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ICPLM–SPEAKERS'ABSTRACTS

The following speakers' abstacts are from the 2005 meeting of the American Association of Clinical Chemistry annual meeting held in Orlando Florida USA during 2005.

2.1 What's new in paediatric microbiology?

Campos JM

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The events of the previous decade have been among the most exciting in the annals of paediatric clinical microbiology. Development of laboratory tests featuring rapid, noncultural approaches has been at the heart of the upheaval. This era of technological change began gathering momentum during the 1970s with the introduction of antigen detection assays. This new breed of tests continued to evolve during the 1980s and 1990s into assays which are faster, much simpler and less dependent upon expensive instrumentation than their ancestors. Many of these tests in their current formats can be performed safely and with reasonable accuracy by individuals in non-laboratory settings, such as physician's offices. Also in the 1990s we witnessed the growing popularity of molecular diagnostic testing – a subject of other lectures at this scientific meeting.

Since the beginning of the new millennium, we have already witnessed the emergence of several new infectious diseases and the development of worrisome antimicrobial resistance trends of concern to paediatric patients.

This session will address the clinical and laboratory characteristics of human metapneumonia virus and a novel human coronavirus (HCoV-NL63) that is distinct from SARS. Trends in the resistance of Staphylococcus aureus to the semisynthetic, penicillinase-resistant penicillins will also be discussed. The goals of this lecture will be:

1. To convey to the audience an understanding of the principles of collecting, processing, and performing laboratory tests on paediatric respiratory tract specimens for diagnosis of viral infections in the clinical microbiology laboratory.

2. To discuss the laboratory aspects of emerging pathogens of current interest in paediatrics.

3. To describe recent trends in the spectrum of antimicrobial resistance in Staphylococcus aureus and to illustrate ocioeconomic families in developing countries.

iH.pylori and non ulcer dyspepsia be treated? What are the indications and goals for therapy?

2.2 Helicobacter pylori - A friend or a foe?

El-Saifi Ahmed, Tayeb Sawsan, Nouh Akram

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Helicobacter pylori is prevalent in more than half the population worldwide and most individuals are asymptomatic.

In children, the incidence of Helicobacter pylori ranges from 6-16% in high-income families with access to hygienic food and clean water and 50-70% in low socioeconomic families in developing countries.

Acquisition of H. pylori in childhood seems to be an important factor in developing peptic ulcer, gastric carcinoma and gastric MALT lymphoma in adulthood. On the other hand, some studies have revealed that eradication of H.pylori is associated with an increased incidence of esophageal cancer, reflux esophagitis and obesity. Is H.pylori a friend or a foe? Should patients with H.pylori and non ulcer dyspepsia be treated? What are the indications and goals for therapy? Diagnosis of H.pylori involves invasive methods (Biopsy) and non invasive methods (Urea breath test, Serology, Stool Antigen assay and culture as well as the detection of the cag A cytotoxin associated H.pylori strains). Specificity and Sensitivity of the different diagnostic tools will be discussed.

2.3 Rotavirus and the need for vaccination

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An estimated 440,000 children die each year from rotavirus (one quarter of diarrhoea deaths). These deaths occur predominantly in developing countries. In contrast, morbidity from rotavirus occurs both in developed and developing countries. Rotavirus causes 30-50% of all diarrhoeal admissions in children and 10–15% of those

treated in the community. This very significant mortality and morbidity emphasises the need for safe and effective rotavirus vaccines. Importantly, rotavirus is not prevented by good sanitation and hygiene. The first available rotavirus vaccine, Rotashield®, was withdrawn shortly of licensure, due to its association with intussusception. Although this was seen as a major setback, the experience led to new opportunities. Two new vaccines, Rotateq® (Merck) and Rotarix® (GlaxoSmithKline), have undergone extensive testing in a range of developed and developing countries and no increased risk of intussusception has been demonstrated. It is hoped that these vaccines will become widely available in the near future with licensing plans underway. However past experience has shown that there have been unacceptable delays in introducing new vaccines to developing countries. The Global Alliance for Vaccines and Immunizations, the World Health Organization and Centers for Disease Control and Prevention are therefore helping to fast-track development a nd introduction of rotavirus vaccines in developing countries. An initial US$30 Million launched the Rotavirus Vaccine Program, which aims to nurture a new paradigm for vaccine introduction. Reliable local disease and economic burden data are extremely important to help policy-makers decide whether or not to support the use of a new vaccine. The Asian Rotavirus Surveillance Network (ARSN), established in 1999, has shown that rotavirus disease burden in Asia is higher than previously reported. The ARSN data, coming from both developed and developing countries, will help determine whether rotavirus vaccines can be introduced into the Asian Region at an early stage.

2.4 The clinical microbiology laboratory in the era of molecular diagnosis

Hannah Sprecher

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The past recent years have witnessed dramatic changes in our diagnostic approach to microbial infections. Although standard cultures still play a pivotal role in the microbiology laboratory, novel molecular techniques have been assessed and shown to represent interesting potential adjuncts in the diagnosis of infectious diseases. Most molecular techniques in microbiology relied upon the use of various types of PCR.

Those techniques are mostly useful in 5 different settings: (1) to establish an accurate diagnosis as rapidly as possible in life-threatening infections (e.g. bacterial meningitis); (2) to identify fastidious organisms in a short time (e.g. mycobacterial infections); (3) to delineate pathogen sensitivity to various antibiotics, simultaneously with pathogen identification (e.g. staphylococcal infections); (4) to trace the spread of pathogens and their intrinsic pathogenicity during local or global outbreaks (e.g. molecular epidemiology); (5) and to detect pathogens not identifiable using standard methods (e.g. so-called culture negative endocarditis). Although those novel techniques are still emerging, and in need of standardization, they provide the clinical microbiologist with powerful tools to address challenging situaations.

2.5 Epidemiology of childhood allergies

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The International Study on Allergies and Asthma in Childhood (ISAAC) has showed large variations in the prevalence of asthma and allergic diseases worldwide. Environmental influences have a part to play in this, with higher rates of prevalence seen in developed countries with a western lifestyle. The pattern of prevalence of allergies in Asia supports this notion. Prevalence of asthma and allergies in urban China is higher than in the rural regions. Immigration data within Asia also supports this concept. Immigrants from lesser developed countries to Singapore were less likely to have symptoms of asthma and allergy. However, prevalence of these symptoms increased with increased duration of residence in Singapore. The trends of these diseases in a developed (Singapore) and developing (Indonesia) nation over 7 years were examined to see if increasing progress and westernization resulted in changing asthma prevalence. Interestingly, the prevalence of asthma symptoms increased in the 13 to 14 year olds, but decreased in the 6 to 7 year olds. This change was similar in both countries. We propose that regional environmental changes have resulted in this decrease in the younger cohort, suggesting that environmental influences in early life are of paramount importance.

