Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Pediatric/Fetal Clinical Chemistry

B-208

Effect of Multiple Transfusions on Lipid Peroxidation in Preterm Infants

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Multiple blood transfusions are commonly used in the course of neonatal intensive care unit (NICU) stay in very low birth weight (VLBW) infants. The number of transfusions received has been associated with the development of prematurity complications (retinopathy, necrotizing enterocolitis, bronchopulmonary dysplasia, intraventricular hemorrhage, and periventricular leukomalacia). The severity of the illness can increase the number of transfusions required, resulting in iron overload which may increase the release of reactive oxygen species. The aim of this study was to determine the relationship between blood transfusions, ferritin levels and oxidative stress in preterm infants.

Preterm infants (n=23, gestational age 28.43±3.50 weeks; birth weight 1180±471g) admitted to the NICU were enrolled. Five of them (21.7%) were never transfused, while 10 cases (43.5%) were transfused less than 5 times, 2 cases (8.7%) 6-10 times, and 6 cases (26.1%) were transfused more than 10 times. Venous blood samples were taken when they were at least 20 days of age in a period free of infection according to clinical signs and laboratory test results. Serum malondialdehyde (MDA) levels were measured by HPLC (Ultimate 3000, Thermo Dionex, USA) with a flourescence detector. Within-run precision values were 1.8-5.5% and between-run precision values were 6.5-9% for 0.40-1.55 µmol/L MDA, according to manufacturer's claim. The lower detection limit was 0.02 µmol/L. Serum iron and iron binding capacity were measured colorimetrically (Cobas 8000 Modular Analytics, Roche Diagnostics, Germany). Ferritin levels were measured with an immunometric test with electrochemiluminescence detection (Modular Analytics E170, Roche Diagnostics, Germany).

There was a significant difference in serum ferritin levels between transfused (median: 457ng/mL, range:108-2717) and non-transfused (median: 203ng/mL, range:102-268) infants (P=0.017). There was a statistically significant correlation between serum ferritin and MDA levels (P<0.001; r=0.693). Also, the correlation between the number of transfusions and serum ferritin levels was statistically significant (P=0.016; r=0.558). Serum MDA levels were significantly higher in infants with serum ferritin levels >450 ng/mL (P<0.001). When the infants were grouped according to prematurity related complications; transfusion numbers, serum ferritin, and MDA levels of those with two or more complications were significantly higher when compared to cases without complications (P<0.001, P=0.001, and P=0.019, respectively).

In conclusion, iron status of VLBW infants has to be monitored to detect iron deficiency and also transfusion-related iron overload. Ferritin can be used to assess the iron status of preterms. Ferritin levels can also reflect lipid peroxidation as we have shown its correlation with MDA, the levels of which were higher in infants with two or more prematurity-related complications. It is important to use restrictive transfusion guidelines in order to protect preterms from iron overload and oxidative stress. Further research is necessary to determine a cut-off level for ferritin to decide when to start iron prophylaxis.

B-209

An Evaluation of the HemoCue Assay for the Rapid Assessment of Plasma Free Hemoglobin in Pediatric Patients Undergoing Extracorporeal Membrane Oxygenation (ECMO)

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Background: Extracorporeal membrane oxygenation (ECMO) therapy on newborns is associated with increased risk of hemolysis which can lead to hemoglobin associated nephropathy and, in some cases, acute renal failure. Accordingly, ECMO patient care guidelines stress the importance of early detection of ECMO associated hemolysis *via* measurement of the levels of plasma free hemoglobin.

Objective: To verify the accuracy and precision of the HemoCue spectrophotometric method for assessment of plasma free hemoglobin (PfHb) on ECMO patients with a focus on values in the lower range of detection.

Methods: HemoCue (HemoCue, Brea, CA) PfHb results were compared to those obtained on the well-established Abbott Architect (AA) c4000 analyzer (Abbott Laboratories, Chicago, IL). The HemoCue method 1) oxidizes hemoglobin to methemoglobin with nitrite, then 2) after conversion to azidemethemoglobin, absorbance is measured at 570 and 880 nm. Potential interferences were assessed by performing a spectrophotometric scan on our Cary 100 (Agilent, Santa Clara, CA) in order to determine the degree of spectral overlap with bilirubin and lipoprotein, the latter being partially corrected using the 880 nm absorbance reading. In addition, analysis for bilirubin and triglyceride was performed. Also, as an adjunct marker for hemolysis, lactate dehydrogenase was measured. Precision studies were based on replicate analysis on patient plasma samples. Assays were also performed on ultracentrifuged samples with results compared to those obtained on neat and filtered samples. Serial dilution recovery studies were performed on a lysed sample with a known/measured hemoglobin value (Unicel DxH800, Beckman Coulter, Brea, CA).

Results: Linear regression analysis showed a significant proportional error with results 29 percent lower on the Hemocue method (Hemocue=0.71*AA + 4.8; R^2 = 0.70, N = 22). Two samples with triglyceride > 400 mg/dL showed spuriously high Hemocue values. No statistical difference in mean values was observed between the two methods (t Stat = 0.165, P = 0.870). Serial dilution recovery on the Hemocue method was 106 percent (Hemocue (7.2 g/dL) versus DxH800 (6.8 g/dL)). The serial dilution curve plot showed an excellent fit with an expected log relationship (Hemocue = -111.5*ln(Dilution Factor) + 594.3). Precision studies showed CV% at seven levels ranging from 9.7- 4.2 percent. LDH showed positive correlation with PfHb values obtained on the AA method (AA = 5.9*LDH + 290; R^2=0.44). As expected, bilirubin levels showed a positive PfHb interference, the latter increasing slightly with the bilirubin level. Neat samples tested versus filtered samples showed no significant difference whereas ultracentrifuged samples consistently gave lower PfHb values.

