
MEETING SUPPLEMENT

Report and Abstracts of the Joint Annual Congress of the AMBQ-CAMB 2009

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The second joint congress of l'Association des Médecins Biochimistes du Québec (AMBQ) and the Canadian Association of Medical Biochemists (CAMB) was held this year from October 7 to 9 in Montreal. The setting was the picturesque Hôtel Place d'Armes, which is situated in the historic Old Montreal district. There were over 60 attendees comprising both Specialists and Medical residents-in-training and representing the breadth of Canada from the Atlantic to the Pacific.

The scientific committee composed of Dr. Jean. Dubé (Centre Hospitalier U. de Sherbrooke), Dr. Bernard Fruteau-de-Laclos (Centre Hospitalier AUQ), Dr. Éline Letendre (Centre Hospitalier U. de Montreal), Dr. Bassam A. Nassar (Capital Health) and Dr. Claude Petitclerc (CHUM) arranged a series of informative and interesting scientific sessions.

Day 1 saw a training session for the medical residents conducted by Dr. Yves Giguère (Centre Hospitalier de l'U. Laval) on Prenatal Screening. A meeting of the specialty committee of the Royal College for

Medical Biochemistry followed this. A major topic of this meeting was the re-alignment of the training requirements.

Day 2 began with the business meeting of the AMBQ. The scientific sessions began later that day with a session on "Pharmacotoxicology and the Role of the Laboratory" chaired by Drs. Andre Mattman (B.C. Children & Women's Health Centre) and Bas-sam A. Nassar. The first speaker, Dr. Margaret Thompson (Hospital for Sick Children), in her talk "Clinical Toxicology – for the Laboratory" reviewed the role of the Ontario Poison Centre, which may serve as a model for the rest of the country. This was followed by Dr. Zulfikarali Verjee (HSC), who is clearly a master of the subject, with his talk, "Challenges in Urine Drug Screens: Ongoing Issues". The morning session ended with Dr. Andre Mattman's presentation "Heavy Metal Toxins – How and Why to Test in the Clinical Laboratory".

Day 3 moderated by Dr. Éline Letendre (CHUM) focused on risk factors for cardiovascular disease. The

first speaker in the morning was Dr. Jacques Genest Jr. (McGill University Health Centre) who reviewed the new Canadian guidelines for the diagnosis and treatment of dyslipidemias. He described in detail the thinking behind the new guidelines. This was followed by a presentation by Dr. Allan Jaffe (Mayo Clinic) who gave the audience a most authoritative description of the soon to be introduced fourth generation high sensitivity assays for troponins. These assays will have a marked impact on the assessment of cardiac damage perhaps even more so than the original introduction of the troponins. The afternoon featured three speakers discussing the pro and cons of high-sensitivity C-reactive protein for the assessment of cardiovascular disease. Dr. Jean Grégoire (Institut de cardiologie de Montréal) presented the pro side of the debate reviewing in particular the recent Jupiter trial. Dr. James Brophy (MUHC) presented the con side of the debate in a most entertaining manner. He even had the audience performing stretching exercises! It was left to Dr. Jean Bergeron (CHUL) to provide a balanced view of the two preceding speakers.

The last day, Day 4, was primarily dedicated to oral and poster presentations by the residents. A jury consisting of Drs. Jean Dubé (CHUS), Yves Guigère (CHUL), and Joël Girouard (CHUL) had the “difficult” task of awarding prizes to the best oral and to the best poster presentations. The winners this year were Dr. Alexis Blaass (U. de Montréal) for the oral presen-

tation entitled “Characterization of a new *LCAT* mutation causing familial *LCAT* deficiency (FLD) and the role of *APOE* as a modifier gene of the FLD phenotype” and Dr. Adell Elsharif (McMaster U.) for the poster presentation entitled “Method Validation Study to Evaluate the Analytical Performance of the STAT-SITE Meter for the Measurement of Serum Beta-Hydroxybutyrate”. The scientific portion of the conference ended with a most comprehensive presentation on smoking cessation, both clinical approaches and therapeutics by Dr. Joanne Provencher (Hôpital Laval). Dr. Provencher reminded us that smoking cessation by an individual could be achieved with the correct support.

