Tuesday, July 29, 2014

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

Clinical Studies/Outcomes

A-068

Cystatin C as a marker of renal function in adult Nigerians with Hypertension and Diabetes

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Background/Objective: Cystatin C (Cys C) has been suggested to be a better marker of the glomerular filtration rate (GFR) compared to the widely used serum creatinine. The aim of this study was to compare the accuracy of Cys C with that of serum creatinine in the assessment of GFR in patients with hypertension and diabetics.

Method: Twenty hypertensives and twenty diabetics were compared with forty age-matched healthy controls. Serum Cys C and serum creatinine were estimated in all study subjects and compared with the actual GFR as estimated by the Cockcroft and Gault's algorithm. The strength of significance of correlation was assessed using Pearson correlation (a p value of< 0.05 was accepted as significant).

Results: The serum Cys C correlated well with serum creatinine ($R = 0.757$, $p<0.01$) and with the estimated GFR ($R = -0.733$ and $- 0.710$ respectively; $p<0.01$). However, in the ROC analysis, the AUC of serum cystatin C (0.727) was found to be superior to that of plasma creatinine (0.539).

Conclusions: Serum Cys C and serum creatinine were well correlated in evaluating GFR in hypertensive and diabetic patients. However, serum Cys C was more closely correlated with the GFR and may therefore be a more accurate test of renal function in hypertensive and diabetic patients.

A-069

The best markers for systemic inflammatory response syndrome (SIRS) criteria

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Background: The present study was undertaken to find out and compare the usefulness of C-reactive protein (CRP) and hematology parameters in patients with SIRS. Patient presenting systemic inflammatory response syndrome (SIRS) exhibit two or more of the following criteria: Temperature greater than 38°C or less than 36°C, Heart Rate greater than 90 beats/minute, Respiratory rate greater than or equal to 20 breaths/minute or $PaCO_2$ less than or equal to 32 mmHg, WBC greater than or equal to 12,000/μL or less than or equal to 4,000/μL, and suspected or proven infection.

Some of these patients were also under treatment of antineoplastic drug. This type of treatment induces bone marrow suppression and therefore influences the immunity response to inflammation and infection. Therefore we examined WBC, LYM#, NEU#, ALY#, LIC#, PLT and CRP as biomarkers in patients with combination of SIRS and/ or antineoplastic drugs in order to evaluate if the bone marrow suppression could affect the predictive value of the different parameters and analyze which would be the most valuable

Methods: We examined 51 patients that we classified into distinct 4 groups depending on the combination of presence or not of SIRS and Antineoplastic drug medication:

5 patients with SIRS(+) and Antineoplastic drug medication (+);

14 patients with SIRS(+) and Antineoplastic drug medication (-).

13 patients with SIRS(-) and Antineoplastic drug medication (+).

18 patients with SIRS(-) and Antineoplastic drug medication (-).The area under the receiver operator characteristic curve (AUROC) was determined with 4 groups. Whole blood patient samples were tested for WBC, LYM#,NEU#,ALY#,LIC#,PLT and CRP using Pentra MS CRP(HORIBA Lid.)

Results: The AUROC value by 14 patients with SIRS(+) and Antineoplastic drug medication (-) and 18 patients with SIRS(-) and Antineoplastic drug medication (-) were 0.81 for CRP, 0.65 for PLT and 0.61 for WBC. And also the AUROC value by 5 patients with SIRS(+) and Antineoplastic drug medication (+) and 13 patients with SIRS(-) and Antineoplastic drug medication (+) were 0.82 for PLT, 0.8 for CRP and 0.77 for WBC. The AUROC of both PLT and CRP was 0.87. The AUROC of both WBC and CRP was 0.80.The ALY# and LIC# did provide useful information for evaluating that the patient had SIRS or not. The LYM# and NEU# had almost the same performance as WBC.

Conclusion: Some patients with SIRS(-) based on SIRS criteria also had inflammatory disease such as cholecystitis and otitis media. We evaluated patients with SIRS(-) by CRP. The results show, patients with SIRS(-) and CRP(>5mg/L) and including inflammatory disease such as cholecystitis and otitis media and patients with SIRS(-) and CRP(<5mg/L) without inflammatory disease. This shows that CRP is an excellent marker for SIRS criteria. Since WBC is affected by bone marrow suppression of Antineoplastic drug medication and CRP is not affected by it. The AUROC combination between CRP and PLT is better than the AUROC combination between CRP and WBC. Therefore, our conclusion is that CRP and PLT provide better results as supportive markers for SIRS criteria.

A-070

A case of Glutaric Aciduria type 1 in a Black South African girl

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Background: Glutaric Aciduria type 1 is an autosomal recessive inborn error of metabolism caused by a deficiency of glutaryl-Co enzyme A dehydrogenase (GCDH). If unrecognised it results in a severe extrapyramidal disease and mental retardation. It has been well described in North American and European Caucasian populations but only recently identified as potentially one of the commonest inherited metabolic disorders in black South Africans. **Methods and Results:** We present a case of a 9 month old Black South African girl who was admitted for investigations of sudden hypotonia following a fall from her mother's back. There was no family medical history of note. She was delivered at term by normal vaginal delivery and had normal developmental milestones until the fall. She had no previous medical history. She had relative macrocephaly (head circumference 46cm - 97th centile) and was globally hypotonic with hyperreflexia. She had normal renal and liver function tests, lactate, ammonia, amino acid profile and negative reducing substances screen. She had high urine glutaric acid at 4061umol/mmol creatinine (reference: <30) and 3-OH glutaric acid at 49 umol/mmol creatinine (reference: < 2.5). MRI of brain showed T2 bilateral hyperintensity of the basal ganglia, cerebral peduncles and central tegmental tracts, symmetrical restricted diffusion of globus pallidus in keeping with acute on chronic changes of pre-existing Glutaric aciduria type 1. **Conclusion:** Macrocephaly is an important clinical sign of many neurometabolic disorders and one of the earliest signs of Glutaric Aciduria type 1 (GA type 1) thus warrants screening in all cases of macrocephaly of unknown origin regardless ethnicity. GA type 1 typically presents with acute cerebral injury precipitated by an intercurrent infectious illness. It is a preventable and treatable disorder requiring patients with GA type 1 to low protein (lysine and tryptophan) diet, riboflavin and L-carnitine supplementation. It has been linked to A293T mutation in the glutaryl-CoA dehydrogenase gene in black South Africans.

A-071

Assessment Of Serum And Urine Sialic Acid In Sickle Cell Anaemia Patients

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Aim and objectives: To assess serum and urine sialic acid and compare their values with other biochemical markers of nephropathy in patients with sickle cell anaemia.

Patients and methods: It was a cross sectional descriptive study that involved adult (18 – 60 years) sickle cell anaemia patients that were clinically stable without sickle cell anaemia crisis. The control participants were healthy volunteers that were aged-matched. Urine and blood specimens were obtained from the participants. The urine albumin analysis was by Lowry method, urine and plasma creatine was by Jaffe', Kinetic method, while the serum and urine sialic acid was by standard Ehrlich (P – dimethyl amino benzaldehyde) method. Data were analysed using SPSS version 16.

Results Sixty eight of the subjects were sickle cell anaemia patients while 30 respondents served as control. Table 1, shows the plasma and urine results of the studied analytes in the sickle cell anaemia patients and controls. There was a negative

correlation between serum sialic acid and urea (r = -0.15, P = 0.41), creatinine (r -0.17, P = 0.34) albumin creatinine ratio (r = -0.19, P =0.81), but these were not significant. However, there was a positive significant relationship between urine sialic acid and albumin creatinine ratio (r = 0.44, P = 0.01).

Conclusion: Serum sialic acid unlike urine sialic acid does not correlate well with other well established marker of nephropathy (creatinine and urea). Monitoring of urinary sialic acid in patients with sickle cell anaemia who are in steady state is therefore an important adjuvant test in detecting the early onset of sickle cell nephropathy.

Table 1: Plasma and urine results of the studied analytes in control (non SCA) and SCA patients

	Control (non-SCA anaemia) respondents (n = 30)	SCA respondents (n = 68)	p-value
Plasma urea (mmol/L)	3.33±0.16	6.43±0.54	<0.001
Plasma creatinine (μmol/L)	73.98±2.95	86.70±6.03	0.06
SSA (mmol/L	1.93±0.67	1.58±0.96	0.04
ACR (mg/mmol)	2.19±0.10	4.50±0.24	<0.001
USA (mmol/L)	0.78±0.04	1.13±0.05	<0.001
USCR (mmol/mol)	60.52±3.39	169.39±13.59	<0.001

ACR = Albumin creatinine ratio

USA = Urine sialic acid

USCR = Urine sialic and creatinine ratio

SCA = Sickle cell anaemia

A-072

Interleukin-18 a Potential Marker of Liver Cirrhosis in Chronic Hepatitis C Patients.

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Background: WHO reported that Egypt has a very high prevalence of HCV and a high morbidity and mortality from chronic liver disease, cirrhosis, and hepatocellular carcinoma. In patients with liver cirrhosis, liver biopsy is the gold standard method to establish the diagnosis. But liver biopsy has many disadvantages, it is invasive, coasty and difficult to be standardized**.** That is why there has been increasing interest in noninvasive assessment of liver cirrhosis by the use of alternative serum markers. IL-18, a proinflammatory cytokine can be synthesized by injured hepatocytes and increases the susceptibility of liver endothelial cells to undergo apoptosis. An increased circulating level of IL-18 is likely to play a pathogenic role in patients with chronic liver disease.

Aim of the work: This study aimed to prove that Interleukin-18 can be used as a potential marker of liver cirrhosis in chronic hepatitis C patients. IL-18 levels were measured in 20 HCV infected patients with cirrhosis and compared to 20 non-cirrhotic HCV patients and 20 healthy controls.

Results: Serum IL-18 levels were significantly higher in cirrhotic (836 pg/ml) and non-cirrhotic CLD patients (751 pg/ml) than in healthy controls (278 pg/ml). IL-18 level is significantly increased with increase in histological stage of liver cirrhosis and its concentration may predict the degree of hepatocellular damage. A Positive correlation founded

between serum IL-18 levels and Child Pugh score suggest that IL-18 can be used as an additional non-invasive marker for monitoring the degree of liver cirrhosis in chronic hepatitis C patients and as a monitoring tool to assess response to therapy. Low cut off value of serum IL-18 at 495 pg/ml (which showed sensitivity of 100%) can be used as a screening value under which most of cases are probably negative cases for liver cirrhosis. While, a high cut off value of serum IL-18 at 875 pg/ml (which showed specificity of 95%) can be used as a diagnostic value above which 95% of cases are probably positive cases for liver cirrhosis.

Conclusions: IL-18 level as a non-invasive marker can be used for

follow up of chronic HCV patients and assessement of the severity of the disease and degree of liver cirrhosis instead of liver biopsy which has been proved to be invasive, coasty and difficult to standardize. Usage of low cut off value of serum IL-18 at 495 pg/ml as a screening value under which most of CLD cases are probably negative for liver cirrhosis and no need for liver biopsy. Usage of high cut off value of serum IL-18 at 875 pg/ml as a confirmatory test with liver biopsy above which 95% of cases are probably positive for liver cirrhosis. Usage of IL-18 level between 495 and 875 pg/ml as an indication for liver biopsy to diagnose cases with liver cirrhosis.

A-073

Risk map in three emergency laboratories

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Background: Patient Safety is considered one of the key aspects of the quality policies of Health Systems The objective is to perform an analysis of potential errors in three emergency laboratories to estimate the impact on patient safety.

Methods: The Clinical Laboratory of Catlab consists of a central laboratory and three hospital emergency laboratories. It is calculated an estimation of the potential risks in each of the three emergency laboratories: Laboratory (1) 609794 clinical analysis/2013, Laboratory (2) 424882 clinical analysis/2013 and Laboratory (3) 256857 clinical analysis/2013.

The Failure Mode and Effect Analysis (FMEA) is the methodology that analyzes the quality, safety and reliability of the functioning of a system, identifying potential errors and their causes from their effects. By using the FMEA, it is calculated the risk priority number (NPR), which is the product of the estimation of the incidence, gravity and detectability (I*G*D). The NPR will allow us to prioritize the importance of the possible errors.

We analyze the possible potential errors that can arise in the different processes that are developed in the laboratories, according to the item that is affected, the causes and their effects.

Results:

Processes	Laboratory(1)	Laboratory(2)	Laboratory(3)
Strategics	27%	17%	31%
Support	10%	6%	10%
Pre-preanalytical	17%	23%	19%
Preanalytical	17%	24%	10%
Analytical	8%	14%	8%
Postanalytical	12%	9%	19%
Post-postanalytical	9%	7%	8%

Conclusion: There are differences between the pre-analytical errors in Laboratory (2) and the Laboratories (1) and (3). The Laboratory (3) makes a greater estimation of the error in the post-analytical processes. It is relevant the importance of the results of the strategic processes in the Laboratories, especially in the Laboratories 1 and 3.

The results obtained using the FMEA will allow us to implement a posteriori improvement actions.

A-077

Value of suspecting undiagnosed G6PD deficiency in very severe hyperbilirubinemia with hepatitis A.

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Background: India has a high burden of both hepatitis A and G6PD deficiency (upto 15%) with no Government funded screening for G6PD. In adults only 2% of infectious hepatitis cases cross a bilirubin value of 30 mg/dL, and we present a case with total Bilirubin more than 60 mg/dL.

History: A 16 year old Indian boy who presented with 7 day history of fever, nausea, vomiting, malaise, anorexia, abdominal pain and yellow urine. On examination he showed deep icterus, and hepatomegaly. Total Bilirubin at presentation was 17.26 mg/dL of which about 60% was conjugated. AST was 7300 IU/L and ALT 6700 IU/L and ALP 640 IU/L. HEV and HAV IgM came back strongly reactive. His Hemoglobin was already 6.3 g/dL when initially checked after admission and two days after it dropped further to 5.3 g/dL and his Bilirubin shot up to 66.95 total of which about 70% was conjugated. next day the transaminases were coming down, with AST 745 mg/dL, ALT 1751 mg/dL, ALP was down to 350 mg/dL. LDH which was not checked initially turned out to be 1364 U/L this time, and Reticulocytes 19%. Putting together the dropping Hb, very severe Bilirubin, and high LDH and Reticulocytosis, an additional hemolytic etiology was suspected. DAT and a G6PD screen were added on in the pre-transfusion sample. DAT came out negative but the G6PD screen was strongly positive for deficiency and a quantitative spectrophotometric G6PD test fetched a level ~30% of lower reference rage even at this hemolytic phase. The patient was also screened for Wilson's disease by Serum Ceruloplasmin and 24 hr Urine copper which all came negative. The patient gradually recovered with transfusion and supportive therapy and his diagnosis of G6PD in addition to HepatitisA.

Conclusion: G6PD deficiency should be suspected in subjects with a history of hemolysis with Hepattis A, even when the majority of fraction of bilirubin is

conjugated . We also suggest to vaccinate all G6PD deficient patients for hepatitis A. Whether to screen blood bag for G6PD before transfusion to Hepatitis patients is of debate. Note Hepatocye damage would be aggraveted due to hepatocellular G6PD deficiency since the deficiencys is not isolated to only Erythroid tissue.

A-079

Neutrophil Gelatinase Associated Lipocalin and atherosclerosis: a study of its association with known cardiovascular risk factors and metabolic syndrome.

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Background: The Neutrophil Gelatinase Associated Lipocalin (NGAL) is a glycoprotein involved in the processes of innate immunity and inflammation. Initially described in neutrophils and epithelial cells, it is also produced by macrophages and smooth muscle cells in atherosclerotic lesions. It activates proteolytic processes linked to metalloproteinase 9 (MMP-9), considered to be responsible for vascular remodeling and rupture of atherosclerotic plaque, leading to clinical presentations of cardiovascular diseases (CVD).

Objectives: To evaluate the associations of NGAL with parameters known as cardiovascular risk factors and criteria for metabolic syndrome.

Methods: Observational, cross-sectional study of a sample (n = 219) of outpatiens assisted by the Family Medicine Program in the city of Niterói, Brazil, aged 45 years and older. All data and samples were collected between August 2011 and August 2012. NGAL serum levels were determined by sandwich ELISA (Bioporto Diagnostics), samples were frozen at -80°C until first thawed. We evaluated its associations with several risk factors.

Results: Of the parameters tested, we observed significant positive correlation between NGAL means and age, creatinine, leukocyte count, Framingham estimated risk, and a negative correlation with HDL cholesterol (Table 1).

Discussion: To stablish preventive strategies for CVD is one important goal of health systems, and it depends on accurate individual risk assessment. There are consistent data from studies in animals and humans that support the importance of NGAL in the patho-physiology of atherosclerosis and CVD. The biological effect of NGAL in atherosclerosis is suggested by the co-localization of NGAL and MMP-9 expression in human carotid atherosclerotic plaques with increased gelatinase activity in these tissues. The quest for understanding the role of this new biomarker in predicting cardiovascular events is of paramount importance, and at this point of the study we could show its association with HDL and to Framingham risk estimates.

Table 1: Mean differences of log NGAL values

category	log NGAL			
	N	mean	SD	*P*
Leukocyte count				
<11.000	200	4.98	0.55	0.001
>11.000	10	5.60	0.48	
Serum creatinine				
<1,2 mg/dL	199	4.97	0.54	0.002
>1,2 mg/dL	19	5.38	0.58	
Low HDL cholesterol (NCEP-ATPIII)				
no	156	4.94	0.55	0.003
yes	62	5.19	0.52	
10 years estimated risk of Myocardial infarction and death by Framingham score				
<10%	129	4.93	0.56	0.039
>10%	69	5.11	0.56	

A-080

Standardization of paroxysmal nocturnal hemoglobinuria assay

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Introduction: Paroxysmal Nocturnal Hemoglobinuria (PNH) is caused by clonal expansion of hematopoietic stem cells that present somatic mutations of the phosphatidylinositol-glycan-class A (PIG-A), resulting of biosynthesis deficiency of the molecule glycol-phosphatidyl-inositol (GPI) and partial or inability to express GPI-anchored proteins**.** Conventional assays to diagnose PNH present high specificity, but low sensibility. Flow Citometry has been used to evaluate the expression of GPI-anchored proteins in different blood lineages, because it is a highly specific and sensitive technique, and it is the gold standard for PNH management and diagnose.

Objective: standardize and validate the PNH immunophenotyping assay using the reagent FLAER (fluorescent aerolysin) for implementation in the routine of a diagnostic laboratory.

Methodology: A total of 80 peripheral blood samples within 24 hours collection from population with ages between 20 and 40 years old were used. Two protocols of surface markers were tested and all the monoclonal antibodies were titrated (Flaer, CD55, CD64, CD24, CD14, CD16, CD45). The acquisition was performed on BD FACSCanto II (BD, San Jose, CA) and the data was analyzed in the Infinicyt (Cytognos S.L., Salamanca -Spain) software.

Results: In the study population, were obtained seven PNH positive samples (9%) and 73 PNH negatives (91%). In all cases the FLAER reagent presented excellent performance in detection of neutrophils and monocytes with PNH phenotype, and there was no conflict between the FLAER staining and GPI-anchored proteins expression. The possibility to differentiate cells type I, II and III in erythrocyte population, when the MEM43 clone from the monoclonal antibody CD59-PE is used was also observed (FI = 2589. 62 negative samples and FI= 954,72 positive samples).

Conclusion: The evaluation of the FLAER reagent performance in detection of PNH clone through Flow Citometry, in this study, presented clear and precise distinction between normal cells and deficiency GPI cells, corroborating with the current literature.

A-081

Effects of Vitamin D Treatments on Arginine Derivatives in patients with Stage 3 and 4 Chronic Kidney Disease

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Background: Vitamin D nutritional status has been linked to chronic kidney disease (CKD) and cardiovascular disease (CVD) in epidemiologic studies. Arginine derivatives, especially symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) have been associated with CKD and CVD. However, data available in literature regarding the relation between vitamin D blood levels and arginine derivatives is conflicting. **Objective:** In this prospective study, our objective was to examine if vitamin D supplementation and increase in 25-hydroxyvitamin D blood levels significantly change arginine, ADMA, SDMA, and the ratios of these biomarkers. **Methods:** This study was approved by our Institutional Review Board. A total of 16 CKD patients (stage 3 and 4) with vitamin D deficiency (<20 ng/mL) and insufficiency (<31 ng/mL) were enrolled in our study. Patients were instructed to take either vitamin D2 or vitamin D3 at 50,000 IU/month for 6 months if they were vitamin D insufficient or 50,000 IU/week for a month then 50,000 IU/month for 5 months if they were vitamin D deficient. 25-Hydroxyvitamin D2, 25-Hydroxyvitamin D3, arginine (ARG), ADMA, and SDMA were measured at baseline and after 24 weeks of supplementation. All analytes were determined by liquid chromatography-tandem mass spectrometry methods. **Results:** Vitamin D supplementation increased 25-hydroxyvitamin D levels from a mean of 24.8 ng/mL at baseline to 39.6 ng/mL after 24 weeks of treatment ($p<0.0001$). Arginine, SDMA, ADMA and their ratios were not significantly different before and after the treatments ($p>0.05$). **Conclusion:** Though vitamin D supplementation in stage 3 and 4 CKD patients significantly improved vitamin D status it did not significantly affect arginine, ADMA or SDMA blood levels.

