Autoimmune disease

Cod: 0079

LEU10PRO (C.869T>C) AND ARG25PRO (C.915G>C) POLYMORPHISMS OF TRANSFORMING GROWTH FACTOR β1 GENE IN HASHIMOTO'S THYROIDITIS

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BACKGROUND: Autoimmune thyroiditis, known also as chronic lymphocytic thyroiditis or Hashimoto's thyroiditis (HT), is the most common organ-specific autoimmune disorder affecting approximately 18% of overall population. The etiopathogenesis of HT has not been clearly elucidated, although the role of chronic inflammation, endothelial dysfunction and imbalance between pro- and anti-inflammatory cytokines has been established. Transforming growth factor β1 (TGFβ1) is required to maintain immune homeostasis, and is implicated in lymphocyte infiltration, production of autoantibody in the thyroid gland and thyrocyte destruction seen in patients with HT. The aim of the present study was to investigate the possible association of Leu10Pro (c.869T>C) and Arg25Pro (c.915G>C) single nucleotide polymorphisms (SNPs) of TGFβ1 gene with the occurence of HT.

METHODS: We analyzed the genotype and allele frequencies of polymorphisms at codon 10 and 25 in 110 patients with established HT diagnosis and 197 healthy controls using PCR-restriction fragment length polymorphism (RFLP) technique.

RESULTS: With regard to TGFβ1 Leu10Pro (c.869T>C) polymorphism, the frequency of C allele was increased in HT patients accoding to controls (p= 0.018, OR=1.5, 95% CI= 0.07-2.11), and combined CT+CC genotypes was associated with 2.9-fold increased disease risk (p= 0.00012, 95% CI= 1.67-5.04). Regarding Arg25Pro (c.915G>C) polymorphism, there was a significant increase of C allele frequency in patients with HT compared to healthy controls (p= 0.013, OR= 1.81, 95% CI= 1.13-2.89). In addition, C allele carying subjects (CG + CC) had 2.23-fold increased risk for developing HT according to GG homozygotes (p= 0.003, 95% CI= 1.32-2.76). No association between polymorphisms and HT phenotypes were observed.

CONCLUSIONS: We suggest that the Leu10Pro and Arg25Pro polymorphisms of TGFβ1 gene may be related to occurrence of HT. However, more studies with larger sample size including other loci of the TGFβ1 gene are necessary to support our findings before any statement can be made about the relationship between HT and TGFβ1 gene polymorphisms.

Autoimmune disease

Cod: 0080

THE ASSOCIATION OF RHEUMATOID ARTHRITIS ACTIVITY WITH LIPID LEVELS AND INSULIN RESISTANCE

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BACKGROUND: Rheumatoid arthritis (RA) is a chronic inflammatory disorder that affects small joints in hands and feet. Although rheumatoid arthritis can occur at any age, it usually begins after age 40. The disorder is much more common in women. It has been recognized that inflammatory conditions, such as RA lead to increased cardiovascular (CV) events and diabetes mellitus. The aim of this study is to examine the relationship of RA activity with lipid levels and insulin resistance (IR).

METHODS: Twenty five patients (22 women and 3 men) were included in pilot-study. RA disease activity was assessed by the Disease Activity Score28 (DAS28). Low or moderate disease activity was defined as DAS28<5.1, and high as DAS28 $\geq$ 5.1. Total, HDL-, LDL- cholesterol, triglycerides, glucose and insulin serum levels were determined by commercial assays. Insulin resistance was calculated with homeostasis model assessment (HOMA) index.

RESULTS: The patients were divided into the two groups: high DAS28 group (DAS28 $\geq$ 5.1) (16 patients) and low/moderate DAS28 group (DAS28<5.1) (9 patients). The two groups were similar in body mass index. There was significant difference ($p<0.05$) between two RA activity groups in HDL-cholesterol concentrations. Patients in high RA activity group had lower HDL-cholesterol level compared with low/medium RA activity group (1.35 mmol/L vs 1.68 mmol/L) There was no significant difference in total- and LDL cholesterol, triglyceride, glucose and insulin levels. The patients group with high RA activity had higher HOMA index compared with low/medium RA activity group (3.57 vs 2.28), but the difference was not statistically significant. Significant association was found between DAS28 index and HDL-cholesterol level ($\rho=-0.40$, $p=0.04$), between IR and glucose ($r=0.66$, $p<0.001$). There was also negative correlation between IR and HDL-cholesterol ($r=0.36$, $p=0.07$).

CONCLUSIONS: In RA patients, HDL-cholesterol level significantly correlated with disease activity. The association of IR and RA activity was identified. Based on these results we can hypothesize that impaired insulin sensitivity and lower HDL-cholesterol levels in high RA activity patients can lead to increased cardiovascular events and diabetes mellitus type 2.

Autoimmune disease

Cod: 0081

DEVELOPMENT OF A KIT FOR RAPID DIAGNOSIS OF AUTOIMMUNE DISEASES

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BACKGROUND: More than hundred human diseases are classified as autoimmune and approximately 5% of the population worldwide suffers from a certain autoimmune disorder. Due to the high degree of morbidity and disability, the social and economic impact of this kind of human diseases is significant, even comparable with that of cancer and cardiac diseases. Therefore, the precise and early detection of specific autoimmune response is of first importance for adequate diagnosis and treatment of the patient, pain relief and prevention of pathogenic complications.

METHODS: Several biomarkers, specific for rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, diabetes, myasthenia gravis, Hashimoto thyroiditis and celiac disease were chosen for detection by combined enzyme-linked immunosorbent assay (ELISA). Purified autoantigens were coated on ELISA plates in different concentrations in order to determine the optimal conditions for detection of autoantibodies in serum samples of patients. Different positive control autoantibody concentrations were tested, as well as different chromogenes for detection.

RESULTS: Our team has developed a new kit for rapid diagnosis of autoimmune diseases, which provides simultaneous detection of several biomarkers, characteristic for different autoimmune conditions. By testing serum samples from healthy individuals and patients with certain autoimmune disorder we were able to determine 100% specificity of the diagnostic assay.

CONCLUSIONS: A new kit for rapid diagnosis of rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, myasthenia gravis, diabetes, Hashimoto thyroiditis and celiac disease was elaborated. The kit also allows risk assessment for development of rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, diabetes, myasthenia gravis, Hashimoto thyroiditis or celiac disease in diagnosed patients with other autoimmune disorder, genetically predisposed individuals or family members of patients with autoimmune pathology.

Autoimmune disease

Cod: 0082

INFLUENCE OF RENAL INSUFFICIENCY ON PHARMACOKINETICS OF METHOTREXATE

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BACKGROUND: Methotrexate (MTX), in low dose is one of the most frequently used antirheumatic drugs in patients with rheumatoid arthritis (RA), because of its benefit and risk profile. Glomerular filtration is the dominant pathway of MTX elimination. Our study want to determine the effects of impaired renal function on the pharmacokinetics of MTX in RA patients and possible hepatotoxicity.

METHODS: 38 RA patients were included in this study. MTX was administered intramuscularly (7.5-15mg). Subjects were divided into three groups, according to their creatinine clearance (CLCR); group 1: CLCR lower than 45 ml/min; group 2: CLCR between 45 and 80 ml/min and group 3: CLCR higher than 80 ml/min. Blood samples were collected from each subjects, 2, 12 and 24 hours after drug administration. We determined concentrations of MTX and transaminase liver enzymes.

RESULTS: MTX concentrations were 1.2 to 1.5-times higher in group 1 than in groups 2 and 3. Total MTX t1/2 eliminations were 23h in group 1, 12.8 hours in group 2 and 10.5 hours in group 3. Linear regression revealed good correlations between clearance values of MTX and creatinine clearance. Elevated ALT/AST levels occurred in 30% patients, 12 hours after MTX therapy in group 1, 10% and 7% of patients in group 2 and 3. Highest level of ALT is 96 IU/L, AST 62 IU/L.

CONCLUSIONS: Eliminations half life was significantly increased and total clearance was significantly reduced with the degree of renal impairment. Longer elimination half life induced increased chance of liver dysfunction.

Autoimmune disease

Cod: 0083

CLINICAL USEFULNESS OF FECAL CALPROTECTIN AS A BIOMARKER FOR THE DIAGNOSIS OF INFLAMMATORY BOWEL DISEASE

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BACKGROUND: The diagnosis of inflammatory bowel disease (IBD) is based on radiological, endoscopic, and histological examination, along with clinical examination and various serological markers. Although gastrointestinal endoscopy and histological examination of biopsy specimens are considered the gold standard for diagnosing IBD and defining subtypes, several studies have shown that quantitation of fecal calprotectin (fCal) may assist in diagnosing and monitoring IBD.

