
 Wednesday, July 30, 2014

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

Pediatric/Fetal Clinical Chemistry

B-255**Increased expression of aquaporin 9 in placenta from pregnant women with gestational diabetes**

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Background: Leptin is expressed in human placenta acting as an autocrine signal for the trophoblast. In fact, leptin is now considered an important regulatory signal in fetoplacental physiology. Placenta leptin expression is increased in pathological pregnancies, such as gestational diabetes, and it may play a role in the overgrowth of placenta, which supplies a growing fetus with nutrients and water. This function of placenta is partly based on the expression of aquaporins/glyceroporins, such as aquaporin 9 (AQP9).

The aim of this study was to analyse the expression of AQP9 in placenta from gestational diabetes compared with that from normal pregnancy. In addition, we studied the in vitro effect of leptin on the AQP9 expression by trophoblast explants from control pregnancies, in order to check the possible leptin regulation of AQP9 expression in human placenta.

Methods: We collected 30 placentas (20 from control pregnancy and 20 from gestational diabetes), after caesarean delivery at term. The expression of AQP9 was determined by quantitative RT-PCR, immunoblot and immune histochemistry. In addition, explants from control placenta were incubated in vitro with increasing concentrations of leptin (0-100 nM) during 6 h and AQP9 expression was also measured. Mean values were analysed by ANOVA followed by Bonferroni's post test.

Results: We have found that AQP9 expression was significantly increased by 30% in placenta from gestational diabetic pregnancy. Western blot confirmed the increased protein level of AQP9 in placenta from gestational diabetic women. Immune histochemistry demonstrated the localization of the overexpressed AQP9 in the syncytiotrophoblast. In vitro incubation of trophoblast explants with increasing concentrations of leptin demonstrated the dose-dependent effect of leptin on the expression of AQP9, with a maximal effect at 10 nM increasing three-fold the basal leptin expression.

Conclusions: Data show that AQP9 expression is increased in placenta from pregnant women with gestational diabetes and this may contribute to supply more nutrients to the fetus. The increased expression of AQP9 in gestational diabetes may be mediated at least in part by leptin, since leptin levels are known to be increased in gestational diabetes and we have found that leptin stimulates the expression of AQP9 in vitro. In conclusion, we have shown that leptin positively regulates the expression of AQP9 in the trophoblast and this effect may mediate the observed increase in AQP9 expression in trophoblast from pregnant women with gestational diabetes.

B-257**Absence of eosinopenia as predictor of procalcitonin < 2 ng/mL in pediatric patients**

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BACKGROUND: The role of eosinopenia as a marker of sepsis has recently been evaluated. The aim of our study was to assess whether eosinophilia was a reliable criteria to predict procalcitonin (PCT) values under 2 ng/mL in pediatric patients presenting to the emergency department to rule out sepsis.

METHODS: From January 2012 to December 2013, a total of 605 consecutive children aged 3 to 36 months (median: 16.5 m; 46.6% male) were enrolled in the study. Procalcitonin levels and eosinophil counts were measured on admission. PCT was measured with a two-step two-site sandwich electrochemiluminescence immunoassay (ECLIA) on the ROCHE cobas e 411 system. An undetectable eosinophil count (<0.01 x 10⁹) or <10/mm³ was considered as eosinopenia.

RESULTS: The absolute eosinophil count in the study population was 30/mm³

(median, range 0-1420). The average value of eosinophils in the group with PCT <2 ng/mL was 103/mm³, versus 28/mm³ in the group with PCT > 2 ng/mL (p <0.01). At a cut-off value of <=0/mm³, the eosinophil count yielded a sensitivity of 51.4% and a specificity of 83.6%, a positive predicted value (PPV) of 17.2% and a negative predicted value (NPV) of 96.3%, with an area under the curve of 0.711 (95% CI: 0.674-0.747). At a cutoff value of 120/mm³, the NPV of the absolute eosinophil count was 98.6%, thus allowing to rule out a PCT >2 ng/mL value in 21.4% (141/568) of children.

CONCLUSION: The absence of eosinopenia can be considered as an inexpensive warning test for predicting PCT <2 ng/mL in pediatric patients. The presence of an absolute eosinophil count > 120/mm³ allows to rule out a PCT >2 ng/mL, thus avoiding PCT determination in 21.4% of children, which is of interest especially in the context of countries with limited resources

B-258**Neonatal umbilical cord blood cardiac troponin as reflecting fetal growth, age and well-being.**

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Background: The advent of highly sensitive assays for cardiac troponin have made possible investigations to better understand the biology and pathophysiology of these cardiac markers. The aim of the current study was to document the umbilical cord blood concentrations of troponins I (cTnI) and T (cTnT) using high sensitivity assays and correlate these with maternal and fetal clinical history.

Methods: Umbilical cord blood was collected immediately following delivery from 416 babies, including 12 sets of twins. Cardiac troponins were assayed using hs-cTnI on Abbott Architect and hs-cTnT on Roche E4111. Clinical history was obtained from clinical notes. Ethics permission was obtained from ACT Health Human Research and Ethics Committee for the study and consent was obtained from mothers for their participation.

Results: All results were above LoD for both assays (1.0 ng/L cTnI; 5.0 ng/L cTnT). Minimum cTnI concentration was 1.2 ng/L (median 6.9n/L; Q1 4.5, Q3 12.1), cTnT 7.0ng/L (Q1 27.1, Q3 51.0). Troponins were statistically significantly correlated [cTnT=21.45 ln(cTnI)-0.82; R² = 0.3418, P<0.001]. Babies who were born before 32 weeks gestation (n=17) had higher median cTnI of 14.7ng/L (Q1 6.7, Q3 22.7) and cTnT of 58.0 ng/L (Q1 56, Q3 102) compared with those born after 41 weeks gestation [n=24, median cTnI 6.0ng/L (Q1 4.5, Q3 10.43); cTnT of 31.2 ng/L (Q1 23.0, Q3 48.6). Babies with the highest cTnI (>48 ng/L, >95%ile) had markedly elevated cTnT (n=6, median 197 ng/L, range 151-297).

