Autoimmune diseases, autoimmunity, allergy

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ATOPY AND ALLERGIC DISEASES IN ALBANIAN STUDENTS (ATOS STUDY)

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BACKGROUND-AIM

An increasing prevalence of allergic diseases and bronchial asthma has been reported all over the world. Lately it is reported that the rise in prevalence of childhood asthma has relented in Western Europe, but persists in eastern regions where it has traditionally been low. The aim of the study was to describe the prevalence of asthma, rhinoconjunctivitis and atopic dermatitis in students in the 4th year of the Faculty of Medicine; estimate the allergy sensitization to any aeroallergen by skin prick test and evaluate the correlation of positive skin prick test (in vivo) with specific IgE (sIgE)

METHODS

The ATOS is a cross sectional study carried out in Tirana, using the ISAAC methodology (The International Study of Asthma and Allergies in childhood). 258 medicine students 21-22 yold completed a modified symptoms questionnaires. Skin prick test (SPT) were performed to all the study group (16 common aeroallergen-Strallergene); the students who resulted positive at least to aeroallergen (wheal $\geq$ 3mm) were referred for the assessment of specific IgE using AlleisaScreen Panel 30 RespA and 30 FoodA by MEDIWISS Analytic GmBH: an immunoblot assay for the quantitative determination of circulating allergen sIgE in human serum.

RESULTS

Response rate was 98% for skin tests and sIgE too. A reported lifetime history of asthma resulted in 4.7% of the students, allergic rhinitis in 12.9% and atopic dermatitis in 7.4%. Prevalence of sensitization to aeroallergen, based in SPT, resulted 24.9 %; 57.8 % of them were polysensitized. This high prevalence was more attributed to the sensitization to house dust mites (D.Pteronyssinus - 22%; D.Farinae - 21. 56%), followed by grass pollen 9,8%. The correlation between the SPT+ and sIgE was 68,2% for D.Pteronyssinus, 72,8% for grasses and 75% for trees.

CONCLUSION

Despite substantial changes in our lifestyle and home environment the prevalence of allergic disease in Albania is lower than the European developed countries although the prevalence of allergic sensitization as measured by SPT is significantly high, comparable with the English speaking countries. Also the correlation between the SPT+ and sIgE among the atopic students resulted 68,2%-75% depending on the tested allergen, confirming once more the value of the SPT in the diagnosis of allergic diseases.

Autoimmune diseases, autoimmunity, allergy

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THE PREVALENCE OF AUTOANTIBODIES IN PARANEOPLASTIC RHEUMATIC SYNDROMES

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BACKGROUND-AIM

Paraneoplastic syndromes are a group of rare disorders caused by a malignancy. It is known that tumor cells express antigens which can induce the formation of specific autoantibodies. The associations between rheumatic manifestations, autoimmunity and malignancy may aid in the diagnosis of underlying pathology. The aim of this study was to assess prevalence and diagnostic value of autoantibodies in paraneoplastic rheumatic syndromes.

METHODS

The study group consisted of 48 patients with paraneoplastic rheumatic syndrome and 45 control group patients with solid tumor. Both groups were matched for sex (male 52% vs. 56 % respectively) and age (60,9±8,9 years vs. 60,5±9,5 years respectively). Characteristics of solid tumors in groups were similar – 49% cases with tumors of prostate, 27% with lung cancer and 24 % with breast tumor. Patients were examined for the presence of autoantibodies: rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (anti-CCP) test was performed by enzyme-linked immunosorbent assay (ELISA); anti-nuclear antibodies (ANA) were tested by indirect immunofluorescence on Hep-2 cells; immunoblotting technique was used to detect autoantibodies to anti-neuronal antibodies (ANNA).

RESULTS

RF was detected in 31% of patients with paraneoplastic rheumatic syndrome and in 20% of control group; it should be noted that 45% of patients with lung cancer had RF positive. ANA were positive in 25% of patients with rheumatic syndrome and in 25% of control group patients. ANNA were positive in 8% paraneoplastic rheumatic syndrome group and in 7% of control group patients. Significant difference wasn't observed comparing frequency of autoantibodies in the groups of patients with tumors of different localization.

CONCLUSION

No significant differences were observed comparing frequency of detecting autoantibodies in the group of patients with paraneoplastic rheumatic syndromes and control group with solid tumors. Rheumatoid factor, anti-cyclic citrullinated peptide, anti-nuclear and anti-neuronal autoantibodies were found to be similarly frequent in the paraneoplastic and the control groups. Detection of autoantibodies and immunology profile is limited in assessing of paraneoplastic rheumatic syndromes.

Autoimmune diseases, autoimmunity, allergy

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EVALUATION OF PERCENTAGE OF REGULATORY T-CELLS (CD4+CD25+, CD4+ CD25HIGH, CD4+CD25HIGHCD127LOW) IN PATIENTS WITH DOWN SYNDROME

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BACKGROUND-AIM

Down syndrome (DS) is an autosomal chromosomal disorder caused by trisomy of all or a critical part of chromosome 21. The autoimmune regulator protein (AIRE), a transcription factor located on a chromosome 21, cell-mediated and humoral immunity play a crucial role in autoimmunity. Children with DS demonstrate an increased risk of developing various autoimmune thyroiditis, coeliac disease and type 1 diabetes. In DS the over-expression of chromosome 21-encoded gene products lead to impaired interaction between immature thymocyte and thymic stromal cells. Thymus has two main functions: deletion of self-reactive T-cells and the production of natural CD4+CD25high. Regulatory T-cells have drawn tremendous interest due to their role in maintaining tolerance by suppressing the immune response. This allows to prevent autoimmune diseases.

