EFFECTS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON ALBUMINURIA RELATIVE TO GLOMERULAR FILTRATION RATE IN HIV-INFECTED PERSONS

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Background: Highly active antiretroviral therapy (HAART), especially tenofovir-containing regimens, has been implicated in albuminuria. Methods: We prospectively evaluated the effects of HAART on albumin to creatinine ratios (ACRs) in 102 antiretroviral-naïve HIV-infected persons treated with Tenofovir/Emtricitabine/Efavirenz (TDF/FTC/EFV), n=33; Zidovudine/Lamivudine/Nevirapine (ZDV/3TC/NVP), n=53; and Zidovudine/Lamivudine/Efavirenz (ZDV/3TC/EFV), n=16. Diabetes mellitus and hypertension were excluded. ACRs and glomerular filtration rates (eGFR) were estimated at baseline, and at 1, 3, 6 and 9 months post-therapy; prevalence of albuminuria (ACR ≥ 300mg/g), and microalbuminuria (ACR 30-300mg/g) were similarly estimated. HAART effects on normal ACR (0-30mg/g) were also monitored.

Results: At baseline, one patient (0.9%) had nephrotic-range albuminuria with ACR of 2450mg/g. Overall, 8 (7.8%) patients had albuminuria; 53 (51.9%) had microalbuminuria; while 41 (40.2%) had normal ACRs, 28 (27.5% of 102) of which had nonalbuminuric renal insufficiency. eGFR and ACRs improved concurrently on HAART (ACR, Wilk’s lambda 0.439, power 0.763, p=0.032); albuminuria improved significantly on all the 3 regimens at 9 months (p=0.006, 0.012 and <0.001 respectively). Microalbuminuria resolved earlier (1 month) with ZDV/3TC/NVP than with TDF/FTC/EFV and ZDV/3TC/EFV (24.31mg/g versus 76.51mg/g and 63.59mg/g; p=0.028, 0.016 respectively). Microalbuminuria relapsed on TDF/FTC/EFV and ZDV/3TC/EFV at 6 months but resolved again at 9 months (66.7 versus 29 mg/g, p=0.006; and 51.2 versus 9.5mg/g, p=0.001 respectively); no relapse on ZDV/3TC/NVP. At 9 months, ZDV/3TC/EFV caused the greatest resolution of microalbuminuria (85.7% decline in ACR from baseline) compared with ZDV/3TC/NVP (72.5% decline) and TDF/FTC/EFV (63.9% decline). In multivariate analyses, predictors of ACR include age (Odds ratio OR 2.8, p= 0.025); female gender (OR, 3.4, p=0.014); CD4+ (OR 0.99, p=0.002).

Conclusions: HIV induces renal impairment. Thus, albuminuria, microalbuminuria and nonalbuminuric renal insufficiency are highly prevalent in antiretroviral-naïve HIV-infected persons but nephrotic-range albuminuria is uncommon. Albuminuria and microalbuminuria and eGFR improve concurrently on HAART (with/without tenofovir). Zidovudine-based HAART (ZDV/3TC/NVP) resolves microalbuminuria earlier, and without relapse, unlike Tenofovir-based regimen and zidovudine with efavirenz (ZDV/3TC/EFV).
ASSOCIATION BETWEEN GRAFT FUNCTION AND SERUM SOLUBLE CD30 (sCD30) LEVELS IN PATIENTS WITH KIDNEY TRANSPLANTATION

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The CD30 molecule is preferentially expressed on human CD4 and CD8 T cells. A soluble form of CD30 (sCD30) is released into the bloodstream when CD30 T-cells are activated. Elevated serum soluble CD30 molecule (sCD30) have been related to acute cellular rejection and poor graft outcomes in kidney transplantation. Recent studies have suggested that sCD30 may predict early graft function after transplantation. This prospective study aimed to assess the relevance of serial postoperative serum sCD30 measurements for predicting graft function and acute rejection after transplantation.

We studied 50 kidney transplant recipients (13 female, 37 male; mean age: 38.12 ± 13.67). Blood samples were collected immediately before and after surgery at day 1, day 7, month 1 and month 3. Serum sCD30 levels were measured by ELISA using a commercial kit (eBioscience Human sCD30 ELISA). Serum creatinine levels were analysed by modified Jaffe method in Cobas 8000 analyser. GFR was estimated by CKD-EPI equation. Patients were assigned to 2 groups: defined slow graft function (SGF) as a reduction in serum creatinine by <70% on day 7 and immediate graft function (IGF) as ≥70%.

Patients were also separated depending on their history of acute rejection (AR) episodes. Data were expressed as mean ± Standard errors. We compared sCD30 levels with between the groups using both Mann-Whitney U and Student’s t tests. Among 50 recipients, 6 had SGF, and 44 had IGF, no patient had delayed graft function. Twelve cases were clinically diagnosed as AR by renal biopsy. Serum sCD30 levels demonstrated consistent significantly decreases after transplantation while GFR values had consistent increases. Pretransplant sCD30 levels were statistically different between IGF and SGF groups (136.84±8.83 ng/mL in IGF and 153.97±24.43 ng/mL in SGF (p<0.05). Serum sCD30 levels at day 1 and day 7 were also significantly higher in SGF compared to IGF. There were no significant differences in serum sCD30 levels between the groups at month 1 and month 3 (p>0.05). Pretransplant sCD30 levels are not different between patients with and without AR.

Our results show that pretransplant serum sCD30 is a good predictor of early graft function in kidney transplantation. The sequential monitoring of sCD30 level may identify the patients at the risk in the early period post-transplant.
Kidney diseases
Cod: T281

**CYSTINE CRYSTAL DETECTION USING A SINGLE QUADRUPOLE LC-MS SYSTEM. A CASE REPORT**

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**Background:**
Cystinuria is a rare condition in which stones made from an amino acid called cysteine form in the kidney, ureter, and bladder. Cysteine is formed when two molecules of an amino acid called cystine are bound together.

**Case report:**
A 33-years-old patient came to the emergency room presenting colic pains in the right lower abdomen. She has had 2 similar episodes since she was 14-years-old with kidney stone expulsion, pyelonephritis in 2013 and cystitis in 2014. Proteinuria was found in 2015 during a medical routine blood test. Repetitive lithiasis episodes were described in family history.

**Material and methods:**
First-hour urine specimen was collected. Aution Max AX-4280 and Sedimax Urine Analyzer of Menarini® and Single Quadrupole LC-MS 2020 system (Shimadzu Corporation, Kyoto Japan) was used. Urine sample was centrifuged for 5 min at 1500 g. Urinary sediment was diluted in methanol (1:25) and ten microliters of sample were injected into the HPLC-MS system. Cystine was separated using a Supelco (Sigma) reverse phase column C18 (150mmx4.6 mmx5 µm) at 35 ºC and detected in a Single Quadrupole LC-MS 2020 system (Shimadzu Corporation, Kyoto Japan). HPLC gradient was generated by mixing water with methanol. Chromatographic method started with 15% methanol for 2 min and changed linearly to 85% over 8 min, hold for 1 min and then decreasing to 15% methanol over 1 min. The total method run time was 14 min and the flow rate was 0.4 mL/min. Cysteine was ionized by electrospray ionization (ESI) in positive-ion mode and was monitored by Selected Ion Monitoring (SIM) mode at m/z 241.0. Hydrochloric acid and Glacial acetic acid were used to observe the crystals solubility.

