

abstracts of papers

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 annual meeting of the Academy of Clinical Laboratory Physicians and Scientists (ACLPS), June 3-5, 2004, in Denver, CO. Authors receiving a 2004 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.

2

Interference of Three Chinese Medicines, Tian-Wang-Bu, An-Shen-Bu-Xin, and Dingxin-Wan, With Serum Digoxin Immunoassays and Neutralization of Free Digoxin-Like Immunoreactive Components by Digibind.

Doina Ivan, Jennifer Jiang, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School.

Chinese medicines are available without prescription, and the general population widely uses such medications. Tian-Wang-Bu and An-Shen-Bu-Xin are popular Chinese medicines used by the older population for stimulation of the heart, and each medication contains 5 to 6 different herbal preparations. Dingxin-Wan is used both as an antidepressant and a heart stimulant. Because people taking digoxin also can use these medications, we studied the potential interference of these medications with 3 commonly used digoxin immunoassays (some components of these medicines have structural similarity with digoxin). We also studied the possibility of neutralizing the free digoxin-like immunoreactive components of these Chinese medicines using the polyclonal Fab fragment of an antidigoxin antibody (Digibind, Burroughs Wellcome).

We prepared aqueous, ethyl alcohol, and ethyl acetate extracts of each of these Chinese medicines (25 mg/mL), and 10 to 20 μ L was added to 1-mL aliquots of drug-free serum (expected in vivo concentrations based on recommended dose and 50% bioavailability). Then apparent digoxin concentrations were measured by the fluorescence polarization immunoassay (FPIA) using the TDx/FLX analyzer (Abbott Laboratories), microparticle enzyme immunoassay (MEIA) using the AxSYM analyzer (Abbott Laboratories), and a chemiluminescent immunoassay (CLIA) using an ACS:180 analyzer (Bayer Diagnostics).

We observed significant digoxin-like immunoreactivity (range, 0.46-0.98 ng/mL) in the ethanol and ethyl acetate extracts of Tian-Wang-Bu. The ethyl acetate extract of An-Shen-Bu-Xin and the aqueous and ethyl acetate extracts of Dingxin-Wan showed significant immunoreactivity. The highest activity was observed with the ethyl acetate extract of Tian-Wang-Bu. These digoxin-like immunoreactivities were observed only with the FPIA assay. The protein binding of digoxin-like immunoreactive components varied between 45% and 55% in these 3 herbs. More interestingly, when digoxin serum pools prepared from patients receiving digoxin were supplemented with extracts of these Chinese medicines, we observed falsely increased serum digoxin concentrations (25%-30% increases) with the FPIA, but apparent digoxin concentrations were falsely lowered (30%-40%) using the MEIA assay. The CLIA assay showed no interference from any of these Chinese medicines. Free digoxin-like immunoreactive components can be neutralized by using Digibind (5-10 μ g/mL) in vitro as evidenced by complete disappearance of free digoxin activity in the presence of Digibind. We conclude that the CLIA assay is free from interference of these Chinese medicines, but the FPIA assay can be used for rapid detection of these Chinese herbs in serum.

6

***Campylobacter concisus* in Unexplained Diarrhea: Identification by 16S Ribosomal DNA Sequencing.**

Roger D. Klein, Susan Bell, and Stephen C. Edberg. Sponsor: Peter Jatlow. Department of Laboratory Medicine Yale University, New Haven, CT.*

We investigated possible relationships between *Campylobacter* species other than *Campylobacter jejuni* and unexplained diarrhea using sequencing of a *Campylobacter*

genus-specific segment of the 16S rRNA gene to identify and speciate *Campylobacter* isolates.

Stool samples from patients who presented to the Yale-New Haven Hospital primary care and emergency departments with and without diarrhea from April to September 2003 were collected in liquid Cary-Blair transport medium. Samples from 225 patients with diarrhea and 67 control subjects were cultured at 37°C on Mueller-Hinton agar with 5% sheep blood in a hydrogen-enriched (5%) microaerobic atmosphere for 7 days. Samples were filtered using 0.65- μ m filters and incubated at 37°C before culture. *Campylobacter* isolates were identified by sequencing a polymerase chain reaction (PCR)-amplified *Campylobacter*-specific segment of the 16S rRNA gene. These identification results were compared with reference identifications that were made using a combination of microscopy, biochemical tests, and full-length 16S rDNA sequencing.

We recovered 40 bacterial isolates from patients and control subjects. Fourteen isolates from the 225 patients and 2 isolates from the 67 control subjects were identified as *Campylobacter* species by genus-specific PCR. Of 16 *Campylobacter* isolates, 16 were identified as *Campylobacter concisus* by sequencing of the *Campylobacter* genus-specific PCR products. All sequences obtained were highly homologous to reference sequences, with the majority (11/16) of isolates demonstrating matches of more than 99%. Identifications by PCR amplification and sequencing of *Campylobacter* genus-specific PCR products were 100% concordant with reference identifications of the 40 isolates. Patients had an approximately 2.08-fold greater likelihood of growing *C concisus* in stool than did control subjects. This difference was not statistically significant (95% confidence interval, 0.46-9.39). There was no statistically significant difference in the rate of recovery of *C concisus* in patients and control subjects, suggesting that *C concisus* might be part of the normal gastrointestinal flora in some individuals. PCR amplification and sequencing of *Campylobacter* genus-specific segments of the 16S rRNA gene might be an efficient method for definitively answering this question in larger studies.

7

Effect of Grapefruit Juice on Bioavailability and Metabolism of the Chinese Medicine, Chan Su.

Syed Zaidi,* Margaret Olsen, Alice Wells, Jeffrey K. Actor, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School.

Chinese medicines are readily available from herbal stores. Chan Su is a Chinese medicine used as a cardiogenic agent. Grapefruit juice contains furanocoumarins, which inhibit cytochrome P-450 and reduce the clearance of certain drugs. Components of grapefruit juice also inhibit P-glycoprotein, thus increasing the bioavailability of specific drugs. By taking advantage of high cross-reactivity of bufalin, the active component of Chan Su, with the fluorescence polarization immunoassay (FPIA) of digoxin (Abbott Laboratories, Abbott Park, IL), we studied the interaction between grapefruit juice and Chan Su in mouse model.

In the first experiment, 5 control mice were fed with aqueous extract of Chan Su (15 mg/kg) using gavage. Five mice in the experimental group received aqueous extract of Chan Su along with 200 μ L of grapefruit juice. Blood specimens were withdrawn at 1-, 2-, 6-, 24-, and 48-hour intervals, and apparent digoxin concentrations were measured using the FPIA assay and

the FLx/TDx analyzer. We observed no significant difference in apparent digoxin concentrations in the control and experimental groups at 1 hour, indicating that when the mice were fed with Chan Su along with grapefruit juice there was no increased absorption of Chan Su. However the half-life of Chan Su was significantly prolonged in the presence of grapefruit juice (no apparent digoxin activity observed after 24 hours in control vs 3 of 5 mice showed measurable digoxin-like immunoreactivity even at 48 hours when Chan Su was administered with grapefruit juice).

In the second set of experiments, 9 mice received grapefruit juice (200 μ L) the night before the experiment, and 5 of 9 mice also received another dose of grapefruit juice 1 hour before feeding with Chan Su. Control mice received no grapefruit juice. Then all mice were fed with same dose of Chan Su, and again blood specimens were withdrawn at 1, 2, 6, 24, and 48 hours. Interestingly, when mice were given grapefruit juice the night before and again 1 hour before feeding with Chan Su, the bioavailability of Chan Su was increased and clearance was decreased compared with the control group. For example, in mouse 5 in group 1 (received grapefruit juice twice), the apparent digoxin concentrations were 2.12, 1.56, 0.94, and 0.34 ng/mL at 1-, 2-, 6-, and 24-hour intervals. In contrast, in mouse 4, control group, the corresponding values were 0.96, 0.78, and 0.28 ng/mL at 1-, 2-, and 6-hour intervals. No level was detected at 24 hours.

We conclude that if Chan Su is fed to mice along with grapefruit juice, the clearance of Chan Su is reduced but bioavailability is not increased. In contrast, if grapefruit juice is administered before the feeding, bioavailability was increased and clearance was reduced. The maximum effect was observed when grapefruit juice was administered twice (the night before experiment and 1 hour before feeding with Chan Su). Such effects might be present in humans, and consumption of grapefruit juice might increase the toxicity of Chan Su.

8

Using a Diluted Thrombin Time as an Indicator of Plasma Argatroban Concentration.

Jason Love,* Chris Ferrell, and Wayne Chandler. Department of Laboratory Medicine, University of Washington, Seattle.

Argatroban is a direct thrombin inhibitor approved for the prophylaxis and treatment of thrombosis associated with heparin-induced thrombocytopenia. Dose adjustment of argatroban typically is achieved by monitoring the activated partial thromboplastin time, but this can be problematic in patients with lupus inhibitors. We evaluated the thrombin time (Diagnostica Stago STA) as a lupus inhibitor-insensitive alternative for monitoring argatroban levels.

First, we developed a dose-response curve for the thrombin time vs plasma argatroban level over concentrations that spanned argatroban's therapeutic range. The therapeutic thrombin time ranged from 135 to 210 seconds. This range may not be useful for all instruments, as some methods do not report thrombin times greater than 100 seconds. Next, we spiked 34 plasma samples with a therapeutic, 1.0- μ g/mL dose of argatroban and measured the thrombin time. The 34 samples included patients with lupus inhibitors, high and low fibrinogen levels, high and normal D-dimer levels, and warfarin treatment, and patients with no known coagulation abnormalities. Based on our dose-response curve, the

expected thrombin time for 1.0 µg/mL of argatroban was 154 seconds. In our 34 samples, the mean thrombin time was 131 seconds, with a coefficient of variation of 23%. The accuracy and precision were unacceptable. Despite spiking with a therapeutic dose, the thrombin time indicated that most samples were subtherapeutic while others were supertherapeutic.

