Wednesday, August 3, 2016

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

Pediatric/Fetal Clinical Chemistry

B-220

Evaluation of GDF-15 and YKL-40 as Early Markers of Subclinical Diabetic Nephropathy and Cardiovascular Morbidity in Young Patients with Type 1 Diabetes Mellitus

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Background: Diabetic nephropathy constitutes a major long-term complication in patients with type 1 diabetes mellitus (T1DM) and its diagnosis is based on microalbuminuria. Growth Differentiation Factor-15 (GDF-15) is a protein belonging to the transforming growth factor beta superfamily that has a role in regulating inflammatory and apoptotic pathways in injured tissues and during disease processes. Chitinase-3-Like Protein 1 (YKL-40) is a protein with the ability to communicate with other signal transduction pathways to modulate various physiologic processes, such as inflammation, apoptosis, tissue remodeling, cell growth, and angiogenesis. An increasing body of evidence exists supporting the involvement of these two proteins GDF-15 and YKL-40 in cardio-renal events, therefore we aimed to investigate in an observational follow-up study their role in unravelling early diabetic nephropathy and their impact as potential risk markers for cardiovascular morbidity. **Patients and Methods**: Fifty-six patients with T1DM, aged 13.1±3.2 years and 49 healthy controls aged 12.8±6.6 years were recruited. Along with standard blood and urine chemistry, measurements of serum Neutrophil Gelatinase Associated Lipocalin (NGAL), Cystatin C, YKL-40 and GDF-15 were performed by means of immunoenzymatic and immunonephelometric techniques. eGFR values were calculated from Cystatin C based e-GFR equations[1]. The measurements were performed at enrolment and after 12-15 months. **Results**: At baseline, mean GDF-15 levels were not significantly different between children with diabetes (289.5pg/mL) and controls (278.6pg/ml). At re-evaluation, mean GDF-15 in patients increased (366.7pg/mL), ($p=0.001$) and was significantly higher than in controls ($p<0.001$). GDF-15 levels correlated negatively with eGFR values ($r=-0.27$, $p=0.04$, $n=56$) and positively with both total Cholesterol ($r=0.29$, $p=0.033$, $n=54$) and LDL-Cholesterol ($r=0.35$, $p=0.009$, $n=54$) at re-evaluation. Mean YKL-40 level in T1DM patients increased from baseline (17.4ng/mL) to re-evaluation (20.5ng/mL), ($p<0.001$), while no significant difference was observed between patients with T1DM and controls ($p>0.19$) at baseline. YKL-40 levels correlated positively with NGAL, GDF-15, total Cholesterol and Triglycerides concentrations at both time-points of evaluation ($r= 0.35$, $p=0.007$; $r=0.55$, $p<0.0001$; $r=0.29$, $p=0.04$; $r=0.46$, $p<0.001$, respectively and $r= 0.31$, $p=0.02$; $r=0.36$, $p=0.006$; $r=0.44$, $p<0.001$; $r=0.47$, $p<0.001$, respectively). A positive correlation was also found between YKL-40 levels and Systolic Arterial Pressure (SAP) values at all evaluation points ($r=0.23$, $p=0.02$). **Conclusions**: To our knowledge, this is the first study to demonstrate a predictive role for serum GDF-15 and YKL-40 as early markers of diabetic nephropathy in children and adolescents with T1DM before severe overt nephropathy occurs. In addition, the associations of these biomarkers with SAP and hyperlipidemia reflect their possible prognostic role on cardiovascular morbidity suggesting their measurement besides microalbuminuria to unravel early renal dysfunction. Defining new predictors as supplementary tests to urinary albumin excretion for the early diagnosis of diabetic nephropathy and cardiovascular morbidity would accelerate effective management and treatment approaches needed to minimize the rates of severe cardio-renal morbidity and mortality in young patients with T1DM. These data should be confirmed by further large-scale longitudinal studies before being integrated in the diabetic nephropathy risk assessment of young patients with T1DM. *[1]Clinical Chemistry, 60:974-986, 2014.*

B-221

Circulating Dickkopf-1 Protein Levels in Normal-Weight and Obese Children: Evidence of Involvement of Canonical Wnt Signaling

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Background: Dickkopf-1 protein (DKK-1) is the founding member of a multigene family of which DKK-1, DKK-2 and DKK-4 are secreted proteins that bind to the extracellular domains of the Low-Density-Lipoprotein (LDL) receptor-related proteins 5 and 6 (LRP5/LRP6) and inhibit Wnt/β-catenin signaling, making DKK-1 a drug target for multiple diseases. DKK-1 was originally identified in *Xenopus* as a molecule that can induce head structures, and this is the basis for the name. Secretion of Wnt proteins by adipose cells plays an important role in the control of adipogenesis. The Wnt antagonist, DKK-1, is secreted by human pre-adipocytes and influences adipocyte maturation and growth. DKK-1 mRNA increases six hours after onset of human adipogenesis and this is followed by an increase in DKK-1 protein. With further differentiation, mRNA and protein levels of DKK-1 progressively decline to undetectable in mature adipocytes. The transient induction of DKK-1 correlates with down-regulation of cytoplasmic and nuclear beta-catenin levels, representing a surrogate marker of canonical Wnt signaling and Wnt/beta-catenin transcriptional activity. Of note, DKK-1 protein has been implicated also in bone remodeling pathways. **Patients and Methods**: In this study we measured the circulating DKK-1 levels in 16 normal-weight and 25 obese girls using immunoenzymatic techniques and we investigated possible correlations of DKK-1 levels with parameters of anthropometric evaluation; insulin resistance; adipose tissue secretory molecules {adiponectin, leptin, retinol binding protein-4 (RBP-4) and lipocalin-2}; bone remodeling biomarkers, (osteoprotegerin (OPG), receptor activator of NF-κB ligand (RANKL), osteocalcin, C-terminal cross-linking telopeptide of collagen type-I (CTX), bone alkaline phosphatase (bALP) and tartrate-resistant acid phosphatase isoform-5b (bone TRACP-5b) and a low grade inflammation marker (hs-CRP). **Results**: We found that: a) DKK-1 levels were significantly higher in normal-weight girls than obese girls 37.5±18.0 vs. 18.6±2.4 pg/mL, $p=0.009$, b) BMI and HOMA index values correlated negatively with DKK-1 levels ($r=-0.508$, $p<0.001$ and $r=-0.380$, $p<0.01$, respectively), c) logDKK-1 values correlated significantly only with adiponectin levels ($r=-0.404$, $p=0.008$), d) DKK-1 and RANKL levels correlated positively with each other, ($r=0.492$, $p<0.001$) and e) hs-CRP and DKK-1 levels correlated, negatively with each other ($r=-0.371$, $p=0.01$). **Conclusions**: These preliminary findings suggest that indices of metabolic syndrome such as obesity, insulin resistance, and low grade inflammation markers are negatively associated with circulating DKK-1 protein levels in children. Obesity is characterized by inappropriate expansion of adipose cells (hypertrophic obesity) and probably by a disrupted ability to recruit and differentiate new precursor cells (pre-adipocytes). Thus, the impairment of adipogenesis observed in obesity could be attributed to disrupted suppression of canonical Wnt signaling via DKK-1. Moreover, the lower DKK-1 levels found in obesity may also explain the connection of obesity with better bone accrual.

