

Speaker Abstracts

ChemP Pres 1: **Homocysteine and cardiovascular disease: Current status and questions.**

Professor Ottar Nygård, University of Bergen, Norway.

Observational studies have reported associations between circulating levels of the amino acid homocysteine (tHcy) and risk of cardiovascular disease (CVD), and several randomized clinical trials have been conducted recent years to evaluate the clinical effect of homocysteine lowering therapy.

Two such studies were conducted in Norway. In the Western Norway B Vitamin Intervention Trial (WENBIT, n=3.090) and the Norwegian Vitamin Trial (NORVIT, n=3.749), patients with ischemic heart disease were randomized to groups receiving 0.8 mg folic acid plus vitamin B12 0.4 mg daily or no such treatment, to assess whether they would benefit from lowered tHcy levels with respect to major adverse clinical events such as myocardial infarction, stroke, CVD death or all-cause death. Using a 2 x 2 factorial design, participants were also randomized to groups receiving vitamin B6 40 mg daily or no vitamin B6. In both studies, B vitamin treatment was not associated with the incidence of major adverse CVD events during in-trial follow-up. During extended follow-up of the combined NORVIT-WENBIT study population, treatment with folic acid plus vitamin B12 was associated with increased risk of long-term CVD mortality among participants with hyperhomocysteinemia at baseline. Exploratory analyses also showed that baseline tHcy was not independently associated with CVD outcomes, whereas tHcy measured after 1-2 months of folic acid plus vitamin B12 treatment was a strong predictor of in-trial major cardiovascular events. Vitamin B6 treatment was not associated with outcomes.

Our findings with respect to cardiovascular outcomes are consistent with the null effects of homocysteine-lowering B-vitamin treatment demonstrated in large randomized controlled trials to date. The data also suggests that elevated tHcy is associated with cardiovascular risk only when the hyperhomocysteinemia is not caused by B vitamin deficiency, and this is contrary to what would be expected if homocysteine has a causal role in CVD progression. Potential explanations for these findings will be discussed.

ChemP Pres 2 : **Determinants of Urinary Betaine Excretion**

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Abnormal urinary excretion of betaine has been demonstrated in patients with diabetes or metabolic syndrome. We aimed to identify the main predictors of excretion in cardiovascular patients and to make initial assessment of its feasibility as a diagnostic marker.

We used data from 2971 patients without severe renal failure participating in the Western Norway B-vitamin Intervention Trial (WENBIT), who delivered \geq two urine and blood samples at baseline and at 1–2 visits during a median 37 months follow-up. Betaine in urine and plasma were measured by liquid-chromatography-tandem mass spectrometry.

The strongest determinants of urinary betaine excretion by multiple regression analysis were diabetes mellitus, serum glucose, age and estimated glomerular filtration rate (GFR). Patients with diabetes mellitus type 2 had higher excretion than those without diabetes. In patients with diabetes mellitus type 2, betaine excretion showed age and gender adjusted associations with serum glucose, diuretic and diabetic medication, estimated GFR and plasma.

The discriminatory power of betaine excretion for diabetes type 2 was very good, and betaine excretion was stable during the follow-up period.

The high betaine excretion in diabetes and its positive association with GFR may reflect impaired renal tubular handling of betaine in diabetic patients. The good discriminatory power for diabetes and the high test-retest stability of betaine excretion should motivate further investigation of betaine excretion as a useful marker for diagnosis and long-term control of diabetes mellitus.

ChemP Pres 3 :Differential effects of Transcobalamin 67A->G and 776C->G polymorphisms on plasma concentrations of holo transcobalamin, Cobalamin, Methylmalonic acid and total homocysteine in a cohort of healthy middle-aged men and women

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Background: The transcobalamin (TC) polymorphisms *TCN2* 776C->G and *TCN2* 67A->G may affect the affinity of TC for cobalamin (Cbl), which in turn may influence cellular uptake and intracellular availability of the vitamin in terms of changed plasma concentrations of methylmalonic acid (MMA) and total homocysteine (tHcy).

Method: We studied the phenotypic effects of two common genetic polymorphisms in the TC gene, the *TCN2* 776C->G and *TCN2* 67A->G polymorphisms, on plasma holoTC in a subgroup of 2414 men and women (mean age 56 years) that had been selected on the basis of these *TCN2*-genotypes from a cohort of 10,601 healthy Norwegians, and evaluated its association with plasma concentrations of Cbl, MMA and total tHcy.

Results: Plasma holoTC was significantly lower in *TCN2* 67AG (54.7 pmol/L) and *TCN2* 67GG (45.5 pmol/L) compared with the AA genotype (62.0 pmol/L) (*P trend* < 0.001), but remained unchanged across the *TCN2* 776C->G genotypes (*P trend* = 0.18). The two polymorphisms interacted significantly as plasma holoTC determinants (*P trend* < 0.001), as plasma concentrations differed significantly across the composite genotypes. Plasma MMA was borderline significant higher in *TCN2* 67A->G variants (*P trend* = 0.06), and this effect was virtually mediated by low plasma holoTC. Plasma tHcy and serum Cbl were not altered by either polymorphism or by their interaction.

Conclusions: The genotype-related decrease in plasma holoTC was not accompanied by impaired intracellular Cbl availability, as indicated by no consistent increase in plasma MMA and plasma tHcy. This weakens the diagnostic utility of plasma holoTC as a diagnostic marker of Cbl deficiency.

ChemP Pres 4 : **MALDI-TOF MS based genotyping of common variants related to one-carbon metabolism**

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Several polymorphisms of genes involved in one-carbon metabolism have been reported. The associations of methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism with folate and homocysteine status are well documented, whereas data on the reported metabolic phenotypes of other polymorphisms are often based on small studies providing inconsistent results.

We have investigated the association between a panel of biochemical parameters (homocysteine (tHcy), folate, vitamin B₁₂ (cobalamin), methylmalonic acid (MMA),

vitamin B₂ (riboflavin), choline, betaine, dimethylglycine (DMG), cystathionine, cysteine, creatinine, methionine and vitamin B₆ (PLP)) and genetics variants related to one-carbon metabolism in several large-scale studies. In order to enable rapid screening for polymorphisms in candidate genes, we have developed a high-throughput genotyping platform based on MALDI-TOF MS. The panel of genetic variants includes: betaine-homocysteine methyltransferase (*BHMT*) 742G>A, cystathionine β-synthase (*CBS*) 844ins68 and 699C>T, cystathionine γ-lyase (*CTH*) 1364G>T, dihydrofolate reductase (*DHFR*) del19, nitric oxide synthase 3 (*NOS3*) – 786T>C and 894G>T, folate receptor 1 (*FOLR1*) 1314A>G, methylenetetrahydrofolate dehydrogenase (*MTHFD1*) –105T>C and 1958G>A, *MTHFR* 677C>T and 1298A>C, methionine synthase (*MTR*) 2756A>G, methionine synthase reductase (*MTRR*) 66A>G and 524C>T, reduced folate carrier-I (*SLC19A1*) 80G>A, serine hydroxymethyltransferase (*SHMT*) 1420C>T, transcobalamin-II (*TCN2*) 67A>G and 776C>G, and thymidylate synthase (*TS*) 1494del6.

We will demonstrate the design and features of our MALDI-TOF MS method and present the main findings from our largest metabolic phenotyping study, the Norwegian Colorectal Cancer Prevention (NORCCAP) cohort including 10.610 healthy subjects. This investigation confirmed the strong associations of *MTHFR* 677C>T with tHcy and folate, but also observed significant ($P \leq 0.01$) changes in metabolite concentrations according to other gene polymorphisms. These include *MTHFR* 1298A>C (associations with tHcy, folate and betaine), *MTR* 2756A>G (tHcy), *BHMT* 742G>A (DMG), *CBS* 844ins68 (tHcy, betaine), *CBS* 699C>T (tHcy, betaine, cystathionine) and *TCN2* 776C>G (MMA). In addition, quantitative analysis of allele signal intensities in MALDI-TOF MS demonstrated two separated clusters of heterozygotes for the *BHMT* 742G>A polymorphism in the WENBIT (Western Norway B-Vitamin Intervention Trial) study population. Significant different levels of DMG according to clusters of heterozygotes suggest a functional copy number variant in the *BHMT* gene.

ChemP Pres 5: Stability of metabolites related to one-carbon metabolism: implications for diagnostics and the design of population studies

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We have developed methods for the determination of metabolites, cofactors and biomarkers that are involved in one-carbon metabolism and used them to study chronic diseases in population-based studies. Because pre-analytical factors may influence the outcome of such studies, analyte stability should be investigated as part of method validation. We collected whole blood with and without anticoagulant from 16 men and women aged 32-72 years. Aliquots of plasma (EDTA, heparin and citrate) and serum were frozen (-80°C) immediately after separation, and after incubation for 1 hour to 8 days at 23°C. Samples were then thawed on ice and analyzed as one batch. LC-MS/MS, GC-MS/MS and microbiological assays were used to quantify 45 analytes, including B vitamins (folate, B₂, B₆ and B₁₂), homocysteine, methylmalonic acid, kynurenines and neopterin. For most, but not all, analytes stability was highest in EDTA-plasma. Results were consistent with a quantitative interconversion between B₂ vitamers.

Similar findings were observed for vitamin B6, and for 5-methyltetrahydrofolate, which could be recovered almost quantitatively as degradation products (hmTHF and pABG). Analyte instability may give rise to random and systematic errors that have implications for diagnostics and epidemiological studies. In multicentre studies, non-uniform sample treatment may introduce serious artifacts, which may affect study outcomes. For some analytes, sums of concentrations of vitamins, or concentrations of degradation products may be useful surrogate markers.

ChemP pres 6: **Urinary Kynurenine and Tryptophan Excretion and Long-Term Prognosis After Elective Coronary Angiography**

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Abstract

Coronary artery disease (CAD) and other manifestations of atherosclerosis are recognized as chronic inflammatory diseases in which activated macrophages and T lymphocytes are centrally involved. CD4 positive TH1 cells within atherosclerotic plaques synthesize the pro-inflammatory cytokine interferon γ (IFN- γ). IFN- γ stimulates conversion of the essential amino acid tryptophan into kynurenine by inducing the rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO). We have recently shown that plasma kynurenine:tryptophan ratio (KTR) predicts risk of future adverse events in patients referred for stable angina pectoris (SAP), participating in the Bergen Coronary Angiography Cohort (BECAC).

Atherogenesis is a generalized process. Indeed, glomerular and renal microvascular inflammatory changes are common in cardiovascular disease even without overt ischemic nephropathy. Despite this prevailing evidence, urinary biomarkers of inflammation have only to a limited extent been evaluated in patients with CAD.

We have assessed urine KTR as a predictor of long term prognosis in 2874 SAP patients who were included in the BECAC study and followed for a median of 55 months.

We first evaluated relations of urine KTR levels to clinical and biochemical characteristics of patients at baseline. Then, using cox proportional hazard regression and generalized additive regression, we assessed the association of urine KTR level to risk of clinical events during follow-up. Multivariable adjustment was performed to control for potential confounders. Finally, we evaluated the incremental prognostic value of this biomarker by calculation of areas under receiver operating characteristics curves and net reclassification improvement.

In the oral presentation at the Pathvine 2010 conference we will present major findings of this study and discuss interpretations of our results.

ChemP Pres 7 : Long and Short-lived Effects of Tobacco Smoking on Circulating Concentrations of B-vitamins

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BACKGROUND: Smoking is associated with decreased levels of several anti-oxidant vitamins. We sought to determine the relation between circulating levels of selected B-vitamins and smoking status, with particular attention to longitudinal associations.

METHODS: We used baseline data from two B-vitamin intervention trials that included 6837 subjects with ischemic heart disease. Smoking habits were ascertained by interview. Vitamins and metabolites, including the nicotine metabolite, cotinine, were measured in plasma or serum by microbiological assays or gas/liquid chromatography-tandem mass spectrometry.

RESULTS: The highest circulating concentrations of folate and pyridoxal 5ÿphosphate (PLP), and lowest total plasma homocysteine (a functional marker of folate status), were observed for self-reported never smokers, followed by ex-smokers, and current smokers ($P_{\text{trend}} < 0.0001$). Cobalamin, and its functional marker, methylmalonic acid, were not associated with smoking status. Based on their low cotinine levels, we were able to identify a group of smokers that had abstained from smoking for 3 days or more. Compared to smokers with high plasma cotinine, smokers with low levels had significantly higher circulating levels of folate, PLP, and riboflavin ($P < 0.005$), and this trend continued for ex-smokers with increasing time since smoking cessation.