METHODS

The study group consisted of 30 children aged 7-12 years with cytogenetically confirmed DS-simple trisomy 21 (47,XY,+21 or 47,XX,+21). To assess the percentages of lymphocytes Treg (CD4+CD25+, CD4+CD25high, CD4+CD25high CD127low) in blood samples, the flow cytometry was used. The control group comprises 27 healthy children.

RESULTS

The percentage (mean±SD) of CD4+CD25highCD127low in the DS group was significantly lower than that in the control group (50.3#17,8 vs 59.8±13.2%, 0,04>p>0.03). There were no significant differences in percentages of CD4+CD25+ and CD4+CD25high between DS children and healthy subjects (5.4#2.8 vs 5.1#5.1%, 0.13>p>0.12 and 1.2±1.0 vs 1.1±0.8%, 0.90>p>0.80, respectively).

CONCLUSION

The reduction of T regulatory lymphocytes count (CD4+CD25highCD127low) can be related to their higher migration towards peripheral infection regions and can be a factor that intensifies autoimmune disorders development.

Autoimmune diseases, autoimmunity, allergy

M153

ULCERATIVE COLITIS AND NEOPTERIN: RELATED ?

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BACKGROUND-AIM

Ulcerative colitis (UC) is a chronic inflammatory bowel disease, which invades the colon mucosa and progresses with remissions and exacerbations. In fact, ulcerative colitis is not only a digestive tract disease, but also a systemic disease with many kinds of non-intestinal retention. In active monocytes/macrophages, neopterin is synthesized from guanosine triphosphate (GTP) via the GTP cyclohydroxylase enzyme, the final product of the pteridine metabolism, as an indicator of the oxidative stress induced by the immunological system. This study aims to show the relationship between the Truelove-Witts activity criteria and the level of neopterin in ulcerative colitis and the usability of neopterin in determining the activity of the disease.

METHODS

Patients who had been followed up between March and June 2012 for ulcerative colitis in the gastroenterology clinic at Istanbul Education and Research Hospital were included the patient group; 34 patients were classified for their ulcerative colitis activity as mild (N=13), moderate (N=18), and severe (N=3) based on the Truelove-Witts activity index, according to the symptoms, physical examination findings and laboratory values. And patients who did not have any autoimmune disease, infectious disease or malignant tumoral disease, and therefore with no history of using medication, and who were evaluated as having normal colonoscopy results made up our control group (N=43). Serum neopterin levels were measured by enzyme-linked immunoassay (DRG Diagnostics, Germany). Statistical analyses were performed with SPSS 11.5 package software.

RESULTS

There was no significant difference in sex between the groups. Analysis revealed no significant difference in the age-adjusted average of reciprocal transformed neopterin levels between the patients (1.80 nmol/L) and controls (1.72 nmol/L; $F = 0.328$; $p = 0.569$). We found no correlation between serum neopterin levels and the disease activity ($rs = 0.025$, $p = 0.891$).

CONCLUSION

These results demonstrated that serum neopterin levels remained unchanged in patients with ulcerative colitis compared to the control group, and age and gender did not have any specific impact on this outcome.

Autoimmune diseases, autoimmunity, allergy

M154

COMPLEMENT C3 AND COMPLEMENT C4 ASSAYS FOR THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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BACKGROUND-AIM

The complement proteins are a group of at least 20 immunologically distinct components in blood and tissue fluids. They interact sequentially with Ag-Ab complexes, with each other, and with cell membranes in a complex but adaptable way to destroy viruses and bacteria and, pathologically, even the host's own cells. Abnormal serum levels of complement proteins may be due to either inherited or acquired diseases. C3 and C4 are weak and late-reacting Acute Phase proteins. Diseases in which Complemet C3 changes can be anticipated include active forms of systemic lupus erythematosus (SLE) and membranoproliferative glomerulonephritis. Genetic Complement C4 deficiency is linked with a high prevalence rate to autoimmune diseases, especially systemic lupus erythematosus (SLE).
Thermo Scientific™ Indiko™ and Indiko™ Plus used in this study are bench top clinical chemistry analyzers, especially suitable for small and medium sized laboratories or as a back-up analyzer for bigger ones. Colorimetric, turbidimetric and ISE methods are well applied and CE marked. The Indiko analyzers are complete easy-to-use systems including the instrument, system reagents, calibrators and controls.

METHODS

C3 and C4 methods are immunoturbidimetric. Specific antiserum is added in excess to buffered samples. The increase in absorbance is caused by formation of immunocomplexes between the measured analyte and the specific antibody. The absorbance is measured at 340 nm when the reaction has reached the end-point. The change in absorbance is proportional to the amount of antigen (Complement C3 or Complement C4) in solution.