Conclusions: The HemoCue method provides rapid and reasonably accurate measurements of plasma free hemoglobin. However, our study suggests that levels in the 10 to 30 mg/dL range should be interpreted with caution. Triglyceride and bilirubin both interfere in a proportional manner and must be considered when interpreting results on unfiltered samples. Adding LDH as a surrogate hemolysis marker appears to be of marginal value.

B-210

Transference of CALIPER Pediatric Reference Intervals to Beckman Coulter AU Clinical Chemistry Assays

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Objective: The CALIPER program has established a comprehensive database of pediatric reference intervals largely using the Abbott ARCHITECT biochemical assays. To expand clinical application of CALIPER reference standards, transference studies have been initiated to transfer data from Abbott assays to other common clinical chemistry platforms based on the CLSI guidelines. Here, we report a transference study aimed to transfer CALIPER reference intervals from the Abbott ARCHITECT to Beckman Coulter AU assays.

Design and Methods: Approximately 200 pooled patient serum specimens were measured on both the Abbott ARCHITECT c8000 and the Beckman Coulter AU systems. Beckman coulter offered more than one assay for the majority of tested analytes. Data analysis and transference were performed in accordance with the CLSI documents C28-A3 and EP9-A2. R^2 values were determined using linear or Deming regression, and quantile-quantile, standardized residual, and Bland Altman plots were used to assess the correlation of the data between the two systems. Analytes with an R^2 value <0.70 were deemed non-transferable. For stringent validation, 100 reference samples from the CALIPER cohort of healthy community children were assayed on the Beckman Coulter AU system. Transferred reference intervals were considered verified when ≥90% of CALIPER values fell within the 95% confidence intervals of the calculated intervals.

Results: Results from the vast majority of Beckman Coulter AU assays (82%; 51/62) strongly correlated (R^2≥0.70) with the corresponding Abbott ARCHITECT assays. Only bicarbonate and calcium results showed poor correlation between both systems. Abbott ARCHIITECHT reference intervals were transferrable to all 51 Beckman Coulter assays. Transferred reference intervals were, in general, similar to previously published CALIPER reference intervals. The vast majority of the transferred reference

intervals were sex-specific. Most [80% (40/51)] of the transferred reference intervals were verified using healthy children reference samples from the CALIPER cohort. This percentage increased to 94% (48/51) if we set the verification cutoff to 80% of CALIPER samples falling within the 95% confidence intervals of the calculated reference intervals. It is important to note that the comparisons performed between the Abbott ARCHITECT and Beckman Coulter systems make no assumption as to which system is more accurate.

Conclusion: The majority of CALIPER reference intervals were transferrable to Beckman Coulter AU assays allowing the establishment of a new database of pediatric reference intervals. This further expands the utility of the CALIPER database to clinical laboratories using the AU assays and should help improve test interpretation in the clinical setting. Laboratories using the assay-specific CALIPER reference intervals reported in the present study should perform further validation on their own testing platform using reference specimens from healthy children in the local population as recommended by CLSI.

B-211

Lecithin-sphingomyelin ratio and phosphatidylglycerol are not superior to lamellar body count when assessing risk for respiratory distress syndrome

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Background: In our practice, lamellar body count (LBC) is the initial laboratory testing for assessing the maturity of the fetal lungs. LBC <15,000 is indicative of an immature fetus, while LBC >39,000 is indicative of a mature fetus. LBC 15,000 – 39,000 is considered indeterminate. Lecithin-sphingomyelin ratio (L/S ratio) and Phosphatidylglycerol (PG) by thin layer chromatography (TLC) used to be considered confirmatory testing for assessing the risk of respiratory distress syndrome (RDS). LBC is easy to perform with a quick turn-around time, and is available 24 hours per day, while the L/S Ratio and PG takes approximately 6 hours and requires tedious sample preparation. The important L/S ratios as related to fetal lung maturity are divided into two categories: immature (L/S<2.0) and mature (L/S ≥2.0). A PG positive result is indicative of mature lung. Recent literature casts doubt on the values of L/S ratio test. We hypothesized that L/S ratio and PG are not better indicators than LBC for assessing the risk of RDS. **Design:** Amniotic fluid was collected via standard clinical practice. LBC was run immediately after sample collection and samples with LBC 15,000-39,000 were performed for L/S ratio and PG at the time of clinical care. Leftover samples with LBC >39,000 and <15,000 were kept at -70°C for later L/S ratio and PG testing. Collection of leftover patient samples and clinical data for this study was approved by the Institutional Review Board. **Results:** Of the 113 samples, 72 samples had LBC >39,000, while 5 had LBC <15,000, and 36 had LBC between 15,000 and 39,000. 29 samples with positive or negative LBC results were randomly selected and analyzed for L/S and PG. In total, there were 64 samples with complete data for LBC, L/S, and PG. Of the 64 patients, 7 babies were born with RDS. Their LBCs ranged from 1,000 – 38,000 (2 had LBC <15,000, the remaining 5 had LBC 15,000 - 39,000). The L/S ranged from 1.4 – 3.4, while 4 out of the 7 samples were negative for PG. 93% of LBC gave correct diagnosis (<15,000 with RDS and >39,000 without RDS), while 80% of L/S ratios gave correct diagnosis (<2.0 with RDS and ≥2.0 without RDS), and only 63% PG results gave correct diagnosis (negative with RDS and positive without RDS). For LBC in indeterminate range (15,000 to 39,000), 77% of L/S ratios gave correct diagnosis, and only 44% of PG results gave correct diagnosis. **Conclusion:** L/S ratio and PG are not superior to LBC for predicting RDS. However, L/S ratio may be used as a follow-up test for patients with indeterminate LBC results.

