P11 – Immunology

P11-01

Comparative analysis of turbidimetry and nephelometry methods for the measurement of immunoglobulin

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Background: The analysis of the protein content in urine samples is often required in the clinical practice. Nephelometry and turbidimetry are alternative methods, frequently used in the laboratory routine, and its choice depends on various factors including the cost, availability and characteristics of the laboratory equipments. A discussion is still ongoing, about the most suitable method, considering its sensibility and specificity, for the measurement of proteins in very low concentration levels, like monoclonal components in urine samples. The aim of this study is to compare the results of Immunoglobulin G (IGG), kappa (CLK) and lambda (CLLl) light chains concentration in urine samples, obtained by turbidimetry and nephelometry.

Materials and methods: Experimental and retrospective study of 24-hour urine random samples, from 51 patients of Pedro Hispano Hospital, collected for monoclonal protein excretion screening. IGG, CLK and CLL analysis were performed by turbidimetry (Abbott Diagnostics, Architect C 8000®) and by nephelometry (Beckman Coulter IMMAGE® equipment for IGG and Siemens Dade Behring BN II equipment for CLK and CLL). Statistic analysis was performed with SPSS (vs 18) software.

Results: Correlation values between results obtained by turbidimetric and nephelometric methods were 93,5%, 99,0%, 99,0% respectively for CLK, CLL and lGG, with p values 0.065 (CLK), 0.570 (CLL), 0.004 (IGG). Significance level was 0.05.

Conclusions: Our results suggest that the performance of both methods is similar for the three parameters under study. Therefore, turbidimetry, the method most commonly used in automated large scale laboratory routine models, like COREIabs, is suitable for this propose.

P11-02

Comparison of rheumatoid factor analysis by nephelometry (BN II) and turbidimetry (ADVIA 2400)

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Background: Detection of rheumatoid factor (RF) is a useful method in the differential diagnosis of rheumatic diseases, primarily used to help diagnose rheumatoid arthritis or Sjögren syndrome. Changes in serum RF levels may be used as indicators of disease activity, but they are also proving useful in monitoring responses to therapy.

Materials and methods: Levels of FR were analyzed in 75 serum samples in parallel by our usual immunonephelometric method in a BNII analyzer (Siemens) and a new immunoturbidimetric method in Advia2400 (Siemens). Data were statistically analyzed using the MedCalc statistical package by agreement kappa statistic, Spearman correlation and regression of Passing-Bablok. To categorize the results were considered negative values RF < 14 IU/mL in Advia2400 method and < 15.9 IU/mL in the nephelometric assay, and other results were considered positive.

Results: The observed agreement was 91% (expected by chance: 55%), yielding a kappa index of 0.795 (95%CI: 0.624-0.966). We calculated Spearman correlation for positive samples (N = 35) be-

tween the ratios (value/upper reference limit) of both methods: 0.810 (0.573-0.922) (P < 0.01). The Passing-Bablok regression equation was: Advia2400 = 3.56 + 1.09 x BnII (95% CI slope: 0.794-1.55; 95% CI intercept:-9.26-9.47), so no bias was evident.

Conclusion: According to the results, the strength of categorical agreement between both methods can be considered "strong" (Ladis-Koch criterion). That is, the results are qualitatively comparable. However, the results are not quantitatively interchangeable. The turbidimetric RF Advia2400 assay is a reasonable alternative to the nephelometric, as it shows a good correlation, besides being cheaper and faster.

P11-03

Evaluation of C3 and C4 immunoturbidimetric assays on ABBOTT Architect ci16200 analyzer

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Background: Our laboratory wished to consolidate C3 and C4 testing by converting from immunonephelometry to immunoturbidimetry. We evaluated analytical performance of Abbotts C3 and C4 immnoturbidimetric assays on Abbott Architect ci16200 chemistry/immunochemistry analyzer.

Materials and methods: Analytical evaluation included determination of within - run (N = 20), between - run (N = 20) imprecision, inaccuracy (N = 20) using control materials and method comparison on human samples (N = 90) comparing Architect ci16200 and Siemens BNProSpec immunonephelometer. For the sample comparison Passing-Bablok and Bland-Altman analyses were performed. For the evaluation of clinical significance, reference-change values (RCV) at two control levels were calculated.