RESULTS

The repeatability (within-run precision) for C3 is 1.5–1.8 % (CV; n=84), and for C4 1.9–2.0 % (CV; n=84). The within device (total) precision for C3 is 2.2–2.8 % (CV; n=84), and for C4 2.6–3.1 % (CV; n=84). The Indiko methods correlated well with the reference methods.

CONCLUSION

With these ready-to-use system reagents, C3 and C4 analysis on Thermo Scientific Indiko and Indiko Plus analyzers is quick and accurate.

Autoimmune diseases, autoimmunity, allergy

M155

RHEUMATOID FACTORS (RF) ASSAY FOR THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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BACKGROUND-AIM

Rheumatoid arthritis (RA) is a common disease (1 - 2% of adult population) and can present at any age and involve any joint. Rheumatoid factor is of more value in prognosis than in diagnosis. RF often precede the onset of the illness, sometimes by many years. The risk of RF positive healthy individuals contracting RA is stated to be 5-40 times higher than in RF negative individuals. Rheumatoid factor refers to the immunoglobulin M antibody, which binds aggregated IgG as its antigen.
Thermo Scientific™ Indiko™ analyzers, Indiko and Indiko Plus, used in this study are bench top clinical chemistry analyzers, especially suitable for small and medium sized laboratories or as a back-up for bigger laboratories. They are applicable for colorimetric and turbidimetric assays as well for electrolytes employing ISE technology. The Indiko and Indiko Plus analyzers are user-friendly complete systems including the instrument, system reagents, calibrators and controls as well the CE marked applications.

METHODS

The method is based on the reaction between rheumatoid factors and microparticles coated with human immunoglobulins G. Specific RF reagent is added to a buffered sample. The increase in absorbance caused by formation of immunocomplexes is recorded when the reaction has reached its end-point. The change in absorbance at 540 nm is proportional to the amount of rheumatoid factors in solution.

RESULTS

The assay measuring range is 15-110 IU/ml extended with automatic dilution up to 440 IU/ml. The repeatability (within-run precision) is 0.5 and 1.0 % (CV) and the within device (total) precision is 1.4 and 2.4% (CV) for samples with RF concentrations of 33 and 92 IU/ml (N=84). A comparison study was performed by the Konelab PRIME 60i Rheumatoid factors method as a reference. Linear regression was $y = 0.12x - 2.8$ and $r = 0.997$ (N=62).

CONCLUSION

The results demonstrate that Rheumatoid factors (RF) can be analyzed reliably and easily using Thermo Scientific Indiko and Indiko Plus clinical chemistry analyzers.

Autoimmune diseases, autoimmunity, allergy

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HIGH PREVALENCE OF ALLERGY SENSITIZATION TO ACARUS SIRO IN 248 ALBANIAN PATIENTS WITH RESPIRATORY SYMPTOMS

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BACKGROUND-AIM

Acarus Siro (formerly known as Tyroglyphus Farinae) is a Storage mite that induces occupational allergy in farmers. Since it has also been found in house dust, flour, cheeses and cereal foods like cookies, oatmeal, cornflakes etc, it causes sensitization in non-farming populations too. In this study we evaluated the prevalence and possible causes of sensitization to Acarus Siro in a group of patients with allergy symptoms.

METHODS

We studied 248 patients with respiratory symptoms suggestive of allergy, who were referred for determination of specific aeroallergens. The patients were divided into two groups: pediatric (0-14 years) and adults (> 14 years). For each patient we determined serum total IgE with Architect ci8200 system and serum specific IgE for Acarus Siro with AlleisaScreen panel of 30 aeroallergens by MEDIWISS Analytic GmbH: an immunoblot assay for the quantitative determination of circulating allergen specific IgE in human serum.

RESULTS

According to the class the results were divided into 3 groups: Not present: Class 0-I; Slight increase: Class II; and High sensitivity: Class III-VI. In 93 pediatric patients we found that 42 (45.1%) were positive for the presence of antibodies to Acarus Siro, of which 37 (39.7%) belonged to class II, and 5 (5.4%) belonged to class III-VI. In the adult group, 191 totals, we found 94 (49.2%) patients sensitized to Acarus Siro, of which 56 (29.3%) belonged to class II and 38 (19.9%) belonged to class III-VI.

CONCLUSION

The high percentage of sensitization to Acarus Siro in 248 patients with respiratory symptoms and the amplification of significant sensitization (class III-IV) from 5.4% in the pediatric group to 19.9% in the adult group, testify for the interference of environmental factors. Damp housing conditions, inappropriate storage conditions of cereal-based processed foods, as well as the long time of storage and eventual creation of mould may be some of the factors that contribute to the amplification of sensitization with the passing of years. The results of this study affect also the consumer's rights and should be used to reinforce and improve the implementation of EU standards in Albania in processed food handling and storage.

Autoimmune diseases, autoimmunity, allergy

M157

CLOSTRIDIUM DIFFICILE AND AUTOIMMUNE BOWEL DISEASE: RELATIONSHIP BETWEEN DISEASE AND INFECTION SUSCEPTIBILITY IN A GENDER PERSPECTIVE.

