypothesize that overexpression of myocardin could drive BMMSCs into SMC lineage. In initial experiments, transfection of myocardin-expressing plasmid into BMMSCs induced SMC marker gene expression, including smooth muscle myosin heavy chain (SMMHC).

A second approach involves signaling modification by factors and inhibitors. Preliminary data demonstrate that MEK inhibition with PD98059 leads to SMC marker gene expression, including SMMHC, presumably through inhibition of Elk-1 signaling.

Studies are underway to develop these early indications of SMC differentiation into functional smooth muscle cells.

Acknowledgments: Supported by the CAP Foundation (K.T.) and NIH-R01GM054739 (A.W.). Myocardin plasmid is a kind gift from Dr Eric Olson (U Southwestern). BMMSCs were provided by the Tulane Center for Gene Therapy.

58

Effects of Hemoglobin C and S Traits on Five Commercial Glycated Hemoglobin (HbA_{1c}) Assays.

Shella K. Mongia,[1] Shawn Connolly,[2] Steve Hanson,[2] Randie R. Little,[2] Curt L. Rohling,[2] Richard F. Roberts,[3] and William L. Roberts.[1,3] [1]Department of Pathology, University of Utah, Salt Lake City; [2]Diabetes Diagnostic Center, University of Missouri, Columbia; and [3]ARUP Laboratories, Salt Lake City.

Glycated hemoglobin (HbA_{1c}) is formed by nonenzymatic attachment of glucose to hemoglobin, primarily at the N-terminus of the β chain. It is used to assess long-term glycemic control in patients with diabetes. The accuracy of GHb methods can be affected adversely by the presence of HbC or HbS trait. At least 10% of African Americans have the HbC or HbS trait. We evaluated the effects of HbC or HbS traits on 5 commercial methods using Primus CLC 330 boronate affinity HPLC as the comparison method.

Whole blood samples from individuals who were homozygous for HbA (n = 40) and heterozygous for HbC or HbS (n = 31 and n = 43, respectively) were analyzed by the following methods/instruments: G7 (Tosoh Bioscience), Direct HbA_{1c} on Olympus AU 400 (Pointe Scientific), Ultra 2 (Primus), Variant II (New) (Bio-Rad Laboratories), and Vitros Fusion 5.1 (Ortho Clinical Diagnostics). For each test method, results for each type of sample were compared with those by the CLC 330 method, which is unaffected by the presence of HbC or HbS trait. Deming regression analysis was performed to determine whether the presence of HbC or HbS trait produced a clinically significant effect on HbA_{1c} results. We chose evaluation limits of 6% and 9% HbA_{1c}. After correcting for possible calibration bias by comparing results from the HbAA sample group, we evaluated method bias attributable to the presence of HbC or HbS trait with a clinically significant difference being more than 10% (ie, 0.6% at 6% HbA_{1c} and 0.9% at 9% HbA_{1c}).

At 6% HbA_{1c}, the Ultra2, Variant II, and Direct HbA_{1c} methods had positive biases for HbC and HbS traits with results ranging between 0.02 and 0.59 for HbC and 0.01 and 0.53 for HbS, whereas the Fusion 5.1 and G7 methods had negative biases of –0.28 and –0.38 for HbC trait and –0.25 and –0.36 for HbS trait, respectively. For HbC, at 9% HbA_{1c}, negative biases ranging from –0.01 to –0.29 were observed for the Ultra2, G7, and Fusion 5.1 methods, whereas positive biases were observed for the Variant II and Direct HbA_{1c} methods (0.29 and 0.20, respectively). For HbS trait at 9% HbA_{1c}, the Variant II and Fusion 5.1 methods had positive biases of 0.47 and 0.15, respectively, whereas the Ultra2, G7, and Direct HbA_{1c} methods had negative biases between –0.03 and –0.82. Although there are small, possibly statistically significant interferences from HbC and HbS traits, we do not consider these clinically significant for any of the methods studied.

60

Pharmacogenetic Factors Affecting INR Control During Warfarin Initiation.

Manish J. Gandhi,[1] Jenine K. Harris,[2] Brian F. Gage,[3] Paul M. Ridker,[4] Elena Birman-Deych,[3] and Charles S. Eby.[1,3] Departments of [1]Pathology and Immunology and [3]Medicine, Washington University, St Louis, MO; [2]Department of Public Health, St Louis University, St Louis; [4]Center for Cardiovascular Disease Prevention and Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Empiric warfarin-dosing algorithms start with a standard dose followed by modifications based on the international normalized ratio (INR) until the target range is achieved. Age, ethnicity, and allelic variations in the CYP2C9 and VKORC1 genes can significantly change the warfarin dose needed to maintain therapeutic anticoagulation. However, if an empiric dosing algorithm can compensate for genetic factors that affect the therapeutic warfarin dose, genetic testing for these variants is unnecessary. Thus, we measured the effect of CYP2C9 and VKORC1 genotypes on the performance of the warfarin-dosing algorithm used in 508 eligible subjects who were part of the Prevention of Recurrent Venous Thromboembolism (PREVENT) trial. Briefly in the PREVENT trial, before randomization to receive warfarin or placebo, eligible patients participated in a 28-day open-label run-in phase designed to ensure that all participants could have their dose of warfarin titrated to a stable INR level between 1.5 and 2.0. Patients were started with 3 mg of warfarin and subsequently had 4 or 5 follow-up visits (3-7 days apart) to evaluate the INR and titrate warfarin dose. By using linear interpolation, we computed the time above and below the target INR range (1.5-2.0) for 7 to 28 days. Most patients (87%) were white, and all had a history of venous thromboembolism.

