PF03
MASS SPECTROMETRIC MEASUREMENT OF URINARY NETRIN-1 IN RENAL TRANSPLANTATION

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Background: Netrin-1 can be a useful early diagnostic biomarker of acute kidney injury (AKI) after renal transplantation. The use of netrin-1 in clinical practice requires that this biomarker be associated with an analytical method that combines specificity, accuracy and robustness. This study aimed to develop an optimized multiple reaction monitoring (MRM) method using ultrafast liquid chromatography coupled with tandem mass spectrometry to measure urinary netrin-1 levels in renal transplant recipients.

Materials and methods: Purified recombinant human netrin-1 tryptic standard was analyzed by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF) MS/MS and LC-MS/MS to select for peptides that provided specificity and adequate response in developing an MRM method for urinary netrin-1 quantification. Human urine samples collected from kidney transplant recipients were isolated, concentrated, precipitated and trypsin digested before mass spectrometric analysis of netrin-1. Netrin-1 levels were also measured in urine samples by enzyme immunoassay.

Results: The tryptic peptide ion $MH^{+2}$of ^{270}DSYFYAVSDLQVGGR^{284} (*m/z* 839) provided an adequate signal and was used for quantification of netrin-1 under conditions employed for LC-MS/MS analysis. MALDI-TOF MS/MS spectra obtained by collision-induced dissociation of the parent MH^{+2} ion ^{270}DSYFYAVSDLQVGGR^{284}resulted in*y*8, *y*9 and *y*11 product ions that were used for quantitative analysis by MRM method. Urinary Netrin-1 content measured by LC-MS/MS after transplantation was significantly higher compared to before transplantation levels. The Spearman correlation coefficient between the two methods was statistically significant. Intra-day and inter-day coefficient of variation provided good repeatability and reproducibility for validation of LC-MS/MS analysis.

Conclusions: LC-MS/MS quantification of Netrin-1 may provide a new reference method to determine changes of this potential biomarker in human kidney transplant patients.

PF04
ASSOCIATION OF METHYLENETETRAHYDROFOLATEREDUCTASE GENE POLIMORPHISMS C677T ON THE HOMOCYSTEINELEVELS IN CORONARY ARTHERYDIASEASE

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Background: The aims of this paper were to determine the concentration of the total homocysteine(tHcy), to find whether the increased level of tHcy is

associated with mutation of the C677T methylenetetrahydro- folatereductase gene (MTHFR). Also, we wanted to show whether the determination of the polymorphisms of this gene is particularly involved in the establishing of the diagnosis of coronary artery disease (CAD), and whether this genetic analysis is the choice for this most common disease.

Subjects and methods: The study included 84 subjects divided into two main groups: 43 healthy subjects as control group and 41 patients with CAD. The concentration of tHcy was determined by a cyclic enzymatic method, and the mutation of the MTHFR C677T gene was examined by a polymerase chain reaction.

Results: The concentration of tHcy plasma in the patients with CAD was significantly higher (18,72 ± 5,31 μmol / L) compared to the control group (11,11 ± 3,23 μmol / L) ($p < 0.001$). The statistical analysis with multiple regression showed that at the genotypes CT and TT of the MTHFR (C677T), the tHcy level is not significantly higher than the CC genotype of MTHFR (C677T) nor in the control group nor in the patients with CAD.

Conclusion: The analysis of the results showed that the polymorphism of the MTHFR (C677T) gene is not associated, in most cases, with the mild to moderate hyperhomocysteinemia.

PF05
MEAN PLATELET VOLUME VALUES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is characterized by inflammatory pathways that lead to proliferation of synovial cells in joints. Like many autoimmune diseases, the etiology of RA is multifactorial. platelet-derived markers of inflammation and thrombogenicity are least studied, and their association with other risk factors in RA still remains obscure. The aim of this study was to investigate the mean platelet volume (MPV) values in this disease.

Materials and methods: Whole blood samples were collected from 60 healthy control and 119 patients with rheumatoid arthritis. The mean age for controls and patients were 45±11 and 45±3 years, respectively. Patients with chronic disease and inflammatory disorders were excluded. MPV levels were calculated with Abbott Cell Dyne heamotolgy analyzer. Statistical analysis was performed with SPSS v15.

Results: The median of MPV values in patients rheumatoid arthritis [9.5 (5.39-12.5)] were significantly higher compared to control group [7.5 (5.91-11.3)] ($p<0.001$).

Conclusions: MPV is a component of the CBC test. Although clinical utility and validity of MPV have not been established yet, some authors argue its use in inflammatory disorders. According to this study's results, MPV values might present the inflammation and must be established in large scale patient populations.