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BACKGROUND-AIM

Autoimmune Bowel Diseases (ABD) affect about 1% of population. C. difficile is the major cause of antibiotic associated diarrhea and colitis because of the release of two toxins: TcdA and TcdB coded by the gene sequence PaLoc. Actually C. difficile infections (CDI) is considered one of the prevalent nosocomial infections.
The aim of this 'gender oriented' study is to demonstrate the relationship between ABD and CDI using Fecal Calprotectin (FC) as a marker for the therapeutic follow up and for the risk stratification.
FC is a protein found mainly in neutrophils, but also in monocytes and macrophages, released during neutrophil activation or death. Released during the inflammatory process, it's considered a specific biomarker of active gastrointestinal inflammation.

METHODS

Two groups of 101 patients have been enrolled into the study. The first group was characterized by controls, the second by patients that had a diagnosis of ABD. CDI have been identified using the loop-mediated isothermal DNA Amplification Assay for the Detection of the pathogenicity locus of toxigenic Clostridium difficile. Amplification is based on primers that specifically amplify a 204 bp region of the conserved 5' sequence of the tcdA gene. FC have been measured using the quantitative lateral flow assay BÜHLMANN Quantum Blue Calprotectin High Range, a sandwich immunoassay based on two monoclonal antibodies.

RESULTS

In our study all the 202 patients enrolled have been tested for FC and CDI. We have found the higher risk of CDI in under 40 years old female celiac patients. In Crohn disease and Ulcerative Colitis there is the same risk of CDI. In Crohn disease the higher risk affects under 40 years old female patients, in Ulcerative Colitis over 60 years old male patients.

CONCLUSION

There is a relationship between ABD and CDI. This study has showed the association between CDI and high values of FC . Finally FC appears to be an excellent biomarker for ABD diagnosis, to evaluate the follow up of disease and to create a gender oriented diagnostic profile.

Autoimmune diseases, autoimmunity, allergy

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IN VITRO IGE SENSITIZATION TO PLANTAGO WEED POLLEN IN ADULT PATIENTS WITH ALLERGIC RHINITIS FROM SOUTHERN ROMANIA

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BACKGROUND-AIM

A recent position statement regarding European patient in vivo assessment of IgE –sensitization to pollen allergens recommended testing for weed pollen from herbaceous plants typical for temperate regions, Ambrosia artemisiifolia and Artemisia vulgaris, and Mediterranean areas, Parietaria officinalis. Other weed pollen extracts are not included in the screening inhalation panel, but some authors suggested that different weed may be of significance in countries like Spain, Italy and Greece.

METHODS

We determined the extensive specific IgE antibody profile to aeroallergens in sera of allergic rhinitis patients. Serum IgE in vitro assessment was performed using new method with membrane strips coated with thin parallel lines of several purified and characterized aeroallergens used as solid phase. The membranes were fixed as onto synthetic foil. If the sample was positive, specific antibodies in the serum sample attached to the allergens coupled to the solid phase. In the second incubation step, the attached specific IgE antibodies react with alkaline-phosphatase-labelled anti-human antibodies, and in the third step, the bound antibodies were stained with chromogen/substrate solution capable of promoting a color reaction. Euroline inhalation profile was used for assessment of serum specific IgE to grass pollen, tree pollen, and weed pollen (w1 common ragweed, w6 mugwort, w9 plantain), along with Dermatophagoides spp mites, cat, dog, and horse epithelia and ascomycetes.

RESULTS

From a total of 196 sera from adults patients with allergic rhinitis from Southern Romania, a region with temperate-continental climate, the rate of in vitro IgE sensitization to Plantago lanceolata weed pollen was 4.59%, while IgE sensitization to Asteraceae weed pollen was higher and important (19.9%), with or without other sensitizations. This supports the recommendations of the pan-European inhalation test panel, but suggests that over weed pollen extracts, such as narrowleaf plantain / ribwort (Plantago lanceolata), may be used depending on local climate characteristics.

CONCLUSION

In this Southeastern Central European region with important plant biodiversity, patients with allergic rhinithis are sensitized in vitro especially to Asteraceae weed pollen, and to a lesser degree to Plantago lanceolata weed pollen.

Autoimmune diseases, autoimmunity, allergy

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THE MOST COMMON FOOD ALLERGENS IN THE PEDIATRIC POPULATION ON THE SLOVENIAN COAST

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BACKGROUND-AIM

IgE antibodies appear in human blood as a result of sensitization to specific allergens. The purpose of the study was to review the specific antibodies IgE in the pediatric population on the Slovenian coast in the period from 2005 to 2014. The medical examination of children and adolescents was carried out in the pediatric clinic. Those individuals who showed clinical signs that could result from allergies were sent to the laboratory confirmation for the presence of IgE antibodies. The pediatric research group included 1625 subjects aged from 27 days after birth to 18 years.

METHODS

Specific antibodies IgE to food allergens were determined by ImmunoCAP Phadia® 100 analyzer (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden). Qualitative measurements of specific antibodies IgE were performed with CAP System FEIA. The presence of antibodies IgE in serums was first analyzed with the screening test fx5. ImmunoCAP multi-test fx5 is the food allergy screening test for common childhood food allergens. It includes the commonest 6 allergy-provoking foods. All serums with positive screening results were assayed for the specific antibodies IgE to cow's milk, hen’s egg white, peanuts, soybean, wheat and codfish.