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Serum bile acid profile in neonates with cholestasis caused by citrin deficiency

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Infants with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) are characterized by conjugated hyperbilirubinemia and markedly high levels of serum bile acids. However, the mechanisms remain unclear. This study aimed to establish a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantification of serum bile acids and compared the compositions in patients with NICCD and infants having citrullinemia but no mutations found in *SLC25A13* gene (non-NICCD). Separation of bile acids were carried out on a 2.1 mm x 150 mm Symmetry C18 column with 3.5 μm particle size using an Agilent 1200 HPLC system. Mobile phase consisted of 5 mM ammonium acetate in H_2O (87.5%), 0.1% NH_4OH (12.5%) and methanol at a flow rate of 0.2 mL/min. An API 5000 tandem mass spectrometer was used for the detection with electrospray ionization source and negative ionization mode. The established LC-MS/MS method measured 15 serum bile acids within 14 minutes and has a liner range of 0.01~1 μM for glycolithocholic acid, taurolithocholic acid and lithocholic acid, 0.1~10 μM for taurocholic acid,

glycochenodeoxycholic acid and taurochenodeoxycholic acid, and 0.05~5 μM for the others (r>0.99). The within-run and run-to-run imprecision (CV) of all bile acids was 1.2~10.9% and 3.1~10.8%, respectively, with the mean recovery of 90.5~112.6%. Compared to non-NICCD, NICCD infants had significantly elevated serum total bile acids (158.5 vs. 31.2 μM, p<0.01), glycocholic acid (13.8 vs. 0.24 μM, p<0.05), taurocholic acid (32.9 vs. 8.3 μM, p<0.001), and taurochenodeoxycholic acid (69.3 vs. 0.3 μM, p<0.01). And the resultant ratios increased in NICCD infants, including primary/secondary bile acids (516 vs. 159, p<0.05), taurine/glycine-conjugated bile acids (2.1 vs. 0.6, p<0.01), and conjugated/free bile acids (326 vs. 70, p<0.05). In summary, we established LC-MS/MS method for serum bile acid profile analysis, and found a distinct bile acid profile in NICCD patients.

B-223

Usefulness of procalcitonin to predict serious bacterial infection in febrile pediatric patients

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Background: Fever is a common symptom in pediatric patients visiting emergency department. Many of them have non-bacterial causes of fever, but some febrile infants and children would have occult serious bacterial infection (SBI) such as bacteremia, bacterial urinary tract infection(UTI), lobar pneumonia, bacterial meningitis, bacterial gastroenteritis and so on. To avoid possible complication, it is important to recognize SBI as early as possible. Various tests are are used in the laboratory evaluation of patients. However, it is still difficult to predict the presence of SBIs with complete certainty. Procalcitonin (PCT) is known as one of the acute phase reactants for identifying invasive bacterial infection. The objectives of this study is to compare the performance of serum PCT with other traditional screening tests such as C-reactive protein(CRP) and absolute neutrophil count(ANC) for detecting SBI in febrile pediatric patients.

Methods: From November 2014 to July 2015, febrile 212 infants and children younger than 7 years old, who visited emergency department, were studied for SBI. Blood, urine and/or CSF cultures were performed in most of patients. Chest radiograph were done in most of patients. Serum PCT levels were compared with CRP levels and ANC between febrile patients with SBI and without SBI.

Results: The overall prevalence of SBI was 6.1 %(13 patients) of 212 febrile infants and children. Of 13 patients with SBI, 4(30.8%) patients had positive blood culture, 7(53.8%) had positive urine culture and 2(15.4%) had pneumonia. Patients with SBI had higher PCT levels (1.3±3.2ng/ml vs 0.4±2.1 ng/ml, p<0.001), higher CRP levels (57.2±114.6 mg/L vs 27.6±92.2mg/L, p=0.005) and higher ANC ($8.22x10^9$ cells±$6.38x10^9$ cells/L vs $5.76x10^9$ cells±$9.15x10^9$ cells/L, p=0.02) than those without SBI. We also assessed the diagnostic properties of the three biomarkers (PCT, CRP and ANC) using receiver operating characteristic (ROC) curve. The area under the curve (AUC) for PCT was largest (0.76, 95% CI=71 to 0.80), followed by CRP (0.74, 95% CI=0.69 to 0.78) and ANC (0.70, 95% CI=065 to 0.75).

Conclusion: PCT had better diagnostic accuracy than traditional screening tests such as CRP and ANC for identifying febrile pediatric patients with SBI. And further study on large cohort is required to definitely determine the benefit of PCT over traditional screening tests for SBI.

B-224

A Multi-Hospital Health System's Experience with Pediatric Reference Ranges

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Establishment of age-specific and sex-specific reference intervals for pediatric population is critical for correct clinical interpretation of the lab results. Laboratories face challenges in establishing pediatric intervals since it is extremely difficult to locate and gather normal pediatric specimens. To overcome this challenge, we used our large database of patient results from our outpatient settings as a "proxy" for normal patients. We selectively looked at multiple pediatric group practices but excluded physician groups that we know would have a high probability of elevated results (e.g., endocrinology, urology, etc.). The vast majority of these outpatient pediatric subjects had routine lab work ordered with mostly normal results and few abnormal results. From this database, we pulled the data from specific sex and age intervals to verify the Canadian Laboratory Initiative for Pediatric Reference (CALIPER) Intervals for analytes such as Calcium, ALP, CO2, Creatinine, FSH, Prolactin, Albumin, Direct HDL and Total Protein measured on the Abbott ARCHITECT platform. We found that the majority of results were normal and verified many of the CALIPER intervals. In most cases, we checked one year of data using EP Evaluator Verification of Reference Interval Module with pass criteria being 10.0% and were able to verify the specific sex and age intervals for these analytes. This study shows how real world data from a five-hospital health system looks when compared to the CALIPER intervals.

We focused on this approach to verification after fielding questions from specific physician's observing what they perceived as a shift of elevated results for specific analytes. Pulling their databases indicated these elevated results. But pulling much larger group practices and excluding specific high disease probability practices showed normal results within the reference range. The elevated results for the questioning physicians were related to their small population size, practice or pre-analytical issues.

When a common reference range is used by multiple instruments at different locations it is essential that analytical quality in relation to the reference range and quality control be robust. We accomplish this using the Sunquest BDUP function (Blind Duplicate). This allows us to check one instrument against the other using established criteria to evaluate the difference in results obtained and graph them on a Levy-Jennings plot. We perform this daily at all sites with two or more instruments and monthly between all sites to help assure that each instrument is turning out similar or correlated patient results.