**Results:**
Cystine crystals were observed in the urinary sediment. Mass spectrum showed an intense peak which coincided with the internal standard of cystine. Crystals were dissolved by adding Hydrochloric acid, they were not by adding Glacial acetic acid.

**Conclusions:**
This procedure is a fast and simple method that provides a high-throughput, robust assay that can be used for the diagnosis and management of cystinuria.
METABOLIC BONE DISEASE IN CHILDREN WITH STEROID RESISTANT NEPHROTIC SYNDROME

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Background: Children with steroid resistant nephrotic syndrome (SRNS) are at a greater risk of metabolic bone disease due to biochemical derangements of renal disease and due to the effects of corticosteroids and immunosuppressant therapy. The present study was undertaken to evaluate the calcium-vitamin D status in children with SRNS.

Methods: A cross-sectional case control study was performed to investigate the calcium-vitamin D status in 50 patients of SRNS and 40 healthy controls. Serum levels of 25 hydroxy vitamin D [25(OH) D], calcium, phosphorus, alkaline phosphatase (ALP) and parathyroid hormone (PTH) were assayed in all subjects. The SRNS patients were further divided into 3 groups according to their Up:Uc ratio.

Results: Vitamin D and calcium levels were significantly lower in the SRNS patients as compared to control (p < 0.0001 and p < 0.001) whereas PTH was significantly elevated (p < 0.01). Lower levels of vitamin D and calcium were found in the relapse phase (p < 0.01 and p = 0.001). PTH and ALP levels were however higher (p < 0.05 and p = 0.001). On correlating with Up:Uc ratio vitamin D and calcium showed a significant negative correlation (p < 0.01 and p < 0.05) whereas a positive correlation was seen for PTH and ALP (p < 0.05 for both).

Conclusion: There is a clear diminution of serum 25 (OH) D in patients with SRNS which reverts rapidly to normal after cessation of proteinuria. It may be associated with severe nephrotoxicity. Prophylactic therapy with vitamin D should be advocated in these patients.
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Kidney diseases

Cod: T283

BINDING OF BROMOCRESOL GREEN AND BROMOCRESOL PURPLE TO ALBUMIN IN CHRONIC KIDNEY DISEASE

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BACKGROUND: Albumin assays based on binding to bromocresol purple (BCP) and bromocresol green (BCG) yield different results in chronic kidney disease. Altered dye binding of carbamylated albumin has been suggested as the cause. In this study, a detailed analysis was carried out in which uremic toxins and Kt/V, a parameter describing hemodialysis efficiency were compared with serum albumin, assayed using BCG and BCP based assays.

METHODS: BCP and BCG assays were run on a Cobas 8000 platform (Roche), uremic toxins were assayed using an HPLC assay. Prior to analysis, serum samples were denaturated (95°C) followed by filtration. For the determination of the free fractions, denaturation was preceded by filtration. The HPLC system consisted of a Waters Alliance 2695 device and 2 serial detectors: a Waters 996 photodiode array detector and a Waters 2475 fluorescence detector. The separation was performed on a XBridge C8 column (Waters) with an Ultrasphere ODS guard column (Beckman).

RESULTS: In vitro carbamylation resulted in a linear decrease of the BCP/BCG ratio. Analysing albumin on 62 specimens originating from dialysis patients, regression analysis of the BCP/BCG ratio with serum urea showed a significant negative correlation. BCP/BCG ratio showed a negative correlation with Kt/V: y (BCP/BCG ratio) = -0.06 (Kt/V) +1.01 (r = 0.70; p < 0.001). Among the uremic toxins investigated, para cresyl and indoxyl sulfate showed a significant correlation with the BCP/BCG ratio. Inflammation, evidenced by serum concentrations of alpha 1 acid glycoprotein and CRP, was associated with a reduced BCP/BCG ratio.

CONCLUSIONS: Among the uremic toxins investigated, a significant correlation was only found between the BCP/BCG ratio and para cresyl and indoxyl sulfate. Determination of the BCP/BCG ratio for albumin is a simple, and less labour intensive method for assessing protein carbamylation in chronic kidney disease.
Kidney diseases
Cod: T284

ARE LABORATORY CREATININE METHODS TRACEABLE? – COMPARISON WITH ID-GCMS REFERENCE METHOD

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Traceability of laboratory results is a recognised need within quality standards based on ISO15189 and ISO17025. Where possible higher order reference methods are used for comparison of participant results with traceable values in the Weqas EQA schemes. With the drive for more traceable creatinine measurements, there is now a trend to the use of more specific enzymatic methods.

8 Pools of human serum covering a creatinine range of approximately 25 to 600µmol/l were distributed on 4 occasions to all Weqas participants. Each had associated creatinine reference target values using a JCTLM listed ID-GCMS Reference Method. Returned results showed an increase in laboratories using enzymatic methods from 18% in 2012 to 37% in 2016. The Jaffe ID-GCMS traceable group showed good correlation with the reference method above 150µmol/L but exhibited a negative bias below this value. The enzymatic methods showed good agreement with the ID-GCMS target value across the range of distributed samples. Using the MAPS (minimum analytical performance specification) bias criteria for creatinine, only the enzymatic method group is within the specified 3.8% desirable limit or 5% achievable limit. However within the enzymatic group the overall mean was influenced by the Roche and Olympus methods, which showed a marked positive bias. The Abbott and Siemens Dimension enzymatic methods showed closer agreement with the ID-GCMS target values. Within each enzymatic method group a large variation was also observed with substantial overlap of the data seen between groups.

Whilst traceability of creatinine methods has improved, even within the ID-GCMS traceable methods, variability has been observed as highlighted by comparison of EQA returns with the ID-GCMS reference method. The use of ID-GCMS reference targets as opposed to comparison with mean data eloquently highlights the variability.
Kidney diseases

**SERUM AND URINE BETA TRACE PROTEIN AS EARLY MARKERS OF DIABETIC KIDNEY DISEASE**

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**BACKGROUND:** Serum and urine concentrations of beta trace protein (BTP) were recently proposed as new markers of glomerular filtration rate (GFR). Diabetic kidney disease (DKD) is one of the most frequent causes of end-stage renal failure. When DKD is detected at the early stage, end-stage renal failure may be effectively prevented. There is a need for novel markers of early DKD. The aim of the study was to compare serum (sBTP) and urine BTP (uBTP) as markers of GFR among patients with early DKD in the course of type 2 diabetes (T2DM).

**METHODS:** 80 patients were included with T2DM at risk and with early stages of DKD (with eGFR >60 ml/min/1.73m²; albuminuria <300 mg/g creatinine); 42 women, 38 men; mean age 61 +/- 12 years; median diabetes duration 8 (2-11) years. sBTP and uBTP, serum cystatin C (cysC), urine transferrin (uTRF) and IgG were measured with immunonephelometry; urine neutrophil gelatinase-associated lipokalin (uNGAL) by immunochemiluminescence. uTRF, uBTP and uNGAL concentrations were corrected for urine creatinine (uTRF/Cr; uBTP/Cr and uNGAL/Cr, respectively).