METHODS: Since cut-off values and factors that affect fCal levels are not clear, we evaluated a reference range and cut-off value for fCal. Fifteen IBD patients (8 with active inflammation and 7 in remission) and 370 controls were enrolled for determination of fCal levels. All control patients underwent duodenoscopy and colonoscopy for the screening of abnormal gastrointestinal lesions. The fCal tests were performed using ELiATM Calprotectin (Phadia GmbH, Germany) according to the manufacturer's instructions.

RESULTS: The median (interquatile range) fCal levels of the IBD group in remission, the IBD group with active inflammation, the total IBD group, the group which were normal by endoscopy, and the total health screening group were 25.0 (19.5 – 29.8), 711.5 (470.0 – 1356.0), 310.0 (26.0 – 721.8), 0.0 (0.0 – 19.0), and 16.6 (0.0 – 62.0) μg/g, respectively. The fCal level of the 95th percentile of normal in the endoscopy group was 55.0 μg/g, which was similar to the cut-off value of the manufacturer. The fCal levels measured in the IBD group with active inflammation were significantly higher than in the IBD group in remission (P=0.001), the all-normal group (P<0.001), and the total health screening group (P<0.001). The receiver operating characteristic area of the fCal levels to predict IBD with inflammation was 0.979 (95% CI 0.959 to 991; P<0.001) with a cut-off of 309.8 μg/g (sensitivity, 100.0%; specificity, 94.4%).

CONCLUSIONS: This study provides evidence for the use of fCal testing in the diagnosis and monitoring of IBD patients. Determinations of fCal values in variable sub-groups will enable understanding of the significance of the fCal value.

Autoimmune disease

Cod: 0084

SERUM CALPROTECTIN LEVEL FOR DIAGNOSIS AND DETECTION OF DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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BACKGROUND: Early identification of RA and timely detection of progression are truly global challenges facing the RA community. However, lack of sensitivity and imprecision of the currently available biomarkers have impaired the ability to implement potentially effective therapies in a timely manner. Aim of this study: is to evaluate the clinical utility of serum calprotectin, a calcium-binding protein secreted predominantly by neutrophils and monocytes at inflamed joints; in diagnosis as well as assessment of rheumatoid arthritis activity.

METHODS: Serum calprotectin level was measured in 60 adult patients with RA and 20 apparently healthy age- and sex-matched subjects serving as a control group. Furthermore, CBC, RF, ESR, CRP were done for the patient group.

RESULTS: Serum calprotectin showed a highly significant elevation in patients with RA when compared with the healthy control group. Moreover its level showed a highly significant increase during disease activity. Also a highly significant positive correlation was found between serum calprotectin and ESR, WBCs, platelets. In addition, a significant positive correlation was found between serum calprotectin and C-reactive protein as well as significant negative correlation between serum calprotectin and Hemoglobin. Meanwhile, a non-significant correlation was recorded with RF. By ROC curve analysis, Serum Calprotectin at a cut- off level of 450 ng /mL, has 75% sensitivity & 90% specificity for diagnosis of RA. The optimum cut-off level of the marker for prediction of disease activity was 950 ng /mL with 80% sensitivity, 76% specificity.

CONCLUSIONS: Serum calprotectin is a promising marker in the diagnosis of rheumatoid arthritis, and can be used to predict disease activity.

Autoimmune disease

Cod: 0085

A NOVEL CYTOBEAD IMMUNOASSAY FOR SIMULTANEOUS DETECTION OF CELIAC-DISEASE SPECIFIC ANTIBODIES AND TOTAL IGA

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BACKGROUND: The novel CytoBead CeliAK assay allows the simultaneous analysis of endomysial IgA-antibodies (EmA), anti-tissue transglutaminase enzyme (tTG)- and anti-deamidated gliadin peptide (DGP) IgA-antibodies as well as the determination of total IgA in one reaction environment.

METHODS: The assay runs on glass slides with tripartite wells, left: two different microbead populations (MP) coated with tTG or DGP, middle: monkey esophagus, and right: MP coated with anti-IgA. The assay was interpreted visually by conventional fluorescence microscope and by the digital imaging platform AKLIDES®. Overall, sera of 377 patients and controls (155 celiac disease (CD) patients, 5 IgA-deficient patients, 127 patients with other diseases, 90 blood donors) were run.

RESULTS: By visual evaluation, positivity of anti-tTG, anti-DGP, EmA, and total IgA in the CD patient group was 99.4%, 91%, 98%, and 100%, respectively. Altogether, each CD patient serum exhibited at least one positive result for anti-tTG, anti-DGP or EmA resulting in diagnostic sensitivity of 100%. All IgA-deficient patients were classified correctly as IgA negative. The diagnostic specificity using blood donors as control group for anti-tTG, anti-DGP, and EmA was 100%, 93.3%, and 100%, respectively. By automated evaluation with AKLIDES, the diagnostic sensitivity for anti-tTG and anti-DGP was 97.4% and 88.3%, respectively and the diagnostic specificity within the blood donor group 100% and 97.8%, respectively. Routine anti-tTG ELISA and anti-DGP ELISA showed a diagnostic sensitivity of 99.4% and 77.9%, respectively, whereas the diagnostic specificity using the blood donor group was 97.7% and 95.5%, respectively.

CONCLUSIONS: Thus, the novel assay provides the unique opportunity to detect CD-specific antibodies and exclude IgA-deficiency simultaneously and shows excellent diagnostic sensitivity and specificity.

Autoimmune disease

Cod: 0086

FLOW CYTOMETRIC IMMUNOBEAD ARRAY TO DETECT PLASMA AUTOANTIBODIES AGAINST PLATELET GLYCOPROTEINS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA

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BACKGROUND: Autoantibodies against platelet glycoproteins (GPs) play an important role in immune thrombocytopenic purpura (ITP). This study aims to develop a flow cytometric immunobead array (FCIA) assay to detect platelet autoantibodies commonly present in plasma of ITP patients.

METHODS: Plasma samples were collected from 71 ITP patients and 136 non-ITP controls and incubated with polystyrene microbeads coated with antibodies against human platelet GPs IX (SZ1), Ib (SZ2), IIIa (SZ21), GPIIb (SZ22), and P-selectin (SZ51). Flowcytometric analysis was performed to detect the platelet antigen- autoantibody complexes using a FITC-labeled antibody. Area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate diagnostic accuracy. The results were analyzed compared to that of a monoclonal antibody immobilization of platelet antigen (MAIPA) assay.

RESULTS: Autoantibodies against GPIb, GPIIb, GPIIIa, GPIX and P-selectin were detected in plasma of ITP patients, as indicated by higher mean fluorescent intensity(MFI) values when microbeads with antibodies SZ1, SZ2, SZ21, SZ22, and SZ51 were used. The ITP group were distinguished from non-ITP and healthy control group by MFI values (SZ1: 3.38 ± 2.26 vs. 2.01 ± 1.87 or 1.91 ± 1.12, P<0.01; SZ2: 4.23 ± 1.30 vs. 2.35 ± 2.10 or 2.33 ± 1.51, P<0.01; SZ21: 2.51 ± 0.94 vs. 1.92 ± 1.47 or 1.69 ± 1.39, P<0.01; SZ22: 2.28 ± 0.84 vs. 1.63 ± 1.05 or 1.49 ± 0.17, P<0.01; and SZ51: 3.04 ± 1.98 vs. 1.16 ± 0.61 or 1.48 ± 1.13, p <0.01). In ROC analysis, with a cut-off value of 3.75, 4.59, 2.91, 2.41, and 2.13, respectively, AUC values were 0.738, 0.828, 0.799, 0.794 and 0.867 respectively. Compared with the previously reported assays, this new FCIA eliminated the need of isolating platelets from ITP patients without compromising assay sensitivity and accuracy in predicting ITP.

CONCLUSIONS: This simplified FICA assay with multiple antibodies against platelet GPs may be more suitable for ITP diagnosis in clinical laboratory settings.

Autoimmune disease

Cod: 0087

LIPID PROFILES AND ATHEROGENIC LIPOPROTEINS IN EARLY RHEUMATOID ARTHRITIS

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BACKGROUND: As compared to the general population, risk of cardiovascular mortality and morbidity are raised two folds in rheumatoid arthritis (RA), be due to traditional risk factors such as dyslipidemia which is thought to be present before clinical onset of RA. The present study investigates the lipid profiles and atherogenic lipoproteins in early RA patients.