The day and the congress ended with a business meeting of the CAMB chaired by the out going president, Dr. Bassam A. Nassar. A new executive was elected: Dr. Elizabeth MacNamara (SMBD-Jewish General Hospital, president), Dr. Yves Guigère (CHUL, vice-president), Dr. Andrew Don-Wauchope (McMaster U. Health Sciences Centre, secretary-treasurer), Dr. Andre Mattman (BCCWHC, councilor), Dr. Brian M. Gilfix (MUHC, councilor), Dr. John Heathcote (Vancouver, councilor), and Dr. Dattily Ooi (Children’s Hospital of Eastern Ontario, councilor),

We all look forward to next year’s combined meeting which is again slated to take place in Montreal in October.

CETP IN RARE DYSLIPIDEMIAS

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Objective: To determine CETP mass and activity in rare disorders of HDL metabolism.

Methods: Patients: Fasting plasma samples were obtained from two individuals with TD and from patients with LCAT deficiency, analphalipoproteinemia, Hepatic lipase deficiency (HL), vasculitis, and hypoalphalipoproteinemia. Their clinical and laboratory findings have been previously reported.

Assay: CETP activity was measured using commercial CETP Fluorescence kit (Roar Biomedical Inc., New York, NY). The kit includes donor (without apoA-I) and acceptor lipoprotein particles. Incubation of donor and acceptor with a CETP source results in the CETP mediated transfer of fluorescent neutral lipid from donor to acceptor, rate of which is determined by the increase in fluorescence intensity as the fluorescent neutral lipid is removed from the donor to the acceptor. The amount of fluorescent substrate transferred was expressed as pmoles of fluorescent substrate transferred within 3 hours. CETP concentration was measured by ELISA using specific rabbit antibody against human CETP.

Results: While CETP mass correlated with serum HDL-C levels, the activity decreased in patients with LCAT deficiency, FED, HL deficiency and vasculitis, but increased in TD. We speculate that the composition of HDL particles in these disorders differs and results in the different CE transfer rates.

Conclusion: CETP mass correlates with the HDL-C concentration but the activity differ widely among the patients with rare disorders of HDL metabolism, probably due to the changes in HDL quality.

CHARACTERIZATION OF A NEW LCAT MUTATION CAUSING FAMILIAL LCAT DEFICIENCY (FLD) AND THE ROLE OF APOE AS A MODIFIER GENE OF THE FLD PHENOTYPE

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Introduction: LCAT (lecithin:cholesterol acyltransferase) is an enzyme which plays an essential role in cholesterol esterification and reverse cholesterol transport. Familial LCAT deficiency (FLD) is a disease characterized by a defect in LCAT resulting in extremely low HDL-C, premature corneal opacities, anemia as well as proteinuria and renal failure.

Method: We have identified two brothers presenting characteristics of familial LCAT deficiency. We sequenced the *LCAT* gene, measured the lipid profile as well as the LCAT activity in 15 members of this kindred. We also characterized the plasma lipoproteins by agarose gel electrophoresis and size exclusion chromatography and sequenced several candidate genes related to dysbetalipoproteinemia in this family.

Results: We have identified the first French Canadian kindred with familial LCAT deficiency. Two brothers affected by FLD, were homozygous for a novel *LCAT* mutation. This c.102delG mutation occurs at the codon for His35 causing a frameshift that stops transcription at codon 61 abolishing LCAT enzymatic activity both *in vivo* and *in vitro*. It has a dramatic effect on the lipoprotein profile, with an important reduction of HDL-C in both heterozygotes (22%) and homozygotes (88%) and a significant decrease in LDL-C in heterozygotes (35%) as well as homozygotes (58%). Furthermore, the lipoprotein profile differed markedly between the two affected brothers who had different *APOE* genotypes. We propose that *APOE* could be an important modifier gene explaining heterogeneity in lipoprotein profiles observed among FLD patients. Our results suggest that a *LCAT*^{-/-} genotype associated with an *APOE* ε2 allele could be a novel mechanism leading to dysbetalipoproteinemia.