B-212

Validation of Minimum Volume Blood Gas Collections

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Background:

We evaluated minimum collection volume in the Smiths Medical Portex 1 mL Line Draw Arterial Blood Sample Syringe kit (Smiths Medical, Keene NH) that would produce reliable arterial blood gas (ABG) and electrolyte results.

Methods:

We collected 0.3 mL blood through an arterial catheter from adult inpatients in a 1 mL Smiths Medical blood gas syringe with a Smiths Medical Filter-Pro device to remove air bubbles; and compared ABG results to those obtained from a full 3 mL Smiths Medical Portex syringe (with Filter-Pro). Analytes measured included pO2, pCO2, pH, hemoglobin, ionized calcium, sodium, potassium and glucose on Radiometer ABL825 and Radiometer ABL90 (Radiometer, Bronshøj, Denmark) blood gas analyzers. We also compared ABG results between minimum volume samples hand-carried to the laboratory vs. those sent via pneumatic tube. Finally, we evaluated 0.3, 0.4, and 0.5 mL collection volumes with mixing of samples for 2 minutes (rather than 30 seconds) prior to analysis on the ABL90 analyzer.

Results:

Minimum volume (0.3 mL) samples analyzed on the ABL825 demonstrated a mean (SD) hemoglobin bias of -0.4 ± 0.3 g/dL, with 3/20 samples demonstrating hemoglobin results > 0.5 g/dL different from the matching 3 mL syringe value. In contrast, minimum volume samples (n=14) analyzed for hemoglobin on the ABL90 demonstrated a mean (SD) bias of -0.1 ± 0.2, with 13/14 within 0.5 g/dL of the matching full syringe value.

pO2 values (n=20) from the 0.3 mL collections demonstrated a mean (SD) bias of 31 ± 25 mm Hg compared to full 3 mL syringe values, with 17/20 failing to meet crosscheck criteria (within 10 mm Hg at pO2 < 100 mm Hg and within 10% at pO2 ≥ 100 mm Hg). Hand-carrying 0.3 mL samples did not significantly impact this bias (n=10), with a mean (SD) bias of 35 ± 38mm Hg and 7/10 failing crosscheck criteria.

Comparison of 0.3, 0.4, and 0.5 mL collection volumes in the 1 mL syringe demonstrated mean (SD) pO2 bias of 9 ± 14 mm Hg (0.3 mL), 12 ± 23 mm Hg (0.4 mL), and 3 ± 11 mL (0.5 mL) when samples were mixed for 2 minutes prior to analysis (rather than 30 seconds). 8/20 (0.3 mL), 7/19 (0.4 mL), and 4/19 (0.5 mL) samples failed crosscheck criteria for pO2 when samples were mixed 2 minutes prior to analysis on the ABL90. No other blood gas or electrolyte analytes demonstrated significant differences between sample volumes or analyzers.

Conclusion:

Oxygen tension and hemoglobin demonstrated sensitivity to sample volume. Use of the ABL90 (rather than ABL825) improved accuracy of hemoglobin measurement for reduced sample volumes. For pO2, significant bias and variability was seen when less than 0.5 mL was collected into a 1 mL syringe. Increasing mixing time to 2 minutes (from 30 seconds) mitigated this bias, though collection of volumes < 0.5 mL still resulted in ± 20 mm Hg variability in pO2 values. Neonatal practices using minimum volume collections should be aware of the potential for variability in pO2 values.

B-213

L-2-Hydroxyglutaric aciduria: a case report

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Introduction: L-2-Hydroxyglutaric aciduria (L-2-HGA) (OMIM #236792) is an autosomal recessive neurometabolic disease. Since its first description by Duran in 1980, only few cases have so far been reported. It occurs mostly in childhood and characterized by slowly progressive neurological dysfunction with cerebellar ataxia, pyramidal signs, intellectual decline, seizure, and extrapyramidal symptoms. Characteristic magnetic resonance imaging findings include signal intensity abnormalities of the subcortical cerebral white matter, putamen, and dentate nucleus. We report two siblings who were diagnosed to have L-2HGA.