METHODS: Forty patients of recently diagnosed RA as per American College of Rheumatology diagnostic criteria 2010, with disease duration of < 6 months and no prior treatment were recruited. Plasma lipids was estimated by conventional methods using Friedwald's formula, Apolipoproteins and Lp(a) by immunoturbidometry, and small dense Low density lipoprotein (sdLDL) (by subtracting HDL-C from the Cholestrol value obtained in the supernatant after precipitation with Heparin/$MnCl_2$) were measured in patients and compared with thirty age and sex matched controls.

RESULTS: The patient group comprised of 33 females and 7 males, ages ranging from 18 years to 51 years, with 27 being seropositive. Total cholesterol values > 175 mg/dl was observed in 35%, with mean value of 138.8±34.9 mg/dl vs 123.4 ± 14.2mg/dl in controls ($p<0.00023$), HDL cholesterol values 42.1±12.2mg/dl vs 50.3 ± 3.22 mg/dl ($p<0.0003$), Triglycerides 136.05 ± 68.08 mg/dl, 32.5% with values >150 mg/dl and LDL-C was in the normal range, 101.0 mg/dl ± 32.8. Lp(a) was increased in 70% of patients, Tg/HDL ratio in patients was 3.50 ± 2.22 vs 3.18 ± 0.778 in controls, ApoB/Apo A1 ratio was 1.03± 0.35 vs 0.87± 0.21 ($p< 0.05$). The mean sdLDL-C level in patients was 61.07± 24.23 mg/dl vs 32± 10 mg/dl which was elevated.

CONCLUSIONS: The presence of increased proatherogenic to antiatherogenic particles as evidenced by the low HDL-C, elevated Lp(a), increased ApoB/ApoA1 ratio and sdLDL particles in early RA patients, perhaps accelerate the atherogenic process. Whether the treatment modalities for RA, modulate the atherogenic factors needs further study.

Autoimmune disease

Cod: 0088

AUTOIMMUNITY: INSULIN RESISTANCE IN THYROID HYPOFUNCTION

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BACKGROUND: Thyroid hormones are involved in metabolic regulations which are altered in thyroid hypo-function. The present study was designed (1) to find out occurrence of insulin resistance in hypothyroid patients and (2) to compare insulin resistance in sub- clinical and overt thyroid hypo-function.

METHODS: One hundred eighteen patients with the diagnosis of hypothyroidism based on their clinical and thyroid function test profile were included in this cross sectional hospital based descriptive study with their informed consent. HOMA-IR as an index of insulin resistance was calculated for each subject from their fasting plasma glucose and serum insulin levels. Autoimmunity against thyroid was evaluated by estimating anti TPO antibodies.

RESULTS: HOMA-IR as an index of insulin resistance was comparable in overt (5.8±3.24) and subclinical hypothyroidism (6.27±3.87) but was above the reference range for this population. Hypothyroid anti TPO positive cases has high TSH compared to negative cases in both overt hypothyroidism and subclinical hypothyroidism.

CONCLUSIONS: Hypothyroidism induces insulin resistance but the degree of insulin resistance is not dependent on severity of thyroid hypo-function however is associated with autoimmunity against thyroid.

Autoimmune disease

Cod: 0089

FREE LIGHT CHAINS: ACTIVITY MARKER IN CELIAC DISEASE?

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BACKGROUND: Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals. In children with signs or symptoms of CD and tissue transglutaminase type 2 (TG2) antibodies with levels >10 times upper limit of normal,the likelihood for villous atrophy is high, this positivity should be verified by endomysial antibody IgA (EMA) and HLA to make the diagnosis of CD without biopsies. Diseases associated with increased B-cell activation have high concentrations of free light chains (FLC). In patients in whom no intestinal biopsy is needed to diagnose CD the existence of a new marker could provide information about disease activity and mucosal recovery after gluten-free diet. Our proposal is to assess the usefulness of FLC summation κ and $\lambda(\Sigma\kappa+\lambda)$ in patients diagnosed with CD compared with the results obtained in a control group without disease and matched ages,and potential use as a marker of disease activity.

METHODS: 76 patients attending the Gastroenterology Consultation with suspected CD:38 patients were diagnosed with CD (24 females, 14 males aged 1 to 13 years): CD group and 38 patients (26 women and 12 men) age-matched group CD without disease:control group. Underwent routine biochemistry, serological markers of CD:TG2 and EMA, FLC κ and λ, The Binding Site, and genetic study. Mann Whitney test.

RESULTS: Serological markers of CD were remarkably positive in CD group.Median $\Sigma\kappa+\lambda$ CD group vs control group was significantly different 31,8 mg/L vs 17,14 mg/L, $p < 0.0001$. Three months after gluten free diet a second control was performed in 4 patients CD group, a significant difference in the concentration of $\Sigma\kappa+\lambda$ respect to concentrations at diagnosis was observed.

CONCLUSIONS: - The differences found in the $\Sigma\kappa+\lambda$ between CD and control groups could be related to the alteration of the intestinal mucosa
- The control performed in 4 patients 3 months after gluten free diet reflects the sharp decline in the $\Sigma\kappa+\lambda$, parallel to the other serological markers but the normalization of serological markers do not always match the normalization of the mucosa
- Determining FLC may potentially be used as a marker, which might reflect clinical recovery of the patient after gluten free diet

Autoimmune disease

Cod: 0090

ANTIBODIES TO PARIETAL CELLS: CORRELATION WITH VITAMIN B12 LEVELS IN THE CONTEXT OF ANEMIA INVESTIGATION

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BACKGROUND: Gastric parietal cell autoantibodies (PCA) represent a serum biomarker for autoimmune (type A) gastritis, a silent and highy prevalent disease which can progress, after 20-30 years, in pernicious anemia. The aim of this study was to investigate the correlation of PCAs and vitamin B12 levels in the context of anemia investigation.

METHODS: In this retrospective study, which was conducted in the University Hospital of Ioannina, data were collected from the Immunology Unit of Microbiology Department, Biochemistry Department and Hematology Laboratory. During three years period (2011-2013), a total of 239 PCA positive patients were included in the study (152 female, 87 male; mean age: 59.52±17.485 years). Patients were found PCA positive using an indirect immunofluorescence assay (NOVA Lite ANA plus, INOVA Diagnostics, San Diego, CA). Additionally, serum levels of vitamin B12 were measured by chemoluminescence immunoassay (Unicel DXI 800, Beckman Coulter) and red blood cell analytic parameters such as Hematocrit (Ht), Hemoglobin concentration (Hb) and Mean Corpuscular Volume (MCV) were also studied (Sysmex XE-500 counter).

RESULTS: An increased prevalence of PCA positivity was observed in women with a median age of 60 years, a finding which is in accordance with literature. Of the 239 PCA positive patients, 10 were found with low vitamin B12 serum levels (4.18%), 68 with low Ht values (28.45%), 64 with low Hb values (26.77%) and 27 with increased MCV (11.29%). Our findings do not support any correlation of PCA positive samples with low B12 serum levels (p 0.884), low Ht (p 0.379), low Hb (p 0.266) or increased MCV (p 0.36).

CONCLUSIONS: Although parietal cell antibodies (PCAs) are diagnostic for atrophic gastritis, they have not been proven to be a prognostic marker for pernicious anemia. However, according to literature, in patients with positive PCAs and atrophic gastritis, factors such as genetic predisposition, T-lymphocytes and alimentation, probably contribute to progression to pernicious anemia.

Autoimmune disease

Cod: 0091

THE REQUEST OF ANTI-DSDNA TEST SHOULD DEPEND ON RESULT OF ANTI-NUCLEAR ANTIBODIES

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BACKGROUND: The presence of anti-nuclear antibodies (ANA) and anti-dsDNA are both autonomous diagnostic criterions for systemic lupus erythematosus (SLE). They are both performed irrespectively of the result of the other criterion. However, anti- dsDNA has its own specific ANA pattern described as homogenous pattern.
Aim. We aimed to study whether we could miss any anti-dsDNA positive results when we always test for ANA first and then proceed with anti-dsDNA only in homogenous ANA positive cases.

METHODS: We studied 294 samples tested for ANA by IIF (HEp 20-10 and primate liver, EUROIMMUN, Germany) and for anti-dsDNA (anti-dsDNA-NcX ELISA, EUROIMMUN, Germany). We comprared ANA patterns with anti-dsDNA results.

RESULTS: ANA was negative in 57 and positive in 237 cases. From these 237 ANA positives, homogenous pattern was found in 168 and non-homogenous pattern in 69 cases. From 57 ANA negatives, anti-dsDNA was negative in 56 cases, the only exception exceeded slightly the cut-off value. Anti-dsDNA was positive in 35% (57/168) ANA positive homogenous cases and 16% (11/69) non-homogenous cases. SLE was diagnosed accordingly in 27/57 and 3/11.