Results and conclusions: Results obtained for within run imprecision: C3 (L1 0.8%, L2 0.8%), C4 (L1 0.8%, L2 0.8%), between run imprecision: C3 (L1 1.9%, L2 1.2%), C4 (L1 2.1%, L2 3.9%) and inaccuracy: C3 (L1 3.7%, L2 4.08%), C4 (L1 2.2%, L2 6.6 %), satisfied desirable specifications for I%, B% and TE% derived from biological variation. Regression equations with 95% CI obtained from Passing-Bablok analysis were as followed: C3 y = - 0.21 (-0.24-(-0.17)) + 1.7 (1.13 – 1.20)x, C4 y = 0.00 + 1.10 (1.13-1.20)x. Bland-Altman plot showed C3 values on average 2.2% lower (limits of agreement -9.7-5.2%) and C4 on average 9.6% higher (limits of agreement 5.1-14.1%) on Architect ci16200. Although Passing-Bablok regression showed statistically significant difference between methods, Bland-Altman plot showed differences clinically insignificant if compared to RCV values.

P11-04

Comparison of serum total IgE tests performed by four different assay systems

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Background: In vitro testing is commonly used to diagnose allergies and predict allergic tendency especially in pediatrics. Intermethod comparisons between four different commercial tests for serum total IgE had only been evaluated by limited studies. To determine whether IgE levels derived from different assays are precise and equivalent to those measured by ImmunoCAP.

Materials and methods: Precision was determined following Clinical and Laboratory Standards Institute EP5-A2 using patients' pooled se-

rum. Two levels materials were analyzed in duplicates at two separate times per day for 20 days in each four systems. We performed interassay comparisons using 132 Korean patients who visited one tertiary care hospital in Seoul. For each deidentified sample, total IgE levels were measured using four different assay systems(bioMérieux VIDAS, Siemens Immulite2000, Roche Cobas e 601, Phadia ImmunoCAP). Results were analyzed to determine whether IgE measurements were comparable.

Results: Four clinically used total serum IgE assays showed excellent precision performance, with coefficients of variation (CVs) below 9%. After 132 paired comparison test, Immulite2000 ($y = 1.02x - 1.0$, $R^2 = 0.9806$) and Cobas e 601 ($y = 1.14x+-0.94$, $R^2 = 0.9913$) showed good correlation with ImmunoCAP assay. Although some minor outliers were noted. VIDAS ($y = -4.92x + 0.91$, $R^2 = 0.9423$) showed significant deviation from linearity with ImmunoCAP by Passing – Bablok analysis.

Conclusions: The Immulite2000 and Cobas e601 total IgE were showed reliable performance and comparable result with ImmunoCAP assay. VIDAS showed underestimated trend in total IgE values. We should take into account the intermethod differences between those assays for clinical applications.

P11-05

Imunonephelometric quantification of monoclonal protein

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Introduction: Hevylite is a new test for imunonephelometric quantification of monoclonal protein. The reagent consists of polyclonal antibodies produced in sheep. Since the target of this antibodies are unique junctional epitopes between the heavy chain and light chain constant regions, antibodies can separately identify the different light chain types of each Ig class (IgG kappa, IgG lambda). Aim of the study was evaluation of new test for monoclonal protein (M protein) quantification.

Materials and methods: Study included 20 samples with already detected M protein. In all samples were measured total imunoglobulin A, G and M (Cobas 6000, Roche) and done capillary zone electophoresis (Capillarys2, Sebia) and immunofixation electrophoresis (Hydrasys, Sebia). After detection, M protein is quantified with The Binding Site test Hevylite on nephelometer Siemens BNII.