RESULTS

The main laboratory research group included 454 serums with positive screening test fx5 results. In 65% of those results we confirmed the increased presence of specific antibodies IgE on milk. 203 individuals (45%) demonstrate an allergic response to egg white. The presence of specific antibodies IgE to peanut and wheat had almost the same percentage of children (22%). Allergic hypersensitivity to soybean had 17% of children in our research group. The lowest proportion of children (3%) had present specific antibodies IgE to codfish.

CONCLUSION

The results of our review show that most allergies to milk appear in early childhood, in the first year of life the most, less in the second and third year. Allergic sensitization to egg white, peanuts, wheat and soybeans also occurs in the first year of life, but at a lower percentage. The presence of specific antibodies IgE to codfish occurs in older children in the coastal region of Slovenia.

Autoimmune diseases, autoimmunity, allergy

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RESULTS OF COMPARISION OF IMMULITE 2000 3G ALLERGY SPECIFIC IGE ASSAY WITH PHADIA IMMUNOCAP SYSTEM FOR SPECIFIC IGE

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BACKGROUND-AIM

Allergy is a malfunction of the immune system that causes a reaction to normally harmless substances (allergens). In vitro testing is commonly used to diagnose and manage allergies.
The aim of this study was to compare allergen specific IgE levels derived from two different assay systems.

METHODS

Forty patients from Pula General Hospital Allergy practice were included. Specific IgE levels were measured on Phadia 100 ImmunoCap system (Thermo Fisher Scientific, Uppsala, Sweden) and Immulite 2000 (Siemens, UK). Specific IgE levels were measured for Dermatophagoides petronyssinus (d1), egg (f1), Dactylis glomerata (g3), Fraximus americana (t15), Ambrozia elator (w1), Cat Dander-Epithelium (e1), Chicken Meat (f83), Peanut (f13), Walnut (f256), Alternaria alternata (m6).

RESULTS

For all tested allergens, values greater than 0.35 kU/L were considered positive. Test results were classified into one of seven classes; Class 0 being negative and class 6 highly positive. For values <0,35 kU/L which were classified as negative and those >100 kU/L which belong to class 6, both systems gave similar results and enable correct classification. The exception was t15 where 3 of the 8 cases that tested negative with Immulite tested positive with Phadia. According to measured values for d1, on both Phadia and Immulite, 23 out of 40 patients were positive, among whome 5 were classified at higher class per Immulite results. For f1, among five positive cases, Immulite classified four at higher and one at lower class. For g3, from eight positive cases, six resultes had same classification as Phadia did and two were classified as higher, per Immulite. For e1, f83, all the positive cases were ranked one class higher, according to Immulite. For f13, m6, values measured on both Phadia and Immulite were classified equally.

CONCLUSION

These results showed differences among measured values of specific IgE levels for some allergens, which tested with Immulite showed slightly increased values, although only among positive cases. Nevertheless, the differences are not decisive in diagnostic procedure.

Autoimmune diseases, autoimmunity, allergy

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OTHER ANTIPHOSPHOLIPIDS ANTIBODIES IN SERONEGATIVE ANTIPHOPSHOLIPID SYNDROME ?

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BACKGROUND-AIM

Antiphospholipid Syndrome (APS) is an autoimmune disease that leads to arterial and/or venous thrombosis, recurrent pregnancy loss and persistently positive aPLs (Lupus Anticoagulant, anti-cardiolipin and anti-beta2glycoprotein1 (aB2GP1)). Many patients with clinical manifestations highly suggestive of APS are persistently negative for 'criteria' aPL. They are classified as having seronegative APS (SNAPS). However, they could have antibodies against other epitopes such as other phospholipids or cofactor/phospholipid complex. Lack of standardisation and sensibility/ specificity of existing assays for detecting relevant aPL bring us to test other epitopes.
The aim of our study is to focus on 2 epitopes to evaluate their utility in SNAPS: the B2GP1 Domain 1 (D1) and the complex Phosphatidylserine/Prothrombin (PS/PT).

METHODS

Our study is based on data from 83 patients selected on following criteria: patients with obstetricals manifestations (recurrent pregnancy loss, intrauterine fetal death (IFD), premature deliveries...), which were negative for the classical aPL (SNAPS, n = 55), patients follow up for known APS (SPAPS, n=28). The aD1 IgG were detected with a Chemiluminescent Assay (CLA) (QUANTAFlash®, Inova Diagnostics). The aPS/PT IgG and IgM were measured with a commercially kit ELISA (QUANTALite®, Inova Diagnostics). Data were compared with the previous results for aB2GPI obtained with the CLA technique (QUANTAFlash®, Inova Diagnostics), other biological criteria and clinical manifestations.