A-084

A study of the parasitic causes of anaemia in children under five(5) years of age in the Bolgatanga municipality, Ghana

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Background: Anemia is a condition affecting children especially those less than 5 years, and pregnant women in the world (WHO, 2000). In tropical and developing countries, anemia is particularly prevalent with 50% or more of pre-school children and pregnant women being moderately or severely anemic (Cheesbrough, 2006). It is against this background that this research is being conducted.

Methods: This study represents the results of a descriptive cross-sectional study to establish the prevalence, magnitude and causes of anemia among children less than

5 years in the Bolgatanga municipality. Simple random sampling technique was used to obtain specimen from 100 participants following informed consent. Specimens analyzed included stool (wet preparation with physiological saline) for intestinal parasites and blood samples for hemoglobin measurement and detection of the presence of malaria parasites using Rapid Diagnostic Technique (RDT) kits. Actual measurement of their hemoglobin (Hb) was done using a Sysmex analyzer. Anemia was defined as haemoglobin concentration less than 11.0g/dl (Cheesbrough, 2006).

Results: The prevalence of anemia among the children was 62%. Of the anemic children, 35 (56%) showed presence of malaria parasites in their bloodstream whiles 18(29%) had intestinal parasites. 42(66%) of the anemic children had Hb between 10.0 and 11.0g/dl (mildly anemic), 13.0 (23%) had Hb between 7.0 and 9.9g/dl (moderately anemic), with 7 (11%) of them being severely anemic (Hb below 7g/dl). All the parasites detected in the stool samples were hookworm. The presence of these intestinal parasites and malaria parasites in the stool and blood respectively had a significant association with low Hb ($p<0.005$). **Conclusion**: Based on the results of the study, the prevalence of anemia was high in the study population. Malaria parasitaemia was a major cause of anemia in our environment followed by hookworm infestation. The study recommends among other things that the Ministry of Health should embark on intensive health education programs in the communities on early detection of malaria and hookworm infestation and enforce their prevention.

A-086

Fecal Biomarker Testing Identifies Exocrine Pancreatic Insufficiency in Patients with Possible Irritable Bowel Syndrome

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Objective: 1) To use fecal biomarker testing to identify a subset of patients with symptoms of irritable bowel syndrome (IBS) that might be explained by the presence of exocrine pancreatic insufficiency (EPI), and 2) to identify within that subgroup additional fecal biomarkers that might suggest a primary disease process capable of causing secondary EPI.

Relevance: IBS, long considered a "diagnosis of exclusion," is now recognized as an "umbrella diagnosis," identifying a heterogeneous group of patients who may in fact have one or more of several underlying, treatable diagnoses. EPI is one such diagnosis, but few studies have examined its prevalence or biological context in the setting of possible IBS. Because EPI may occur secondary to another gastrointestinal problem such as inflammation or parasitic infection, fecal biomarkers suggesting such processes may further clarify the diagnostic picture in patients presenting with symptoms consistent with IBS. A fecal biomarker panel with appropriate components may thus represent an important role for clinical laboratory medicine in diagnosis and management of conditions producing symptoms consistent with IBS.

Methods: One-year analysis of de-identified stool testing data for fecal pancreatic elastase-1 (FPE1) in all patients for whom stool specimens were submitted for testing bearing at least one of 13 ICD-9 codes indicating the potential for IBS. FPE1 testing was conducted using a standard dual monoclonal antibody ELISA technique with binding sites for two unique epitopes on the human PE-1 molecule. When biomarker data on other conditions were available on the same specimen, they were analyzed for significant associations with FPE1 status using Fisher's Exact Test, with significance set at $p < 0.05$.

Validation: A total of 9112 records were identified that included at least one of the 13 IBS-related ICD-9 codes and also a result of FPE1 testing. FPE1 in the range 200 mcg/g defined normal. Definitions of abnormal for other biomarkers were: calprotectin >120 mcg/g, eosinophil protein X (EPX) > 7 mcg/g, H. pylori, B. hominis, and other parasites = present.

Results: Objective 1: Some degree of EPI was suggested in 858 (9.4%) of all records, with FPE1 <100 mcg/g stool in 289 (3.2%) and FPE1 100 to 199 mcg/g in 569 (6.2%). Objective 2: FPE1 of <100 was significantly associated with abnormal results for fecal calprotectin and EPX, as well as with evidence of infection with Blastocystis hominis, non-Blastocystis parasites, H. pylori, and entamoeba. FPE1 of 100-199 was significantly associated with parasitic infection as well.

Conclusions: Among patients in whom IBS was a consideration, fecal biomarker testing revealed that 9.4% had indications of some degree of exocrine pancreatic insufficiency by FPE1. Fecal biomarker testing was also more likely to suggest presence of an inflammatory or parasitic gastrointestinal condition in patients with abnormal FPE1, suggesting that in some cases EPI itself might be secondary to such underlying processes, and indicating potential lines for further evaluation of possibly treatable diagnoses. Further prospective studies with definitive patient follow-up are recommended.

A-087

Association between the Delta Estimated Glomerular Filtration Rate and the Prevalence of Monoclonal Gammopathy of Undetermined Significance in Korean Males

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Background: The prevalence of monoclonal gammopathy of undetermined significance (MGUS) varies with age, gender, and ethnicity. We investigated the association between the reduction in the estimated glomerular filtration rate (eGFR) and the prevalence of MGUS in healthy Korean males.

Methods: We enrolled 723 healthy Korean males who visited the hospital for regular health checkups. Serum creatinine concentration, serum electrophoresis, serum immunofixation, and the serum free light chain assay were performed. Data, including age, date of health checkup, and previous serum creatinine concentrations were obtained from electronic medical records. We calculated delta eGFR per year and the prevalence of MGUS was compared based on the delta eGFR per year and age group.

Results: Thirteen (1.8%) of seven hundred and twenty-three participants exhibited the monoclonal band on serum immunofixation. Prevalence of MGUS by age group was 0.00% (0/172 for 40s), 1.63% (6/367 for 60s), and 3.80% (7/184 for > 60-years). The median decrease in delta eGFR per year was 5.3%. The prevalence of MGUS in participants in their 50s with > 5.3% decline in delta eGFR per year was significantly higher than those with < 5.3% decrease in delta eGFR per year (3.16% vs. 0.00%; $p = 0.049$). The prevalence of MGUS in participants in their 50s with > 5.3% decrease in delta eGFR per year was similar to that of healthy males in their 60s.

Conclusion: Using the rate of reduction in delta eGFR per year in healthy Korean males who had their serum creatinine level checked regularly may increase the MGUS detection rate in clinical practice.

A-090

An Update on a Candidate BK Virus DNA Standard Reference Material

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Background: The polyomavirus, BKV, is widespread in the population due to primary infection during childhood and largely remains latent in the majority of individuals. However, illnesses as a result of BKV occur in immunocompromised patients, organ transplant, HIV/AIDS- infected, or diabetic patients. Two common illnesses resulting from BKV, hemorrhagic cystitis and nephropathy, occur in transplant patients, as prescribed anti-rejection medications can weaken the immune system. This often leads to renal allograft failure. To treat BKV, accurate quantitation of viral load is necessary for proper adjustment of anti-rejection medications in an effort to improve an individual's immune function. However, current BKV standards are only available for the Ia genotype of the virus, and it has been well-documented that using assays specific to Ia can lead to underestimation of viral load of other genotypes, due to primer and/or probe binding site polymorphisms. Therefore, we propose a candidate reference material panel of BKV genotypes to aid in precise measurements of viral load.

Methods: Viral DNA was extracted from six available clinical isolates and distinct genotypes (Ia, Ic, III, IV, V, VI) of the BK virus. Single site restriction digestion was performed to linearize each viral genome and long-range PCR was performed to amplify entire genomes. Each amplicon was ligated into a plasmid backbone, transformed into, and subsequently propagated in, XL-10 Gold E. coli cells. DNA was extracted, purified, and re-linearized to enable sequencing and accurate copy number measurement. Using the Illumina MiSeq platform, whole genome sequencing was performed for each genotype. New primer and probe sets, specific to each genotype, were designed using this sequence information and optimized for quantitative PCR (qPCR) and digital PCR (dPCR).

Results and Future Goals: We have amplified and sequenced six full-length BKV genomes. Next-generation sequencing information allowed us to design and optimize efficient (between 90-110%) primer and probe sets for both qPCR and dPCR platforms. Homogeneity and stability studies will be performed for each DNA construct using the newly developed assays, and the materials will be certified for genome copy number using dPCR.

Conclusion: The availability of a panel of BKV DNA genotypes as potential reference materials will enable traceability for manufacturers of calibrant materials. This will ultimately improve upon the consistency and accuracy of viral load quantitation measurements across laboratories, leading to improved dosing regimens in infected patients.

A-092

Can Procalcitonin or Lactate help with Sepsis Evaluation in Cancer Patients?

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Objective: Severity of illness due to sepsis in patients with cancer is a major clinical complication and contributes to high mortality in this patient population. We evaluated two biomarkers used to help discriminate between bacterial infection and noninfectious acute-phase reactions: lactate (LA) and procalcitonin (PCT).

Methods: A cohort of 40 random patients with malignant disease admitted to Memorial Sloan Kettering Cancer Center was studied. Out of these 40 patients, 10 were considered as "controls" using the sepsis diagnostic criteria of lactate < 2.0 mmol/l, and negative bacterial cultures: and PCT< 0.5 ng/mL per manufacturer's recommendations. Six out of the forty patients had viral infections. LA and PCT were measured at the time of admission followed by an additional sample collection for each patient within 24 hours. Heparinized blood samples were obtained and the plasma used for LA analysis on the Nova pHOX and for PCT determined by an Enzyme Linked Fluorescent Antibody analysis on the mini Vidas (BioMérieuxS, France). Concordance studies between LA and PCT were done.

Results: Our study demonstrated that elevated LA and PCT levels have only a 50% concordance. PCT was elevated in 50% of the patients and demonstrated a lack of concordance with plasma Lactate. Additionally, we observed that the PCT was elevated in patients who were negative for bacterial infection, based on microbiological cultures, and did not correlate well with LA concentrations >4 mmol/L. The six patients with viral infection had significantly elevated PCT levels and the LA levels were < 4 mmoL/L. In order to examine the possible effect of pre-analytical factors that may explain the LA/PCT discordance, we analyzed LA stability in five healthy volunteers at different time points at room temperature. Within 15 minutes, the LA in heparinized blood increased steadily, and by 30 minutes, LA concentrations were almost 40 % higher than the initial LA measurement. Conclusions: Our preliminary findings suggest that PCT levels were consistently high in cancer patients without sepsis. Our data also indicates a poor correlation between LA and PCT in cancer patients evaluated for suspected bacterial infection and neutropenia. Increased LA concentrations could be due to pre-analytical variation, oxygen deficit due to mitochondrial oxidative phosphorylation, inhibition by interleukins (ILs), tumor necrosis factor (TNF) or a combination of the latter. These data support the already known poor specificity of LA as an accurate indicator of sepsis. Further clinical investigation is also needed to explain elevations in PCT in the absence of bacterial infection in patients with cancer.

Based on these data, the significance of PCT in cancer patients without infection is unclear and requires further investigation. Our data also suggests a need to investigate the relationship between plasma LA and PCT in the clinical evaluation of sepsis in cancer patients.

A-095

Urinalysis/Microscopy Reflex to Urine Culture on i R I C E L L® Complete Urinalysis™ Workcells: Are We There Yet?

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Background: Urinary Tract Infection (UTI) is serious health problem, it is very common among the geriatric population, it is the second most common type of infection (second only to respiratory tract infection),and can cause serious complications and it is a significant cause of morbidity and death, with the expected death rate as high as 3% in those who develop pyelonephritis. The high mortality rate is largely due to delayed presentation and the development of bacteremia/sepsis.

Urinalysis (UA) is one of the most frequently requested tests; it is estimated that between 200 and 300 million UA are performed yearly in the United States. It is an invaluable tool in the diagnosis of UTI, malignancy, calculi, and detecting systemic diseases affecting the kidneys.

Methodology: 1,634 specimens were collected for urinalysis and culture from residents in Long-Term Care Facilities over a period of 2 weeks. Urinalysis and microscopy was done using iRICELL™ automated system. All the specimens were cultured; the culture was done using MicroScan Walkaway96 conventional panels. We used urine culture as the reference standard, no growth or <10,000 colony-forming unit/mL were considered negative, cultures with > 50,000 colonies colony-forming unit/mL were considered positive. The urinalysis instrument generates a "urine culture candidate report" when at least one of the five bacteriuria parameter (Nitrite, Leucocyte Estrase, and microscopy values: white blood cells, bacteria and all small particles); the report was used to assess its effectiveness for urine culture screening.

Results: 801 (49.0%) had positive culture. 1344 (82.3%) specimens were identified as urine culture candidates. Of the 1,344 that were identified as a candidates, 772 (57.4%) had positive culture, 547 (40.7%) were negative, 25 (1.9%) were contaminated. 290 (17.7%) specimens were not selected as culture candidates because of the negative urinalysis; of these specimens 29 (10%) had positive culture and 259 (89.3%) were negative.

Conclusion: using the candidate for urine culture would have avoided urine culture on about 15% of the patients but would have missed the diagnosis for 10% of the patients that were considered as having normal specimens. Urinalysis and microscopy still lack the sensitivity and specificity to be used alone to diagnose urinary tract infection, more work needed to establish better parameters and algorithm to be used when screening patients for UTI using urinalysis.

A-096

Interleukin-6 (IL-6) Combined with Clinical and Demographic Parameters Predicts Death in Critically Ill Patients with Systemic Inflammatory Response Syndrome

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Background: Increasing sepsis severity is associated with significant mortality. Currently, there are few prognostic biomarkers for septic patients. Objective: To determine the prognostic utility of 5 inflammatory biomarkers, clinical and demographic data to predict mortality among Medical ICU (MICU) patients with systemic inflammatory response syndrome (SIRS). Methods: This retrospective cohort study enrolled 201 MICU patients with SIRS. Among them, 91 were septic and 45 died during their admission. TNFα, IL-6, IL-10, CRP and LBP were measured on the Immulite 1000 (Siemens Healthcare Diagnostics) in residual plasma collected on the day of SIRS and 1 or 2 days prior to meeting SIRS criteria (days -1 and -2). Procalcitonin (PCT) was measured on the Vidas B•R•A•H•M•S® Assay (Biomeriéux). The prognostic strength of individual biomarkers and logistic regression models that combined baseline co-variables (SIRS criteria, age, and sex; Model A) and biomarker concentrations (Model B) were evaluated. The change in serial biomarker concentrations (days -2, -1 and 0) and the difference in median concentrations for each day were determined for survivors and non-survivors by two way ANOVA and Mann-Whitney tests respectively. Results: Table 1 lists the areas under the receiver operating characteristic curves (AUCs) for individual biomarkers and models. The evolution of IL-6 over time was significantly different in survivors and non-survivors (time-survival interaction p=0.02). Median IL-6 concentrations were significantly different in both groups during all study days (p<0.01, <0.001, and <0.0001 on days -2, -1, and 0 respectively). Conclusions: IL-6 was the best single predictor of mortality. PCT showed limited prognostic ability. When combined with baseline demographics and clinical data, IL-6 showed improved ability to predict death. Additional biomarkers (model AB) did not improve the model's prognostic strength. Serial measurement of IL-6 combined with other clinical parameters accurately predicts mortality in patients with systemic inflammatory response syndrome.

A-098

Fatigue in rheumatoid arthritis and its relation to interleukin-6 serum level

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Objectives: This work aimed to investigate the occurrence and level of fatigue and its relation to IL-6 serum level in rheumatoid arthritis (RA) patients. **Patients and methods:** The study included 60 patients with RA diagnosed according to the 2010 ACR-EULAR classification criteria for RA and 30 healthy controls. Patients were included if they were above 18 years and fulfilled a score P6 over 10 of the 2010 ACR-EULAR classification criteria for RA. Disease activity was assessed using 28 joint disease activity score (DAS28), erythrocytes sedimentation rate (ESR) and C-reactive protein (CRP). Fatigue was assessed using the Bristol Rheumatoid Arthritis Fatigue Multidimensional Questionnaire (BRAF-MDQ) and serum IL-6 level was measured in patients and controls using enzyme-linked immunosorbent assay (ELISA) technique. **Results:** The BRAF-MDQ was significantly higher among patients (mean =50.6± 15.2) than controls (mean= 7.8± 3.7) (p< 0.001). Patients' mean IL-6 serum level was 35.05 ±21.23 pg/ml and 4.72 ±3.09 pg/ml among control subjects (p< 0.001). DAS 28 ranged between 4.33 and 7.67. Mean 1st hour ESR was

43.57 mm and CRP was positive in 76.7% of patients. Significant correlations were found between BRAF-MDQ score and serum IL-6 level (r = 0.947, p <0.001), ESR (r =0.509, p< 0.001) as well as CRP positivity (r =0.411, p=0.005) in RA patients. Serum IL-6 level correlated with ESR (r = 0.463, p< 0.001) and CRP (r =0.376, p= 0.01) among patients. **Conclusion**: Fatigue is a common symptom and scores higher among RA patients than healthy controls and should be measured in all RA patients with simple fatigue questionnaires matching with different cultures. Fatigue becomes more prominent as serum IL-6 level increases independently of the disease duration and activity.

A-099

Ferritin in sera and CSF: Its importance as both predictive and etio-diagnostic biomarker in ischemic stroke, single center prospective study

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Background:Iron and ferritin are known to have an important role in stroke as well as in other disorders. This prospective study was designed to determine whether determination of CSF ferritin levels might help to estimate the severity and prognosis of stroke.Methods:Thirty-two patients with a diagnosis of acute stroke due intrinsic atherosclerotic vessel pathology were included in the study within 24 h from onset of symptoms. Plasma and CSF ferritin were assayed; and correlated them with the known marker Aβ1-42 at admission. Clinical status was determined by the Canadian Stroke Scale at admission and on day 21.

Results:Serum ferritin level was found to be higher in patients with large lesion size (P < 0.01), deteriorated neurologic status during clinical follow-up (P = 0.03) and ICAD stroke patients (P < 0.01). CSF and Serum ferritin level were correlated with neurologic deficit (r = 0.50, P < 0.001). No correlation was found between Aβ1-42 and ferritin levels (r = 0.07, P = 0.7). Serum ferritin level (P = 0.007; OR = 1.02; 95% CI, 1.01-1.03) and large size of lesion (P = 0.021, OR = 11.92; 95% CI; 1.46-197.12) were independently associated with stroke due to ICAD pathology, Increased serum ferritin levels correlate to severity of stroke and the size of the lesion.

Conclusion:our results supported that a raised level of CSF and serum ferritin may imply a poor prognosis in terms of neurologic deterioration or ICAD induced stroke patients.

A-100

A Novel Missense Mutation in the Androgen Receptor Gene Causes the Complete Androgen Insensitivity Syndrome

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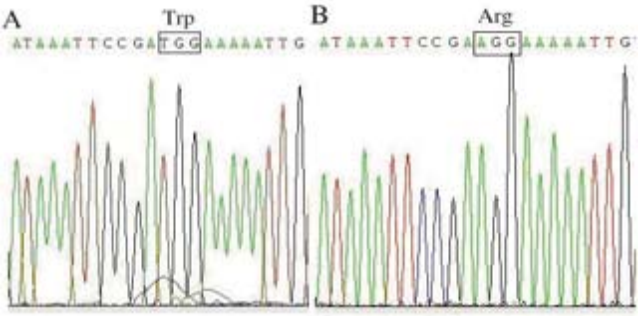
A 22-year-old, phenotypically female individual was presented at hospital because of primary amenorrhea. The physical examination revealed normal female external genitalia, sparse pubic hair and developed breasts with juvenile nipples and pale areolas. A mass was found in the left inguinal canal. The gynecological examination revealed a short, blind-ending vagina 7.0 cm in length. Ultrasonography of the pelvis showed no uterus, fallopian tube, or ovaries. The hormonal profiles were as follows: follicle-stimulating hormone (FSH) 23.4 IU/L, luteinizing hormone (LH) 39.6 IU/L, testosterone (T) 23.0 nmol/L, estradiol (E_2) 0.177 nmol/L. FSH and LH were above the upper normal limit. T was elevated compared with the normal female level and was in the normal range for males. E_2 was in the normal range for females. Cytogenetic analysis from the peripheral blood lymphocytes revealed a 46,XY karyotype in the patient.

DNA (genomic DNA was extracted from peripheral blood of the patient) sequencing of polymerase chain reaction (PCR) products revealed a single-nucleotide substitution (A→T) at position 2184 in exon 3, resulting in the conversion of arginine (AGG) to tryptophan (TGG) at amino acid position 608 in the DNA-binding domain of the AR (Figure 1). The novel missense mutation of exon 3 in the AR gene, resulting in the nonfunctional protein, is responsible for the clinical symptoms of CAIS.