Next we diluted each of the argatroban-spiked samples 1:4 with normal plasma and remeasured the thrombin time. Based on our dose-response curve, the therapeutic range for diluted specimens was 55 to 100 seconds, a level more suited for thrombin time measurement on most instruments. The expected thrombin time for a 1.0-µg/mL dose after 1:4 dilution was 73 seconds. In our 34 samples, the mean thrombin time was 68 seconds with a coefficient of variation of 9%. No values fell outside the therapeutic range. It is likely that the normal plasma dilution supplemented deficient factors or diluted out interfering factors, allowing the assay to be a more specific measure of argatroban concentration.

We conclude that an undiluted thrombin time is too sensitive and too variable in its response to argatroban for use in therapeutic monitoring. The diluted thrombin time is an accurate indicator of argatroban level suitable for argatroban monitoring in most patients, including those with lupus inhibitors.

9

Comparison of Chemiluminescent and Enzyme Immunoassays for the Detection of Hepatitis B Core Antibody.

*Kim D. Jewell, David A. Black, and Daniel D. Bankson.
Departments of Pathology and Laboratory Medicine, Veterans Affairs Puget Sound Health Care System and University of Washington Medical Center, Seattle.*

The presence of antibodies to hepatitis B core antigen (anti-HBc) is a useful marker of hepatitis B virus (HBV) exposure. In certain subsets of patients, anti-HBc might be the only indicator of HBV infection. An automated enhanced chemiluminescent assay for anti-HBc is now available for use by US laboratories. The object of the present study was to compare anti-HBc results from the VITROS Immunodiagnostic Products Anti-HB Core Reagent Pack (Ortho-Clinical Diagnostics, a Johnson and Johnson Company, Rochester, NY) with our current Abbott Corzyme EIA (Abbott Laboratories, Abbott Park, IL). Both assays use competitive immunoassay techniques with the amount of anti-HBc present inversely proportional to the signal detected. The VITROS ECi assay uses horseradish peroxidase-labeled mouse anti-HBc for competition and a chemiluminescent luminol derivative with electron transfer enhancement to generate a light signal.

We assayed 40 human serum samples by both methods, with 100% agreement. All 21 samples for which no anti-HBc was detected by the Abbott assay also were negative by the VITROS ECi. Similarly, 19 Abbott-positive samples were positive by VITROS with no indeterminate results. In comparison with the Abbott assay, the VITROS assay was 100% sensitive and specific. In our experience, the automation of the VITROS ECi system has proven time-efficient, decreasing both sample handling time and time to result reporting. The advantage of bar-coded patient sample identification and automation became apparent to us during this study. Testing using the VITROS ECi system revealed a false-positive test by the Abbott method secondary to a technologist transcription error that occurred in the more manual Abbott assay. In addition, the VITROS ECi is a random access, STAT-capable platform

with decreased calibration and repeated testing requirements in comparison with the Abbott Corzyme method.

This study demonstrates excellent agreement of anti-HBc results for the Ortho VITROS ECi with the Abbott Corzyme EIA. Also, the Ortho VITROS ECi offers the advantages of automation and decreased risk of transcriptional errors that might lead to erroneous result reporting.

11

Memory-Specific Transcripts in CD8+ T Cells Are Enriched in Long-Term Hematopoietic Stem Cells.

Chance John Luckey,^{1,2} Ananda W. Goldrath,² Christophe Benoist,² and Diane Mathis.² Sponsor: David B. Sacks.*

¹Department of Pathology, Brigham and Women's Hospital, and ²Joslin Diabetes Center, Harvard Medical School, Boston, MA.

Memory T cells have an essential role in protection from reexposure to most viral, parasitic, and bacterial infections. Memory T cells specific for a pathogen can be detected for many years after pathogen eradication, and their persistence requires an active process of self-renewal. Our goal was to identify the molecular pathways essential for memory T-cell self-renewal.

To gain an unbiased view of the genes uniquely expressed in memory T cells, we developed a well-defined CD8+ T-cell receptor transgenic system combined with Affymetrix GeneChips to compare memory with naive and effector populations. GeneChip data were analyzed independently using either Affymetrix MAS 5.0 software or the RMA algorithm in the Affy statistical analysis package developed by the collaborative, open source project www.bioconductor.org.

The probe level analysis of RMA demonstrated a significant reduction in the statistical noise of our data and was used to identify transcripts that were enriched uniquely in memory T cells. We then validated 21 selected memory-enriched transcripts via quantitative polymerase chain reaction (PCR) with 20 of the genes demonstrating a fold change equal to or greater than that predicted in the GeneChip analysis. In fact, the vast majority of transcripts were expressed at much higher relative levels in memory cells when measured by PCR. Finally, we looked at relative expression of our memory cell-enriched genes in various stem cell populations. Interestingly, many of these memory T cell-enriched transcripts also are enriched in long-term hematopoietic stem cells when compared with short-term and common progenitor populations. This suggests that some of the pathways active in memory T cells are shared by other self-renewing cells in the hematopoietic system. Given the role of memory T cells in immune responses ranging from effective childhood vaccination to destructive autoimmune diseases, we hope that the further elucidation of these pathways will provide targets for the enhancement or reduction of T-cell memory as needed in particular clinical situations.

12

Translating 16S rDNA Sequencing Identification Into Cost Effective Phenotypic Testing.

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

Although sequencing of bacterial 16S rDNA is the "gold standard" for positive bacterial identification, the technique is

technically and fiscally impractical for routine incorporation at the clinical bench. Our objective was to devise an inexpensive and readily accessible identification scheme that would allow identification with accuracy equal to 16S rDNA sequencing. The development of such phenotypic identification schemes is especially poignant for clinically significant species belonging to groups with overlapping biochemical features.

In our study, we used a large group of *Streptococcus milleri* clinical isolates previously sequenced to species: 17 *Streptococcus anginosus* (SA), 16 *Streptococcus constellatus* (SC), and 12 *Streptococcus intermedius* (SI). Isolates were tested by the Vitek1.0, Vitek2.0, and Fluo-card milleri identification systems. In addition, an innovative method (triplate method) of identification using hyaluronidase production and hemolysis on sheep and human blood agars was evaluated.

The Vitek1.0 correctly identified 0%, 19%, and 0% of SA, SC, and SI, respectively. The Vitek2.0 correctly identified 88%, 38%, and 100% of SA, SC, and SI, respectively. Fluo-card milleri correctly identified 81%, 38%, and 67% of SA, SC, and SI, respectively. Finally, the triplate method correctly identified 94%, 88%, and 100% of SA, SC, and SI, respectively.

We found the use of a large group of sequenced strains as a powerful means of evaluation of bench, commercial, and novel methods of bacterial identification, especially groups composed of member species with overlapping biochemical features. Of the commercial products assessed, the Vitek1.0 provided the least reliable results, while the Vitek2.0 proved more reliable than Fluo-card and Vitek1.0. Initial results of the triplate method are promising, providing the most consistent identification of the *S milleri* group species when compared with the commercial methods studied.

13

Black Cohosh Interferes With Estradiol and Progesterone Measurement In Vitro and in Female Mice In Vivo.

Catherine McNeese, Margaret Olsen, Alice Wells, Jeffrey K. Actor, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School.

Black cohosh is a popular alternative to estrogen replacement therapy for relief of menopausal symptoms, primarily hot flashes. Studies indicate that this herbal remedy is effective in alleviating menopausal symptoms. Phytoestrogen cimicifugoside, cyclolanostanol xylosides, and terpene glycosides are present in black cohosh.

We studied potential interference of 2 black cohosh products (product 1, black cohosh 65% grape alcoholic extract, Electric Institute, Sandy, OR; product 2, black cohosh dry extract in capsules, Nature's Herb, UT) with estradiol and progesterone assays. Two standard solutions of dry powder of black cohosh were made. One solution was made in water (25 mg/mL) and another in absolute alcohol (25 mg/mL). Then 2.5 or 5 μ L of liquid extract of black cohosh or 5 or 10 μ L of aqueous or alcohol extract of black cohosh (from standard solutions, product 2) was added to 1 mL of serum. The estradiol level was measured by using the microparticle enzyme assay (Abbott Laboratories) on the IMx analyzer or the chemiluminescent assay (Bayer Diagnostics) on the ACS:180 Plus analyzer. The progesterone level was measured by using a chemiluminescent assay (Immulate, DPC) and also by using the Bayer assay on the ACS:180 Plus analyzer. Three different serum pools were studied for in vitro experiments.

We observed significant positive interference of the liquid extract of black cohosh (product 1) and the aqueous extract of product 2. No effect was observed with the alcohol extract of product 2. Product 1 and product 2 (aqueous and alcoholic extracts) interfered with the progesterone assay. For example, the concentration of estradiol in pool 1 was 61.4 pg/mL. In the presence of 5 μ L of product 1, the value was increased falsely to 91.8 pg/mL. The value also was increased falsely to 74.7 pg/mL with 5 μ L of aqueous extract of product 2, but the value was 63.8 pg/mL in the presence of 5 μ L of the alcoholic extract of product 2. Similarly, progesterone values increased from a control value of 0.57 to 1.20 ng/mL in the presence of 5 μ L of product 1. The progesterone value also was increased to 0.85 ng/mL in the presence of 5 μ L of the alcoholic extract of product 2. Product 1 demonstrated more significant interference with both assays than did product 2. Interestingly, the Bayer Diagnostics estradiol assay was unaffected by both black cohosh products, but a significant negative interference was observed in the progesterone assay manufactured. Such effects also were present in vivo when we fed female mice with black cohosh. Mice were bled the night before the experiment to obtain the baseline, and blood was obtained from each mouse 1 hour after feeding with black cohosh.

We conclude that black cohosh interferes with the estradiol assay manufactured by Abbott Laboratories, but such interference can be avoided by using the Bayer estradiol assay. In contrast, both progesterone assays showed significant interference.