Other helpful tools are using inter-individual Biological Variation (CV_I) to define an Optimal (0.25 X CV_I), Desirable (0.50 X CV_I), and Minimal (0.75 X CV_I) quality control precision for these assays. In addition, we used Biological Variation and analyte precision to calculate Reference Change Value (RCV). RCV defines the critical difference that if exceeded between two sequential results indicates a significant change in patient condition. Likewise, the Index of Individuality is helpful in determining when to use the reference range (i.e., Index ≥1.0) and when to use RCV (Index ≤1.0).

B-225

Biochemical diagnosis of mitochondrial Respiratory Chain disorders in clinically suspected Egyptian children

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Background and objective: Mitochondrial Respiratory Chain (RC) disorders are a growing group of disorders with a large variety of clinical presentations ranging from well defined clinical syndromes to non specific manifestations as failure to thrive and seizures. This study aimed to describe the clinical, biochemical and histochemical spectrum of 23 Egyptian patients with confirmed mitochondrial RC disorders as the first pilot study for biochemical measurement of RC in Egypt.

Patients and Methods: Twenty three Patients clinically and radiological suspected to have mitochondrial RC disorders were referred to the Inherited Metabolic Disease Unit laboratory, Cairo University Children's hospital. Using muscle biopsy homogenate, histochemical staining of Cytochrome Oxidase and Succinate dehydrogenase and spectrophotometric assay of RC complexes were done.

Results: Eleven patients confirmed to have isolated complex I deficiency (48%), two patients had combined complex I and complex II deficiency (9%), two patients had combined (complex I, II+III, complex IV) deficiencies (9%), one patient had combined complex I & III (4%), two patients had isolated complex II deficiency (9%), one patient had isolated complex IV deficiency (4%), and four patients had normal respiratory chain enzymes activities (17%).

Conclusion: To the best of my knowledge, this is the first study reported on the Egyptian children with the clinical suspicion of mitochondrial disease. The presence of 19 positive cases out of 23 cases confirmed to have RC deficiency points to their high prevalence these disorders among Egyptian paediatric patients. With the advent of next generation sequencing technology, mitoexome and whole exome sequencing represents an appealing approach for elucidation of the molecular basis of these disorders among Egyptian patients.

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Mitochondrial respiratory chain complex activities in high risk pregnancies

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Background:

Mitochondria have a central role in the energy metabolism and provide ATP in most cells. Mitochondrial oxidative phosphorylation is also the key energy source for placental functions and fetal growth. The placenta is a very important multifunctional

transient organ, essential for the healthy development of the fetus. The purpose of this study was to investigate the function of placenta by measuring respiratory chain complex (RCC) activities in high risk pregnancies, in addition to evaluate the correlation between double test risk ratio and RCC activities.

Methods:

The placenta samples were collected from 50 pregnant women following elective cesarean section; 20 normal pregnancies (controls), 6 preeclampsia (PE), 6 intrauterine growth restriction (IUGR), 6 advanced age (over age 35), 6 twins and 6 preterm deliveries were included in the study. Complex I, II-III, IV and citrate synthase (CS) enzyme activities were measured by kinetic spectrophotometric assays. Immulite 2000 and Prisca software was used for estimation of double test risk ratio.

Results:

Complex I activity was 31.43 ± 4.17 U/g protein, Complex II-III activity was 23.55 ± 5.2 U/g protein, Complex IV activity and CS activity were 144.39 ± 21.9 U/g protein and 79.9±8.81U/g protein in normal placenta, respectively. Complex I, II-III and IV activity were significantly lower in the study group than the controls ($p<0.05$). Especially, Complex I and II-III activity were significantly reduced in placenta of preterm deliveries compared to the controls ($p<0.003$). The mean activity values of Complex I in IUGR, PE, twins, and advanced age groups was also lower compared with the controls ($p>0.05$). Similarly, reduced complex II-III activity was observed in PE, twins, IUGR and preterm deliveries than the controls ($p>0.05$). Increased Complex IV activity was observed in 33% of advanced age pregnancies.

Then mitochondrial RCC complex activities were expressed per CS activity for standardization of the assay. Mean values of mitochondrial complex I/CS activity and complex II-III/CS activity was found below in all study groups than controls. Mean values of mitochondrial complex IV activity / CS activity was found below in all study groups than controls, except advance age groups.

Double test was performed for all pregnant women between 10-14th weeks of gestation. Double test risk ratio was above the cut-off limit (1:300) in 43% of the study group; Complex I and Complex II/III activity was reduced in 76% and 30% in this group, respectively.

Conclusion:

Impaired placental mitochondria RCC functions can lead to adverse pregnancy outcomes such as PE, IUGR and preterm delivery. This is the first report that documents a positive association between decreased placental mitochondrial complex activities and high double test risk ratio. Pregnant women with high risk in double test should be monitored carefully in terms of PE, IUGR and preterm delivery.

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Primary Human Cytomegalovirus (HCMV) Infection in Pregnancy: Unrevealing the Metabolic Network

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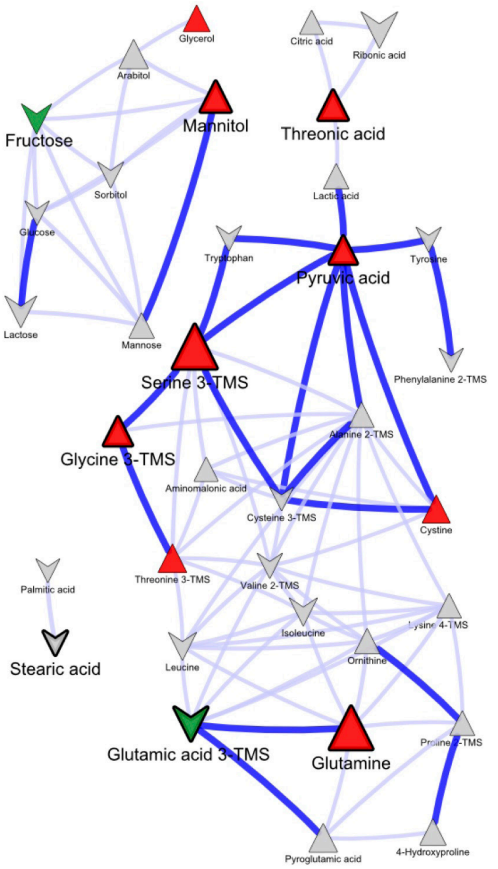
BACKGROUND: Maternal human cytomegalovirus (HCMV) infection during pregnancy represents one of the most frequent risk of birth defects and long-term sequelae. The severity of the infection depends on the clinical features: symptomatic or asymptomatic; with or without complications. **OBJECTIVE:** Our aim was to study the metabolic profile of amniotic fluid (AF) by means of metabolomics networks in HCMV-infected fetuses compared with controls. **METHODS:** we enrolled 20 pregnant women with diagnosis of primary HCMV infection developed in the neonate transplacentally (transmitters), and 23 non-infected subjects undergoing amniocentesis for cytogenetic-based diagnosis. An AF DNA level $>10^5$ copies/mL was considered a reliable predictor for symptomatic congenital infection. AF samples were analyzed by using an Agilent 5975C platform interfaced to the Gas Chromatograph (GC) 7820 equipped with a DB-5ms column. The resulting chromatograms were identified using the database NIST08 (National Institute of Standards and Technology's mass spectral database). 150 target compounds were identified; they were included into a own devoted library. Multivariate statistical analysis was done and a partial least squares discriminant analysis (PLS-DA) model was created, including transmitters and controls. Subsequently, the model was exploited for network mapping by using the software MetaMapR, in order to identify the relationship among the discriminant metabolites. **Results:** As showed in Fig.1, where red triangle means increase; green means decrease; and the size is an index of importance, PLS-DA model showed separation between the two groups (Accuracy=0.88 R2=0.75 Q2=0.58; Cross validation (CV) method: 10-fold CV, Performance measure: Q2 $p<0.01$). Eleven compounds were found responsible for the discrimination. The metabolic network analysis demonstrated that glutamine and glutamate; pyrimidine; purine; alanine-aspartate glutamate; arginine and proline; cysteine and methionine; glycine-serine threonine were the most influenced metabolic pathways in transmitters when compared with controls. **Conclusion:** Metabolomics may be considered an effective strategy in searching of useful information on pregnancy-related pathologies.