The study extends the spectrum of exon 3 mutations in the AR gene.

Figure 1. DNA sequence of exon 3 of the androgen receptor(AR) of the patient(A) and the normal sequence (B). The mutant sequence shows conversion of arginine (AGG) to tryptophan (TGG).

A-101

Association Between Serum Uric Acid Levels and Non-Alcoholic Fatty Liver Disease

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Background: Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of conditions ranging from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH) and cirrhosis. It has become one of the most common forms of chronic liver disease affecting 20-30% of the Western population. Elevated serum uric acid (SUA) levels have been suggested to be an independent risk factor for the development of NAFLD in healthy adults. In this study, we aimed to investigate the relationship between SUA and liver histology in non-diabetic and normotensive patients with NAFLD.

Materials and Methods: A total of 242 male patients, 102 with non-alcoholic steatohepatitis (NASH) and 140 with non-NASH were included. The histopathological examination of the cases were carried out according to Kleiner NAFLD activity score (NAS) combining steatosis, lobular inflammation and ballooning hepatocyte degeneration. Hyperuricemia was diagnosed as SUA more than 7 mg/dL.

Results: The prevalance of hyperuricemia was 33.4%. SUA levels in patients with NASH were significantly higher than those of non-NASH (p= 0.035). There was also no difference between the NASH and non-NASH groups in terms of hyperuricemia prevalence (p= 0.107). Univariate and multivariate analyses both demonstrated that hyperuricemia had a significant association with younger age (OR, 0.930; 95% CI, 0.884-0.979) (p= 0.005), higher body mass index (OR, 1.173; 95% CI, 1.059-1.301) (p= 0.002) and hepatocellular ballooning (OR, 1.678; 95% CI, 1.041-2.702) (p= 0.033). Area under the curve for hepatocellular ballooning was OR, 0.626; 95% CI, 0.544-0.708) (p= 0.005) and NAS was OR, 0.579; 95% CI: 0.507-0.652) (p= 0.035). Hyperuricemia was insignificant in predicting other hepatic necro-inflammatory changes.

Conclusions: SUA level seems to be a useful metabolic parameter in the differentiation of NASH and non-NASH. In addition, hepatocellular ballooning, which is considered to be an earlier predictor of hepatocyte injury, was the unique histological parameter associated with hyperuricemia. It is also thought to be a biochemical evidence in the pathogenesis of liver damage. Further studies on the involvement of SUA in NAFLD will not only expand our understanding of the mechanism of NAFLD, but will also assist in the eventual development of new prevention and treatment strategies for NAFLD by modulating the SUA levels.

A-102

The Elecsys® Periostin assay as a companion diagnostic for the novel asthma drug lebrikizumab

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Objective: The Elecsys® Periostin assay is being developed as a companion diagnostic for the anti-IL13 drug lebrikizumab in asthma. The current clinical trial assay was used to evaluate the precision performance according to CLSI EP 5-A2 under field conditions. The assay was used in the Phase IIb lebrikizumab trials (LUTE and VERSE) to stratify asthma patients by baseline serum periostin levels.

Methods: The Periostin assay is an automated electrochemiluminescence immunoassay. The assay requires a total of 18 minutes to perform. It employs two monoclonal antibodies targeted to Periostin via the sandwich principle. The assay is used for the in vitro, quantitative determination of periostin in human serum.

Imprecision testing was conducted using a total of eight human serum pools covering the entire measuring range (10-160 ng/mL). This experiment was carried out at three external routine laboratories, on three **cobas e** 601 analyzers. The experimental setting included two replicates in two runs per day for twenty-one days (EP-5), using two reagent lots. Using this design, repeatability, intermediate precision and reproducibility were estimated based on a variance-components model.

It has been shown that high serum Periostin levels (≥ 50ng/ml) are associated with benefit from lebrikizumab in asthma (Phase II lebrikizumab trial MILLY, Corren et.al. NEJM, 2011). LUTE and VERSE were randomized, multicenter, double-blind, placebo-controlled, parallelgroup clinical trials. Patients were aged 18 years and older whose asthma remained uncontrolled despite daily treatment with inhaled corticosteroids (ICS) therapy and a second controller medication. Patients were randomized in a 1:1:1:1 ratio to receive subcutaneous lebrikizumab at 250 mg, 125 mg or 37.5 mg dose or placebo, administered every 4 weeks. The primary endpoint was reduction in exacerbation rate and the main secondary endpoint was percent change in forced expiratory volume (FEV_1). Efficacy analysis for each endpoint was assessed in subgroups of periostin high (≥50 ng/ml) and low (<50 ng/ml) patients.

Results: Repeatability for the Periostin assay varied from 1.24% to 1.64% CV, intermediate precision varied from 2.30% to 2.56% CV and overall reproducibility varied from 3.10% to 3.95% CV. The precision obtained fulfilled the assay specification and met the requisite target to segment subjects properly to the correct periostin stratum.

A total of 463 patients were enrolled in both studies, of which 42% of patients were classified as periostin-high. Compared with placebo, the exacerbation rate was reduced by 60% (95% CI: 18, 80) in periostin-high patients (combined dose levels) and by 5% (95% CI: -81, 47) in periostin-low patients. FEV_1 at 12 weeks increased in the periostin-high group by 9.1% (95% CI: 2.2, 15.9) over placebo, compared with a 2.6% (95% CI: -2.7, 7.9) increase over placebo in the periostin-low group.

Conclusion: The Periostin assay demonstrated good precision and was used for clinical sample testing to stratify patients in the lebrikizumab Phase IIb trials. Lebrikizumab treatment reduced the exacerbation rate and increased FEV1 in patients with uncontrolled asthma on ICS and a second controller, particularly in those who were periostin-high, confirming the findings from the Phase II trial MILLY.

A-103

Equivalence between high sensitivity CRP and low sensitivity CRP tests in infants

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Background: C-reactive protein (CRP) is an acute-phase protein that rises in response to inflammatory processes. Most clinical laboratories perform two different assays for measuring CRP, low sensitivity CRP (ls-CRP) and high sensitivity CRP (hs-CRP). The hs-CRP is usually used in combination with other biomarkers to assess risk of developing myocardial infarction in patients presenting with acute coronary syndromes and risk of cardiovascular disease in adults who do not manifest disease at present. Along with the monitoring of inflammatory process, ls-CRP is also used in the diagnostic evaluation of sepsis in neonates and infants. CRP values in healthy infants and neonates vary from a range of 0.2 mg/dL to 1.0 mg/dL. Therefore, for the diagnosis of sepsis, a concentration >1.0 mg/dL is considered abnormal and serial testing is frequently performed. The objective of this study is to verify whether the hs-CRP method is equivalent to ls-CRP in monitoring inflammatory processes in infants.

Methods: For this study, 33 serum samples with ls-CRP higher than 0.7 mg/dL from a patient population of <1 year old were collected. These serum samples were analyzed by both ls-CRP and hs-CRP assays on the UniCel DxC800 Systems by turbidimetric immunoassays. In both methods, CRP reacts with a specific antibody to form insoluble antigen-antibody complexes. The turbidity was measured at 340 nm for ls-CRP with an analytical measurement range of 0.7mg/dL to 20 mg/dL and at 940 nm for hs-CRP with the range of 0.02 mg/dL to 6 mg/dL. Equivalence of these two methods for the chosen population was evaluated with an allowable total error of 0.6 mg/dL or 25 %, whichever is higher. Also, 18 samples with ls-CRP values <0.7 md/dL were analyzed by both methods.

Results: 33 specimens were compared over a range of 0.22 to 23.35 mg/dL by ls-CRP. The difference between the two methods was within the allowable error for 31 of 33 (94 %) specimens, with a linear regression equation y=0.886x+0.3713 and the correlation coefficient (R) of 0.9908.The error index was determined by the ratio of the difference between two methods (hs-CRP – ls-CRP) to the allowable total error and the average error index was -0.13 with a range of -1.03 to 1.25. For 12 samples with concentrations < 2.4mg/dL by ls-CRP, the mean bias was -0.16 mg/dL with a standard deviation of 0.39 mg/dL, out of which 2 samples had error >0.6 mg/dL. For 21 samples with concentrations > 2.4mg/dL by ls-CRP the mean bias was -1% with a standard deviation of 12%. Also, all the 18 samples with CRP concentrations <0.7mg/dL by ls-CRP presented values <0.7 mg/dL by hs-CRP method.

Conclusions: CRP concentrations in the patient population of <1 year old infants can be analyzed by both Beckman Coulter UniCel DxC ls-CRP and hs-CRP methods to monitor inflammatory processes. Overall, the hs-CRP assay is equivalent to the ls-CRP method for the majority of patients (94%). However, in about 17% patients with CRP < 2.4 mg/dL, the bias may be greater than the allowable total error.

A-105

SDMA Outperforms Serum Creatinine-Based Equations in Estimating Kidney Function Compared with Measured GFR

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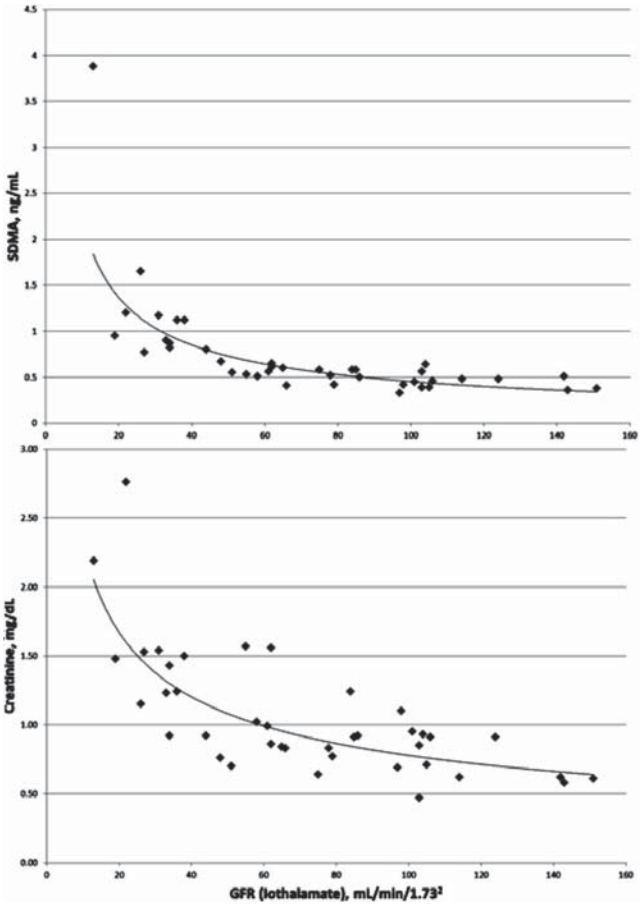
Background: Symmetric dimethylarginine (SDMA), a catabolic product of post-translational modified arginine containing proteins, is an emerging biomarker for renal function. Recent studies have shown that there is a correlation between SDMA and renal function in chronic kidney disease (CKD) patients. However, there are no published reports that have investigated the performance of SDMA versus creatinine in estimating glomerular filtration rate (GFR) in both CKD and non CKD patients. The objective of this study was to determine the correlations of measured GFR with SDMA levels, creatinine levels, eGFR (MDRD), and eGFR (CKD-EPI). **Method:** EDTA plasma samples were obtained from 21 male and 19 female adult subjects who were 21-76 years old. These subjects had GFR measured by radioactive iothalamate renal clearance, ranging from 13-151 mL/min/1.73^2. Arginine, asymmetric dimethylarginine (ADMA), and SDMA in EDTA plasma were assayed using a previously validated LC-MS/MS method. **Results:** A power regression analysis showed R^2=0.7544 and R^2=0.5827 the correlation of measured GFR with SDMA and creatinine, respectively. A linear regression analysis was used for the correlation of measured GFR with eGFR by MDRD and eGFR by CKD-EPI with R^2=0.6534 and R^2=0.7183, respectively. **Conclusion:** Correlation of measured GFR with SDMA was higher than those with creatinine, eGFR (MDRD), and eGFR (EPI) in a population of CKD and non CKD patients. In Conclusion: SDMA outperforms creatinine and creatinine-based equations in estimating kidney function compared with measured GFR.



A-106

Prevalence of Metabolic Syndrome and its Components according to Different Definitions among Nepalese Type 2 Diabetic Patients

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Objective: The objective of this study is to determine the prevalence of the metabolic syndrome (MetS) and its components according to WHO (1998), NCEP ATP III (2005), IDF (2005) and newly introduced Harmonized (2009) criteria and determine their agreement and disparity in diagnosing MetS among Nepalese patients with type 2 diabetes mellitus (DM).

Methods: Patients with type 2 DM without any other acute or chronic illness were recruited for the study from the Manipal Teaching Hospital (MTH), Pokhara, Nepal. Clinical data were obtained by interviewing the patients with structured questionnaire, anthropometric and blood pressure measurements and biochemical analyses of the blood samples. The analyzed biochemical parameters included fasting serum glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), triglycerides and high density lipoprotein cholesterol. Statistical analysis included usage of Student's t and Chi square tests, kappa statistics and 95% confidence intervals.

Results: 1061 (male: 589, female: 472) type 2 diabetic patients aged between 30-89 years were included in the study. The total age adjusted prevalence was 69.9 %, 73.9%, 66.8% and 80.3% according to WHO, NCEP ATP III, IDF and Harmonized definitions, respectively. The Harmonized definition outperformed other definitions in diagnosing MetS. Prevalence generally increased with the age and remained highest in the age range of 50-69 years in both the sexes. It was significantly higher in females (p=0.000) according to WHO, NCEP ATPIII and Harmonized definitions. Patients of Dalit community had the highest prevalence according to NCEP ATP III (p=0.033) and Harmonized (p=0.037) definitions whereas patients of Mongol and Newar communities outnumbered other communities according to WHO (p=0.001) and IDF (p=0.037) definitions, respectively. The overall agreement was substantial between Harmonized and NCEP ATP III (κ=0.62), moderate between Harmonized & IDF (κ=0.54) and NCEP ATP III & WHO (κ=0.51), fair between NCEP ATP III & IDF (κ=0.33) and Harmonized & WHO (κ=0.37) and slight for WHO & IDF definitions (κ=0.26). The most frequent component of MetS was central obesity according to the WHO (98.8%), IDF (99.9%) and Harmonized (85.6%) definitions respectively. However, decreased HDL was the most frequent component (93.5%) according to NCEP ATPIII definition. On the other hand, hypertension was the least frequent component according to all four definitions (WHO: 66.2%, NCEP ATP III: 72.1%, IDF: 65% & Harmonized: 67%). The overall diagnostic potential of the NCEP ATP III definition was the best among others [sensitivity: 90.8%, specificity: 98.9%, positive predictive value: 99.9% and negative predictive value: 49.9%] while comparing with Harmonized definition as the gold standard.

Conclusion: The prevalence of MetS in type 2 DM is very high in both genders irrespective of the definitions used and increases with age thus posing a potential threat of increased cardiovascular diseases in this group of patients. The patient education and control of the modifiable risk factors for MetS should be given due priority by the clinicians in the management of subjects with type 2 DM.

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Serum 25-Hydroxy Vitamin D3 Levels in Patients with Psoriasis

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Background: Vitamin D has classically been associated with phosphorus-calcium metabolism and bone physiology. However, the finding of vitamin D receptors at different sites suggests that vitamin D also has important extraskeletal functions. Vitamin D plays a pivotal role in modulating dendritic cell function and regulating keratinocytes and T-cell proliferation. Psoriasis is considered a prototypic T helper (Th) 17-mediated disease with a putative role played by vitamin D deficiency in its pathogenesis. The most efficient laboratory approach consists of a well-defined evaluation of immune response in order to help diagnosis, to monitor evolution, and to evaluate the effects of individualized therapeutic treatments. Vitamin D has been found to regulate immune function in a number of inflammatory and autoimmune disorders. The objective of this study was to find out the serum vitamin D levels in patients with psoriasis.

Methods: Blood samples were collected and serum was seperated with centrifugation from 51 healty and 93 patients with psoriasis. Patients with chronic disease and calcium metabolism disorders were excluded. This study was approved by local ethic committee. 100 µL internal Standard (d6-25-hydroxyvitamin D3) and 1000 µL acetonitrile were added to 250 µL of serum, calibrator, control for protein precipitation, vortexed for a minute and centrifuged at 13.000 rpm for 10 minutes. 40 µL of supernatant was injected into HPLC analytical column for chromatography. Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an atmospheric pressure chemical ionisation (APCI) operating in positive mode. This method's coefficient of variation and % bias values were 9.35,1.29; 3.81,3.21 and 2.29,2.60 for 10, 32 and 150 µg/L, respectively. Statistical analysis was performed with SPSS v16.

Results: Serum 25-hydroxy vitamin D3 levels were significantly lower in patient group (10.3 ±5.6) compared to control group (13.7 ±7.8) (p=0.004) according to Mann-Whitney U test. Also, there was no statistically significant correlation between Psoriasis Area Severity Index (PASI) and serum vitamin D levels (p=0.99) according to Spearman correlation analysis.

Conclusion: Vitamin D may play an important role of psoriasis pathogenesis. Vitamin D has been used to treat psoriasis in the topical form with great success. Low levels of vitamin D may also have important implications in the pathogenesis of psoriasis. Vitamin D3 acts mainly on the vitamin D receptor to regulate keratinocyte growth and differentiation, but also

has an influence on immune functions of dendritic cells and T lymphocyte. Vitamin D deficiency may be common in patients with psoriasis. Screening for vitamin D deficiency may be useful for comprehensive management.

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CEDIA® Cyclosporine Applications for the Beckman Coulter AU480, AU680 and AU5800 Analyzers

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Background: Cyclosporine is an immunosuppressant used in the treatment of autoimmune diseases and the reduction of tissue rejection following organ transplantations.

Although its mechanism of action is still under investigation, cyclosporine appears

to affect the metabolism of T-helper lymphocytes and T-suppressor lymphocytes, resulting in the inhibition of the immune system. Although cyclosporine is safely used over an established narrow range of concentrations, inadequate dosing may lead to organ rejection, while overuse may lead to a number of adverse effects including nephrotoxicity, and hepatotoxicity. The probability of adverse effects on users' health increases with drug concentration. Therefore, it is crucial to monitor cyclosporine levels to achieve optimal immunosuppressive effects in patients.

Methods: The CEDIA Cyclosporine PLUS Assay is based on the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. Cyclosporine in the human whole blood sample competes with cyclosporine conjugated to one inactive fragment for antibody binding site. Once cyclosporine in the sample binds to antibody, inactive enzyme fragments reassociate to form active enzymes. The amount of active enzyme results in an absorbance change that is directly proportional to the amount of cyclosporine in the sample, and is measured spectrophotometrically. The Beckman Coulter AU480/AU680/AU5800 analyzers are new applications for the CEDIA Cyclosporine PLUS Assay. Performance measures of the CEDIA Cyclosporine PLUS Assay on the Beckman Coulter AU480/AU680/AU5800 analyzers were conducted in low-range (25-450 ng/mL) and high-range (450-2000 ng/mL) assay studies. Analyzer performance was determined for precision, linearity, limit of detection, and accuracy. Correlation studies using the AU480/AU680/AU5800 analyzers were conducted against the reference analyzer, Hitachi 911.

Results: All studies were evaluated using CLSI guidelines. Five levels of cyclosporine controls were used in the studies. The precision ranged from 7.1 to 3.1%CV for within-run and 12.7 to 8.6%CV for total run. Low range linearity was measured and confirmed over a range of 9.08-427.85 ng/mL on the AU480/AU680/AU5800. High range linearity was measured and confirmed over a range of 590.1-2027.6 ng/mL on the AU480/AU680/AU5800. The limit of detection on the AU480/AU680/AU5800 yielded 8.3 ng/mL. Accuracy was measured using patient correlation against the reference analyzer Hitachi 911, which yielded a Deming's Regression for each analyzer. (Low Range Cyclosporine): AU480 = 1.03*(Hitachi 911) - 0.40 (N = 115, r = 1.00), AU680 = 0.97*(Hitachi 911) + 13.00 (N = 100, r = 1.00), AU5800 = 1.00*(Hitachi 911) - 0.50 (N = 116, r = 0.99). High Range Cyclosporine: AU480=1.03*(Hitachi 911) + 90.96 (N = 126, r = 0.97), AU680=1.07*(Hitachi 911) + 30.23 (N = 126, r = 0.97), AU5800=1.05*(Hitachi 911) + 9.00 (N = 126, r = 0.97).

Conclusion: All measured studies demonstrated acceptable performance, validating the use of the CEDIA Cyclosporine PLUS Assay on the Beckman Coulter AU480/AU680/AU5800 analyzers, and will provide an effective monitoring system for patients receiving cyclosporine therapy.