CONCLUSIONS: It is justified to detect ANA first and not to proceed with anti-dsDNA testing in ANA negative cases. Samples from parients with suspected SLE and positive ANA need additional anti-dsDNA testing, in spite of ANA pattern.

Autoimmune disease

Cod: 0092

ROLE OF ETANERCEPT IN THE TREATMENT OF OSTEOPOROSIS FOR RHEUMATOID ARTHRITIS AND SPONDYLOARTHROPATHY PATIENTS

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BACKGROUND: TNFa is a cytochine which take part in the immune and inflammatory responses. It plays an important role in the rheumatoid arthritis and spondyloarthropathy. Increased concentrations of the same are found in art and pac with AR and SPA. TNFa touches bone metabolism stimulating osteo-classic development and activity. Anti TNFa (etanercept) are connected to them and neutralize the biological activity of this inflammatory cytochine. Etanercept (Enbrel) is a protein created by the fusion of a chain of TNFp75 receptor with Fc protion of the humane immunoglobulin IgG1, receptor's part is connected to TNF alfa extracellular molecules neutralizing it, while Fc part serves for the prolongation of 1/2nd plasmatic life of the agent. It is indicated in the treatment of AR and SPA patients.

METHODS: For all the patients under study the bone density was evaluated using T-score measured with DXA in the lumbar region initially, in the 24th and 48th week of the treatment with Etanercept. At the same time for the patients was measured the level of seric calcium, phosphor, and ALP, 25(OH) vitD3, TSH and PTH. All patients were treated with Etanercept(Enbrel) 50mg/sc 1xweek.

RESULTS: The average value of T-score measured by the beginning of the treatment resulted -3.2 ± 0.7. In the 24th week of the treatment, the average value of T-score for the group under study, resulted -2.5 ± 0.5 and in the 48th week of the treatment the average value of T-score resulted -1.5 ± 0.3.

CONCLUSIONS: Etanercept is presented efficient in osteoporosis treatment for AR and SPA patients. It showed an increase of bone mineral density lowering the risk for pathological fractures. It offers a new opportunity in the osteoporosis treatment for the AR and SPA patients who present weak tolerance for standard treatments of osteoporosis.

Autoimmune disease

Cod: 0093

COMPARISON OF ANTINUCLEAR ANTIBODIES DETECTION ON THE AUTOMATED NOVA VIEW® IMAGE ANALYSIS SYSTEM TO CONVENTIONAL FLUORESCENT MICROSCOPY

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BACKGROUND: Although various alternative methods for anti-nuclear antibodies (ANA) screening have been developed so far, indirect immunofluorescence (IIF) on HEp-2 cells is regarded as the gold standard method. However, disadvantages of the conventional IIF testing of ANA, including lack of standardization and subjectivity of interpretation, still remains a problem. Introduction of automatic slide preparation and digital reading could eliminate such problems and enhance overall diagnostic performance of the ANA IIF testing.

METHODS: ANA HEp-2 test was performed on 141 clinically defined human sera using two different reagents (Zeus and INOVA). Results of digital image reading on NOVA View on INOVA reagent were compared to those of conventional fluorescent microscopic reading on Zeus reagent. Agreement of negative/positive interpretation and pattern recognition between two testing platforms were compared. Accuracy of automatic titration by NOVA View was assessed by comparing manual finial dilution titer. We also compared the NOVA View output to the visual human interpretation of the same archived digital image by the NOVA View.

RESULTS: Concordance rate for positive/negative interpretation between two methods was comparable (92.9%, 131/141). Ten samples with discordant results were all negative by human interpretation (Zeus), but showed positive interpretation with low titer by NOVA View reading (INOVA). Agreement for ANA patterns was 77.0% (47/61), showing majority of discordant samples included low ANA titer. Agreement within one titer difference between automatic titration and manual dilution was 75.4% (46/61). Agreement for positive/negative interpretation between NOVA View output and visual human interpretation of the same image was 97.8% (138/141).

CONCLUSIONS: Automated IIF reading using the NOVA View digital microscope system could be reliably used as the alternative for the conventional manual microscopic reading. While decreasing laboratory workload by eliminating dilution step for end-point titration and reducing interpretation time, it could facilitate ANA standardization by providing quantified results with nuclear LIU values.

Autoimmune disease

Cod: 0094

RELATION OF PARIETAL CELL ANTIBODIES WITH AUTOIMMUNE DISEASES OTHER THAN TYPE A GASTRITIS

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BACKGROUND: Autoimmune diseases are a heterogeneous group of chronic diseases of unknown etiology. A systematic follow-up of patients with autoimmune diseases has shown that these diseases can be part of complex organ-specific and systemic clinical syndromes. The aim of the study was the identification of other classes of autoantibodies in patients with gastric parietal cell autoantibodies (PCA), the biomarker of autoimmune gastritis.

METHODS: A total of 239 PCA positive patients (152 women, 67 men) were included in the study performed at University Hospital of Ioannina over a three-year period (2011-2013). All these patients were also screened for antinuclear antibodies (ANA), anti-thyroid peroxidase antibodies (anti-TPO), antimitochondrial antibodies (AMA) and anti-smooth muscle antibodies (ASMA). Detection of PCA, ANA, AMA and ASMA were performed using an indirect immunofluorescence assay (NOVA Lite, INOVA Diagnostics, San Diego, CA) while anti-TPO detection was performed with a chemiluminescence immunoassay (UniCel DxI 800, Beckman Coulter).

RESULTS: Among the 239 PCA positive patients, 86 were also found ANA positive (29.2%), 19 AMA positive (1.8%) and 24 ASMA positive (8.1%). Of the 152 PCA positive women, 122 (80.3%) had also anti-TPO antibodies. Additionally, 50 out of the 87 PCA positive men 57.5%) were anti-TPO positive (female/male ratio: 1.74:1).

CONCLUSIONS: We found no statistically significant relation between PCAs and ANA, AMA or ASMA. On the contrary, the incidence of PCA positivity was high in patients with autoimmune thyroiditis as our study confirmed the correlation between thyroid and gastric autoimmunity (autoimmune polyglandular syndrome type III).

Autoimmune disease

Cod: 0095

SERUM CONCENTRATION METALLOPROTEINASE-3 (MMP-3)IN PATIENTS WITH RHEUMATOID ARTHRITIS IN DIFFERENT TREATMENTS

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BACKGROUND: The irreversible destruction of the cartilage, tendon, and bone that comprise synovial joints is the hallmark of rheumatoid arthritis (RA).RA is an autoimmune disease afflicting numerous joints throughout the body. In RA disease, inflammatory cytokines such as interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) stimulate the production of matrix metalloproteinases (MMPs), enzymes that can degrade all components of the extracellular matrix(ECM). Literature reports suggest that between all the MMPs the type 3 (MMP-3) is a useful diagnostic and prognostic marker in patients with RA.However, based on these data, MMP-3 could represent a novel candidate for in vitro and in vivo studies as a molecular target in RA physiology. The purpose of this study is to analyze the correlations between serum concentrations of the matrix metalloproteinase (MMP-3), and clinical markers of disease activity in patients with RA, expecially the early forms, and to assess the clinical value of serum matrix metalloproteinase MMP-3 in evaluating joint destruction and therapeutic effects.

METHODS: We have analyzed MMP3 concentration levels from N.=30 patients affected by rheumatoid arthritis in different treatment:
1) N.10 early R.A. patients
2) N.10 early R.A patients treated by Methotrexate in conventional way
3) N.10 R.A. patientstreated by biological drug (etanercept 50mg/week)
50 healthy donners were evaluated as control.
Serum MMP-3 concentration was determined by commercial ELISA immunoassay (Aesk Diagnostics GRIFOLS,ITALY)

RESULTS: We found reference range 148ng/ml (Healty controls) for MMP-3 serum.The distributions results Gaussian with M± S.D. = 68±40.Concentration is modified in the different patiets group studied.

CONCLUSIONS: The aim of the study is to determine if matrix metallo-proteinases 3 (MMP-3) might be useful clinical marker of disease activity especially in early rheumatoid arthritis and determine if serum MMP-3 are a good laboratory index to evaluate the joint injury status and therapeutic effect, hoping that this marker could be superior to other traditional and routine laboratory indexes.

Autoimmune disease

Cod: 0096

CELLULAR PHENOTYPES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS, UNDER TREATMENT

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BACKGROUND: Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disorder with an etiology not yet well understood. The pathogenesis of the disease is extremely complex, with several studies showing alterations of lymphocyte phenotypes, but there are no studies of these changes in the Brazilian population yet. The aim of this study was to determine alterations in NK, T and B cells in patients with SLE under treatment and compare such alterations in the active and inactive forms between themselves, and with the controls.