2.6 The genetic basis of the hygiene hypothesis in allergy

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Innate immune cells recognize pathogen-associated molecular patterns, leading to the differentiation of naive T-helper (Th) lymphocytes into Th1 instead of Th2 cells. In the absence of early-life microbial stimulation, the adaptive immune system skews toward Th2-mediated immunity. Such individuals are more susceptible to atopy during childhood. This concept is known as the 'hygiene hypothesis'. Recent farming studies suggest early endotoxin exposure to be protective against asthma. CD14 acts in concert with Toll-like receptors (TLRs) to mediate these effects. Atopic disorders were associated with important genetic targets of innate immunity, with CD14 being the most extensively investigated. We reported recently that the presence of -159C in CD14 was associated with serum total IgE concentration in atopic children, whereas others found such association in non-atopic Caucasians. Single nucleotide polymorphisms (SNPs) in CD14 were also linked to the susceptibility for atopic eczema. Investigators also examined the relation between TLR SNPs and atopic disorders. Although several studies could not detect such association, TLR4 Asp299Gly was found to affect atopy severity in Caucasians. TLR10 SNPs were also associated with asthma and airway hyper-responsiveness. In two Caucasian studies, SNPs in nucleotide-binding oligomerization domain protein 1 modulated the development of asthma, atopic eczema and serum total IgE. Besides, variants in human defensin ?-1 gene contributed in a gender-specific way to asthma susceptibility. Interestingly, we found recently that

mannose-binding lectin haplotypes protected against asthma and atopy in Chinese children. To complicate the issue, a recent study found atopy to be less common in hepatitis A-seropositive subjects carrying an insertion variant of TIM-1, encoding the cell surface receptor for hepatitis A virus. This finding suggests an important gene-environmental interaction in mediating atopy susceptibility. In conclusion, there is a strong genetic basis of the hygiene hypothesis that links innate immunity to both the development and severity of allergic diseases.

2.7 Cytokine and chemokine Immunopathology in allergic asthma

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T-helper lymphocyte type 2 (Th2) and inflammatory cytokines have been implicated for playing important roles in the induction and maintenance of the inflammatory cascade in allergic asthma. We compared, in plasma and whole blood culture supernatant, the inflammatory cytokines interleukin (IL)-17, IL-18, IL-6 and IL-12, Th2 cytokines IL-10 and IL-13, chemokines IL-8, regulated upon activation normal T cell expressed and secreted (RANTES), IFN-inducible protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1) and monokine induced by IFN-gamma (MIG), and intracellular interferon-? (IFN-?) and IL-4 in Th cells of 41 patients with allergic asthma and 30 sex- and age-matched health control subjects.

Chemokines, intracellular cytokines and cell surface expressions of CC chemokine receptor (CCR)3 and CCR5, and CXC chemokine receptor (CXCR)3 on peripheral blood mononuclear cells (PBMC) were analysed by flow cytometry.

Cytokines were measured by ELISA. Plasma IL-18, IL-12, IL-10 and IL-13 concentrations were significantly higher in allergic asthmatic patients than normal control subjects (all $p < 0.05$). Allergic asthmatic patients showed higher plasma IL-17 and IL-6 concentrations than normal controls. Plasma level of Th2 RANTES concentration and cell surface expression of CCR3 receptor were significantly higher but Th1 chemokine IP-10 was significantly lower in asthmatic patients ($p < 0.01$).

Whole blood assay indicated that there was a significant decrease in Th1 chemokine IP-10 and MIG production in phytohaemagglutinin and lipopolysaccharide activated blood cell culture ($p < 0.05$). The percentage of IFN-?-producing Th1 cells was significantly higher in normal control subjects than asthmatic patients ($p < 0.001$), but the percentage of IL-4 producing Th2 cells did not differ ($p > 0.05$). Consequently, Th1/Th2 cell ratio was significantly higher in normal subjects than asthmatic patients ($p < 0.001$). Allergic asthma is characterized by a Th2 predominance with the elevation of both inflammatory and Th2 citokines and chemokines.

2.8 Monitoring of Inflammatory markers in allergic Rhinitis

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Allergic rhinitis is an inflammatory disease of the nasal mucosa. The typical symptoms of allergic rhinitis are itching, multiple episodes of sneezing, watery nasal discharge and nasal obstruction. The pathogenesis of the nasal allergic reaction initially involves the interaction of allergens with specific IgE bound to the surface of mast cells and basophils in the nasal mucosa. This leads to the cross-linking of these antibodies resulting in cellular activation and mediator release, which are responsible for the allergic symptoms. Moreover, trans-endothelial migration of inflammatory cells and their activation within the reactive tissue are characteristic features, which represent the result of a complex network of interactions between various mediators, cytokines, chemokines and cell adhesion molecules.

Our studies demonstrate that a significant increase in the concentrations of histamine, tryptase, and LTC4 in nasal secretions occurred within seconds or minutes after NAC, and this was accompanied by itching, sneezing, rhinorrhea and nasal obstruction.

This response has been described as the early phase reaction (EPR). For the symptoms of itching and sneezing, the EPR may start as early as seconds and often last for only a few minutes after NAC. However, if one considers nasal obstruction and rhinorrhea, it may last more than 1 hour.

The infiltration and activation of eosinophils are found to be the predominant condition during the late-phase reaction (LPR), which is mainly characterized by unilateral and/or bilateral nasal obstruction with little sneezing and rhinorrhea. The term “late phase reaction" in fact covers a phenomenon of chronic inflammation in the nasal mucosa of an allergic individual following NAC. For hours or sometimes days after the challenge, nasal obstruction is the clinically predominant symptom.

When assessing the inflammatory cells and mediators in nasal secretions, the infiltration and activation of eosinophils are found to be the predominant mechanisms in the pathogenesis of this inflammatory condition. The pathophysiological condition of LPR is found to be very common in patients with ongoing allergic rhinitis. Therefore, a combined assessment of the EPR (e.g., histamine, tryptase and LTC4) and LPR markers (e.g., eosinophil count and ECP concentration) in nasal secretions is a very useful model for monitoring and assessing chronic nasal inflammation in patients with allergic rhinitis and their response to therapies.

2.9 Paediatric obesity: a healthcare challenge

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Obesity and overweight are global health problems affecting both adults and children. In Canada, the combined rates of overweight and obesity among school-aged children increased from 15% in 1981 to 35.4 % and 29.2% respectively. These figures mirror that from the United States, Europe and other developing countries. This growing worldwide problem will have health consequences as these children get older. They face an increased risk of developing diabetes, heart disease, and orthopaedic problems as well the psychological challenges related to their obesity. One factor in this growing epidemic is a shift to a positive energy balance, the energy intake being greater than the energy expended. While we can modify our food intake and exercise more; some of us cannot regulate our weight gain as much as others. Genetics, metabolism and environment are also important contributors. A brief overview of this health problem will be presented.