14

Evaluation of a Specific Oxycodone Assay for Urine Drug of Abuse Screening.

Kim H. Allison, David A. Black, James Garbin, and Daniel D. Bankson. Departments of Pathology and Laboratory Medicine, Veterans Affairs Puget Sound Health Care System and University of Washington Medical Center, Seattle.

Oxycodone is a semisynthetic opioid prescribed for the management of moderate to severe pain that has increasingly become a drug of abuse. Various immunoassays are commercially available for the detection of opiates in urine, but these assays are not designed for the specific detection of oxycodone and its metabolites oxymorphone and noroxycodone. Other nonimmunoassay techniques include thin-layer chromatography, high-performance liquid chromatography, and gas chromatography-mass spectrometry (GC-MS), but they are time-consuming and less appropriate for the mass screening of urine.

The objective of this study was to evaluate a new enzyme immunoassay, DRI Oxycodone Assay (Microgenics, Fremont, CA), for its use in the qualitative or semiquantitative detection of oxycodone in human urine. The assay uses specific antibodies to detect oxycodone and oxymorphone by an enzyme-mediated immunologic technique. The assay was run on a Hitachi 911 (Roche Diagnostics, Indianapolis, IN). We selected a positive threshold cutoff of 300 ng/mL.

Urine samples confirmed positive by GC-MS (n = 16) all were positive by the DRI Oxycodone Assay. Twenty urine samples that were negative on the DRI Opiate Assay screen (Diagnostic Reagents, Sunnyvale, CA) also tested negative by the DRI Oxycodone Assay. In addition, 2 patients prescribed oxycodone in 5-mg doses 3 times a day had levels below the 300-ng/mL cutoff for detection. Another patient dosed at 5 mg 6 times a day screened

positive. Between-run precision measurements ($n = 10$) at 225 and 375 ng/mL had coefficients of variation (CVs) of 2.1% and 1.8%, respectively. Within-run precision ($n = 10$) evaluated at 700 ng/mL had a CV of 2.3%. The assay was linear up to at least 10,000 ng/mL. In addition, high urinary concentrations of other opiates (morphine, codeine, hydrocodone, levorphanol, methadone, and morphine-3-gluconide) and additional commonly prescribed drugs did not produce semiquantitative results above our 300 ng/mL cut-off. To test the usefulness of this new assay as part of our comprehensive urine drug screen, we used it to screen 99 consecutive patient urine samples from an alcohol and drug abuse treatment program. Two of these samples tested positive for oxycodone with the 300 ng/mL threshold, both of which were negative on concurrent DRI Opiate Assay. The DRI Oxycodone Assay is a new sensitive and specific screening test for detection of urinary oxycodone and may be considered an addition to a urine drug of abuse screening panel or as a separate stand-alone test.

16

Assay-Specific Differences in Lipemic Interference in Native and Intralipid-Supplemented Samples.

Joshua A. Bornhorst,^{1*} Richard F. Roberts,² and William L. Roberts.¹ ¹Department of Pathology, ²ARUP Institute for Clinical and Experimental Pathology, University of Utah Health Sciences Center, Salt Lake City.

Lipemia is a potential cause of interference with chemistry measurements in the clinical laboratory. The lipid emulsion Intralipid frequently is used to simulate lipemia in interference studies.

A series of experiments was performed to compare interference produced by native lipemic patient samples with interference produced by supplementing samples with Intralipid. Sixteen patient samples with varying levels of lipemia were assayed simultaneously in duplicate for lipemic index, triglycerides, α_1 -antitrypsin, ceruloplasmin, prealbumin, transferrin, and haptoglobin, using a Roche Systems Modular Analytics P analyzer. The samples subsequently were subjected to high-speed centrifugation in an Airfuge for 5 minutes. The infranate then was reassayed in duplicate for the same set of analytes. A substantial reduction in L-index and triglyceride concentrations was observed. Intralipid then was added to each sample to produce L-index values equivalent to or exceeding those observed in the original native lipemic patient sample and assayed in duplicate for the same set of analytes. The ratios of the measured concentrations of the analytes in each sample following centrifugation and following Intralipid supplementation relative to the measured analyte concentrations in the native patient sample were calculated.

The correlation coefficients observed for triglyceride concentrations and L-indices in Intralipid-supplemented samples ($r = 0.84$) and native lipemic samples were comparable ($r = 0.88$). Increasing L-index and triglyceride levels in native patient samples were associated with substantial depression of the observed ceruloplasmin and prealbumin concentrations. The degree of interference varied roughly linearly with measured triglyceride levels. The ratio of measured analyte concentration relative to that in the centrifuged sample was approximately 0.05 for ceruloplasmin and 0.2 for prealbumin for a triglyceride concentration of 2,000 mg/dL (L-index of ~350). Transferrin concentrations of native patient samples were

depressed to lesser extent (ratio of 0.8) for transferrin measurements. High concentrations of triglycerides in native samples had little effect on the measured concentrations of α_1 -antitrypsin and haptoglobin. In contrast with the lipemic interference effects observed in native samples, supplementation of patient samples with Intralipid to triglyceride levels of 1,600 mg/dL (L-index of ~400) had little effect on all analyte measurements. Thus, the use of samples supplemented with Intralipid does not provide a universally accurate method to estimate endogenous assay interference in lipemic patient samples.

17

A Reagent Wash Improves the Precision of an EMIT Assay Measuring Free Phenytoin.

Danelle R. Beaudoin, Michael Ka, and Petrie M. Rainey.
Department of Laboratory Medicine, University of Washington and Harborview Medical Center, Seattle.

When the Abbott TDx Free Phenytoin reagent became temporarily unavailable, we investigated Dade-Behring Syva EMIT reagents to measure free phenytoin. These reagents are free of metabolite cross-reactivity problems known to be present with the Abbott reagent. Our objective was to modify the EMIT total phenytoin method to give accurate and precise measurements of free phenytoin concentrations using an Olympus AU400.

Serum ultrafiltrates prepared using Amicon Centrifree devices and in-house standards prepared in phosphate-buffered saline (PBS) were used for free phenytoin measurements. Modifications were made to the manufacturer-recommended EMIT procedure for total phenytoin. Accuracy and precision were determined for each candidate method. Abbott free phenytoin calibrators were used.

The serum ultrafiltrates gave consistently lower results on the EMIT assay than on the TDx assay. Deming regression on 30 specimens ranging from 0.8 to 2.8 $\mu\text{g/mL}$ (TDx) gave a correlation equation of Y (EMIT) = $1.108X$ (TDx) - 0.523 ($R^2 = 0.71$). Using the unmodified total phenytoin procedure on phenytoin in PBS gave unacceptable imprecision of about 9%. Attempts to optimize phenytoin concentrations in the final reaction mixture by increasing the sample volume produced little to no improvement. Incorporating an "R2 push" (a 10- μL flush of the R2 reagent added to the reaction mix) assured consistent final concentrations of reagents and more than halved imprecision. Final conditions using 10 μL of sample and the R2 push gave coefficients of variation of 1.6% to 5.7% and recoveries of 93% to 110% at concentrations from 1 to 4 $\mu\text{g/mL}$.

The lower free phenytoin results found with the EMIT method compared with the TDx method are consistent with an absence of metabolite cross-reactivity. An R2 push on the AU400 effectively improved the precision of the EMIT assay. The R2 push merits investigation for improving the precision of other EMIT methods for therapeutic drug monitoring.

19

Haptoglobin Phenotype in Systemic Lupus Erythematosus.

Yasi Saffari,^{*} Jafar Bozorgmehr, and S.M. Hossein Sadrzadeh.
Department of Laboratory Medicine, University of Washington, Seattle.

Systemic lupus erythematosus (SLE) is a multisystem disease of autoimmune origin. It is a chronic, relapsing, remitting disease that involves joints, skin, serosal membrane, central nervous system, kidney, and the cardiopulmonary systems. The cause of SLE remains unknown. Genetic factors are believed to be of importance in the etiology of SLE. Also, it is now established that patients with SLE have increased oxidative stress. Iron and iron-rich compounds like hemoglobin have been known to be potent oxidants. Haptoglobin (Hp), a plasma protein that removes free hemoglobin from circulation to prevent renal damage, also may be involved in the etiology of SLE. Hp has 3 phenotypes (Hp 1-1, Hp 2-2, and Hp 2-1) in which type 1-1 is biologically the most active and type 2-2 is the least active. Hp phenotypes have been shown to have significant impact on the extent of tissue injury following myocardial infarction. That is, patients with type 2-2 had the most and those with 1-1 had the least myocardial damage following acute myocardial infarction.

We investigated the role of Hp in SLE by correlating plasma Hp phenotypes and total antioxidant activity in patients with SLE. Leftover specimens from 25 patients who visited Harborview Medical Center (HMC) or the University of Washington Medical Centers (UWMC) were selected randomly. Control subjects were healthy subjects without liver or hemolytic disorders who visited HMC or UWMC for routine checkups. Hp was phenotyped by gel electrophoresis, and total antioxidant status was assessed by using a colorimetric method.

Our results showed strong correlation between plasma Hp phenotypes and SLE. That is, 60% of patients with SLE had Hp 2-2. Only 29% of patients had Hp 2-1, and 11% had Hp 1-1. In contrast, the Hp phenotype type distribution in control subjects was as follows: Hp 2-2, 35%; Hp 1-1, 20%; and Hp 2-1, 45%. Also, plasma total antioxidants were lower among patients compared with control subjects regardless of the Hp type (mean \pm SD, 0.87 ± 0.36 vs 1.45 ± 0.35 mg/dL, patients vs control subjects, respectively; $P < .05$). Our preliminary results show that patients with SLE have less antioxidant defense against oxidative injury, and the majority of these patients had Hp 2-2. Hp 2-2 might be a risk factor for developing SLE and might be used as a marker to identify individuals at risk for this disorder.

20

Association of Haptoglobin Phenotypes, Oxidative Stress, and Epilepsy.