METHODS: The active and inactive forms of the disease were classified using the SLEDAI-2K and scores > 4 were considered active form for SLE while scores ≤ 4 were considered inactive form. We have studied 12 patients with SLE with active form (SLE-A), 18 patients with inactive form (SLE-I) and, as controls, 11 subjects without the disease. NK, T and B cells markers such as CD3, CD4, CD8, CD16, CD19, CD25, CD56, HLA-DR, NKG2D and FoxP3 were assessed in lymphocytes from peripheral blood through flow cytometric assays.

RESULTS: NKt cells showed to be decreased in patients with SLE-A compared to control (p= 0,007; Mann-Whitney) and compared to SLE-I (p= 0,015; Mann-Whitney). However, T CD4+ showed to be decreased in patients belonging to both SLE-A (p = 0,048; T-Student) and SLE-I (p= 0,003; T-Student) groups compared to control. Similarly, regulatory T-cells population showed to be decreased in patients in the groups SLE-A (p= 0,018; T-Student) and SLE-I (p= 0,049; T-Student) compared to control. B cells population also showed to be decreased in patients in the groups SLE-A (p =0,029; Mann-Whitney) and SLE-I (p< 0,001; Mann-Whitney) compared to control. Conversely, T CD8+ cells population showed to be increased in patients in the groups SLE-A (p= 0,009; T-Student) and SLE-I (p <0,001; T-Student) compared to control. The expression of HLA-DR in T CD8+ cells also showed to be increased in patients in the groups SLE-A (p <0,001; Mann-Whitney) and SLE-I (p= 0,043; Mann-Whitney) compared to control.

CONCLUSIONS: Despite the patients to be under treatment, the data taken together allow to confirm the characteristic immune system dysfunction of the SLE, as well variations in the cellular phenotypic profile according to the disease status. Support: CNPq, CAPES and FAPEMIG (Brazil).

Autoimmune disease

Cod: 0097

FREQUENCY OF ANTI-MITOCHONDRIAL ANTIBODIES IN AN ADULT POPULATION GROUP-COMPARISON OF TWO IMMUNOASSAYS

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BACKGROUND: Anti-mitochondrial antibodies (AMA) are the most important serological markers of Primary Biliary Cirrhosis. The aim of the present study was to determine the frequency of AMA in a general adult population, by the use of two immunoassays.

METHODS: During a three-year period (2011-2013), a total of 2143 patients were included in the study, which performed at Ioannina University Hospital. AMA were determined by the use of an indirect immunofluorescence (IIF) assay (Nova Lite ANAPlus, Inova Diagnostics, SanDiego, CA). In order to confirm AMA-reactivity, all F-AMA (fluorescent anti-mitochondrial antibodies) positive sera were further examined using an immunoblotting assay (Euroline Liver Profile, Euroimmun AG, Lübeck, Germany). All patients were also screened for antinuclear antibodies (ANA) by an IIF assay (NovaLiteHep-2 ANA, Inova Diagnostics).

RESULTS: F-AMA positives were found in 49 patients, 37 female (75.5%) and 12 male (24.5%). Sixteen out of 49 positive samples showed a low AMA titer (≤ 1/80) and 33 a high titer (≥ 1/160). F-AMA positive samples with a high titer were confirmed with immunoblotting. In all 33 samples a cytoplasmic staining was detected by IIF on Hep-2 cells. Two samples exhibited a mixed cytoplasmic/fine speckled nuclear, 2 a mixed cytoplasmic/nucleolar homogeneous and another 2 a mixed cytoplasmic/centromere pattern. Among the 16 F-AMA positive samples with a low fluorescence titer, 11 were found negative by the immunoblotting assay (M2 negative), 3 in the borderline zone and only 2 positive. Furthermore, 8 out of 16 samples were found ANA negative, 2 displayed a speckled nuclear and the rest 6 a cytoplasmic pattern on Hep-2 cells.

CONCLUSIONS: From a total number of 2143 samples, 33 (1.5%), which corresponding to an equal number of patients, were confirmed as AMA positive. A higher incidence in Gastroenterology patients was observed with a greater proportion in women. Our data indicate that there is an excellent concordance between the two immunoassays (IIF and immunoblotting) for samples with high fluorescence titers. Low concordance for low fluorescence titers requires further investigation. Finally, future studies are needed in order to establish the correlation between laboratory data and clinical symptoms.

Autoimmune disease

Cod: 0098

TISSUE-TRANSGLUTAMINASE ANTIBODIES AS INDICATOR OF BIOPSY IN CELIAC DISEASE

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BACKGROUND: In 2012, new diagnostic criteria for Celiac disease (CD) were published by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), suggesting that in symptomatic children with high serum transglutaminase antibody (TGA) values (at least 10 times the upper limit of normal), CD diagnosis could be established without histological confirmation when HLA and antiendomysium antibodies are positive and they have a good clinical response to a gluten free diet. The aim of this study was to examine positive results of TGA in patients with clinical suspicion of CD and determine if biopsy could be avoided in subjects with high antibody titers.

METHODS: Between January 2011 and January 2014 a total of 146 individuals with positive TGA were enrolled in the study (33 men, 66 women, 47 kids). Samples were processed by enzyme immunoassay with a reagent for anti-tTG IgA (Evolis, Bio-Rad, cutoff 15 U / mL). A review of medical records was performed in order to verify the result of the biopsy expressed by Marsh classification and the final diagnosis. Statistical analysis of data was performed using SPSS (v15.0). Associations between variables were studied using the Spearman correlation coefficient. Statistical significance was set at $p<0.05$.

RESULTS: Data analysis showed that only 39% of the adult subjects had the biopsy performed. In the children group, 17% had biopsy and 26% had been diagnostic by the new diagnostic approach in agreement with ESPGHAN. A final diagnosis of CD was made in 42 patients (29% of the referrals). Serum TGA levels in patients with CD differ significantly from those without CD (median (IQR), 300.0 (49.6) vs 32.3 (78.6) UI; $p<0.001$). Correlation study in adults showed a significant association between TGA concentrations and biopsy results according to Marsh criteria ($r=0.786$, $p<0.001$).

CONCLUSIONS: Results suggest that high levels of TGA are associated with hyperplasic lesions and villous atrophy (Marsh II and III), so performing biopsy would not be necessary in adult patients with high levels of TGA, as already recommended in pediatric population by the ESPGHAN guide. However, studies including a large number of patients should be conducted to confirm these results and to establish a TGA cutoff from which this invasive test could be avoid.

Autoimmune disease

Cod: 0099

ASSOCIATION STUDY BETWEEN GENE POLYMORPHISM IL-17, IL-23 AND TGF-B AND ANTIPHOSPHOLIPID SYNDROME

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BACKGROUND: To investigate the serum concentrations of cytokines IL17, IL23 and TGFß and gene polymorphisms as susceptibility markers for Primary Antiphospholipid Syndrome (PAPS) in Serbian population. Controlling the expression and production of these cytokine may be a new approach to the treatment of PAPS and gene polymorphisms can influence the risk of illness.

METHODS: We analyzed samples of fifty patients with Primary Antiphospholipid Syndrome (PAPS) and fifty healthy controls. Serum concentrations of IL17, IL23 and TGFß were measured by commercial ELISA kits. The SNP rs2275913 (IL17A), rs763780 (IL17F), rs11209026 (IL23) and rs1800471 (TGFß) was genotyped using commercial pre-synthesized TaqMan allelic discrimination assay.

RESULTS: The levels of IL17, IL23 and TGFß were significantly higher in PAPS patients than in the control group. No statistically significant differences were observed in the distribution of genotypes and alleles of the rs2275913, rs763780, rs11209026 and rs1800471 variants in patients with PAPS compared to healthy subjects.

CONCLUSIONS: Data from future association studies may be added to the meta-analyses to obtain more precise estimates of effect sizes.

Autoimmune disease

Cod: 0101

RELATIONSHIP BETWEEN (HLA)-DRB1 ALLELES AND SUSCEPTIBILITYOF RA IN ETHNIC ALGERIAN PATIENTS

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BACKGROUND: Rheumatoid arthritis (RA) is an inflammatory degenerative rheumatic chronic frequent pathology which affects 1% of the world's adult population. RA is more prevalent in women with frequent beginning between the ages of 30 and 50. The exact cause of RA is still unknown. It is believed that Genetic predisposition as well as environmental triggers seems to play an important role. Among the identified susceptibility genes to RA HLA, class II genes are the most studied. The aim of this study is to explore the relationship between human leukocyte antigen (HLA)-DRB1 alleles and susceptibility, clinical and biological features of RA in an Algerian patients population.