Conclusion:

The relationship between cTn and current objective measures of fetal well-being are assessed to determine whether cTn measurement in this clinical setting is of value.

B-259**Dynamic changes in circulating amino acids and acylcarnitines in children and adolescents: A CALIPER study of healthy community children and new pediatric reference intervals**

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Objective: An up-to-date and comprehensive database of pediatric reference intervals is essential for the accurate management of children with metabolic disease. The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) program has established pediatric reference intervals from healthy community children for a comprehensive list of common biomarkers relevant to the diagnosis and management of inborn errors of metabolism (IEM).

Methods: Healthy children and adolescents from birth to 19 years were recruited based on informed parental consent. A cohort of over 500 healthy individual samples was used to calculate pediatric reference intervals for 36 acylcarnitines (LC-MS/MS) and 37 amino acids (Waters MassTrak amino acid analyzer). Over 100 healthy individual samples were focused in the 0 - 2 week age range to ensure reliable reference intervals were established in the newborn period. Reference intervals were calculated using non-parametric statistics according to CLSI-C28-A3 and partitioned based on age and sex.

Results: The majority of analytes demonstrated reference intervals requiring 2 - 4 age dependent partitions, with the most common being within the newborn period of 0 - 2 weeks. Also, several analytes displayed a unique reference interval during puberty, some of which demonstrated sex-based partitions. These were often related to energy metabolism and growth in the muscle. Finally, the minority of analytes demonstrated one reference interval for all ages and sexes.

Conclusions: The reference intervals established here for acylcarnitine and amino acid profiles will aid in the accurate diagnosis and monitoring of children suspected of IEM. Furthermore, reference intervals extending to 19 years of age will aid in the management of children and adolescents treated for metabolic disease. Importantly, these reference intervals are established for the indicated instrumentation and should be validated on other platforms and for local populations as recommended by CLSI.

Table 1. Pediatric reference intervals for biomarkers used in children with suspected metabolic disease. (BHB-beta-hydroxybutyrate).

Analyte	Age	Number of samples	Lower limit (95% CI)	Upper limit (95% CI)
Valine (umol/L)	0-<2wk	145	92 (73-96)	326 (299-349)
	2wk-<13yr	253	126 (123-139)	356 (339-364)
	13yr-<19yr	♂ : 46	166 (157-179)	301 (289-309)
		♀ : 51	155 (145-162)	259 (246-269)
BHB (mmol/L)	0-<1yr	91	0 (0-0)	1.73 (1.16-2.13)
	1yr-<19yr	219	0 (0-0)	0.12 (0.11-0.13)
Total carnitine (umol/L)	0-<1yr	67	21 (17-26)	78 (74-81)
	1yr-<12yr	136	28 (27-31)	61 (55-74)
	12yr-<19yr	♂ : 53	32 (30-33)	59 (57-61)
		♀ : 56	24 (21-27)	53 (50-54)

B-260

Closing the gaps in pediatric population reference values for cancer biomarkers: A CALIPER study of healthy community children

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Background: The CALIPER (Canadian Laboratory Initiative in Pediatric Reference Intervals) program, a national research initiative aimed at closing the gaps in pediatric reference intervals, sought to develop a database of covariate-stratified reference value distributions for 11 key circulating tumor markers including those used in assessment of patients with childhood or adult cancers.

Methods: Healthy community children from birth to 18 years of age were recruited to participate in the CALIPER project with informed parental consent. Participants completed questionnaires and were assessed according to established inclusion and exclusion criteria. Serum samples from approximately 400-700 children were analyzed on the Abbott Architect ci4100 and reference intervals were established for Alpha-Fetoprotein (AFP), Ant-Thyroglobulin (Anti-Tg), Human Epididymis Protein 4 (HE4), Cancer Antigen 125 (CA125), Cancer Antigen 15-3 (CA15-3), Cancer Antigen 19-9 (CA19-9), Pro Gastrin-Releasing Peptide (ProGRP), Carcinoembryonic Antigen (CEA), Squamous Cell Carcinoma Antigen (SCC), as well as Total and Free Prostate Specific Antigen (PSA) according to CLSI C28-A3 statistical guidelines.

Results: Significant fluctuations of biomarker concentrations by age and/or gender were observed in 10 of 11 biomarkers investigated. Results for three of the markers examined (CA19-9, CA125 and SCC) are shown in Table 1. Age partitioning was required for CA15-3, CA125, CA19-9, CEA, SCC, ProGRP, Total & Free PSA, HE4 and AFP while gender partitioning was also required for CA125, CA19-9, Total & Free PSA.

Conclusion: The establishment of pediatric reference intervals for tumor biomarkers will not only aid in harnessing the full potential of tumor markers in a pediatric population but also in research aimed at determining the value of tumor marker use in various cancers.