CONCLUSION: Smoking lowered circulating levels of folate, PLP, and riboflavin, but concentrations increased significantly after a few days of smoking cessation. These effects may be related to acute smoking-induced oxidative stress. We suggest that the longer-lasting effects among ex-smokers may reflect changes in diet, and/or restoration of tissue-vitamin levels during the first few months to years after smoking cessation.

ChemP Pres 9: Association of the Integrin ð3 (PIA1/A2) Polymorphism with Body Mass Index (BMI)

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Aims: The platelet integrin ð3 P1^{A1/A2} polymorphism has been associated with obesity-related disorders such as type 2 diabetes and coronary artery disease (CAD). The aim of our study was to investigate whether the association of this polymorphism with diabetes is due to an effect on body anthropometry.

Methods: Anthropometric measurements were obtained from a total of 300 Indian subjects. This cohort included 70 diabetic subjects. Genotyping of the $\beta 3$ PI^{A1/A2} locus was accomplished using restriction fragment length polymorphism (RFLP) analysis.

Results: The presence of the PI^{A2} allele (heterozygote combined with homozygote) was not associated with an increased risk of diabetes (odds ratio [OR]=0.84 [95%CI, 0.39-1.80], p=0.65) compared with the PI^{A1/A1} genotype. However, the PI^{A2} allele was associated with an increased risk of overweight/obesity (OR=2.25 [95%CI, 1.11-4.57], p=0.02). The frequency of the PI^{A2} allele in lean subjects was 0.06 whilst in overweight/obese subjects it was 0.13 (p=0.01).

Conclusions: The $\beta 3$ PI^{A2} allele is not associated with an increased risk of diabetes but it is associated with an increased risk of overweight/obesity in Indian subjects. The polymorphism lies within the $\beta 3$ integrin section of the fibrinogen receptor. The mechanism by which this gene affects body weight is not known however, studies have shown that $\beta 3$ integrin is expressed within preadipocytes.

ChemP Pres 10: **Association of Leptin-melanocortin genes with obesity and waist circumference in South African learners.**

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Background: Obesity is a multifactorial disease that is rarely associated with mutations in genes of the leptin-melanocortin pathway. Due to their direct cause-effect relationships with morbid obesity, these genetic defects are relevant in the study of the mechanisms underlying the common polygenic obesity. The present study investigated the contribution of the leptin-melanocortin genes in the development of obesity in South African children and adolescents.

Methods: School learners (110 Mixed Ancestry and 79 Black Africans) were screened for the presence of *CART*, *GHRL*, *LEP* and *LEPR* polymorphisms by sequencing PCR amplified gene fragments. Genotype/allele association analysis was conducted to determine the effect of identified polymorphisms on the development of obesity.

Results: The following polymorphisms were detected *LEP* (19A>G, Lys36Arg, and Val94Met); *LEPR* (Lys109Arg, Gln223Arg, and Lys656Asn); *GHRL* (Leu72Met); *CART* (g.1281G>A, c.499delA, and g.517A>G). In an optimal model containing all polymorphisms, age, gender, and ethnicity, learners with the *LEPR*-Lys109Arg *g* allele had 0.51 odd ratio of being obese than the major allele carriers. The minor alleles (*c* and *g*) of the *LEPR*-Lys656Asn and *CART*-g.517A>G were associated with increased obesity (odd ratio= 1.77 and 5.98, respectively than the major allele carriers). After adjusting for age, gender and ethnicity, *LEPR*-Lys656Asn and *CART*-g.517A>G were individually (1.94 and 3.80 odd ratio, respectively)

significantly associated with increasing obesity, while the *LEPR*-109Arg lowered the waist circumference.

Conclusion: The present study identified polymorphisms in the leptin-melanocortin genes and their association with obesity (body mass index and waist circumference, confirming the contribution of more than one polymorphisms in weight regulation.

ChemP Pres 11: **Physical activity and insulin resistance in adults residing in a low-income community in Cape Town .**

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Insulin resistance has shown to be a precursor to a number of lifestyle-related chronic diseases and abnormalities in adults and is affected by a number of factors including genetics, age, physical activity and acute exercise, diet, obesity and body fat distribution and medication. Physical activity has shown to have marked effects on improving sensitivity to insulin through various physiological mechanisms and numerous correlational studies have identified a relationship between these two variables, suggesting the beneficial role exercise has on insulin resistance. This study aimed to identify a relationship between current levels of physical activity, physical fitness and insulin resistance in adults between the ages of 35 and 65 years of age residing in a low-income, previously disadvantaged community in Cape Town.

A total of 186 volunteers participated in this study aged 35 to 65 years of age and ranging from healthy individuals to those with diagnosed chronic conditions. Insulin resistance (determined by the homeostasis model assessment of insulin resistance [HOMA-IR]), physical activity (measured by the Global Physical Activity Questionnaire) and health-related physical fitness were measured. These five components included body composition, determined by body mass index (BMI) and waist circumference, cardiorespiratory fitness, determined by the 3-minute step test, muscle strength, determined by the handgrip strength, muscle endurance, determined by the crunch test, and flexibility, determined by the sit-and-reach test. Spearman correlation was used to identify the relationship between HOMA-IR, body composition and physical activity and fitness. Results indicated a weak relationship between age ($r = 0.15$), body mass index ($r = 0.78$; $r = 0.17$), waist circumference ($r = 0.71$; $r = 0.22$), GPAQ ($r = -0.07$; $r = 0.18$), cardiorespiratory fitness ($r = 0.19$; $r = 0.27$), handgrip strength ($r = 0.58$; $r = 0.11$), muscle endurance ($r = 0.28$; $r = 0.10$), flexibility ($r = -0.25$; $r = 0.27$) and insulin resistance for both men and women respectively. Significant correlations were only identified in BMI, waist circumference and handgrip strength for males and muscular endurance for females. This suggests that there are other potential mediators influencing this relationship; participants did not answer the questionnaire truthfully or lacked understanding of the questionnaire; or the HOMA-IR formula was not suitable for this population. Further investigation is recommended to identify possible causes for this weak relationship.

ChemP12: **Homocysteine and lifestyle factors**

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Plasma total homocysteine (tHcy) is influenced by a variety of lifestyle factors, including coffee consumption, smoking, physical activity, diet and intake of B-vitamins. A Western diet, rich in processed meat, pizza and snacks is associated with high tHcy whereas prudent diets containing fruits, vegetables and fish are associated with low tHcy. In general, healthy lifestyle factors reduce plasma tHcy concentration whereas adverse factors increase tHcy. B-vitamins, in particular folate and vitamin B12, are strong determinants of plasma tHcy, and changes in tHcy according to lifestyle are to some extent attributable to their effects on B-vitamin status. This has been most clearly shown for smoking and coffee consumption, which increase tHcy and lower folate, B6 and B2, but not B12. Notably, quantile regression analysis demonstrated tail effects with no difference in B-vitamin concentrations between coffee consumption categories at low vitamin concentrations but a progressive increase in the difference at higher concentrations. A complementary (and opposite) effect profile was demonstrated for tHcy. Recent data demonstrate that folate, B12, B6, B2 and betaine are interactive determinants of tHcy, and the effect size for one substance is largest when the levels of the others are low, and most pronounced in subjects with the MTHFR 677-TT genotype. This observation forms the basis of the concept of the B-vitamin cross-taking network. Thus, multiple lifestyle, nutritional and genetic factors interact and affect the concentration of tHcy, and among these folate status is a strong predictor. A combined analysis of two large intervention trials with folic acid in CVD patients (WENBIT and NORVIT) demonstrated that the tHcy level measured after one month of intervention was a stronger predictor than base-line tHcy of major adverse cardiovascular events (MACE; cardiovascular death, acute myocardial infarction or stroke) during trials, and cardiovascular mortality during extended follow-up. Future studies on tHcy and disease risk should place more emphasis on folic acid non-responsive fraction of tHcy.

ChemP Pres 13: **The Genetics of Obesity**

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Obesity is the classic example of a human phenotype that depends on both a genetic and an environmental input. Evidence for the genetic aetiology of obesity is overwhelming, with the identification of single gene knock-out mutations that cause morbid obesity, being one of the strongest. These mutations all seem to occur in genes that encode proteins that are expressed in the hypothalamus and which act to regulate appetite e.g. leptin, leptin receptor, proopiomelanocortin (POMC), and melanocortin 4 receptor (MC4R). However, these rare mutations explain very little of the variance observed in body mass index (BMI) in the human population, strongly suggesting that BMI is a polygenic trait. Efforts to find the gene polymorphisms that explain the majority of this variance have recently been boosted by rapid advances in genetic screening methodologies. These new techniques allow millions of single nucleotide

polymorphisms (SNPs) to be genotyped in a single blood sample. This has led to the identification of a number of new genetic loci that may contribute to determining adipose tissue mass. The most thoroughly investigated of these polymorphisms is that at the FTO locus. The protein coded by this gene is a DNA demethylase and has been shown to be expressed in many tissues, including the hypothalamus. Along with FTO, a number of other gene polymorphisms have been confirmed to contribute to body fat mass in genome wide association studies (GWAS) performed in large cohorts of human subjects. The identified genes include those expressed within the brain and within adipocytes, suggesting that the aetiology of polygenic obesity involves changes in both appetite regulation and adipocyte metabolism. Despite the success of GWAS in identifying polymorphisms associated with obesity, these genetic factors still explain only a small percentage of the variation in human body adiposity. The continued search for the 'missing' large effector gene variants will no doubt consume the efforts of geneticists for some time to come!

ChemP Pres14 : **MODY in Africa – does it exist?**

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Maturity-onset diabetes of the young (MODY) is the term given to a group of autosomal dominantly inherited disorders of non-insulin-dependent diabetes with onset typically before the age of 25. These are the most common forms of monogenic diabetes, accounting for an estimated 1 – 2% of all diabetes cases. MODY is often misdiagnosed as either type 1 or type 2 diabetes.

Heterozygous mutations in at least six different genes have been identified to date as causative factors of MODY. Identifying cases of monogenic diabetes is important as it directs appropriate treatment: some MODY patients need no treatment, whereas others are very sensitive to low dose sulphonylureas.

MODY seems to have a worldwide distribution and many studies have been published on MODY mutations found in France, Britain, China and Spain. To our knowledge such studies have not been reported in South African or African populations. Although some genetic studies were conducted in South African Black and White diabetic, none have reported on monogenic types of diabetes.

The international Diabetes Federation (IDF) Diabetes Atlas reported a prevalence figure of 3,4% for the 24 million South Africans between the ages of 20 and 79 (2003), with an expected increase to 3,9% by 2025. Since recent studies suggested that 1 – 2% of diabetes cases are due to monogenic causes and that 2 – 5 % of patients with Type 2 diabetes may in fact have MODY, it is obvious that even though no data is available on MODY in South Africa and Africa, it does indeed exist.

We are therefore forced to ask the question: Where are all the MODY patients we should be diagnosing? The most logical answer is that patients identified with possible MODY cannot be diagnosed because genetic testing for MODY causing mutations are not readily available.

ChemP Pres 16: **Investigation of an atypical protoporphyric family in South Africa**

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Background: Erythropoietic protoporphyria (EPP) results from an inherited, partial deficiency of the final enzyme in the haem pathway, ferrochelatase, which facilitates the incorporation of ferrous iron into protoporphyrin IX to form haem. Here we investigated members of a South African family who presented with photosensitivity and raised red cell protoporphyrin levels. Although a diagnosis of EPP was considered, atypical features were noted. These included highly penetrant disease, more severe liver involvement, a preponderance of protoporphyrin in its zinc-chelated (as opposed to free) form, a therapeutic response to iron supplementation, and an absence of mutations in the ferrochelatase gene. Objectives & Methods: This form of atypical porphyria was investigated by reviewing selected clinical and biochemical data from this family and establishing a fluorometric ferrochelatase enzyme assay to measure ferrochelatase activity in subjects and controls. Results: We present results associated with porphyrin accumulation in tissues of affected family members. The assay was successfully established in our laboratory and no significant difference was noted between the ferrochelatase enzyme activity of affected subjects and controls, suggesting that the defect is located elsewhere. Discussion: Whilst this study was in progress, a collaborative effort between colleagues and our laboratory led to the discovery of gain of function mutations in the X chromosome erythroid specific 5-aminolevulinic acid synthase 2 (ALAS2) gene, catalysing the first committed step in haem biosynthesis. These were disease-causing in our family and in other (European) families with similar atypical features. We present data from further mutational analysis of ALAS2 from the local family. Conclusion: The atypical features observed in the studied family are in keeping with a disease locus distinct from ferrochelatase and is confirmed by the normal ferrochelatase enzyme activity recorded. The ability to detect the ALAS2 disease-causing mutation allows us to assist in clinical assessment and explain the pathophysiology of the disease.