METHODS: A case / control study was performed on 134 RA cases filling at least 4 of 7 criteria of the ACR and 132 controls without any inflammatory rheumatic pathology. Using polymerase chain reaction – sequence specific primers (SSP), 134 RA patients and 132 healthy controls were genotyped for HLA-DRB1 and HLA-DRB1*04 subtypes.

RESULTS: HLA-DRB1*04 was found to have increased frequency in the RA group compared to controls ($P < 0.001$, OR = 3.14), and was associated with anti-citrullinated protein antibodies positivity (ACPA) ($P = 0.01$, OR = 2.35). In contrast, HLA-DRB1*07 was found to have a decreased frequency in patients compared to controls ($P = 0.003$, OR = 0.44) and significant decrease was observed in patients with the rheumatoid factor (RF) positivity subgroup ($P = 0.009$, OR = 0.29). HLA-DRB1*04:05 was associated with RA ($P = 0.005$, OR = 3.41), whereas, HLA-DRB1*04:02 showed a protective effect against RA ($P = 0.003$, OR = 0.20).

CONCLUSIONS: HLA-DRB1*04 was associated with increased risk for RA and ACPA positivity, while HLADRB1* 07 was associated with reduced risk for RA and RF synthesis in Algerian patients.

Autoimmune disease

Cod: 0102

PREVALENCE AND PATTERNS OF ANTINUCLEAR ANTIBODIES IN PATIENTS OF A RESPIRATORY DISEASES HOSPITAL

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BACKGROUND: Antinuclear antibodies (ANA) are important tools in the diagnosis of systemic autoimmune diseases. They are usually more demanded in multidisciplinary medical centers where exist departments of internal medicine, kidney diseases and rheumatology. In this study we aimed to determine their prevalence and specificity in samples sent to the immunology lab of a respiratory diseases hospital.

METHODS: Between January 2012 and December 2013, we analyzed the samples of 1067 patients (sex ratio : 0.56 mean age 50.8 years) for ANA detection by IIF on Hep2 cells (initial dilution of 1/80). Positive samples were further tested for specific antinuclear reactivities by an immunodot(ANA profile 3 Euroimmun) and /or by IIF on crithidia luciliae.

RESULTS: 185 patients were ANA positive (17.3%) with titers ranging from 1/80 to 1/20480. The most frequent clinical symptoms that justified the search for ANA were pleural and pericarditis effusions, bronchiectasis, arthralgias and interstitial lung disease. ANA positivity was more frequent in females than in males ($p<0.01$). Fluorescent patterns were homogenous (46%), speckled (31%), cytoplasmic (22%) and nucleolar (13%). A positive result by immunodot was found in 56% of the patients with positive ANA. Anti SSA and anti Ro/52kDa antibodies were the commonest specificities. ANA with titers higher than 1/640 correlated well with the presence of ENA antibodies.

CONCLUSIONS: This study conducted in a respiratory diseases hospital showed a prevalence of 17.3% of ANA positivity. In other studies done in larger hospitals, frequencies of ANA vary between 23% to 42%. These differences may be related to the populations studied (age, gender, and particularly the pathologic conditions) or to the choice of initial screening dilution. Our study like many others found more frequent ANA positivity in females and a correlation between high titers ANA with anti ENA positivity, on the other hand, low titers ANA with no defined specificities were less likely to be clinically meaningful.

Autoimmune disease

Cod: 0103

ASSOCIATION OF BAFF AND APRIL SERUM LEVELS AND THE EXPRESSION OF BAFF-R, BCMA AND TACI ON B CELL SUBSETS WITH CLINICAL MANIFESTATIONS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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BACKGROUND: Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by B cell hyperactivity. B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) binds with different affinities to receptors expressed by B cells termed BAFF-R, BCMA, and TACI. This signaling pathway has an important role in the selection, maturation and survival of B cells. The aim of the study was to evaluate the expression of BAFF/APRIL receptors on B cell subsets in patients with SLE compared to healthy controls (HC).

METHODS: 30 patients with SLE and 15 HC were evaluated. The study included 3 newly diagnosed patients who had not received pharmacological treatment. The disease activity and organ damage were evaluated by Mexican SLE disease activity index (Mex-SLEDAI) and SLICC damage index. Peripheral blood B cell subsets were assessed using multicolor flow cytometry using CD19, CD27 and CD38 staining. Expression of BAFF-R, TACI and BCMA was analyzed on each subset. BAFF and APRIL concentrations were measured by a sandwich ELISA. Statistical analysis included Kruskal-Wallis test, Mann-Whitney U tests and Spearman's r-test.

RESULTS: Distribution of peripheral B cells subsets was disturbed in SLE compared to HC, with an increased proportion of memory ($p<0.05$) and plasma B cells ($p<0.01$). Serum BAFF levels were increased in SLE compared to HC (3.19±4.26 vs 0.97±0.21 ng/mL, $p<0.01$). APRIL levels did not differ between SLE and HC. Serum BAFF levels correlated with the numbers of CD19+ B cells ($rs= 0.407$, $p<0.05$) and Mex-SLEDAI ($rs= 0.584$, $p<0.01$). We observed decreased expression of BAFF-R in SLE patients, on immature, naïve and plasma B cell subsets ($p<0.05$), whereas TACI expression was decreased on both memory and plasma B cells ($p<0.05$). BCMA expression was decreased between the SLE on naïve, memory and plasma B cell subsets ($p<0.05$). These differences were explained by a decreased expression of BCMA among newly diagnosed patients and/or with severe activity (Mex-SLEDAI$\geq$8) compared to patients in remission (Mex-SLEDAI$\leq$2) and HC.

CONCLUSIONS: Elevated serum levels of BAFF in SLE may promote B cell survival and disease activity. Decreased BCMA expression was associated with renal activity, hemolytic anemia, serositis, and with organ damage.

Autoimmune disease

Cod: 0104

ANTISPERM ANTIBODIES AFTER HERNIA MESH REPAIR

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BACKGROUND: There are immunological reasons of infertility in some men after hernia repair. The aim was to study the influence of mesh hernia repair on antisperm antibodies production.

METHODS: A prospective interventional longitudinal cohort study on 82 male patients without exclusion criteria who had an inguinal hernia. Patients underwent mesh hernia repair. Main outcome measures the quantitative value of antisperm antibodies (ASA) in serum (IU/ml) before and after operation. The antisperm antibodies were analyzed by enzyme-linked immunoadsorbent assay.

RESULTS: The antisperm antibodies (ASA) value increased by an average of 4.75 U/ml postoperatively ($Z=3.67$, $p<0.001$). Antisperm antibodies (ASA) significantly increased postoperatively but stayed in normal range in all patients except one patient who had testicular ischemia as postoperative complication.

CONCLUSIONS: Mesh hernia repairs without complication caused an increase of the antisperm antibodies (ASA) value but without clinical significant autoimmune reaction.

Autoimmune disease

Cod: 0105

CYTOBEAD ANA - A NOVEL INDIRECT IMMUNOFLUORESCENCE TEST FOR THE SIMULTANEOUS DETECTION OF ANTI-NUCLEAR ANTIBODIES

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BACKGROUND: The detection of anti-nuclear antibodies (ANA) by indirect immunofluorescence (IIF) on HEp-2 cells has been evolved as the standard screening assay for autoimmune diagnostics. Nevertheless, this technique is characterized by high variation due to poor standardization and lack of automation. Thus, enzyme immunoassays (EIA) with corresponding antigenic targets are used to confirm the findings of HEp-2 IIF. The aim of this study was to develop a novel immunoassay for the serological diagnosis of systemic autoimmune diseases that combines screening for ANA using HEp-2 cells and the confirmation of ANA testing by analyzing the reactivity against corresponding nuclear antigens coated on fluorescent microparticles via IIF simultaneously in one reaction environment (CytoBead ANA).

METHODS: The antigens Ro60, Ro52, La, CENP-B, RNP-Sm, Sm, dsDNA and Scl-70 were each coated onto red fluorescent microparticles that can be differentiated according to their size (9 µm and 15 µm). Finally, the novel assay combines the screen for ANA on HEp-2 cells by fixation of the cells in the middle part with confirmative testing using antigen coated microparticles which are clockwise immobilized in four compartments around the middle part. Reference sera with clinical background (n=170) and healthy controls (n=30) were tested for ANA by IIF with AKLIDES®. Results obtained were compared to those of routine IIF and EIA.