**RESULTS:** Median sBTP was 0.65 (0.53-0.77) and uBTP 2.09 (0.70-4.19) mg/L. sBTP significantly correlated with routine markers of GFR: serum creatinine (R=0.52), cysC (R=0.75), and estimated GFRs based on creatinine and cysC (R=-0.58 and R=-0.70, respectively) (p<0.001). Also, sBTP correlated with urine IgG (R=0.29), serum urea (R=0.40), uric acid (R=0.43) and age (R=0.50), (p<0.05). Patients with newly diagnosed diabetes had lower sBTP (median 0.42 vs 0.65 mg/L). Weak correlations were observed between uBTP and serum creatinine (R=0.31) and urea (R=0.33) (p<0.05). uBTP/Cr positively correlated with uNGAL/Cr (R=0.37) and uTRF/Cr (R=0.37) (p<0.05). There were no correlations between sBTP and uBTP; or between BTP and diabetes duration, glycated hemoglobin or albuminuria.

**CONCLUSIONS:** In early DKD, increased sBTP indicates glomerular impairment and is a better marker of GFR than uBTP. However, uBTP seems associated with early tubular impairment in T2DM patients. Our preliminary results indicate that both sBTP and uBTP concentrations provide valuable information about renal function in early DKD. More studies are needed to compare BTP with currently used renal markers like cystatin C and uNGAL.
Kidney diseases

Cod: T286

ESTIMATION OF GLOMERULAR FILTRATION RATE (GFR) BASE ON CYSTATIN C VALUES. ITS EVALUATION IN DIABETIC PATIENTS

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Diabetic nephropathy (DN) is the most frequent pathology in hemodialysis admission. To detect DN in early stages it is recommended to measure albumin excretion. However, decline in glomerular filtration rate (GFR) can occur without albuminuria, and DN can present only a reduced GFR (type 2 Diabetes Mellitus). KDIGO guidelines recommend CKD-EPI2009 formula to estimate GFR, using CystatinC based formula whenever a confirmatory test is required, or when creatinine measurement is not reliable.

The objective was to verify the existence of significant differences in GFR estimated with CKD-EPI2009, CKD-EPI CystatinC and CKD-EPI creatinine-cystatinC, comparing them with MDRD-4IDMS formula, according to KDIGO2012 guideline recommendations.

156 diabetic patients (age range 40-70 years) with glycated hemoglobin HbA1c ≥ 6.5%. Creatinine, CystatinC, glycated hemoglobin and urinary creatinine and albumin were measured with Cobas 6000, Roche.

The formulas used to estimate GFR cystatinC, recategorized some of patients. This has an important clinical impact in patients with GFRcrea between 45 and 75 mL /min/1.73m2. 10 patients were confirmed to have Chronic Kidney Disease and other 5 patients were reclassified to a different stage of Chronic Kidney Disease.

The use of Cystatin C as a confirmatory test when GFR values are slightly and/or moderately disabled is useful to define the forecast risk.
BIOMARKERS IN EARLY DIAGNOSIS OF ACUTE KIDNEY INJURY

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Background
Several novel urinary and serum biomarkers have shown promise in the early detection and diagnostic evaluation of acute kidney injury (AKI). The diagnosis and prognosis utility of novel and traditional AKI biomarkers such as NAG, α-GST, NGAL and Cystatin C and evaluated during a prospective study of 68 adults undergoing kidney transplantation.

Patients and Methods
In a prospective cohort study 68 adult who underwent kidney transplantation from living or deceased donors between 2010 and 2014 were included. Patients divided into two groups based on the presence or absence of graft dysfunction. Urinary NAG, α-GST, NGAL and serum creatinine and Cystatin C were all measured at preoperative baseline, postoperatively and the time of the initial clinical diagnosis of AKI. Receiver operative characteristic curves were generated and the areas under the curve (AUC) were compared. All biomarkers was measurement used standardized techniques.

Results.
Of all investigated patients 24.5% developed AKI in stage1, 5.2% of whom progressed to stage3. Preoperative concentration of α-GST and uNGAL are basis to predict the future development of stage 1 and stage 3 of AKI. Urinary NAG (AUC-ROC = 0.89) and NGAL (AUC-ROC = 0.93) was exceptional early markers in the first hours after the development of AKI (expressed in stage 3)

Similar significant changes are proved in the concentration of α-GST.

The best sensitivity and specificity for AKI detection by urinary NGAL observed at 2 hour after transplantation (cut-off point 280 ng/ml) and urinary NAG at 4 hour (cut-off point 7.5 U/mmol creatinine). In the same time the concentrations of serum Cystatin C showed significantly values (3.85+/-1.55 v c.g.0.65+/-0.11 mg/L; AUC-ROC=0.95); and urinary α-GST:19.24+/-1.87 v c.g.3.9+/-.2.3 µg/L(AUC-ROC=0.93).

Conclusion
Urinary biomarkers especially NAG and NGAL may improve to ability to detect early AKI (in the first hours) and determine the clinical prognosis at the time of diagnosis.
Kidney diseases

Cod: T288

DEVELOPMENT OF A 4-PLEX BIOCHIP ARRAY FOR THE EARLY DETECTION OF CHRONIC KIDNEY DISEASE

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Background. Chronic Kidney Disease (CKD) involves the progressive loss of kidney function over a period of time and currently, methods of diagnosing early stage CKD are lacking. Late diagnosis of CKD can ultimately lead to end-stage renal disease requiring kidney dialysis or transplantation. The availability of rapid screening tests for the detection of early stage CKD biomarkers is advantageous to identify individuals at risk of progressive renal disease. Biochip array technology enables the detection of multiple analytes from a single sample and this study reports the development of a 4-plex biochip array for the determination of C3a des Arg, C-Reactive Protein (CRP), Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Cystatin C.

Methods. Simultaneous sandwich chemiluminescent assays, defining discrete test sites on the biochip surface and applied to the Evidence Investigator biochip analyser were employed. Assay sensitivity and precision were evaluated. Correlation studies were conducted by assessment of stage 3 CKD and normal serum samples using this 4-plex biochip array and other available methodologies.

Results. Assay evaluation showed sensitivity values of 0.41 µg/mL (C3a des Arg), 0.55 µg/mL (CRP) 0.01 µg/mL (NGAL), 0.03 µg/mL (Cystatin C) (assay ranges: 0-100 µg/mL, 0-60 µg/mL, 0-2 µg/mL, 0-5 µg/mL, respectively). Intra-assay precision, expressed as CV (%) was < 12 % for all the assays. The correlation studies from the assessment of stage 3 CKD (n=19 for CRP and cystatin C, n=18 for NGAL) and normal serum samples (n=17 for CRP and cystatin C, n=18 for NGAL) showed, following linear regression analysis, R² values > 0.938 to other available methodologies.

Conclusions. The results indicate optimal analytical performance of the developed 4-plex biochip array for the simultaneous determination of C3a des Arg, CRP, NGAL and Cystatin C. The biochip based immunoassays correlated favourably with other existing methodologies. This 4-plex biochip array is a reliable multi-analytical tool in the identification of early CKD biomarkers.
Kidney diseases

Cod: T289

**BODY FLUID VOLUMES AND SCALING OF MEASURED GLOMERULAR FILTRATION RATE - GENDER DIFFERENCES**

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Background: Normalized glomerular filtration rate (GFR) plays a primary role in defining reference ranges with age across patient physical demographics. Body surface area (BSA) is widely used clinically, but its physiologic relevance has been questioned, compared with body volumes [(total body water (TBW) and extracellular fluid volume (ECV)]. The aim of the study was to evaluate the difference between normalized GFR value depending on which of the scaling methods used: (1) scaling GFR to BSA [(unadjusted GFR standardized to a BSA of 1.73 m² (GFR BSA)]; (2) scaling GFR to ECV (GFR - ECV); (3) scaling GFR to TBW of 40 L, (GFR-TBW regression method)].