Jafar Bozorgmehr,* Yasi Saffari, and S.M. Hossein Sadrzadeh.
Department of Laboratory Medicine, University of Washington, Seattle.

Epilepsy, one of the most common neurologic disorders, affects approximately 2 million Americans. The biochemical etiology of seizure disorders is still unknown. It is well established that iron and iron compounds can cause oxidative damage to tissues such as brain. Hemoglobin is the richest source of iron in the body that has been suggested to be involved in hemorrhagic brain damages. Haptoglobin (Hp) is a plasma glycoprotein that binds free hemoglobin and removes it from circulation to prevent renal damage. Hp has 3 phenotypes (Hp 1-1, Hp 2-2, and Hp 2-1). Type 1-1 is biologically the most active, and type 2-2 is the least active. Recently, we showed that Hp 2-2 was overrepresented in epilepsy.

The goal of this study was to investigate the association of Hp phenotypes and oxidative stress in recurrent epilepsy. Blood

samples were obtained from 14 patients with idiopathic epilepsy with more than 1 tonic clonic seizure attack per month and 26 healthy control subjects. Oxidative status was assessed by measuring plasma thiobarbituric acid reactive substances (TBARs; aldehydic by-product of lipid peroxidation), β -carotene, and total antioxidant capacity (TAOC). Hp phenotypes were determined by gel electrophoresis.

The distribution of Hp phenotypes in our patients was as follows: Hp 2-2, 66%; Hp 2-1, 22%; and Hp 1-1, 12%. The distribution of Hp phenotypes in control subjects was as follows: Hp 2-2, 37%; Hp 2-1, 49%; and Hp 1-1, 14% ($P < .001$). The mean plasma TBARs was higher in patients than in control subjects (mean \pm SEM, 0.88 ± 0.06 vs 0.64 ± 0.07 μ mol TBARs/mg protein, patients vs control subjects, respectively; $P < .05$). Importantly, the mean TBARs was highest in patients with Hp 2-2 (0.98 ± 0.08 μ mol TBARs/mg protein) and lowest in those with Hp 1-1 (0.8 ± 0.03 μ mol TBARs/mg protein). Also, the mean TAOC was significantly lower in patients than in control subjects (mean \pm SEM, 1.21 ± 0.03 vs 1.508 ± 0.05 mmol/L, patients vs control subjects, respectively; $P < .001$). Notably, patients with Hp 2-2 had the lowest (1.181 ± 0.04 mmol/L) and those with Hp 1-1 had the highest (1.44 ± 0.01 mmol/L) TAOC. In addition, β -carotene levels in patients were significantly lower than in control subjects (mean \pm SEM, 0.35 ± 0.03 vs 0.53 ± 0.05 μ g/dL, patients vs control subjects, respectively; $P < .05$). Mean β -carotene levels were lowest in patients with Hp 2-2 (0.28 ± 0.05 μ g/dL) and highest in patients with Hp 1-1 (0.47 ± 0.05 μ g/dL). Our results suggest that association of Hp 2-2 with epilepsy might result in enhanced oxidative stress and low antioxidant defense in patients with epilepsy. Thus, Hp 2-2 might be useful as a marker for early diagnosis and prognosis for Hp 1-1 in individuals susceptible to epilepsy.

21

Development and Implementation of an Enterprise-Scalable, Multi-institutional Platform Supporting the Prospective Banking of Clinical Specimens.

Lynn Bry, Neil Herring, Milenko Tanasijevic, and David Weinberg.
Clinical Laboratories and Division of Pathology Informatics, Brigham and Women's Hospital, Boston, MA.

We are building a platform to develop consented, deidentified or anonymized repositories of clinical specimens that link to associated phenotypic data from the clinical record. Our approach has addressed data warehousing and operational issues concerning the functional and prospective banking of discarded and excess clinical samples for institutional review board (IRB)-approved projects. Partners Healthcare has been at the forefront of supporting such endeavors, including the development of multi-institutional databases and query tools to harness terabytes of clinical information for ongoing research and clinical trials.

A collaborative effort among the Brigham and Women's Hospital Department of Pathology, Harvard-Partners Center for Genetics and Genomics, Massachusetts General Hospital Laboratory of Computer Science, and Allez Software, Lexington, MA, has resulted in the operational infrastructure, software, and IT platform to actively bank clinical materials for IRB-approved studies, while tying into existing electronic resources of phenotypic information from the clinical record. Key pieces of this infrastructure include the following: (1) a robust query engine by which to identify samples for collection from among the thousands

accessioned daily; (2) LIMS functions to assist with banking, inventory management, and further sample processing; and (3) study management and IRB interfaces to ensure HIPAA compliance and stringent standards for protecting patient privacy. Furthermore, we anticipate this platform will provide an operational component that prospectively might populate existing networks such as SPIN (Shared Pathology Informatics Network) with clinical materials and we hope will form the basis for an available source of software, standards, and methods to coordinate prospective banking activities among institutions.

22

Breast Lymphoma: A Review of 18 Cases.

Virginia E. Duncan, Vishnu V.B. Reddy, Nirag C. Jhala, David C. Chhieng, and Darshana N. Jhala. Department of Pathology, University of Alabama at Birmingham.

The literature addressing breast lymphoma is sparse. In patients with clinically suspected breast malignancy, it is important to distinguish carcinoma from lymphoma owing to significant differences in their treatment and prognosis. This study retrospectively evaluated the clinical features, morphologic features, and incidence of breast lymphoma during 13 years at the University of Alabama, Birmingham. The usefulness of fine-needle aspiration (FNA) was evaluated in the cases for which results were available.

Eighteen cases of breast lymphoma during a period of 13 years were reviewed. These patients presented with a strong suspicion of breast carcinoma based on clinical history or mammographic findings. FNA, tissue biopsy or resection, or both were performed to determine the diagnosis. Patients' ages ranged from 33 to 91 years (mean age, 60.5 years); 17 were female and 1 was male. FNA was performed in 7 of 18 patients, and tissue diagnosis was available for all 18 cases.

The FNA diagnosis was consistent with the tissue diagnosis in 6 (86%) of 7 cases. Of 6 cases, 1 was diagnosed as an atypical lymphoproliferative process, favor benign (14%), which subsequently was diagnosed on biopsy as diffuse large B-cell lymphoma. Tissue diagnoses revealed that most cases (12 [67%]) represented diffuse large B-cell lymphomas and 3 cases (17%) were follicular lymphomas. The remaining 3 cases (17%) were diagnosed as plasma cell neoplasm, T-cell neoplasm, and precursor B lymphoblastic lymphoma. Flow cytometry and gene rearrangement studies supported the diagnosis of lymphoma in 10 cases. Although rare, breast lymphoma should be considered in the differential diagnosis of a breast mass. A high index of suspicion for lymphoma should be maintained because it can facilitate the accurate triage of breast biopsy specimens for ancillary studies. FNA, along with ancillary studies, is useful for the initial diagnosis of breast lymphoma because it can facilitate appropriate triage of patients for further management.

25

Detection of Small Peptides in Serum Specific to Acute Myocardial Infarction by Surface-Enhanced Laser Desorption/Ionization Mass Spectrometry.

Kenneth C. Clark, Mohamed A. Virji, and Jorge L. Sepulveda. Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

The annual incidence of acute myocardial infarction (AMI) in the United States is approximately 1.1 million, with almost half of these cases resulting in death within 1 year of the event. While sensitive and specific biochemical markers of cardiomyocyte damage such as cardiac troponins and creatine kinase MB (CK-MB) have greatly enhanced the ability to diagnose AMI, symptoms often precede their detection by as much as 4 to 8 hours, which can result in delayed or suboptimal treatment. Our objective was to identify smaller peptides released from the damaged myocardium into the serum that might be detectable earlier than current tests for cardiac markers.

By using surface-enhanced laser desorption/ionization mass spectrometry (SELDI), the serum proteomic profiles from 13 control subjects (non-AMI hospital patients), 35 AMI patients with elevated cardiac troponin levels, and a pooled sample from more than 100 healthy individuals were analyzed. Peak cluster analysis was performed with the Biomarker Wizard of the Ciphergen ProteinChip Software.

Of 298 peak clusters found, 128 were significantly different ($P \leq .05$) between the patients and control subjects using a 2-tailed t test. Eighteen of these clusters, with mass to charge (m/z) ratios between 1,063 and 16,700, had mean intensity ratios between patients and control subjects greater than 1.8 and diagnostic sensitivities ranging from 25% to 71% with specificity of 90% or more. A combination of 4 selected clusters achieved 100% sensitivity and specificity for AMI. Peptides smaller than cardiac troponin and CK-MB are released into the serum during an AMI. Further identification of these peptides might lead to methods for earlier detection of AMI.

26

Function and Evolution of the Pregnane X Receptor: A Nuclear Hormone Receptor That Regulates Drug and Endobiotic Metabolism.

Matthew D. Krasowski, Kazuto Yasuda, Erin G. Schuetz, and Anna Di Rienzo. Sponsor: Jonathan L. Miller. Department of Pathology, University of Chicago, Chicago, IL.*

The pregnane X receptor (PXR; NR1I2) is a nuclear hormone receptor that regulates a coordinated metabolic response to facilitate the elimination of toxic concentrations of drugs (xenobiotics) and endogenous compounds (endobiotics). Ligands as diverse as rifampin, hyperforin (from St John's wort), pregnane steroids, and bile acids activate human PXR, which then leads to increased expression of enzymes and transporters involved in metabolic detoxification and excretion (eg, cytochrome P-450 enzymes). The PXR gene shows extensive sequence diversity between different animals, higher than other nuclear hormone receptors. Our purpose was to study the spectrum of compounds that can activate human PXR and to perform cross-species comparisons of PXR function in fish, amphibians, birds, and mammals.