ChemP Pres 17 : **Case report: Undetectable serum ALP activity in a patient with 'Wilsonian crisis'**

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A 21-year-old woman presented to her general practitioner, with a 3-day history of general malaise, upper abdominal pain, nausea and vomiting. On examination she appeared acutely ill, with jaundice, tenderness over the right hypochondrium, but no neurological signs. She had previously been well and there was no history of risk factors for, nor family history of liver disease. Viral hepatitis was considered to be the most likely diagnosis, but rapid clinical deterioration necessitated emergency hospital admission a day later. Serology for acute Hepatitis A, B and EBV was negative, and FBC revealed a macrocytic anaemia. Liver function tests demonstrated hyperbilirubinaemia, raised GGT and AST with normal ALT. Serum ALP was notable for being undetectable at less than 5 IU/L. Haemolysis was suggested by an elevated serum LDH and low haptoglobin. The combination of haemolysis and low ALP alerted the clinician to the possibility of Wilson's disease (WD). The diagnosis was subsequently confirmed by finding a >50-fold elevation in urinary copper excretion of 29.8 umol/day (normal <0.55), and cirrhosis with hepatocyte copper deposition on histological examination of a liver biopsy specimen. A clinical diagnosis of 'Wilsonian crisis', comprising fulminant hepatic failure and acute haemolysis, was made. Following treatment with plasmapheresis, the patient recovered sufficiently to be discharged from hospital. She continues to receive chelation therapy and will be worked up for orthoptic liver transplantation. Both liver disease and haemolysis in WD is believed to be due to oxidative damage by reactive oxygen species, generated as a result of copper excess. Low ALP has similarly been ascribed to oxidative damage by hydroxyl free radicals. These aspects will be further elucidated in the case discussion.

ChemP Pres 18 : **Assessment of CD26 and ADA as surrogate markers of the activation status of the immune system in chronic HIV-infection as correlates of risk of disease progression**

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Recent studies have highlighted that chronic immune activation and inflammation are key driving forces behind the loss of CD4 T cells and progression of the disease. During acute HIV-infection, the GIT mucosal integrity is disrupted which results in ongoing translocation of bacterial products such as LPS across the epithelial lining. This provides a source for constant priming of the immune system and a chronic pro-inflammatory environment ensues. Cost-effective and clinically applicable markers of inflammation will therefore add value to the CD4 count and viral load tests. Chronic inflammation is closely associated with tissue hypoxia. Adenosine, an inflammatory regulator, is released into the extracellular environment from inflamed

and hypoxic tissues and inhibits inflammation via specific receptors, A2AR, on immune cells. A2a receptors are up-regulated during inflammation through cytokines such as TNF- α and IL-1 α as well as the bacterial product, LPS, and exhibit a strong anti-inflammatory and immunosuppressive effect. Adenosine has a short half-life and is rapidly catabolized by the enzyme adenosine deaminase (ADA) to inosine. ADA is localized extra-cellularly to the cell surface via binding to its receptor CD26. During hypoxic states, there is increased production of both CD26 and ADA. Although an acute increase in adenosine levels prevents excessive inflammation through its anti-inflammatory action, chronically elevated levels may be harmful. ADA plays an important role in limiting chronically elevated levels of adenosine. This pilot study will correlate levels of circulating ADA with levels of expression of CD26 on CD4 T-cells in 30 patients with HIV-infection versus 20 uninfected matched controls. These levels will in addition be correlated with CD4 counts and viral loads. We postulate that surrogate markers of immune activation, such as ADA and CD26, may be implemented to identify patients in need of earlier access to treatment despite acceptable CD4 counts or viral loads.

ChemP Pres 19: **Discovery proteomics-Application to HIV infection**

Prof T.S. Pillay
Dr D. Haarbarger

The (re)application of proteomic techniques in the 1990s has provided the researcher with a set of new tools to help understand biological processes at the molecular level. The HIV pandemic has had a major impact, especially in Africa. Although tremendous progress has been made over the years, understanding of the pathogenesis and treatment of the infection is still not optimal. It is therefore logical to ask if there is a role for proteomics in increasing our understanding of HIV or in the management of HIV patients?

Proteomic methods can be applied in various ways to study HIV. The proteome of the virus itself can be analysed. Cells infected with HIV can be compared with normal cells to determine the effect on intracellular proteins. The cell culture medium of cultured HIV-infected cells can be analysed (the secretoome), or plasma can be used to assess whole body changes in HIV infection. The various proteomes can be monitored for changes in response to infection or to changes related to treatment.

In this presentation, I will review examples of research into HIV infection performed using proteomic methods and to illustrate the diversity of the research. I will then present the results of research from our laboratory into the identification of new biomarkers in the serum of patients infected with HIV. In our study we used proteomic methods to analyse the serum of stage I HIV positive subjects, in order to identify novel protein changes that are induced in the human serum proteome following HIV infection. We analyzed serum from HIV positive and HIV negative groups by 2D-gel electrophoresis. 7 659 spots were detected and 562 matches were made by mass spectrometry and database searching. 11 spots were found to be

significantly different between the two groups. Nine spots were significantly decreased and two spots were significantly increased in the HIV positive group compared to the HIV negative group. The positive identification of these markers provides the basis for future studies where changes in these can be analysed sequentially as part of clinical laboratory monitoring of the progression of this disease.

ChemP Pres 20: Anti-retroviral therapy- getting to the HAART of the problem.

J A George

Antiretroviral therapy (ART) has resulted in substantial improvement in the mortality and morbidity associated with human immunodeficiency virus (HIV) infection. As this population ages, cardiovascular disease is becoming an increasingly important health burden. Many factors are involved in the development of this problem, with traditional risk factors (smoking, dyslipidaemia, diabetes, family history, hypertension) the main contributors. ART and HIV itself can modify the risk of cardiovascular disease through effects of dyslipidaemia and insulin resistance; there is some evidence that it may have effects on atherosclerosis and endothelial dysfunction. Several antiretroviral drugs have been shown to increase cardiovascular risk. As newer therapies become available the cardiovascular impact of these will need careful evaluation. Current published guidelines suggest regular monitoring of cardiovascular risk both before and after commencing ART. Existing risk assessment tools have not been fully validated in an HIV setting and need to be used with caution. Lifestyle modification and pharmacological intervention are important management strategies. Initiating or switching to, ART with a lower potential for metabolic derangement should also be considered.

ChemP Pres 21 Free light chains and human Immunodeficiency virus

**Zemlin, Department of Chemical Pathology, National Health Laboratory
Annalise E Services and the University of Stellenbosch**

South Africa is currently facing an enormous challenge with its Human Immunodeficiency Virus (HIV) and tuberculosis epidemics. Sub-Saharan Africa has the highest prevalence of HIV in the world and South Africa carries a large percentage of the global HIV burden.

HIV infection induces immune dysfunction with abnormalities in both cellular and humoral immune systems. Infection with HIV is associated with a gradual decline of CD4+ T cells as well as several abnormalities of B cells. HIV is associated with numerous B-lymphocyte abnormalities; monoclonal gammopathies and non-Hodgkin lymphoma being two of them. The B-cell abnormalities may be due to inappropriate communication between CD4+ T-cells and B-cells amongst others. A decrease in CD4+ T-cells is one of the markers of HIV infection and gives an indication of when to start antiretroviral therapy. Monoclonal gammopathies refer to an immunoglobulin molecule produced by a clone of plasma or B-cells. HIV-infected persons have been

found to have an increased incidence of monoclonal gammopathy when compared to the general population.

Antibody molecules consist of two identical heavy and light chains, each containing variable and constant domains. The variable domains of each light chain/heavy chain combines with the antigen binding site. Light chains are of 2 types, kappa (κ) and lambda (λ), and any given antibody has either light chain but not both. Increased free light chain levels and an abnormal ratio are due to imbalances in heavy and light chain products. Plasma proliferative disorders that produce monoclonal gammopathies also have increased free light chain concentrations and an abnormal free light chain ratio. Although HIV is associated with an increased incidence of monoclonal gammopathies, there is a paucity of data available studying free light chains in HIV-infected subjects.

Chemp Pres 22: Point of Care Testing for Pediatric and Adult Patient Care: Challenges and Opportunities

**Sharon Geaghan, M.D. Chief, Pathology
Lucile Packard Children's Hospital at Stanford
Co-Director, Stanford Hospital and Clinics Clinical Laboratory Services
(Pediatrics)**

Needs Assessment:

The Point of Care testing arena is one of the two fastest growing areas in laboratory diagnostics, according to industry analysts. Increasingly, laboratorians are being asked to entertain requests for point of care testing, analyze technical performance characteristics as compared to conventional laboratory testing, and do so for a variety of specialized applications. Outcomes-based research is available, but is not keeping up with the explosion of technology. This model of distributed laboratory services brings forth a host of considerations, such as accuracy; connectivity and informatics support; cost-benefit analyses and health economics.

Learning Objectives:

The participants will learn how to analyze requests for Point of Care testing using defined criteria; learn how to approach analytic performance requirements for Point of Care testing; become familiar with a wide variety of applications for Point of Care testing; see examples of outcomes research in Point of Care testing; be provided a framework for cost-benefit analysis for Point of Care; and be made aware of key trends in, and the importance of informatics in Point of Care diagnostics. This session will bring together the current knowledge in the field and practice experience so that laboratorians can insure a sound and successfully implemented Point of Care testing program in their practice setting.

Expected Outcomes:

At the end of this session, the participants will be able to:

- 1) Identify the principles of assessing Point of Care tests as alternatives to conventional laboratory testing;
- 2) Define an approach to analytic performance

requirements for Point of Care testing ; 3) List the principal applications of Point of Care testing in a variety of settings, both inpatient and outpatient, as well as specialized settings such as patient transport, emergency department, operating room, ICUs, sports medicine, primary care and specialty clinics ; 4) Identify outcomes-based practices in Point of Care testing ; 5) Determine an approach to analysis of the health economics aspects of Point of Care Testing ; 6) List key principles of informatics in implementation of Point of Care testing.

ChemP Pres23: Cardiac Troponin T Quantitative Assay Failure due to Antibody Interference

Philip Fortgens UCT/NHLS

Fierdoz Omar UCT/NHLS

Cardiac troponin is widely used as a specific and sensitive marker of myocardial injury. Measurement of cardiac troponins is achieved by immunoassay which, despite extensive experience with this methodology, is still subject to interfering substances compromising accuracy. We report a case of assay failure using the CARDIAC Troponin T Quantitative reader (Roche Diagnostics).

A 61-year-old female was seen in the emergency unit with chest discomfort, prompting a request for cardiac troponin T (cTnT) levels. Repeated attempts at obtaining a cTnT measurement, using the Roche CARDIAC Troponin T Quantitative cardiac reader, failed, as evidenced by the absence of a positive control line on test strips. This analyser utilizes the sandwich principle on a test-strip using two murine monoclonal anti-cTnT antibodies. The presence of an interfering immunoglobulin G was identified by protein A-Sepharose affinity chromatography, as the cause for this measurement failure. This autoantibody was not heterophilic in nature, but was most likely directed against cTnT. Further investigation showed no interference by this autoantibody on the Roche Elecsys E170 automated troponin T platform, which also uses two murine monoclonal antibodies. To the best of our knowledge, this is the first report of interfering substance-mediated cTnT assay failure on the CARDIAC Troponin T reader platform.

ChemP Pres 24 : Inaccuracy identified by external quality assurance – a case report

Kuti M, Roux P, Hoffman M, Erasmus RT

Chemical Pathology, NHLS Tygerberg and University of Stellenbosch

Participation in external quality assurance schemes is a must for all laboratories. It is a important requirement by accreditation agencies. The Tygerberg Academic Laboratory subscribes to the Clinical Chemistry programme of Thistle QA. The scheme involves the analysis of certified reference materials by participating laboratories. The performance of the laboratory is assessed by the proximity to the certified value and the percentage deviation and the precision of test results.