Age- and Sex-Specific Pediatric Reference Intervals for 3 Tumor Biomarkers

Analyte	Age	Male Reference Interval	No. Of Samples	Confidence Interval	Female Reference Interval	No. Of Samples	Confidence Interval
CA19-9	0 - < 1 year	< 2.00, 54.40	59	< 2.00, (46.39, 58.90)	< 2.00, 37.6	56	< 2.00, (31.04, 42.63)
	1 - < 14 years	< 2.00, 27.40	172	< 2.00, (22.80, 32.60)	< 2.00, 27.40	172	< 2.00, (22.80, 32.60)
	14 - < 19 years	< 2.00, 12.02	66	< 2.00, (10.32, 13.13)	< 2.00, 12.02	66	< 2.00, (10.32, 13.13)
CA125	0 - < 4 months	2.4, 22.2	56	(2.0, 3.0), (20.2, 24.4)	2.4, 22.2	56	(2.0, 3.0), (20.2, 24.4)
	4 months - < 5 years	7.0, 32.6	191	(4.9, 8.0), (31.6, 35.3)	7.0, 32.6	191	(4.9, 8.0), (31.6, 35.3)
	5 - < 11 years	4.7, 28.5	174	(4.4, 5.8), (24.4, 29.4)	4.7, 28.5	174	(4.4, 5.8), (24.4, 29.4)
	11 - < 19 years	5.5, 27.7	122	(5.4, 6.5), (23.5, 30.2)	5.5, 39.1	128	(3.9, 6.7), (30.4, 41.0)
SCC	0 - < 1 week	> 70.0	44	> 70.0	> 70.0	44	> 70.0
	1 week - < 1 year	0.4, 6.7	114	(0.4, 0.5), (5.6, 7.9)	0.4, 6.7	114	(0.4, 0.5), (5.6, 7.9)
	1 - < 8 years	0.4, 1.7	81	(0.3, 0.5), (1.6, 1.8)	0.4, 1.7	81	(0.3, 0.5), (1.6, 1.8)
	8 - < 19 years	0.5, 2.1	142	(0.4, 0.5), (2.0, 2.2)	0.5, 2.1	142	(0.4, 0.5), (2.0, 2.2)

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Sex-based differences in gestational age at lung maturity as determined by lamellar body counts

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Introduction: In late gestation, as fetal lungs prepare to transition to an air environment, alveolar epithelial cells called type II pneumocytes synthesize and store a mixture of phospholipids and proteins known as pulmonary surfactant. Around week 32 of gestation, surfactant release into the amniotic fluid begins in the form of structures called lamellar bodies (LBs). Adequate surfactant is an important predictor of fetal lung maturity, especially in cases of prematurity.

Lamellar bodies can be quantified in amniotic fluid using the platelet channel of automated hematology analyzers due to similarity in size. Inadequate surfactant production is associated with respiratory distress syndrome, which is more likely to occur in patients with lower lamellar body counts (LBCs).

Respiratory distress is more common in male late preterm infants than in females, possibly related to sex-based differences in lung maturity. Female fetuses have demonstrated earlier development of lung maturity than males in previous studies using other markers of lung maturity, such as the lecithin/sphingomyelin ratio, presence of phosphatidylglycerol, and loss of phosphatidylinositol; however, sex differences in lamellar body counts have not been assessed in the literature. This study aimed to determine if female fetuses demonstrate evidence of lung maturity using LBCs at an earlier gestational age than males.

Methods and Analysis: The population for this retrospective cohort study included all pregnant women who had amniocentesis with LBC analysis on the Advia Hematology System at our institution from 2003-2012 and subsequently delivered within 72 hours. Data were collected from our laboratory database of amniotic fluid LB counts. Gestational age at the time of the amniocentesis, fetal sex, and additional demographic and outcome data were collected from medical records. Lung maturity was defined as a LB count >35,000. Linear regression analysis of the data was done using Microsoft Excel, with statistical analysis performed by analysis of covariance (ANCOVA) to evaluate the relationship between gestational age, lamellar body count, and sex.

Results: 263 deliveries were included for analysis, with 128 female (49%) and 135 male (51%) infants. Gestational ages ranged from 30-41 weeks and lamellar body counts from 4332 to >200,000. Lung maturity with LBC>35,000 was demonstrated in 105/128 female (82%) and 107/135 male (79%) infants. The regression line of LBC versus gestational age for females was y=10188x-311669, with r=0.47, and for males it was y=10391x-317262, with r=0.47. The two lines did not differ significantly in either slope (p=0.93) or intercept (p=0.95).

Conclusion: Our study did not demonstrate a significant difference in gestational age at lung maturity by fetal sex as measured with LBCs. These results differ from prior data showing a higher degree of lung maturity in female than male infants using other indices, despite larger sample sizes in our study. Broad inclusion criteria and relatively wide scatter of data may have contributed to the non-significant results. Sex differences in LBCs were not found to explain increased prevalence of neonatal respiratory distress in males.

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Pediatric reference intervals for 29 endocrine and special chemistry biomarkers on the Beckman Coulter DxI Immunoassay System: A CALIPER study of healthy community Children

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BACKGROUND: Appropriate interpretation of laboratory test results requires carefully established reference intervals based on a healthy population. Growth and development can markedly influence circulating concentrations of biomarkers, so accurate reference intervals established on the basis of a healthy pediatric population are essential for test result interpretation. The CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) program, a national research initiative aimed at closing the gaps in pediatric reference intervals, sought to develop a database of covariate-stratified reference intervals for endocrine and special chemistry markers on the Beckman Coulter DxI Immunoassay System.

DESIGN AND METHODS: Healthy children and adolescents were recruited as part of the CALIPER study. After informed parental consent was obtained, participants filled out a questionnaire including demographic information and provided a blood sample. We measured 29 proteins using the Beckman Coulter DxI Immunoassay System utilizing 443 - 636 samples per assay. The variance, age- and sex-specific concentrations of analytes were visually inspected from scatterplots of protein concentration as a function of age for both genders. Pediatric reference intervals were calculated according to Clinical Laboratory Standards Institute (CLSI) C28-A3 guidelines. Partitions based on age and/or sex were determined and statistically evaluated using the Harris and Boyd method. After removal of outliers, reference intervals were calculated using the non-parametric rank method, with values ranked and the 2.5th and 97.5th percentiles calculated.

RESULTS: We observed a complex pattern of change in most protein concentrations from the neonatal period to adolescence. The changes in concentration observed for each of the examined proteins were classified into 1 of 5 categories: (a) high variance and high concentration within the neonatal period that decreases abruptly shortly after birth: AFP, ferritin and prolactin; (b) high variance at birth that is significantly reduced around 1 year of age: free T4 and thyroglobulin (c) high variance and high concentration within the neonatal period that decrease gradually with age: SHBG and vitamin B12; (d) high variance at birth that decreases abruptly around 1 year of age and increases again in adolescence: cortisol, DHEAS, folate, testosterone (males), FSH (especially females) and LH (especially females); and (e) constant variance throughout life but variable concentration according to age: free T3 and total T4. Estradiol (and progesterone to a lesser extent) concentrations and variance were low from birth, then increased in females during adolescence. Insulin increased slightly with age for both genders. Ostase (bone-specific alkaline phosphatase) displayed constant variance and concentration, with a sharp decrease in adolescence, especially in females.