Material and methods: In 60 patients [30 female, median age -56.3 (53 – 62); 30 male, median age 54 (47.1 – 59.3) years] with newly diagnosed hypertension radionuclide-based GFR measurement (slope - intercept method, 99mTc-DTPA, 37 MBq, Captus 3000, Capintec) was done. Only patients with measured GFR greater than 60 mL/min/1.73 m² were included. Anthropometric measurements were used for estimation of Fat Mass (FM), TBW, ECV and BSA.

Results: There was no significant differences between male and female patients in respect to systolic (P=0.21) and diastolic (P=0.16) blood pressure, unadjusted GFR (P = 0.32), as in GFR BSA (P = 0.46) and GFR ECV (P=0.16). TBW (46.4 +/- 6.3 vs. 33.3 +/- 2.7, P = 0.01), and ECV (23.2 +/- 3.9 vs. 17.9 +/- 1.8, P = 0.00) were significantly higher in male compared to female patients. GFR-TBW in female was significantly higher compared to GFR BSA (110.2 +/- 18.3 ml/min/40L vs 90.21 +/- 11.7 ml/min/1,73 m² P = 0.00), while significant differences between GFR - TBW vs GFR - BSA was not observed in male patients (91.4 +/- 10.6 ml/min/40L vs 89.4 +/- 9.51 ml/min/1,73 m², P = 0.31).

Conclusion: TBW could provide more precise normalisation of GFR, especially in female patients with GFR greater than 60 mL/min/1.73 m².
EFFICIENT REPORTING OF ESTIMATED GLOMERULAR FILTRATION RATE WITHOUT HEIGHT IN KOREAN CHILDREN

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Background: The updated bedside Schwartz equation is a recommended creatinine-based formula to estimate glomerular filtration rate (eGFR) in children. It requires constant, serum creatinine concentration and height to calculate eGFR. Unlike the serum creatinine level, obtaining height information from the laboratory information system (LIS) is often not available in clinical laboratory. Recently height-independent eGFR equations including Q-age, Simple height-independent (Simple-HI), and Full age spectrum (FAS) equations have been introduced. We aim to evaluate the performance of three height-independent eGFR equations in Korean children.

Methods: A total of 250 children who underwent 51-chromium-ethylenediaminetetraacetic-acid (51Cr-EDTA)-based GFR measurements were enrolled. The 51Cr-EDTA GFR was defined reference GFR. Serum creatinine concentration was measured by isotope dilution mass spectrometry (IDMS) traceable Jaffe method, and then recalibrated the Jaffe results to an IDMS-traceable enzymatic method. Bias (estimated GFR-measured GFR), precision (root mean square errors, RMSE), accuracy (P30) of three height-independent eGFR equations were compared to that of the updated Schwartz equation. P30 was defined the percentage of patients whose estimated GFR within ±30% of measured GFR.

Results: The median (first quartile, third quartile) age and measured GFR of study population were 10 (6, 13) years and 115 (97, 142) mL/min/1.73m 2. The mean±SD bias (mL/min/1.73m 2) of four eGFR equations were 8.7±43.0 for updated Schwartz, 5.8±42.9 for Q-age, 0.7±42.8 for Simple-HI and 4.2±42.6 for FAS equations, respectively. Three height-independent equations showed significantly lower bias than that of updated Schwartz equation. The RMSE and P30 were 43.8 and 64.4% for updated Schwartz, 43.2 and 66.8% for Q-age, 42.7 and 66.0% for Simple-HI, and 42.7 and 66.8% for FAS equations, respectively.

Conclusions: Three height-independent eGFR equations were less biased and as accurate as the updated Schwartz equation in Korean children. Use of the height-independent eGFR equations will enable efficient reporting of eGFR though LIS in clinical laboratories.
KLOTHO IN SERUM - A POTENTIAL MARKER OF ACUTE KIDNEY INJURY AFTER SURGERY

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Background:
Acute kidney injury (AKI) is defined and classified by criteria like KDIGO, but these criteria are insufficient for early diagnosis of AKI as they mainly rely on serum creatinine. In this pilot study, we investigated the role of serum Klotho as a potential early marker in diagnosis of AKI after cardiac surgery.

Methods:
Patients were included into three groups (non-AKI, AKI-1 and AKI-2) according to AKI stages using KDIGO criteria. The levels of creatinine and Klotho in serum were measured before surgery and at four additional time-points after surgery.

Results:
The pilot study was conducted on 46 patients. Baseline levels of serum Klotho were not significantly different between the groups. Two hours after cardiopulmonary bypass (CPB) Klotho levels were significantly higher in AKI-2 group (p=0.040) but not in AKI-1 group (p=0.231) when compared to non-AKI group. The levels were not significantly different between the groups 24 and 48 hours after surgery. The significant difference in creatinine levels was measured as late as the first day after surgery, when the levels were higher in AKI-2 group (p=0.019) than in non-AKI group.

Conclusion:
Our pilot study suggests that serum Klotho may have potential to identify patients at risk of developing more severe kidney injury after cardiac surgery using CPB already in the first hours after surgery. However, the role of serum Klotho as an early marker of AKI after surgery still needs to be confirmed in larger groups of patients.
Kidney diseases

Cod: T292

ESTIMATION OF KIDNEY FUNCTION IN PATIENTS WITH β-THALASSEMIA MAJOR USING DIFFERENT LABORATORY METHODS

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Background-Inulin clearance is the most accurate test for GFR measurement. In patients with β-thalassemia major, Inulin clearance measurements were shown to be 40% lower than estimated GFR (eGFR), calculated by different creatinine-based equations, when creatinine was measured by the Jaffe reaction (Creat-J). Since inulin clearance cannot be routinely used, the present study examines whether serum creatinine measurement using enzymatic method (Creat-E) or Cystatin C could provide a better assessment of kidney function than Creat-J.

Methods-Blood samples were drawn from β-thalassemia major patients for serum Creat-E and Creat-J calibrated to isotope-dilution mass spectrometry traceable, Cystatin C, and thyroid function tests. To calculate eGFR, creatinine and Cystatin C values were placed in the following equations: Cockcroft-Gault (CG), MDRD study, 2009 CKD-EPI creatinine, 2012 CKD-EPI creatinine-cystatin C, and 2012 CKD-EPI cystatin C. Statistical analysis was performed using Spearman’s correlation test and Wilcoxon non-parametric paired test. Data are presented as means ± SD. P<0.05 was considered to be statistically significant.

Results-22 adult patients with a mean age of 38±10 yrs were included. The results demonstrated a significant difference between paired values of creatinine. Mean Creat-J was 0.45±0.3 mg/dl, median 0.37 mg/dl (0.18-1.23 mg/dl); mean Creat-E was 0.63±0.3 mg/dl, median 0.55 mg/dl (0.27-1.35 mg/dl), p<0.001, which comprises a 32±18% and 33% lower mean and median Creat-J vs. Creat-E, respectively. Using Spearman’s test, correlation between Creat-E and Creat-J was 0.75 (p<0.001). Using 2009 CKD-EPI Creat-E as a reference, most equations showed statistically significant overestimation of eGFRs. Correlation coefficients showed best agreement of eGFR values based on CKD-EPI creatinine and MDRD Creat-E, r=0.954 and between CKD-EPI Creat-E and CKD-EPI Creat-J = 0.854, p<0.01. eGFR based on Cystatin C did not improve correlation vs. CKD-EPI Creat-E (0.733), yet when combined with Creat-E, in 2012 CKD-EPI creatinine-cystatin C formula, the coefficient was 0.892, p<0.01.