Cloned cDNAs of human, rabbit, mouse, rat, chicken, frog, and zebrafish PXRs were used to create luciferase-based reporter assays. The cDNA constructs were expressed in HepG2 liver cells by calcium phosphate transfection. Xenobiotics and endobiotics were tested at concentrations from 1 nmol/L to 200 μ mol/L.

PXR function shows dramatic differences between the animal species. A broad range of xenobiotics, steroids, and bile acids activate mammalian PXRs at micromolar concentrations, with human PXR

showing the broadest ligand specificity. In contrast, fish and frog PXR are activated by a more restricted range of endogenous ligands and are not activated by xenobiotics such as rifampin. The zebrafish PXR is activated particularly well by species-specific bile alcohols (but not mammalian bile acids) and by testosterone metabolites. The tuberculosis drug rifampin, which produces marked induction of human metabolic enzymes, activates human PXR well but does not activate rodent PXR, consistent with the inability of rifampin to up-regulate rodent drug metabolism. Human PXR shows very broad ligand specificity and responds to toxic levels for a range of xenobiotics and endobiotics. PXR from a variety of species demonstrate a progressive broadening of ligand specificity in the evolution from fish to amphibians to mammals. PXR originally may have functioned to respond to toxic levels of endobiotics, and its role expanded in mammals to respond to toxic dietary compounds. Certain prescription and herbal drugs activate this "toxic chemical surveillance" system, which leads to clinically important drug-drug interactions.

27

Outcome of Transfusion of CMV-Seronegative Immunosuppressed Cancer Patients With CMV Seropositive Granulocytes.

Fernando Martinez, Kathleen Sazama, Aida B. Narvios, and Benjamin Lichtiger. Division of Pathology and Laboratory Medicine, the University of Texas M.D. Anderson Cancer Center, Houston.

This study addressed the question of whether cytomegalovirus (CMV)-seronegative immunosuppressed patients should receive only CMV-seronegative granulocyte transfusions. The design was a case-control study. Our question was that the CMV serology was independent of CMV antigenemia (pp65) after transfusion with CMV-seropositive granulocytes. CMV serology was the independent variable. Cases were immunosuppressed cancer patients with CMV-seronegative status. Controls were immunosuppressed cancer patients with unknown CMV serology. We searched the files of the M.D. Anderson Cancer Center for patients who received granulocyte transfusions between January 2002 and December 2003. Data were analyzed using Epi Info 3.01 (CDC).

We identified 120 patients: 18 were CMV-seronegative, and 42 had unknown CMV serology. Because of lack of pp65 ordered after transfusion, we excluded 9 CMV-seropositive patients and 20 CMV patients with unknown serology. Randomly, we selected 9 unknown serology patients as control subjects. In total, there were 9 controls and 9 cases. Sixteen patients had leukemia (84%), 2 had MDS (11%), and 1 had Hodgkin disease (5%). Fungal pneumonia was the most common complication (35%). Prophylactic antivirals were given to 15 patients (79%). One case had a positive pp65 after transfusion (5%). This was detected 5 days after transfusion with 1 U of CMV-seropositive granulocytes. No controls had positive pp65 after transfusion. No patient developed CMV disease.

There was no difference in the odds of developing CMV antigenemia between cases and controls. One CMV-seronegative patient had a positive pp65 after transfusion, but none from the CMV unknown serology. The lapse of 5 days after transfusion and the time of the positive pp65 agrees with a precursor monocyte as a

reservoir of CMV because granulocytes have a shorter half-life. Owing to CMV ubiquity, we cannot rule out natural infection. We concur with previous studies in that in our population there is not an added risk of developing CMV infection when transfusing CMV-seropositive granulocytes. This may be the result of antiviral prophylaxis. Previous studies with opposite results measured CMV disease and not antigenemia. This is, nevertheless, a small study limited to a very specific population of patients, and larger studies are needed. We consider that CMV serology is not a predictor of CMV disease in immunosuppressed patients with cancer requiring transfusion of granulocytes.

28

The Effect of Anticoagulant Therapy on a Partial Thromboplastin Time-Based Lupus Inhibitor Assay.

Donald A. Bergstrom, Chris Ferrell, and Wayne Chandler.*

Department of Laboratory Medicine, University of Washington, Seattle.

We evaluated a partial thromboplastin time (PTT)-based lupus inhibitor assay in the presence of several anticoagulants. A lupus inhibitor in the Staclot LA assay (Diagnostics Stago, Asnieres, France) is based on the difference in the PTT measured in the absence and presence of hexagonal phase phospholipid (δ value): borderline positive, 8 to 13 seconds; moderately positive, 14 to 27 seconds; and strong positive, more than 28 seconds. In our laboratory, normal plasma has a δ value of ± 2 seconds. The Staclot LA reagent includes polybrene (a heparin inhibitor) and, therefore, should be insensitive to heparin in the sample. We performed the Staclot LA assay on pooled normal plasma spiked with 0 to 1 U/mL of heparin, 0 to 2.5 $\mu\text{g/mL}$ of argatroban, or 0 to 6 $\mu\text{g/mL}$ of lepirudin.

The assay was unaffected by heparin up to 1 U/mL. In the presence of lepirudin, the PTT was prolonged more than 150 seconds at the 6- $\mu\text{g/mL}$ dose, while the δ value became more negative with increasing lepirudin concentrations, from -2 seconds with no lepirudin to -20 seconds for 6 $\mu\text{g/mL}$ of lepirudin. A similar phenomenon was observed for argatroban, with the 2.5- $\mu\text{g/mL}$ dose resulting in a δ value of -8 seconds. We were concerned that because normal plasma had a more negative δ value in the presence of direct thrombin inhibitors, we would lose the ability to detect positive lupus inhibitors in the presence of direct thrombin inhibitors. To address this, we tested plasma from patients with known lupus inhibitors in the presence of 3 $\mu\text{g/mL}$ of lepirudin or 2 $\mu\text{g/mL}$ of argatroban. Contrary to our expectations, in the presence of lepirudin, the δ value was increased relative to no drug. For a borderline positive lupus inhibitor, the δ value increased from 8 seconds in the absence of drug to 48 seconds with lepirudin. The δ values of 2 specimens with moderate lupus inhibitors increased from 11 and 18 seconds to 45 and 106 seconds, respectively, when lepirudin was added to the plasma. In a specimen with a strong positive lupus inhibitor, the Staclot assay failed in the presence of lepirudin because the clotting times were longer than allowed by the instrument (>300 seconds). Similar results were obtained for argatroban, for which the δ values were prolonged relative to no drug, and the aforementioned 3 specimens were all identified as strong positive lupus inhibitors by our current criteria.

We conclude that the Staclot LA assay is unaffected by up to 1 U/mL of heparin; however, in the presence of argatroban or lepirudin,

the Staclot LA assay cannot be interpreted using our current criteria for characterizing lupus inhibitors.

30

Evaluation of the Reference Interval for Free (Nonceruloplasmin-Bound) Serum Copper.

Gwendolyn A. McMillin,* James J. Travis, William E. Owen, Francis M. Urry, Shannon Swenson, and William L. Roberts. Department of Pathology, University of Utah and ARUP Institute for Clinical and Experimental Pathology, Salt Lake City.

Free (nonceruloplasmin-bound) serum copper is one of several biomarkers used for screening and diagnosis of Wilson disease (WD), and it also is used to monitor copper chelation therapy. The conventional reference interval for free serum copper (Cu_F) is 0 to 10 $\mu\text{g/dL}$.

This reference interval was evaluated with 2 data sets. Total serum copper (Cu_T , $\mu\text{g/dL}$) and ceruloplasmin (Cp , mg/dL) values were obtained by ICP-MS and turbidimetric immunoassay (Roche), respectively. The Cu_F was estimated mathematically: $Cu_F = Cu_T - (3 \times Cp)$. Results were determined with serum obtained from a population of healthy volunteers ($n = 112$; age, 19-61 years; 45 men). Archived results for Cu_F that were reported by a reference laboratory between September 1997 and January 2004 ($n = 944$) also were evaluated.

Range, median, and mean values were similar when data were grouped by age. Median and mean values of Cu_F were approximately 10% lower in males in the database population and 40% lower in males in the healthy adult population. The distribution of Cu_F is nongaussian, and, thus, a nonparametric rank-based approach was taken to determine a reference interval. Based on the 5th and 95th percentiles, reference intervals of 0 to 28 $\mu\text{g/dL}$ for the database population and 0 to 23 $\mu\text{g/dL}$ for the healthy adult population were determined. Using the conventional reference interval, 30% of the healthy adults had Cu_F of more than 10 $\mu\text{g/dL}$, as did 25% of the database results. The prevalence of WD is low, approximately 1:30,000. A cutoff value of 10 $\mu\text{g/dL}$ applied to WD screening would generate a large number (approximately 25%) of false-positive results, which may lead to unnecessary testing. Changing the cutoff value to 23 $\mu\text{g/dL}$ produces fewer false-positive results (approximately 5%). To assess clinical sensitivity of 10- and 23- $\mu\text{g/dL}$ cutoff values, Cu_F values obtained from untreated WD patients that were published in 4 studies (38 patients) were evaluated. In this affected population, 97% of Cu_F values were more than 10, and 74% were more than 23 $\mu\text{g/dL}$; the 5th and 95th percentiles were 13 and 93 $\mu\text{g/dL}$. Thus, clinical sensitivity is compromised and clinical specificity is improved by raising the upper limit of normal for Cu_F . The data presented herein suggest that the conventional reference interval for Cu_F should be reconsidered; specifically, the upper limit of normal should be raised and the impact of sex further evaluated.

32

Soluble CD40 Ligand in Elderly Patients Undergoing Diagnostic Cardiac Catheterization.

Anna M. Halldorsdottir,^{1*} Joshua M. Stolker,² and Charles S. Eby.^{1,2} ¹Departments of Pathology and Immunology and ²Medicine, Washington University, St Louis, MO.