2.10 Epidemiology, lifestyle, nutrition and treatment

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Type 2 diabetes results from a decline in insulin action due to insulin resistance and relative insulinopenia. Even in the absence of frank diabetes, insulin resistance is associated with many factors that increase risk for premature cardiovascular disease: hyperinsulinism, hypertension, dyslipidaemia and hypercoagulability. The metabolic syndrome, the consequence of obesity and insulin resistance, is the most common endocrine disorder to affect children, adolescents and adults. Throughout the world's westernized societies, the incidence of type 2 diabetes in adults has reached epidemic proportions. The dire consequences of type 2 diabetes threaten to shorten average life span, produce severe morbidities and increases health care costs. As a consequences of high caloric diets and inactivity, a similar epidemiologic explosion of type 2 diabetes are further predicted. At the conclusion of this presentation the attendee will be able to :

1) describe the relationship between obesity, insulin resistance and type 2 diabetes,

2) enumerate the metabolic effects of insulin resistance and their relationship to risk for cardiovascular disease,

3) explain why paediatric type 2 diabetes is on the rise and

4) summarize the current state of knowledge of the genetics of type 2 diabete

2.11 Asian perspective, endocrinology and type 2 diabetes

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Obesity and type 2 diabetes are becoming more prevalent in children and adolescents. It is likely that many obese children and adolescents may have developed components of the metabolic syndrome in an early age. Recent screening studies performed in Taiwan and Japan confirmed that type 2 diabetes is increasing in children and adolescents. The diabetic children had higher BMI, cholesterol and blood pressure than non-diabetic children. As many of the Asian countries are undergoing rapid development and westernization, it is highly likely that the problem of obesity and type 2 diabetes will become increasing common in the region. Studies in young adults with type 2 diabetes have confirmed that they are at high risk of developing long-term complications. Population-based data on the prevalence of metabolic syndrome in Asian children are limited. Recently, we have studied a random community sample of 2116 schoolchildren aged 11-20 years Anthropometric data including height, weight, waist circumference, blood-pressure were obtained at the schools. Fasting blood samples and urine samples were also obtained for the assessment of lipid profile, plasma glucose and urinary microalbumin assay. The presence of metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP) of the United States. The overall prevalence of metabolic syndrome (having at least 3 criteria) was 2.0% (2.5% in boys and 1.6% in girls). 10.9% of children had elevated triglycerides (>1.25 mmol/L). 2.4% had low HDL-C (<1.03 mmol/L). Among the subjects, 24.8% and 6.9% had one and 2 components of the metabolic syndrome. This study highlighted the problem of metabolic syndrome in Asian adolescents. Lifestyle and dietary modifications are necessary in order to control the anticipated epidemic of obesity and type 2 diabetes in Asia.

2.12 New research on obesity: Lessons from Animal models and insights Into underlying mechanisms

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Insulin resistance and type 2 diabetes are rapidly emerging as major disorders of childhood and adolescence. This appears to be closely linked to a rapid rise in the prevalence of obesity in the paediatric population. The development of insulin resistance appears to lead to a "metabolic syndrome", which includes a number of major complications such as dyslipidaemia and hypertension. Childhood metabolic syndrome promotes the development of premature atherosclerosis and significantly increases cardiovascular disease risk early in life. The mechanisms linking obesity, insulin resistance, and metabolic dyslipidaemia are not fully understood. This lecture will attempt to discuss some of the key mechanistic issues surrounding insulin resistance and its association with metabolic dyslipidemia.

Most of the recent progress in this field has come from the use of genetic and diet-induced animal models of insulin resistance. New data from these animal studies particularly the fructose-fed hamster, a model of metabolic syndrome and dyslipidaemia, will be reviewed. Evidence from both animal and human studies suggest a key role for insulin sensitive tissues such as adipose tissue, liver, and intestine in the development of an insulin-resistant state and its associated lipid and lipoprotein disorders. An important but not well-appreciated dietary change has been the substantial increase in the amount of dietary fructose consumption from high intake of sucrose and high fructose corn syrup, a common sweetener used in the food industry. Emerging evidence from recent epidemiological and biochemical studies clearly suggests that the high dietary intake of fructose has rapidly become an important causative factor in the development of the metabolic syndrome. The trends in fructose consumption, the metabolic consequences of increased fructose intake, and the molecular mechanisms leading to fructose-induced lipogenesis, insulin resistance and metabolic dyslipidaemia will be discussed.

2.13 Recent advances in childhood cancer: insights from biology and future promise

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Cancer in the paediatric age group which spans the period from the neonate to adolescence differs considerably from cancer in adults. These differences extend from diagnostic type which ultimately influences etiology, biology, and natural history, relative incidence and distribution, frequency, clinical presentation and manifestations, and response to therapy and outcome. In the past four decades, cancer has gone from a nearly uniformly fatal disease to one in which more than 75% of children can be expected to enjoy disease-free survival in excess of five years, and who are presumably cured of their disease. Pivotal to this success has been the results from hypothesis-driven clinical trials performed for the most part by paediatric cancer cooperative groups. Currently >90% of children <15 years of age receive their care in a paediatric cancer program which is a member of the Children's Oncology Group (COG) the single NCI-sponsored cooperative clinical trials group. Comprehensive multidisciplinary treatment has been instrumental in improving outcomes. Despite the dramatic improvements in outcome, cancer remains the leading cause of death from disease in children and adolescents. The search for the genetic origin of cancer has resulted in observations of genomic alterations consisting of point mutations, viral insertions, amplification, deletions, and rearrangements, each of which may alter the process of normal cell growth and development. Exploiting specific genetic alterations to design novel treatments for paediatric cancer is critical to the planned success in achieving or exceeding target childhood cancer survival rates in the next decade. Improved outcome for childhood cancer requires the coordinated integration of correlative biology studies with well-controlled clinical trials and the translation of basic molecular genetics to refinement of risk groups, development of risk-adjusted therapy, and ultimately therapy directed to specific molecular lesions.

2.14 Improving Treatment of Childhood Acute Lymphoblastic Leukaemia Therapy In Singapore

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Tremendous progress has been made in curing children afflicted with acute lymphoblastic leukaemia. In Singapore, the 5-year event-free survival (EFS) has improved from 5% in the 1960s to 80% in our recent 1997-2002 cohort. Further improvement in EFS can only come from more accurate tailoring of the intensity of therapy to the patients' predicted risk of relapse. This tailored therapy is extremely important because the excellent survival has revealed long-term toxicity of therapy on growing children who survives cancer therapy.