Results and conclusions: Monoclonal protein were detected even when immunoglobulins were in reference ranges. When detected M protein is IgA class, densitometric determinated gamma globulins can not be usefull in quantification of M protein. Numerical results of total immunoglobulin concentrations and M protein are not comparable. Ratio kappa/lambda may be a good indicator of clonality. Unquestionable is importance of capillary zone electroforesis in identifying monoclonal gammapathies as simple and inexpensive routine technique in clinical laboratory. But, sometimes when the M protein (expecially IgA) migrates in the beta reagion, with either transfferin or C3, only quantification of the M protein will provide adequate follow-up. Considering the price of reagent the test probably will not find use in screening for monoclonal protein but will be usefull in monitoring the disease course.

P11-07

YKL-40 correlates with pro-inflammatory cytokines in rheumatoid arthritis

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Background: The aetiology of rheumatoid arthritis remains unknown, although it is estimated that autoimmune mechanisms play a major role in the pathogenesis of the disease. YKL-40 is a candidate for a novel inflammatory marker. It is secreted by activated macrophages and neutrophils, synovial cells, arthritic chondrocytes and cancer cells. In this study we evaluated YKL-40 levels in serum and synovial fluid of patients with rheumatoid arthritis in comparison with the concentration of pro-inflammatory cytokines.

Materials and methods: We examined serum and synovial YKL-40, IL-1β and TNF-α levels in 37 rheumatoid arthritis patients, aged 53.14 ± 2.73. The concentrations of these markers were measured by ELISA.

Results and conclusions: The levels of YKL-40, IL-1β and TNF-α in patients were remarkably higher compared to the healthy group ($P < 0.01$). A significant correlation between serum and synovial YKL-40 levels and concentrations of IL-1β and TNF-α in patients with rheumatoid arthritis was observed ($P < 0.01$). These pro-inflammatory cytokines are involved in the pathogenesis of rheumatoid arthritis and are targets for the therapeutic treatment. It is shown that IL-1β and TNF-α could induce secretion of YKL-40 by chondrocytes. We determined a strong correlation between serum YKL-40 concentration and the conventional biochemical marker for assessment of disease activity - C-reactive protein (CRP) ($P = 0.004$; $r = 0.582$). Our data suggest potential involvement of YKL-40 in inflammation and disease activity of rheumatoid arthritis. Acknowledgments The study is supported by grants NO–1/2009 and NO–1/2010 from Medical University– Plovdiv.

P11-08

Epstein-Barr virus infection resembling autoimmune liver disease in 17-year-old girl – case report

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We report a case of a 17-year-old girl admitted to Department of Pediatric Gastroenterology and Hepatology for chronic intermittent diarrhea with significant weight loss. Biochemical liver lesion confirmed with imaging techniques was found characterized with cholangitic pattern. High value of fecal calprotectin and hypoalbuminemia suggested inflammatory bowel disease with liver lesion as an extraintestinal manifestation. Autoantibody profile corresponding to autoimmune hepatitis type I was found including positive antinuclear (ANA) and smooth muscle (SMA) antibodies. Antimitochondrial antibodies (AMA) were negative on immunofluorescence test but high reactivity was observed using line immunoassay with both native AMA-M2 antigen and recombinant fusion protein AMA-M2-3E. Infective serology on admission revealed recent primary Epstein-Barr virus (EBV) infection with viral capside antigen (VCA) IgM and IgG positive, EBV early antigen (EA) IgG negative and EBV nuclear antigen (EBNA) IgG positive. Primary suspicion of ulcerative colitis was rejected regarding the normal endoscopic and histopathological findings of ileocolonic mucosa and spontaneous resolution of clinical symptoms, biochemical and ultrasonographic abnormalities. Repeat testing after 3 months revealed persistently positive ANA and SMA but absence of reactivity with AMA antigens on line immunoassay. Serologic tests documented seroconversion further supporting the diagnosis of EBV primoinfection. Repeated PCR analysis was negative for EBV DNA.

EBV infection as trigger for autoimmune disease has been attributed to occurrence of cross-reactive antibodies, due to the mimicry of epitopes between host and EBV proteins. We presented an example of EBV primoinfection resembling autoimmune liver disease although follow up is suggested due to the persistently positive ANA and SMA.