Case report: An 11-year-old boy was referred for extrapyramidal movements and learning disabilities. He was born to 2nd degree consanguineous parents and had an uneventful perinatal period. He had normal development until the age of 5 years, when he presented with afebrile seizures and social withdrawal. This became progressively worsened. On examination, he had extrapyramidal movements consisting of ataxia, tremors and dyskinetic movements. He was able to speak short sentences with meaning. Cranial nerve examination was normal but had mild spasticity of all four limbs. He had normal occipito-frontal circumference. Blood counts, renal and liver function tests were normal. Cranial MRI showed generalized polymicrogyri and white matter changes involving the cerebrum and cerebellum with a subcortical distribution and changes of the basal ganglia. Electroencephalogram showed frequent beta activity diffusely but within normal limits. Urinary organic acids done by gas chromatography/mass spectrometry (GC-MS) showed elevated 2-OH glutaric acid levels with normal levels of glutaric acid, ethyl malonic acid and isovaleryl-glycine. His older sibling had similar neurological manifestations but with milder learning disabilities. Urinary organic acid profile of the older sibling also revealed elevated levels of 2 hydroxyglutaric acid. Acylcarnitine profile, plasma amino acids and chromosome study were normal. In view of the clinical picture and elevated levels of 2-hydroxy glutaric aciduria, enantiomeric analysis was done and that confirmed the diagnosis of L-2-HGA. Molecular analysis confirmed the homozygous mutation

c.844C>T of the L2HGDH gene. Our patients were started on Riboflavin and follow-up have shown improvement in the dystonia.

Conclusion: L-2-Hydroxyglutaric aciduria is a neurometabolic disorder which should be considered as a differential diagnosis in patients with neurodevelopmental regression, extrapyramidal signs and characteristic MRI findings.

B-215

Utility of full gene analysis in the diagnosis of cystic fibrosis

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Background:

Over 1500 mutations in the CFTR gene have been identified. The current gold standard for diagnosing cystic fibrosis (CF) is the sweat test. Additionally, multiple genetic testing options are available including several mutation panels as well as a full gene analysis (CFTR sequencing). The advantage of full gene analysis over mutation analysis, especially in patients that have indeterminate sweat chloride results, has not been well documented. Additionally, the correlation between genotype and phenotype is extremely variable.

Methods:

This was a single-institution, retrospective clinical study. We identified all sweat chloride tests ordered at St. Louis Children's Hospital from July 1, 2012 through June 31, 2014. For each patient with a sweat chloride >30 mMol/L or higher, a chart review was conducted to obtain genetic testing results and final diagnosis.

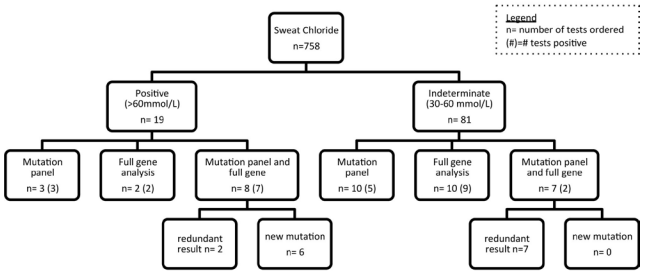
Results:

Of the 758 sweat tests conducted within the 2 year period, 19 (2.5%) resulted positive (>60mmol/L) and 81 (10%) indeterminate (30-60mmol/L). Of the 19 patients that had positive sweat chloride, 13 underwent genetic testing. 12 (92%) were positive for CFTR mutations. In this group, full gene analysis identified a new mutation that was classified as clinically significant in 3 of the 10 cases (S489X, E1371X, and I618T); however their identification was not diagnostically or therapeutically useful.

Of the 81 patients that had an indeterminate sweat chloride, 27 underwent genetic testing. 16 (59%) patients were positive for CFTR mutations. All 7 instances were both a full gene and mutation panel was requested resulted in redundant results. In this group, full gene analysis identified a new mutation in 2 out of 17 cases (I1139V and P750L); however this knowledge did not aid in diagnosis.

Conclusion:

At this point, full gene analysis does not seem to offer a diagnostic advantage in both sweat chloride positive and indeterminate patient populations.



B-216

A six-month survey of meconium and umbilical cord drug testing results between July and December of 2014

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Background: The concern of substance-exposed newborns and neonatal abstinence syndrome is rising. Meconium and umbilical cord are unique neonatal specimen types that provide longer detection window for drug testing than maternal/neonatal urine. Due to ethical considerations, data of drug deposition and concentration in neonatal matrices addressing *in utero* substance exposure is limited.

The objective was to survey the drug classes found in unmatched meconium and umbilical cord samples from at-risk newborns submitted to our laboratory for forensic purposes.

Methods: Meconium and umbilical cord specimens were aliquoted at 0.5g for drug screening by validated immunoassays. Presumptive positive samples were then aliquoted again for confirmation by validated chromatography-mass spectrometry-based methods to identify and quantify drugs and metabolites. Results were collected between July and December 2014 and summarized in the Table.

Results: During the 6-month period, 8,169 meconium samples and 16,985 umbilical cord samples were received. The two matrices showed similar positivity rates or prevalence ranking for most drug classes. Cotinine, carboxy-THC, opiates (codeine, morphine, hydromorphone, and hydrocodone), and buprenorphine (BUP) are the four classes with highest positivity rate seen in both matrix types, followed by other pharmaceutics oxycodone/oxymorphone (OXY), methadone, and benzodiazepines (BZP). Amphetamines and cocaine are the next two prevalent illicit drug classes found in both matrix types. Some discrepant positivity rate of each drug class between the two matrices may be due to distinct sampling sizes, analytes of choice, and analytical sensitivity. For example, oxazepam was the only BZP analyte confirmed in meconium, whereas nordiazepam, diazepam, alprazolam, midazolam, temazepam were additionally confirmed in umbilical cord. BUP and OXY had doubled positivity rate in umbilical cord compared with meconium, possibly due to much lower limits of quantitation.