The early detection of patients with apparently stable coronary artery disease but who are at increased risk for future cardiovascular events is important. Specific biomarkers could be especially important in elderly patients who are at increased risk for complications associated with cardiac catheterization. Soluble CD40 ligand (sCD40L) has been shown to be an independent prognostic factor for death or myocardial infarction in patients with acute coronary syndromes. CD40L is a member of the TNF superfamily and exists in 2 active forms, soluble and membrane-bound. It is expressed on a variety of cells, including CD4+ T cells, vascular endothelial cells, macrophages, and platelets. Accumulating evidence has linked CD40 signaling with atherogenesis and thrombosis.

This prospective, observational pilot study involved 147 subjects 60 years old or older referred for diagnostic cardiac catheterization. Blood samples drawn before catheterization were analyzed for levels of sCD40L, using a commercial enzyme-linked immunosorbent assay (ELISA) kit, as well as CRP and BNP. Clinical outcomes were analyzed at 3 months and will be assessed again at 6 months after catheterization. The mean sCD40L level was $678 \pm 1,632$ pg/mL . However, 69% of values were less than 250 pg/mL . There was no association between sCD40L and age, sex, race, smoking status, diabetes, or the intake of aspirin, Plavix, or statins. There was no correlation between sCD40L levels and CRP or BNP levels. Twelve patients with cardiovascular events at 3 months did not have higher mean sCD40L levels than subjects without such events (135 ± 167 vs $710 \pm 1,671$ pg/mL). At 3 months, this pilot study does not indicate that sCD40L is helpful in risk stratification of elderly patients referred for diagnostic catheterization.

During method evaluation, several preanalytic factors were identified that affect accurate measurement of sCD40L: specimen type (serum greater than plasma), processing conditions (prolonged time and elevated temperature-dependent increase of sCD40L), and centrifugation (residual platelets increase sCD40L). Furthermore, in a subset of patients with elevated sCD40L concentrations, serial dilutions produced nonlinear results. These technical issues might limit the clinical usefulness of the sCD40L ELISA.

33

Human Fetuin-A Levels and Calcification Risk Index as Novel Indicators of Cardiovascular Disease and Mortality in End-Stage Liver Disease and End-Stage Renal Disease.

Nicole M. Nilson,* Scott Mullaney, Patricia Thistlethwaite, Ping Gao, David Herold, and Paul Wolf. Department of Pathology and Laboratory Medicine, Department of Medicine Division of Nephrology, Department of Surgery, Division of Cardiothoracic Surgery, University of California and VA Medical Centers San Diego; Epitope Diagnostics, San Diego.

Fetuin-A is a circulating glycoprotein synthesized by hepatocytes. It is well known that fetuin-A regulates calcium metabolism and osteogenesis. Previous investigations have demonstrated that fetuin-A is the major calcification inhibitor found in serum, and it interferes with ectopic calcium precipitation, especially in the arterial vascular system. The object of this study was to determine fetuin-A levels and the calcification risk index (CRI) in a diverse group of patients, including healthy subjects, patients with end-stage liver disease (ESLD), patients with end-stage renal disease (ESRD) undergoing dialysis, and patients with coronary artery bypass grafts for severe atherosclerotic coronary artery disease.

Serum fetuin-A levels were determined by using an enzyme-linked immunosorbent assay technique. The assay uses a 2-site "sandwich" technique with 2 selected specific polyclonal antibodies that bind to different epitopes of human fetuin-A. Other serum analytes that were determined by using an automated clinical chemistry instrument were total calcium, phosphate, albumin, C-reactive protein. CRI is a ratio of CA \times P product divided by fetuin-A concentration.

The results showed that the serum fetuin-A level was decreased dramatically in all patients with ESLD and in a moderate number of patients with ESRD undergoing dialysis. CRI was elevated (>100) in 86% of ESLD and ESRD patients. Moreover, a significant increase of the CRI level (>300) was found in all patients with coronary artery disease. The conclusions based on the results are that the patients with ESLD had markedly decreased levels of fetuin-A due to decreased synthesis of this protein. Patients with ESRD had moderately decreased levels or a level in the lower normal range of serum fetuin-A with a remarkable increase in CRI. Increased C-reactive protein in our patient group indicated that the patients had an inflammatory illness. The cause of this is increased levels of interleukin-6, which is the chief stimulator of the acute phase protein response. Fetuin-A is a negative acute phase protein. Thus, decreased fetuin-A levels or an increased CRI is associated with increased ectopic calcification in the arterial vascular system and with a risk of developing atherosclerotic cardiovascular disease.

34

Comparison of Serum and Heparinized Plasma Samples for Measurement of Common Chemistry Analytes.

Rodney R. Miles,¹ Richard F. Roberts,² and William L. Roberts.²
¹Department of Pathology, University of Utah Health Sciences Center and ²ARUP Institute for Clinical and Experimental Pathology, Salt Lake City.

Although serum and heparinized plasma specimens are considered equivalent for many assays, differences in results between these 2 sample types have been reported for common chemistry analytes, including glucose. The aim of this study was to compare results from serum and heparinized plasma samples for 41 different chemistry tests.

Serum and heparinized plasma specimens were drawn from 20 apparently healthy volunteers who had been fasting for 12 to 14 hours. The samples were centrifuged, serum and plasma were separated from cells, and 1-mL aliquots were stored at -70°C . Before analysis, the aliquots were thawed and mixed well. Matched aliquots of serum and heparinized plasma were analyzed on a Roche Modular P analyzer. Differences in the mean values for the 2 sample types were considered clinically significant at 2% for sodium; 3% for cholesterol, calcium, chloride, and glucose; 4% for potassium; 5% for high-density lipoprotein; and 10% for all other analytes tested.

Relative to serum samples, heparinized plasma samples showed clinically significant decreases in bile acids (-64.0%), potassium (-6.0%), and rheumatoid factor (-24.5%). Clinically significant increases were seen in aldolase (40.0%), angiotensin converting enzyme (ACE; 21.6%), C-reactive protein (CRP; 62.9%), and lactate dehydrogenase (LD; 20.6%). According to assay package inserts, both serum and heparinized plasma samples are acceptable for bile acids, aldolase, ACE, and CRP. Only serum samples are acceptable for the rheumatoid factor assay. Previous reports

have shown differences in LD values between serum and heparinized plasma samples; however, there is disagreement in this area, and both sample types are acceptable according to the package insert. Serum potassium values are known to be elevated relative to plasma values, so separate reference ranges are required. In contrast with a previous report and current recommendations, values for glucose concentrations were comparable between the 2 sample types. Our data suggest that serum and heparinized plasma samples give results that differ enough to alter clinical decision making in some assays, including several for which the 2 specimen types are considered equivalent. Only serum samples should be accepted for these particular assays.

35

Lupus Anticoagulant Testing in the Presence of Direct Thrombin Inhibitors.

Jonathan R. Genzen* and Jonathan L. Miller. Department of Pathology, Pritzker School of Medicine, University of Chicago, Chicago, IL.

Lupus anticoagulants (LAs) are antibodies that prolong phospholipid-dependent clotting assays and predispose patients to thrombosis, recurrent fetal loss, and thrombocytopenia. Despite the increased use of direct thrombin inhibitors in the hospital setting, no studies have specifically investigated whether these agents can alter the results of LA testing.

In vitro studies, therefore, were conducted to determine whether the commercially available direct thrombin inhibitors argatroban and lepirudin might interfere with the results and interpretation of LA testing. Heparin also was investigated, because it commonly is present in samples sent to the coagulation laboratory. Concentration-response curves were generated by adding increasing amounts of anticoagulant to normal plasma to determine the in vitro concentration of anticoagulant that prolongs the activated partial thromboplastin time (aPTT) to 75 seconds (2.5 times the baseline aPTT). Corresponding concentrations of anticoagulant were 0.4 U/mL of heparin, 1.24 $\mu\text{g}/\text{mL}$ of argatroban, and 1.66 $\mu\text{g}/\text{mL}$ of lepirudin. These individual concentrations of anticoagulant then were added to the plasma to check for potential effects on dilute Russell viper venom tests (DRVVTs) and PTT-based platelet neutralization procedures (PTT-PNPs).

For argatroban, the percent correction of the DRVVT in the presence of a high concentration phospholipid was not elevated, and no false-positive results were detected. As anticipated, the presence of a heparin-neutralizing agent in the DRVVT reagent mix prevented heparin from affecting the percent correction. Lepirudin, however, increased the percent correction to values normally considered positive for LAs, suggesting that false-positive interpretations might be made in the absence of accurate a priori knowledge of a patient's drug regimen. A small number of false-negative DRVVT results were detected using known weakly LA-positive plasma in the presence of argatroban, although no false-positive results were identified under these conditions. In PTT-PNP assays, the addition of argatroban or lepirudin did not produce false-positive results with normal plasma. Moreover, in almost all cases, the degree of correction for LA-positive plasma in the PTT-PNP assay actually was enhanced in the presence of argatroban or lepirudin. Under the conditions present in this study, we found the following: (1) Lepirudin in plasma precludes use of the DRVVT in testing for LAs. (2) Lepirudin in the PTT-PNP assay seems to improve the

separation of results between LA-positive and LA-negative plasma samples. (3) Argatroban acts similarly to lepirudin in the PTT-PNP assay but might mask a weak LA in the DRVVT.

36

Lamellar Body Counts Are Affected by Specimen Storage Conditions.

Nikola A. Baumann, Charles Eby, and Ann M. Gronowski.
Department of Pathology, Division of Laboratory Medicine,
Washington University School of Medicine, St Louis, MO.*

The lamellar body count (LBC) in amniotic fluid (AF) is an assay used to evaluate fetal lung maturity. Lamellar bodies, consisting primarily of densely packed circular arrays of phospholipid membrane, are produced by type II alveolar cells and represent the storage form of pulmonary surfactant. The LBC in AF increases as gestation advances and provides an objective estimate of the quantity of surfactant. While AF specimens commonly are frozen and stored for research and laboratory quality control purposes, little has been done to determine the stability of LBCs in AF. The objectives of this study were to examine the stability of LBCs in AF on storage at varying temperatures (room temperature, 4°C or -20°C) and to develop methods for specimen storage that would maintain LBC integrity.