Figure 1.



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Optimization of Estradiol Measurements to Improve Utility in the In Vitro Fertilization Setting

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Background: Measurement of estradiol (E2) plays an important role in clinical management for women undergoing ovarian stimulation for *in vitro* fertilization (IVF). However, inconsistent or unreliable estradiol values can pose a challenge in monitoring and management of patients. We performed a comparison study of different immunoassay platforms compared to liquid chromatography-tandem mass spectrometry in an effort to improve IVF success rate for our patient population at the Family Fertility Center.

Methods: Comparison studies were performed using estradiol assays on ADVIA Centaur CP system, Architect i1000 (Abbott) analyzer and AB Sciex 5500 LC/MS/MS system. The first part of the comparison studies were carried out on thirty-seven patient samples (ranging from 124 pg/mL to 4000 pg/mL) using ADVIA Centaur CP analyzer and Architect i1000 analyzer. The second part of the study included analysis of fifteen selected samples on the Tandem Mass Spectrometer (LC/MS/MS). Samples

were analyzed on ADVIA Centaur CP system within 1-2 hours of collection. Then they were aliquoted and stored frozen (-80 ºC) until testing on latter two instruments. Analysis of results for comparison studies was performed using the EP Evaluator Data Innovation (EE 10) program. In addition, ovarian follicle size and number were obtained via transvaginal ultrasound.

Results: Results of comparison studies between ADVIA Centaur CP analyzer and Architect i1000 analyzer showed that Centaur had a significant positive bias of 20%. Comparison studies between Tandem Mass Spectrometer (LC/MS/MS) and ADVIA Centaur CP analyzer also resulted in a significant positive bias of 20% with Centaur. The same fifteen patient samples had negative bias of 0.3% when comparison studies were carried out between Tandem Mass Spectrometer (LC/MS/MS) and Architect i1000 analyzer. Results were then analyzed (between ADVIA Centaur CP analyzer and Architect i1000 analyzer) for low concentrations of estradiol (<1000 pg/mL, n=16) and Centaur resulted in an 18% bias while the bias was much greater at high concentrations of estradiol (1000-2000 pg/mL, n=13) at 26%. In addition, using the Architect i1000 estradiol values corresponded to better prediction of ovarian stimulation based on ultrasound measurement of follicular number and size.

Conclusions: The Architect estradiol assay shows excellent precision and very little bias compared to the gold standard, LC/MS/MS at all ranges of estradiol studies. Therefore results indicate that our fertility center would benefit measurements of estradiol levels using Architect i1000 analyzer for monitoring ovulation stimulation and further clinical management for in vitro fertilization.

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Efficiency of the Lecithin-Sphingomyelin Ratio and Phosphatidylglycerol in Comparison to Lamellar Body Count for Testing Fetal Lung Maturity

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Background: In fetus, immature lungs may lead to respiratory distress syndrome (RDS). Lamellar body count (LBC) is the primary laboratory test to assess the stage of lung maturity in our institution that utilizes the following ranges: <15,000 (immature fetus), 15,000-39,000 (indeterminate) and >39,000 (mature fetus). Lecithin-sphingomyelin ratio (L/S ratio) is used to test the indeterminate results of LBC testing which utilizes ranges: <2.0 (immature), and ≥2.0 (mature). Phosphatidylglycerol (PG), which is determined during the L/S ratio test, is also used to determine maturity based on whether it is present or absent. A PG positive result is indicative of mature lung. LBC is run on an automated hematology analyzer with a quick turn-around time, and is available 24 hours a day. L/S ratio and PG is a labor intensive thin layer chromatography which requires tedious sample preparation and takes ~6 hours to perform. We hypothesized that the L/S ratio and PG testing do not provide significant supplementary information when determining fetal lung maturity in comparison to the LBC testing. **Method:** Amniotic fluid was collected from 92 patients via standard clinical practice. LBC was tested immediately post sample collection. Samples with indeterminate LBC values had L/S ratio and PG performed at time of clinical care. Leftover samples with LBC >39,000 and <15,000 were stored at -70°C for L/S ratio and PG testing. Collection of leftover patient samples and clinical data for this study were approved by the Institutional Review Board. **Results:** 10 of the 92 patients were diagnosed with RDS. LBC and L/S ratio testing were compared based on their ability of predicting RDS. LBC, L/S, and PG all had sensitivity of 20%, 30%, and 40%, respectively. In terms of specificity, LBC had 60% specificity for predicting RDS, while LS Ratio had 89% and PG had 76% specificity. Positive predictive value (PPV) and negative predictive value (NPV) were also calculated. LBC had a PPV of 50% and a NPV of 94%. L/S and PG both had high NPV of 91%, but low PPV. L/S PPV was 25%, while PG PPV was 17%. **Conclusion:** L/S ratio did not improve prediction of RDS. Based on these findings and taking into account the complexity of L/S ratio testing, L/S ratio testing is not recommended in a clinical setting.

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Prognostic value MR- proadrenomedullin appendicitis in pediatric population

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Background: mid-regional proadrenomedullin (MR-proADM) is a precursor of the active peptide adrenomedullin produced by the adrenal and renal tissues under stressing situations

Objective: evaluate the diagnostic and prognostic value of MR-proADM in identifying children with acute appendicitis (AA).

Methods: observational, prospective and analytical study.

170 patients were recruited from November 2013 to April 2014.Children between 3 and 16 years, 56% of males that were admitted in the emergency department with acute abdominal pain and that after initial evaluation were suspicious for AA. Patients excluded from the study (34): appendectomy or recent surgery (3 months prior), immune disease, chronic respiratory or cardiovascular disease, inflammatory bowel disease, patients who had been treated with antibiotics or steroids in the last month.