Conclusion: We showed that cotinine, THC, several opioids, and benzodiazepines are the most commonly seen drug types tested in both meconium and umbilical cord.

Positivity rate and minimum-maximum (median) concentrations of drugs in meconium and umbilical cord

Meconium positivity rate	Min-Max (median) ng/g	Umbilical cord positivity rate	Min-Max (median) ng/g
Cotinine 20.9% (14/67)	11 - 133 (55)	Cotinine 49.9% (384/769)	2.2 - 505 (58)
THC 16.1% (1315/8169)	40 - 3,033 (156)	THC 15.2% (2581/16985)	0.04 - 75 (0.9)
Opiates 8.8% (719/8169)	41 - 16,806 (239)	Opiates 12.2% (2071/16985)	0.2 - 414 (2.6)
Buprenorphine 6.6% (134/2031)	8.6 - 5,250 (238)	Buprenorphine 11.4% (956/8405)	0.13 - 61 (2.6)
Methadone 4.7% (254/5428)	84 - 141,256 (6,027)	***Oxycodone 4.6% (554/11994)***	0.2 - 1,826 (2.8)
Amphetamines 4.1% (337/8169)	41 - 36,829 (979)	Methadone 3.7% (529/14236)	0.8 - 403 (57)
Cocaine 2.6% (215/8169)	40 - 6,226) 279	***Benzodiazepines 3.1% (414/13234)***	0.9 - 384 (4.6)
Oxycodone 1.9% (64/3437)	92 - 1,631 (385)	Amphetamines 2.9% (495/16985)	2.0 - 2,877 (42)
Tramadol 1.3% (44/3437)	65 - 25,136 (1,872)	Cocaine 1.9% (322/16985)	0.2 - 6,435 (15)
Meperidine 1.2% (41/3437)	47 - 1,531 (325)	Barbiturates 1.7% (247/14236)	1.9 - 7,989 (237)
Barbiturates 1.0% (53/5428)	263 - 28,889 (1,708)	Meperidine 1.3% (161/11994)	1.0 - 545 (33)
Benzodiazepine 0.1% (5/4333)	62 - 310 (203)	Tramadol 1.2% (148/11994)	1.4 - 2,384 (115)
PCP 0.09% (7/8169)	34 - 1,112 (258)	PCP 0.02% (3/16985)	7.4 - 34 (9.7)
Propoxyphene 0.02% (1/4333)	(1,405)	Propoxyphene 0.01% (1/13234)	(82)

B-217

Serum amyloid A (SAA) values in healthy newborns and infants

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Background: Serum amyloid A (SAA) is important diagnostic biomarker including peadiatric diseases. The aim of the study was to evaluate the reference intervals of SAA in healthy newborns and infants.

Methods: Serum levels of SAA were investigated in group of 50 healthy children aged from 1 day to 1year (30 boys and 20 girls), CRP (C reacting protein) less than 3 mg/l. We used commercially available immunonephelometric assay on an Beckmann Immage 800 analyzer. Children were divided into three groups according to the age (1st group - 10 newborns with the age 1 - 30 days; 2nd group - 30 children with the age 31 - 180 days; 3rd group - 10 children with the age of 181 days - 1 year). Simple nonparametric bootstrap procedure was used for the evaluation of the reference ranges (2.5th and 97.5th percentile) in selected groups.

Results: Serum amyloid A (SAA) reference ranges in groups of newborns and infants were as follows (expressed as 2.5th and 97th percentile): 1st group: 1.00 - 8.93 mg/l; 2nd group: 0.99 - 10.56 mg/l, 3rd group: 0.99 - 3.73 mg/l.

Conclusion: We evaluated SAA reference intervals in healthy newborns and infants.

B-218

Evaluation of Cotton Balls for Urine Collection for Measurement of Homovanillic Acid and Vanillylmandelic acid Acid

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Background: Homovanillic acid (HVA) and vanillylmandelic acid (VMA) are measured in the diagnosis and follow-up of neuroblastoma that are most common cancer type in infants and young children. Urine is a preferred sample for the measurement of HVA and VMA. Although 24 hour urine sample collection is probably the best, random urine collection with normalization of HVA and VMA results by creatinine concentration are acceptable for both diagnosis and follow-up of neuroblastoma. Urine collection in children could be challenging and it often needs use of bags for sample collection. This method is cumbersome and time consuming. Alternate ways of sample collection such as urine collection on filter paper have been used. We investigated the possibility of urine collection on cotton balls as they are easy to use and widely available. We evaluated 4 different types of cotton balls as an alternate way of urine collection for the measurement of HVA and VMA.