Conclusions-Our results suggest that in thalassemia major patients, creatinine should be measured using a standardized enzymatic method. Furthermore, based on correlation and paired evaluation results, we suggest applying the 2009 CKD-EPI Creat-E or 2012 CKD-EPI CreatinineE-cystatin C equations to estimate GFR in this subset of hematologic patients.
Kidney diseases

Cod: T293

COMPARATIVE CLINICAL EVALUATION OF TWO SECOND AND ONE THIRD GENERATION PARATHYROID HORMONE ASSAY IN BOTH HEALTHY AND HEMODIALYSIS PATIENTS

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BACKGROUND

For the diagnosis and management of chronic kidney disease-mineral and bone disorder (CKD-MBD), the measurement of parathyroid hormone (PTH) is essential. Recent studies have shown that the “intact PTH” assays of the second generation detect both PTH1-84 and PTH7-84 fragments, whereas recently developed third generation (or “whole PTH”) assays do not recognize the PTH7-84 fragment. The purpose of the study is to compare two commercial second generation assays and one third generation assay and evaluate if their results are comparable in healthy and hemodialysis population.

METHODS

10 healthy subjects (5 male and 5 female) and 25 hemodialyzed patients (15 male and 10 female) selected by our hospital’s dialysis centre, were included in the study. Serum PTH-intact and whole- concentrations were measured using two 2nd generation assays (Roche Elecsys and Diasorin Liaison) and a 3rd generation assay, Elecsys PTH1-84, respectively.

RESULTS

As expected, hemodialyzed patients have significantly higher PTH levels compared to healthy individuals, irrespective of the assay used (Roche Elecsys: 485.52 vs. 40.83, p=0.005; Diasorin Liaison: 568.15 vs. 40.59, p=0.0096, Elecsys PTH1-84: 271.1 vs. 40.67, p=0.0068). Using the pooled sample, the wPTH assay yields lower values compared to the iPTH ones (Roche Elecsys: 205.41 vs. 358.39, p=0.0001; Diasorin Liaison: 205.41 vs. 417.42, p=0.0005). However, when restricted to the healthy individuals all assays yield comparable results (p=0.5503 and p=0.8510 for the pairwise comparisons, respectively). Both second generation assays were found to be strongly correlated with the wPTH assay, with R² equal to 0.9917 (p<10⁻⁴) for Roche Elecsys and 0.9857 (p<10⁻³) for Diasorin Liaison. In both cases however this result was independent of the disease status. Nevertheless, when used as diagnostic tests all assays yield the same sensitivity and specificity.

CONCLUSIONS

Our results show that both second generation assays correlate well with each other for both healthy and HD patients. Moreover, both second and third generation PTH assays are strongly correlated in healthy subjects as well as in hemodialysis patients. However, when PTH values are high, the mean wPTH levels are almost half compared to iPTH. More research is needed in order to assess the utility of third generation PTH assays in hemodialysis subjects.
ASSOCIATION BETWEEN 1,25-DIHYDROXYVITAMIN D3 LEVELS WITH INORGANIC PYROPHOSPHATE, FETUIN A, OSTEOPROTEGERIN, BONE MORPHOGENIC PROTEIN-2 AND ALKALINE PHOSPHATASE IN RENAL TRANSPLANT PATIENTS

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We aimed in our study that examine the relationship between plasma 1,25-dihydroxyvitamin D3 levels with BMP-2 and ALP which activators of vascular calcification, and fetuin-A, OPG and PPi which inhibitors of vascular calcification in renal transplantation patients.

35 renal transplantation patients were included in the research. Assays were performed in the collected blood samples before (PrTx) and after 6 months than operation (PTx). 23 patients were taken lateral lumbar radiograph and made pulse wave velocity (PWV) measurements. By considering the mean age of patients (40.30±12.86 years), 7 m/s was accepted the normal PWV value. Than 2 groups were performed which are PWV<7 and PWV≥7 m/s.

PTx, that was observed significant increase in plasma 1,25-dihydroksivitamin D3 levels compared to the PrTx (p=0.0001). There was a significantly increase in serum calcium levels (p=0.0001) and decrease in serum phosphorus1 with ALP2 levels compared to the PrTx (p1=0.001, p2=0.011). Increase in PPi, Fetuin A and BMP-2 levels with decrease in OPG levels were not statistically significant compared to PrTx. 1,25-dihydroksivitamin D3 levels correlated with OPG1 and BMP-22 in both PrTx* and PTx** (r1*=0.925 ve r2*=0.762; r1**=0.574, ve r2**=0.515). Both period, BMP-2 showed significant correlation with OPG (r*= 0.700; r**=0.684).

That can be considered to increase the calcification inhibitors against of increasing calcification risk due to the protection of system as endogenous. In this case, the possibility of vitamin D is calcification inhibitör should not be excluded. It is expected that normalized levels of vitamin D can show a protective effect in CKD patients.
Kidney diseases
Cod: T295

NON-DSA ANTIBODY AND ANTIBODY-MEDIATED REJECTION TRANSPLANT GLOMERULOPATHY: A CASE REPORT

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Background
Anti-HLA (Human Leucocyte Antigen) donor-specific antibodies (DSAs) increase the risk of antibody-mediated rejection and graft failure after kidney transplantation, but also non-DSA antibodies are involved in transplant outcomes. Luminex® Technology for HLA antibody screening before and after organ transplantation resulted crucial for a better understanding of transplantation immunology and improvement in clinical practice. Here we described a case of non-DSA antibody and the occurrence of acute antibody-mediated rejection (AMR) in a kidney transplant recipient.

Methods
Complement-dependent cytotoxicity (CDC) cross-matches between donor and recipient were performed, pre- and post-transplant sera were analyzed in order to identify anti-HLA IgG and IgM antibodies, using Luminex® Technology (LSA, Luminex® Single Antigen class I and class II, One Lambda). Recipient and donor were typed for HLA-A*, HLA-B*, HLA-C*, HLA-DRB1*, HLA-DQB1* antigens at low/high resolution level.

Results
A 65-year-old man with end-stage renal disease, due to unknown nephropathy, underwent ABO-incompatible unrelated living donor kidney transplantation. Among mismatches between donor and recipient there were: A*24, A*68. Cross-match assay was negative on T and B cells. On day 7 post-transplant he developed a biopsy-proven AMR. Anti-donor blood group antibodies and DSAs were negative, while anti-A*11:02 IgG antibody was strongly positive with MFI (mean fluorescence intensity) values up to 20000. He was treated with eculizumab for resistant AMR. During the next 2 years he suffered from several relapses of AMR, each time associated with an increased of A*11:02 MFI. Based on HLAMatchmaker software and HLA Epitope Registry the presence of A*11:02 antibody could be the expression of shared eplets between the real DSAs (A24, A68) and the antibody revealed by Luminex® assay.