The report of cycles 39 - 41 showed that TAL results for ALT exceeded the +3SD limits on numerous occasions. This was in comparison with the appropriate method class. Results for all other parameters were all less than 2SD. An in-house troubleshooting check was carried out over several months. Notably, the internal quality control procedures showed no bias nor increased imprecision. The manufacturer was contacted. The review by the manufacturer revealed that the reagents of the ALT which came in pairs were not being replaced appropriately: one of the reagent pairs was found to exhaust earlier and the practice in the laboratory was to replace only the exhausted reagent, leaving its pair on board. The practice in the laboratory was changed to replace reagents in pairs. Subsequent to this, the results from the EQA have ranged consistently below the +3SD limits.

The above case illustrates the unique ability of external quality assurance programmes to monitor the ongoing accuracy of laboratory analysis. They may serve to expose inappropriate methodology practices which can be amended resulting in improved quality service delivery.

ChemP Pres25 : **Renal insufficiency does not affect levels of ischaemia modified albumin**

Angidi Pillay Mauree Medical Student, UCT

John Christian Stanfliet UCT, Division of Chemical Pathology and NHLS

Tahir Pillay UCT, Division of Chemical Pathology and NHLS

Aim Our aims were to assess whether the presence of renal insufficiency significantly altered the levels of ischaemia modified albumin (IMA).

Introduction IMA is currently being investigated as a potential marker of myocardial ischaemia in patients presenting to Emergency Departments with chest pain. It performs very well as a “rule-out” test for suspected acute coronary syndrome. However, some aspects of its basic pathophysiology remain unknown. This includes the impact of renal insufficiency on IMA levels.

Methods We examined a total of 44 samples. 19 were from patients adjudged to have renal insufficiency, and 25 were control patients. The levels of IMA were analyzed using a well-described cobalt binding assay. Creatinine, albumin and urea levels were measured on the Roche Modular.

Results Creatinine, urea and albumin levels were significantly different between the two groups. There was no increase in IMA levels in patients with renal insufficiency even though they had lower albumin levels. After adjusting for albumin, there still was no difference in IMA levels between the two groups.

Conclusions IMA levels are not increased in people with renal insufficiency.

ChemP Pres 27: **Low anti-oxidised LDL antibodies in individuals with hyperglycaemia.**

TE Matsha¹, **MS Hassn**¹, **Esterhuyse J**¹, **Erasmus RT**².

¹ **Biomedical Sciences and Nursing/Radiography, Cape Peninsula University of Technology, Cape Town, South Africa.** ² **Chemical Pathology University of Stellenbosch, Cape Town, South Africa.**

Background: Circulating oxidized low-density lipoproteins (OxLDLs) are associated with subclinical atherosclerosis in adults, particularly those with hyperglycaemia whilst, antioxidants (anti-OxLDL) antibodies have been suggested to be protective against its development. We measured the levels of anti-OxLDL in hyperglycaemic and normoglycaemic individuals.

Methods: From an epidemiological study that screened a high risk urban population for diabetes, 98 hyperglycaemic and 79 normoglycaemic subjects were selected for this study. An enzyme immunoassay was used to determine human IgG autoantibodies against OxLDL. Oral glucose tolerance test, anthropometric variables, blood pressure, hs-CRP, urine cotinine, and lipids were performed on all the subjects.

Results: Significantly lower anti-OxLDL were observed in the hyperglycaemic group compared to controls, $p = 0.02$, particularly the diabetic group, $p = 0.0005$. The anti-OxLDL showed significant negative correlation with hs-CRP, age, plasma glucose levels, total cholesterol and triglycerides. In hyperglycaemic subjects, further negative correlations were found between anti-OxLDL and urine cotinine and LDL-cholesterol.

Conclusion: Hyperglycaemia is consistently associated with oxidative stress, consequently leading to an increased risk of cardiovascular diseases. The oxidation of LDL is an important mechanism leading to atherosclerosis. Our results suggest that in hyperglycaemic subjects, low anti-OxLDL may play an important role in the development of atherosclerosis.

ChemP Pres 28: **Characterization of interference by Efavirenz in urinary screening immunoassays for Tetrahydrocannabinol**

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Aims: Therapy with the antiretroviral drug, Efavirenz (EFV), has been shown to yield false positive results for tetrahydrocannabinol (THC) by several urine screening immunoassays. In the literature, this interference has been attributed to EFV-8-glucuronide (EFV-8-G), one of two major urinary metabolites. The aim of our study was to characterize EFV cross-reactivity using immunoassays frequently employed for testing in South Africa.

Methods: Random urine samples were obtained from thirty patients attending the antiretroviral clinic at the Tshwane District Hospital. All participants were over the age of 18 years and had been taking a daily dose of 600 mg EFV for at least 14 days. Samples were analyzed for THC metabolites by two POCT devices (ACON Laboratories and Rapid Response Drugs of Abuse test strips) and two automated

immunoassays (Roche Diagnostics Cannabinoids II and Beckman Coulter SYNCHRON systems THC2). THC confirmatory testing was performed by gas-chromatography mass spectrometry (GC-MS).

Results: Using the Rapid Response test strips, 28 out of 30 samples yielded positive results. All results generated by the ACON test strips and automated immunoassays were negative. THC metabolite concentrations measured by GC-MS were undetectable in all samples, confirming that the Rapid Response test strips had yielded false positive results. On re-analysis of samples by the Rapid Response test after addition of glucuronidase, the positive results reverted to negative, supporting the hypothesis that interference was due to EFV-8-G.

Discussion: Given the low molecular weight of cannabinoids, linkage to carrier proteins is required in order to produce effective immunogens for antibody generation. By manipulating the position of carrier protein linkage, antibodies with variable selectivity for different parts of the THC molecule can be generated. This may be an important contributor to EFV-8-G cross-reactivity in some immunoassays.

ChemP Pres29: **A Complicated case of Hyperemesis Gravidarum**

Careen Hudson NHLS Tygerberg/University of Stellenbosch

Lourens Jacobsz NHLS Tygerberg/University of Stellenbosch

Rajiv Erasmus NHLS Tygerberg/University of Stellenbosch

A 22 year old nulliparous female at 12 weeks, presented to the Tygerberg Obstetrics admissions department complaining of severe nausea and vomiting. Her symptoms had worsened progressively over a two week period and during the past 48 hours she remarked on a noticeable yellowing of her skin and eyes. She had no abdominal pain but on systemic review did report occasional palpitations, sweating and poor heat tolerance. Examination was unremarkable apart from a tachycardia and obvious scleral jaundice. Her liver was not palpable and she had no external features of autoimmune thyroid disease. A bedside ultrasound was performed which confirmed a single, viable intra-uterine pregnancy with no evidence of molar tissue.

Biochemistry revealed a low potassium (2.8 mmol/L), and mild dehydration (urea 6.1 mmol/L, creatinine 68 umol/L). Liver functions were deranged with a hyperbilirubinaemia a (92 umol/L) which was predominantly conjugated (82 umol/L). ALP was 78 U/L, GGT 152 U/L, ALT 481 U/L and AST 251 U/L. The patient was thyrotoxic with a free T4 of 65 pmol/L, and a TSH of 0.02 mIU/L. Her hCG was markedly elevated at 350 753 IU/L. TSH receptor antibodies proved negative as did a hepatitis screen. The patient was treated conservatively with fluids, potassium replacement and anti-emetics. Her condition rapidly improved. One week following admission her free T4 had dropped to 15.1 pmol/L. Serum bilirubin had normalised, although transaminases remained moderately elevated (ALT 215 U/L and AST 87 U/L). Her hCG had fallen to 215 488 IU/L.

What follows is a discussion on the possible aetiologies of hyperemesis gravidarum and an exploration of the pathophysiology behind the complications experienced in our case. Through a better understanding of these mechanisms it is hoped that we may

discover novel forms of treatment and prevention of this common complication of pregnancy.

ChemP Pres 30: Audit of Inherited Metabolic Disease in South Africa and Screening Approaches applicable to the South African Healthcare Environment

George F van der Watt ,Red Cross Childrens Hospital, Division of Chemical Pathology, NHLS and University of Cape Town, Cape Town, South Africa.

This presentation will discuss the spectrum of inherited metabolic disease (IMD) seen in South Africa with an emphasis on disease profiles prevalent in different population groups. Current data suggests that the black African population in Southern Africa has relatively low genetic diversity with high carrier frequencies for a small number of mutations representing relatively few disorders that are not well recognized or managed by current healthcare systems. This is largely due to the historical and current contrast in the quality and accessibility of clinical and laboratory services available to different population groups in South Africa. Two of these disorders, namely glutaric aciduria type 1 and galactosaemia are especially prevalent and of particular concern as major morbidity is preventable at low cost in both disorders, provided a diagnosis is made soon after birth. Strategies to expand screening for these and other disorders in South Africa will be presented and discussed in the light of these disease profiles together with the current major constraints on healthcare spending and critical infrastructure shortcomings present in this developing country.

ChemP Pres 31: The Case For Newborn Screening

Cartwright JD and Knoll DP.

ChemP Pres 31: The Case For Newborn Screening

Cartwright JD and Knoll DP.

Screening identifies a disease before the complications of that disease develop. Many successful screening programmes have benefited communities, but newborn screening takes it to another level, of diagnosis and intervention before signs and symptoms have developed, prevention of complications resulting in a normal life. It has been described as the greatest advance since the vaccination programmes that have saved so many lives. Every community has 1 to 2 affected infants per 1000 live births but the disorders encountered differ between the communities. The criteria of Wilson and Junger for screening are adequately fulfilled in a correct NBS programme. NBS is safe, widely accepted by parents, and cost effective. The technique of obtaining the dried blood spot is easily learnt, and the introduction of the Tandem Mass Procedure into laboratories has enabled the public health laboratories in most of the world to test for 8-30 disorders on a single blood spot in less than a minute, thus 100 specimens a day can be processed. The programme is entirely suited to countries or areas with a high birth rate such as South Africa.

We do not know with any certainty what disorders we have here but preliminary data suggest that in the African congenital hypothyroidism is rare, MCAD is yet to be identified ,yet galactosemia is not uncommon and glutaric aciduria type 1 has a significant incidence ,whereas in white infants the incidence of disorders is the same as in Caucasian babies in Europe.

We report on a NBS programme carried out in Gauteng and Limpopo Provinces from 1999 to 2005 and describe the difficulties encountered. A successful programme has 6 parts, education of professionals and public, collection and handling of the samples, obtaining and confirming results, communicating both normal and abnormal results to the parents, treating the affected infant, and genetic counselling and long term follow up of the family and the affected child.

Posters

ChemP Post 1: HIV Protease Inhibitors induce Insulin resistance via the NFκB pathway

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Judy A King National Health Laboratory Service South Africa
Tahir S Pillay University of Cape Town South Africa

Objective: The aim of this study was to elucidate the molecular basis of insulin resistance induced by HIV protease inhibitors (HPIs). Chinese hamster ovary cells transfected with high levels of human insulin receptor (CHO-IR) and 3T3-L1 adipocytes were used to elucidate the mechanism of this side effect.

Methods: CHO-IR cells and 3T3-L1 adipocytes were treated with indinavir, a prototypic HPI, for 16 h followed by insulin stimulation for 5 min, and then lysed and analysed by immunoblotting using antibodies against phosphotyrosine, insulin receptor (IR) β-subunit, protein tyrosine phosphatase 1B (PTP1B), suppressor of cytokines signalling-1,-3 (SOCS-1,-3), insulin receptor substrate 1 (IRS-1), phosphoIRS-1 serine residue 307 (S307), Src homology 2B (SH2B), adapter protein with a pleckstrin homology domain and an SH2 domain (APS) and actin. CHO-IR cells were also pre-treated with sodium salicylate (NaSal) (5mM) for 1h to inhibit nuclear factor kappa B (NFκB).

Results: Indinavir induced a significant decrease in tyrosine phosphorylation of the IR β-subunit and a significant increase in the phosphorylation of IRS-1 on S307 in both CHO-IR cells and 3T3-L1 adipocytes. Concomitantly, levels of PTP1B, SOCS-1 and -3, SH2B and APS were not altered significantly. In CHO-IR cells pre-treated with NaSal, the effects of indinavir on tyrosine phosphorylation of the IR β-subunit and phosphorylation of IRS-1 at S307 were abrogated.