Methods: Four different cotton balls were evaluated: Walgreens Studio 35 Beauty, Wal-Mart White Cloud, Target Up &Up and Kendall Curity. A total of 22 patient urine samples, commercial controls purchased from Bio-Rad Diagnostics and spiked urines were used for this study. These samples were tested for creatinine, HVA and VMA concentrations prior to the addition of cotton balls. One cotton ball from each source was saturated with 2-5 mL of each patient, control and spiked urine and then processed at 6 hour and 18 hour intervals for creatinine, HVA and VMA analyses. Creatinine was measured using a Syva V-twin chemistry analyzer. HVA and VMA were extracted from urine using ethyl acetate. The extracts were derivatized, and HVA and VMA were measured by gas-chromatography mass spectrometry using deuterated internal standards. HVA and VMA concentrations were expressed as mg/g creatinine.

Results: No significant difference was noted either in creatinine or HVA and VMA concentrations in the samples incubated with cotton balls as compared to straight samples. Mean creatinine concentrations were 121, 123, 122, 124 and 122 mg/dL for direct sample, and samples incubated with cotton balls from Walmart, Walgreens, Target and Kendall respectively. Also, no significant difference was found in HVA and VMA concentrations among direct samples or samples incubated with cotton balls. Mean HVA concentrations (mg/g creatinine) were 15.2, 15.1, 15.0, 15.3, and 15.4 respectively. Mean VMA concentrations were 12.7, 12.7, 12.8, 12.9, 12.9 mg/g creatinine respectively.

Conclusion: The cotton balls tested demonstrated no adverse affect on HVA, VMA or creatinine concentrations, and, therefore, can be used for urine collection as necessary for the measurement of HVA and VMA.

B-219

CLSI-based Transference of CALIPER Pediatric Reference Intervals to the Roche Cobas 6000 and the Roche Modular System

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Background: Correct interpretation of laboratory tests requires

accurately established reference intervals (RIs). Many gaps in pediatric

reference intervals currently exist and CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) has begun to address these limitations by establishing pediatric age- and gender-specific RIs for over 80 biochemical markers on the Abbott ARCHITECT system. However, this database was only directly applicable for Abbott ARCHITECT assays. In 2013, CALIPER expanded the scope of this database by transferring RIs to biochemical assays from other major manufacturers, including the Roche Cobas 6000, as well as Beckman, Ortho, and Siemens systems. This current study further broadens the application of the CALIPER database by performing further transference and validation studies for additional analytes on the Roche Cobas 6000, and the Roche Modular System.

Methods: Approximately 200 serum samples from pediatric outpatients attending SickKids Hospital (Toronto, Canada) were analyzed on the Roche Cobas 6000, the Roche Modular System, and the Abbott ARCHITECT ci8200 systems. Statistical analysis was performed using Excel (Microsoft) and R software. According to CLSI C28-A3 and EP9-A2 guidelines, CALIPER RIs established on the Abbott ARCHITECT were transferred to assays performed on the Roche Cobas 6000 and the Roche Modular System. Specifically, the correlation between the analyzers was assessed and the line of best fit was calculated by the least squares approach or Deming regression, depending on the r^2 value. The appropriateness of the linear model was assessed using Q-Q, standardized residual, and Bland-Altman plots. The equation of the line of best fit was then used to transfer the CALIPER RIs to the Roche systems. 95% confidence intervals were calculated using the root of the mean-squared error (RMSE), calculated as reference limit ± 1.96*RSME. Calculated RIs were validated on these systems using 100 reference specimens from the CALIPER biobank of healthy children. RIs were considered validated if >90% of the reference samples fell within the transferred RIs, inclusive of the 95% confidence intervals.

Results: Most assays were transferable from the Abbott ARCHITECT to the Roche Cobas 6000 (12 out of 16 analytes) and the Roche Modular System (31 out of 36 analytes). Carbon dioxide and magnesium were not transferable to either system due to poor correlation ($r^2 < 0.70$). The hsCRP assay was not transferable to the Roche Cobas 6000 due to failure to meet criteria of the normality plots. Eight of the 12 transferred reference intervals were verified following analysis of reference specimens from healthy children on the Roche Cobas 6000, and 19 of 31 transferred reference intervals were verified on the Roche Modular System.

Conclusion: This study extends the utility of the CALIPER pediatric reference interval database for laboratories using the Roche Cobas 6000 and the Roche Modular Systems, enabling further implementation of CALIPER reference intervals across Canada and worldwide. CALIPER RIs for different analytical platforms can later be collectively analyzed by future studies in an attempt to develop common RIs across all clinical chemistry instruments and standardize laboratory test interpretation in diagnosis and monitoring of pediatric disease.

B-220

Copeptin in pediatric patients

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Background: Copeptin is a carboxy-terminal peptide cleaved from pre-pro-vasopressin (AVP). It is a stable surrogate biomarker for AVP. It is produced in a 1:1 ratio with AVP, has no known physiological function, a longer plasma half-life, and is more stable in serum/plasma. In patients with heart failure (HF), elevations are associated with increased risk of death or need for cardiac transplantation independent of B-type natriuretic peptide (BNP) and cardiac troponin concentrations. Gender differences in copeptin values have been reported in healthy adults, newborns and patients with myocardial infarction. Gender differences have not been described previously in a large pediatric population.

Objective: We sought to determine reference intervals among pediatric patients.