Conclusions
The introduction of new sensitive techniques, as Luminex® Technology, had a significant influence on the clinical approach to HLA antibodies analysis. It is possible that the presence of this non-DSA can influence allograft outcome, by interacting with some 3dimensional donor structures. If confirmed, the identification of a causative structural epitope opens new horizons in the characterization of the HLA antibody response.
Kidney diseases

**Cod: T296**

**ARE ALBUMIN-CORRECTED CALCIUM LEVELS USEFUL TO ASSESS CALCEMIA IN CHRONIC KIDNEY DISEASE PATIENTS? EVALUATION OF TWO NEW DERIVED FORMULAS WITH IMPROVED ALBUMIN MEASUREMENT**


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**Background**

Disorders of mineral and bone metabolism (MBD) are prevalent in patients with chronic kidney disease (CKD). Spanish Society of Nephrology (SEN) Guidelines for the management of CKD-MBD recommend serum calcium to be regularly measured in patients with CKD stages 3-5D. They also state that the measurement of ionized calcium (iCa) is preferred and that if total calcium (tCa) levels are used instead, then they should be adjusted in the setting of hypoalbuminemia. Because the method for measurement of albumin may affect the calcium correction formula, we compared the ability of the non-corrected and albumin-corrected tCa levels, calculated by using two recently derived formulas measuring albumin with an improved bromocresol purple (BCP) method, to identify low, normal or high iCa levels.

**Methods**

**Study population:** 40 patients (mean age: 70 years (interquartile range: 55-78), range: 23-89 years, 17 female/23 male) with stages 3 to 5 CKD from Nephrology Department monitored for CKD-MBD, including the measurement of tCa, iCa and albumin levels, were included.

**Laboratory assays:** 50 blood samples were collected from the CKD patients. tCa and albumin levels were measured in venous samples in Dimension Vista analyzer using complexon and BCP methods, respectively. Arterial samples were collected in lithium heparin syringes for iCa measurement in an ABL 800 analyzer by using ion-selective electrodes.

Three different previously reported formulas were used to predict corrected tCa (ctCa):

- **Formula 1:** ctCa (mg/dL) = tCa (mg/dL) + 0.8 (4 – Albumin (g/dL))
- **Formula 2:** ctCa (mg/dL) = tCa (mg/dL) + 0.75 (3.5 – Albumin (g/dL))
- **Formula 3:** ctCa (mg/dL) = tCa (mg/dL) + 0.7 (4 – Albumin (g/dL))

Formulas 2 and 3 were derived by using BCP method for albumin.

**Statistics:** The agreement between iCa levels and the other estimators of tCa levels was evaluated by using kappa (κ) coefficient.

**Results**

The study included 50 samples from the CKD patients. The prevalence of hypercalcemia (iCa level > 5.2 mg/dL) was 4%; that of hypocalcemia (iCa level < 4.6 mg/dL) was 46%. Agreement between iCa levels and the other estimators of blood calcium concentration was only fair:

- iCa vs. tCa: κ: 0.394
- iCa vs. ctCa (formula 1): κ: 0.352
- iCa vs. ctCa (formula 2): κ: 0.323
- iCa vs. ctCa (formula 3): κ: 0.352

**Conclusion**

Neither non-corrected nor albumin-ctCa predicts correctly iCa levels in patients with stages 3 to 5 CKD. An accurate assessment of blood Ca concentration requires the measurement of iCa.
Kidney diseases

MYO-INOSITOL OXYGENASE AS A NOVEL MARKER IN DIAGNOSIS OF ACUTE KIDNEY INJURY

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Background: Due to the lack of diagnostic efficiency of serum creatinine in acute kidney injury (AKI), development of novel diagnostic markers is an emerging need for AKI. Therefore, we investigated markers of myo-inositol oxygenase (MIOX), neutrophil gelatinase-associated lipocalin (NGAL) and cystatin c whether applicable in the diagnosis of acute kidney injury. Material and Methods: We enrolled a total of 39 (19 female, 20 male) AKI patients and a total of 38 (18 female, 20 male) healthy controls in the study. Diagnosis of AKI is defined by an increase in serum creatinine levels by ≥ 0.3 mg/dl in following 48 hours or an increase of serum creatinine levels by ≥ 1.5-fold from a known or assumed baseline or a decrease of urinary output to less than 0.5 ml/kg/hour in following 6 hours. We compared levels of serum MIOX, NGAL and cystatin c measured in two groups.

Results: We found that concentrations of serum creatinine, blood-urea-nitrogen (BUN), MIOX and cystatin c were higher in AKI group. ROC curve analysis showed AUC for MIOX and cystatin c were respectively 0.694 (95% CI 0.579 to 0.794), 0.976 (95% CI 0.912 to 0.997). For MIOX, if Cut-off concentration is set to 77.3 pg/ml, then diagnostic sensitivity and specificity is to be 53.8 % (95% CI 37.2 – 69.9), 81.5 (95% CI 65.7 – 92.3) respectively. For cystatin c, if Cut-off concentration is set to 1.4 mg/dL, then diagnostic sensitivity and specificity is to be 94.8 % (95% CI 82.7 – 99.4), 94.7 % (95% CI 82.3 – 99.4) respectively. Concentrations of serum NGAL measured in two groups were not different.

Conclusion: Measurement of serum MIOX and cystatin c levels is valuable in the diagnosis of AKI patients. MIOX as a kidney-specific enzyme in early diagnosis of AKI needs for more studies and more focusing in future.
Kidney diseases

PERFORMANCE EVALUATION OF THE VITROS® NEPHROCHECK® TEST*

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Acute kidney injury (AKI) is a complex disorder with high mortality due to comorbidities and management challenges, especially in the critically ill patient. The VITROS® NEPHROCHECK® Test quantitatively measures Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) and Insulin-like Growth Factor Binding Protein 7 (IGFBP-7) to generate an AKI risk index (AKIRISK™ Score). Patients with AKIRISK™ Score less than 0.3 are at low risk of developing AKI while those with values ≥ 0.3 are at high risk. We have evaluated the performance of the VITROS NEPHROCHECK Test on the VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System. The test is linear across the range of 0.6 to 34.1 ng/mL for TIMP-2 and 2.2 to 482.2 ng/mL for IGFBP-7 resulting in an AKIRISK™ Score range of 0.001 to 16.4. Limits of Blank were determined to be 0.008 ng/mL and 0.006 ng/mL for TIMP-2 and IGFBP-7, respectively. Limits of Quantitation were determined to be 0.086 ng/mL for TIMP-2 and 0.106 ng/mL for IGFBP-7 respectively, resulting in an AKIRISK Score of 1.7x10-4. A 20-day precision study with pooled patient samples at mean AKIRISK Scores of 0.13, 0.51, 4.44, 10.16 resulted in within-laboratory percent coefficients of variation (%CV) of 7.5%, 5.9%, 5.5%, and 5.8% respectively on the VITROS 3600 and %CV of 5.7%, 6.3%, 8.5%, and 7.5%, respectively on the VITROS 5600 Integrated System. Potential interfering substances including acetoacetate, acetone, ammonia, albumin, creatinine, hemoglobin, myoglobin, pH (4.0 – 8.0), urea and uric acid were tested and shown not to interfere in the assay. The accuracy of the test was evaluated with 99 patient specimens spanning the assay measuring range against the Astute Medical NEPHROCHECK Test System (Astute) and the following linear regression statistics were obtained: VITROS = 1.14*Astute + 0.0126; (r) = 0.96. Fifty samples adjudicated for AKI risk were assayed using the VITROS NEPHROCHECK Test, and the data was analyzed for clinical performance. The sensitivity of the test was determined to be 90.0%, specificity of 60.0% with positive predictive value of 60% and negative predictive value of 90% for the Test. In conclusion, the assay demonstrated good precision, and acceptable clinical performance. (* under development)
Kidney diseases