RESULTS

Our results show no significant differences between the aD1 and the classical aB2GPI (9 positive vs 14 positive, respectively). We find 3 new positive with aD1: 2 SPAPS, 1 SNAPS.
For aPS/PT, our data don't show significant difference with other aPL (10 positive vs 14 respectively for the IgG; 14 positive vs 14 for the IgM). We find 3 new positive for IgG (3 SPAPS) and 4 new positive for IgM (2 SPAPS, 2 SNAPS). About the two SNAPS positive for aPS/PT, they presented obstetricals manifestations (IFD, pre-eclampsia, respectively, both with vascular placental abnormalities).

CONCLUSION

These results show that we are able to improve the diagnosis in only a few cases of SNAPS. Further more studies might be necessary to confirm, or not, the clinical relevance of these two tests.

Autoimmune diseases, autoimmunity, allergy

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MULTIPLE SCLEROSIS LIKE-DISEASE IN SJÖGREN'S SYNDROME: CLINICAL ASSOCIATION OR MIMICRY?

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BACKGROUND-AIM

Risk factors for CNS (central nervous system) involvement in primary Sjögren's syndrome (SS) are poorly known. We have studied the correlations of CNS involvement with demographic, clinical and laboratory features in SS.

METHODS

One hundred and six consecutive SS patients (2002 American-European criteria) aged 18-69 years were evaluated in a cross-sectional study. Patients with other associated connective tissue diseases and/or lupus-specific autoantibodies (anti-dsDNA, anti-Sm and anti-Rib-P) were excluded. At study entry, all patients were assessed using standardized clinical and laboratorial protocols, including anti-Ro(SS-A)/anti-La(SS-B), rheumatoid factor, anti-alpha-fodrin, IgG/IgM anticardiolipin, IgG/IgM anti-beta2-glycoprotein-I and lupus anticoagulant. Patients with CNS involvement were also examined by an expert neurologist, and they underwent brain/spinal cord magnetic resonance imaging and cerebrospinal fluid analysis.

RESULTS

Seven of 106 patients (6.6%) had CNS involvement, all of them with focal/multifocal manifestations- multiple sclerosis-like disease (n=3), myelitis (n=1), stroke with antiphospholipid syndrome (n=2) and optic neuritis plus hypophysitis (n=1). Comparison of patients with and without CNS involvement revealed similar mean age (47.7±13.4 vs. 48.2±10.5 years, p=0.902), female predominance (100 vs. 97.0%, p=1.000) and disease duration (p=0.837). Frequencies of SS involvements (parotiditis, arthritis, cutaneous vasculitis, small airways disease, renal tubular acidosis, lymphoma) and cardiovascular risk factors were also comparable in the both groups (p>0.05). Conversely, livedo reticularis (LR) (57.1 vs. 7.1%, p=0.002) and lupus anticoagulant (42.9 vs. 5.1%, p=0.009) were more frequent in those with CNS involvement.

CONCLUSION

The association between CNS involvement in SS and the presence of LR observed herein suggests that a vascular injury may have a role in the pathogenesis of CNS damage. Supporting this notion, LR was observed in more than one third of patients with connective tissue disorders referred for multiple sclerosis like-disease to a neurological clinic, and SS was the most frequent diagnosis in these patients.

Autoimmune diseases, autoimmunity, allergy

M163

RARE AUTOANTIBODIES ASSOCIATED WITH DIFFUSE SYSTEMIC SCLEROSIS AND AGRESSIVE INTERSTITIAL LUNG DISEASE.

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BACKGROUND-AIM

Scleroderma (SSc) is an autoimmune disease characterized by the formation of scar tissue in skin and fibroblastic disorders. Localized scleroderma (LS) is associated to the presence of anti-centromere (ACA), anti-PM-Scl and anti-Th/To antibodies, while diffuse scleroderma (DS) is associated with the presence of anti-Scl70, and less frequently to anti-PM-Scl of 75 kDa, anti-RNA polymerase III (RNApIII), anti-NOR90 and anti-fibrillarin, which also must be taken into account. Our goal is to present the importance of the study of all the antibodies associated with a given condition clinically suspected and the observation of a characteristic pattern by indirect immunofluorescence (IF) and immunoblotting (IB) assays.

METHODS

24 year old woman who comes to the emergency department with disnea and cough with off-white expectoration. She reported the feeling of skin tightness on her face and a mouth opening capacity reduction. Two years ago she was hospitalized for pneumonia accompanied by hypopigmentated lesions on her upper body.

RESULTS

The presence of anti-NOR90 and anti-RP11 antibodies was foud by IF and IB.
Discussion: Anti-RNA polymerase antibodies are directed against the protein complex of RNApIII. RP11 and RP155 are antigenic recombinant proteins from the complex of RNApIII. Antibodies against these proteins are associated to serious forms of LS with pulmonary affectation, skin disease and sclerodermic renal crisis (SRC). Anti-NOR90 antibodies are very rare, even though their presence is associated with scleroderma pulmonary complications. In our case, the presence of the antibodies anti-NOR90 and anti-RP11 was associated to an aggressive ILD, leading to an early death. This was probably the reason why she did not develop a SRC, which is the classical clinical complication associated to the presence of anti-RP11.

CONCLUSION

It is important to determine all the antibodies related to SSc whenever there is a clear clinical suspicion, and not just to limit the study to the most frequent antibodies, such as ACA or anti-Scl70.