Methods: Sera from 240 healthy children (40 each male and female in three age groups: 2-6 years, 7-12 years and 13-17 years) were identified from an institutional pediatric biobank and obtained in compliance with the Institutional Review Board. Any patient with a diagnosis of anemia, autoimmune disease, hematologic disease/bleeding, circulatory/heart failure, kidney or liver disease, malignancy, malnutrition, diabetes, or pregnancy were excluded. Copeptin was measured using the B.R.A.H.M.S Kryptor Compact Plus (Kryptor / Thermo Fisher, Waltham, MA) with Copeptin Ultra-Sensitive (US) Immunoassay kit (802R.050) Non-parametric analysis was used to establish the 95th percentile reference interval between genders.

Results: The overall mean serum copeptin (±SD) was 14.6±43 pmol/L. Concentrations were not significantly associated with age. The large variation in normal values prompted a more detailed investigation into medication histories. Active fentanyl prescriptions were identified in 48 (20%) subjects. Serum copeptin was significantly elevated among these patients (39.9±91 pmol/L vs. 8.3±5.4 pmol/L; p=0.0102). Patients prescribed fentanyl had diagnoses of digestive disorders (n=15), infectious respiratory disorders (n=13), skin concerns (n=4), urinary problems (n=4) and musculoskeletal complaints (n=15) but there was no association between fentanyl and specific comorbidities. Copeptin concentrations also were not associated with any specific comorbidity. After excluding patients prescribed fentanyl, the mean serum copeptin was significantly higher in boys (9.3±5.9 pmol/L) compared to girls (7.3±4.8 pmol/L; p =0.0116). The 95th percentile cutoff for normal was 18.4 pmol/L (95CI 16.2 - 38.2) for boys and 20.0 pmol/L (95CI 15.1 - 21.3) for girls. In our adult validation, copeptin was similarly significantly higher among men (7.18±5.53 pmol/L) than women (4.46±2.43 pmol/L, p=0.003) in a healthy cohort aged 23 - 80 (n=230). The

95th percentile cutoff for normal among adults was higher at 13.0 pmol/L (95CI 2.9 - 23.2) for men and 8.3 pmol/L (95CI 6.5 - 10.0) for women.

Conclusions: Serum copeptin is significantly but modestly higher among boys compared to girls. The considerable overlap of confidence intervals at the 95th percentile may limit the diagnostic importance of this gender difference. Serum copeptin is elevated in patients receiving fentanyl. Additional studies are required to determine if fentanyl is the reasons for this effect but its use must be taken into account when calculating normal values.

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Drug Excretion into Breast Milk: are all drugs contraindicated for breastfeeding?

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Background:

Epidemiological research provides strong evidence for health benefits associated with breastfeeding, including reduction in infant mortality, infection and development of chronic diseases, as well as positive impacts on cognitive development. Studies have shown that 66-80% of women are on medication during the postpartum period. Although not all drugs may be considered contraindicated while breastfeeding, there remains little data on this topic. Methotrexate(MTX) is the first line of treatment for rheumatoid arthritis(RA), which has a high incidence in women of childbearing age. We developed a sensitive and specific LC-MS/MS method to quantitate MTX and its metabolite in human milk and applied it to patient samples. We also calculated the relative infant dose of MTX to determine the risk to the infant.

Methods:

A simplified drug extraction method using hexane, methanol and acetonitrile facilitated efficient drug extraction from breast milk. Methotrexate was measured using an IONICS 3Q 210 mass spectrometer. Detection was performed by multiple reaction monitoring mode using electrospray ionization in positive ion mode. Settings: ESI Voltage 5000; Nebulizer Gas, 400; Drying Gas, 120; Heating Gas, 350; Source Temp(°C), 250; MTX MRM 455.1/308.0 and 455.1/134.0. Liquid chromatrographic separation was performed on a Shimadzu Prominence UFLC. A 5 µL sample was injected into an Imtakt C8 column (2.0x75 mm, 3 µm) at room temperature. The method was fully validated in terms of selectivity, linearity, accuracy, precision, stability and recovery according to standard clinical laboratory protocols. Comparison using patient samples was also performed. Patients receiving MTX therapy for RA were recruited through the SickKids Motherisk Program for the DLAC Project or through the Rheumatology Clinic at Southlake Regional Health Centre in Newmarket, Canada. Whole breast milk samples were aliquotted and stored at -20°C until sample preparation, extraction and analysis.

Results:

Results from the method validation will be presented. Pharmacokinetic profiling of methotrexate and its metabolite in breast milk were determined following a subcutaneous dose of 25 mg/mL of methotrexate, once weekly. Breast milk samples were obtained at the following 7 timepoints: pre-dose (time zero), 1 hr, 12 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs post-dose. Both foremilk and hindmilk were measured. We found that MTX is excreted into breast milk, but with no notable differences in drug concentrations between foremilk and hindmilk. The highest drug concentrations occurred between 1-12 hours post-dose; the concentration steadily decreased between 12 - 48 hours, with small but detectable levels from 48 - 96 hrs. Methotrexate is excreted into breast milk at significant concentration within the first 24 hrs post-dose. However, no notable differences in drug concentrations between foremilk and hindmilk were observed

Conclusion: Due to the difficulty in obtaining foremilk and hindmilk, this is the first study to measure and compare drug levels in this sample type. This data provides the foundation to establish a TDM system for measuring drug concentrations in breast milk, with the aim to carry out population-based pharmacokinetic analysis to determine safety guidelines on drug excretion into breast milk as well as breast feeding guidelines.