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Chemical composition of urinary tract calculi assessed in a Caribbean teaching hospital

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Background: Urolithiasis is a heterogeneous and fairly common urological disorder with an estimated lifetime prevalence of between 5 to 10%, with the risk being higher in men. Environmental and genetic factors contribute to calculi formation. Research regarding kidney stones has gained increased attention in light of its complex molecular genetic basis as well as recent associations between urolithiasis and cardiovascular diseases. Importantly, the proper management of patients with renal stone disease involves the analysis of urinary calculi by varied methods. The main objective of this study was to assess the chemical constituents particularly inorganic minerals of urinary tract stones observed at the University Hospital of the West Indies over a 4-year period. The study is validated by the observation that there is a paucity of data on the types and composition of calculi recovered from patients within this region.

Methods: The study was conducted on 288 urinary tract stones sent to the chemistry laboratory for analysis from both male and female patients. Samples were washed with deionized water, air-dried and powder obtained via pulverization in an agate mortar. Qualitative chemical analysis of the stones for calcium, magnesium, phosphate, oxalate, uric acid, cystine and bicarbonate was done based on standard methods. Carbonate content was determined by the effervescence test by exposing the stone powder to concentrated hydrochloric acid. After the mixture was boiled, the filtrate was used for detection of calcium with ammonium oxalate, magnesium with potassium phosphate and ammonia, phosphate with ammonium molybdate and oxalate with calcium chloride. The powder was also boiled with N-potassium hydroxide and Folin's uric acid reagent with sodium cyanide added to the filtrate to detect uric acid. Cystine was detected with sodium nitroprusside and sodium cyanide.

Results: The incidence of stones from males and females was in the ratio of 1.4:1. Calcium, the main constituent was present in 96.2% of the stones followed by phosphate 67.7%, oxalate 56.3%, magnesium 28.2% and uric acid 17.3%. Mixed calcium phosphate accounted for 42.4% of the stones while there were 25.4% pure calcium phosphate stones. Mixed calcium oxalate accounted for 43.4% of the stones while there were 12.9% pure calcium oxalate stones. Mixed uric acid was present in 16.3% and urinary stones containing bicarbonate accounted for 11.8%. One cystine stone was recorded.

Conclusion: The study revealed that a relatively high proportion of the urinary tract stones in the sample population consisted of both pure and mixed calcium phosphate, followed by calcium oxalate and uric acid. The main contributory factor to the frequency of these stones seems to be hypercalciuria resulting from hypercalcaemia. Results show that males are more likely to present with urolithiasis. Ongoing studies are geared at garnering more detailed information on both the composition and structure of the urinary tract stones using solid state nuclear magnetic resonance spectroscopy and X-ray diffraction crystallography.

A-110

Screening for Hemoglobin Variants [HVs] While Measuring Hemoglobin A1c (HA1c).

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Context: As part of an effort to develop an in-lab protocol for the reporting of HA1c results in the presence of HVs, we sought to assess the frequency and identities of HVs in the VAMC population.

Methods: Over seven weeks, 5188 patients were assayed for HA1c using HPLC [Tosoh; South San Francisco, CA] according to manufacturer's directions. Specimens with an HV flag were referred (Lab Corp; Raleigh, NC) for hemoglobin ID [H-ID]. Estimates of the frequency of AS and AC (8 and 2.8% respectively) among African-American [A-A] and the population prevalence of DM (9.5%) are literature derived. A total of 40,648 HA1c assays were done in calendar year 2012.

Results: A total of 208 (4%) HV flags were generated. The median age was 61 YO [27, 90], 188 (90.4%) were male and 192 (92.3%) A-A while 6 were of unknown race (UR). Of these, 181 [87%] had sufficient specimen for H-ID and results included AS (118/113 A-A, 4 UR), AC (42/40 A-A, 1 UR) while the remaining 21 comprised HPHF (9), D-LA (3), SC (2), G-Norfolk (1), Kokomo (1), A2' (1) and no HV identified [NVI] (4). Of a total of 57,680 veterans registered at the VAMC, 35.7% are self-identified as A-A. Diabetic patients (5480) were assumed to be tested 3 times (on average) in any 12 month period and the remaining 24,208 assays were attributed to once-tested patients. Thus in any given 12 month period 29,668 patients out of the total of 57,680 (51.47%) patients would be tested. Requests were assumed to be uniformly distributed over 52 weeks. Making all these assumptions the expected number of AS/A-A patients was [29,668]•[0.357]•[0.08]•[7/52] = 114 [actual=113]. A similar calculation yields an expected 40 instances of AC trait [actual=40]. HPHF and Kokomo specimens generated an HV flag but not a numeric result for HA1c.

Conclusion: In our population that was 35.7% AA, 4% of patients assayed for A1c generated an HV flag. More than 90% occurred in AAs and 85% were S and C trait. Four (2.2%) specimens were NVI. The observed and (crudely) estimated number of AA patients with S and C trait were in close agreement [chi-sq. = 0.084; p>.9].

A-111

Tubular Damage Is Present In Patients with MGUS and Asymptomatic Multiple Myeloma Even in the Absence of Impaired Estimated Glomerular Filtration Rate; Alterations of Neutrophil Gelatinase-Associated Lipocalin and Cystatin-C in Myeloma Patients Post IMiD- and Bortezomib-Based Regimens

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Background: We have recently shown that urinary and serum Neutrophil gelatinase-associated lipocalin NGAL were elevated in the vast majority (90% and 70%, respectively) of newly diagnosed patients with multiple myeloma (MM), while serum cystatin-C (CysC), an accurate marker of GFR, was elevated in 70% of them. However, there is no information for the value of these markers in patients with MGUS, asymptomatic MM (AMM), as well as in symptomatic MM post treatment.

Patients and Methods: Thus, we measured urinary and serum NGAL and serum CysC in 40 patients with MGUS; 36 with AMM and 120 healthy controls. Furthermore, we measured serum NGAL and CysC in 39 newly diagnosed symptomatic MM patients before and after frontline therapy with novel agents. Serum and urinary NGAL was measured using an immunoenzymatic technique, while CysC was by means of immunonephelometry. The estimated GFR (eGFR) was calculated using the CKD-EPI equation. Patients were divided into the 5 CKD stages of the KDIGO classification, according to eGFR.

Results: Urinary NGAL was elevated in patients with MGUS (median: 14ng/ml, range 0.5-31ng/ml) and AMM (22.3ng/ml, 0.9-78ng/ml) compared to controls (5. ng/ml, 0.7-9.8ng/ml, $p<0.001$ for both comparisons). Similarly, serum NGAL was elevated in patients with MGUS (106ng/ml, 74.9-205.5ng/ml) and AMM (94.2ng/ml, 29.5-306.4ng/ml) compared to controls (63ng/ml, 37-106ng/ml, $p<0.01$). There was no difference between MGUS and controls or MGUS and AMM regarding CysC serum values, indicating that traditional indices of renal function could not detect early renal damage. However, 22 (55%) patients with MGUS and 24 (66%) with AMM had higher urinary NGAL values than the higher value of the controls. Similarly, 9 (22.5%) MGUS and 11 (30%) AMM patients had higher levels of serum NGAL than the higher value in the control group. As expected, patients with symptomatic MM had elevated serum NGAL and CysC ($p<0.001$). NGAL strongly correlated with CysC ($r=0.675$, $p<0.001$) and CKD stage (mean±SD values for stages 1/2, stage 3 and stages 4/5 were: 97±57ng/ml, 144±79ng/ml and 205±124ng/ml, respectively, $p=0.014$). CysC also correlated with CKD stage (0.96±0.29mg/l, 1.54±0.32mg/l and 2.51±1.00mg/l respectively, ANOVA $p<0.001$). Among patients with eGFR <50ml/min at baseline (n=22), 4/4 who received bortezomib-based regimens and 5/18 who received IMiD-based regimens achieved at least minor renal response. After 4 cycles of therapy, serum NGAL increased in patients who received IMiD-based therapy compared to baseline (255±264ng/ml vs. 147±104ng/ml, $p=0.021$), but not in patients who received bortezomib (119±68ng/ml vs. 159±111ng/ml $p=0.520$), regardless of myeloma response to treatment.

Conclusions: We conclude that the high levels of urinary and serum NGAL in MGUS and AMM indicate the presence of subclinical renal damage in these patients early in the course of their disease, when other markers of renal function, such as sCr or even the more sensitive CysC indicate that renal function is preserved. Thus, NGAL may be useful as an early marker that predicts the development of renal damage and the progression of the disease in these patients. NGAL seems also to increase in patients with renal impairment who receive IMiD-based regimens.

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Association of Interleukin (IL-18) -607A/C and -137C/G Polymorphisms With Early Graft Function In Renal Transplant Recipients

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Background: Recent studies have suggested that increased levels of IL-18 in serum and renal parenchyma may predict acute rejection in patients with renal transplantation. IL-18 mediates a wide range of inflammatory and oxidative responses including renal injury, fibrosis and graft rejection. It has been reported that the promoter -607 and -137 polymorphisms of IL-18 influence the level of cytokine IL-18 expression. This prospective observational study aimed to assess the relevance of serial postoperative serum/urine creatinine, cystatin C and IL-18 measurements for monitoring early graft function in renal transplant recipients and to evaluate the pro-inflammatory property of IL-18 by measurement of serum IFN-γ and CRP. We also determined the effect of IL-18 -607 A/C and IL-18-137 C/G polymorphisms on graft function.

Methods: This study included 75 renal transplant recipients (28 female, 47 male; mean age: 38.28 ± 13.03) from living related donors. Blood and urine samples were collected immediately before and after transplantation at day 7 and month 1. Serum IFN-γ, IL-18, creatinine, cystatin C, CRP and urinary IL-18, cystatin C and creatinine levels were measured. Polymorphisms of the promoter region of the IL-18 gene, IL18-607A/C and -137C/G were determined by analysis of "real-time PCR/Melting curve". GFR values were estimated by Modified Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and some cystatin-C-based formulas (Larsson, Rule, Hoek). SPSS 20.0 software was used for statistical analysis.

Results: Serum creatinine, cystatin C, CRP, IFN- γ, IL-18, urine cystatin C levels and urinary cystatin C/creatinine, urinary IL-18/creatinine ratios were significantly decreased after transplantation ($p<0.005$). Serum cystatin C and IL-18 levels were significantly higher in patients with IL-18-137 GG genotype before transplantation. While pretransplant levels of serum creatinine and IL-18 were found significantly higher in patients with IL-18-607 CC genotype, we also observed significantly higher serum IFN-γ levels and estimated GFR (MDRD and CKD-EPI) values in CA genotype ($p=0.012$). Receiver operating characteristic (ROC) analysis was performed to quantitate the accuracy of the different markers to detect changes in GFR. Posttransplant serum creatinine and cystatin C demonstrated a significantly greater AUC (area under the curve), sensitivity and specificity values than IL-18 and IFN- γ.

Conclusion: In this study, although we observed significantly differences in serum IL-18 levels and some GFR markers according to genotypes, the influence of polymorphisms on early graft function has not been clearly shown. Future larger studies are needed to confirm the association of cytokine gene polymorphisms with graft function. Prior to transplantation, screening of genetic predisposition which may have deleterious effect on graft function could lead to the development of new treatments for better graft survey and ultimately improve the outcome of renal transplantation.

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May routine urine analysis reduce the number of unnecessary culture requests

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Background: Urinary Tract Infection (UTI) is the most common bacterial infection in the society. Bacterial infections can lead to the leukocytosis. Urine analysis is one of the most common tests for assessing urinary-tract infections. Urine culture is still the 'gold standard' for the detection of urinary tract infection, however, it is time- and labor-intensive and has a high number of unnecessary cultures. The aim of this study was to evaluate diagnostic performance of infection-related (associated) parameters of urine preliminary analysis (leukocyte esterase, nitrite, bacteria and leukocyte) in comparison to urine culture as the reference method and to investigate whether the presence of UTI cause leukocytosis.

Methods: The electronic database of our laboratory was searched between July 2013 and December 2013. Our hospital is a tertiary central hospital with 671 beds. Approximately 239.029 urinalyses were requested while the majority of these requests were from outpatients and emergency patients. A total of 3427 patients (1980 women, 1447 men, mean age 49.23±18.42) which were request urinanalysis, CBC and urine culture on the same day were enrolled in the study. All results were retrospectively reviewed. Leukocyte esterase and nitrite in chemical analysis were measured with fully automatic urine analyzer (IRIS iQ200 Diagnostics, USA). Pyuria (WBC) and bacteriuria in microscopy, leukocyte of CBC (Beckman Coulter LH780, USA) parameters were compared with urine culture. Diagnostic performance of parameters for detection of UTI were evaluated.

Results: Urine cultures were positive in 413 patients (%11.9). E. coli was the most frequently isolated bacteria (70.4%). Ratios of positive dipstick results for leukocyte esterase and nitrite in culture positive patients were 85% (n=352) and 40 % (n=166) respectively. The positive microscopy results for bacteria and leukocyte were 31 % (n=127) and 75 % (n=310), respectively. Positive predictive values of leukocyte esterase, nitrite, pyuria and bacteriuria tests were 69 %, 97 %, 74 %, and 91 % while negative predictive values were 80 %, 62 %, 75 %, 58 % respectively. The highest specifity rate was nitrite (99 %). Leukocytosis rate in patients with a positive urine culture were 23 % (n=96). A relatively high correlation was found between LE and microscopic WBC count ($r = 0.827$; $P < 0.001$).

Conclusions: Routine urine analysis is thought to be reliable in preliminary diagnosis of UTI and start empirical treatment without waiting for culture results. Also urine

analysis is easy to apply and quick test that could reduce unnecessary requests with predicting culture results. Dipstick urine analysis and urine microscopy can rule out UTI in considering most samples have no or unsignificant growth. With a systematic algorithm, laboratory workload, cost and unnecessary antibiotic prescriptions could be reduced.

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Enhanced Liver Fibrosis (ELF) Score indicates progressive fibrosis in pre-clinical stages of Primary Biliary Cirrhosis

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Background: Liver fibrosis is a common consequence of most chronic liver diseases. The assessment of liver fibrosis is usually made by histologic analysis of liver biopsy samples. Percutaneous liver biopsy has inherent risks to the patient. Primary biliary cirrhosis (PBC) is a slowly progressive cholestatic disease associated with the development of cirrhosis and liver failure that may justify liver transplantation. PBC diagnosis is based on completion of at least two of the following criteria: characteristic histological findings; elevated alkaline phosphatase serum levels for over six months; and circulating anti-mitochondria autoantibodies (AMA). AMA is detected in roughly 95% of PBC patients, with a specificity of at least 98%. Occasionally AMA-positive (AMA⊕) subjects with normal liver enzymes are identified by the characteristic cytoplasmic pattern in the regular antinuclear antibody indirect immunofluorescence assay on HEp-2 cells. Biochemically normal (BN) AMA-positive individuals may represent earlier stages of CBP and may require confirmation by liver histology analysis. The Enhanced Liver Fibrosis (ELF) score is an alternative approach that combines three serum markers in an algorithm able to generate a score correlated with liver fibrosis state (mild, moderate and severe). **Aim:** To investigate liver fibrosis status, as assessed by ELF score, in BN AMA-positive individuals along a 4.5-year follow-up period.

Methods: ELF score was determined for 37 PBC patients, 68 BN/AMA-positive individuals, and 172 age- and gender-matched blood donors. BN/AMA-positive individuals and PBC patients had two samples obtained 4.57 (1.37 - 16.04) and 4.75 (1.91 - 8.37) years apart, respectively. ELF score was calculated according to a pre-established algorithm based on the serum levels of hyaluronic acid, procollagen III and inhibitor of metalloproteinase determined by chemiluminescence (Siemens Healthcare Diagnostics).

Results: ELF score was higher in baseline PBC samples (mean 9.63; 95%-CI: 9.09-10.17) than in baseline BN/AMA-positive samples (9.14; 95%-CI: 8.92-9.37) ($p=0.038$) and blood donor samples (8.91; 95%-CI: 8.81-9.02) ($p<0.001$), with no significant difference between BN and blood donors ($p=0.284$). There was a significant increase in ELF score in the follow-up of PBC patients ($p=0.010$) and BN/AMA-positive individuals ($p<0.001$).

Conclusion: PBC patients presented evidence of more severe liver fibrosis than BN/AMA-positive individuals did. The progression in liver fibrosis status in BN/AMA-positive individuals along the years indicates a subclinical inflammatory process in these individuals. ELF score appears to be a sensitive parameter for evaluation of the progression of liver involvement in BN/AMA-positive individuals.

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Cardiac troponin I and B-type natriuretic peptide predict clinical outcomes in stable renal transplant recipients

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Background: Cardiac troponins and natriuretic peptides are becoming established as predictors of clinical outcomes (CO) in patients with cardiovascular (CV) disease; however their value in renal transplant recipients has not been established. Methods: Using a case-cohort design, we tested baseline specimens from 1114 stable renal transplant recipients enrolled in the FAVORIT study (The Folic Acid for Vascular Outcome Reduction In Transplantation) using the B-type natriuretic peptide (BNP) and high sensitivity troponin I (hsTnI) assays (both Abbott). CO included all-cause death, dialysis-dependent kidney failure (DDKF) and CV outcomes. The relationship between BNP and hsTnI values and CO were assessed via Cox regression models. Quartiles (Q) of the two biomarkers were included in models adjusted for age, gender, race, treatment, history of smoking, coronary heart disease, diabetes mellitus, A/C ratio, eGFR, BMI, blood pressure, lipid levels, graft vintage and donor type. Combinations of Q4 of BNP and hsTnI (BNP high, hsTnI high) and Q1-3 of BNP and TnI (BNP low, hsTnI low) were also evaluated. Results: Median concentrations and interquartile ranges for TnI and BNP were 5.6 (3.3, 10.5) ng/L and 56 (22, 129) pg/L. Both BNP and TnI levels were associated with age, donor type, history of CHD and diabetes mellitus, systolic BP, eGFR and urine ACR. Hazard ratios (HRs) for each fatal and non-fatal endpoint increased significantly with increasing quartiles of BNP after adjustment and remained significant after adding TnI to the model. HRs for the BNP/TnI combinations were strongly associated with all CO studied (Table). Conclusion: BNP is predictive for death, CV as well as renal outcomes in stable renal transplant recipients. Simultaneous elevation of BNP and TnI is relatively common and strongly predictive of CO.

Adjusted hazard ratios for combinations of low and high BNP and hsTnI results

Clinical outcome	BNP low/ hsTnI low (63%)	BNP low/hsTnI high (13%)	BNP high/hsTnI low (13%)	BNP high/hsTnI high (12%)
All-cause mortality	Ref	1.68 (1.03-2.72)	1.97 (1.19-3.26)	3.26 (1.92-5.54)
Mortality and DDKF	Ref	1.21 (0.77-1.91)	1.73 (1.09-2.76)	3.34 (2.04-5.48)
DDKF	Ref	0.75 (0.39-1.44)	1.32 (0.70-2.49)	2.64 (1.31-5.29)
CV outcomes	Ref	1.14 (0.67-1.92)	2.17 (1.27-3.69)	2.46 (1.38-4.36)
Mortality and CV outcomes	Ref	1.41 (0.90-2.20)	2.12 (1.33-3.36)	2.67 (1.63-4.38)

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Results of Sample Testing for the Determination of Reference Intervals in Apparently Healthy Pediatric Subjects for ADVIA Centaur® Systems, Dimension Vista® and Dimension EXL® Systems Thyroid Assays

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Background: Age-specific reference intervals are necessary for appropriate interpretation of thyroid hormone measurements in the pediatric population and may vary due to methodological differences. A challenge for establishing pediatric reference intervals has been the availability of well-characterized samples from healthy pediatric subjects. Of the few studies that provide this information, most are based on specimens from patients who were hospitalized or required medical care. This study used methodology consistent with CLSI guidelines to pedigree and collect samples from apparently healthy pediatric subjects presenting for regular well child care.

Objective: To test well-characterized specimens from healthy pediatric subjects to establish pediatric reference intervals for various assays and instruments.

Methods: Eight US sites prospectively collected samples from apparently healthy pediatric subjects, under institutionally approved consent/assent procedures. Subjects were normal according to CDC weight- and height-based growth charts, were free of chronic and acute diseases, were not on medication, had no family history of thyroid dysfunction, no visible or palpable goiters, and were negative for anti-thyroglobuin and anti-thyroid peroxidase antibodies. Three age subgroups were analyzed with approximately equal numbers of males and females. Samples were shipped to a central laboratory and tested in singleton using multiple Siemens immunoassay systems. The lower and upper reference limits were defined as the 2.5^{th} and the 97.5^{th} percentiles of the distribution of test results for each of the two older subgroups. For the infant subgroup, a robust method (Horn and Pesce) was used to calculate the reference intervals.