In our current multi-centre ALL study (MA-SPORE ALL 2003), we use the early response of therapy as the primary modality of disease stratification. Using highly sensitive markers of leukaemia cell, we can accurately measure the early response to therapy and accurately predict the patient's risk of relapse within 3 months of therapy. Our preliminary analysis showed that in standard risk patients (40% of patients), with good clearance of disease, a mild deceleration of therapy did not adversely affect the outcome while high risk patients, comprising of 20% of cases, further intensification appear to improve the EFS from 20% to 50%.

The MASPORE ALL 2003 study brings together an Internet-enabled data repository, multifaceted ALL study in children encompassing gene expression, proteomic and pharmacogenetic studies.

2.15 Disease modifier genes in thalassaemia

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Thalassaemia is the most common genetic disorder worldwide. Defects in the globin genes result in impaired globin chain synthesis leading to chronic anaemia and other pathology.

b-Thalassemia is clinically very heterogeneous in severity. The primary modifier include the broad spectrum of ?-globin gene mutations. Patients with mild b+-thalassemia genes are less severe than those with b0-thalassemia.The secondary genetic factors include those involved in the imbalance globin chain synthesis in the red cells. The coinheritance of a-thalassemia and genetic factors enhanced higher HbF production are the two major modifying factors. The tertiary genetic factors are those not related to the globin chain production but related to certain complications common in thalassaemia such as the genes involved in iron absorption, bone and bile metabolism. However, there may be some other uncharacterized genetic factors involved in the determination of different severity in thalassaemia.

We are searching for such modifying genetic factors in over 1000 b-thalassaemia/Hb E patients. Patients are divided into mild, intermediate and severe cases using strict scoring criteria.

Genome-wide search for disease modifying genes by SNPs analysis using MALDITOF technique in 200 patients, each with mild and severe cases. Most of these SNPs are located within 10 kb of 96 percent of the genes. After second stage replication screening we finally pick up around 500 SNPs that showed significant odd ratio and p value different among the mild and severe cases for confirmation by individual genotyping. We expect to find the SNPs that associate with disease severity within the near future.

2.16 Haemochromatosis: a paediatric disease

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Haemochromatosis, a genetic disorder of iron accumulation and overload, has traditionally been considered to be a single disease, predominantly occurring in older men. Homozygosity for a single genetic mutation, C282Y, in the HFE gene, identified in 1996, was initially thought causative in around 90% of adults diagnosed with haemochromatosis. The discovery of 4 other distinct genetic loci for iron transport proteins, also implicated in haemochromatosis, as well as new proteins involved in iron metabolism, has led to a revised classification with five distinct forms of haemochromatosis. In three of these five forms, iron overload may begin very early in life. Juvenile haemochromatosis is an especially severe early onset form of iron overload that may be life-threatening if not identified and treated early. Cardiac failure and hypogonadism are prominent in this form of haemochromatosis. Biochemical findings of haemochromatosis are characterised by high serum ferritin and transferrin saturation. However, an autosomal dominant form of haemochromatosis differs, in that transferrin saturation is usually not elevated. This presentation will define haemochromatosis, detail current concepts of iron metabolism, describe the expanded list of proteins of iron transport and regulation, and, using representative family case-histories, present the current classification of haemochromatosis. The importance of recognising and treating haemochromatosis in a paediatric environment will be emphasized.

2.17 Using pharmacogenetic data for dosage individualization in paediatrics

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Scientific evidence indicates that pharmacogenetics, the study of drug interactions with an individual's genetic makeup, may improve patient safety. Pharmacogenetic testing for some medications adds genetic information to the usual considerations of patient age, weight, disease process, use of other medications, health behaviors and environment. The additional information gained from pharmacogenetics can help physicians and nurse practitioners choose and dose medication that best meets the needs of the individual patient.

Current pharmacogenetic (PG) activities at Cincinnati Children's cover the areas of fundamental, translational PG research and a clinical Genetic Pharmacology Service (GPS). One of the ongoing NICHD sponsored studies is a Pharmacokinetics (PK) and Pharmacogenetics of Risperidone in Children with Pervasive Developmental Disorder (PDD). Risperidone is metabolized by CYP2D6 and CYP3A4 into an active enantiomeric metabolite mixture of (+)- and (-)-9-hydroxy-risperidone. One of the goals of the study is to evaluate the impact of CYP polymorphisms on risperidone PK, adverse events and efficacy in PPD patients participating in this population PK study. Preliminary data from this clinical study will be discussed. The Genetic Pharmacology Service (GPS) for children and adults at Cincinnati Children's Hospital Medical Center uses pharmacogenetics to customize patient care. This service was started mid 2004. GPS offers genetic pharmacogenetic testing for drugs metabolized by major cytochrome (CYP) P450 drug metabolizing enzymes (CYP2D6, CYP2C9, CYP2C19) and common variants of the thiopurine-S-methyltransferase (TPMT) gene. As of January 2005 a drug panel for psychiatric drugs is offered. Results are reported as phenotypes (poor, intermediate, extensive, and ultra metabolizer) and include a dosing recommendation based on the specific phenotype. Preliminary data and cases highlighting the impact of the service will be presented.

2.18 Tdm in paediatrics

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Accurate measurement and proper interpretation of drug concentrations (TDM) can greatly improve medical diagnosis and management of individual patients. TDM is useful for all drugs in some specific clinical situations. TDM is also almost always useful for those drugs with a well-defined relationship between concentrations and effects (either therapeutic or toxic), wide inter- or intra-individual differences in drug distribution or clearance, and no readily available method to clinically assess therapeutic or toxic effects. TDM has special value in paediatrics because of more rapid developmental changes, more limited communication, and greater dosing uncertainty in children versus adults. However, proper TDM requires much more than just measurement of drug concentrations. Proper TDM is a process by which accurate measurement of drug concentrations is combined with knowledge of pharmacokinetics and pharmacodynamics of the drugs as well as patient specific analytical and clinical data. Some important general principles of paediatric TDM will be discussed including some practical and theoretical differences between children and adults. Some specific clinical examples of antiepileptic drug (AED) monitoring will then be discussed. Antiepileptic drugs (AEDs) are a model of how properly done TDM can improve efficacy while decreasing toxicity. Some studies published over the past 25 years from our group will be reviewed to illustrate how properly collected, analyzed and interpreted TDM data can contribute to the use of paediatric AED's. These data demonstrate the wide inter-individual differences in AED clearance in children and illustrate why proper TDM requires more than simply reporting "numbers".

2.19 Population pharmacokinetics – a way forward for optimal dosage prediction in children

Susan E Tett.