Discussion: Sodium salicylate inhibits the upstream activator of NFκB, the IκB kinase β (IKKβ), resulting in decreased phosphorylation of IRS-1 on S307. Serine phosphorylated IRS-1 acts to inhibit phosphorylation of the insulin receptor. We show for the first time that blockade of IKKβ by NaSal abrogates the effect of indinavir on the tyrosine phosphorylation of the insulin receptor by decreasing phosphoIRS-1 S307.

Conclusion: HIV protease inhibitors act via the NFκB pathway to induce insulin resistance in vivo. Salicylates may provide a novel therapy for HIV protease inhibitor-induced lipodystrophy.

ChemP Post 2 : Presence of the CYP2B6 516G>T polymorphism and increased plasma Efavirenz concentrations in South African HIV-infected patients

Verena Gounden NHLS, University of the Witwatersrand, Medical School
Chantal van Niekerk NHLS, University of the Witwatersrand, Medical School
Tracy Snyman NHLS, University of the Witwatersrand, Medical School
Jaya George NHLS, University of the Witwatersrand, Medical School

The 516G>T polymorphism in exon 4 of the CYP2B6 gene has been related to increased plasma EFV levels. EFV levels are associated with therapeutic efficacy, the likelihood of developing adverse CNS effects and treatment non-compliance. The aims of this study were to a) determine the presence of the 516G>T and other CYP2B6 exon 4 polymorphisms in a South African group of HIV-infected individuals and b) to investigate the relationship between the EFV plasma concentrations, the CYP2B6 516G>T polymorphism and the occurrence of CNS related side effects in this group of patients.

Data from 80 patients is presented. Genetic polymorphisms in exon 4 of the CYP2B6 gene were identified using PCR amplification of these regions followed by sequencing of the amplification products. EFV levels were analysed by UPLC-MS/MS. Assessment of the presence of CNS related side effects following EFV initiation were elicited with the use of a questionnaire together with physical examination.

23 % of patients were TT homozygous for the 516G>T polymorphism. These patients had significantly higher EFV levels versus those with the wild (GG) genotype ($p < 0.005$). Those who experienced no side effects had significantly lower EFV plasma concentrations vs. the group, which experienced the most severe side effects ($p < 0.05$). Plasma EFV concentrations displayed high inter-individual variability amongst subjects with levels ranging from 94 $\mu\text{g/l}$ to 23227 $\mu\text{g/l}$

Genotyping for the 516G>T polymorphism and other clinically relevant polymorphisms together with therapeutic drug monitoring of EFV levels may assist clinicians in choosing and adjusting treatment dosages to allow for maximal therapeutic efficacy with minimal toxicity.

ChemP Post 3 : Metabolomic analysis of cell culture supernatants from 3T3-L1 Adipocytes treated with HIV protease inhibitors

Wan Iryani Wan Ismail	Universiti Teknologi MARA	Malaysia
George F van der Watt	National Health Laboratory Service, Red Cross Children's Hospital	South Africa
Judy A King	National Health Laboratory Service, Groote Schuur Hospital	South Africa
Tahir S Pillay	University of Cape Town	South Africa

Introduction and Objective: Insulin resistance is a prominent side-effect of antiretroviral drugs. HIV protease inhibitors (HPIs) interfere in the metabolism of adipose tissue at multiple levels, thereby inducing various side-effects. In this study, metabolomics was used to elucidate how HPIs may affect the cellular metabolic state of cultured differentiated 3T3-L1 adipocytes.

Methods: 3T3-L1 adipocytes were treated with 50 μM indinavir, a prototypic HPI, for 16 h and then stimulated with insulin for 5 min. The cell culture supernatants were analysed for amino acids and organic acids using gas chromatography-mass spectrometry (GC-MS). The data were analysed using the MetaboAnalyst web server.

Results: Twenty-seven amino acids and derivatives were detected. Indinavir did not alter the levels of amino acids. However, indinavir with insulin stimulation increased the levels of 26 amino acids. The most significantly altered amino acids were: aspartate and hydroxylysine. Sixty organic acids and derivatives were identified. Indinavir increased the levels of eight organic acids. When indinavir was combined with insulin, there were significant increases in the levels of 12 organic acids in the treated supernatants. The most significantly altered organic acids were: butane, 3-OH butyrate, succinate and isovalerate.

Conclusion: Metabolomic analysis is emerging as a powerful tool for analysing disease conditions. The results from this study suggest that HIV protease inhibitors induce alterations in the metabolism of adipocytes. Future studies will address the metabolomic profiles of the various protease inhibitors.

ChemP Post 4: Personalised intervention in demyelinating diseases using pathology supported genetic testing.

Susan J van Rensburg NHLS and University of Stellenbosch

Maritha Kotze University of Stellenbosch

Dinie Hon Cape Peninsula University of Technology

Mahjoubeh Jalali University of Stellenbosch

RT Erasmus NHLS and University of Stellenbosch

Marius de Klerk Chemical Pathology, Tygerberg Hospital

Megan Rensburg NHLS, Chemical Pathology

INTRODUCTION:

Demyelination may have an impact on brain function due to dysfunction of neuronal pathways. We have developed a system of personalized intervention to optimize myelin protection and recovery in people genetically predisposed to demyelinating diseases such as multiple sclerosis (MS). The intervention is determined by the results of biochemical and mutation analysis. Biochemical deficiencies are addressed within the context of published evidence. The research study, which has Ethical Approval from the University of Stellenbosch, includes a structured interview with each patient involving family history and dietary questionnaires, biochemical tests and genetic testing. Results are entered into a data base for research purposes after informed consent was given by patients.

AIM OF SUB-STUDY:

To investigate whether there is a relationship between iron parameters and folate metabolism in patients with MS.

PATIENTS AND METHODS:

In 20 patients visiting our research facility, iron parameters were compared with MTHFR mutations. The following iron parameters were determined: haemoglobin, serum iron, ferritin, transferrin (Tf) and Tf saturation. Plasma samples were collected on ice for homocysteine determination. Mutation analysis was done for hemochromatosis (HFE) and 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C.

RESULTS:

Patients who had no mutations in MTHFR (wildtype, n=8) had significantly (P=0.04) higher serum iron levels ($19.08 \pm 1.63 \mu\text{mol/l}$) than patients with A1298C mutations (heterozygotes, n=9, $13.23 \pm 1.16 \mu\text{mol/l}$, and homozygotes, n=3, $13.03 \pm 3.68 \mu\text{mol/l}$).

CONCLUSION:

Deficiencies of iron and folate are both related to anaemia, which may cause fatigue and impaired myelin production and maintenance in MS patients. The present preliminary study revealed a possible effect of a mutation in the MTHFR gene impacting on serum iron concentration.

ChemP Post 5: **Sweat testing at Tygerberg Hospital for the diagnosis of Cystic Fibrosis**

Hoffmann, Mariza	Department of Chemical Pathology, National Health Laboratory Services and the University of Stellenbosch
Zemlin, Annalise E	Department of Chemical Pathology, National Health Laboratory Services and the University of Stellenbosch
Erasmus, Rajiv T	Department of Chemical Pathology, National Health Laboratory Services and the University of Stellenbosch

Introduction

Cystic Fibrosis (CF) is the most common lethal genetic disease in Caucasians. It has a diverse clinical presentation and is caused by numerous genetic mutations, making a clinical and genetic diagnosis difficult.

Quantitative sweat analysis is the most discriminatory test for diagnostic purposes, but is technically demanding, time consuming and often fails. Sweat conductivity provides an alternative with a faster turnaround time and lower failure rate, but borderline and abnormal results need to be confirmed with quantitative testing.

Objectives

To determine the clinical scenarios in which sweat testing was requested and whether clinical and laboratory practices were in accordance to international guidelines.

Methods

This was a retrospective audit performed at Tygerberg Hospital, South Africa. Patient records and laboratory data were reviewed on all sweat tests reported during 2009.

Results

Ninety-four patients were tested for CF. Conductivity results were reported on 91 patients and 6 patients had quantitative sweat chloride results. Most patients presented with respiratory symptoms (n=28;30%), gastro-intestinal problems (n=15;16%), or a combination of the two (n=34; 40%). Only 1 patient had a positive family history of CF.

Of the 91 conductivity results, 9 (10%) had borderline or high values using manufacturers cut-offs. Of these, only 4 (44%) had confirmatory testing as per

guideline recommendations. When guideline cut-offs were used, an additional 10 (11%) patients for follow-up testing were identified.

A total of 5 patients were diagnosed with CF using quantitative sweat chloride determination. Although all 5 patients had genetic testing, a mutation was detected in only one (20%) patient.

Conclusion

Our audit identified several problems in sweat testing practices at our hospital. Using manufacturer's cut-offs for conductivity, 11% of potential CF cases could be missed. Additionally, 66% of borderline and abnormal conductivity results are not confirmed. Genetic testing missed 80% of cases diagnosed using sweat test analysis.

Chemp Post 6: **Standardised and unstandardised serum creatinine: construction of a normogram for Schwartz formula estimated GFR at Red Cross Children's Hospital**

Judy King **National Health laboratory Service and University of Cape Town**
Ryan Benjamin **National Health Laboratory Service and University of Cape Town**

Introduction: The Schwartz formula estimates the glomerular filtration rate (eGFR) in children. The empiric formula is based on unstandardised creatinine. A formula based on the standardised Jaffe creatinine is pending. In the interim, Red Cross Children's Hospital is introducing the standardised creatinine assay. The eGFR calculated from standardised and unstandardised serum creatinine differ. This study constructs a normogram to allow calculation of this difference. **Methods:** A method evaluation and least squares fit was performed to determine a formula that relates standardised to unstandardised serum creatinine on the Beckman-Coulter DxC (n=171) across the serum creatinine concentration range 15 µmol/L to 1000 µmol/L. For an arbitrary unstandardised serum creatinine result (x), the corresponding standardised serum creatinine result (xs) was calculated algebraically. A formula for the difference between the algebraic eGFR based on standardised creatinine and that for unstandardised creatinine was derived. An additional sample (n=38) was used to validate the difference formula. The difference formula evaluation used linear regression and Bland-Altman plots. The statistical analysis was formalized using a QQ-plot and the Shapiro-Wilk test for normality. A paired student t-test compared actual and expected percentage change. **Results:** The relationship between unstandardised and standardised serum creatinine is linear with $x = 0.915x_s + 20.148$ µmol/L (r=0.973). The percentage difference from the unstandardised result is $-8.5 + 2014.8/x_s$ where x_s is measured in µmol/L. A Gaussian distribution of differences could not be excluded (p = 0.44). A statistically significant difference between expected and actual percentage change in eGFR for the standardised serum creatinine, across the measured range of this study, could not be confirmed (p = 0.72). **Conclusion:** Paediatric nephrologists at Red Cross Children's Hospital are encouraged to use the normogram to ensure that their eGFR results are consistent with those prior to the standardisation of the serum creatinine assay.

ChemP Post 7: Monounsaturated fatty acids, n-9 and n-7 in blood cells from patients with multiple sclerosis

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Introduction: The underlying cause of multiple sclerosis (MS) is unknown but an autoimmune and/or infectious component has been implicated. Fatty acids, especially PUFAs are precursors for inflammatory substances such as the eicosanoids. On the other hand, the n-9 MUFAs are thought to have anti-inflammatory effects whilst C16:1n-7 has been shown to have anti-viral effects.

Aim: The aim of this study was to investigate levels of MUFAs in blood cells from MS patients, and to correlate these with C-reactive protein and the Kurtzke Expanded Disability Status Scale (EDSS) and Functional System Scores (FSS).

Methods: The phosphatidylcholine (PC), phosphatidylethanolamine (PE), Phosphatidylserine (PS) and phosphatidylinositol (PI) MUFA composition in RBC and PBMC membranes from 26 patients with MS and 25 healthy control subjects were determined by gas chromatography.

Results: PBMC PE C18:1n-7 was significantly decreased in patients. Total n-9 MUFAs, C18:1n-9, C20:1n-9, and C24:1n-9, showed significant inverse correlations with the FSS. PC C24:1n-9 in RBC membranes showed a positive correlation with the FSS. In contrast, C18:1n-7, total C16:1n-7 and total n-7 MUFAs in RBC and/or PBMC membranes showed significant positive correlations with the EDSS/FSS. The CRP showed significant positive correlations with control PC C16:1n-7 and total C16:1n-7, and significant positive correlations with MS PE C18:1n-7, total C18:1n-7 and total n-7 MUFAs.