Results:

System	Assay[a]	Infant	n	Children	n	Adolescent	n
System	**Assay[a]**	**≥1mo to <24mo**	**n**	**≥2yr to <13yr**	**n**	**≥13yr to <21yr**	**n**
ADVIA Centaur	FT3	3.28-5.19	72	3.34-4.80	190	3.04-4.65	129
ADVIA Centaur Dimension VISTA	FT4	0.94-1.44	72	0.86-1.40	190	0.83-1.43	129
	T3	1.17-2.39		1.05-2.07		0.86-1.92	
	T4	6.03-13.18	82	5.50-12.10	191	5.50-11.10	148
	TSH	0.741-5.24		0.628-3.90		0.438-3.98	
Dimension VISTA Dimension EXL	FT3	3.34-5.24	82	3.31-4.88	191	2.91-4.53	148
	FT4	0.88-1.48		0.81-1.35	190	0.78-1.33	147
	T4	7.4-14.3	75	6.8-12.5		6.0-11.6	
	TSH	0.781-5.72		0.704-4.01	185	0.516-4.13	147
Dimension EXL	FT3	3.47-5.29	75	3.35-4.82	185	2.91-4.70	147
	FT4	0.93-1.45	77	0.82-1.40	187	0.78-1.34	
	T4	6.6-13.4	75	5.8-11.8	186	5.4-10.6	

[a]Assay units: FT3, pg/mL; FT4, ng/dL; T3, ng/dl; T4, µg/dL; TSH, µIU/mL

Conclusion: Pediatric reference intervals were established for ADVIA Centaur, Dimension Vista and Dimension EXL thyroid assays using rigorously pedigreed samples. These data will assist with the appropriate interpretation of thyroid measurements in infants, children and adolescents.

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Evaluation of six different formulas and equations for estimate low density lipoprotein cholesterol (LDLc) concentration.

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Background: The National Cholesterol Education Program Adult Treatment Panel (ATP III) identified the serum LDLc concentrations as the primary criterion of diagnosis and treatment of patients with hyperlipidemia and also as one of the most important parameter for evaluation of coronary heart disease and cardiovascular risk assessment. Most of Peruvian clinical laboratory used the Friedewald formula and some LDLc precipate method for estimate serum LDLc concentration, but the Friedwald formula used has some limitations especially with extreme low and high triglycerides values (> 400 mg/dL). The aim of the study was compare six different proposal formulas and equations: Friedewald, Anandaraja, Chen, Puavilai, Vujovic and Cordova for estimate value of serum LDLc compared to LDLc precipitate method. **Methods:** A descriptive cross-sectional study was conducted and 220 serum samples from adolescent students of medical technology at the Peruvian University Cayetano Heredia were collected during the annual clinical evaluation performed on November 2012. Serum samples on fast conditions for lipid profile were obtained, the measurement of LDLc cholesterol and HDLc cholesterol were performed by precipitation methods (Wiener Lab, Argentina), Total Cholesterol (TC) and Triglycerides (TG) were measurement by enzymatic colorimetric endpoint method (Wiener Lab, Argentina), using a BT 3000 Plus automatic photometer analyzer at the Clinical Laboratory of Cayetano Heredia Clinic - Universidad Peruana Cayetano Heredia. Linear regression analysis and Blant Altman plots were performed to evaluate six different formulas and equations for LDLc estimation and compared to LDLc precipate method, using MS Excel and MedCalc Statistical Software. TG and LDLc obtained values for the statistical analysis and clinical classification were divided in levels according to ATP III. **Results:** Linear regression and Blant Altman plots showed a significant correlations and agreements, respectively. The correlation coefficient for serum sample group of TG ≤ 149 mg/dL were to Friedewald, Chen, Puavilai, Vujovic (R^2>0.95), Cordova (R^2=0.94), Anandaraja (R^2=0.81), respectively. For serum sample group of TG between 150-199 mg/dL were Friedewald, Puavilai (R^2=0.88), Chen, Vujovic (R^2=0.89) and Cordova (R^2=0.91), whereas TG (200-400 mg/dL) Friedewald, Chen, Anandaraja, Puavilai , Vujovic (R^2=0.87) and Cordova (R^2=0.86). Using Friedewald formula n=18 (8.18%), Anandaraja n=30 (13.63%), Chen n=44 (20%), Puavilai n=14 (6.36%), Vujovic n=24 (10.91%) and Cordova n=106 (48.18%) patients show a probably misclassification for hyperlipidemia. **Conclusion:** Six differents formulas showed significant correlations and agreements according Lineal regression analysis and Blant Altman plot. The Puavilai equation/formula showed good performance to estimate LDLc in all triglycerides levels and shows less misclassification of patients for hyperlipidemia compared to LDL precipitate method used in this study.

A-119

A Hope for Healing Using Amniotic Membrane and Stem Cells

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Objectives: Testing a new technique for treatment of chronic non-healing ulcers using amniotic membrane (AM) alone or in combination with autologous mesenchymal stem cells (MSCs) as an application of clinical laboratory medicine in regenerative medicine.**Methodology:** After Institutional Ethical Committee approval, each patient signed informed written consent. The study was conducted on **37**chronic leg ulcers. Patients were randomly divided into **4**groups; **Group I**: (control group **11**ulcers), ulcers were treated with conventional wound dressings that were changed day by day for **8**weeks. **Group II**: (**14**ulcers) AM was placed in contact with ulcer and held in place with 2ry dressing; which was changed day by day. **Group III**: (**6**ulcers) autologous BM derived MSCs were injected into ulcer bed and ulcer edges. **Group IV**: (**6**ulcers) Autologous MSCs were injected into ulcer bed and ulcer edges, and freshly prepared AM was placed in contact with ulcer and held in place with 2ry dressing.**Patients were subjected to:*I. Assessment of ulcer healing and wound measurement:*-1. Percentage of the healed wound area and healing rate:Healed wound area%= (**Original wound size-final wound size) /Original wound size×100 **Healing rate in cm^2/day**= (Original wound size-final wound size) /Time consumed to reach final wound size**-2. Wound size:** Greatest length and greatest width were measured, and surface area was calculated in cm^2.**-3. Wound grading**: Based on Pressure Ulcer Scale for Healing (PUSH Tool) Ulcers classified as **Mild**, **Moderate, and Severe**. ***II- Pain Assessment:***On a scale from 1 to10, pain level is classified as (**No pain, Mild, Moderate, & Severe**).***III- Follow up:***-Healing rate and ulcer size-Pain Assessment -Taken of AM graft (Day2).-Ulcer images (Day0, and weekly till end of the study)**Statistical Analysis** It was performed with SPSS software version15.0 for Windows (SPSS Inc., CR). **Results:**Significant improvement in patients of groups **II**,**III**, and **IV** regarding pain &ulcer size .There was significant difference in healing rate &reduction in ulcer size between **group I** and **(group II &IV)** with (**P<0.001**) where wounds showed an overall decrease in wound size and improvement in wound bed with healthy granulations**. Group II** results showed complete healing of **14**ulcers in**14-60**days with mean of **33.3±14.7**, healing rate was **0.064 –2.22**and mean of **0.896 ±0.646**cm^2/day with **100%** reduction in ulcer size. In **group III**; **4**ulcers (**66.7%)** showed complete healing in **45-60**days with mean of **50.5±6.7**, while **2**ulcers **(33.3%)** showed partial healing with healthy granulations. Ulcers' healing rate was **0.084 – 0.787**cm^2/day, with mean of **0.303 ±0.254**. Reduction in ulcer size ranged from **50.4%** to**100%**, with mean of

83.9% ±24.9%. In **group IV**- in the combined treatment with AM and autologous MSCs- **3**leg ulcers **(50%)** showed complete healing in **14- 25**days with mean of **17.7±6.35** and **3**ulcers **(50%)** showed partial healing with healthy granulations. Range of Ulcers' healing rate was **0.359–1.23**cm^2/day with mean of **0.759 ±0.361**. Range of reduction in ulcer size in group **IV** was **46.5% – 100%**, and mean of **78.95%±24.2%**. **Conclusion:**Using AM alone or in combination with autologous MSCs represents promising simple, safe, effective, and novel therapeutic approach for closing and healing of persistent non-healing chronic leg ulcer.

A-120

Inflammatory Markers in Polycystic Ovary Syndrome

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Background: Previous studies have demonstrated polycystic ovary syndrome (PCOS) associated with a proinflammatory state. The clinical spectrum of PCOS includes components of the metabolic syndrome, such as central obesity, insulin resistance, dyslipidemia and hypertension. All these disorders are epidemiologically related to cardiovascular disease, most probably through low-grade intravascular chronic inflammation. We aimed to investigate whether inflammatory markers, including C-reactive protein (CRP), procalcitonin and ischemia modified albumin (IMA) are related and altered in polycystic ovary syndrome.

Methods: A case-control study including fifty women diagnosed with PCOS,

according to Rotterdam criteria, and thirty-three controls, matched for body mass index (BMI) and age. Serum samples for CRP, procalcitonin and IMA were collected from these women under spontaneous menstrual cycles between the third and seventh days and between 08:00 and 10:00 after an overnight fast, but at random times if they suffered severe oligo- or amenorrhoea. CRP levels were measured by immunoturbidimetric method (ADVIA® 2400 Chemistry System, Siemens Healthcare Diagnostics Inc.Tarrytown USA). Procalcitonin levels were measured by sandwich immunoassay method (ADVIA® Centaur CP System, Siemens Healthcare Diagnostics Inc. Tarrytown USA). MA levels were measured by spectrophotometric method. The $p<0.05$ was considered as statistically significant.

Results: PCOS patients had increased levels of testosterone and luteinizing hormone (LH) compared to healthy BMI matched controls. Mean CRP, procalcitonin and IMA levels were higher in patients with PCOS than controls, but these differences were not statistically significant.

Conclusion: In PCOS women, plasma levels of CRP, procalcitonin and IMA were not significantly increased when compared with age- and BMI-matched controls. Several previous studies suggest that chronic low-grade inflammation in PCOS is an important risk factor for long-term situation including cardiovascular complications, metabolic disorders and ovarian dysfunction. The precise mechanisms underlying these associations require additional studies to clarify the state of the cardiovascular system in women with PCOS compared with controls in large numbers of patients to determine the relative contribution of different factors including insulin resistance, androgen status and BMI.

A-121

Recommendations of U.S. Preventive Services Task Force for Health Screenings that Include Use of Laboratory Tests

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Background: Health screening tests greatly impact the public's health because they involve testing of asymptomatic populations for diseases or conditions where interventions may impact health outcomes. U.S. Preventive Services Task Force (USPSTF) is the leading independent panel of experts that conducts rigorous and impartial assessments of scientific evidence for effectiveness of clinical preventive services.**Methods:** All screening recommendations are rated by USPSTF into 1 of 5 grades: 'A' for strong recommendation with high certainty that net benefit is substantial, 'B' for recommendation with high certainty that net benefit is moderate or moderate certainty that net benefit is moderate to substantial, 'C' for individualized decision making with at least moderate evidence that net benefit is small, 'D' against screening with moderate to high certainty that net benefit is zero or harms outweigh benefits, and 'I' is a statement that no recommendation can be made due to insufficient, poor quality, or conflicting evidence, with the balance of benefits and harms of screening undetermined.**Results:** Laboratory screening tests for 35 diseases/conditions have been included in USPSTF's evaluations for different target populations, resulting in a total of 60 currently active recommendations for laboratory testing. Of these, there were 20 'A' and 'B' recommendations including: cervical cancer in women aged 30-65 years; chlamydial infection in sexually active women aged ≤24 years and older pregnant women at risk; colorectal cancer in adults aged 50-75 years; diabetes mellitus in adults with sustained hypertension, gonorrhea infection in sexually active women at increased risk, hepatitis B virus (HBV) infection in pregnant women; hepatitis C virus infection of persons at high risk and 1-time screening of those born in 1945-1965; HIV screening of pregnant women, 1-time HIV screening of adolescents and adults aged 15-65 years; risk of coronary heart disease in men aged ≥35 years, as well as both men aged 20-34 years and women aged ≥45 years who are at increased risk; and syphilis infection in pregnant women. The most common recommendation grades were D and I (17 each). D recommendations included: cervical cancer in women aged <21 years, those aged >65 years not at high risk and with adequate prior screening, those with hysterectomy leading to removal of cervix and no history of high-grade or cancerous lesion, and human papilloma virus screening of women aged <30 years; colorectal cancer in adults aged >85 years; genital herpes simplex virus infection in adults, pregnant women, and adolescents; gonorrhea infection in men and nonpregnant women at low risk for infection; HBV infection in men and nonpregnant women; hereditary hemochromatosis in adults; lead poisoning in children aged 1-5 years at average risk and pregnant women; ovarian cancer in women excluding those with known genetic mutations that show increased risk; pancreatic cancer; prostate cancer; and syphilis infection in nonpregnant persons not at increased risk.**Conclusion:** While common perception is that screening is recommended for major and prevalent diseases, the UPSTF either recommended against (28%) or insufficiently supported (28%) use of more than half of the evaluated laboratory markers.

A-122

Performance of the Elecsys® Vitamin D Assay in a Multicenter Evaluation

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Background: Increased awareness and investigation into the importance of vitamin D for many physiological processes has resulted in dramatically increased demand for vitamin D testing. New automated methods to measure 25-hydroxyvitamin D (25-OHD) levels have been developed to help laboratories accommodate this surge. The fully automated Roche Elecsys® Vitamin D assay, which allows for the quantitative determination of total 25-OHD in human serum and plasma, was recently introduced to the US market. The Elecsys Vitamin D assay is standardized against a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method using National Institute of Standards and Technology (NIST) reference materials for calibration. The current study evaluated the performance of the Elecsys Vitamin D assay across several laboratories in the United States to determine its suitability for use in clinical practice. Functional sensitivity, imprecision, and clinical concordance with a well-established NIST standardized LC-MS/MS method were assessed. **Methods:** Functional sensitivity was assessed using 8 pooled samples covering a range of 0-16 ng/mL, which were tested once a day for 10 consecutive days. Precision was assessed, following CLSI EP5-A2/EP15-A2, from 5 pooled samples in the 10- to 50-ng/mL range. Deming regression analysis was used to compare the results of the Elecsys Vitamin D assay obtained at 2 sites on the cobas e411 analyzer with LC-MS/MS performed at Mayo Clinic. Four sample cohorts were used: subjects referred for routine vitamin D testing were included in cohort I, while predialysis patients (cohort II), pregnant women (cohort III), and patients in the intensive care unit (cohort IV) represented unique subpopulations. The clinical performance of the 2 methods was evaluated using 20 ng/mL and 30 ng/mL as cutoffs for vitamin D sufficiency. **Results:** The functional sensitivity of Elecsys Vitamin D, assessed at 2 sites, was 3.52 ng/mL and 3.37 ng/mL with coefficient of variation (CV%) of 15% and 16%, respectively. Elecsys Vitamin D demonstrated consistent reproducibility across lots (N=2), across the 2 sites tested, with CV% of 5.66% to 7.12% (SD for concentrations below 15 ng/mL; 1.40 ng/mL). Consistent repeatability (CV% of 1.30% to 5.48% [0.40 to 0.72 ng/mL]), within site/lot precision (CV% of 1.94% to 7.47% [0.49 to 1.26 ng/mL]), and within site/across 2 lots precision (CV% of 4.06% to 7.78% [1.00 and 1.10 ng/mL]) was demonstrated. Overall, acceptable consistency between Elecsys Vitamin D assay at both sites, and LC-MS/MS was observed for regression parameters in both the routine and specialty cohorts with a slope range of 1.03 to 1.22 and Pearson's r range of 0.857 to 0.932. The LC-MS/MS sufficient/insufficient classification based on the 20-ng/mL cutoff over cohort I (routine adults) was 67.3% sufficient/32.7% insufficient compared with 68.8%/31.2% observed with the Elecsys method at 1 site. Using the 30-ng/mL cutoff over cohort I, the LC-MS/MS classified 36.7%/63.3% as sufficient/insufficient, respectively, and the Elecsys showed agreeable numbers at 38.3%/61.7%. **Conclusion:**

The Elecsys Vitamin D assay showed robust technical performance, with good functional sensitivity and low imprecision. Analysis of multiple cohorts showed high clinical concordance with NIST standardized LC-MS/MS, supporting the suitability of the assay for use in clinical practice.

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Homogeneous immunoassay for estradiol based on blue-emitting upconverting nanophophors and luminescence resonance energy transfer

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Objective: The purpose of this study was to demonstrate a competitive homogeneous assay for 17β-estradiol (E2) based on photon upconversion luminescence. $NaYF_4$: Yb,Tm upconverting nanophosphor (UCNP) with strong emission at 475 nm under 980 nm excitation was used as a donor in upconversion luminescence resonance energy transfer (UC-LRET) with small molecular conventional fluorescent dyes energy acceptors.

Methods: Upconverting nanophosphors (UCNPs; $NaYF_4$: 20% Yb, 0.5% Tm) of 25-30 nm in size were synthesised using oleic acid as chelating agent and shape modifier.[1] The nanophosphors functionalized silica shell comprising carboxylic acid groups, which were covalently conjugated to anti-E2 Fab antibody fragments (Fab S16) using carbodiimide chemistry. E2 conjugated Alexa Fluor 488 (E2-AF488) and fluorescein isothiocyanate (E2-FITC) dyes were used as acceptor conjugates. The luminescence

spectrum of the donor (emission peak around 475 nm) overlapped with the excitation spectrum of the acceptors. In the homogeneous E2 assay, E2 dilutions in buffer were first incubated together with the Fab S16 coated upconverting donor, then E2-AF 488 or E2-FITC were added to the reaction and the sensitized acceptor emission was measured at 565 nm with anti-stokes luminescence plate reader (Hidex Oy, Turku, Finland) under 980 nm infrared excitation.

Results: By using 0.006 mg/ml UCNP donor with 16 nM E2-AF488 acceptor conjugate or 0.01 mg/ml UCNP donor with 4 nM E2-FITC acceptor conjugate in the assay reaction, standard curves with IC_{50} values of 2 nM and 1.7 nM were obtained, respectively. The working range of the assay was from 0.80 nM to 3.10 nM E2 concentrations with both the acceptors. Signal levels for the homogeneous assay using the E2-FITC acceptor conjugate were two times higher than by using the E2-AF488 acceptor conjugate. However, the ratio between maximum and minimum signals, or the signal-to-background ratio, was 12 for E2-AF488 and 8 for E2-FITC acceptor.

Conclusions: In this study, we introduced a blue emitting upconverting nanophosphor as an efficient donor in UC-LRET and demonstrated a competitive homogeneous upconversion based immunoassay for E2. Photon upconversion and luminescence resonance energy transfer enable sensitive homogeneous immunoassays, which eliminate the autofluorescence background with simple instrumentation and render UCPs an attractive reporter technology for clinical diagnostics.

References:1. Kale, V; Soukka, T; Hölsä, J, Lastusaari, M. J. Nanopart. Res.2013, 15, 1850.

A-124

Enhanced Liver Fibrosis (ELF) Score for the Evaluation of Liver Fibrosis in Autoimmune Hepatitis

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Background: Evaluation of fibrosis is very important in chronic liver diseases, in order to define prognosis and therapeutic options and to establish strategies of follow-up. Liver biopsy has been considered the gold-standard for the evaluation of fibrosis. However, is an invasive method and prone to sampling error. These problems have motivated the search of non-invasive and more reliable methods for detecting and staging liver fibrosis. The ELF (Enhanced Liver Fibrosis) score is based on an algorithm of three markers: (hyaluronic acid, pro-collagen III e inhibitor of metalloproteinase 1) and has been evaluated in different etiologies of liver diseases. However, ELF score has not yet been evaluated in patients with autoimmune hepatitis (HAI). **Aim**: to evaluate the performance of ELF score in patients with AIH, using the liver biopsy as a gold-standard.

Methods: Patients with HAI and a liver biopsy performed in less than 12 months were included and had a serum sample collected for the ELF score (Siemens Healthcare Diagnostics). Patients with hepatitis B or C were excluded, as well as patients with alcohol consumption > 20g/day.

Results: 109 patients were evaluated and 89 were included (90% female, mean age 34y). According to the ELF cut-offs proposed by the manufacturer, 8% of patients had absent/mild fibrosis, 42% moderate fibrosis and 50% advanced fibrosis. ELF score overestimated fibrosis in 28% of cases and underestimated in 33%. The accuracy of ELF (AUROC) for significant fibrosis ($F \geq 2$) was 0,64 (CI 95%: 0,52-0,77; p= 0,05), for advanced fibrosis ($F \geq 3$) was 0,59 (CI 95%: 0,47-0,71; p= 0,17) and for cirrhosis was 0,66 (CI 95%: 0,54-0,77; p= 0,009). Coeficient of correlation for ELF and fibrosis was 0,24 (p=0,22), for periportal necroinflammatory activity was 0,28 (p= 0,009) and for lobular necroinflamatory activity was 0,43 (p<0,001).

Conclusion: ELF score had not a good performance as a marker of liver fibrosis in patients with AIH. Using proposed cut-offs, there was overestimation as well as underestimation of liver fibrosis in many cases when comparing to liver biopsy. It is possible that the biomarkers included in the ELF score could have been influenced by the intense necroinflammatory process observed among AIH patients. In fact, ELF score was more intensely associated to lobular activity (p< 0,001) then to liver fibrosis (p= 0,22). It is interesting to note that 4 patients with acute hepatitis and no fibrosis had a high ELF score, confirming that intense inflamation is a relevant confounder of indirect liver fibrosis assessment.

A-125

Organisms cultured from the synovial fluid of infected prosthetic joints.