P11-09

Concentration of IgE antibody in nasal lavage in allergic rhinitis and non-allergic rhinitis

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Background: Molecular and cellular inflammation mechanisms in nasal mucosa in allergic rhinitis (AR) and non-allergic rhinitis (NAR) have not yet been clarified. To contribute to their understanding and developing of differential diagnostics, concentration of total IgE was measured in nasal lavage in subjects with allergic rhinitis and non-allergic rhinitis. The objective of this study was to measure concentration of IgE antibody in nasal lavage and determine possible correlations.

Materials and methods: Study included 60 patients of both sexes aged 18-65. Patients were divided in two groups, according to diagnosis of allergic or non-allergic rhinitis (N = 30). Nasal lavage samples were taken by modified Nacleir method. Nasal cavity was flushed with 3 mL of saline solution (0.9% NaCl). The concentration of total IgE was measured by fluoro enzyme immunoassay (FEIA) on UniCAP 100 (Phadia AB, Uppsala, Sweden). The results were evaluated by non-parametric Mann-Whitney U-test. The values $P < 0.05$ were considered statistically significant.

Results: Results were expressed as median and interquartile range (Q1 and Q3). The concentration of total IgE was higher in patients with allergic rhinitis than in patients with non-allergic rhinitis: 40.60 kU/L (23.78-54.43) *vs*. 2.55 kU/L (2.05-5.80), respectively; $P < 0.001$.

Conclusions: The results showed significantly higher concentration of IgE antibody in nasal mucosa in patients with allergic rhinitis than in patients with non-allergic rhinitis. Local IgE-mediated inflammation plays important role in allergic rhinitis and measuring the concentration of local IgE antibodies in nasal lavage may contribute to diagnostics of allergic disease.

P11-10

Immunologic laboratory diagnostic tests for patients with chronic diarrhea

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Background: Patients with chronic diarrhea are difficult to diagnose and treat. Along with the conventional clinical and instrumental tools for elucidating the diagnosis there are contemporary immunologic examination for immunomediated intestinal diseases such as celiac disease (CeD), Crohn`s disease (CD) and ulcerative colitis (UC). The aim of the study was to evaluate clinical significance of antibodies against deamidated gliadin peptide (anti-DGP), antibodies against Saccharomyces cerevisiae (ASCA), fecal calprotectin (FC) and fecal lactoferrin (FL) as diagnostic tools for establishing the diagnosis of CeD, CD and UC.

Materials and methods: We examined 137 patients – 37 with CD, 58 with CeD, 42 with UC. Sera and stool samples were tested. We also tested 25 healthy persons as control groups. The sera samples were evaluated with ELISA for anti-DGP antibodies (IgG) and ASCA (IgA), and the stool samples for FC and FL with a rapid Card test, lateral flow assay with Quantum Blue reader and ELISA for FC.

Results: Anti-DGP antibodies were positive in 100% of patients with CeD and in no one of UC and CD. ASCA were positive in 14,8% of CD and 25,9% of UC patients. FC was positive in 83% of UC, 87% of CD and 37.5% of CeD patients. FL was positive in 52% of UC and 54% of CD patients.

Conclusion: We found that these new immunologic markers are promising in diagnosis: anti-DGP is highly specific for CeD, ASCA are not very sensitive and discriminative for CD and UC. FL and FC are typical for active neutrophil inflammation.

P11-11

Polymeric forms of free light chains: two case reports

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Background: Urinary Bence-Jones protein (BJP) refers to urinary excretion of monoclonal light chains. Light chains appear in the urine when the metabolizing capacity of nephron is exceeded. Negligible urinary BJP in light chain multiple myeloma patients due to polymeric forms have been described previously.

Materials and methods: Serum and 24-hour urine of two patients with multiple myeloma were studied. Capillary electrophoresis (Capillarys®) was used to detect and measure monoclonal protein (MP). Serum free light chains (FLC) were measured by immunonephelometry in an Immage 800 (Beckman Coulter®) analyzer. MP was identified by Immunofixation.