Demographic data (sex and age), clinical history and analytical data: leukocyte count (µl/mm3), neutrophil count (µl/mm3), c-reactive protein, CRP (mg/dL) were collected. The Pediatric Appendicitis Score, PAS, was also calculated for all patients. The final diagnosis of AA was determined by histologic confirmation.

MR-proADM of all samples was measured in plasma EDTA tubes. Samples were centrifuged in the first two hours after their collection at 3.000 rpm for 15 minutes, the samples were frozen at -80ºC until their analysis. Determinations were made in a BRAHMS MR-proADM KRYPTOR analyzer by means of an immunofluorescent technique using a sandwich method with polyclonal antibodies. Statistical analyses were performed with SPSS 17.0 and MedCalc 11.2.1

Results: Of the 136 children included, 44 were diagnosed with appendicitis, 74 with unspecific abdominal pain, 5 with mesenteric adenitis and 13 with others diagnostics. Mean concentration of MR-proADM for AA, nonspecific abdominal pain and mesenteric adenitis were respectively: 0.52 nmol/L (IC: 95 % 0.46-0.57), 0.37 nmol/L (IC: 95% 0.35-0.40) y 0.50 nmol/L (IC: 95% 0.17-0.85). p<0.001.

The diagnostic accuracy of the different analytical markers studied (leukocyte count, neutrophil count, CRP, MR-proADM and PAS score) was calculated.The areas under the ROC were for MR-proADM of 0.75 (95% CI 0.67-0.82), for CRP 0.72 (95% CI 0.64-0.79), for neutrophil counts 0.86 (95% CI 0.79-0.92), and for leukocyte count 0.88 (95% CI 0.81-0.93).Value for PAS score was 0.87 (95% CI 0.80- 0.92).

The cutoff point for pro-ADM was: 0.34 nmol/L (sensitivity: 93.18 % and specificity: 45.65%) and 0.3 mg/dL for PCR (sensitivity and specificity: 68.18 %, 68.48%). Patients with pro-ADM >0.34 nmol/L, no appendicitis 50 and 41 did it suffered. Patients with pro-ADM ≤0.34 nmol/L, no appendicitis 42 while 3 yes they had. The positive predictive value, PPV for pro-ADM was 45.1% and the negative predictive value, NPV was 93.3%. Considering pro-ADM and PCR together, patients with pro-ADM>0.34 and PCR>0.3 mg/dL did not have appendicitis 22 and 27 finally did it suffered. PPV= 55.1%. Patients with pro-ADM ≤0.34 nmol/L and PCR≤0.3 mg/dL did not have appendicitis 35 while 0 yes they had. VPN=100%.

Conclusion: the combination of CRP≤0.30 mg/dL and MR-proADM≤0.34nmol/L showed a negative predictive value of 100%, with 61% of specificity. This combination could be useful for excluding AA in children admitted to the emergency department.

B-232

Tanner Stage-Stratified Pediatric Reference Intervals for Dihydrotestosterone

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Background: 5α-Dihydrotestosterone (DHT) is the most potent androgen hormone. It is generated in the body through reduction of testosterone by cholestenone 5α-reductase. Clinical utilities of DHT include workup of incompletely virilized males for 5α-reductase deficiency/pseudohermaphroditism, as well as monitoring of androgen action during androgen replacement therapy. A pediatric DHT reference interval study was conducted on 1502 well-characterized subjects, using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Children from 7-17 years old (748 males and 754 females) were recruited via community advertisements. Exclusion criteria included known medical conditions, medication use, or lack of parental consent. Physical exams, including Tanner staging, were completed by a single individual per sex, to reduce subjectivity. DHT values were generated using LC-MS/MS. Non-parametric reference intervals were established for each sex separately, using StatisPro. For both male and female reference intervals, the number of subjects in Tanner Stage 5 partition was less than 120 (and showed no significant difference in DHT with Tanner Stage 4), and was therefore combined with Tanner Stage 4 to allow nonparametric analysis.

Results: Based on Tanner Stage partitioning, the following proposed reference intervals were determined. (Table below)

Conclusion: Tanner Stage-specific DHT reference intervals largely overlap between males and females at Tanner Stage 1. However, at all other stages, the upper reference limit is substantially higher in males than females. Due to the significant differences observed between sexes and Tanner Stages, this study underscores the importance

of having a large number of healthy subjects to establish nonparametric reference intervals, and the developmental differences in DHT concentration.

Tanner Stage-specific DHT reference intervals						
Sex	Tanner Stage	Age	n	95% reference interval (pg/mL)	Lower reference limit 90% CI*	Upper reference limit 90% CI*
Male	1	7 - 12	276	1.0 - 49.4	0.4 - 1.6	40.3 - 94.7
	2	8 - 14	133	3.5 - 397.9	0.9 - 5.5	220.0 - 450.0
	3	12 - 18	135	14.8 - 574.6	10.1 - 31.3	468.0 - 791.0
	4 and 5	13 - 18	204	44.9 - 511.8	4.2 - 99.8	479.0 - 693.0
Female	1	7 - 15	294	1.0 - 64.3	0.8 - 1.4	53.5 - 104.0
	2	10 - 16	121	5.5 - 95.9	5.0 - 8.1	77.1 - 110.0
	3	11 - 18	133	11.4 - 158.3	7.3 - 20.0	139.0 - 208.0
	4 and 5	12 - 18	206	18.7 - 196.8	10.6 - 23.2	173.0 - 346.0
CI* = confidence interval						

B-233

Rapid Diagnosis of Niemann-Pick Type C patients with Plasma Colestane-3β,5α,6β-triol and 7-ketocholesterol by LC-ESI-MS/MS

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Background: Niemann-Pick Type C (NP-C) is a rare autosomal recessive lysosomal storage disorder caused by impaired intracellular transport of unesterified cholesterol and glycolipids due to mutations in either *NPC1* or *NPC2* gene. NP-C is usually underdiagnosed due to a variable age of onset and heterogeneous age-dependent clinical manifestations. Moreover, definitive diagnosis is based on genetic investigations which are time consuming and not always conclusive. Development of novel therapies for NP-C in recent years emphasized the urgent need for a reliable biomarker in early laboratory diagnosis. Recently, colestane-3β,5α,6β-triol and 7-ketocholesterol that result from non-enzymatic oxidation of cholesterol have been shown to be elevated in plasma of NP-C patients. We explored the usage of plasma colestane-3β,5α,6β-triol and 7-ketocholesterol as powerful diagnostic biomarkers for rapid diagnosis of NP-C. **Methods:** Immediately separated 50 µL plasma was sufficient for the analysis. Analyses were performed on a triple quadrupole mass spectrometer (Shimadzu 8040 LC-MS/MS, Japan) equipped with an ESI source and a reversed phase column after derivatization of oxysterols with dimethylglycine esters. Eight point calibrators and 3 levels of QC were used. Statistical analysis was performed with MEDCALC. **Results:** Both colestane-3β,5α,6β-triol and 7-ketocholesterol levels in NP-C patients were significantly elevated compared to healthy individuals. Mean colestane-3β,5α,6β-triol levels was 20.9±8.4 ng/mL and 7-ketocholesterol was 31.2±14.5 ng/mL for 70 healthy individuals. Mean colestane-3β,5α,6β-triol levels was 128.3±67 ng/mL and 7-ketocholesterol was 216.9±125.4 ng/mL for 8 NP-C patients. ROC analysis yielded AUC of 0.99 and 1.00 for colestane-3β,5α,6β-triol and 7-ketocholesterol, respectively. At the cut-off of 39 ng/mL, colestane-3β,5α,6β-triol demonstrated a specificity of 98.6% and a sensitivity of 100%. Both a specificity and a sensitivity of 100% at a cutt-off of 72 ng/mL was observed for 7-ketocholesterol. NP-C patients were confirmed with genetic analyses. **Conclusion:** Our data demonstrates that plasma colestane-3β,5α,6β-triol and 7-ketocholesterol fulfills the need of rapid and reliable biomarkers for NP-C.