AF specimens sent to the laboratory for physician-ordered fetal lung maturity analyses were used. Specimens were obtained within 24 hours of collection, LBCs were measured on a Beckman-Coulter LH 750 automated hematology analyzer, and aliquots of the samples were stored at room temperature, 4°C or -20°C for up to 60 days to determine the effect of temperature on LBCs over time. We analyzed 20 specimens under each condition. The effect of repeated freeze-thaw cycles on LBCs also was determined. Finally, we examined the effect of the addition of glycerol to AF specimens stored at -20°C. Precision studies also were performed.

The LBCs in AF specimens were stable for 10 days at room temperature and 50 days at 4°C. Storage for longer periods at room temperature or 4°C resulted in increased LBCs. We found that single and repeated freeze-thaw cycles had dramatic effects on sample stability, causing the LBC to decrease by an average of 15% (range, 0%-39%). Interestingly, the addition of 10% glycerol to the AF stabilized the LBC, thus allowing the specimens to be frozen and thawed without affecting LBC integrity. Our data suggest that patient AF samples be stored for no longer than 10 days at room temperature or 50 days at 4°C. For LBC quality control materials, patient samples can be pooled and stored frozen with 10% glycerol. LBCs from samples frozen without glycerol will be significantly lower than prefreeze counts.

37

Separation of Urine Uroporphyrin and Coproporphyrin Type I and III Isomers by HPLC.

Mark E. Miller, Mary L. McGowen, and Elizabeth L. Frank.
Department of Pathology, University of Utah, Salt Lake City.*

Porphyria cutanea tarda (PCT) can be identified in the clinical laboratory by observation of a characteristic pattern of carboxylated porphyrin compounds following separation by high-performance liquid chromatography (HPLC). Some samples

exhibit unusual patterns that cannot be interpreted readily. To distinguish PCT from acute porphyrias in urine specimens submitted to the laboratory for analysis, we developed chromatographic conditions to separate porphyrin isomers of the I and III series. Type I isomers are derived enzymatically from the normal heme biosynthetic pathway, whereas type III isomers form spontaneously owing to accumulation of intermediates when enzymes are defective or absent as occurs in the porphyrias.

Urine was applied directly to a C-30 column with a deuterio-porphyrin internal standard. The analytes were eluted using a mobile phase gradient from 50% ammonium acetate/acetonitrile (90:10) to 100% methanol/acetonitrile (85:15). Porphyrins were detected fluorometrically and quantified by comparison of peak area to peak area of standard porphyrin solutions.

Separation of uroporphyrin and coproporphyrin type I and type III isomers was achieved in 16 minutes; total run time was 27 minutes. The retention times (minutes) were as follows: uroporphyrin I, 3.7; uroporphyrin III, 4.2; coproporphyrin I, 13.7; coproporphyrin III, 15.4. Results were compared with our existing assay for unfractionated porphyrins. Specimens previously tested by routine HPLC analysis were subjected to these separation conditions. Unaffected patient samples showed a predominance of type I isomers. Samples from patients with acute intermittent porphyria showed predominantly type III isomers. Samples from patients with PCT showed similar amounts of the type I and III isomers. The chromatographic method described for measurement of isomers of carboxylated porphyrin compounds provides additional information useful for the characterization of porphyrin disorders.

40

Using Corrected Laboratory Reports to Identify the Clinical Impact Associated With Problems in Microbiology Testing.

Shan Yuan, Michael L. Astion, Jeff Schapiro, and Ajit P. Limaye.
Departments of Laboratory Medicine and Internal Medicine,
University of Washington School of Medicine, Seattle.*

We developed a strategy to identify errors in clinical microbiology testing and characterize the clinical impact associated with these errors. During a 6-month period, we used corrected microbiology laboratory reports identified by the laboratory information system as the source of potential cases and prospectively reviewed 458 corrected laboratory reports, or approximately 0.6% of all reports issued by the 2 participating laboratories during this period. In an initial screening, 172 cases (37.5%) were judged to represent laboratory errors of potential clinical significance. Subsequent medical record review could not exclude significant clinical impact in 148 cases, or 32.3% of the total. Within 24 to 48 hours after the corrections were made, these 148 cases were investigated by telephone interviews with the involved clinicians.

In 28 cases (6.1% of all corrected reports; 18.9% of the cases investigated), there was undesirable clinical impact associated with the laboratory error. These cases were analyzed to determine the nature of their clinical impact, responsibility for the errors, preventability of the errors, and the testing phase involved. Among these 28 cases, the most commonly associated outcomes were delayed therapy (17 cases [60.7%]), unnecessary therapy (7 cases [25.0%]), and inappropriate therapy (5 cases [17.9%]). In 2 cases, laboratory error resulted in complications necessitating consultation with infectious diseases specialists. In 1 case, surgery was required for treatment of empyema resulting from delayed

therapy. Presence of transient morbidity entirely attributable to the laboratory error was difficult to assess because of the complex medical conditions of many of the patients but was likely to be present in a significant fraction of these cases, as delayed or inappropriate therapy was common.

The laboratory was entirely responsible (24 [85.7%]) or partially responsible (4 [14.3%]) for the 28 cases in which clinically significant errors were detected. The majority of these cases (23 [82.1%]) were preventable errors of the cognitive type. Most occurred during the analytic phase (27 [96.4%]), with 19 cases (67.9%) involving problems interpreting Gram stains. We used corrected reports to identify clinically significant laboratory errors, most of which were amenable to educational interventions or increased supervision of laboratory personnel. The method can be implemented easily in other laboratory settings. The information obtained helps laboratories identify and prioritize areas that need improvement and guides the selection of interventions that improve patient safety.

41

Genotyping of Hepatitis C Virus by Melting Curve Analysis: Analytic Characteristics and 18 Months' Experience in Analysis of 586 QC and Clinical Samples.

Grant C. Bullock,* David E. Bruns, and Doris M. Haverstick.
Department of Pathology, University of Virginia, Charlottesville.

We have proposed a rapid-cycle, real-time polymerase chain reaction (PCR) assay with melting curve analysis for genotyping of hepatitis C virus (HCV) (*Clin Chem.* 2002;48:2147-2154). The objective of the present study was to determine the analytic characteristics of the proposed assay and its performance in routine use.

The assay has been used in 70 runs on 3 different LightCyclers by 6 technicians during the last 18 months. No assay failures occurred in the 70 runs, and the Tms for the studied genotypes remained in discrete, nonoverlapping ranges. The mean (range) Tms for QC samples were 64.1°C (63.2°C-64.9°C; CV = 0.49%; n = 88) for type 1; 59.5°C (58.8°C-59.8°C; CV = 0.44%; n = 28) for type 2a/c; 52.5°C (51.8°C-53.0°C; CV = 0.57%; n = 29) for type 2b; and 50.2°C (49.5°C-50.7°C; CV = 0.64%; n = 30) for type 3.

Among 411 patient samples, typing was successful in 399 (97.1%). One sample produced a Tm of 45.3°C which is below the known ranges for genotypes 1, 2, 3, and 4, and there was insufficient sample to repeat the melting curve analysis or obtain sequence information. Eleven samples were reported as none detected; 4 of these samples had viral loads of less than 10,000 IU/mL, confirming the level of sensitivity originally published. The remaining 7 samples had no quantitative results available. Five samples had coinfections (with types 1 and 2a/c in 2 patients, types 1 and 2b in 2 patients, and types 2a/c and 4 in 1 patient). For the remaining 394 patient samples, 301 were type 1, 19 were type 2a/c, 30 were type 2b, 40 were type 3, and 4 were type 4. The ranges of Tms showed no overlap, with mean (range) Tms of 64.2°C (63.2°C-64.8°C; CV = 0.43%) for type 1; 59.5°C (58.8°C-59.9°C; CV = 0.53%) for type 2a/c; 52.5°C (52.1°C-52.8°C; CV = 0.41%) for type 2b; 50.3°C (49.2°C-51.1°C; CV = 0.74%) for type 3; and 54.6°C (53.9°C-55.1°C; CV = 0.95%) for type 4. To expand previously available information on rarer genotypes, the first 10 patient samples that were identified as genotype 2 (n = 9) or 4 (n = 1) were genotyped by DupliType sequencing (Quest); sequence data confirmed the genotype in each case. We conclude

that real-time PCR with melting curve analysis is a precise, reliable, and robust approach to HCV genotyping.

43

Differential Expression of Secretoglobins in Breast Tumors.

Alison Woodworth,* Kelly P. Peterson, and Mark A. Watson. Sponsor: Mitchell G. Scott. Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO.

The secretoglobin gene family includes a set of five related genes (SCGB1A1, SCGB1D1, SCGB1D2, SCGB2A1, and SCGB2A2) that are clustered on human chromosome 11q12.2. These genes encode small polypeptides of unknown function that are expressed in a limited number of epithelial tissues and form covalent homodimers and heterodimers with each other. One secretoglobin polypeptide, mammaglobin (SCGB2A2), heterodimerizes with a second family member, lipophilin B (SCGB1D2), and is expressed mainly in breast epithelial cells. SCGB2A2 also is overexpressed frequently in human breast tumors, making it an attractive diagnostic marker for the detection of occult tumor cells. We hypothesized that distinct but overlapping transcriptional regulatory machinery controls expression of secretoglobin genes in breast epithelial cells and that their combined expression profile might be associated with unique molecular features of breast cancer.

We examined the expression of the secretoglobin transcripts in 45 primary human breast tumors by real time RT-PCR and found that the tumors clustered into distinct groups based on the expression of these 5 genes. SCGB2A2 and SCGB1D2 were highly correlated across the majority of the tumors examined, suggesting that they are coregulated by a common signaling pathway. To identify other genes that might be affected by the same upstream signaling events or the regulatory genes themselves, we examined several sets of our own and previously published gene expression microarray data profiles of primary human breast tumors.