CONCLUSIONS: This study shows the complex expression profiles of several endocrine and special chemistry biomarkers as a function of age and gender. This allowed the calculation of age- and sex-specific reference intervals for 29 endocrine and special chemistry markers, specific to the Beckman Coulter DxI Immunoassay System. The completion of this study will aid in accurate diagnosis and laboratory assessment of children being monitored by Beckman Coulter immunoassays in healthcare institutions worldwide. It is however important that these reference intervals be validated for the local pediatric population as recommended by CLSI.

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Using the clinical laboratory's database for indirect estimation of the reference intervals of serum creatinine.

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Background: Reference intervals (RI) age-specified of serum creatinine are important for triage, diagnostics and monitoring of chronic kidney disease. Ideally, RIs must be determined by sampling a healthy population. There are two methods of sampling: a direct one and an indirect one. The direct method, that follows the recommendations of CLSI and IFCC, involves the selection of reference individuals. The aim of this study is to estimate the RIs of serum creatinine through the indirect sampling method, suggested by Horn.

Methods: Transversal study conducted in 44.592 individuals, ages between 1 and 74, both genders, which performed serum creatinine (enzymatic method) in Advia 2400

equipment, in a private laboratory, between September 2013 and February 2014. The statistics included Box-Cox transformation, Tukey's test, Kolmogorov-Smirnov test and estimation of RI by non-parametric method of the percentiles. The confidence interval was determined as 90% for the reference limits (percentile 2.5 and 97.5). The statistics were calculated by MedCalc software.

Results: Tukey's test was used in the transformed data and it identified 2.39% of individuals as outliers. All results considered as outliers were excluded from the original data. The RIs were determined by non-parametric method of the percentiles of residual data (Table 1).

Table 1. Reference intervals for creatinine concentrations in serum (mg/dL) calculated with a nonparametric method.

Age group	n	Males (%)	outliers	Lower limit	Upper limit
				2.5 percentil (90% CI)	97.5 percentil (90% CI)
1 to < 3 years	347	48.86	5	0.19 (0.18 to 0.20)	0.38 (0.36 to 0.40)
3 to < 5 years	417	51.86	13	0.26 (0.25 to 0.26)	0.46 (0.44 to 0.50)
5 to < 7 years	434	44.92	9	0.30 (0.29 to 0.31)	0.53 (0.51 to 0.55)
7 to < 9 years	496	40.76	7	0.33 (0.32 to 0.34)	0.58 (0.56 to 0.60)
9 to < 11 years	501	36.40	10	0.37 (0.35 to 0.37)	0.62 (0.61 to 0.62)
11 to < 13 years	482	37.37	13	0.41 (0.41 to 0.42)	0.71 (0.69 to 0.72)
13 to < 15 years	613	37.04	8	0.46 (0.44 to 0.47)	0.86 (0.83 to 0.89)
Women (18 to < 75 years)	25536	n.a.	600	0.50 (0.50 to 0.50)	0.94 (0.94 to 0.95)
Men (18 to < 75 years)	14700	n.a.	401	0.70 (0.69 to 0.70)	1.25 (1.24 to 1.25)

Conclusion: The direct method of sampling is preferred. However, the difficulty to obtain a representative number of reference individuals, especially for pediatric population, can be overcome by using the indirect method. This method demonstrated to be an excellent alternative method to determine the RIs, which for serum creatinine are compatible with the main description in the literature.

B-264

A Urine-based Immunoassay for Urocortin 3 and Diagnosis of Sleep Apnea

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Introduction: Obstructive sleep apnea (OSA) affects 2-3% of children in the US and is characterized by obstruction of the upper airway during sleep resulting in disruption in ventilation, hypoxemia, and sleep fragmentation. Children suffering from OSA are more likely to develop behavior difficulties, learning disabilities, pulmonary/systemic hypertension and decreased growth. Currently, the gold standard for diagnosing OSA is an overnight sleep study which is labor intensive, limited by availability and expensive. Development of a non-invasive test using a urine biomarker would provide an inexpensive and more accessible test to diagnose OSA. In a previous study, proteomic analysis by mass-spectrometry revealed a 4 kilo Dalton peptide in urine which was significantly increased in children with OSA compared to controls (Pub Med #). The peptide was identified as the stress-coping peptide Urocortin 3 (UCN3); 38 amino acids in length and is expressed in kidney tubules, heart and brain. UCN3 is involved in modulating stress responses, osmoregulation, and in regulating the hypothalamus-pituitary-adrenal axis. The objective of this present study was to develop a urocortin 3 (UCN3) two-site immunometric assay for eventual use as a rapid non-invasive diagnostic screening tool for OSA in children

Methods: To develop monoclonal and polyclonal antibodies targeting the mature human peptide, we immunized mice and rabbits with synthetic UCN3 constructs conjugated with KLH. Following conventional prime and boost immunization strategies, the mice were sacrificed, the spleens were harvested and immortalized. ELISA was used to identify the clones with reactivity for unconjugated UCN3. Full length UCN3 and UCN3 fragments were coated in 96-well plates and assayed with hybridoma supernatants (neat and 1:10 dilution) to determine reactivity. The ideal clones were defined as those that yielded the highest signal to full length UCN3 and to either the N-terminal or C-terminal half of the peptide. All of the antibodies were affinity purified and used to develop a two-site immunometric assay. Antibody pairs were identified using checkerboard ELISA and affinity characterization using Biacore (GE life science). The highest affinity mAb was used for antigen capture and the polyclonal was used for detection.