Cod: T299

COMPARISON BETWEEN WHOLE AND INTACT PARATHYROID HORMONE ASSAYS

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Parathyroid hormone (PTH) is a linear peptide of 84 amino acids produced exclusively by the parathyroid glands whose main function is to regulate calcium levels in the body. Parathyroid hormone 1-84 (1-84 PTH) is the biologically active hormone. The current available assays for PTH also measures PTH (7-84) at the same time, leading to misclassification of the patients. Now, 3rd generation methods measuring only PTH (1-84) have been developed and are clinically used as a whole PTH method. The aim of our study was to examine the correlation between 1–84-PTH and intact-PTH in patients on haemodialysis (HD). A total of 120 hemodialysis patients were enrolled from dialysis services of our hospital. The serum PTH level was evaluated by both intact and whole PTH assays, using ADVIA® Centaur Intact Parathyroid (iPTH) assay on ADVIA Centaur XP system and Lumipulse G whole PTH, a 3rd generation kit with 100% cross reactivity with PTH1-84 and 0% with PTH7-84 on Fujirebio Lumipulse G600II instrument, respectively.

When we compared to intact PTH, the whole- PTH method yielded over 50% lower values and the difference remained constant through the entire range of PTH results. In fact the median level of Lumipulse G whole PTH was 96.8 pg/mL and that of Centaur iPTH was 229.5 pg/mL. Despite different absolute results whole PTH assay had a very high correlation with the iPTH assay ($r= 0.950, P<0.001$). The comparison of methods yielded the following Passing–Bablok regression line: $\text{PTHi Fujirebio} = 0.9454 + 0.3692 \text{ PTH Centaur}$ (95% confidence interval [CI] of slope: 0.35–0.38; 95% CI of intercept: -2.43-3.42). Bland–Altman plot revealed that the average bias between the assays was -201.8 pg/mL, using this plot analysis, we could see that as the serum PTH level increased, there was a large difference between two assays. The presence of metabolites is associated with an overestimation in PTH assessment particularly in the case of patients with renal failure requiring dialysis because the measured value don’t accurately reflect the functional status of parathyroid. A whole PTH method that uses highly specific antibodies and that is calibrated against the Gold standard (NIBSC 95/646), can be of great help in the diagnosis and clinical interpretation of PTH status in these patients.
Kidney diseases
Cod: T300

RENAL COMPLICATION OCCURS IN SPITE OF INCREASED LEVELS OF SOLUBLE RECEPTORS FOR ADVANCED GLYCATION END PRODUCTS

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Background: Advanced glycation end products (AGEs) interacts with its cell receptors RAGE (receptor for AGE) resulting in the activation of nuclear factor kappa-B, increased expression of proinflammatory cytokines and formation of oxygen radicals. These factors have been involved in the pathogeneses of numerous diseases. Soluble receptor for AGE (s RAGE) circulating in the blood acts as a decoy for the AGE and hence it protects the tissue damage from interaction of AGE with RAGE. In chronic renal disease the levels of sRAGE are elevated. One would have expected that increased sRAGE levels would have protected the renal damage.

Hypothesis: It is hypothesized that the increases in the levels of AGEs are greater than the increases in the levels of sRAGE and hence the protective effects of sRAGE overridden by AGE.

Objectives: The objectives are to determine: 1) if the levels of serum AGE, and sRAGE are elevated in the end stage renal disease; 2) if the increases in the levels of AGEs are greater than the increases in the levels of sRAGE.

Methods: The study subjects comprised of 88 patients with end stage renal disease (ESRD) and 22 control subjects. Blood samples were collected for measurement of AGE and sRAGE using commercially available ELISA kits. Protocol was approved by Ethic Committee. The data are presented as mean ± SE. The data analysis was made using 2-tailed paired student “t” test.

Results: The serum levels of AGE in control subjects were 1.042 ± 0.053 µg/ml while the levels were 7.04 ± 0.51 µg/ml in patients with ESRD. These values were 6.77 times greater in patients with ESRD than in control. The values of serum sRAGE in control subjects were 802.44 ± 63.49 pg. /ml while these values for patients with ESRD were 1990.38±155.25 pg. /ml. The serum levels of sRAGE in patients with ESRD were 2.45 times higher than the in control subjects.

Conclusions: The data suggest that the serum levels of both AGE and sRAGE are elevated in patients with ESRD and that the increases in the levels of AGE are 2.77 times higher than the increases in the levels of sRAGE. The renal damage may be due to greater increases the levels of AGE than the increases in the levels of sRAGE.
Background: Renal fibrosis is the common anatomical characteristic of most renal diseases, irrespective of their etiology, leading to chronic kidney disease. It is a multifactorial process where many cellular and molecular mediators are involved. As more than 10% of the general population suffers from renal fibrosis, it is imperative to focus on the discovery of novel, early markers for this disease.

Methods: Proteomic analysis of the renal parenchyma from a rodent model of fibrosis (Unilateral Ureteral Obstruction,UUO) and a cultured renal cell line was performed. From the identified differentially expressed proteins calreticulin and the family of 14-3-3 proteins were further studied. Western blot and qPCR confirmed the proteomic results at protein and mRNA level. In order to evaluate the clinical significance of these findings archived biopsy material was used from patients suffering from IgA nephropathy (N=19), membranous nephropathy (N=12), lupus nephritis (N=11) and ANCA-associated glomerulonephritis (N=11). Healthy renal tissue was used as control (N=5). Biopsies were studied for the presence of 14-3-3 proteins using immunohistochemistry. The ImageJ software was used for the quantification of the stained area. Results are given as mean±SD.

Results: Proteomic analysis data revealed that calreticulin and the family of 14-3-3 proteins are critically involved in the fibrotic process. Both proteins were significantly up-regulated at the mRNA and protein level specifically in tubular epithelial cells in the animal model. In all different nephropathies tissue area (in $\mu m^2 \times 10^3$) stained with 14-3-3 proteins showed a statistically significant increase (p<0.01) compared to control samples (control: $11\pm6$; IgA: $177\pm93$; membranous: $156\pm74$; lupus: $191\pm61$; ANCA: $177\pm72$). Moreover, co-localization of calreticulin and 14-3-3 proteins was observed specifically in distal tubular epithelial cells.

Conclusions: Our studies, extending from basic to clinical research, suggest that calreticulin and the family of 14-3-3 proteins are potential indicators of renal damage and could be used to evaluate the integrity of the tubulointerstitial compartment of the kidney.

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Kidney diseases

Cod: T302

LUMIPULSE® WHOLE PTH 3RD GENERATION ASSAY: REFERENCE VALUES IN A HEALTHY POPULATION AND CLASSIFICATION OF SECONDARY HYPERPARATHYROIDISM IN CKD5

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Background
Third generation PTH assays detect only the bioactive total PTH 1-84 fragment and require to establish new reference values and to assess the impact in medical care of mineral and bone disorders in CKD5 patients.

Methods
Serum PTH levels were measured with Lumipulse whole PTH chemiluminescent enzyme immunoassay on LUMIPULSE G600 II from Fujirebio in a healthy population. We enrolled 249 subjects (124 women, 125 men) normophosphaticemic, normocalcemic, with 25-OH vitamin D levels >30 ng/ml, and eGFR >60 ml/min/1.73m².