Autoimmune diseases, autoimmunity, allergy

M164

ASSOCIATION OF CD36 T188G POLYMORPHISM WITH ATHEROGENIC INDEX IN MEXICAN PATIENTS WITH RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects synovial joints and the main causes of death are cardiovascular complications. CD36, a type B scavenger receptor, is postulated as a key molecule in the development of atherosclerosis. A polymorphism in exon 10, the T188G allele, results in decrease of CD36 and increase of total cholesterol (TC) and atherogenic index. The aim of this study was analyze association of CD36 T188G polymorphism with atherogenic index, cytokines and CD36 membrane expression on monocytes in RA mexican patients.

METHODS

Transversal analytical study. We included 62 mexican mestizo RA patients who underwent lipid profile, quantifying TNF-α and IL-6 and monocyte CD36 expression. The identification of polymorphism was performed by PCR- RFLP with Nde I enzyme. Comparisons between means were performed using T test and was considered significant $p<0.05$.

RESULTS

T188G CD36 polymorphism was in Hardy-Weinberg equilibrium in mexican mestizo population. The genotype frequencies for TT, TG and GG were 62.9%, 37.1% and 0%. Significant differences between the TG and TT genotypes were found in TC mg/dL (180.84 ± 53.50 vs. 246.12 ± 42.82 p= 0.01), HDL mg/dL (54.16 ± 12.81 vs. 43.84 ± 5.95 p<0.001), atherogenic index TC/HDL (3.92 ± 1.09 vs. 5.48 ± 2.20 p < 0.001), TNF-α pg/mL (26.03 ± 5.38 vs. 32.77 ± 12.30 p=0.04), IL-6 pg/mL (18.54 ± 14.09 vs. 45.07 ± 34.95 p=0.01) and mean fluorescence index for CD36 (89.56 ± 65.62 vs. 68.53 ± 68.53 p=0.05).

CONCLUSION

Mexican RA patients carriers of TG genotype of CD36 T188G polymorphism exhibit high atherogenic index that may be associated with decreased of CD36 expression on monocytes and increase levels of proinflammatory cytokines TNF-α and IL-6.

Autoimmune diseases, autoimmunity, allergy

M165

COMPARISON OF THE RELIABILITY OF CELIAC DISEASE SEROLOGY TO REFLECT INTESTINAL DAMAGE

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BACKGROUND-AIM

In view of the increasing importance of the serological biomarkers for the screening and diagnosis of celiac disease, their differential performance, and the lack of head to head comparison, the reliability of those isolated or combined antibodies to reflect the intestinal damage in children with CD was evaluated.

METHODS

95 pediatric CD patients (mean age 8.3), 45 nonspecific abdominal pain children (AP) (mean age 7.3), 99 normal children (NC) (mean age 8.5) and 79 normal adults (NA) (mean age 28) were tested by the following ELISAs, detecting IgA, IgG or both, IgA and IgG: AESKULISA® Gliadin (AGA), AESKULISA® tTg (tTG; RUO), AESKULISA® DGP (DGP) and AESKULISA® tTg New Generation (tTg complexed to gliadin=tTg-neo). The results were compared to the degree of intestinal injury, using revised MARSH criteria. Scatter diagrams and regression analysis comparing the 12 antibodies' optical density (OD) activities to the degree of the intestinal damage were correlated.

RESULTS

Most of the assays were able to differentiate patients with low and high degree of intestinal damage. Comparing the different correlations between CD associated IgA and IgG antibodies' isotypes, the tTg-neo IgA ($r2=0.968$, $p<0.0025$) and tTg-neo/DGP IgGs ($r2=0.989$, $p<0.0001$/ $r20.985$, $p<0.0001$, respectively) stood out, significantly, as the best indicators of the intestinal damage in CD.
The highest OD values (medium 2.94±1.2, $p<0.0001$) were achieved by using the tTg-neo IgA ELISA in patients with MARSH 3c.

CONCLUSION

It is suggested that tTg-neo IgA/IgG antibodies should be preferably used to reflect intestinal damage during screening, diagnosing and monitoring compliance in childhood CD.

Autoimmune diseases, autoimmunity, allergy

M166

INDUSTRIAL FOOD ADDITIVE MICROBIAL TRANSGLUTAMINASE IS IMMUNOGENIC IN CHILDREN WITH CELIAC DISEASE

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BACKGROUND-AIM

Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules. It is a family member of human tissue transglutaminase (tTg), involved in CD. Despite declarations of mTg safety, direct evidence for immunogenicity of the enzyme is lacking.

METHODS

The serological activity of mTg, tTg, gliadin complexed mTg (mTg neo-epitope) and gliadin complexed tTg (tTg neo-epitope) were studied in: 95 pediatric celiac patients (CD), 99 normal children (NC) and 79 normal adults (NA). Sera were tested by ELISAs, detecting IgA, IgG or both IgA and IgG: AESKULISA® tTg (tTg), AESKULISA® tTg New Generation (tTg neo-epitope (tTg-neo)), microbial transglutaminase (mTg) and mTg neo-epitope (mTg-neo). MARSH criteria were used for the degree of intestinal injury.