Results: Analysis of the clones yielded twenty hybridomas reactive towards UCN3, with five clones exhibiting absorbance >1.0 to full length and either the N- or C-terminus of UCN3. Checkerboard and Biacore analysis using these five clones and an anti-UCN3 polyclonal antibody (pAb) identified that mAb 4D3 was the ideal capture antibody and the anti-UCN3 pAb as the detection antibody. These antibodies generated an assay with a linear range from 100 to 3100 ng/mL, an intra-assay CV of 2.7 and 3.7% (N=2) at 100ng/ml and 3160ng/ml, respectively. Recovery of UCN3 spiked into urine was 127% at 100 ng/mL and 100% at 3100 ng/mL (N=3).

Conclusions: The set of monoclonal and polyclonal antibodies developed provides a Urocortin 3 ELISA-based assay with analytical characteristics suitable for subsequent studies to test the clinical predictive value measuring UCN3 in urine for the diagnosis of OSA in pediatric subjects.

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CLSI-based transference of the CALIPER database of pediatric reference intervals to Beckman Coulter Clinical Chemistry Assays

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OBJECTIVES: Reference intervals represent the range of results that are commonly observed in a population of healthy individuals. These intervals are defined as the range that encompasses the central 95% of the distribution of test results from reference individuals sampled from a healthy reference population. Comparison of a given test result to an appropriate reference interval enables proper clinical assessment. Accurate pediatric reference intervals obtained in healthy community children are essential for the accurate diagnosis and management of diseases in children. The CALIPER program has established a comprehensive database of age- and sex-stratified pediatric reference intervals for over 70 common biochemical markers, proteins, lipids, and enzymes, as well as endocrine markers and fertility hormones. However, this database was only directly applicable for assays performed on the Abbott ARCHITECT ci4100 system. We therefore sought to expand the scope of this database to biochemical assays performed on the Beckman Coulter Synchron DxC800 chemistry platform, allowing for a much wider application of the CALIPER database.

DESIGN AND METHODS: Based on CLSI C28-A3 and EP9-A2 guidelines, CALIPER pediatric reference intervals were transferred to Beckman Coulter chemistry assays performed on the Synchron DxC800 instrument, using specific statistical criteria. First, 200 pediatric pooled patient serum specimens were analyzed on the Abbott ARCHITECT ci4100 and the Beckman Coulter DxC800, and the data was subjected to regression analysis, standardized residual, Bland-Altman, and quantile-quantile (Q-Q) plots. A total of 34 chemistry, enzyme, lipid/lipoprotein and protein markers were assessed. Regression analysis was performed to determine correlation of values between analytical systems, resulting in determination of R2 for each analyte. Analytes with R2 values equal to or greater than 0.95 were deemed transferable to the Beckman Coulter DxC800 platform. To assess the validity of the transferred reference intervals, 100 serum samples from the CALIPER cohort (healthy community children) were assayed on the Beckman Coulter DxC800 and validation was assessed based on CLSI C28-A3 criteria.

RESULTS: Most (22) of the analytes showed good correlation between the Abbot and Beckman systems with R2 values equal to or greater than 0.95. Ten analytes showed strong correlation, with R2 values between 0.77 and 0.94. Two analytes (carbon dioxide and calcium) showed poor correlation, and were not transferable to Beckman Coulter DxC800. Most transferred reference intervals determined using the Beckman system were validated through the analysis of CALIPER reference samples.

CONCLUSIONS: The current study allows successful transference of a large number of routine and special chemistry markers from the CALIPER database to the assays on the Beckman Coulter DxC800 analytical systems. This will greatly extend the utility of validated CALIPER reference intervals which will be directly applicable for assays performed on the Beckman Coulter DxC800 chemistry platform. Validation of the reference intervals should facilitate the broad application of CALIPER reference intervals at pediatric centers worldwide.

B-266

Development of a Pregnant Subject Biospecimen Bank

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Background: A need exists in the research community for serum, urine, cord blood, placenta and other tissues from pregnant women in order to study disorders that affect both the mother and fetus and to establish gestational age-specific reference intervals.

Objective: To create an infrastructure to recruit pregnant subjects, collect biospecimens throughout pregnancy and make them available to researchers along with clinical information and outcomes.

Methods: In 2008, a system was put into place to consent subjects and track them across a major medical center throughout their pregnancy. Biospecimens, including

serum/plasma, urine, vaginal swabs, follicular fluid, placenta, cord blood, semen, and infant heel stick blood are collected. Clinical data is gathered and stored in a database. A business plan was developed and a cost-recovery fee structure was implemented. Specimens are provided to researchers immediately or frozen and stored for short or long periods of time, at the researcher's discretion. Long term storage utilizes an already established university specimen repository.

Results: Funding has been obtained to support this structure for six years. The cost to launch a similar Biobank is ~\$200,000 per year and to maintain this infrastructure has been ~\$250,000 per year and includes 3.5-5.0 FTE. Average enrollment is 12 women/week from 4 recruitment sites. Over 4,700 samples have been distributed to 11 researchers from 5 different university departments, and over 40,000 samples have been banked for future research. Major clinical outcomes are shown in the Table. The Biobank has helped university researchers receive grant funding including R01, SCOR, ICTS, and March of Dimes.

Conclusions: Here we describe the successful formation of a biorepository for specimens collected from subjects longitudinally throughout pregnancy. Over a six year period, we demonstrate that: funding can be obtained, enrollment is sufficient, accumulation of clinically significant outcomes can be achieved, and the Biobank allows researchers to fund and conduct research.

Table. Outcome data from subjects included in biobank

	Number	%
Pregnant	1669	84.9
Not pregnant	297	15.1
Status of Pregnant patients (if known)		
Delivered	1353	81.1
Loss	37	2.1
Unknown	279	16.7
Complications		
Preeclampsia	165	9.9
Pregnancy induced hypertension	139	8.3
Intrauterine growth restriction	101	7.5
Macrosomia	87	6.4
Fetal anomalies	84	6.2
Chorioamnionitis	102	6.1
Gestational diabetes	92	5.5

B-267

Evaluation of a Discriminatory Zone for Serum Beta-human chorionic gonadotropin (βhCG) in Early Pregnancy

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Background: The beta-human chorionic gonadotropin (hCG) discriminatory zone is the concentration of serum hCG at which a gestational sac should be visible on sonography in a normal intrauterine pregnancy (IUP) and has been used to aid in management of women presenting with pain and/or vaginal bleeding in early pregnancy. The reliability of an hCG discriminatory zone has been debated in the literature. In addition, large inter-individual variation of serum hCG concentrations and the lack of standardization between assays prohibits the use of a universal hCG cut-off. The aim of this project was to perform a retrospective study to determine a discriminatory zone for the serum hCG assay used in our institution.