UNE HYPERLIPIDÉMIE SECONDAIRE A NE PAS OMETTRE

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Introduction: Devant une perturbation du bilan lipidique chez un enfant dont l'un des parents est connu pour hypercholestérolémie familiale (HF), le diagnostic repose sur deux arguments : les LDL > 95^e percentile et la présence de xanthomes tendineux chez le parent. Toutefois, une hyperlipidémie type IIb peut orienter le diagnostic vers une cause secondaire.

Objectif: Reconnaître une cause secondaire d'hyperlipidémie mixte dans un contexte d'histoire familiale d'hypercholestérolémie familiale (HF).

Observation: Nous rapportons l'observation de deux frères d'âge respectifs de 8 et 10 ans, d'origine canadienne française, ayant une histoire familiale d'HF. L'enfant de 10 ans asymptomatique, présentait une hyperlipidémie type IIa compatible avec un diagnostic d'HF hétérozygote alors que l'enfant de 8 ans présentait un type IIb. Un bilan sanguin d'extension chez ce dernier orientait le diagnostic vers une cause secondaire. L'absence de mutation du récepteur à LDL renforçait encore plus ce diagnostic. Après traitement étiologique de trois mois, normalisation du bilan lipidique sans que le patient ait recours à une statine. Cette évolution favorable est consolidée depuis trois mois.

Conclusion: Une dyslipidémie de cause secondaire peut mimer une HF chez un patient avec une histoire familiale positive. Le traitement de l'étiologie corrige la dyslipidémie sans recours aux statines.

METHOD VALIDATION STUDY TO EVALUATE THE ANALYTICAL PERFORMANCE OF THE STAT-SITE METER FOR THE MEASUREMENT OF SERUM BETA-HYDROXYBUTRATE

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Objectives: To evaluate the analytical performance of the STAT-Site meter (Stanbio laboratory USA) for the measurement of serum beta-hydroxybutyrate (β OH-B) concentration.

Methods: The precision was evaluated using two levels of quality control materials (low and high). Within run and between run precision studies were performed. CVs (coefficient of variation) compared against accepted standards. Fifty-one leftover patient samples with previously reported β OH-B concentrations were used for the method comparison. The accuracy was evaluated and compared to our current laboratory method, (Wako Autokit 3-HB, Wako chemical USA). Slope, intercept, correlation coefficient, Deming regression and paired t-test were calculated using Analyse-it® software

Results: The meter showed reasonable precision for the measurement of β OH-B with CV of 0.0% and 6% for within run precision study and 8.82% and 10.07% for between run precision study which are acceptable according to two CAP surveys. Further these are comparable to our current method CV and the manufacturer claimed CV's. Deming regression analysis showed a linear relationship between the two methods: The slope of the regression equation was 0.98 (95% CI, 0.87-1.08); intercept 0.10 (95% CI 0.03-0.16) and correlation coefficient of 0.979. There was no bias detected by the visual inspection of the difference plot, this was confirmed by the calculation of the paired t-test ($p = 0.19$).

Conclusion: The STAT-Site meter is a practical, rapid method with wide analytical range that meets precision and accuracy criteria. The method is suitable for the use in a research context until it gets approval for clinical use.

EVALUATION OF THE UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICE 2008 (UK NEQAS 2008) GUIDELINES FOR THE DIAGNOSIS OF SUBARACHNOID HEMORRHAGE USING SPECTROPHOTOMETRIC XANTHOCHROMIA: A RETROSPECTIVE STUDY

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Objective: Evaluation of the UK NEQAS 2008 guidelines for the interpretation of spectrophotometric xanthochromia.