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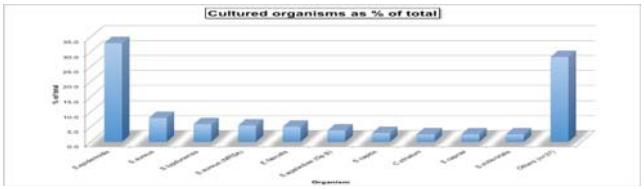
Objective:Prosthetic joint infection (PJI) occurs after primary joint replacements at a rate of 1.0-3.0% and after revision joint replacements at a rate of 2.0-20.0%. Synovasure™ is a laboratory developed test (LDT) for the detection of alpha-defensins in the synovial fluid (joint fluid), which have been shown to be elevated in infected samples. There are two main objectives of this study; first to describe the variety and distribution of organisms infecting joint replacements nationally, and second to evaluate for organism-specific differences in the synovial fluid alpha-defensin level.

Methods:1698 synovial fluid specimens from 40 states were sent to our laboratory for the SynovasureTM alpha-defensin assay and were also cultured using the BacT/ALERT® FAN FA/FN culture bottles for recovery of aerobic and anaerobic organisms (Biomerieux). The organisms were identified and evaluated for susceptibilities using the VITEK® 2 ID/AST system (Biomerieux), a fully automated system that provides rapid microbial identification and susceptibility testing.

Results:47 different organisms from 237 culture-positive samples were identified (distribution summarized in graph). The data indicate that Staphylococcus epidermidis and Staphylococcus aureus account for 46% of the organisms cultured. Of S. aureus isolates, 41% were methicillin-resistant. In addition, there were no statistically significant organism-specific differences in the synovial fluid alpha-defensin levels in the culture-positive samples.

Conclusion:In a large national survey, we have identified Staphylococcus epidermidis (33%), Staphylococcus aureus (13%) and Staphylococcus lugdunensis (6%) as the most common organisms isolated from synovial fluid. The 40% rate of methicillin-resistance among S. aureus isolates is quite concerning given the poor outcomes associated with the treatment of these prosthetic infections. The elevation of alpha-defensin in the synovial fluid of infected joint arthroplasties does not appear to be influenced by the organism type.



A-126

Predictive roles of N-terminal proBNP and high sensitive Troponin T levels in determining mortality in chronic renal failure patients

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Background: The mortality in chronic renal failure (CRF) patients is higher than that in normal population. Cardiovascular events are the major contributors in increased mortality rate in CRF patients since more than 50% of deaths are due to cardiovascular events. Cardiac troponins are highly specific and sensitive markers of myocardial damage. Increments in troponin levels in CRF are related to adverse clinical outcomes. B-type natriuretic peptide (BNP) is a cardiac neurohormone predominantly released from the ventricles in response to left ventricular volume expansion and pressure overload. It is released as a preproBNP and is cleaved into proBNP and a signal peptide. ProBNP is subsequently cleaved into BNP and the inactive N-terminal proBNP (NT-proBNP) peptide. Levels of BNP or NT-proBNP are known to be elevated in patients with left ventricular dysfunction. BNP may serve as an important plasma biomarker for cardiac stress and ventricular hypertrophy in patients with CRF. In fact, elevated levels of BNP indicate an increased risk of cardiac events and mortality in patients undergoing peritoneal dialysis. Markers with capacity to identify CRF patients with high mortality risk early in the course of disease may help in clinical management of these patients improving outcomes. In the present study we investigated the predictive values of NT-proBNP and high sensitive Troponin T (hsTNT) levels separately and in combination in determining all-cause mortality in CRF patients on peritoneal dialysis.

Methods: A total of 51 CRF patients (21 female, 30 male, mean age: 50.9 ± 16.5 years) and 41 healthy control subjects (22 female, 19 male, mean age: 45.7 ± 11.6 years) were included in the study. Serum levels of NT-ProBNP and hsTnT (ECLIA method) were measured in all study subjects. Optimal prognostic cut-off points for NT-ProBNP and hsTnT were determined. Overall survival rates were determined by using the Kaplan-Meier method with log-rank test for comparison among groups with different cut-off points of NT-ProBNP and hsTnT. **Results:** CRF patients had significantly higher levels of NT-ProBNP (5382.00 ± 1115.00 vs 77.00±9.99 pg/mL, p<0.00001) and hsTnT (0.070 ± 0.010 vs 0.004 ± 0.001 ng/mL, p<0.00001) than control subjects. Of the 51 patients enrolled, 24 died and 27 survived during a median follow-up period of 56.4 months (P25-P75: 32.3-57.3). Optimal prognostic cut-off points for NT-ProBNP and hsTnT were ≥ 1950 pg/mL and ≥ 0.050 ng/mL, respectively. Patients with elevated hsTnT levels had higher risk than patients with elevated NT-proBNP levels when compared with the group which had low levels of both markers (HR 4.00 [95% CI, 1.57−10.20], *P* =0.004 and HR 3.31 [95% CI, 1.40−7.82], *P* = 0.006], respectively). Patients with elevated levels of both hsTnT and NT-proBNP had a markedly increased all-cause mortality risk (HR 10.38 [95% CI, 2.82-38.27], *P* <0.0001). **Conclusion:** Our results have demonstrated for the first time that determination of both hsTnT and NT-proBNP levels is better at predicting CRF patients with high mortality risk than determination of either biomarker alone.

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Enhanced Liver Fibrosis (ELF) Score for the Evaluation of Liver Fibrosis in Shistossomiasis Mansoni

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Background: Schistosomiasis mansoni has a worldwide distribution and affects over 70 million people, constituting a major cause of fibrogenic liver disease. The assessment of periportal fibrosis by ultrasound is the gold-standard for staging fibrosis in patients with schistosomiasis. However, although simple and non-invasive, ultrasound examination is not always available in endemic areas. **Aim**: to correlate the ELF score with patterns of periportal fibrosis obtained by ultrasonography in patients with Schistosomiasis mansoni.

Methods: Patients of both genders, aged> 14 years, coming from an endemic area for schistosomiasis with a history of exposure to contaminated water were included. An ultrasonography was performed by a single examiner, with unit Siemens Acuson X 150 with 3.5 MHz convex transducer. Periportal fibrosis was classified according to the six patterns of Niamey (graded from A to F, with A = no liver fibrosis and F = very advanced fibrosis). Exclusion criteria were the presence of serum markers of viral hepatitis, ethanol consumption > 210 g/week and steatosis. All patients had serum samples collected for determination of ELF score on the same day of ultrasonography. The evaluation of ELF markers (hyaluronic acid, procollagen III and inhibitor of metalloproteinase 1) was performed by chemiluminescence (Siemens Healthcare Diagnostics).

Results: 78 patients were evaluated; 50 (64%) were female, mean age 50 ± 14 years. According to Niamey classification, patients were divided into three groups: 7 patients (9%) presented pattern A/B of fibrosis, 33 (42%) pattern C/D and 38 (49%) pattern E/F. Groups showed the following ELF scores, according to the pattern of fibrosis: A/B = 8.55 ± 0.40, C/D = 9.31 ± 1.23, E/F = 9.52 ± 0.93. Significant difference was observed between groups A/B and E/F (p = 0.01).

Conclusion: In patients with Schistosomiasis mansoni the ELF score was able to discriminate patients with patterns A/B (absent or mild fibrosis) of those with E/F (advanced fibrosis).The test can be an useful tool for the diagnosis and for monitoring fibrosis progression in patients with Schistosomiasis mansoni in situations where ultrasound is not available.

A-128

Circulating blood platelet function in acute liver allograft rejection

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Background: Platelet surface receptor expression is increased in acute vascular events in various clinical settings. Endothelial injury is associated with increased adhesion and aggregation of platelets. In the present study, we investigated platelet aggregation, and markers of platelet activation in liver allograft recipients with acute rejection.

Methods: The whole blood samples from 20 recipients with biopsy-confirmed acute rejection (rejection group), 20 recipients with stable graft function (stable group) after liver transplantation, and 20 healthy volunteers (control group) were collected. To examine platelet function we measured platelet aggregation tests by using platelet aggregometer, platelet expression of glycoprotein (Gp)Ib, GpIIIa, GpIIb/IIIa, and P-selectin (granule membrane protein 140 [GMP140]) under unstimulated and activated conditions by quantitative flow cytometry, and plasma soluble P-selectin (sP-selectin) by enzyme-linked immunoadsorbent assay.

Results: When the platelet membrane GpIb, GpIIIa, and GpIIb/IIIa expression of rejection group, stable group, and control group were compared in a resting state and in a agonist [thrombin receptor-activating peptide (TRAP)]-activated state, no significant differences were observed among three groups ($P>0.05$). Platelet expression of P-selectin and sP-selectin levels were significantly increased in rejection group and stable group compared with control group ($P<0.05$). A significantly increased sP-selectin level was found in rejection group compared with stable group ($P<0.01$), and platelet P-selectin expression in a TRAP activated state ($P<0.05$). Platelet maximum aggregation values induced by all agonists (adenosine diphosphate, arachidonic acid, collagen, epinephrine) were higher in rejection group than stable group, but the difference did not reach statistical significance ($P>0.05$).

Conclusion: Acute rejection induces platelet activation without inducing circulation platelet aggregation, which reveals the state of ongoing pathogenesis of immune and inflammatory processes.

A-129

Interferences by hemolysis, lipemia and bilirubin on routine parameters on chemistry analyzers in a pediatrics hospital

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Background: Clinical laboratory assays can be affected by different interferences, most commonly, hemoglobin, lipids, bilirubin, auto-antibodies, and heterophile antibodies. Our goal was to study the effect of interference by hemolysis, lipemia and bilirubin on routinely performed parameters on various chemistry analyzers including VITROS 5600, BN Prospec, Dimension Xpand, Architect i1000SR, and Advia Centaur using pediatric samples in the Texas Children's Hospital laboratory. Further we wanted to establish cut-off indices above which these interferences confound analysis of pediatric samples. **Methods:** We tested the effect of hemolysis on K+, AST, LDH, TBIL, ALT, CK, Mg++, ALB/TP, ALKP, Fe, Lipase,NH3+ and phosphorus on VITROS 5600. Similarly for lipemia interference we analyzed HIV-1/2,Testosterone, Progesterone, Ceruloplasmin,Haptoglobin,C3 / C4, IgG / IgM / IgA, Vitamin D,AFP,aHBC,HCG,CK-MB,TSH, Albumin,Ferritin and Glucose. Lastly, for icteric interference we performed analyses for Estradiol, Folate, ALB/TP, ALT, GGT, Glucose, Na+, Cortisol, C4, Haptoglobin and HbA1c on the respective analyzers. Experiments were carried out with test samples from 3 different serum pools spiked with increasing concentrations of hemolysate (0.75g/l, 1.5g/l, 3.0g/l, 6.0g/l,), 20% Intralipid (400mg/dl, 1000mg/dl, 2000mg/dl) and commercially available Bilirubin (100uM, 250uM and 500uM). These were then analyzed on the various instruments in laboratory: VITROS 5600, Architect i1000SR, Prospec, Xpand, and Centaur. The Hemolysis-(H), Lipemia-(T) and Icterus- (I) indices were measured and documented on VITROS 5600. Lastly, for lipemic interference (>2000mg/dl) we further treated the samples with Lipoclear(1:5 ratio) and then re-analyzed the samples.

Results: We categorized hemolysis by H-index of 101-250 as mild, 251-500 as moderate, 501-999 as significant and H-index of >1000 as grossly hemolysed. Similarly, for Lipemia T-index of < 100 was considered mild,101-500 as moderate and T- index of >501 as severely lipemic. For Icteric evaluation I-index of 0-5 was characterized as normal, 5.1-9.9 as mild, 10.0 -19.9 as moderate and ≥ 20 as grossly icteric. For significant hemolysis, the main parameters affected and which shouldn't be reported included AST, LDH, TBIL, and NH3+. All the measured analytes were compromised by gross hemolysis and these samples should be rejected. None of the analyzed parameters were significantly affected by the level of Icterus. With regards to lipemia, mild lipemia (<400mg/dl) did not affect the assays while Ceruloplasmin, Haptoglobin, C3, C4, and Immunoglobins (IgG, IgA, IgM) were significantly affected by moderate (400-1000 mg/dl) and severe lipemia (>2000mg/dl), and hence should not be reported. Vitamin D also showed a significant decrease in moderately lipemic samples (decrease of 10-15%). However, since most samples are drawn in a postprandial state, we tested the effect of Lipoclear. Addition of Lipoclear to moderately lipemic samples significantly attenuated the effect of lipemia interference on the above mentioned analytes.

Conclusion: Accurate reporting of pediatric samples for the analytes affected by common interferences will lead to better clinical interpretation. Our results can be applied to other laboratories for the analysis and reporting of these parameters.

A-130

Serum Neopterin Levels in Patients with Pulmonary Embolism

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Background: Neopterin (D-erythro-1-2-3-trihydroxypropylpterin) is produced from guanosine triphosphate by activated human monocytes, monocyte derived dendritic cells, and macrophages. Release and production of neopterin is stimulated mainly by interferon-c (IFN-c) released by activated Th1- lymphocytes during the cellular immune response. Pulmonary thromboembolism (PTE), is an extremely common medical problem. Yet despite its frequency, much remains to be learned regarding the pathogenic mechanisms that initiate pulmonary thromboembolism. Marked activation of endothelium, platelets, and leukocytes are key events in thrombogenesis in pulmonary thromboembolism. The role of clinical laboratories is to give accurate, fast and reliable results to clinicians. Also, early detection of diseases as pulmonary thromboembolism is important to take care before the serious complications. The aim of this study was to determine serum neopterin concentrations as an early biomarker on patients with pulmonary thromboembolism.

Methods: Blood samples were collected from 41 healthy control and 38 patients with pulmonary embolism. Patients with chronic disease and inflammatory disorders were excluded. This study was approved by local ethic committee. Serum neopterin levels were analyzed with flourometric detection by high perfomance liquid chromatography. Briefly, 100 µL trichloroacetic acid was added to 500 µL serum sample, vortexed and centrifuged at 10000 g for 10 minutes. Supernatant was injected to C18 chromatographic column on Agilent HPLC system. Chromatographic detection was perfomed at 353 and 438 nm excitation and emission wavelength. This method's coefficient of variation and % bias values were 4.21,3.50; 6.89,5.62 and 8.14,6.64 for 0.5, 5 and 10 µmol/L, respectively. Statistical analysis was performed with SPSS v16.

Results: Serum neopterin levels were significantly lower in control group (5.72 ±2.29 µmol/L) compared to patient group (7.83 ±4.45 µmol/L) (p=0.049) according to Mann-Whitney U test.

Conclusions: Activated T cells and macrophages synthesise and release a number of cytokines whose main function is immunoregulation. As a marker of cellular immune response activation depending on IFN-γ release, neopterin may better reflect the disease. Our findings suggest that neopterin levels may be used as an immunological marker in follow-up the disease.

A-131

How non-standardized serum creatinine-based definitions of acute kidney injury contribute to disparate reported incidence rates of contrast-induced nephropathy

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Background- Prior controlled studies of intravenous contrast-induced nephropathy (CIN), acute kidney injury (AKI) resulting from iodinated contrast material administration, have reported incidence rates ranging from 2% to 40%. This discrepancy may be partially explained by the lack of a standardized serum creatinine (SCr)-based definition of AKI. To date, there is little evidence regarding how different definitions of SCr-defined AKI and baseline renal function affect the incidence of AKI, and which, if any, of these definitions provide a reliable means to distinguish between CIN and contrast-independent AKI. In the current study, we examined how using different SCr definitions of AKI and baseline renal function affect AKI incidence rates in a propensity score-matched cohort of contrast-enhanced and unenhanced CT scan recipients.

Methods- All contrast-enhanced and unenhanced abdominal, pelvic, and thoracic CT scans performed at our institution from 2000-2010 and accompanying pre- and post-scan SCr results were identified. Contrast and noncontrast scan recipients of similar clinical and demographic characteristics were compared following propensity score-based 1:1 matching. AKI was defined using absolute (SCr ≥ 0.5mg/dl and ≥ 0.3mg/dl over baseline), relative (SCr ≥ 25% and ≥ 50% over baseline), and hybrid definitions (SCr ≥ 0.5mg/dl or ≥ 25%, and ≥ 0.3mg/dl or ≥ 50% over baseline). Baseline renal function was defined by using either the mean SCr result 24 hours or 7 days prior to scan. The incidence of AKI was compared between groups using Fisher's exact test.

Results- Following 1:1 matching, 21,372 patients were identified who underwent a contrast enhanced (N=10,636) or unenhanced CT scan (N=10,636). Using the six definitions of AKI, the incidence of contrast-dependent AKI ranged from 2% to 15% while the incidence of contrast-independent AKI ranged from 1% to 14%. Regardless of AKI definition, the rates of contrast-independent AKI were statistically similar to the rates of contrast-dependent AKI (ORs ranged from 0.91 (95% CI 0.66-1.24), p=.58 to 1.84 (0.91-3.75), p=.08). Higher incidences of AKI were observed if baseline renal function was defined using the mean 7-day pre-scan SCr result compared to using the mean 24-hour pre-scan SCr result.

Conclusion- Differing definitions of AKI and baseline renal function contribute to disparities in the reported incidence of AKI. However, no definition could extricate CIN from contrast-independent AKI causes in our cohort.

A-133

Intravenous Contrast Material Exposure is not an Independent Risk Factor for Dialysis or Mortality

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Background – Contrast-induced nephropathy (CIN), defined as acute kidney injury (AKI) occurring immediately after exposure to iodinated contrast material, is generally reported to be a self-limited phenomenon. However, concern remains that CIN can cause irreversible nephrotoxicity resulting in dialysis and death, particularly in patients with specific pre-existing comorbidities including renal failure, diabetes mellitus, and congestive heart failure. In the current study, we sought to determine the true incidence of short-term dialysis and mortality following intravenous contrast administration among individuals with closely matched demographic and clinical characteristics using propensity score analysis.

Methods - All contrast-enhanced and unenhanced abdominal, pelvic, and thoracic CT scans performed at our institution from 2000-2010 and related pre- and post-scan serum creatinine (SCr) results were identified. Contrast and noncontrast scan recipients were compared following propensity score-based 1:1 matching to reduce intergroup selection bias. Patients with pre-existing diabetes, congestive heart failure, or chronic or acute renal failure were identified as high-risk patient subgroups for nephrotoxicity. The effects of contrast exposure on the incidence of AKI (SCr ≥ 0.5mg/dl above baseline within 24-72 hours of exposure), and dialysis or death within 30 days of exposure were determined using odds ratios and covariate-adjusted Cox-proportional hazard models.

Results - 1:1 propensity score matching yielded a cohort of 21,346 patients (10,673 contrast-enhanced exams/10,673 noncontrast exams). Within this cohort, the risk of AKI (O.R.=0.94 (95% CI: 0.83-1.07), p=.38), emergent dialysis (O.R.=0.93 (0.52-1.65), p=.89), and 30-day mortality (H.R.=0.97 (0.87-1.06), p=.45) was not significantly different between contrast and noncontrast groups. Although patients who developed AKI had overall higher rates of dialysis and mortality, contrast exposure was not an independent risk factor for either outcome (dialysis: O.R.=0.89 (0.40-2.01), p=.78; mortality: H.R.=1.03 (0.82-1.32), p=.63). Similar findings were observed among the subgroups of patients with renal failure, diabetes, and congestive heart failure.

Conclusion - Intravenous contrast material administration was not associated with excess risk of AKI, dialysis, or death, even among patients with comorbidities reported to predispose them to nephrotoxicity.

A-134

Neutrophil gelatinase-associated lipocalin (NGAL) and Pentraxin -3 (PTX-3) Levels in Chronic Renal Failure and their relationship with Inflammation

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Background: Early diagnosis of Chronic Renal Failure (CRF) provides to initiate the effective treatments that can decelerate the progress of the disease and improve prognosis. However, lack of early predictive biomarkers presents difficulties in early diagnosis. Therefore, finding biomarkers that considerably increases in the early stages of CRF and demonstrates correlation with the disease progress is necessary.

NGAL is a known biomarker of acute kidney injury that is secreted in response to renal tubular epithelial cells damage. Increased NGAL levels are determined in the blood and urine following acute renal injury. Pentraxin-3 (PTX-3) is an inflammatory mediator. In contrast to other members of the pentraxin family such as CRP and

Serum Amiloid Protein, PTX-3 is produced in the inflammation site and shows close correlation with the level of tissue damage. Inflammation plays an important role in the progression of the CRF and contributes to increased mortality and morbidity observed in CRF.

In this study, we investigated serum NGAL and PTX-3 levels and their relationship with the severity of renal damage in patients with early stage CRF.

Method: 20 (2 male and 18 female) non diabetic Stage 1 and 34 (17 male and 17 female) Stage 2 CRF patients were included in this study. Average age in the Stage 1 CRF group was 42.55±7.00, and in the Stage 2 CRF group 47.44±7.47. In both groups, serum NGAL and PTX-3 were measured by using the ELISA method from Bioscience Human and Aviscera Bioscience, respectively.