Conclusion: Contrary to previous reports MUFAs in RBC and PBMC membranes from patients with MS were not increased. The inverse correlations between the n-9 MUFAs and the EDSS/FSS is of importance as olive oil supplementation has been shown to have a beneficial effect in inflammatory diseases such as rheumatoid arthritis. The major component of olive oil is oleate (C18:1n-9) (79 %). The positive correlations between the n-7 MUFAs and the EDSS/FSS and especially the CRP suggested that the n-7 MUFAs could play a role in the inflammatory conditions in patients with MS.

ChemP Post 8 **HbA1c OF 6.5% to diagnose Diabetes Mellitus – Does it work for us? The Belville-South Africa study**

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Background: Currently diabetes is diagnosed according to either ADA or WHO criteria; both require a fasting glucose determination. Apart from being inconvenient, glucose has a large biological and diurnal variation. HbA1c has been the gold standard for glycaemic control follow-up for several decades. Previous problems with assay standardization meant that HbA1c could not be used as a screening tool. In 2009, the ADA proposed an HbA1c level of 6.5% - the value after which the incidence of retinopathy increases – as a diagnostic level for DM diagnosis. The objective of this study was to validate this cut-off in our local community.

Methods: Nine hundred and forty six participants were recruited in a community-based study. When those with known DM (121) and those with uncertain diagnosis (6) were excluded, 819 participants were included. All participants were screened for DM according to both ADA and WHO criteria. The ADA diagnostic cut-off for HbA1c of 6.5% was tested separately for each group. A receiver operator characteristic (ROC) curve for both criteria was generated to establish an optimal cut-off.

Results: One hundred and eighty two (22%) males and 637 (78%) females were included. Using ADA criteria, 117 (14%) were diagnosed with DM with 50% having an HbA1c value of $\geq 6.5\%$. Using WHO criteria, 147 (18%) were diagnosed with DM with 46% having an HbA1c value of $\geq 6.5\%$. Using ROC curves, a level of 6.1% was found to be ideal when using both the ADA and WHO criteria (AUC 0.85 and 0.82 respectively). The sensitivities at this cut-off were 80% and 75% respectively and the specificities 77% and 78% respectively.

Conclusions: We established an HbA1c level of 6.1% as optimal for the diagnosis of DM and our results emphasise the need for evidenced based values to be established in various population groups.

ChemP Post 9: **Elevated High-sensitivity CRP levels in South African Adolescents with Metabolic Syndrome**

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Aims:

Metabolic syndrome (MetS) and its associated cardiovascular risk are on the increase in children. High-sensitivity C-reactive protein (hs-CRP) has emerged to be a useful marker for inflammation associated with atherosclerosis and cardiovascular disease. The purpose of this study was to examine the levels of hs-CRP in South African adolescents with MetS and to investigate metabolic traits that may be associated with higher CRP levels.

Methods:

We studied the association of hs-CRP with MetS variables in 324 adolescent learners, aged 15-18 year old from different racial groups. The NCEP ATP III for ages 15-18 year olds were used to define the MetS. Anthropometric variables, blood pressure, fasting blood glucose and lipids were performed on consenting learners. Overweight and obesity were determined according to age and gender as defined by the International Obesity Task Force.

Results:

The prevalence of MetS and obesity was 3.7%. Hs-CRP levels were significantly higher in subjects with MetS (2.56mg/l) and in those with a waist-circumference > 90th percentile (P <0.01). Ethnic and gender differences were seen.. Higher hs-CRP levels were seen in obese learners with the MetS, but were significantly lower in those with MetS and normal weight. Median hs-CRP levels increased with increasing number of metabolic abnormalities and exceeded 3 mg/ l in 60 (19 %) of adolescents of which 33 (55%) had a normal BMI.

Conclusion:

Hs-CRP levels are associated with metabolic syndrome variables in South African adolescents and these findings suggest that obesity appears to be a major mediator of CRP levels.

ChemP Post 10: **Colon cancer cases at CH Baragwanath Hospital**

DIPUO RAMOLODI NHLS S.A

Objective

- The objective of the study is to highlight the importance of chemistry tests in assisting in the diagnosis or management of colon cancer
- It is also to make an awareness of the mortality rate of colon cancer

Background

Colon cancer¹, also called colorectal cancer or large bowel cancer includes cancerous growth in the rectum, colon and appendix

- It is the 3rd leading cause of cancer related deaths in the western world
- The risk of developing cancer increases with age, but however cases in young people have been reported, especially when influenced by certain risks such as smoking and physical inactivity
- A study was done by Dr Jeffrey A Meyerhardt on the 5th of July 2006 to establish whether regular physical activity decreases the likelihood of colon cancer, the results found were that being physically active can reduce the risk of colon cancer and this is so provided that there are no other risk factors

Method

- Collecting data of patients who have been diagnosed with colon cancer
- Providing clinical history and personal information of the patients
- Comparing the dates of diagnosis/ biopsy with the date in which patient's samples were screened for colon cancer
- Highlighting whether chemistry tumour markers (eg CEA) used for the management of the cancer do correlate with the health improvement of the patients

Results/ conclusion

- 80% of patients diagnosed with colon cancer were found to have been screened using chemistry tests before
- CEA was found to be the most common test used in the management of colon cancer, and occult blood test to be the preferred screening test.

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ChemP Post 11: An evaluation of CRP request patterns and turnaround times following the provision of an after hours laboratory services for this analyte

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Background: CRP levels are often requested urgently, especially in paediatrics. Until October 2009; CRP was performed at Immunology at NHLS Tygerberg. Demand for a 24 hour service led to the test being transferred to Chemical Pathology. We investigate the impact of an after-hours service on the number of CRP requests and its effect on turnaround time (TAT). We also investigate the clinical impact of delayed CRP results on patient care.

Methods: Data searches conducted on the NHLS DISA system identified CRP test requests and their TAT for Immunology in February 2009 and Chemistry in February 2010. TAT was further divided by time taken to arrive at reception, time to registration, time to analysis and time to review. Thirty paediatric folders with the poorest TAT were examined for possible clinical impact.

Results: A total of 2714 CRP tests were requested in February 2009 and 2862 in February 2010. TAT showed a significant improvement following the provision of the after-hours service. Improvements were seen in all laboratory controlled parameters including analysis time and time to review. Delayed CRP results had a clear negative clinical impact in 50% of the 30 folders reviewed, including delay in diagnosis and initiation of treatment, unnecessarily prolonged IV antibiotic treatment, delayed discharge and unnecessary repeat testing.

Conclusions: The implementation of an after-hours service for CRP testing has resulted in a significant improvement in TAT. As we found that delayed CRP results had a definite impact on patient care, it can be surmised that this improved TAT will be of benefit to patient care and customer satisfaction.

ChemP Post 12: An evaluation of the reasons for rejected samples in the Chemistry and Haematology Laboratories at Tygerberg Academic Hospital

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Background: Recent publications emphasize the importance of pre-analytical laboratory errors and report that up to 70% of total laboratory errors occur in this phase. Identification of errors detects specific problems and highlights local pre-analytic processes susceptible to errors. The rejection of unsuitable samples can lead to delayed turnaround time and affect patient care.

Methods: A retrospective audit was conducted investigating the rejection rate of routine specimens received at our chemistry and haematology laboratory over a 2 week period. The reasons for rejection and potential clinical impact of these rejections, as well as their influence on turnaround time, were investigated. Thirty patient files were randomly selected and examined to assess the impact of these rejections on clinical care.

Results: A total of 32910 specimens were received in the specified time period, of which 481 were rejected, giving a rejection rate of 1.46%. The main reasons for rejection were clotted specimen (30.6%) and insufficient sample volume (22.5%). Only 51.7% of rejected samples were repeated and the average time for a repeat sample to reach the laboratory was 5 days (121 hours). Of the repeated samples, 5.1% had results within critical values. Examination of patient folders showed that in 40% of cases the rejection of samples had a definite impact on patient care.

Conclusions: The evaluation of pre-analytical processes in our laboratory, with regard to sample rejection, allowed us to identify problem areas where improvement is necessary. Rejected samples had a definite impact on patient care and can thus affect customer satisfaction.

Chemp Post 13: **Glutaric Aciduria Type 1 in South Africa – High Incidence of Glutaryl-CoA Dehydrogenase Deficiency in Black South Africans**

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Background: Glutaric Aciduria type 1 (GA1) is an inherited disorder of lysine and tryptophan catabolism that typically manifests with acute cerebral injury associated

with an intercurrent illness. Of the thirteen cases of GA1 known to us in South Africa eleven occurred in unrelated black Africans and eight were diagnosed over a period of 25 months in our laboratory. Methods: Urinary glutaric acid and 3-hydroxyglutaric acids were determined by gas chromatography-mass spectrometry and glutaryl-CoA dehydrogenase (GCDH) activity measured in cultured fibroblasts using ¹⁴C-glutaryl-CoA as substrate. The GCDH gene was sequenced using standard molecular methods. The carrier frequency of the A293T mutation in the Black South African population was estimated. Results: Biochemically, all tested patients demonstrated elevated levels of urinary 3HGA with fibroblast GCDH enzyme activity <5% of normal. Glutaric acid excretion, however, was variable and five patients had urine GA levels <50 μmol/mmol creatinine that could be described as low excretors of GA. Molecular results showed that the patients of African descent were homozygous for the A293T mutation whereas a single patient of mixed ancestry tested heterozygous for A293T and R402W. A patient of Asian ancestry from a consanguineous relationship and was homozygous for a novel Q59P mutation in the GCDH gene. Allele frequency of A293T was determined in 750 African newborns resulting in a carrier frequency of 1 in 36 individuals and a predicted prevalence rate for GA1 of 1 in 5184. Conclusion: If this data is predictive, GA1 may well be the most common and unrecognized inherited metabolic disorder in South Africa. Until such time as effective population-wide newborn screening becomes available for this treatable disorder, we urge clinicians working in South Africa to maintain a high index of suspicion GA1, especially in black children with associated acute or chronic neurology or in cases of suspected non-accidental head injury.

ChemP Post 14: **Determining aldosterone:renin concentration ratio cut-off and plasma renin stability**

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Introduction: The aldosterone: renin activity ratio (ARR) is a useful screening tool for primary hyperaldosteronism, with numerous cut-offs having been described. In our laboratory, a cut-off of 1000 (pmol/L per ng/ml/h) was used. The renin activity assay, being cumbersome, has been replaced in many laboratories by the measurement of renin concentration. As the units and nature of these assays differ, different cut-off values (preferably assay-specific) are required for the plasma aldosterone: renin concentration ratio (ARC) when screening for primary hyperaldosteronism. Renin is an unstable analyte due to cryoactivation, and special precaution is required in the handling and storage of specimens. However, it is necessary to re-analyse samples in

dilution when levels exceed the measuring range, necessitating a second freeze-thaw cycle. Aim: The objectives of this study were to determine the ARC cut-off for primary hyperaldosteronism, using the Diasorin GammaCoat® Plasma Renin Activity and Cisbio Renin III Generation assays, by determining the relationship between these assays; and to ascertain whether renin concentration is stable after two freeze-thaw cycles. Methodology: 20 samples were analysed for renin activity, renin concentration and aldosterone concentration (Siemens Coatcount Aldosterone RIA kit). A further cohort of samples was analysed for renin concentration, and re-analysed after a second freeze-thaw cycle. Results: Plasma renin activity (x) correlated significantly with renin concentration (y) ($y = 6.1388x + 6.2522$; $r^2=0.9877$), with the relationship between ARR (x) and ARC (y): $y = 0.041x + 12.953$ ($r^2 = 0.9762$). The ARC cut-off was calculated as 54pmol/mIU. Renin levels were stable after two freeze-thaw cycles i.e. did not differ significantly from original results ($y = 0.9708x - 0.5144$; $r^2 = 0.9906$). Conclusions: When the ARC substitutes ARR as a screening tool for primary hyperaldosteronism, using the above-mentioned assays, the cut-off of 54pmol/mIU may be used. Plasma renin concentration is stable after two freeze-thaw cycles.