Method: A search of the laboratory database for all the xanthochromia test results between May 1st 2008 and May 1st 2009 was performed. Medical charts were reviewed for patients of Hôpital de l'Enfant-Jésus (HEJ) that had at least one detectable pigment (bilirubin, oxyhemoglobin, or methemoglobin). Xanthochromia results obtained with 4 different criteria (Chalmers original, Modified Chalmers, Duiser and UK NEQAS 2008) were compared.

Results: We reviewed 41 medical charts (2 patients with duplicate lumbar punctures (LP) for a total of 43 LP). For these 41 patients there were 11 positive xanthochromia results, 5 of which were in concordance with a final diagnosis of subarachnoid hemorrhage (SAH). The diagnosis of the 6 other positive xanthochromia results were as follow: meningeal spread of a lymphoma, cerebral amyloid angiopathy, exertional headache, viral encephalitis with a possibility of petechiae on the cerebral CT and second LP. Interpretation (negative/positive) of 40/43 LP was identical for the 4 methods. 2 LP were positive with Duiser and UK NEQAS 2008 but negative with Chalmers approaches (final diagnosis: SAH and cerebral amyloid angiopathy). 1 LP was positive only by the Duiser method (viral encephalitis).

Conclusions: UK NEQAS 2008 guidelines identified all SAH but are sensitive to traumatic and pathologic meningeal lesions. Except for a case of viral encephalitis with a suspicion of cerebral petechiae on CT, UK NEQAS 2008 gave xanthochromia results similar to the one in use at HEJ (Duiser). Chalmers original and Modified Chalmers methods missed one of the five SAH.

RE-EVALUATION OF THE CORRECTION FACTOR FOR ETHANOL IN THE CALCULATION OF THE OSMOLAL GAP

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Background/objectives: It is well-known that ethanol (EtOH) demonstrates non-ideal solute behaviour in plasma. This is reflected by its larger than expected contribution to the plasma osmolality. Published multiplicative correction factors for the EtOH contribution range from 1.20 to 1.25. The objective of this study is to determine an optimal correction factor specific to the instrumentation at Vancouver General (VGH) and St. Paul's (SPH) Hospitals.

Methods: Laboratory data from patients presenting to the two respective emergency department between August 01, 2007 and November 30, 2008 were extracted from the Sunset database. Plasma sodium, urea, glucose, and EtOH were measured using the two high-volume chemistry analyzers employed at the sites: the Siemens (previously Dade) RXL (VGH) and the Siemens (previously Bayer) Advia 1650 (SPH). Plasma osmolality was measured by freezing-point depression and calculated (excluding the EtOH contribution) using the following standard formula (in SI units): $2 [Na] + [Urea] + [Glucose]$.

Patients without EtOH data or who had undetectable EtOH were excluded as were patients with methanol or ethylene glycol present. Standard regression statistics were employed.

Results: Twelve hundred and fifty-three patient samples (n=823 from SPH and n=430 from VGH) were included. Empirical correction factor m, satisfying, $Osmol\ gap\ (mmol/kg) = m[EtOH]\ (mmol/L)$ was consistently found to be 1.15 for VGH, SPH and both combined.

Conclusions: The correction factor of 1.15 for ethanol from the current study appears to be more representative and reliable. Further studies to evaluate its validity in other hospital sites as well as its utility in screening patients with known toxic alcohol ingestion will be warranted.

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) MULTIPLEX – PCR METHOD

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Introduction: Gene polymorphisms in human *methylene-tetrahydrofolate reductase (MTHFR)* have been linked to the disorders of folate metabolism, and may contribute to such disease as neural tube defect, coronary heart disease, venous thrombosis, and several types of cancers.

Methodology: We have modified our current real-time PCR method (*Clin Biochem* 2000;33:535-539.) for the detection of the *MTHFR* c.677C>T variant to a real-time multiplex-PCR method for detection of *MTHFR* c.1298A>C and c.677C>T variants on a LightCycler 1.2. Our multiplex method is partly based on that of *Agarwal et al.* (*J Mol Diag* 2007;9:345).