Methods: Inclusion criteria included females with clinician-ordered ultrasound (US) performed and serum hCG measured within 48h between June 2010 and December 2012 (n=554). The Roche Cobas intact hCG+β assay was used to measure hCG on a Cobas e immunoassay analyzer (Roche Diagnostics). Chart review was performed on a subset of unique patients with serum hCG concentrations between 1,000-5,000 mIU/mL to determine pregnancy outcomes (n=106). Ultrasound reports were reviewed and presence of embryonic structures recorded. Serum hCG concentrations were correlated with presence or absence of gestational sac (GS) and pregnancies were categorized as ectopic or IUP. The IUP group was further categorized into viable or non-viable pregnancies. ROC curve analysis was performed.

Results: In the subset of chart-reviewed women, the median age was 31 years old (range:18-51) and serum hCG concentration was 2320±973 mIU/mL (mean±SD). Serum hCG concentrations were independent of estimated weeks of gestation (p=0.9611). GS was present in 66 cases (hCG 2412±1030 mIU/mL) and not visible by US in 39 cases (hCG 2180±870 mIU/mL). Of those cases with no GS present, chart review revealed that 15 were ectopic (hCG 2372±902 mIU/mL) and 24 were IUP (hCG 2060±846 mIU/mL). Serum hCG concentrations were not different between ectopic or IUP (2446±1128 mIU/mL versus 2287±933 mIU/mL, respectively, p=0.7056). Serum hCG concentrations between viable and non-viable IUPs were similar (2397±943 mIU/mL versus 2220±929 mIU/mL, respectively, p=0.3198). ROC curve analysis identified that in the absence of a GS, a serum hCG concentration of 1700 mIU/mL would be 87% sensitive and 63% specific in differentiating an ectopic pregnancy from an IUP (AUC=0.63, p=0.2783), and a cut-off of 3900 mIU/mL would provide a sensitivity of 13% and a specificity of 95%.

Conclusions: While defining an hCG discriminatory zone would prove valuable to clinicians, especially in the absence of visible gestational sac on sonography, we found a large overlap of serum hCG concentrations in females with ectopic and IUP. The use of a single measurement of serum hCG below or above a given threshold does not provide enough sensitivity or specificity to definitely diagnose ectopic pregnancy. However, when used in conjunction with clinical judgment and sonography, a higher hCG cut-off of 3900 mIU/mL provides increased specificity and may aid in the diagnosis and management of patients.

B-269

Quantitative amino acid analysis using liquid chromatography tandem mass spectrometry and aTRAQ reagents. Do we have a new gold standard?

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Background: Defects in the metabolism or the transport of a specific amino acid or a group of amino acids leads to disorders generally referred to as inherited disorders of amino acid metabolism. Accurately quantifying amino acids in biological fluids (plasma, urine, or cerebrospinal fluid) is essential for the diagnosis and follow up of amino acidopathies, as well as useful for the nutritional assessment of patients with non-metabolic conditions. Amino acid analysis is conventionally performed on an ion-exchange chromatography (IEC) based amino acid analyzer, which provides excellent separation and reproducibility with minimal sample preparation. The IEC method has several disadvantages: long run time, large sample volume, and lack of analyte specificity due to interfering substances. To address our large clinical load and improve specificity, we have optimized the aTRAQ method (AB SCIEX) with ion-pairing reverse-phase liquid chromatography and tandem mass spectrometry, and transferred the assay in our clinical lab.

Methods: Samples were labeled with aTRAQ reagents prior to instrument analysis by API 4000 in conjunction with SHIMADZU HPLC. The chromatographic separation was performed using the ABSciex amino acid analysis column or the Phenomenex Gemini-NX column, C18, 4.6x150mm, 5um. Amino acid quantitation was obtained using 6-point external calibration with stable isotope dilution. MultiQuant software was used for data analysis.

Results: Our method allowed accurate quantification of 47 physiological amino acids and related compounds, including the isomers alloisoleucine and isoleucine, and four additional analytes: sulfofysteine, agininosuccinic anhydrides, formiminoglutamic acid, and glycylproline. The assay analytical performance was evaluated using standard solutions, spiked samples of varying concentrations, and de-identified clinical specimens. The assay was linear from 1 umol/L to 2500 umol/L. The total imprecision was less than 10% and the recoveries were between 90 and 110% for most amino acids. The assay was compared with IEC method with 115 plasma samples and 38 urine samples, run in parallel. The comparison yielded good correlation for most amino acids [slopes between 0.93 and 1.04, y-intercepts between -7.0 and +7.0, R² greater than 0.96]. In urine, as expected, correlation was poor for several amino acids due to interfering substances that cannot be separated by IEC. Reference intervals were established with a focus on the pediatric population. De-identified clinical samples from patients with known disorders of amino acids metabolism or transport were also analyzed with this method and were 100% concordant with IEC.

Conclusion: We have optimized the aTRAQ procedure for amino acid analysis to achieve lower imprecision and better batch to batch reproducibility. Compared with the IEC method, this assay has shorter instrument run time and increased specificity. We have made the assay robust and ready for implementation in the clinical lab. We are able to report 47 amino acids and related compounds from a single sample analysis. This represents a broad, all-inclusive panel for amino acids analysis that, in our opinion, could replace ion-exchange chromatography in the clinical lab.