Results: Patient 1: A MP Lambda was detected in serum (13.2 g/L). Bence-Jones protein was identified and measured in urine (300 mg/24 hour). Serum κ and λ FLC were measured with a concentration of 10 mg/L and 18500 mg/L, respectively. Patient 2: Serum MP was detected. Immunofixation showed two different MPs, κ FLC and IgG κ. The concentration was 2.6 g/L and 9 g/L, respectively. No Bence-Jones proteinuria was detected. The concentration of κ and λ FLC was 9060 mg/L and 7 mg/L, respectively. Both patients had a normal renal function.

Conclusions: The presence of serum MP of FLC with absence or low levels of urinary BJP suggests a polymeric form of the MP with a large molecular size that prevents normal excretion by kidney. The difference between serum MP and FLC could be due to overestimation in the nephelometric measurement by the presence of polymeric forms of FLC that may have produced a more intense immunoprecipitation reaction.

P11-12

Eosinophilic cationic protein (ECP) and total IgE concentration in children with atopic diseases

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Background: Eosinophilic cationic protein (ECP) is a basic protein located in the granules of eosinophil granulocytes and released during eosinophil activation. ECP serum levels are associated with the intensity of allergic inflammation. Elevated total IgE concentration is common to all atopic diseases. The aim of the study was to compare ECP and total IgE concentration in sera of patients with asthma, allergic rhinitis and atopic dermatitis, their values with values obtained from healthy children

and to determine the relationship between these analytes in the mentioned groups of patients.

Materials and methods:The study included 43 children with asthma, 20 with allergic rhinitis, 20 with atopic dermatitis and 30 healthy children as controls. ECP and total IgE serum values were determined using an automated fluorescence enzyme immunoassay (FEIA) on an UniCAP R 100 immunoanalyzer.

Results: Although the ECP concentration median was highest in the group of asthmatic patients (15.3 µg/L) and lowest in the group of atopic dermatitis patients (10.4 µg/L), there was no significant difference among the groups of patients, while total IgE concentration was higher in the groups of patients with asthma and allergic rhinitis ($P < 0.001$). ECP and total IgE concentrations were significantly higher in patients with asthma ($P < 0.001$ for both) and allergic rhinitis ($P = 0.018$; $P < 0.001$) compared to controls. Weak positive correlation between these analytes was found in asthmatic patients ($r = 0.478$, $P = 0.001$).

Conclusion: The results indicate that ECP values can be used as a marker of inflammation in asthmatic patients and those with allergic rhinitis. ECP and total IgE concentrations were weakly correlated only in asthmatic patients.

P12 – Kidney diseases

P12-01

The performance of compensated serum creatinine in pediatric samples

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Background: According to current recommendation serum creatinine measurement in adults should be performed by compensated Jaffe method. The enzymatic method is recommended for pediatric population but high commercial price is limiting factor for its implementation in routine practice. We estimated proportion of compensated serum creatinine concentration below measuring range in children; following to method comparison analysis of compensate Jaffe *vs*. enzymatic creatinine method.

Materials and methods: A total of 58 pediatric serum samples were included in the study (median age 44 months, age range 2 days-18 years). The measurement of creatinine by enzymatic method (measuring range 0-2700 µmol/L) was done on the Cobas c311 analyzer (Roche Diagnostic) and by compensated Jaffe method (measuring range 18-2200 µmol/L) on the Olympus AU400 analyzer (Beckman Coulter).

Results: In 36 out of 58 samples (group 1, median age 66 months, age range 2 days-18 years) concentrations of compensated creatinine were in measuring range (median 34 µmol/L, range 18-195 µmol/L) but it was not a case in 22 out of 58 samples (group 2, median age 12 months, age range 15 days-6 years) with compensated creatinine concentrations below measuring range. There was a significant difference between two groups regarding age (t-test, $P < 0.001$). The Passing and Bablok regression analysis showed ($N = 36$) intercept -4.42 (95%CI 6.03 to -3.00), slope 1.02 (95%CI 1.00 to 1.06); $r = 0.99$; range tested 18-195 µmol/L.

Conclusion: A high proportion of creatinine concentrations under measuring range was unacceptable, concerning younger children. Method comparison analysis revealed underestimation of pediatric serum creatinine by compensated Jaffe method.