This meta-analysis identified 10 additional genes (7 known genes and 3 ESTs) whose expression patterns were correlated highly with SCGB2A2 and SCGB1D2 expression. Real-time RT-PCR analyses of all 10 genes in an independent set of 48 breast tumors validated the results obtained *in silico*. Not surprisingly, one of the genes identified was SCGB2A1 (mammaglobin B), a secretoglobin gene whose proximal promoter shares more than 95% sequence homology with that of SCGB2A2. Interestingly, most of the other identified genes are associated with the transforming growth factor (TGF) β signal transduction pathway, suggesting that this pathway might influence the coexpression of secretoglobins in breast tumors. Ongoing studies are focused on demonstrating a correlation between specific patterns of secretoglobin gene expression and clinical and pathologic features of breast cancer. In fact, differential expression of these genes might represent a diagnostic molecular profile of a tumor's response (and, hence, potential therapeutic vulnerability) to extracellular stimuli such as TGF- β .

44

A Complex Cytogenetic Abnormality in a Congenital/Infantile Fibrosarcoma.

Michelle Dolan, Daniel Mulrooney, and Jose Jessurun. Sponsor: John Eckfeldt. Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis.

Congenital/infantile fibrosarcoma (CFS) is a malignant fibroblastic tumor that typically arises in children younger than 2 years. Cytogenetic analyses of these tumors have shown a recurring chromosomal translocation, t(12;15)(p13;q25), as well as a number of recurring trisomies, particularly of chromosomes 8, 11, 17, and 20. The 12;15 translocation results in fusion of the *ETV6* gene at 12p13 to the *NTRK3* gene at 15q25, and the critical fusion has been shown to be on the derivative chromosome 15.

We describe a case of CFS with a complex rearrangement of chromosomes 12 and 15 resulting in joining of the same break-points as those seen in the recurring 12;15 translocation. The patient, a newborn girl, presented with a mass compressing the spinal cord. Cytogenetic analysis performed on tissue from a needle biopsy revealed a hyperdiploid karyotype with 52 chromosomes including gains of chromosomes 8, 10, 11, and 20 and a dicentric chromosome containing the long arms, centromeres, and proximal short arms of 1 chromosome 12 and 1 chromosome 15. DAPI staining and multicolor fluorescence in situ hybridization (FISH) confirmed that this rearrangement was dicentric and composed of chromosomes 12 and 15. This dicentric rearrangement, joining 15q25 to 12p13, would be expected to yield a fusion gene with the same orientation as the critical fusion resulting from the more common simple reciprocal 12;15 translocation. The complex 12;15 rearrangement seen in this case, not previously documented in the literature, thus underscores the importance of 12p13/15q25 fusion in the pathogenesis of CFS. Further follow-up will be necessary to determine whether this unusual chromosomal finding will be associated with an atypical clinical course.

46

Superwarfarin Poisoning Should Be Considered in Patients With Vitamin K Deficiency Unresponsive to Therapy.

Tracy M. Rauch* and Marisa B. Marques. Department of Pathology, University of Alabama at Birmingham.

Brodifacoum, a member of the superwarfarin family, commonly is used as rodenticide. This compound has a terminal phase half-life of at least 128 days. Because of its long half-life, identification of superwarfarin intoxication is necessary to ensure the appropriate length of treatment. We report 2 cases of brodifacoum poisoning in which coagulopathy persisted despite multiple doses of vitamin K and units of fresh frozen plasma (FFP).

Case 1 involved a 59-year-old woman who presented to the hospital with easy bruising, headache, and myalgia. The physical examination revealed soft tissue bleeds in all extremities without mucosal bleeding. Her prothrombin time (PT) was more than 100 seconds (INR >12), partial thromboplastin time (PTT) was 172 seconds, fibrinogen level was 746 mg/dL, and D dimer was 952 ng/mL. Her hematocrit was 27%, and the platelet count was $218 \times 10^3/\mu\text{L}$. PT and PTT mixing studies corrected with addition of normal plasma. The differential diagnosis included severe vitamin K deficiency and liver failure. A normal factor V level confirmed the former diagnosis, and therapy was started with vitamin K and FFP. At discharge, the symptoms had improved, and the INR was 3 after 45 U of FFP during a period of 13 days. On further questioning, the patient reported having handled rat poison approximately 1 week before admission.

Case 2 involved a 38-year-old man with a history of venous thromboembolism who presented with bruising and myalgia. His PT and PTT were more than 100 seconds (INR >12) and more

than 200 seconds, respectively, and corrected in the mixing studies. The hematocrit was 24%, and the platelet count was normal. Although he denied taking warfarin, his condition improved with vitamin K and FFP, and he was discharged against medical advice 2 days later. A possible history of rodenticide ingestion was elicited from the family.

Warfarin, dicumarol, and difenacoum were undetectable by HPLC in both patients' serum samples. However, testing for the superwarfarin, brodifacoum, was positive at 200 ng/mL (case 1) and 36 ng/mL (case 2). Superwarfarin intoxication should be in the differential diagnosis of patients with coagulopathies consistent with vitamin K deficiency that are refractory to treatment. The recognition of this condition is crucial for proper long-term management and follow-up.

47

Thrombosis Risk Test Utilization in an Academic Center.

Charles A. Mayfield, George A. Fritsma, Jeroan J. Allison, Bart Prevallet, Brian K. Adler, and Marisa B. Marques. University of Alabama at Birmingham.

The demand for thrombosis risk testing grows as clinicians recognize the prevalence of thrombophilia. As in other conditions, pathologists know that test utilization often is inappropriate. We reviewed all antithrombin (AT), protein C (PC), protein S (PS), antiphospholipid antibodies (APA), activated protein C resistance (APCR), and factor V Leiden (FVL) orders received in our laboratory from July 1999 through September 2002, to assess their appropriateness. We defined inappropriate utilization as any instance in which: (1) PC or PS was ordered without APCR/FVL; (2) PC or PS was ordered without APA; (3) APA was ordered without APCR/FVL; or (4) PC and PS were decreased, implying current oral anticoagulant therapy or acute thrombosis.

Physicians of 17 specialties tested 1,763 patients during this period, excluding orders from rheumatology and obstetrics/gynecology. The patients' diagnosis codes were many and included deep vein thrombosis, pulmonary embolism, etc. The highest percentage of patients was tested for APA alone, 27%, followed by 19% tested for all 5 conditions, 15% tested for all except AT deficiency, and 23 other combinations in smaller percentages. The rates of instances of inappropriate orders were as follows: PC and PS ordered without APCR/FVL, 36%; PC and PS ordered without APA, 25%; APA ordered without APCR/FVL, 63%; and PC and PS were both low, 13.7%. These data demonstrate a need for improved physician understanding of how to appropriately workup patients for hypercoagulability. We propose that pathology-initiated interventions at the point of test ordering have the potential to improve utilization patterns as thrombophilia awareness increases.

49

Acute Myeloid Leukemias Bearing the *Ft3* Internal Tandem Duplication Have a Characteristic Immunophenotype Not Shared by Acute Myeloid Leukemias With Activating Point Mutations in *Ft3* or *ras* Genes.

Joel E. Mendelin,^{1*} Derek L. Stirewalt, MD,² Hye Son Yi,¹ Kayla M. Sheets,² Jerald P. Radich, MD,² and Brent L. Wood.¹ Sponsor: Steven J. Kussick.¹ ¹Department of Laboratory Medicine, University of Washington, and ²Fred Hutchinson Cancer Research Center, Seattle.

Our objective was to determine whether characteristic immunophenotypic features of the myeloid blasts that we have observed in acute myeloid leukemias (AMLs) bearing internal tandem duplications (ITDs) of the *Flt3* gene are present in AMLs bearing activating point mutations in the *Flt3* tyrosine kinase domain (TKD) or in the *N-ras* or *K-ras* genes.

Four-color flow cytometry was performed on fresh or cryopreserved peripheral blood or bone marrow specimens from 106 patients with de novo or relapsed AML. Antigens evaluated included CD34, CD38, CD45, CD123, and CD133 in all cases and many other antigens in most cases. Screening for *Flt3* ITD was performed by DNA polymerase chain reaction (PCR) and capillary electrophoresis and confirmed by single-strand conformational polymorphism (SSCP) analysis and DNA sequencing. Screening for the major *Flt3* TKD mutations at codon 835 or 836 was performed by DNA PCR and *EcoRV* digestion to look for mutation-associated loss of this restriction site. Screening for activating *N-ras* and *K-ras* point mutations at codons 12, 59, and 61 was performed on most cases using SSCP.

Flt3 ITDs were identified in 26% of cases, activating TKD point mutations in 9.4% cases, activating *N-ras* mutations in 13% of cases, and activating *K-ras* mutations in 7% of cases; 46% of all *Flt3* ITD+ cases and 68% of the ITD+ cases expressing CD34 demonstrated a characteristic dyssynchronous pattern of CD34 and CD38 coexpression, which was present in only 1.6% of cases with wild-type *Flt3* genes and in none of the cases with *Flt3* TKD or activating *N-ras* or *K-ras* mutations. The positive predictive value of dyssynchronous CD34/CD38 expression for the *Flt3* ITD was 93%. In addition, 75% of all *Flt3* ITD+ cases expressed CD123 with little or no CD133, in contrast with 31% of wild-type *Flt3* cases ($P < .001$), 33% of cases with *ras* mutations ($P < .025$), and 40% of the *Flt3* TKD cases (not statistically significant). Based on the expression of CD34, CD38, CD123, and CD133, AMLs with *Flt3* ITDs can be distinguished from AMLs with *Flt3* TKD mutations, activating point mutations in *N-ras* or *K-ras*, or wild-type *Flt3* or *ras* genes. It is possible that this unique immunophenotype reflects the unique biologic behavior of AMLs with the *Flt3* ITD.