Results: NGAL levels in patients with Stage 2 CRF were higher than that of the patients with Stage 1 CRF ($p<0.01$). ROC analysis and diagnostic tests used for the calculation of a diagnostic cut off value for Stage 2 CRF. For the diagnostic cut - off value of 1037 pg/ml, sensitivity was 73.55%, specificity 70.00%, positive predictive value 80.65%, and negative predictive value 60.87%. Area under the curve was estimated as 73.3% and its standard erreor was 6.9%.In the patients with NGAL levels higher than 1037pg/ml, the probability of having Stage 2 CRF was found to be 6481 times higher than that of the patients with NGAL levels less than 1037 pg/ml.In Stage 1 and 2 CRF groups , 19.4% and 80.6% of the patients had NGAL levels higher than 1037, respectively. In Stage 2 cases, there was a positive correlation between serum creatinin and NGAL concentrations (r=0,362; p0.05). In Stage 1 and 2 CRF patient groups, CRP and PTX-3 concentrations did not show correlation with eGFR values. There was no statistically significant correlation between NGAL and PTX-3

Conclusion: The serum NGAL concentrations were found to be higher in Stage 2 CRF than Stage1 CRF group. Our results indicate that NGAL could be used as a strong and independent diagnostic biomarker for the early stage CRF and a risk for disease progression. We did not observe statistically significant correlation between serum NGAL and PTX-3 levels.

A-135

Serum Lipoprotein-Associated Phospholipae A2 and Methylarginine Levels in Patients with Pulmonary Embolism

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Background: Pulmonary thromboembolism (PTE) is a preventable disease with higher mortality and morbidity characterized by diagnostic difficulties and recurrence risk. Obstruction of pulmonary arteries is developed from the detached fragments of thrombus in deep veins of the lower extremities. Plasma lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet activating factor acetylhydrolase, is produced by inflammatory cells, co-travels with low-density lipoprotein (LDL), and hydrolyzes oxidized phospholipids, thereby propagating inflammation and potentially thrombosis. ADMA is derived from the catabolism of proteins containing methylated arginine residues. Higher ADMA concentrations have been measured in many cardiovascular and metabolic diseases. There has been limited markers for laboratory evaluation of pulmonary thromboembolism. The aim of this study was to find out the serum methylated arginine and lipoprotein-associated phospholipase A2 levels in patients with pulmonary embolism.

Methods: Blood samples were collected from 41 healthy control and 45 patients with pulmonary embolism. Patients with chronic diseases were excluded. This study was approved by local ethic committee. Serum lipoprotein-associated phospholipase A2 test was analyzed with a colorimetric kit on automated system (Abbott C16000). Reported intra- and inter-assay CV values for 171 and 456 ng/mL were 1.3;0.6 and 3.6;4.9, respectively. Method was linear up to 486 ng/mL. Serum methylated arginine levels were analyzed with liquid chromatography tandem mass spectrometry on ABSCIEX API 3200 system. Briefly, 1 mL deuterated d7-ADMA containing methanol was added to 200 µL serum sample for protein precipitation, vortexed and centrifuged on 13000 g for 10 minutes. 40 µL supernatant was injected to Shimadzu LC-20AD HPLC system. Chromatographic seperation was performed on C18 column for 5 minutes. This method's coefficient of variation and % bias values were 15.6,10.2; 9.72,7.88 and 6.45,6.02 for 0.4, 0.8 and 1.6 µmol/L, respectively. Statistical analysis was performed with SPSS v16.

Results: Serum phospholipase A2 levels were significantly lower in patient group (158.3 ±49.8 ng/mL) compared to control group (206.5 ±38.4 ng/mL) ($p<0.001$) according to independent sample t test. Serum asyymetricdimethylarginine levels were significantly lower in patient group (0.44 ±0.17 µmol/L) compared to control group (0.60 ±0.18 µmol/L) ($p<0.001$). Serum syymetricdimethylarginine levels were significantly lower in patient group (0.54 ±0.20 µmol/L) compared to control group (0.70 ±0.17 µmol/L) ($p<0.001$). Serum arginine levels were significantly lower in patient group (163 ±81 µmol/L) compared to control group (285 ±127 µmol/L) ($p<0.001$). Serum citrulline levels were significantly lower in patient group (9,14 ±6,20 µmol/L) compared to control group (21,81 ±7,26 µmol/L) ($p<0.001$). Serum arginine/ADMA ratio was significantly lower in patient group (379 ±145 µmol/L) compared to control group (477 ±205 µmol/L) ($p=0.013$).

Conclusion: ADMA has been evaluated in several different classes of pulmonary hypertension. In previous studies, no significant difference was reported between pulmonary embolism and healthy controls. Also, in this study serum methylated arginines and phospolipase A2 levels were found to be statsitically lower compared to control group. This might be due to venous characteristic of this disease. Serum asymmetric dimethylarginine levels may be affected by several factors. Arginine/ADMA levels may provide accurate data and be a reliable marker compared to single ADMA measurements.

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Prevalence of metabolic syndrome among hypertensives in Ghana

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Background: Metabolic syndrome can be found in approximately one-third of patients who do not have diabetes but have hypertension. There are numerous correlations between the metabolic syndrome and hypertension, although this is not always the case. As metabolic syndrome and hypertension are independent risk factors for the same disease process, namely cardiovascular disease, it is possible that patients suffering from both these disease entities may have a compounded risk. Our study will therefore attempt to determine the prevalence of metabolic syndrome and investigate the proposed association between these two disease entities.

Methods: This cross-sectional study was conducted at the Hypertension Clinic of the Department of Medicine, Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana between April 2009 and November 2010. Informed consent was obtained from 300 participants consisting of 200 hypertensives (diagnosed by a Consultant Physician based on WHO – International Society of Hypertension Guideline of blood pressure ≥140/90 mmHg or use of antihypertensive) and 100 apparently healthy normotensives served as control. Blood samples were collected in the morning after an overnight fast of at least 12 hours. Serum and plasma were stored at -80ºC after centrifugation at 2000g for 5 minutes until assayed. Fasting blood glucose (FBG), Apolipoprotein A-1 (Apo A-1), Apolipoprotein B (Apo B), Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein (HDL) were measured on an Auto-Analyzer (Flexor junior, Vital Scientific N.V., The Netherland).

Results: The prevalence of MetS among the hypertensive patients were significantly higher than the normotensive control (56.5% vrs 9.0%, 54.5% vrs 5.0% and 65.5%vrs15.0%, $p<0.001$) using NCEP ATP III, WHO and IDF criteria respectively. Irrespective of the criteria applied, all the components of MetS were significantly higher among the hypertensive patients as compared to the normotensive control. Among the hypertensive patients, the highest prevalence of cardiovascular risk factor was abdominal obesity as measured by WHR (77.0%), followed by reduced HDL-cholesterol (74.0%). From the univariate analysis, females were at about 3 times at risk of developing hypertension as compared to the male counterpart (OR = 2.7; 95% CI = 1.6-4.4; p = 0.0000). Reduced apolipoprotein A1 served as a risk factor (aOR = 13.4; 95% CI = 1.5-121.4; p = 0.0210) whilst high apolipoprotein A1 protects the individual from developing hypertension (aOR = 0.1; 95% CI = 0.0-0.2; p = 0.0000). High apolipoprotein B poses about 9 times risk of developing hypertension as compared to the normal level (aOR = 9.3; 95% CI = 4.2-20.9; p = 0.0000). Both Impaired fasting glucose and diabetes each pose more than 10 times risk of developing hypertension as compared to normoglycaemia.

Conclusion: The study demonstrated that, hypertension is more than just elevated blood pressure in our setting; it is intimately associated with the metabolic syndrome. There is therefore the need for metabolic screening of all hypertensives and increase awareness on the critical importance of public health strategies aimed at reducing risk factors in the entire population. Early detection and treatment of the global risk profile should thus become a priority.

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CEDIA® Mycophenolic Acid Applications on the Beckman Coulter AU480, AU680 and AU5800 Analyzers

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Background: Mycophenolic Acid (MPA) is metabolized from Mycophenolate Mofetil (MMF) or Mycophenolate Sodium. It is an immunosuppressant used in the prevention of tissue rejection for patients who have undergone renal and heart transplants. MPA is a specific inhibitor of inosine-monophosphate dehydrogenase (IMPDH) - an enzyme used by B and T lymphocytes for de novo purine synthesis. This repression of B and T cell proliferation results in the desired immunosuppressive effects.

Methods: The CEDIA MPA Assay is based on the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. MPA in the human plasma patient sample competes with MPA conjugated to one inactive fragment for antibody binding site. Once MPA in the sample binds to antibody, inactive enzyme fragments reassociate to form active enzymes. The amount of active enzymes results in an absorbance change that is directly proportional to the amount of MPA in the patient sample. This change is measured spectrophotometrically for a quantitative value of concentration. The Beckman Coulter AU480/AU680/AU5800 analyzers are new applications for the CEDIA MPA Assay. Analyzer performance was determined for precision, linearity, limit of detection, and accuracy on the Beckman Coulter AU480/AU680/AU5800 clinical chemistry analyzers, over the range of the assay (0.3-10 µg/mL). Results were measured against the reference analyzer Hitachi 917.

Results: All studies were evaluated using CLSI guidelines. Three levels of MPA controls were used in the studies. The precision ranged from 6.1-2.2%CV for within-run and 7.7-2.5%CV for total run. Linearity was measured and confirmed over a range of 1.8-11.9 µg/mL. The limit of detection on the AU480/AU680/AU5800 yielded 0.07 µg/mL. Accuracy was measured using patient correlation against the reference analyzer Hitachi 917, which yielded a Deming's Regression for each analyzer: AU480 = 0.992*(Hitachi 917) - 0.096 (N = 107, r = 0.998), AU680 = 0.995*(Hitachi 917) - 0.043 (N = 107, r = 0.998), AU5800 = 0.993*(Hitachi 917) - 0.089 (N = 107, r = 0.998).

Conclusion: All measured studies demonstrated acceptable performance, validating the use of the CEDIA MPA Assay on the Beckman Coulter AU480/AU680/AU5800 analyzers, and will provide an effective monitoring system for patients receiving MMF or Mycophenolate Sodium therapy.

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Fructosamine and Glycated albumin with Risk of Coronary Heart Disease and Death

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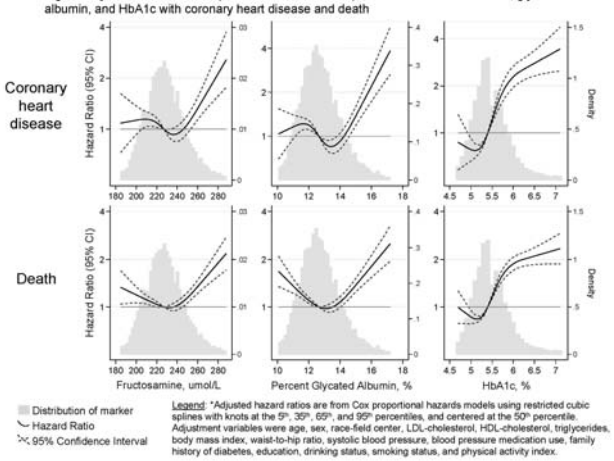
Background: HbA1c is the standard measure to monitor glucose control and is now used for diagnosis of diabetes. Fructosamine and glycated albumin are markers of short-term glycemic control that may add complementary information to HbA1c. However, the associations of fructosamine and glycated albumin with cardiovascular outcomes are uncharacterized.

Methods: measured glycated albumin and fructosamine in 11104 adult participants (792 with a history of diabetes) of the community-based ARIC Study without cardiovascular disease at baseline (1990-1992). We evaluated the associations of fructosamine and glycated albumin with incident coronary heart disease and total mortality. We compared these associations to those for HbA1c.

Results: Baseline HbA1c was highly correlated with fructosamine (Pearson's r =0.82) and glycated albumin (Pearson's r=0.86). During over two decades of follow-up there were 1,032 new cases of coronary heart disease and 2,594 deaths. In Cox proportional hazards models adjusted for traditional cardiovascular risk factors, elevated baseline levels of fructosamine and glycated albumin were significantly associated with coronary heart disease and total mortality (**Figure**). After additional adjustment for HbA1c, the associations were attenuated but remained significant, particularly at diabetic levels of fructosamine and glycated albumin. The associations with death were J-shaped, with an elevation of risk also apparent at the lowest levels of each biomarker, as has been previously observed for HbA1c.

Conclusion: The acceptance of new measures of hyperglycemia is partly dependent on establishing their association with long-term outcomes. We found that fructosamine and glycated albumin were associated with coronary heart disease and mortality and that these associations were similar to those observed for HbA1c. The elevated risk of death at very low levels of fructosamine, glycated albumin, and HbA1c deserves further examination.

Figure. Adjusted* hazard ratios (95% confidence interval) association of fructosamine, glycated albumin, and HbA1c with coronary heart disease and death



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Study of the Association between Endothelial Nitric Oxide Synthase G894T Gene Polymorphism and Heart Failure Severity

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Objectives: The aim of the study was to investigate the association between endothelial nitric oxide synthase (eNOS) G894T gene polymorphism and severity of congestive heart failure (CHF) in a group of Egyptian patients.

Methods: This study was conducted on 30 consecutively selected Egyptian patients admitted to the Cardiology department at Alexandria Main University Hospital suffering from non valvular CHF which was documented clinically and by ECG. Thirty age & sex matched healthy individuals were also included in the study as a control group. eNOS G894T gene polymorphism was studied using polymerase chain reaction-Restriction fragment length polymorphism-(PCR-RFLP) **Results:**. Genotype distribution of eNOS G894T gene polymorphism among patients revealed 13 (43.3%) patients of GG genotype (wild type), 11 (36.7%) of GT genotype (heterozygote) and 6 (20%) were of the TT genotype (homozygote for the mutant allele). Among the controls, 16 (53.3%) were GG genotype, 12 (40%) were GT genotype and 2 (6.7%) were TT genotype. Thus, no statistically significant difference in genotype distribution between patients and controls was observed. In addition, the present study showed no statistically significant difference between different eNOS patients' genotypes as regards NYHA classes, ejection fraction and six-minute walk test. The prevalence of TT genotype was significantly higher in ischemic cardiomyopathy patients when compared to those who had dilated cardiomyopathy and also in hypertensive patients as compared to normotensive patients.

CONCLUSIONS: Although the mutant eNOS G894T allele is associated with both hypertension and dilated cardiomyopathy in Egyptian heart failure patients, it was not correlated to disease occurrence or severity. 1

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Seasonal Variation of Vitamin D Deficiency in a Large Rural Health System: Effect of Assay Change on Deficiency Prevalence

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25 hydroxy Vitamin D (25OH Vit D) continues to be a commonly ordered test to assess bone health in the large, rural Geisinger Health System outpatient population in central Pennsylvania USA (latitude 40-41 degrees N). It is well known that seasonal variation of 25OH Vit D levels exists in temperate latitudes as sunlight exposure varies. Our objective is to report practical impact of monthly surveillance of 25OH Vit D seasonal deficiency/sufficiency prevalence during 2009 to 2013 (N=228,714) using clinical cutoffs of <20, 20-30, and 30-100 ng/mL to judge Deficiency, Insufficiency, and Sufficiency, respectively.

Monthly test volumes increased dramatically in 2009 when the test was first performed in house (2300/month in July 2009 to 4000/month in Jan 2010). Since then the volume has steadily increased to 5500/month. Our osteoporosis management program reports that patients are uniformly monitored with 25OH Vit D and are at

steady state as far as population enrollment stands. Steady increases of 25OH Vit D test volumes (approximately 15%/year) may be attributed to generally increasing numbers of outpatients rather than significant changes in utilization.

Over the course of July 2009 to December 2013, cyclical seasonal variation was documented by monthly prevalence plots, with deficiency rates lowest in July-August-September (16.5%) and highest in Jan-Feb-March (27.7%). Inversely, sufficiency rates were highest in Jul-Aug- Sept (48.5%) and lowest in Jan-Feb-Mar (40.5%). Because deficiency rates repeatedly increase from summer to winter by an average of 68%, it is important to advise clinicians to interpret 25OH Vit D levels and prescribe supplementation taking the month of the year into account. Although common practice is to supplement 25OH Vit D in patients with deficient or insufficient levels (i.e. < 30 ng/mL), a systematic study of prescribing patterns has not yet been undertaken.

In November 2012, the 25OH Vit D immunoassay was changed from Diasorin to Roche. Correlation between the two assays using patient specimens (N=152) was acceptable; Deming slope= 0.963; correlation coefficient=0.8455. Ongoing monthly deficiency prevalence, although similar in amplitude and periodicity, changed somewhat with Roche Jan- Feb-Mar 2013 deficiency prevalence trending 11% higher and Roche Jul-Aug-Sept 2013 deficiency prevalence trending 24% lower than prior comparable monthly Diasorin results. It is too early to tell if this difference is physiological , analytical, or coincidental. Although the population surveillance data are unsorted by patient clinical status, the assay change per se may have an effect on shifting population outcome data, and should be more extensively studied along with more traditional specimen correlation.

We conclude that seasonal variation of 25OH Vit D significantly affects deficiency prevalence and has the potential to change treatment on a population basis. The season of testing should be factored into treatment and other clinical considerations based on limited duration cross-sectional studies. Additional studies are needed to determine if different assays perform differently in populations exposed to different amounts of sunlight and if these differences may be due to levels of Vit D precursor or binding protein.

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Evaluation of circulating levels of inflammatory and bone formation markers in Axial Spondyloarthritis

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Background: Studies have demonstrated the important role of bone remodelling and osteoimmunology in the progression of inflammatory lesions in Axial Spondyloarthritis (SpA) disease.

Objective: This study was conducted to evaluate the inflammatory response by analysis of the serum levels of pro-inflammatory and new bone formation markers in patients with SpA who were treated or not treated with anti-tumor necrosis factor-α (anti-TNF-α) or non-steroidal drugs (NSAIDs) and to correlate these markers with the clinical evaluation scores of the disease activity.

Methodology: The serum levels of myeloperoxidase (MPO), adenosine deaminase (ADA), nitric oxide metabolites (NOx), bone alkaline phosphatase (BAP), Dickkopf-1 (DKK-1), and osteoprotegerin (OP) were measured in 52 SpA patients who were treated or not with anti-TNF-α or NSAIDs and in 26 healthy controls using colourimetric and enzyme immunoassays. The activity and the severity of illness in patients with SpA were assessed using questionnaires (BASMI, Bath Ankylosing Spondylitis Metrology Index; BASFI, Bath Ankylosing Spondylitis Functional Index; and BASDAI, and Bath Ankylosing Spondylitis Disease Activity Index). The data were expressed as the mean ± standard deviation of the mean (SD) for symmetric distribution or in percentage. Comparison of clinical parameters and bone biomarkers between all groups were done with analysis of variance (ANOVA) followed by Bonferroni post hoc test for multiple comparisons among the groups. $P < 0.05$ was considered to be statistically significant. Results: A significant difference between the controls and the patients without medication was observed in relation to NOx, BAP, and OP ($p < 0.01$). When the patients were compared with regard to their treatment, there were no clinically significant differences between the groups ($p > 0.05$).

Conclusion: The NOx, BAP, and OP are emerging as an important inflammatory pathway in Axial Spondyloarthritis. Also the anti-TNF-α or non-steroidal drugs reduce the inflammation and destruction, however these treatment does not modify the serum levels of these biomarkers.

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Suspected Lassa Fever (LF) Case Outcomes: A Comparison to a Non-Febrile Population in Sierra Leone

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Background: Lassa virus (LASV) is the causative agent for Lassa fever (LF), causing an estimated 100,000 - 300,000 cases annually. The precise factors resulting in fatal outcome in LF patients are still largely uncharacterized, although hypovolemic shock is thought to ultimately result in death of afflicted subjects. Additionally, signs of acute renal failure are consistently noted preceding fatal outcomes. Evaluating the difference in clinical and laboratory outcomes between LF cases and non-febrile controls is important to better characterize the clinical presentation of patients with LASV infection and potentially select patients with a higher pre-test probability of infection for diagnostic testing.

Methods: This is a case controlled study of patients suspected of LASV infection, identified in Sierra Leone, West Africa. Cases include both suspected and confirmed LF patients who had a temperature of ≥38°C for <3 weeks and displayed clinical signs and symptoms of a LASV infection upon examination. Controls include non-febrile Sierra Leoneans with a temperature of ≤37.5°C. We measured five outcomes and compared results between the confirmed LF case group (n = 57) and the non-febrile control group (n = 118). We measured BMI, Pulse, BUN, Cr, and BUN:Cr ratio and correlation of clinical parameters with diagnosis and other clinical parameters. Other outcomes such as the metabolic panel and hemoglobin were collected and evaluated as well.

Results: Using the Wilcoxon-Mann-Whitney nonparametric test, we found that confirmed LF cases exhibit elevated BUN (p<.0001) and Cr (p=.0002) measurements (BUN: 10.5 mM/L, Cr: 216. 3 μM/L) compared to non-febrile controls (BUN: 3.4mM/L, Cr: 84.6μM/L). Confirmed LF cases also had a significantly higher respiratory rate (p<.0001) than the non-febrile controls (39.6 breaths per minute versus 21.6).