Multivariate analysis of variance indicated a significant association between the VKORC1 and CYP2C9 genotypes with INR values above and below the target INR (1.5-2.0) on days 7 to 28 of warfarin therapy. For the CYP2C9 genotype, patients with wild type

(WT) spent an average of 16% of their time with an INR or more than 2 compared with 25% and 33% for patients with the *2 and *3 alleles, respectively; 27% of patients with WT compared with 16% of patients with *2 and *3 alleles spent their time with an INR of less than 1.5. Similarly for the VKORC1 haplotype, patients with BB (high dose) spent an average of 15% of their time with an INR of more than 2 compared with 26% for patients with AB (intermediate dose) and patients with AA (low dose), whereas 30% of patients with BB compared with 19% and 20% of patients with AB and AA, respectively, spent their time with an INR of less than 1.5.

A stepwise regression model to determine which of the clinical and demographic variables were associated with the percentage of time spent above and below the target INR was performed. The model demonstrated that independent predictors of time above were the CYP2C9*3 allele and VKORC1 haplotype, whereas for time below, predictors were the WT CYP2C9 allele, younger age, and VKORC1 haplotype group B.

CYP2C9 and VKORC1 genotypes correlated strongly with the INR values on days 7 to 28 of warfarin therapy, suggesting that pharmacogenetics testing may be useful even if results are not available when warfarin is initiated. CYP2C9*3 seems useful not only for estimating the initial warfarin dose, but also for identifying patients at risk for elevated INR during induction.

61

Determination of HbA_{1c} in the Presence of Unusual Hemoglobin Variants: A Comparison of Results From the Bayer DCA-2000 and the Bio-Rad Variant II.

Kelly A. Lloyd, Marcia Doersch, Linda Y. Mamiya, and Daniel E. Sabath. Department of Laboratory Medicine, Red Cell Disorders Laboratory, University of Washington, Seattle.

The level of hemoglobin A_{1c} (HbA_{1c}) reflects blood glucose levels during the previous 2 to 3 months and is a useful tool in the management of patients with diabetes. High-pressure liquid chromatography (HPLC) is the preferred method for measuring HbA_{1c} owing to lower cost and better accuracy than an alternative method, the Bayer DCA-2000 immunoassay. However, the presence of a hemoglobin variant can interfere with accurate HbA_{1c} measurement by HPLC. In the presence of variant hemoglobins, the immunoassay is more reliable because it detects the glycated amino terminus of the β-globin peptide and is not affected by hemoglobin variants other than those of the amino terminus of the β-globin chain. We compared HbA_{1c} measurements by the Bayer DCA-2000 immunoassay and the Bio-Rad Variant II HPLC method in patients with unusual hemoglobin variants to determine which variants interfere with the HPLC assay.

In specimens obtained in the 2001-2006 period, we made 102 HbA_{1c} measurements by HPLC and immunoassay from patients with 11 different hemoglobin variants. Identification of hemoglobin variants was made by directly sequencing the variant genes or by isoelectric focusing (IEF) and/or HPLC comparison with a variant previously identified by sequencing (with the exception of hemoglobin B_2/A_2',which was identified by IEF/HPLC). The results were analyzed and compared using 2-tailed, paired t tests.

We found that the HPLC and immunoassay results were comparable for hemoglobins B_2/A_2' (n = 12; $t = 2.91$; $P = .014$), D (n = 25; $t = 3.41$; $P = .002$), G-Philadelphia (n = 9; $t = 3.81$; $P = .005$), HaSharon (n = 24; $t = 5.35$; $P < .001$), Manitoba (n = 3; $t = 4.62$; $P = .04$), and Toulon (n = 8; $t = 3.02$; $P = .02$). HPLC results did not correlate with immunoassay for hemoglobins Constant Spring (n = 6; $t = 2.07$; $P = .09$), G-Coushatta (n = 4; $t = 0.23$; $P = .83$), Grady (n = 4; $t = 2.10$; $P = .13$), J-Broussais (n = 4; $t = 0.14$; $P = .90$), or LeLamentin (n = 3; $t = 2.65$; $P = .12$). Therefore, an accurate measurement of HbA_{1c} can be made by Bio-Rad Variant II HPLC in patients with the hemoglobin B_2, D, G-Philadelphia, HaSharon, Manitoba, or Toulon trait, whereas an alternative method, such as the Bayer DCA-2000 immunoassay, is required to measure HbA_{1c} in patients with the hemoglobin Constant Spring, G-Coushatta, Grady, J-Broussais, or LeLamentin trait.