Results: The genotyping results of c.677C>T variant analyzed by both methods were in agreement. All samples analyzed for the c.1298A>C variant by the Multiplex-PCR method gave clear-cut results. The LightCycler multiplex method was validated with 14 specimens previously genotyped by restriction fragment length polymorphism at Montreal Children's Hospital. All results were in agreement except for one sample that failed to amplify. There was no significant difference in *T_m* between the Multiplex-PCR procedure and the previously reported *T_m*'s for both variants. When run alone but not as a multiplex, the progress curve of the c.1298A>C variant exhibited the "hook effect". Asymmetric amplification was found to reduce this effect.

Conclusions: The multiplex assay is reliable, economical, fast and simple method to perform as compared with RFLP technique. Asymmetric PCR is a helpful tool to minimize the hook effect.

ASSESSING THE FREQUENCY OF CA-125 MEASUREMENTS WITHIN HAMILTON HEALTH SCIENCES AND ST. JOSEPH'S HEALTHCARE

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Objective: To determine if the number and frequency of CA-125 measurements per patient reflect the 2008 NACB (National Academy of Clinical Biochemist) practice guidelines.

Methods: We collected data retrospectively on CA-125 over a period of one year as part of our ongoing practice in monitoring the tumor markers for quality assurance. The number of CA-125 results per patient as well as the time interval between the 1st and 2nd measurements was noted. To enrich this population for the likelihood that the measurement of CA-125 was used for monitoring or for detection of recurrence, we divided the population into patients from the Jurvainski Cancer Centre (JCC) and the rest of the sites.

Results: The JCC patients contributed the majority of CA-125 results, representing over 75% of all results (n= 3057 results from 998 patients), whereas the remainder of the sites only yielded 959 results from 920 patients. Further analysis of the JCC patients indicated 2 main subgroups: Group A – patients with 1 or 2 results (n=624 patients); Group B - patients with 3 or more results. Overall, less than 4% of patients at the JCC had a time interval between the 1st and 2nd specimens of less than 2 weeks.

Discussion: This initial analysis would indicate that physicians are ordering CA-125 in agreement with the NACB guidelines. To further improve compliance to the guidelines and to prevent subsequent measurements of CA-125 too close, we propose restricting CA-125 orders that are less than 14 days apart to only those that receive Biochemist's approval.

A NEW CAPILLARY ZONE ELECTROPHORESIS METHOD FOR THE SCREENING OF CONGENITAL DISORDERS OF GLYCOSYLATION (CDG)

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Background: The Congenital Disorders of Glycosylation (CDG) are an expanding group of metabolic diseases with a broad clinical presentation. We sought to validate a new Capillary Zone Electrophoresis (CZE) method (Sebia CAPILLARYS™ CDT) to screen for CDG.

Methods: We analyzed 119 serum samples from children of varying ages and of both sexes to establish a reference range of transferrin glycoforms including CDT (Carbohydrate Deficient Transferrin). We then studied serums from 8 known CDG patients and compared the CZE results to the isoelectric focusing (IEF) profiles. We also analyzed serums after extraction from spotted Guthrie cards.

Results: The mean (SD) percentage of transferrin glycoforms is 18.5 (4.4), 78.5 (4.2), 2.5 (1.3) and 0.6 (0.3) for penta-, tetra-, trisialotransferrin and CDT, respectively. There is no statistically significant difference between the different age groups analyzed (0-5, 6-11, 12-15, 16-18, and > 18 years) or between sexes. We observed a good correlation between the CZE and IEF profiles with both fresh serum and serum extracted from Guthrie cards.

Conclusions: The Sebia CAPILLARYS™ CDT system is a simple and reliable method to screen for CDG in pediatric and adult patients with an unexplained clinical syndrome, particularly when the nervous system is involved.