Oxysterol levels in NP-C patients				
Genes	**Mutation**	**Consequence of Mutation**	**Colestane-3β,5α,6β-triol (ng/mL)**	**7-ketocholesterol (ng/mL)**
NPC1	c.3160G>A	A1054T	200	243
NPC1	c.1831-1836 delGATGAA/c.3734-3735delCT		190	425
NPC1	c.3067G>T	V1023F	40	83
NPC1	c.3557G>A	R1186H	61	135
NPC1	c.1123A>C	T375P	110	86
NPC1	c.1073-1073delT	L358R	97	153
NPC1	c.3019C>G/c.3246-3247delTG	P1007A	220	259
NPC2	c.352G>T	E118Stop	108	352

B-234

Marked influence of body mass index (BMI) on biochemical markers of the metabolic syndrome in the CALIPER cohort of healthy children and adolescents

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Background: Reference intervals (RI; i.e. normative values), essential to accurately interpret laboratory tests, are severely lacking in pediatrics, potentially causing erroneous interpretation and misdiagnosis. To address this critical gap, the CALIPER project developed a comprehensive database of over 100 age- and sex-specific pediatric RIs (www.caliperproject.ca). However, body mass index (BMI) is another key covariate that may significantly affect analyte levels. The objective of this study was to determine the effect of BMI on lipid/lipoprotein, inflammatory, and nutritional markers of the metabolic syndrome (MetS) in a healthy pediatric population. If unhealthy levels manifest early in overweight/obese children/adolescents, identifying and treating these patients early may help reverse damage due to adiposity and prevent future disease.

Methods: Lipid/lipoprotein, inflammatory, and nutritional MetS biomarkers were measured in the healthy CALIPER cohort (n=998 or n=681 depending on the analyte) using the Abbott Architect chemistry assays. Exclusion criteria included history of chronic illness or use of prescription medication. Children (2-<10 years) and adolescents (10-<19 years) were analyzed separately, with each sex analyzed separately for adolescents. Variables with a skewed distribution were log transformed to achieve normality. Analyte levels were compared between normal weight (NW), overweight (OW) and obese (OB) children (based on CDC classification) using one-way ANOVA and Bonferroni's Post Hoc test. Independent Sample T-Test determined differences between NW and OW/OB combined.

Results: OW/OB adolescent males but not females had elevated ALT and ferritin, and decreased HDL-C levels compared to NW subjects. Triglycerides, apoB, and CRP were elevated in OW/OB adolescents, although more pronounced in males. Vitamin B12, C3, and C4 were elevated in OW/OB adolescents compared to NW. In children, triglycerides were higher in OW/OB (1.55mmol/L ± 0.07) compared to NW (1.24mmol/L ± 0.03) individuals, $p<0.01$, and C3 was significantly higher in OW/OB (1.31g/L ± 0.02) compared to NW (1.12g/L ± 0.01), $p<0.01$.

Conclusion: Dyslipidemia in insulin resistant states increases the risk of developing cardiovascular disease (CVD) in type 2 diabetes (T2D). Increased triglycerides and apoB (marker of atherogenic lipoproteins) and decreased HDL-C in OW/OB adolescents suggests lipid abnormalities manifest early, prior to developing insulin resistance. Inflammatory proteins, C3, C4, and CRP were elevated in OW/OB adolescents, and C3 was elevated in OW/OB children, suggesting children/adolescents with an increased BMI are in a state of chronic low-grade inflammation. Vitamin B12 levels were lower in OW/OB adolescents suggesting poor nutrition as a result of an unbalanced diet lacking in micronutrients. Critical in homocysteine metabolism, low vitamin B12 levels could result in hyperhomocysteinemia and subsequent increased CVD risk. Ferritin, an acute phase reactant and marker of iron stores, was elevated in OW/OB adolescents, possibly identifying individuals at high risk of T2D. Thus, MetS marker levels are altered in apparently healthy OW/OB pediatric subjects, suggesting T2D and CVD risk factors can be monitored early to help prevent disease development. As such, RIs should either be partitioned by BMI or OW/OB subjects need to be excluded if increased levels predict disease development.

B-235

Reference Intervals of Diagnostic Tests for Mucopolysaccharidoses in Turkish Population

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Background: Mucopolysaccharidoses (MPSs) are a group of inherited lysosomal storage disorders caused by deficiency of lysosomal hydrolases required for glycosaminoglycan (GAG) catabolism. To date, 7 main types with subtypes of MPSs have been identified. Cumulative incidence for all MPSs is 1/25000. The incidence is estimated more in in Turkish population with a high rate (21%) of consangineous marriages. Owing to the great variability of clinical manifestations in patients, clinical diagnosis is difficult and definitive diagnosis is carried out by specific enzyme activity measurements in leukocytes or genetic investigations. Currently, laboratory diagnostic tests become prominent with the approval of enzyme replacement therapies (ERT) for some types of MPSs. The aim of this study was to establish reference values for gold standard diagnostic tests of MPSs as the most common inherited metabolic disorders in Turkish population.

Methods: Reference interval study was performed according to CLSI C28 guideline from a total number of 150 healthy volunteers in both gender under 18 years, 20-30 years, 30-40 years and over 40 years in equal numbers. Measurements of leukocyte specific enzyme activities were carried out with fluorometer (Molecular Devices SpectraMax M2 Microplate Reader, USA) by using fluorogenic substrates. The normality of distribution and extreme outliners were tested by D'Agostino Pearson's and Tukey method, respectively. Outliers and extreme values were shown by box-plot analyses. Statistical analyses were carried out with SPSS v.21.0 program.

Results: Reference intervals for MPSs types were shown in table. A total number of 41 MPS patients were diagnosed with leukocyte specific enzyme activity analyses (13 MPS III, 13 MPS IVA, 13 MPS VI, 2 MPS II patients).

Conclusion: Specific enzyme activity measurements from leukocytes are gold standard for the diagnosis of MPSs. Essential reference values for diagnostic tests of MPSs were determined in Turkish population with a high rate of the disorder.