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It is often difficult to obtain the accurate pharmacokinetic parameters in children that are essential for designing optimal dosage regimens. The number of blood samples that can be collected in neonates or children is limited (so called 'sparse' drug concentration-time samples) and accurate interpretation of these often depends on mathematical modeling. There are software packages available now, which enable accurate modeling of such 'sparse' data. The pharmacokinetic parameters obtained are specific for children (rather than extrapolations as 'mini-adults') and other factors can be explored (such as organ maturity and function, pharmacogenetics) to determine whether they explain any of the variability and need to be included in the subsequent dosage prediction. As an example, for some of the immunosuppressant drugs, tacrolimus and cyclosporine, pharmacogenetic differences between individuals leading to different forms of cytochrome P450 metabolising enzymes or P-glycoprotein drug transporters can now be identified. These may contribute significantly to between-subject variability and may be an important covariate to include in population pharmacokinetic modeling and dosage prediction to enable safe and accurate dosing of these medicines in children. A number of examples will be discussed in the presentation.

2.20 Problems with immunoassays – the expanding role of tandem mass spectrometry in the clinical laboratory

Steven J Soldin,

Children's National Medical Center and Georgetown University

We will discuss the author's experiences with immunoassays detailing some of their shortcomings. Assays involved in this discussion will include digoxin, carbamazepine, phenytoin, amphetamines, opiates, barbiturates, immunosuppressive drugs, hCG etc. The expanding role of tandem mass spectrometry in the clinical laboratory will also be addressed, covering the areas of steroids, thyroid hormones, immunosuppressive drugs and HIV/AIDS drugs.

2.21 Why are diagnoses missed in the newborn period?

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Inherited metabolic disorders are individually rare and yet collectively common. Their presentation in the newborn period shows marked heterogeneity, even within the same disease type and provides a significant challenge for the analytical laboratory offering a diagnostic service. Evidence obtained from the study of external quality assurance schemes together with observations from practice indicates that diagnoses can easily be missed. Data from the ERNDIM proficiency schemes and urinary organic acid scheme suggests that, when the biochemical features are clearly obvious, 100% of specialist laboratories will make a correct diagnosis. However, when the analyses are technically more demanding or the biochemical disturbances are more subtle, up 60% of cases may be missed. Evidence from the urinary organic acid scheme strongly supports the view that the actual performance achieved by individual laboratories shows a significant consistency year on year and that key factors related to workload, staffing and training tend to determine the effectiveness of the service provided. The style and content of the reports issued is also extremely variable and may be an important determinant of the clinical action taken in some cases. Using objective data, this presentation seeks to identify generalisable factors that predispose to diagnostic reliability in the detection of inherited metabolic disorders.

2.22 Childhood lead poisoning in asian countries and the steps taken to evaluate and prevent in the past ten years

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Lead poisoning in the Asian region is due to poor environmental and nutritional conditions. Lead poisoning has been one of the most neglected areas concerning public health policies in the region. Prior to 1995 population data on the blood lead levels was not available due to lack of reliable methodologies or the resources. Sources and pathways of lead were not known. Poverty in this region became the business opportunity to the developed world. Much of the secondary smelting of lead and simultaneous lack of awareness and its impact on health added to the seriousness of the problem. Air, water and land in cities were found to be polluted with lead and the use of lead for various purposes was on rise. Gasoline used till recently in some of these countries contained abnormal amount of lead and over 93% of paints used contained lead. The George Foundation (TGF) studies carried out during 1997-2000 in seven major cities in India the average blood lead levels in children below 12 years of age was found higher than 10mcg/dl resulting in reduced IQ.

Traditional medicines contained lead. Food was adulterated with a variety of lead salts. Since March 2000 unleaded gasoline (containing some lower amount of lead) was introduced in Indian cities as result of the study reports from the TGF. Newer governmental policies were put in place. Several laboratories were setup for the evaluation of blood lead with state of art methodologies such as DPASV. By 2005 environmental monitoring by the lead based industries were seen in addition to lead acid battery certification program to minimize the health hazard due to lead. Average blood lead levels in children moved downwards. Workers developed greater concern. Studies in the Indian region promised lead safe society in days to come.

2.23 Study on inherited metabolic disorders (imds) and need for neonatal screening in Singapore?

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In the early 1960s, studies were performed to identify the cause of hyperbilirubinemia, kernicterus and death common in neonates at that time. A high incidence of G6PD deficiency was found and led to the implementation of a screening program to detect deficiency states in newborn infants. In the early 1980s, studies were conducted to assess the thyroid status in newborn infants because of reports indicating the need for early detection of congenital hypothyroidism, an important cause for mental retardation. Subsequently, a program for routine screening of newborn was introduced in mid-1990.

The National Advisory Council of the Disabled has been concerned with the problems and welfare of the physically and mentally handicapped who are a serious burden and cause of severe stress not only to the family, but also to the society at large. The Council would like to know the cause of congenital disabilities and whether early diagnosis and treatment can be effective in preventing premature death and development of disabilities. Hopefully once diagnosis is known, early treatment and counselling of parents can be provided. Where possible, prenatal investigations may be provided for future pregnancies. At the recommendation of the Council, the Ministry of Health provided substantial research funds for the establishment of a national laboratory for the study of inherited metabolic disorders (IMDs) other than G6PD deficiency, thalassemias and hypothyroidism, which already have their respective screening programs. The primary objective was to determine the incidence and diversity of other metabolic disorders especially those leading to mental retardation, physical disability and other severe clinical consequences. The strategy for prevention of IMDs lies in the definitive identification of these diseases, genetic counseling for the affected families and prenatal diagnosis where possible. Results of the study would help determine the need for a neonatal screening program.

It is neither practical nor cost-effective to provide the full range of tests necessary for diagnosis of the extremely wide range of IMDs documented in the literature. Therefore we had to choose a repertoire of laboratory tests that is likely to give us maximum return of positive findings while incurring lowest possible cost. For the newly established national laboratory for IMDs, instruments and procedures were selected for diagnosis of IMDs affecting amino acid, organic acid and mucopolysaccharides metabolism. Existing laboratory facilities were able to diagnosis other selected disorders.

Results of our 13 years' experience are presented. A total of 3589 patients were investigated and 124 (75 males, 49 females, M/F ratio = 1.53) were found to have an IMD, giving a positive detection rate of 3.5%. The ethnic distribution of the positive cases is as follows: 68 (54.8%) Chinese, 24 (19.4%) Malayan, 14 (11.3%) Indian, 14 (11.3%) others, and 4 (3.2%) unknown. Compared with the current ethnic distribution (76.0% Chinese, 13.8% Malayan, 8.4% Indian and 1.8% others), there appears to be a higher prevalence of IMDs among the non-Chinese.

The distribution of IMD patients according to metabolic disorders is summarised as follows: 40 (32.3%) organic acidurias, 33 (26.6%) amino acidemias/acidurias, 13 (10.5%) urea cycle defects, 15 (12.1%) mucopolysaccharidoses, 6 (4.8%) carbohydrate disorders, and 17 (13.7%) others. The age at which the patient was referred ranged from 1 day to 56 years. Twenty-three (18.5%) manifested severe symptoms and were diagnosed during the neonatal period while 90 (72.6%) were diagnosed before puberty (=< 13years). Results show that IMDs are not uncommon in Singapore. An appropriate laboratory testing service and expertise in interpretation of test results are definitely necessary for their detection. In the absence of such a service, patients will remain undiagnosed or misdiagnosed. However, in view of the wide range of disorders, high cost compared with other tests, and limitation of existing instruments capability, the Ministry of Health has been hesitant in supporting a routine neonatal screening program for apparent healthy newborn infants.