ChemP Post 15: **Macro-Creatine Kinase Confounds a Diagnosis of Acute Coronary Syndrome**

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Introduction Macro-enzymes are high molecular weight serum enzyme complexes occurring in pathological and physiological conditions. They result from either the association of enzymes with variable immunoglobulin classes (or plasma components) or enzyme self-polymerization. The mechanisms underlying macro-enzyme formation are incompletely understood. Their presence has been described in many routinely measured serum enzymes including creatine kinase (CK). Their inefficient renal clearance causes an increased enzyme activity. Macro-enzymes can cause spurious biochemical interpretation and diagnostic/therapeutic errors – thus making timeous detection important! Case A 67 year old male presented with chronic diarrhea, significant weight loss, abdominal distension and chest pain. Physical examination identified hepatomegaly and ascites. His cardiac examination was normal. Abdominal ultrasound confirmed a 22cm hepatomegaly and ascites. Liver biopsy identified metastatic adenocarcinoma infiltration, supported by liver function testing. Alpha-foeto protein was within reference interval values (RIV). His electrocardiographic (ECG) study was normal. His troponin-T levels did not suggest myocardial injury however his CK-MB isoenzyme (immuno-inhibition activity assay) was > 100% of his total-CK (CK within RIV). Repeat testing of CK-MB (mass assay) was within RIV. CK-isoenzyme-electrophoresis identified a type-2 macro-CK pattern. Serial testing of troponin-T and CK-MB (mass) were congruent with repeat ECGs and his evolving clinical presentation. **Discussion and conclusion** The diagnosis of acute coronary syndrome (ACS) can be confounded by CK-MB immuno-inhibition activity assays thus CK-MB mass assays are preferred. Furthermore, clinical presentation and ECG are critical criteria in the diagnosis of ACS. Macro-CK-type-2 (mitochondrial CK oligomeric complexes) is associated with malignancies and accounts for the grossly abnormal CK-MB activity level observed. CK-isoenzyme-electrophoresis can

identify the presence macro-CK enzyme patterns. The suspicion of macro-CK requires early detection and communication with the attending clinician to aid a correct ACS diagnosis.

ChemP Post 16: **Apolipoprotein E, S100B protein and Head Injury**

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BACKGROUND

Variability in the outcome after traumatic head injury is intriguing especially in cases that are clinically graded to have similar injuries. This variability has put the focus on the inherent pre-injury factors possibly environmental and genetic as these patients are managed under similar conditions. Early diagnosis of the presence of traumatic brain injury (TBI) has not translated to reduction in morbidity or mortality.

We focused on S100B protein, present in nervous tissue that is increased in biological fluids during brain damage and secondly apolipoprotein E, a lipoprotein produced by astrocytes and microglia and is responsible for transporting lipids to injured neurons.

OBJECTIVE: This study was performed to correlate the levels of S100B protein in blood and urine and the influence of the different Apolipoprotein genotypes on the outcome and prognosis in patients with head injuries.

METHODS: Blood and urine were used for the determination of the APOE genotype and S100B protein in patients admitted at Chris Hani Baragwanath Hospital who presented with head injury. Patients were grouped into three; mild, moderate and severe based on clinical conscious Glasgow Coma score assigned by the admitting clinician. The APOE-epsilon allele and S100B protein levels was correlated with the outcome at a minimum of 3 months of follow up.

RESULTS: In traumatic head injury, APOE-epsilon4 allele had a poor prognosis and generally took longer to recover. S100B protein levels showed some direct relationship to the extent of injury based on the admission Glasgow Coma Scale but could not independently predict the prognosis and outcome. In normal renal function, inspection of blood to urine S100B ratio offers some promise in approximating time of head injury.

CONCLUSIONS: We are reporting a relationship between Apo E lipoprotein and S100B protein and prognosis and outcome in head injury .

ChemP Post 17: **Apolipoprotein E, S100B protein and Meningitis**

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BACKGROUND

Healing follows injury and the basic healing process is the same, we looked at the factors that have been associated with prognosis in traumatic head injury as having a role to play in the healing process in meningitis. Variability in meningitis healing can

be due to inherent pre-injury factors possibly environmental and genetic as seen in traumatic head injury. We focused on S100B protein and Apolipoprotein E.

OBJECTIVE: This study was performed to correlate the levels of S100B protein in blood and urine and the influence of the different apolipoprotein E genotypes on the outcome and prognosis in patients with different types of meningitis.

METHODS: Patients with laboratory diagnosis of meningitis was retrieved daily from the data management system and sample archiving numbers were used to retrieve appropriate samples daily. Demographic data was used to age and gender match participants. Only those without a history of head injury were selected for the study and grouped under the three main causes of meningitis, viral meningitis, bacterial meningitis and fungal. Investigations were carried out without direct involvement of the patients, in that patient relevant data was obtained from the patient folder. Blood sent for haematological investigations and urine collected within 24 hours of admission were retrieved for the determination of the APOE genotype and S100B protein respectively. The APOE-epsilon allele and S100B protein was correlated with outcome, being recovery death. Univariate and multivariate analyses were performed to determine independent risk factors.

RESULTS: Different APOE lipoproteins have different influences on the outcome in patients with meningitis. B100S protein levels showed some direct relationship to the number polymorphonuclear cells counts.

CONCLUSIONS: We report a relationship between Apolipoprotein E epsilon and meningitis. The study also confirmed previous findings on B100S protein and in meningitis in other populations.

ChemP Pot 18: Apolipoprotein E Apo and Dyslipidaemia in HIV

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Background Before the advent of highly active antiretroviral therapy (HAART), patients with human immune deficiency virus HIV were reported to have abnormal lipograms. The marked increase in dyslipidaemia that followed the use of HAART and the possible cardiovascular complications associated with dyslipidaemia resulted in intense research on the pathophysiology of dyslipidaemia in human immune deficiency virus /acquired immune deficiency syndrome (HIV/AIDS). Apolipoprotein E influences lipoprotein metabolism with some variants resulting in dyslipidaemia due increased remnant lipoproteins accumulation under metabolic stresses. In this study we looked at the distribution of Apolipoprotein E variants in those patients who developed dyslipidaemia. **Methods** Lipograms from the HIV clinics were selected. The sample archiving number was used to retrieve appropriate EDTA samples daily and stored apolipoprotein E genotyping. The cumulative function was used to obtain the base line lipograms and the patient drug management was obtained from the folders. Age and gender matched controls were selected from HIV negative patients with abnormal lipograms. **Results** Results were classified into three 1) predominantly hypercholesterolemia 2) predominantly hypertriglyceridaemia and 3) mixed i.e., both cholesterol and triglyceride being elevated. **Conclusion** We report the frequency and distribution of apolipoprotein E in HIV patients and the pattern of dyslipidaemia associated with different HAART drug regimes.

ChemP Post 19: **Dyslipidaemia in the Black Population**

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BACKGROUND Secondary dyslipidaemias outweigh primary dyslipidaemias by far and it is the one that has been encountered in the black population. However the near epidemic rise in cardiovascular disease among younger Africans than those encountered among the Caucasian counterparts requires a look at possible underlying genetic factors. The absence of dyslipidaemia among other patients with similar primary underlying pathology at the same disease stage and grade despite being exposed to similar environmental factors is another reason why possible genetic predisposition must be excluded in the 'secondary' dyslipidaemia. **MATERIALS AND METHODS** A two year study between September 2006 and September 2008 that looked at lipograms for patients with total cholesterol ranging between 5.2 mmols/l and 30 mmols/l were examined. The secondary dyslipidaemias were grouped according to the underlying pathology i.e. diabetes, obesity etc and further subdivided according to the stage or grade of disease. Controls were selected from non dyslipidaemic patients who were, age, gender and disease grade or stage matched. Documented laboratory evidence of secondary dyslipidaemia i.e. thyroid dysfunction, liver or kidney disease was an inclusion criteria for both participants and controls. **RESULTS** Of the 10 000 patients 28% had elevated cholesterol, 10% elevated Triglycerides with 6% displaying a mixed picture. Diabetes, renal pathology and hypothyroidisms featured as the major underlying pathology in secondary dyslipidaemias. Dysbetalipoproteinaemia due to apolipoprotein e polymorphism is the major underlying genetic predisposition in most patients with secondary dyslipidaemia. **CONCLUSION** The study has shown that secondary dyslipidaemia in the black population should be investigated to exclude underlying genetic predisposition. This is important especially in those who present with persistent or recurrent dyslipidaemia after resolution of the underlying pathology or dyslipidaemias that does not show parallel or corresponding improvement to the underlying pathology.

ChemP Post 20: **Profile of Females with Rheumatoid Arthritis at Chris Hani Baragwanath Hospital**

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BACKGROUND

Rheumatoid arthritis (RA) is a world wide chronic inflammatory disease with a wide array of multisystem comorbidities that affects women more than men. Even though the exact cause of RA is unknown, it is however known that it is an autoimmune

disease resulting predominantly in inflammation and subsequent joint damage. Current data is based on the studies done mainly among the Caucasian population with the ethnic difference being studied in the northern hemisphere. This study looked at the profile of women with RA arthritis in our population.

MATERIALS AND METHODS

A retrospective study that looked at only female patients with abnormal RA test results like rheumatoid factors (RF), antibodies to citrullinated peptides (ACPA) and antinuclear antibody/ANA) were selected from the data management system. Patients were grouped into several categories like positive blood tests, age of onset, blood group, parity, weight, other medical conditions and positive family history were utilised to formulate a profile.

CONCLUSION

The study has shown an arthritis profile in African women. However larger studies should be done to come up with a profile that can be utilised to design preventive measures

ChemP post 21 : **Audit of GC-MS Based Metabolic Disease Tests at Red Cross Children's Hospital 2006 – 2010.**

Introduction: An audit evaluation of two, high capital expenditure, specialized, GC-MS instruments placed within a routine chemistry laboratory with the purpose of improving local metabolic disease testing service delivery. Materials and methods: Two Agilent 7890A/5975C Gas Chromatography Mass Spectrometry (GCMS) instruments were placed in the routine chemistry laboratory in 2007 and SANAS accredited methods developed for the following previously outsourced tests: Urine Organic Acids (UOA), Plasma and Urine Amino Acids (PAA and UAA) and Leukocyte Cystine (LC). All methods were implemented to facilitate processing by rotationally trained technologists including an after-hours service and pathologist reporting/review. Sample sources, volumes and test turnaround times (TAT) were compared to established metabolic disease test panels namely Gilson Cooke Sweat testing (SWT) and urine reducing substances/IMD metabolite screening (URED) for 1 year prior (2006/7) and 3 years after (2007/08 - 2009/10) the implementation. Results: Volumes of all GC-MS tests increased significantly over the period: UOA 335%, PAA 713%, UAA 370%, LC 420% with an associated mean reduction in TAT of 62%. In contrast, SWT volumes increased by 147% with an associated increase in TAT of 220%. For URED, volumes increased by 233% with a 54% decrease in TAT. Regarding sample source, there was a shift to increased sample volumes received from private laboratories together with a progressive increase in samples sourced from other provinces over the corresponding period. Conclusion: The introduction of specialized test methods within a routine laboratory was associated with significant increase in sample volumes together with a widened source base of referred work. Turnaround times improved for all tests incorporated. The growth in volumes exceeded initial projections used to formulate test costing parameters. Routine laboratories with the appropriate expertise should consider retaining specialised testing in favour of outsourcing in those cases where service delivery can be improved upon.

ChemP Post 22: **A Fast Simple GC-MS method for Urinary Homovanillic Acid**

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Relevance: Homovanillic acid (HVA), the metabolite of dopamine is measured as a tumour marker for neuroblastoma, the most common extra-cranial tumour in children. Patients with neuroblastoma excrete large amounts of HVA in urine. Due to the low specificity of our current colorimetric method, a fast and accurate Gas Chromatography – Mass Spectrometry (GC-MS) based method was developed to overcome this issue.