RESULTS: A first evaluation using microparticles coated with Ro60, Ro52, La, CENP-B, RNP-Sm, Sm, dsDNA and Scl-70 with reference sera showed comparable results to those obtained with routine EIA. The intensities of the microparticle-based testing of low and high reactivity sera correlated well with the EIA findings. Overall, the relative specificity of the CytoBead ANA, compared with EIA, was 96 % and the relative sensitivity was 97 %.

CONCLUSIONS: The new test system can replace the two-stage analysis by combining IIF screening with multiplex confirmative testing. The test is suitable for automation with AKLIDES® as well as for manual ANA screening. A further evaluation with an extended panel of patient sera will provide more information concerning the assay performance and accuracy.

Autoimmune disease

Cod: 0106

CELLULAR PHENOTYPES IN PATIENTS WITH LUPUS NEPHRITIS

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BACKGROUND: Lupus nephritis (LN) is the most relevant clinical manifestation in systemic lupus erythematosus (SLE). In the kidney, immune complexes accumulate leading T cells to infiltrate and to secrete proinflammatory cytokines, thereby contributing to increased damage through the recruitment of polymorphonuclear cells. The aim of this study was to determine alterations in NK, T and B cells in patients with SLE under treatment and compare such alterations in patients groups with and without LN between themselves and with the controls.

METHODS: Patients with and without LN were confirmed by renal biopsy. We studied 12 patients with LN, 18 patients without LN and, as controls, 11 subjects without the disease. NK, T and B cells markers such as CD3, CD4, CD8, CD16, CD19, CD25, CD56, HLA-DR, NKG2D and FoxP3 were assessed in lymphocytes from peripheral blood by flow cytometric assays.

RESULTS: T CD4+ cells showed to be decreased in patients in the groups with LN ($p < 0,001$; T-Student) compared to controls. The expression of HLA-DR in T CD4+ cells showed to be increased in patients with LN compared to patients without LN ($p= 0,039$; Mann-Whitney) and to control ($p= 0,004$; Mann-Whitney). Similarly, the expression of NKG2D in CD3+CD56- cells showed to be increased in patients with LN compared to patients without LN ($p= 0,047$; T-Student) and to controls ($p= 0,014$; T-Student). Regulatory T-cells population showed to be decreased in patients without LN ($p= 0,006$; T-Student) compared to control. B cells population also showed to be decreased in patients with LN ($p =0,004$; Mann-Whitney) and without LN ($p< 0,003$; Mann-Whitney) compared to controls. Conversely, T CD8+ cells population showed to be increased in patients in the groups with LN ($p< 0,001$; T-Student) and without LN ($p <0,011$; T-Student) compared to controls. Similarly, the expression of HLA-DR in T CD8+ cells showed to be increased in patients in the groups with LN ($p <0,013$; Mann-Whitney) and without LN ($p= 0,008$; Mann-Whitney) compared to controls.

CONCLUSIONS: In spite of the patients to be under treatment, the data taken together allow to confirm the characteristic immune system dysfunction of the LN, as well variations in the cellular phenotypic profile according to the manifestations of SLE. Support: CNPq, CAPES and FAPEMIG (Brazil).

Autoimmune disease

Cod: 0107

MULTIPARAMETRIC PHENOTYPIC ANALYSIS OF PERIPHERAL BLOOD B-CELL SUBSETS BY FLOW CYTOMETRY IN SYSTEMIC SCLEROSIS

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BACKGROUND: Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by vascular injury, fibrosis of skin and visceral organs, and immune activation. B-cell hyperactivity and autoantibody production associate with SSc, while evaluation of other B-cell alterations may contribute to appropriate classification of disease subsets, assessment of disease activity and to elucidate prognosis. Therefore our purpose was to characterize SSc patient-derived peripheral blood B cells according to their cell surface phenotype using different algorithm, and to compare their proportions to normal controls.

METHODS: B cells were isolated by ficoll gradient centrifugation followed by CD19 magnetic separation. Combined multiparametric flow cytometry analyses were performed with the following monoclonal antibody combinations: CD22/CD23/CD80-FITC, IgD-PE, CD27-PE-Cy5, CD38-APC. Disease activity was evaluated with standard scoring activity index (EScSG) and also the 12-point index recently constructed in the Department of Rheumatology and Immunology.

RESULTS: Our preliminary results showed that proportions of switched memory B cells and centrocytes were significantly lower in SSc patients than in controls, more markedly in active compared with non-active SSc subgroup. B-cell activation analysis also showed significant differences between diffuse (dcSSc) and limited cutaneous SSc (lcSSc): the percentage of CD80+ switched memory B cells was higher in dcSSc, and the proportion of CD80- and CD95- naive B-cells was increased in lcSSc.

CONCLUSIONS: Extended phenotypic analysis of peripheral blood B cells may be a new useful tool in correct determination of disease subsets, assessment of disease activity and prediction of prognosis.

Autoimmune disease

Cod: 0108

SIMULTANEOUS SCREENING AND CONFIRMATION OF ANCAS AND DETECTION OF ANTI-GBM ANTIBODIES WITH CYTOBEAD ANCA® ASSAY

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BACKGROUND: The novel CytoBead® technology combines autoantibody analysis by cell-based screening with the confirmation of corresponding autoantigen reactivities by multiplex microbead technology using immunofluorescence technique (IFT) in one reaction environment.

METHODS: CytoBead® ANCA allows the simultaneous detection of anti-neutrophil cytoplasmic antibodies (ANCA) on ethanol-fixed neutrophils for screening and confirmation thereof using proteinase 3 (PR3) and myeloperoxidase (MPO) coated microbeads. Furthermore, the detection of anti-GBM antibodies is integrated by adding glomerular basement membrane (GBM) coated microbeads. Anti-GBM autoantibodies occur in 10% of rapid progressive glomerulonephritis patients together with ANCA and are required for the differential serological diagnosis in routine diagnostics. This assay format can be interpreted with a standard fluorescence microscope (FITC channel) for semi-quantitative and with the automated interpretation system Aklides® for quantitative analysis. The performance of the CytoBead® ANCA assay was investigated using sera of 666 individuals including 118 patients with ANCA-associated vasculitis, 162 healthy controls, 352 disease controls and 34 anti-GBM positive sera. Receiver operating characteristics and inter-rater agreements (kappa) were used to compare the results of novel CytoBead® ANCA assay with routine autoantibody investigation.

RESULTS: The comparison of classical ANCA screening with ANCA screening by the novel CytoBead® ANCA assay showed very good agreement for pANCA and cANCA patterns (kappa= 0.862, 0.868; respectively). The results of anti-PR3, anti-MPO, and anti-GBM detection by this novel method compared to anti-PR3-, anti-MPO as well as anti-GBM by ELISA revealed good to very good agreement (0.78,0.72,0.87; respectively).

CONCLUSIONS: Consequently, CytoBead® ANCA assay is an attractive alternative to classical time-consuming single parameter ANCA and anti-GBM antibody detection and is therefore applicable as a clinical diagnostic tool for emergency situations.

Autoimmune disease

Cod: 0109

RELATION OF PARIETAL CELL ANTIBODIES WITH AUTOIMMUNE DISEASES OTHER THAN TYPE A GASTRITIS

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BACKGROUND: Autoimmune diseases are a heterogeneous group of chronic diseases of unknown etiology. A systematic follow-up of patients with autoimmune diseases has shown that these diseases can be part of complex organ-specific and systemic clinical syndromes. The aim of the study was the identification of other classes of autoantibodies in patients with gastric parietal cell autoantibodies (PCA), the biomarker of autoimmune gastritis.

METHODS: A total of 239 PCA positive patients (152 women, 67 men) were included in the study performed at University Hospital of Ioannina over a three-year period (2011-2013). All these patients were also screened for antinuclear antibodies (ANA), anti-thyroid peroxidase antibodies (anti-TPO), antimitochondrial antibodies (AMA) and anti-smooth muscle antibodies (ASMA). Detection of PCA, ANA, AMA and ASMA were performed using an indirect immunofluorescence assay (NOVA Lite, INOVA Diagnostics, San Diego, CA) while anti-TPO detection was performed with a chemiluminescence immunoassay (UniCel DxI 800, Beckman Coulter).

RESULTS: Among the 239 PCA positive patients, 86 were also found ANA positive (29.2 %), 19 AMA positive (1.8 %) and 24 ASMA positive (8.1 %). Of the 152 PCA positive women, 122 (80.3 %) had also anti-TPO antibodies. Additionally, 50 out of the 87 PCA positive men 57.5%) were anti-TPO positive (female/male ratio: 1.74: 1).

CONCLUSIONS: We found no statistically significant relation between PCAs and ANA, AMA or ASMA. On the contrary, the incidence of PCA positivity was high in patients with autoimmune thyroiditis as our study confirmed the correlation between thyroid and gastric autoimmunity (autoimmune polyglandular syndrome type III).