Participation in external proficiency testing programs conducted by the Human Genetics Society of Australasia, Australasian Society of Inborn Errors of Metabolism and the College of American Pathologists have been helpful in evaluating not only our analytical performance but also improve our ability in interpreting results. It has enhanced our confidence in providing a good quality laboratory diagnostic service for our clinical colleagues. Our experience with these programs will be presented.

2.24 Maternal Graves' Disease And The Effects On The Newborn

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Approximately 2 in every 1000 pregnancies are affected by maternal thyrotoxicosis, and Graves' disease is the most common cause. Maternal thyrotoxicosis has significant impact on foetal and neonatal outcome, associated with prematurity, small for gestational age and intrauterine death. Uncontrolled hyperthyroidism, duration of thyrotoxicosis during pregnancy, TSH receptor antibody level, duration and age of onset of Graves' disease are risk factors. The abnormal thyroid functions seen in babies of thyrotoxic mothers include neonatal hyperthyroidism, transient neonatal primary hypothyroidism, and transient central hypothyroidism. The underlying causes of the various abnormal thyroid functions in the newborn include the free placental transfer of thyrotrophin stimulating hormone (TSH), receptor antibodies (stimulating and blocking), transfer of antithyroidal drugs, and excessive transfer of free thyroxine (fT4) across the placenta. Only 1 in 70 pregnant women with Graves' disease delivers an infant clinically affected with neonatal hyperthyroidism, which is usually transient and resolves when maternal antibody levels drop by 4-6 months. Transient primary hypothyroidism commonly occurs due to transfer of antithyroid drugs across the placenta, and is related to the last few weeks of the gestation. It is important to keep the maternal drug dose to the minimum so as to minimize the amount of anti-thyroid drugs transferred to the fetus in utero, which can induce fetal hypothyroidism.

Transient central hypothyroidism is a unique entity that is not widely recognised, manifested by low free thyroxine levels with inappropriately normal or low TSH levels in the cord blood, day 1 and day 5. The thyroid function of these infants will subsequently normalised over a variable period of time. It would therefore be prudent to continue L-thyroxine replacement therapy until 2 to 3 years of age to optimise neurological development.

2.25 Recent Advances In The Assessment Of The Growth Hormone-Igf- I Axis

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Disorders of growth hormone (GH) in childhood are characterized by short stature of varying severity, and slow growth caused by abnormalities in the GH and IGF-1 axis. Recent advances have identified abnormalities in the production, regulation, secretion and bioactivity of GH, as well as abnormalities in IGF-1 secretion and the IGF-1 receptor. Molecular abnormalities of the GH receptor are increasingly identified as the cause for the GH insensitivity (GHI) syndromes, which include the Laron dwarf, partial GHI, GH-GH receptor signal transduction failure, IGF-1 synthetic defects and IGF-1 receptor defects. These abnormalities have helped elucidate the physiological components governing normal growth.

The evaluation of growth failure encompasses clinical assessment and biochemical investigations. Since GH secretion is pulsatile, with the usual concentration being low, random GH samples are not helpful unless they are elevated. The dynamic tests of GH secretion use different stimuli to assess adequacy of GH secretion. However, these tests can be problematic in cases of moderate to milder forms of GH deficiency (GHD). The measurement of IGF-1 and IGFBP-3 for the diagnosis of GHD has been proposed as a functional bioassay, since there is no circadian variation. However, there is variability with regard to age, sex and nutritional status. Some children may have low IGF-1 levels with borderline GH responses to provocation tests, and may have some abnormality of the GH-IGF-1 axis, although they are not classically GHD. The identification of new genetic causes of GHD or GHI has broadened the range of etiologies responsible for GH disorders. Since the interpretation of classical endocrine tests is not always clear, analysis of appropriate candidate genes can contribute to a more precise definition of the pathogenesis of the growth disorder.

2.26 Advances in the diagnosis of neurometabolic disorders

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Inborn errors of metabolism (IEMs) result from the absence or abnormality of an enzyme or its cofactor, leading to either accumulation or deficiency of a specific metabolite. A child with an IEM most commonly presents with neurological symptoms and signs such as acute encephalopathy, hypotonia, seizures, developmental delay or regression.

This is not surprising as Mendelian disorders are most commonly expressed in organs derived from the ectoderm, such as the skin and the nervous system.Advances in genetics and metabolic testing have transformed the practice of paediatrics and neurology in the past 15 years. Formerly mysterious hereditable disorders are being genetically defined at a rapid pace. Several hundred genes responsible for neurological disorders have been localized and a growing number of these genes identified. Improved diagnostic abilities have actually broadened the clinical phenotype or spectrum of the diseases in question. This presentation will cover the clinical features of a variety of neurometabolic/neurogenetic disorders, as well as the general principles of metabolic and genetic testing. Some of the pitfalls a neurologist or paediatrician may encounter in specialized testing will also be covered. Special management issues may also arise as certain metabolic or genetic tests may have their own unique methodological and interpretative issues. The availability of genetic tests for various neurometabolic disorders has greatly simplified the diagnostic testing for these conditions. However, the ability to provide an accurate and specific diagnosis often moves clinicians into the realm of genetic medicine, where families and individual patients need care and counselling. Clinicians will have to become familiar with discussing the use of gene tests for predictive, prenatal and carrier testing, while appreciating the limitations of both the laboratory methods involved in testing and the interpretation of the results.

2.27 Early Detection: Challenges In Screening For Reduced Gfr In Children

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In the paediatric nephrology population we sought to determine:

a) which estimated glomerular filtration rate (eGFR) formula is most accurate,

b) the utility of eGFR formulae in detecting renal insufficiency, and

c) to consider an alternative to eGFR reporting: height and weight independent, age and gender specific creatinine reference intervals.

A retrospective review of all patients referred to the BCCH nephrology service over a five- year period identified patients with both a nuclear medicine measurement (99mTc-DTPA) of GFR (nGFR) and a serum Cr measurement (Vitros 950/250) were available. Age, sex, height, weight data and diagnosis were collected. Altogether 267 patients (473 paired nGFR/ creatinine measurements) were identified. Published and novel eGFR formulae were evaluated using the nGFR measurement as the gold standard. A model set (n=180) was used to develop laboratory specific constants for published eGFR formulae, to derive two novel eGFR formulae, and to establish age and gender specific creatinine 'cut-offs' which correspond to nGFR values of 60 mL/min/1.73m2. These formulae were subsequently compared in a validation set (n=87). A novel BCCH eGFR formula, the Schwartz formula, and the Counahan Barratt formula were all found to be reliable predictors of nGFR. In the validation group (n=83), these eGFR formulae agreed with nGFR results (within 30%) approximately 80% of the time and identified 86% of nGFR measurements that were < 60 mL/min/1.73m2 (specificity = 96%). Age and gender specific creatinine 'cut-offs' were as accurate and almost as specific (sensitivity = 87%, specificity = 93%).