Materials and Methods: Extraction and derivatisation: Acidified urine spiked with vanillic acid (VA) internal standard was double extracted into ethyl acetate and diethyl ether using a rapid low volume micro-extraction technique and the supernatant dried down with nitrogen prior to bis(trimethylsilyl)trifluoroacetamide and pyridine derivatisation at 80°C for 30 minutes. Analysis: A Single Ion Monitoring method was used for HVA quantitation using ions m/z 326 for HVA and m/z 297 to identify the VA internal standard on an Agilent 7890A5975C GC-MS system using a 30m, ID 0.25µm, HP-1 MS column. Method Evaluation: The method was found to be linear across the range 0.78 – 100µmol/L with a slope coefficient and coefficient of determination (r^2) of 1.180 and 0.98 respectively. Within run and total imprecision (n=20), determined by analysis of variance was 3.0 and 3.3% at 50µmol/L respectively. The accuracy of the method was evaluated by recovery experiments and yielded recoveries of 96, 97 and 83% at 25, 50 and 200µmol/L respectively. In addition 4 Bio-Rad urine external quality scheme controls resulted within 2 standard deviations of the scheme median. Total method run time approximated 70 minutes Conclusion: The use of a low volume micro-extraction method together with a low cost non-isotopic internal standard allows rapid efficient screening for urine HVA by GC-MS with acceptable method performance parameters. Specificity will be evaluated but should be high as GCMS ion profiles are specific to each analyte. Clinical evaluation will follow.

Chem P Post 23: **Metabolic Syndrome and glucose tolerance in an urban community from Cape Town, South Africa**

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Background and Aims: The incidence of diabetes and obesity has dramatically increased globally and South Africa is no exception. South Africa is a multi-racial country with a population distribution of 80% black, 9.5 % white, 8 % mixed ancestry (Coloured) and 2.5% % Indian. A high incidence of obesity and diabetes in the mixed ancestry population of South Africa has been reported previously with recent studies suggesting an upward trend. We examined the association between the metabolic syndrome (MetS) and glucose tolerance in a population from an urban Coloured community residing in Cape Town, South Africa

Methods: Out of 945 subjects between the ages of 16 – 95 years, 904 had complete data including an oral glucose tolerance test for the diagnosis of metabolic syndrome. Metabolic syndrome was defined according to NCEP ATP III criteria and diabetes according to WHO (1999) criteria.

Results: Metabolic syndrome was present in 351 (39%) subjects, 254 (including 115 self-reported) had diabetes mellitus (DM) and 466 (52%) had normal glucose tolerance. Only 17 (3.65%) of subjects with normal glucose tolerance had MetS whilst in diabetic, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) subjects, MetS was respectively, 39%, 23.5% and 12%. Though the BMI of subjects with IGT (30.8 kg/m²) and DM (31.6 kg/m²) was significantly higher than that of normoglycaemic (28.6 kg/m²) subjects, no significant differences were observed between those with IFG (30.4 kg/m²), normoglycaemia and diabetes.

Conclusion: The high prevalence of the metabolic syndrome, diabetes and obesity is particularly alarming given the strong association between these factors and the development of cardiovascular diseases. Results from this study portend that adverse effects of MetS in the near future might take epidemic proportions in this population group.

Chem P Post 24 : **Physical activity and insulin resistance in adults residing in a low-income community in Cape Town.**

Bartel C, Bassett S, Matsha T, Hassan S, Erasmus R

Insulin resistance has shown to be a precursor to a number of lifestyle-related chronic diseases and abnormalities in adults and is affected by a number of factors including genetics, age, physical activity and acute exercise, diet, obesity and body fat distribution and medication. Physical activity has shown to have marked effects on improving sensitivity to insulin through various physiological mechanisms and numerous correlational studies have identified a relationship between these two variables, suggesting the beneficial role exercise has on insulin resistance. This study aimed to identify a relationship between current levels of physical activity, physical fitness and insulin resistance in adults between the ages of 35 and 65 years of age residing in a low-income, previously disadvantaged community in Cape Town.

A total of 186 volunteers participated in this study aged 35 to 65 years of age and ranging from healthy individuals to those with diagnosed chronic conditions. Insulin resistance (determined by the homeostasis model assessment of insulin resistance [HOMA-IR]), physical activity (measured by the Global Physical Activity Questionnaire) and health-related physical fitness were measured. These five components included body composition, determined by body mass index (BMI) and waist circumference, cardiorespiratory fitness, determined by the 3-minute step test, muscle strength, determined by the handgrip strength, muscle endurance, determined by the crunch test, and flexibility, determined by the sit-and-reach test. Spearman correlation was used to identify the relationship between HOMA-IR, body composition and physical activity and fitness. Results indicated a weak relationship between age ($r = 0.15$), body mass index ($r = 0.78$; $r = 0.17$), waist circumference ($r = 0.71$; $r = 0.22$), GPAQ ($r = -0.07$; $r = 0.18$), cardiorespiratory fitness ($r = 0.19$; $r = 0.27$), handgrip strength ($r = 0.58$; $r = 0.11$), muscle endurance ($r = 0.28$; $r = 0.10$), flexibility ($r = -0.25$; $r = 0.27$) and insulin resistance for both men and women respectively. Significant correlations were only identified in BMI, waist circumference and handgrip strength for males and muscular endurance for females. This suggests that there are other potential mediators influencing this relationship; participants did not answer the questionnaire truthfully or lacked understanding of the questionnaire; or the HOMA-IR formula was not suitable for this population. Further investigation is recommended to identify possible causes for this weak relationship.

Chem Post 25: A comparison of subjectively measured physical activity and objectively measured physical fitness with insulin resistance.

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Subjective measures of physical activity such as self report have characteristically been used in population based studies due to their low cost, feasibility and ability to determine physical activity by domains (types of activity) as well as frequency, intensity and duration. However, self-report may produce unreliable recall and bias, may fail to identify all activities and result in random error. Typically, energy intake is under reported and physical activity over reported. In particular, reports have shown that over-reporters of energy expenditure tend to be obese and also have a lower educational level. The use of objective measures such as pedometers and accelerometers has increasingly become more popular in population-based studies but don't determine intensity, in the case of pedometers, and are expensive, in the case of accelerometers. Physical fitness testing, including cardiorespiratory fitness and muscular strength, then becomes a viable alternative to gauge an individual's fitness levels.

The purpose of this study was to compare subjective and objective measures of physical activity against each other as well as their associations with insulin resistance. Associations were examined using data from 186 participants aged 35 to 65 years. Body composition, physical fitness, physical fitness and insulin resistance were measured. The Global Physical Activity Questionnaire (GPAQ) was administered for physical activity and cardiorespiratory fitness (3-minutes step test), muscular strength (handgrip strength test), muscular endurance (1-minute crunch) and flexibility (sit-and-reach) were measured for physical fitness. Insulin resistance was

presented as HOMA-IR. Spearman correlation was used to identify relationships between physical activity, fitness and HOMA-IR. Results for the subjective and objective associations were weak, indicating -0.28 ($p = 0.12$) for cardiorespiratory fitness, 0.16 ($p = 0.86$) for muscular strength, 0.18 ($p = 0.05$) for muscular endurance and 0.17 ($p = 0.07$) for flexibility. Associations between subjective and objective measures and HOMA-IR indicated strongest with physical fitness, in particular muscular strength in men ($r = 0.59$; $p = 0.02$). The association between GPAQ and HOMA-IR were very weak for males ($r = -0.07$) and females ($r = 0.18$).

Chem P Post 26 : **A new fructosamine reference range for the PathCare private pathology group, Somerset West, South Africa.**

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Background: Serum fructosamine are stable carbohydrate – protein complexes formed by an almost non irreversible enzymatic reaction between glucose and serum proteins, mainly albumin. Fructosamine together with glycated haemoglobin (HbA1c) are used to monitor the state of hyperglycaemia in diabetics. It provides the clinician with an index of glycaemia over a shorter period of time than HbA1c, about 4 weeks due to the high turnover of human serum albumin in blood and the degree of glycation in serum proteins. The evolution of automation in Clinical Chemistry necessitates that each pathology laboratory provides relevant sets of reliable reference values that are population and analyzer/method specific. Currently, the reference range for fructosamine at PathCare ranges between 200 to 285 $\mu\text{mol/L}$.

Methods: Four hundred and forty two (120 white females, 116 white males, 112 coloured females, 94 coloured males) healthy patients visiting the Somerset Pathcare practice were recruited for this study. Fructosamine, random blood glucose, HbA1c, total protein, albumin, and lipid profile was performed on all individuals. Nonparametric methods, whereby the sample 2.5 and 97.5 percentiles are used to form the 95% reference interval, were used to determine the reference values for fructosamine.

Results: Though no significant differences ($p = 0.086$) were observed between males and females in the total population group the coloured males had significantly higher fructosamine levels ($p = 0.01$) compared to their female counterparts. The reference range of the entire sample was 224 – 294, however differed in the different population groups (white females = 230 - 289; white males = 226 – 292; coloured females = 223 – 275; coloured males = 225 – 301).

Conclusion: The new fructosamine reference range is higher than the one currently in use at PathCare (200 – 285). Our results further strengthen the recommendations by pathology bodies that laboratories must establish reference values that are representative of local populations.

ChemP Post 27 : **High risk Cardiovascular Score in Young Mixed Ancestry Adults**

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Background: Various mathematical equations that incorporate the major risk factors (age, sex, high blood pressure, smoking, dyslipidemia, and diabetes) have been developed for the assessment of CVD risk over a 10-year period in general populations. However, the 10-year timeframe of these models has been criticised because an individual's lifetime risk may be high whilst the 10-year risk prediction may be low, therefore delaying efforts to modify that risk. Recently, an algorithm that allows for 30-year risk assessment for individuals with any combination of risk factors has been developed.

Methods: Sex-specific prediction for CVD risk was calculated using the 30-year CVD interactive risk calculator in a total of 583 subjects without a history of CVD. All participants were drawn from an urban community of the Bellville South suburb of Cape Town.

Results: Though the BMI and waist circumference of females were significantly elevated than that of males ($p < 0.0001$), the CVD risk was significantly higher in males, $p < 0.0001$. Generally, diabetes, hypertension and percent CVD risk increased with age, but even in those subjects younger than 35 years some had CVD risk of 20% or more. In the best subsets linear regression analysis the significant predictors of CVD were sibling history of diabetes, triglyceride levels, low density lipoprotein cholesterol and glycated haemoglobin, $p < 0.001$.

Conclusion: The results of our study have important public health implications as CVD is often underestimated in the young. The high lifetime risk in younger subjects may be considered as a warning that CVD in the near future might take epidemic proportions in this country.

ChemP Post 28 : **The prevalence of chronic kidney disease in an adult population of the Western Cape.**

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Background: Chronic kidney disease (CKD) is a condition marked by gradual decline of kidney function over time, and is strongly associated with cardiovascular diseases. The three leading causes of CKD are diabetes, hypertension and glomerulonephritis. The prevalence of chronic kidney disease has only been reported in developed countries, but in developing countries including South Africa, the prevalence is largely unknown.

Methods: In a cross-sectional community survey to study the prevalence of chronic disease in the adult population from Bellville-South, Cape Town, 819 subjects without diabetes participated. The diagnosis of CKD was based on the presence of proteinuria assessed by albumin creatinine ratio and an estimated GFR of <60 ml/min/1.73m² using the Modification of Diet in Renal Disease (MDRD) to estimate GFR from serum creatinine measurements. In addition all participants underwent an oral glucose tolerance test and diabetes was diagnosed using the WHO criteria.

Results: Overall CKD was present in 214 (26.1%) of subjects. One hundred and forty seven subjects (18%) were found to be diabetic and CKD was present in 50 (34%) of these subjects. Three (6%) of the subjects with unknown DM were found to have stage 3 (GFR, 15 - 29 ml/min/1.73m²) of the disease. There were 483 (59%) with normoglycaemia and CKD was present in 106 (21.9%) of these subjects whilst in those with non-diabetic hyperglycaemia (189), CKD was found in 58 (30.7%).

Conclusion: The findings of this study are alarming, but further strengthen the fact that CKD is intricately intertwined (via a network of traditional and non-traditional cardiovascular risk factors) with diseases such as ischaemic heart disease and diabetes, and thus deserves equal attention.

ChemP Post29: **A neuro-metabolic disorder**

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Metabolic disorders pose difficulties to both clinicians & laboratorians. However a good practical approach, including being clinically astute and screening procedures, assists in reaching diagnoses for this group of disorders.

Two siblings present almost one & a half years apart with macrocephaly, delayed milestones and hepatomegaly. Both presented non acutely without hyperlactataemia, hypoglycaemia nor hyperammonaemia.

These siblings will highlight the use of various biochemical analyses and the need for access to further testing including genetic and antenatal testing in affected families.