B-270

Fetal male lineage determination by analysis of Y-chromosome STR haplotype in maternal plasma

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Background: The paternity testing is increasingly becoming a clinical laboratory test and there are some situations that are necessary to perform fetal kinship analysis

before the delivery. The prenatal paternity testing is invasive and most frequently done by testing of chorionic villi or amniotic fluid, which are associated with a stressful sampling that bears a small but existing risk for both mother and child. Actually, with the availability of SNP microarrays and next-generation sequencing, the prenatal paternity testing is been performed non-invasively by analysis of the cell-free fetal DNA in maternal plasma. However, such new methodologies are associated with extras know-how, equipment, and reactions costs. In this way, it is highly desirable to perform non-invasive fetal kinship analysis by using the legacy paternity testing technology. However, the higher maternal DNA background limits the detection of fetal DNA markers (STRs). So, it is necessary to explore the fetus and mother genetics differences (e.g. the Y chromosome in case of male fetus). Thus, the aim of this study is to determine the fetus Y-STR haplotype in maternal plasma during pregnancy and estimate, non-invasively, if the alleged father and fetus belong to the same male lineage.

Methods: The study enrolled couples with singleton pregnancies and known paternity. All participants signed informed consent and the local ethics committee approved the study. Fetal gender was determined by qPCR targeting DYS-14 in maternal plasma and it was confirmed after the delivery. The first consecutive 20 and 10 mothers bearing male and female fetuses, respectively, were selected for the Y-STR analysis. The median gestational age was 12 weeks (range 12-36). Peripheral blood was collected in EDTA tubes (mother) and in FTA paper (father). Maternal plasma DNA was extracted by NucliSens EasyMAG (Biomerieux). All DNA samples were subjected to PCR amplification by ampFLSTR Yfiler (Life Technologies), PowerPlex Y23 (Promega) and an in-house multiplex, which together accounts for 27 different Y-STR. The PCR products were detected with 3500 Genetic Analyzer (Life Technologies) and they were analyzed using GeneMapper-IDX (Life Technologies). Fetuses' haplotypes (in Y-Chromosome Haplotype Reference Database standard format that consider only 15 of the 27 tested Y-STR) were compared to other 5328 Brazilian haplotypes available on www.yhrd.org.

Results: Between 22 and 27 loci were successfully amplified from maternal plasma in all 20 cases of male fetuses. None of the women bearing female fetuses had a falsely amplified Y-STR. The haplotype detected in maternal plasma matched the alleged father haplotype in all cases. One case showed a mutation in the DYS438 locus, which was confirmed after the delivery. Seventeen fetuses' haplotypes were not found in YHRD and three of them occurred twice, which corresponded to paternity probability of 99.981% and 99.944%, respectively.

Conclusion: High discriminatory fetal Y-STR haplotype could be determined from maternal plasma during pregnancy starting at 12 weeks of gestation. Moreover, all male fetuses could be attributed to the alleged father male lineage early in pregnancy. This strategy is an alternative for fetal kinship analysis before the delivery. The main limitation is that it's only applied for mothers bearing a male fetus.

B-271

Pediatric reference value distributions for vitamins A and E in healthy community children: Establishment of new age-stratified reference intervals from a CALIPER cohort

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Objective: Vitamin A (retinol) and vitamin E (alpha tocopherol) are fat soluble micronutrients measured in the pediatric population to monitor deficiencies due to malabsorption secondary to gastrointestinal (GI) disorders. A major challenge of vitamin A and E testing is lack of reliable pediatric reference intervals (RI) which limits accurate interpretation of patient results. We report new pediatric RI for both vitamins as part of the Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER). **Methods:** Healthy community children were recruited with parental consent and whole blood samples collected from 342 healthy children 1 day to 19 years of age. Retinol and alpha tocopherol were extracted from serum using hexane before concentrations were measured with high performance liquid chromatography. Age and sex-specific RI were calculated using non-parametric and robust methods based on CLSI C28-A2 guidelines. **Results:** Comparison of vitamin A and E levels in males and females demonstrated a tight correlation and did not reveal any significant differences requiring no sex partitioning. Further analysis by age demonstrated distinct partitioning patterns. Both vitamin A and E showed age partitioning at 0 to <1 years with levels determined as early as the first day of life. Interestingly, vitamin A exhibited a complex pattern necessitating 4 distinct age partitions trending toward a general rise in levels over time. Vitamin E required 2 age partitions. Levels rose within the first year of life but were reduced slightly after this period requiring only one broad partition between 1 to <19 years. Ratios of vitamin E to cholesterol and triglyceride

were also calculated, correlating well to vitamin E levels. Conclusions: This study establishes pediatric RI for vitamin A and E in a healthy population from neonates to early adulthood. These values will be beneficial in assessing accurate vitamin status when monitoring children with GI disorders or malnutrition.

Analyte (micro mole/L)	Age (years)	Lower Limit	Upper limit	Samples (n)	Higher confidence interval	Lower confidence interval
Vitamin A	1 - < 11	0.97	1.58	100	0.92,1.01	1.54,1.61
	11 - < 16	0.93	2	71	0.82,1.02	1.90,2.09
Vitamin E	16 - < 19	0.82	2.53	50	0.65,1.01	2.33,2.70
	0 - < 1	5.92	51.36	85	1.86,9.67	48.26,54.58
	1 - < 19	14.5	33	245	14.3,78	6.45,6.92
Vitamin E/cholesterol	1 - < 19	3.7	6.7	82	3.53,3.78	6.45,6.92
Vitamin E/triglyceride	1 - < 19	8.53	44.48	83	7.51,9.52	40.37,47.76

B-272

Diagnosis of Primary Hyperoxaluria Type III, A Novel Hereditary Disorder of Hydroxyproline Metabolism, By Gas Chromatography-Mass Spectrometry

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Background and Objectives: Primary hyperoxaluria type III (PH3) is a newly discovered disorder caused by deficiency of mitochondrial 4-hydroxy-2-oxoglutarate (HOG) aldolase, which catalyzes the final step in the metabolism of hydroxyproline. The condition is characterized by the infant to childhood onset of recurrent nephrolithiasis and progressive nephrocalcinosis. Timely detection of primary hyperoxalurias, in particular PH3, remains a significant challenge. Patients have often reached end-stage renal disease by the time they are diagnosed. Here we describe a novel method for the detection of urinary metabolites in primary hyperoxaluria types I, II, and III by gas chromatography-mass spectrometry. We also summarize the clinical and laboratory features of 9 known (i.e., mutation confirmed by sequence analysis) and 2 novel cases of PH3 uncovered by our assay.