In summary, these results confirm that eGFR measurements are effective at screening for renal insufficiency in the paediatric population. Furthermore, they are also effective tools for monitoring change in renal function over time. Age and gender specific creatinine cut-offs do not rely on height measurements and are a practical yet reliable substitute for eGFR reporting.

2.28 Advances in newborn screening

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The range of tests being screened for in the newborn period varies greatly from country to country. The main factors determining this include the disease prevalence, availability of technology and expertise as well as financial constraints. The common diseases that are currently screened for include congenital hypothyroidism, galactosemia, maple syrup urine disease, homocystinuria, biotinidase deficiency, haemoglobinopathies, congenital adrenal hyperplasia, cystic fibrosis, glucose-6-phosphate dehydrogenase deficiency, tyrosinemia, hearing loss and various other inborn errors of metabolism.

In the last decade, there have been many advances in newborn screening. There is a move to replace screening of aminoacidopathies by inhibition assays with tandem mass spectrometry. The latter will be covered in another talk. This talk will cover the evolving philosophy as to what constitutes a disease that deserves screening in the newborn period. It will cover the designs of the various programmes and their outcomes, the legal and ethical issues of informed consent versus informed descent, carrier detection and its disclosure and the storage of blood spot specimens.

2.29 What can the laboratory offer in neonatal intensive care testing?

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As Royal North Shore Hospital (RNS) is not a specialised children's hospital, our laboratory provides general biochemist and some specific tests for neonatal intensive care (NICU) and the Birthing Unit (BU). To meet required turn around times for neonatal specimens, some testing has moved from the central laboratory to the bedside by using blood gas analysers (BGA).

These are managed by the laboratory and measure blood gases, Na+, K+, Ca2+ and glucose and lactate. Bilirubin was recently added to the instrument in NICU because of the reported increase in the incidence of kernicterus.

Lactate measured on scalp samples is available in the BU to facilitate early detection of hypoxaemia. The BGAs are connected to the laboratory via an intranet, enabling laboratory monitoring of QC and instrument problems. The workload for these two instruments has increased nearly 300% in the past three years indicating the importance of providing this on-site service. We have assessed the comparability between the results from the BGAs and the laboratory instruments (Roche Modular and Dimension RxL) using samples collected within 30 minutes of each other. A bias greater than the allowable limits of error (ALE) for the Royal College of Pathologists of Australasia Quality Assurance Program was considered significant. The bias for Na+, K+, HCO3- and glucose were within an ALE. However, the bias of -17.7 for bilirubin was large enough to add a correction factor to the BGA in NICU, resulting in now acceptable comparison. The laboratory also manages the quality assurance program for the 7 blood glucose meters within the two units. An example of the laboratory providing direct support is the measurement of ammonia on a 24 hr basis. This is pivotal to diagnosis and management, as a moderately raised ammonia has generally signified immediate transfer to the specialised children's hospital.

2.30 Poct: the challenge of managing multiple locations

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A primary objective of running a multi-system for point-of-care testing (POCT) is to ensure uniformity of practice in all locations. Instead of having just the physician users of laboratory information, nurses and laboratorians of one institution getting involved in decisions, those from all locations need to become involved. While there clearly has to be a leader of the program, every institution must feel involved in making decisions. A single approach needs to be established with a committee with representatives from all the vested interests from all the institutions having overall responsibility for policy setting and performance monitoring. While much of a POCT Oversight Committee's work is focused on glucose point-of-care testing, the Committee should also function as a court of appeal to which individuals wishing to do point- of-care testing can go, when their request has been denied by the Director of the Laboratory where the requester is based. By maintaining control over what and where POCT is done, and by whom, the Director can ensure optimal use of resources and provision of the best laboratory monitoring for patients. With patients traveling from one facility to another of a Health System, it is necessary to ensure that all point-of-care laboratory data are integrated into a format that can be accessible from any site regardless of different medical record numbers. To minimize differences in practice, all potential point-of-care testers are screened for colour-blindness through a single web-based approach and a uniform approach is adopted to assess the skill levels of the testers, both through knowledge oriented web-based questionnaires and by self-assessment questionnaires oriented to actual practice. Other audits of actual practice are carried out by the various hospitals' Quality Improvement staff. A single approach is used to terminate the right of an individual or group to continue to perform POCT. A centralized Purchasing Department can be used to monitor and curb attempts by unauthorized users to do POCT.

In summary, these results confirm that eGFR measurements are effective at screening for renal insufficiency in the paediatric population. Furthermore, they are also effective tools for monitoring change in renal function over time. Age and gender specific creatinine cut-offs do not rely on height measurements and are a practical yet reliable substitute for eGFR reporting.

2.31 Expanded newborn screening using tandem mass spectrometry

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In Singapore, mass newborn screening is done for G6PD deficiency, congenital hypothyroidism and hearing impairment. The inborn errors of metabolism (IEM), though rare individually, are not uncommon when seen as a single group. Early identification of the metabolic disorder by screening and commencement of therapy before the baby suffers metabolic decompensation would prevent neurodevelopmental disability. Investigation of IEM has been revolutionised by the introduction of tandem mass spectrometry (MS/MS). For newborn screening, a small disk of 3 mm blood spot (equivalent to 3 micro litre of blood) from the neonatal heelstick filter paper is eluted, derivatized with butanolic hydrochloride, and redissolved in an appropriate solvent for injection into the TMS. Isotopically labeled internal standards are added to the sample during preparation. Samples are subjected to a soft ionization procedure (e.g. electrospray) and passed through the first quadruple, which separates them on the basis of their m/z ratios. The ions then enter the collision cell, where they undergo fragmentation. These smaller fragments are analyzed in the second quadruple, and data are interpreted with the aid of a computer. MS/MS can quantify the presence of butyl esters of amino acids and acylcarnitines in the same sample virtually simultaneously using different scan functions. A typical run assays 25 – 30 analytes in approximately 2 minutes, making MS/MS ideal for handling a high volume required for newborn screening.

The disorders that can be detected by MS/MS, include aminoacidopathies, organic acidaemias, fatty acid oxidation defects, carnitine cycle defects and urea cycle disorders. The predictive value IEM screening using MS/MS is quite good with sensitivity 96%, specificity 99.8% and recall rate between 0.2 – 1.3%. However, one limitation of the method is the inability to differentiate between metabolites of identical molecular masses. This is of major importance for leucine, which has three major isomeric forms.