Autoimmune disease

Cod: 0110

ANTIBODIES TO PARIETAL CELLS: CORRELATION WITH VITAMIN B12 LEVELS IN THE CONTEXT OF ANEMIA INVESTIGATION

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BACKGROUND: Gastric parietal cell autoantibodies (PCA) represent a serum biomarker for autoimmune (type A) gastritis, a silent and highy prevalent disease which can progress, after 20-30 years, in pernicious anemia. The aim of this study was to investigate the correlation of PCAs and vitamin B12 levels in the context of anemia investigation.

METHODS: In this retrospective study, which was conducted in the University Hospital of Ioannina, data were collected from the Immunology Unit of Microbiology Department, Biochemistry Department and Hematology Laboratory. During three years period (2011-2013), a total of 239 PCA positive patients were included in the study (152 female, 87 male; mean age: 59.52±17.485 years). Patients were found PCA positive using an indirect immunofluorescence assay (NOVA Lite ANA plus, INOVA Diagnostics, San Diego, CA). Additionally, serum levels of vitamin B12 were measured by chemoluminescence immunoassay (Unicel DXI 800, Beckman Coulter) and red blood cell analytic parameters such as Hematocrit (Ht), Hemoglobin concentration (Hb) and Mean Corpuscular Volume (MCV) were also studied (Sysmex XE-500 counter).

RESULTS: An increased prevalence of PCA positivity was observed in women with a median age of 60 years, a finding which is in accordance with literature. Of the 239 PCA positive patients, 10 were found with low vitamin B12 serum levels (4.18%), 68 with low Ht values (28.45%), 64 with low Hb values (26.77%) and 27 with increased MCV (11.29%). Our findings do not support any correlation of PCA positive samples with low B12 serum levels (p 0.884), low Ht (p 0.379), low Hb (p 0.266) or increased MCV (p 0.36).

CONCLUSIONS: Although parietal cell antibodies (PCAs) are diagnostic for atrophic gastritis, they have not been proven to be a prognostic marker for pernicious anemia. However, according to literature, in patients with positive PCAs and atrophic gastritis, factors such as genetic predisposition, T-lymphocytes and alimentation, probably contribute to progression to pernicious anemia.

Autoimmune disease

Cod: 0111

DEVELOPMENT OF A TURBIMETRIC IMMUNOASSAY FOR THE MEASUREMENT OF SERUM COMPLEMENT C1Q COMPONENT

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BACKGROUND: C1q is a 400kD hexameric subunit of C1, the first component of complement. The binding of C1q to IgM, or aggregated IgG in immune complexes initiates the classical pathway of complement activation, by activation of C1r and C1s. C1q can also bind directly to apoptotic cells and debris, aiding in their clearance. C1 deficiency generally leads to severe immune complex disease with features of systemic lupus erythematosus (SLE) and glomerulonephritis. Approximately 90% of C1q deficient patients have SLE. Reduced serum C1q is also associated with increased risk of fulminant infections with encapsulated bacteria. Objective: To develop a latex enhanced turbidimetric immunoassay on the SPAPLUS analyser for the determination of C1q levels in serum.

METHODS: The assay uses latex beads coated with F(ab)2 fragments of sheep polyclonal antibodies specific to C1q. The beads were used to develop an automated, turbidimetric immunoassay on the SPAPLUS analyser for the quantification of C1q in serum. The assay is standardised against a Normal Human Serum (NHS) pool quantified by C1q Radial Immuno Diffusion (RID) assay (The Binding Site Ltd.)

RESULTS: The assay took 10 minutes and was read at endpoint. The assay range was 8.5-275mg/L using a 1/10 sample dilution, with a sensitivity of 0.85mg/L using neat sample. Intra-assay precision Coefficients of Variation with neat sample at three C1q levels, 2.9, 13.5 and 24.7mg/L, were 6.0% (low sample), 2.1% (medium sample), and 0.44% (high sample). Inter-assay CVs were 16.0%, 4.2% and 3.4%, respectively, for the same 3 samples. A plot of expected versus observed results of diluted NHS resulted in linear regression line y=1.00x-0.21; R2=1. Values from 20 sera were compared using RID and SPAPLUS assays. A plot of SPAPLUS versus RID values resulted in the regression line y=0.89x+9.4; R2=0.94. Spearman's rank correlation (rho) for the 2 data sets was 0.85, P<0.0001. Serum was artificially depleted of C1q, as confirmed by Western blot. When measured on the 2 assays it was found to be depleted by both.

CONCLUSIONS: We have developed and validated an automated immunoassay for the quantitation of serum C1q levels. This assay provides a rapid alternative to RID, and offers similar sensitivity for the detection of C1q deficient patients.

Autoimmune disease

Cod: 0112

FREQUENCY, ETIOLOGY, AND PREVENTION OF STROKE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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BACKGROUND: The main symptoms of CNS lupus can be diffuse (generalized seizures, psychosis) or focal (stroke, peripheral neuropathies). Neuropsychiatric symptoms often occur in the first year of SLE, but are rarely the presenting symptoms.

METHODS: We prospectively and retrospectively reviewed, from 1998 to 2010, the incidence etiology and the prevention of stroke in 69 hospitalized patients with systemic lupus erythematosus (SLE).

RESULTS: Stroke occurred in 10 (14%) of our patients with documented SLE; six (60%) of the 10 had multiple cerebral infarcts. Factors associated with stroke were: systemic thrombosis, elevated partial thromboplastin time, age over 60 years, transient ischemic attacks, previous stroke, and cardiac valvular disease. The major period of risk for the first stroke was during the first 4,5 years of SLE. The most frequent etiology was a cardiogenic embolus, with cerebral vasculitis occurring (one patient) only in association with infection. Because of the decreased fibrinolysis seen in patients with SLE, anticoagulant therapy may be the most effective preventive treatment currently available. Anticoagulant therapy seemed to prevent recurrent focal cerebral ischemia in our patients and was associated with relatively few and minor complications. Patients with a history of transient ischemic attacks or cardiac valvular lesions are at high (50% and 75%, respectively) risk of stroke. Patients who have had a stroke are at high (63%) risk for a recurrent stroke.

CONCLUSIONS: Most CNS events in patients with SLE are transient, benign and we recommended for all of these patients anticoagulant therapy.

Autoimmune disease

Cod: 0113

ANTIOXIDATIVE ENZYMES IN LYMPHOCYTE CULTURE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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BACKGROUND: This lymphocyte culture study was done to assess in vitro the ability of lymphocytes to produce certain mediators and to react to different mitogens. In lymphocyte culture supernatant from the patients with systemic lupus erythematosus (SLE) and healthy controls we measured the activity of SOD and its isoenzymes CuZn SOD, Mn SOD, GPx activity and glutathione concentration. Antioxidative status was assessed before and after stimulation with concanavalin A and Phorbol 12-myristate 13-acetate. Basal antioxidative and post-stimulation status of lymphocytes was thus assessed.

METHODS: The study involved 55 examinees (47 women and 8 men) with SLE in the acute exacerbation stage and 20 healthy examinees, from which we took the samples of heparinized plasma and separated and stimulated the lymphocytes. Peripheral lymphocytes were stimulated with concanavalin A (10µg/ml), (Con A), and Phorbol 12-myristate 13-acetate (10 ng/ml), (PMA), which were added to the incubation medium. After stimulation for 72 h, cell supernatant was separated, in which we determined the activity of SOD, MnSOD, CuZnSOD, and GPx. Commercial test kits by Randox (Ransod i Ransel) were used. After the inhibition of samples with 4 mmol/L KCN (ratio 1:1), the activity of CuZnSOD was calculated as the activity difference of total SOD and MnSOD.

RESULTS: In the studied lymphocyte cultures, an increase of SOD activity was observed: by 39% in the PMA stimulated and by 36.5% in ConA stimulated lymphocytes, which was probably the result of a strong release of superoxide anion radicals. SOD isoenzyme profile was changed too. In the PMA stimulated lymphocytes an increase by 72.5% was observed compared to the corresponding PMA control, while in ConA stimulated lymphocytes the increase was higher in the MnSOD fraction, by 54%. There were no significant changes in the GPx activity and glutathione concentration.

CONCLUSIONS: Lupus lymphocytes demonstrated an increased response to membrane stimulation as shown by an increased production of superoxide anion radical, one of the factors in the onset of vasculitis and tissue damage. The cells of certain tissues exposed to oxidative stress activate local genes for Mn-SOD, a SOD isoenzyme, which can be induced or suppressed by certain cytokines as well.