Reference Intervals for MPSs			
MPS type	**Enzyme**	**Female**	**Male**
MPS II	Iduronate-2-sulfatase (nmol/4 h/mg protein)	7.9-52.5	9.7-55.6
MPS IIIB	N-acetyl-α-D-glucosaminidase (nmol/h)	4.9-19.3	4.6-21.3
MPS IVA	Galactose 6-sulfatase (nmol/17 h/mg protein)	46.3-330.6	40.9-323.2
MPS VI	Aryl sulfatase B (nmol/h/mg protein)	9.7-81.9	11.7-92.1
MPS VII	β-glucuronidase (nmol/h/mg protein)	19.3-171.8	18.5-180.5
Reference enzyme	β –galactosidase (nmol/h/mg protein)	70.8-343.1	63.4-375.1

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CALIPER Pediatric Reference Intervals for Ortho Vitros 5600 Immunoassays

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Background: Correct interpretation of laboratory test results requires accurately established reference intervals. As growth and development can profoundly influence circulating biomarker concentrations, the pediatric population requires unique reference intervals that are appropriately stratified by age and sex. The CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) program is a Canada-wide research initiative that has made considerable strides in addressing the gaps in pediatric reference intervals. This current study expands the CALIPER database by establishing covariate-stratified reference intervals for immunoassay-based tests on the Ortho Vitros 5600 System.

Methods: Healthy children and adolescents recruited from the Greater Toronto Area (GTA) and Hamilton regions as part of the CALIPER study completed a health questionnaire and provided a blood sample. Children with acute or chronic illness, or use of prescription medication within the past week were excluded. Several biomarkers were measured using the Ortho Vitros 5600 Immunoassay System, utilizing approximately 300-600 serum samples per assay. Analyte concentrations were visually inspected and statistically relevant age/sex-based partitions were determined. Outliers were removed using the Tukey or adjusted Tukey test for normally distributed and skewed data, respectively. Age- and sex-specific reference intervals with corresponding 90% confidence intervals were calculated using CSLI C28-A3 guidelines.

Results: AFP, prolactin, rubella IgG, and beta-hCG levels were fairly consistent over the pediatric age range, with no differences observed between sexes. Testosterone, progesterone, LH, FSH, ferritin, estradiol, and CEA all showed sex differences, the majority of which were seen after pubertal age. Folate and vitamin B12 both required age partitioning; however, no differences were seen between sexes.

Conclusion: Complex expression profiles were observed for several immunoassays, allowing calculation of age- and sex-specific reference intervals for the Ortho Vitros 5600 Immunoassay System. This will enable accurate diagnosis and laboratory assessment of children monitored by immunoassays run on this platform in healthcare institutions worldwide. However, we recommend further validation for the local pediatric population and specific analyzer prior to clinical implementation based on CLSI guidelines.

B-237

A Quantitative Mehtod for the Measurement of Dried Blood Spot Amino Acids using Ultra Performance Liquid Chromatography©

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Objective: Measurement of amino acids in dried blood spots has been extensively utilized for the detection of newborns with various inborn errors of amino acid metabolism including phenylketonuria (PKU) and maple syrup urine disease (MSUD). Whereas blood spot amino acid measurement has been invaluable for initial diagnosis, the relative insensitivity of blood spot measurement has found limited use in lifelong monitoring of patients with these disorders. We wanted to test if a blood spot assay was sufficiently sensitive to provide accurate monitoring of patients with amino acid disorders. The work described here outlines our evaluation of blood spot amino acids using ultra-performance liquid chromatography (UPLC).

Relevance: Most patients are currently monitored using plasma samples and measurement by ion-exchange chromatography, a process that can take up to two hours per sample. Many of the patients whom we monitor live several hours away from the hospital and find great inconvenience to travel this distance for routine monitoring purposes. We have previously found that UPLC can provide a more rapid turnaround time for analysis and routinely implement this procedure in our laboratory for plasma amino acid analysis.

Methodology: Plasma amino acids from dried bloods spots were obtained from patient samples and compared to the corresponding plasma measured using the UPLC methodology. Amino acids were extracted from dried blood spots by sonication in methanol. The eluent was dried and resuspended in 50uL of 50:50 acetonitrile:water before derivatization using 20uL of the reconstituted blood spot sample, 60uL 0.042mM norvalline in a borate buffer, and a proprietary reagent, AccQTag®. After incubation for 10 minutes at 55°C, the sample was loaded onto the UPLC with a Waters MassTrak AAA 2.1x150mm column. The derivatized samples were separated using UPLC with UV detection (260 nm) with a cycle time of 45 minutes per sample. To examine the stability of blood spots when exposed to changes in temperature, blood spots were exposed for 3 days at 4°C or 3 days at 65°C, followed by overnight storage at 4°C or room temperature.

Validation: 318 samples were collected for this study. Intra- and inter-assay imprecision (mean CVs) for alloisoleucine, leucine, isoleucine, valine, phenylalanine, and tyrosine ranged from 0.1% to 4.3% and 4.2% to 20.2%, respectively. Recoveries were lower at high levels, an observation that was not previously appreciated.

Results and Conclusions: For phenylalanine and tyrosine, dried blood spot analysis had a very slight negative bias, resulting in lower concentrations of phenylalanine and tyrosine compared to plasma amino acid analysis. For valine, leucine, isoleucine, and alloisoleucine, dried blood spot analysis had a moderate negative bias, resulting in lower concentrations of these amino acids as compared to analysis using plasma amino acids. The results of this study demonstrate that the blood spot filter papers are stable despite temperature and humidity changes, and demonstrate less bias when stored at room temperature before testing. This UPLC based method can reliably measure significant amino acids in dried blood spots and finds the method to be sufficiently sensitive for accurate long-term monitoring of patients with amino acid disorders.

B-238

Pediatric reference intervals for 1,25-dihydroxyvitamin D in the CALIPER Cohort of Healthy Children and Adolescents

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Background: 1,25 dihyroxyvitamin D ($1,25(OH)_2D$) is the most biologically active metabolite of vitamin D. $1,25(OH)_2D$ is essential to childhood growth and development and plays a role in calcium homeostasis and bone growth. Despite its importance, no $1,25(OH)_2D$ reference interval exists for the pediatric population. Traditional $1,25(OH)_2D$ assays require complex manual preparation, however Diasorin has developed a new, fully automated *in vitro* chemiluminescent immunoassay (CLIA) to measure $1,25(OH)_2D$ levels, requiring no sample pretreatment or preparation. In alignment with CALIPER [Canadian Laboratory Initiative for Pediatric Reference Intervals], we aimed to establish age- and sex-specific reference intervals.

Methods: 405 blood samples were collected from apparently healthy children and adolescents aged 0-18 y. Those aged 1-18 y were from the CALIPER cohort, while those aged 0-1 y were Mount Sinai Hospital outpatient samples. $1,25(OH)_2D$ levels were measured using Diasorin Liaison XL, an *in vitro* non-competitive three step sandwich CLIA. Statistical analysis was performed using R software, in accordance with CLSI C28-A3 guidelines. Age- and sex-specific reference intervals with corresponding 90% confidence intervals were calculated.