For screening to merit adoption, as with all preventive services, health and economic benefits ought to outweigh programme costs. Cost-effectiveness has been demonstrated in screening programmes limited to specific disorders.

2.32 Adolescence: a challenge to the clinical laboratory

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Adolescence is traditionally viewed as a time of good health. In fact, during adolescence, a number of health problems can occur, some of which are more common today than they were 20 to 30 years ago. Laboratory professionals play a major role in identifying many of these conditions and, in doing so, lay the groundwork for appropriate treatment. I will discuss some of the more common of these conditions, including teenage pregnancy, sexually-transmitted diseases, adolescent-specific infections, drugs of abuse, obesity, eating disorders, accidents, and attempted suicides. I will show that adolescents are not as healthy as one would like to believe. I will discuss the role of the clinical laboratory, working closely with paediatricians, in assessing these problems and helping solve them.

2.33 Laboratory endocrine aspects of normal and disordered adolescence

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The endocrine system orchestrates the transition of a child through puberty and towards maturity and adulthood. The process is initiated by an increase in frequency and amplitude of pulsatile gonadotrophin releasing hormone (GnRH) resulting in an increase in luteinising hormone (LH) secretion with consequent elevation of sex steroid levels, elevated sex steroid levels act to augment growth hormone (GH) and insulin-like growth factor 1 (IGF-1) levels. Baseline levels of hormones and hormonal response to dynamic tests in healthy children are affected by a number of biological parameters, including age, gender, pubertal status, height, intake of nutrition, body composition, intercurrent illness and ethnicity.

Consequently distinguishing normal from disordered adolescence when interpreting baseline and dynamic test results requires care. The GnRH test can be used to differentiate between gonadotrophin dependent and independent puberty in a child presenting with precocious puberty. In gonadotrophin dependent puberty the LH response will be marked whereas in the prepubertal child or gonadotrophin independent puberty the LH response is limited or suppressed. If GnRH agonists are initiated to halt the progression of puberty the GnRH test is used to ensure adequate gonadotrophin suppression. In childhood a dynamic test of GH secretion may be difficult to interpret if the child is not primed with sex steroids (ie not started puberty) as stimulated GH levels may be naturally low prepubertally falsely suggesting growth hormone deficiency (GHD). Priming with exogenous sex steroids may allow a more accurate assessment of GH status. Retesting at final height is important to identify those that fulfill the criteria for GHD in late adolescence.

I Measurement of IGF-1 is used during the assessment of a child for the presence of GHD and to monitor the efficacy of GH replacement therapy. In either case, robust, normative data are required to allow IGF-1 values to be expressed as standard deviation scores, enabling comparison between individuals and assessment of change over time. However, the generation of such data requires the collection of samples from significant numbers of healthy children. It is imperative for the clinician to understand the performance characteristics and limitations of the IGF-1 assay used and to be aware of the source and quality of the control data. In conclusion the laboratory assessment of the endocrine system in adolescence is challenging. Any interpretation of laboratory results requires knowledge of normal adolescence and contributing biological factors, awareness of the limitations of the test or assay and where available comparison with good quality normative data.

2.34 Pathophysiology and laboratory diagnosis of cardiac diseases in the adolescent

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The heart and vascular system is not just a transport system. It is an integral part of the body. Systemic disease usually affects heart and circulation (e.g. sepsis, thyrotoxicosis). On the other hand pathologic changes of the heart or vascular system have a major impact on the body and therefore on laboratory results. A myocarditis for example presents elevated cardiac enzymes besides humoral active substances, such as brain natriuretic peptide (BNP). BNP is a marker for the competence of the heart and compensatory mechanism. BNP is often used to support the diagnosis of cardiac failure and its prognosis. Another humoral system, the renin-aldosterone-adrenergic system will also be stimulated by pulmonary hyper-circulation and decreased systemic circulation, as observed in large persistent ductus arteriosus. Similar changes have also been found in cardiac failure and been correlated to the overall prognosis. The interaction of circulation, heart and body is also found in valvular heart disease with high velocity flow. This can lead to a destruction of larger molecules such as the von Willebrand factor. Pulmonary hypertension with a decreased pulmonary capillary network can lead to similar changes, even without high velocity jets. The most extreme flow changes are seen in total-cavo-pulmonary-connections. Changes in pressure and flow in the caval vein and the pulmonary arteries often lead to increased liver enzymes, a decreased liver function shown in decreased liver dependend coagulation factors. A totally different approach offers a genetical work up in the diagnosis of "syndromes" with related cardiac disease. Laboratory values will not predict a cardiac anomaly or circulatory state on its own but can show the impairment of cardiac or circulatory changes. They are always an important adjunct in the clinical diagnostic work-up.

In summary, these results confirm that eGFR measurements are effective at screening for renal insufficiency in the paediatric population. Furthermore, they are also effective tools for monitoring change in renal function over time. Age and gender specific creatinine cut-offs do not rely on height measurements and are a practical yet reliable substitute for eGFR reporting.

2.35 Laboratory diagnosis and follow-up of changes in bone metabolism during adolescence

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Biochemical markers of bone metabolism have been used since about 15 years mainly in adults for the diagnosis and monitoring of metabolic bone diseases, however, the markers have gained increased acceptance by paediatricians too. Due to the rapid skeletal growth, the biochemical markers of bone metabolism are mainly used in paediatrics to measure the bone modelling and remodelling activity. In addition, rare genetic diseases show alterations in the concentration of markers of bone metabolism. Markers of bone formation: Alkaline phosphatase (AP) is the most widely used serum marker of bone metabolism. Until puberty the bone isoenzyme represents 77-87% of the total AP activity. The concentration of all formation markers increases in children around the age of puberty, and corresponds with growth spurts in both sexes. A disadvantage of the measurement of the bone isoenzyme of the AP (BAP) is the ~10% cross-reaction of liver AP in the BAP assay. In addition, the BAP reacts slowly, e.g. it takes months in children on glucocorticoids to detect decreases of the BAP, whereas with the other serum markers of bone formation, osteocalcin (OC) and procollagen type I N-terminal propeptide (PINP, a marker of collagen type I formation), in children on glucocorticoids decreasing concentrations are observed within days.

Markers of bone resorption:

The most widely used markers in paediatrics are:

- tartrate-resistant acid phosphatase 5b (TRACP 5b), a serum marker of osteoclast activity,

- ß-CrossLaps (CTX), a marker of collagen type I resorption (preferably measured in EDTA plasma), and

- pyridinium crosslinks (pyridinoline and deoxypyridinoline) preferably measured in a morning spot urine and related to urinary creatinine (or measured in a 24h collection).

Normal ranges in children and adolescents are given for the markers mentioned above.