Conclusion: Early evaluation of BUN, Cr, and respiratory rate in febrile patients may aid in selecting patient populations at high risk for LF and improve diagnostic accuracy.

BMI, Pulse, BUN, Cr, and BUN:Cr compared between confirmed LF cases and non-febrile controls.

	Confirmed LF Cases	Non-febrile Controls	p-value
BMI	18.6	21.9	0.0006
Pulse	93.8	81.4	0.0004
BUN mM/L	10.5	3.4	<0.0001
Cr μM/L	216.3	84.6	0.0002
BUN:Cr mg/dL	19.2	12.7	0.0008

A-144

A comparative study of the prevalence of Salmonella typhi infection in the wenchi municipality using the Widal and culture methods

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Background: *Salmonella typhi* is a Gram negative bacterium that can cause typhoid fever. Worldwide, typhoid fever is a serious public health problem, with an estimated 22 million cases, resulting in 200,000 deaths. The burden of the disease lies mainly in developing countries including Ghana where the provision of sanitary conditions may be inadequate. However, not much research work has been done to explore the problem confronting citizens and ways to eliminate them. Yearly report from Methodist Hospital's Laboratory, Wenchi (2012), indicates that 45.8% and 47.5% of In-patients and Out-patients respectively reacted to the Widal test. The total percentage of reactive cases in 2012 was 47.1%.This study was then carried out to determine the prevalence of typhoid fever and propose recommendations on the appropriate way of diagnosing the disease in Wenchi municipality.

Methods: Non-interventional exploratory study using a purposive sampling for the selection of respondents in March and April 2013. The study comprised 100 participants who were suspected of having typhoid fever and referred to the laboratory for diagnosis. Informed consent was obtained from each participant and then blood and stool specimens were collected. The blood was centrifuged and *Salmonella*

typhi H and O antibody titre determined following serial dilution with the Widal kits (*Salmonella typhi* Antigens H and O). After emulsification with physiological saline, the stool samples were then cultured directly onto Desoxycholate Citrate Agar (DCA). The plates were then incubated at 37 °C in for 24 hours and observed for bacterial growth. Culture results were then compared with the Widal test for each participant.

Results: About 41% of participants had high antibody titre to *S. typhi* H antigen while 12% also reacted to *S. typhi* O.The majority of the respondents were female (61%) and had the lowest reaction to the Widal *Salmonella typhi* H (42.6%) and O (11.5%) test but with no bacterial growth on the Agar. The males (29%) had the highest reaction to the Widal Salmonella typhi H (51.7%) and O (17.2%) test and also with no bacterial growth on the agar. Irrespective of the antibody titre shown in the Widal test, there was no corresponding bacterial growth by culture.

Conclusion: The high antibody titres recorded by the Widal technique with no corresponding bacterial growth by culture may point to the existence of circulating antibodies established during previous exposure. Therefore relying solely on the Widal test may lead to over diagnosis of the infection and concomitant abuse of antibiotics which may contribute the emergency of resistant strains in our region. Proper diagnosis of Salmonella typhi should thus be based on bacteria culture and sensitivity testing.

A-145

Performance of REBA MTB-XDR to detect Extensively Drug-resistant Tuberculosis in a High burden country

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Setting: Multidrug-resistant/extensively drug-resistant tuberculosis (MDR/XDR-TB) is a serious problem worldwide. The early diagnosis and treatment of MDR/XDR-TB is very important. The REBA MTB-XDR (REBA-XDR) line probe assay has been developed recently for the detection of resistance to ofloxacin, kanamycin, and streptomycin in Korea.

Objective: The aim of this study was to evaluate the diagnostic accuracy of the REBA-XDR in detecting XDR-TB in acid fast bacilli (AFB) smear-positive sputum specimens.

Design: We prospectively enrolled 104 patients with AFB smear-positive specimens between July 2010 and January 2013. Mycobacterium tuberculosis was cultured in all samples. The performance characteristics were compared between the REBA-XDR and the conventional drug susceptibility test (DST), GenoType MTBDR*sl* assay, and DNA sequencing analysis results. The conventional DST results were considered to be the gold standard.

Results: Among the 104 specimens, MDR-TB was found in 29.8% (31/104) and XDR-TB in 7.7% (8/104). The sensitivity of the REBA-XDR in detecting resistance to ofloxacin, kanamycin, and streptomycin was 66.7%, 90.9%, and 60.0%, and the specificity was 93.3%, 93.5%, and 85.4%, respectively. The positive predictive values were 62.5%, 62.5%, and 40.9%, and the negative predictive values were 94.3%, 98.9%, and 92.7%, respectively. The accuracy was 89.4%, 93.3%, and 81.7%, respectively. Discordant results between REBA-XDR and conventional DST were found for ofloxacin in 11 samples (10.6%), for kanamycin in 7 samples (6.7%), and for streptomycin in 19 samples (18.3%). Two of 10 (20%) discordant REBA-XDR results for ofloxacin resistance and all 6 (100%) discordant specimens for kanamycin resistance corresponded to the results of the gold standard DNA sequencing test. Five of 10 (50%) discordant results for ofloxacin resistance and 2 of 6 (33.3%) discordant results for kanamycin in the MTBDR*sl* test corresponded to the results of the DNA sequencing test.

Conclusion: REBA-XDR seems to be a useful kit to "rule in" XDR-TB in a high-risk group for drug resistance, especially for the detection of kanamycin resistance.

A-146

Circulating Presepsin (Soluble CD14 Subtype) in Patients with Severe Sepsis and Septic Shock. Data from the Albumin Italian Outcome Sepsis (ALBIOS) Study

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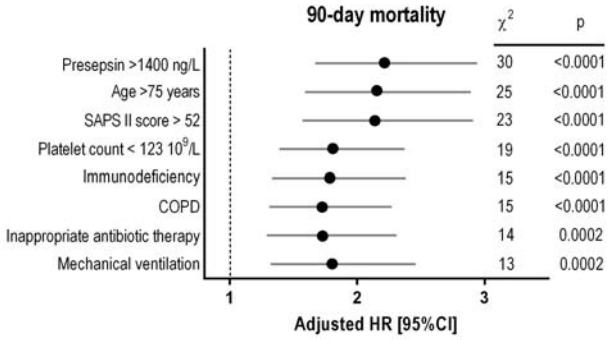
Background: The cornerstone of the emergency treatment of severe sepsis and septic shock is an early, goal- directed therapy. Presepsin, a soluble fragment of CD14 that participates in the innate recognition of pathogens, has been proposed as a novel diagnostic and prognostic marker in sepsis.

Aims: To validate presepsin as a prognostic marker in a large, representative cohort of septic patients.

Methods: Blood samples were collected 1, 2 and 7 days after enrolment in 997 patients with severe sepsis or septic shock enrolled in the multicenter Albumin Italian Outcome Sepsis (ALBIOS) trial (NCT00707122). Presepsin was measured in a central laboratory with a chemiluminescent enzyme immunoassay (PATHFAST Presepsin, Mitsubishi Chemical). The relation between presepsin concentration and mortality in Intensive Care Units (ICU) or at 90 days was assessed with Cox proportional hazards multivariable models. Prognostic discrimination was tested with reclassification metrics.

Results: Concentration of presepsin on day 1 was 946 [492-1887] ng/L (median [Q1-Q3]) and increased gradually with the number of organ dysfunctions and the number of newly developed organ failures in ICU. Circulating presepsin rose in decedents over 7 days in ICU while it markedly decreased in survivors ($p<0.0001$ for time-survival interaction). Presepsin on day 1 independently predicted ICU and 90-day mortality (Figure). Addition of presepsin measured on day 1 on top of all risk factors significantly improved prognostic discrimination and correctly reclassified the risk of 90-day mortality (c-statistics from 0.77 to 0.79, $p=0.004$; integrated discrimination improvement IDI = 0.03 [0.02-0.04], $p<0.0001$; continuous net reclassification index NRI = 0.53 [0.40-0.65], $p<0.0001$).

Conclusion: Presepsin is a robust predictor of mortality in patients with severe sepsis and septic shock. It provides incremental information on top of widespread clinical risk factors and may help in early risk stratification.



A-147

Neutrophil-Gelatinase -Lipocalin in adult cardiac surgery patients: beyond AKI

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) has been largely described as an early marker of acute kidney injury. We report the association of urinary NGAL (uNGAL) values with different adverse outcomes in adult cardiac surgery patients, particularly the need for continuous venous hemofiltration (CVVH), cardiac mechanical assist devices and low cardiac output syndrome

Materials and methods: fresh urine sample of 137 patients (34 females and 103 males; mean of age 64 y) undergoing cardiac surgery (coronary artery bypass, artificial heart valve, heart transplants, complex cardiac surgery) have been collected before, immediately after surgery and then 24 h and 48 h after. AKI was defined as an increase in plasma creatinine levels (more than 50% or more than 0.3 mg/dL (26.5 umol/L) in comparison to the preoperative value during the first 48 h after surgery. uNGAL has been evaluated using a chemiluminescent microparticle immunoassay (CMIA, Architect, Abbott Diagnostic) showing during the study a total imprecision (CV) ranging from 3.85 to 2.35% (20 ug/L and 1218 ug/L, respectively). The reference ranges adopted are 2-120 ug/L for uNGAL and 53-97 (females), 62-115 umol/L (males) for plasma creatinine respectively.

Results: Mean uNGAL levels peaked immediately after cardiac surgery (630 ± 890(SD) ug/L), and remained significantly higher 24 and 48 hours after surgery (565 ±842 and 293±455 respectively, p=0.0003). 24 patients (17%) developed AKI (mean of basal creatinine values and 48 hours after cardiac surgery 106±30.9 and 346.5±127.57 respectively, p<0.001)

Accuracy of u NGAL (AUC) as a predictor for AKI immediately after cardiac surgery and 24 and 48 hours later was 0.775 (95% confidence interval [CI], 0.660 to 0.890), 0.864 (95% CI, 0.788 to 0.940) and 0.838 (95% CI, 0.749 to 0.926), respectively. uNGAL levels correlated significantly with the need of CVVH, cardiac mechanical assist devices and low cardiac output syndrome being AUC 0.862 (95% CI, 0.779 to 0.945), 0.913 (95% CI; 0.840 to 986) and 0.910 (95% CI; 0.840 to 0.981), respectively.

Conclusions: Our study confirms the clinical usefulness of NGAL measurement for the early diagnosis of AKI but underlines that relevant clinical informations on adverse outcome of patients undergoing cardiac surgery may also be obtained.

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Diabetes, pre-diabetes and incidence of subclinical myocardial injury

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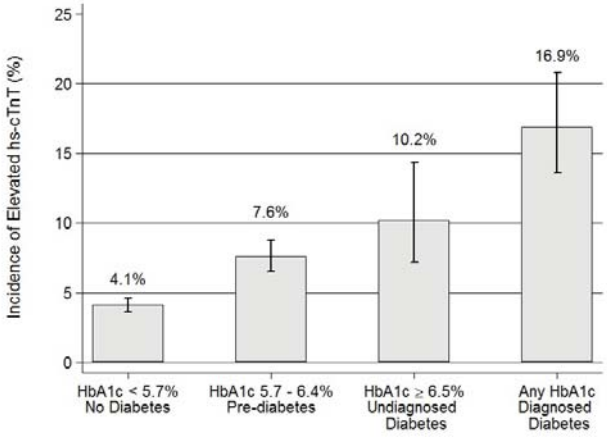
Background: Persons with pre-diabetes and diabetes are at high risk for cardiovascular events. However, the relationships of pre-diabetes and diabetes to development of subclinical myocardial damage are unclear. Our objective was to characterize the associations of pre-diabetes, undiagnosed diabetes, and diagnosed diabetes with 6-year incidence of subclinical myocardial injury, as assessed by a novel highly sensitive assay for cardiac troponin T (hs-cTnT).

Methods: We measured hs-cTnT at two time points, 6 years apart, among 8692 participants of the community-based Atherosclerosis Risk in Communities (ARIC) Study without a history of heart disease, silent MI by ECG, or stroke at baseline (1990-92). The primary outcome was incidence of elevated hs-cTnT (≥14 ng/L) at 6 years of follow-up.

Results: Cumulative probabilities of elevated hs-cTnT (≥14 ng/L) at 6 years among persons with no diabetes, pre-diabetes (HbA1c 5.7-6.4%), undiagnosed diabetes (HbA1c ≥6.5%), and diagnosed diabetes were 4.1%, 7.6%, 10.2%, and 16.9%, respectively. Across these same categories and compared to persons with no diabetes and HbA1c <5.7% (reference), the adjusted relative risks for incident elevated hs-cTnT were 1.40 (95%CI 1.13, 1.73), 1.85 (95%CI 1.24, 2.75), and 2.85 (95%CI 2.14, 3.75).

Conclusion: Pre-diabetes and diabetes were strongly associated with the future development of elevations in troponin T far below the threshold for a diagnosis of myocardial infarction. The results from this community-based prospective study provide evidence for a deleterious effect of hyperglycemia on the myocardium, possibly reflecting a microvascular etiology. Our findings underscore the importance preventing progression to early hyperglycemic states and development of diabetes.



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Robust measurement of branched chain amino acids on the Vantera Clinical Analyzer and the clinical association of NMR-measured valine with type 2 diabetes

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Background: Metabolomic studies have shown that branched chain amino acid (BCAA) levels are independently associated with insulin resistance and type 2 diabetes (T2D). NMR technology has been employed for years to measure lipoprotein particle concentrations in a clinical laboratory setting. However, the information-rich nature of the NMR spectrum lends itself to the measurement of other clinically useful metabolites; therefore, assays were developed to quantify the BCAAs, valine, leucine and isoleucine.

Methods: Proton NMR spectra were collected on fasting serum samples using the Vantera Clinical Analyzer, a 400MHz NMR platform with automated fluidics sample handling, data processing and analysis. The NMR spectra were deconvoluted using proprietary software with models containing reference spectra from serum proteins and lipoproteins. For method comparison purposes, NMR-measured BCAAs were compared with mass spectrometry quantified BCAAs collected from the same serum samples. Valine concentrations were quantified from NMR spectra previously captured for participants in the Multi-Ethnic Study of Atherosclerosis (MESA) and multivariable logistic regression analyses were performed to interrogate the association of valine with the development of T2D.

Results: The levels of valine, leucine and isoleucine quantified in serum samples using NMR and mass spectrometry, were highly correlated (e.g. valine R^2=0.96). For the valine NMR assay, the coefficients of variation (CVs) for the inter-assay and intra-assay precision data were 2.7-5.9%. NMR-measured valine concentrations in MESA subjects (n=3309), who were non-diabetic and whose fasting plasma glucose was <110 mg/dL, were strongly associated with incident diabetes and made a statistically significant contribution to a logistic regression model containing age, gender, race and glucose (see Table).

Conclusions: Levels of the BCAAs valine, leucine and isoleucine can be obtained from the same NMR spectra acquired for lipoprotein particle concentrations. Similar to published BCAA literature, NMR-measured valine is strongly associated with incident diabetes.

	MESA (n=352/3309) (incident diabetes/total # subjects)	
	Wald [2]	p
Age	11.4	0.0007
Gender	20.8	<0.0001
Race	20.1	0.0002
Glucose	218.8	<0.0001
Valine	32.9	<0.0001

A-150

Different genotypes of a functional polymorphism of the TSHR gene are associated with the development and severity of Graves' and Hashimoto's diseases

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Background: The disease severities of autoimmune thyroid diseases (AITDs), such as Hashimoto's disease (HD) and Graves' disease (GD), can vary among patients. ST4 is one of the splicing variants of thyrotropin receptor (TSHR). Increased ST4 transcription may enhance the generating of a shed A subunit, which results in the production of thyroid-stimulating antibody (TSAb). ST4 expression was higher in the AA genotype of *TSHR* rs179247 polymorphism compared to the GG genotype.

Methods: We genotyped this polymorphism in 98 HD patients including 44 patients developed hypothyroidism before the age of 50 years and were treated with thyroxine (severe HD) and 33 HD patients over the age of 50 years were left untreated and demonstrated euthyroid (mild HD), in 112 GD patients including 50 GD patients who had been treated with methimazole and were still positive for TRAb (intractable GD); and 33 GD patients in remission (GD in remission), and in 56 healthy volunteers.

Results: The AA genotype was more frequent in GD patients compared to control subjects (p=0.0201). In contrast, the frequency of the GG genotype was higher in HD patients compared to control subjects (p=0.0186). The A allele was more frequent in GD patients compared to the HD patients (p=0.0010). The AA genotype and A allele were more frequent in intractable GD patients compared to GD patients in remission (p=0.0024 and 0.0005, respectively). The GG genotype was more frequent in severe HD patients compared to control subjects (p=0.015). The proportion of patients who developed GD under 50 years of age was significantly higher in GD patients with the AA genotype compared to those with the AG/GG genotypes (P=0.0251).

Conclusion: The AA and GG genotypes of the rs179247 polymorphism of the *TSHR* gene were susceptible to GD and HD, respectively and were also associated with the intractability of GD and the severity of HD, respectively.

Summary of results		
Genotype	ST4 expression	Associated with
AA	HIGH	Development of **Graves' disease** Intractability of **Graves' disease**
GG	LOW	Development of **Hashimoto's disease** Severity of **Hashimoto's disease**

A-151

Tubular Damage is Ubiquitous in Newly-Diagnosed Patients with Multiple Myeloma: Comparison of Three Urinary and Two Serum Markers of Kidney Injury

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein overproduced by proximal tubular cells in response to kidney injury, while kidney injury molecule-1 (KIM-1) is a type 1 transmembrane glycoprotein that is overexpressed in dedifferentiated proximal tubule epithelial cells after ischemic or toxic injury. Urinary NGAL and KIM-1 have never been evaluated in MM patients.

Patients and Methods: To assess the value of these molecules in MM, we measured urinary and serum NGAL, urinary KIM-1, urinary and serum cystatin-C (Cys-C) in 48 newly diagnosed symptomatic MM patients (27M/21F, median age 65 years). The estimated GFR (eGFR) was calculated using the CKD-EPI equation. (proposed by the CKD Epidemiology Collaboration and is widely accepted in renal impairment). Serum and urinary NGAL was evaluated using immunoturbidimetric assay (BioPorto Diagnostics A/S, Denmark) with a protocol applied in the Siemens Advia 1800 Clinical Chemistry System. Serum and urinary Cys-C was measured on the BN ProSpec analyzer (Siemens Healthcare Diagnostics, Liederbach, Germany), while urinary KIM-1 was also measured using an ELISA (R&D Systems, Minneapolis, MN, USA). For the urinary measurements, a 24h urine collection was used.

Results: The median values (range) for the studied markers in MM patients and in 120 healthy controls were: for urinary NGAL 36ng/ml (0.5-2512ng/ml) vs. 5.3ng/ml (0.7-9.8ng/ml), p<0.001; for serum NGAL 162ng/ml (53-576ng/ml) vs. 63ng/ml (37-106ng/ml), p<0.001; for urinary KIM-1 1.1ng/ml (0.13-4.87ng/ml) vs. 1.3ng/ml (0.1-5.3ng/ml), p=0.345; for urinary Cys-C 0.05mg/l (ND-13.9mg/l) vs. non-detectable, p<0.01; and for serum Cys-C 1.0mg/l (0.4-3.2mg/l) vs. 0.7mg/l (0.3-0.9mg/l), p<0.01. Almost all patients (93%) had higher levels of urinary NGAL than the higher value of the controls; the respective frequency for the other markers was: 68% for serum NGAL and serum Cys-C, 50% for urinary Cys-C and only 10% for urinary KIM-1. All studied markers correlated with eGFR: serum Cys-C (r=-0.758, p<0.001), serum NGAL (r=-0.627, p<0.001), urinary Cys-C (r=-0.498, p=0.008), urinary NGAL (r=-0.430, p=0.01) and urinary KIM-1 (r=-0.369, p=0.021). Only serum Cys-C strongly correlated with the involved serum free light chain (r=0.806, p<0.001). Urinary NGAL correlated also with urinary Cys-C (r=0.880, p<0.001), serum NGAL (r=0.503, p=0.002), 24-h proteinuria (r=0.431, p=0.01) and ISS stage (mean±SD values for ISS-1, ISS-2 and ISS-3 were: 31±29ng/mL, 47±52ng/mL and 408±695ng/mL, respectively; p=0.03). Serum Cys-C correlated also with ISS stage (the values for ISS-1, ISS-2 and ISS-3 were: 0.85±0.19mg/L, 0.94±0.24mg/L and 2.15±0.98mg/L, respectively; p=0.01), while urinary Cys-C correlated with 24-h proteinuria (r=0.564, p<0.001).

Conclusions: Our data suggest that almost all newly diagnosed symptomatic MM patients have tubular damage as assessed by elevated urinary NGAL suggesting that renal impairment is present very early in the disease course. Measurement of urinary NGAL and serum Cys-C offers valuable information for the kidney function of MM patients and their measurement may help in the identification of patients with high risk for the development of acute renal function. The value of KIM-1 seems to be very low in myeloma reflecting the differences in the pathogenesis of myeloma-related renal dysfunction than toxic acute renal injury of other etiology.