Results: There was a significant age-dependent decline in $1,25(OH)_2D$ levels over the first few years of life requiring data partitioning and calculation of reference values for three age groups: 0-<6m, 6m-<3y, and 3-<19y (shown in Table 1). Sub-analysis did not suggest a seasonal effect on $1,25(OH)_2D$ levels in our study group (p=0.364 based on the Mann Whitney U-Test) and sex-partitioning was not necessary.

Conclusion: This study provides, for the first time, robust pediatric reference intervals for the $1,25(OH)_2D$ Diasorin Liaison assay and will improve the accuracy of pediatric test result interpretation for this active form of vitamin D.

Age- and sex-specific pediatric reference intervals for 1,25-dihydroxyvitamin D

Age	Sample Size	Lower Limit	Upper Limit	Lower Confidence Interval	Upper Confidence Interval
0-<6 months	68	92	416	(76,111)	(385,453)
6 months - <3 years	121	104	439	(101,113)	(342,460)
3 -< 19 years	185	108	246	(104,110)	(225,355)

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Comparison of Cord Blood Gas Values and Sampling Errors Pre-and Post-Universal Collection

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Background

Intrapartum asphyxia can lead to fetal metabolic acidosis which, if severe and prolonged, can result in damaging acidosis and encephalopathy. Analysis of acid-base status in umbilical cord (UC) blood can provide valuable information on the metabolic condition of neonates at birth, particularly when fetal distress is suspected during delivery. The umbilical vein and artery are located in close and intertwined proximity so analyses should be performed on both the UC artery and vein to ensure correct sampling. Oxygenated venous blood (from placenta to fetus) should have higher pH and lower pCO2 compared to the deoxygenated arterial blood (from fetus to placenta). The newborn is at higher risk of complications if the umbilical artery pH is <7.0 and base deficit ≥ 12 mmol/L. Prior to October 2014, our institution collected and performed blood gas analysis on cord blood samples only upon physician request. To improve the process for UC sample collection and avoid collection and transport errors resulting in unacceptable specimens or misleading results, a universal collection protocol was implemented in October 2014 requiring blood gas analysis on all infants at birth. We studied the impact of universal cord blood collection on the distribution of pH and base excess values, and the rates of unacceptable samples.

Methods

UC blood gas results were collected retrospectively from 9 months before (n=678) and 6 months after (n=2415) universal collection was implemented. The percentages of births involving severe metabolic acidosis (pH < 7.0 and/or base deficit ≥ 12 mmol/L) in the pre- vs. post-universal screening cohorts were compared. Data from each time period was evaluated for differences between arterial and venous cord sample pH and pCO2 results to assess accuracy of sample collection. Additionally, we calculated test cancellation rates presumably due to collection issues.

Results

Severe metabolic acidosis (pH < 7.0 and/or base deficit ≥ 12 mmol/L) was observed in 15/678 (2.2%) of samples collected pre-universal screening and 16/2415 (0.7%) post-universal screening. Four samples (0.6%) in the pre cohort and one sample (0.0004%) in the post cohort met both criteria for acidosis.

In the pre-universal collection cohort, 127/678 (18.7%) samples required cancellation, most often due to insufficient quantity or clotted sample. In the post-protocol cohort, 140/2415 (5.8%) required cancellation.

As an indication of correct source sampling, 219 of the 251 (87.3%) paired arterial/venous UC samples received pre-protocol had a difference in pH of >0.02, and 217 pairs (86.5%) had a difference of >4

mmHg pCO2 between the arterial cord- and venous cord-labeled samples. After universal collection protocol, 1027/1073 pairs (95.7%) showed a difference in pH of >0.02 and 952/1073 (88.7%) showed a difference in pCO2 >4 mmHg.

Conclusion

Collection technique (and presumably correct sample handling and transport) and correct source sampling improved after implementing universal umbilical cord blood acid-base screening as evident by a decrease in the frequency of sample cancellation and an increase in paired samples with appropriate differences in pH and pCO2. Implementation of universal cord blood screening decreased collection and sample handling errors, but did not appear to increase detection of severe metabolic acidosis in neonates.

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A Technique to Enhance the Stability of a Pediatric Bilirubin Control

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Background: Bilirubin is a degradation waste product of hemoglobin, released during the breakdown of Red Blood Cells. Through a series of enzymatic steps, the heme in hemoglobin is catabolized to bilirubin which is transported to the liver where it becomes conjugated to glucuronic acid. Conjugated bilirubin is excreted from the liver in bile into the intestines where bacteria convert it into urobilinogen to be excreted by the kidneys as urobilin or in the feces as stercobilin. Bilirubin circulates in the blood stream as indirect (unconjugated) and water-soluble direct (conjugated) forms. Current clinical assays can measure total bilirubin (TBIL) and direct bilirubin (DBIL).

Elevated levels of bilirubin in the blood are clinically significant and often result in jaundice, a yellowish discoloration of the skin and eye, and may indicate various hepatic conditions, gallstones, cancer, hemolytic anemia or hepatotoxic drugs. Elevated bilirubin in newborns is a medical emergency due to the immaturity of the blood-brain barrier which may lead to irreversible brain damage. Bilirubin is very sensitive to light and oxygen and quality control materials have notoriously short open vial stability limitations, primarily due to exposure to atmospheric oxygen upon opening the vial. A technique to remove control sample without subjecting it to excessive oxygen would be advantageous to preserve the stability of the control.

Objective: To evaluate the use of a syringe and needle technique to enhance the stability of a pediatric bilirubin control.

Methods: The Quantimetrix Pediatric Bilirubin control, lots 33491 and 33501, Levels 1 and 2, were subjected to an 18 day stability protocol. In one arm, vials were uncapped and a pipette was used to remove 150µL sample before assaying TBIL and DBIL in duplicate on the Siemens Dimension® ExL. In the second arm, vials remained sealed and a 1cc syringe with 18 gauge needle was used to remove the 150µL sample through the rubber stopper. The samples were tested in this manner 9 times over an 18 day period. A linear regression was used to determine the day to failure (DTF) using ±10% cutoff.

Results: For lots 33491 and 33501 lots, the DTF for Level 1 DBIL open vial arm was extrapolated to 22.2 and 26.3 days and the syringe arm to 61.5 and 69 days respectively. The DTF for Level 2 DBIL open vial arm was extrapolated to 42.7 and 61.5 days and the syringe arm to 61 and 64 days respectively. TBIL values showed no appreciable degradation.

Conclusion: Level 1 DBIL values were most affected by oxidation from exposure to atmospheric oxygen. Using a syringe resulted in a marked increase of stability of about 24 to 65 days. This trend was less apparent in Level 2, where using the syringe resulted in an increase of about 52 to 63 days. TBIL values were virtually unaffected by this open vial protocol, actually showing a slight upward trend. Interestingly the current open vial stability claim for this Quantimetrix control is only 5 days while this data set shows excellent stability over the 18 day period across both arms.