Methods: Patient samples and de-identified clinical information were obtained in collaboration with the Mayo Clinic Hyperoxaluria Center and Rare Kidney Stone Consortium. Samples analyzed included urine from unaffected controls, patients with PH1-3, and patients with hyperoxaluria of unknown etiology. Urine specimens were methoximated and extracted with 4:1 (v/v) ethyl acetate/propan-2-ol then evaporated to dryness and derivatized with BSTFA+TMCS in pyridine. Samples were re-extracted in iso-octane and analyzed on an Agilent 5975 series, using hydrogen as the carrier gas. The following commercially available internal standards were used: glycolate-D₂, oxalate-¹³C₂, and glycerate-D₃, in addition to custom-synthesized HOG-¹³C₂.

Validation: The linear range of detection for HOG was 0.024 - 600 µg/mg creatinine (Cr). Pooled normal, intermediate, and elevated HOG urine samples (n=30 for each) were used to determine intra-assay precision (%CV = 2.83, 0.73, and 4.73, respectively) as well as inter-assay precision (%CV = 0.89, 0.48, and 2.60, respectively). In the absence of a comparison method for the analysis of HOG, the accuracy of this test was validating by correlation of test results with clinical findings. Blinded results from analysis of one hundred normal and 34 abnormal specimens were submitted for interpretation; 100% interpretive concordance was achieved for the 134 samples.

Results: The reference range of HOG concentrations in controls was 0.01 - 2.21 µg/mg Cr. In patients with PH1, the range was 0.01 - 0.96 µg/mg Cr. Patients with PH2: 0.01 - 3.10 µg/mg Cr. Testing of urine samples from patients with hyperoxaluria of unknown etiology (i.e., negative for PH1 and/or PH2 by molecular testing) uncovered two male infants with elevated levels of HOG, which were 116.86 and 261.78 µg/mg Cr, respectively. Mutation analysis of the HOGA gene in one of these patients revealed homozygosity for a known pathogenic mutation.

Conclusions: In contrast to previously published methods, our assay can rapidly detect all analytes of clinical utility for the diagnosis of primary hyperoxaluria types I-III. Patients with PH1 and PH2 do not have significantly increased excretion of HOG as compared to unaffected controls. Analysis of urine specimens from patients with hyperoxaluria of unknown etiology can potentially lead to a diagnosis of PH3.

B-273

Pediatric reference intervals for specialty endocrine and chemistry biomarkers on the Abbott Architect ci4100 System: A CALIPER study of healthy community children

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BACKGROUND: Appropriate interpretation of laboratory test results requires carefully established reference intervals based on a healthy population. Growth and development can markedly influence circulating concentrations of biomarkers, thus accurate reference intervals established from a healthy pediatric population are essential for test result interpretation. The CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) program, a national research initiative aimed at closing the gaps in pediatric reference intervals, sought to develop a database of covariate-stratified reference intervals for a number of endocrine and special chemistry markers.

METHODS: Healthy children and adolescents were recruited as part of the CALIPER study. After informed parental consent was obtained, participants filled out a questionnaire including demographic information and provided a blood sample. We measured a number of specialty and endocrine and biochemical markers (Alpha-1 antitrypsin, AGP, Amylase P, Anti-CCP Anti-TPO, Beta 2 microglobulin, C Peptide, Ceruloplasmin, Cholinesterase E, hs-CRP, Cystatin C, DHEA-Sulfate, Glucose, IgE, Insulin, SHBG, Testosterone (2nd GEN, and Bioavailable/Free Testosterone indexes) using the Abbott ARCHITECT ci4100 system and reference intervals were established utilizing 367 - 763 samples per assay. The variance, age- and sex-specific concentrations of analytes were visually inspected from scatterplots of analyte concentration as a function of age for both genders. Pediatric reference intervals were calculated according to Clinical Laboratory Standards Institute (CLSI) C28-A3 guidelines. Partitions based on age and/or sex were determined and statistically evaluated using the Harris and Boyd method. After removal of outliers, reference intervals were calculated using the non-parametric rank method if the sample size was larger than 120, with values ranked and the 2.5th and 97.5th percentiles calculated. If the sample size was between 40 and 120, the robust method was used in reference interval calculation.

RESULTS: We observed a complex pattern of change in most analyte concentrations examined from the neonatal period to adolescence. The changes in concentration observed for each of the examined proteins were classified into 1 of 4 categories: (a) high variance and high concentration within the neonatal period that decreases abruptly shortly after birth: Beta-2-microglobulin, high-sensitivity CRP and Cystatin C; (b) gradual concentration increase with age: albumin BCG, albumin BCP and pancreatic amylase; (c) high variance and high concentration within the neonatal period that decrease gradually with age: Sex hormone-binding globulin (SHBG) and cholinesterase (less pronounced); and (d) high variance at birth that decreases abruptly around 1 year of age and increases again in adolescence: C-peptide, DHEA-S and 2nd GEN testosterone (males). Ceruloplasmin showed a unique expression pattern with a sharp increase in concentration during the neonatal period followed by a gradual decrease over time.

CONCLUSIONS: This study shows the complex expression profiles of several endocrine and chemistry biomarkers as a function of age and gender. This allowed for establishment of age- and sex-specific reference intervals for these biomarkers, which will aid in accurate diagnosis of pediatric patients monitored by immunoassays on the Abbott ARCHITECT ci4100 in healthcare institutions worldwide. It is however important that these reference intervals be validated by each laboratory for the local pediatric population as recommended by CLSI.