B-222

Free light chains in the response assessment of celiac disease patients under gluten free diet

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Background:

Celiac disease (CD) is a chronic immune-mediated small intestinal enteropathy, triggered by exposure to dietary gluten in genetically predisposed individuals and frequently diagnosed during childhood. Confirmatory duodenal biopsy can be avoided if suggestive clinical symptoms are accompanied by positive tests for CD-specific antibodies. New CD-biomarkers would increase confidence on the diagnosis and the number of patients exempting biopsy. Increased serum free light chain levels (sFLC) have been observed in patients with auto-immune diseases making it a potential new test for CD diagnosis and response assessment after initiation of the gluten-free diet (GFD). We seek to assess the utility of sFLC levels as markers of intestinal mucosa alterations in CD patients.

Methods:

165 CD patients with serum samples at diagnosis, of which 21 had follow-up samples at 6 months post-GFD initiation. As control group, 52 patients with initial suspicion of CD that was later ruled out were included. Serum biomarkers: antibodies IgA anti-transglutaminase (TG2) and anti-endomysial (Menarini diagnostics), and FLC (Freelite®)

Results:

CD patients showed median levels of κ+λ sFLC significantly higher than the control group (30.2mg/L vs 18.0mg/L, p<0.0001, Fig.1). Additionally, samples obtained 6 months post-GFD show a significant decrease of summated sFLC levels compared to those at diagnosis (33.6mg/L vs 19.3mg/L, p=0.0016): median decrease of 1.5 fold (0.9-3.6). In fact, after GFD initiation, there is no longer a statistical difference between this group and the non-CD control group (18mg/L vs 19.3mg/L, p=0.28). Finally, 19 of the 20 follow-up samples with available TG2 data show a reduction of its values at 6 months of GFD.

Conclusion:

The statistical difference between the studied groups shows that summated serum FLC levels are good indicators of disease response, possibly reflecting normalization of the intestinal mucosa. The decrease of the TG2 values upon GFD initiation supports this hypothesis but validation from patients with available biopsy is necessary.

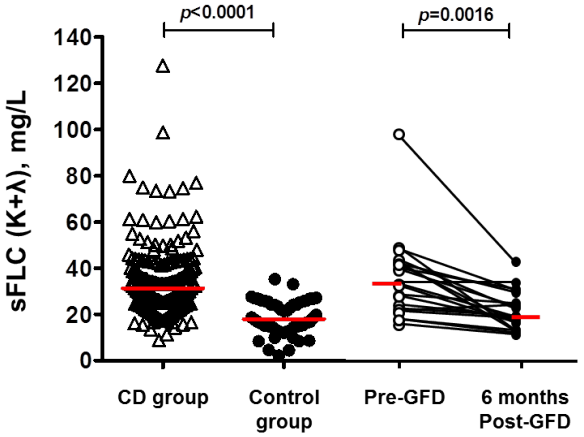

Figure 1. Summated serum free light chain values (sFLC) of 165 CD patients and 52 controls. CD: Celiac disease; GFD: Gluten free diet. The horizontal red lines represent median values. Mann-Whitney statistical test.

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CAPILLARY BLOOD SAMPLING KIT FOR HBA1C VERSUS VENOUS PUNCTURE ON CAPILLARYS 2 FLEX PERCING

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Background:

Capillary blood sample collected from finger prick presents many advantages over venous puncture: low volume, less invasive for the patient, better patient's compliance with monitoring recommendations. The present study was designed to compare the measurement of capillary blood hemoglobin A1c levels with venous blood hemoglobin A1c levels using the Capillarys 2 Flex Piercing system (C2FP) (Sebia, France) on a large range of HbA1c values and with different storage conditions.

Methods:

Data was collected from samples of 60 volunteer patients and covering a wide range of HbA1c values (4.7% - 14% NGSP). Both venous and capillary blood samples obtained simultaneously from each subject were tested using the C2FP system. After an initial assessment of venous HbA1c at J0, capillary and venous samples were stored at room temperature (Room T°) and 4°C respectively, away from light, and re-analyzed together at J5 on the same C2FP system in duplicates. To test stability, 4 different samples were simultaneously taken from venous puncture and finger prick, and stored at different T° (-20°C, 8 days; 2-8°C, 8 days; Room T°, 8 days; 30°C, 3 days). Respective duplicates values were compared to capillary and venous (reference) result at J0.

Results:

The trendline of J5 values using mmol/mol IFFC units (slope: y=0.9904x + 0.1387; R2=0.997) or %NGSP units (slope: y=0.9896x + 0.046; R2=0.997) showed a good correlation. Bland Altman plots showed a 0.4mmol/mol IFCC and 0% NGSP mean differences. All values were included in the recommended +/-6% bias on the bias plot.

Room T° storage during 5 days resulted in a small additional peak of degradation but HbA1c value was still accurate. Reproducibility was assessed using the mean biases between the NGSP duplicates and showed the same 0% for venous and capillary results. Stability study on low, medium and high HbA1c levels showed that ideal conservation was 4°C. Room T° and -20°C give rise to degradation without alteration of HbA1c result. After 3 days at 30°C, only one sample result was slightly out of uncertainty of measurement.

Conclusion:

The Sebia capillary sampling kit offers full automation and full positive ID. We have demonstrated a good correlation with venous sample results. Storage study showed a sufficient robustness for usual sample delivery to central laboratory.