In addition, intact PTH (iPTH) levels were determined using a 2nd generation assay on Cobas e602 from Roche and a 3rd generation whole PTH assay from Fujirebio in 70 hemodialysis patients and classified according to the KDIGO recommendation which suggests that PTH should be maintained in the range of approximately two to nine times the upper normal limit for the assay.

Results
In the 249 healthy subjects, median PTH was 17 ng/ml without differences according to gender and age (younger or older than 60 y.o.). The normal PTH range in serum, defined with the 95% CI after elimination of the outliers according to the Horn estimation method was 8.7 – 31.5 pg/ml.

A good correlation (Lumipulse wPTH = 0.46 * Roche iPTH + 10; r = 0.99) was revealed between the wPTH and iPTH assays. Hemodialysis patients with a PTH concentration below 2-fold of the upper limit, within the KDIGO range, and above 9-fold of the upper limit are respectively 13% - 61% - 26% using Fujirebio’s 3rd generation assay and 10%, 57% and 33% using Roche’s 2nd generation assay (upper limit 50 pg/ml). There is no significant difference in the distribution between the two methods (X² p<0.05).

Conclusion
PTH values determined using the 3rd generation Lumipulse whole PTH assay are lower than the 2nd generation assays. The upper limit of the reference values was determined to be 31.5 pg/ml. However, despite changes in the absolute values of PTH, classification of mineral and bone disorders according to KDIGO definition is similar using 2nd and 3rd PTH assays.
THE EFFECTIVENESS OF VITAMIN D THERAPY IN MAINTENANCE HEMODIALYSIS PATIENTS: ITS IMPACT ON BONE HEALTH

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Background: Bone and mineral disorder (BMD) represents a frequent complication of chronic kidney disease (CKD), affecting most of hemodialysis (HD) patients. Bone-specific alkaline phosphatase (BALP) directly reflects osteoblastic activity, and is therefore considered the most reliable indicator of bone turnover. Current evidence suggests that vitamin D3 analogs represent an effective tool in CKD-BMD management, aiming to regulate secondary hyperparathyroidism. The aim of the present study was to evaluate the effect of vitamin D therapy on bone and mineral metabolism in HD patients and to examine the level of bone turnover depending on patients’ age and dialysis vintage.

Materials and methods: 71 HD patients (43 men and 28 women) were included in the study. The average hemodialysis vintage was 9 years and 5 months. Beckman-Coulter Access Ostase commercial immunoassay was used to measure BALP concentration in order to assess the level of bone turnover. Patients were divided into two groups regarding vitamin D therapy. 32 patients were subjected to vitamin D treatment, alone or in combination with phosphate binders, while 39 patients received only phosphate binders or no therapy at all.

Results: It was determined that dialysis vintage positively correlated with BALP concentration. Patients who have been on HD program longer than 30 months had markedly higher BALP levels compared to those with shorter dialysis vintage (p=0.001).

A significant association with BALP concentration was also observed for patients’ age. Participants aged 60 years or older had lower BALP levels than younger patients (p=0.01).

BALP levels were significantly higher in a group of patients treated with vitamin D analogs, compared to patients who received no vitamin D (p=0.001).

In addition to that, a significant difference was demonstrated regarding the type of vitamin D analogs consumed. Patients who received Paricalcitol showed significantly higher BALP values in comparison to patients treated with Rocaltrol (p=0.024).

Conclusions: Our results indicate the positive effect of vitamin D administration in HD patients, suggesting that vitamin D therapy may be important for bone and mineral metabolism’s preservation and the prevention of low turnover bone disease. Our results also imply Paricalcitol’s superior characteristics compared to Rocaltrol therapy.
URINARY CYSTATIN-C: A NEW AUTOMATED PARTICLE-ENHANCED IMMUNE TURBIDIMETRIC TEST FOR THE ROUTINE EVALUATION OF KIDNEY TUBULAR FUNCTION

Background: Cystatin-C is an inhibitor of cysteine proteases with a molecular mass of 13 kDa. Due to its physical properties it is freely filtrated through the glomeruli then reabsorbed and almost completely catabolized by the proximal tubular cells. It is a normal component of urinary proteins in very low concentration. Recent studies showed that elevated levels of urinary cystatin-C (u-CYSC) reliably indicates tubular dysfunction. Because commercial u-CYSC test is not available at present, we optimized and validated an automated serum immune turbidimetric test for u-CYSC measurements. Furthermore, our aim was to investigate u-CYSC levels in diseases which can cause acute or chronic kidney injury.

Methods: A particle-enhanced immune turbidimetric assay for serum CYSC (DiaSys Gmbh) was adapted for a Cobas 8000/c502 automated analyzer (Roche) to measure u-CYSC. Spot urine samples of healthy volunteers (n=84), individuals with chronic hypertonia (n=43) and septic patients with acute kidney injury (n=35) were analyzed. We expressed our data in u-CYSC/creatinine ratios (mg/mmol).

Results: The detection limit was determined to be 0.017 mg/L u-CYSC. The intra- and inter-assay imprecision expressed as CV% and also the inaccuracy were found to be less than 6%. Reference range for u-CYSC/creatinine ratio was established to be 0.007 (0.004-0.015) mg/mmol [median (2.5-97.5 percentiles)]. Compared to the control group, u-CYSC/creatinine ratios showed an approximately 44-fold elevation in sepsis-related acute kidney injury (p<0.001). The u-CYSC/creatinine values of the chronic hypertonia patient group did not differ significantly from those of controls.

Conclusions: We adapted a highly sensitive, precise and accurate turbidimetric assay for CYSC determination in urine. Our fully automated method is ideal for routine lab testing and our findings confirm that u-CYSC levels sensitively reflect the tubular damage in acute kidney injury.
ABSENCE OF ASSOCIATION BETWEEN S19W POLYMORPHISM OF THE APOLIPROTEIN APOA5 GENE AND TRIGLYCERIDE LEVELS IN DIABETIC NEPHROPATHY IN NORTH IRANIAN POPULATION

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Background: Diabetic nephropathy (DN), a severe complication of type 2 diabetic (T2D), is progressive and susceptibility to DN varies among T2D patients. Some single nucleotide polymorphism in apo A5 gene (ApoA5) revealed that is strongly associated with TG levels and proposed as a predisposing factor for DN. Regarding the high prevalence of T2D in Iran we analyzed the influence of c.56C>G (S19W, rs3135506) polymorphisms of ApoA5, on lipids indicators in population from north of Iran (Mazandaran province).

Materials and Methods: This study comprised patients with established nephropathy (DN+, n = 90), without nephropathy (DN-, n = 71) and controls (n = 58).

Genotyping of APOA5 S19W polymorphisms was performed by PCR–RFLP. Lipids and lipoproteins were assessed by enzymatic methods.

Results: The genotypes frequencies were 91.4 % CC, 8.6 % CG, 0 % GG in controls, 90.1 % CC, 9.8 % CG and 0 % GG in DN- and 95.6 % CC, 4.4 % CG in DN+ patients. The CC genotype was overrepresented among three groups. There is no significant difference in TG levels between diabetic patients and controls with regard to CC, CG or GG genotypes.

Conclusions: These results suggest that APOA5 - c.56C>G (S19W, rs3135506) polymorphisms does not affect lipid levels in T2D patients in our population.