RESULTS

Comparing pediatric CD patients with the 2 normal groups: mTg-neo IgA, IgG and IgA+IgG antibody activities exceed the comparable mTg ones ($p<0.0001$). All mTg-neo and tTg-neo levels were higher ($p<0.001$). tTg IgA and IgG+IgA were higher than mTg IgA and IgA+IgG ($p<0.0001$). The levels of tTg-neo IgA/IgG were higher than tTg IgA/IgG ($p<0.0001$). The sequential antibody activities, reflecting best the increased intestinal damage, going from MARSH 0 to MARSH 3c were: tTg-neo IgG ≥ mTg-neo IgG > mTg-neo IgA+IgG > tTg-neo IgA. Taken together, mTg-neo IgG and tTg-neo IgG correlated best with intestinal pathology ($r2=0.989$, $r2=0.989$, $p<0.0001$, $p<0.0001$, respectively).

CONCLUSION

mTg is immunogenic in children with CD and by complexing to gliadin its immunogenicity is enhanced. Anti-neo-epitope mTg antibodies correlate with intestinal damage to the same degree as anti-tTg. Further studies are needed to explore the pathogenic potential of anti-mTg antibodies in CD.

Autoimmune diseases, autoimmunity, allergy

M167

CLINICAL FEATURES OF IGG4-RELATED DISEASE: A DESCRIPTIVE STUDY

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BACKGROUND-AIM

IgG4-related disease (IgG4-RD) is an increasingly recognized syndrome of unknown etiology comprised of a collection of disorders that share specific pathologic, serologic, and clinical features; most often occurring in middle-aged and older men.Is a potentially multiorgan disorder, the commonly shared features include tumor-like swelling of involved organs, a lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells, variable degrees of fibrosis that has a characteristic "storiform" pattern. In addition, is characterized by elevated serum IgG4 concentrations in the majority of cases (60-70%). Objective: to report the clinical and epidemiological characteristics in patients with high serum IgG4 level.

METHODS

Measurement IgG4 levels in serum samples by Immunonephelometry (BNII (SIEMENS®).Whose values were above the normal range(8.00-140.00 mg/dL) were included into the study.Statistical analysis with SPSS.

RESULTS

753 samples were measurement from our usual laboratory routine, mainly: Internal Medicine, Oncology and Digestive Services.9.69% (n=73) had high IgG4 levels (>140mg/dL)(mean level:319.26 mg/dL)
1) Demographic characteristics: a) female :40.27 % (n = 29); mean age 52 years b)male: 59.72% (n=43); mean age 48 years.
2) Clinical features:
a. Allergic disease (rhinitis, chronic eczema,bronchial asthma): n=19 (26.03%)
b. Lymphomas: n=12 (16.43%)
c. Lung disease: n=10 (13.69%)
d. Liver disease (hepatic steatosis and cholestasis):n = 9 (12.32%)
e. Infiltrated gastric (chronic gastritis): n=8 (10.95 %)
f. Lymphadenopathy: n=8 (10.95 %)
g. Urogenital disease (hyperplasia of prostate, testicular and prostate carcinoma): n=8 (10.95 %)
h. Thyroid disease: n=7 (9.58%)
i. Dermal disease (psoriasis, dermal infiltrate): n=6 (8.21%)
j. Pancreatic disease (pancreatitis, carcinoma): n=6 (8.21%)
k. Kidney disease: n=5 (6.84%)
l. Vascular pathology: n=5 (6.84 %)
m. Retroperitoneal Pathology: n=3 (4.10 %)
n. Other (rheumatoid arthritis, osteoarthritis, valvular disease, abortion repetition): n=11 (15.06%)

CONCLUSION

Our study shows results agree with another autors: IgG4 RD is a chronic, systemic and multiorgan inflammatory disorder.Due to its emerging and newly discovered character more studies are needed to better define the set of clinical and epidemiological manifestations

Autoimmune diseases, autoimmunity, allergy

M168

ONCONEURONALES ANTIBODIES AND ITS ASSOCIATION WITH PARANEOPLASTIC SYNDROMES AND TUMORS.

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BACKGROUND-AIM

The paraneoplastic neurologic syndromes (PNS) define a series of alterations of the nervous system associated with tumors,it affect less than 1% of cancer patients and represent from 1 to 7% of its neurological disorders, but not as a result of metástasis, complications or side effects of cancer treatment, but as a result of immunological disorders associated with specific antibodies call onconeuronales antibodies(OAB). There is a group that can be defined as well characterized which can limit its differential spectrum and to facilitate early diagnosis and treatment since they may precede the diagnosis of neoplasia in more than half of the cases. The aim is select positive OAB from the total number of requests received from June 2010 to April 2014 and its possible association with a clinical syndrome, tumor or PNS diagnosed.

METHODS

Inmuno Dot Blot (ravo Diagnostika®) for the detection of OAB: anti-HU, anti-Yo, anti-Ri, anti-CV2,anti- amphiphysin, anti-Ma1 and anti-Ma2, in serum samples analyzed by prior dilution and incubation with IgG Conjugate and substrate solution.

RESULTS

We analyzed 368 samples and 8 (2,2%) were positive with the following results:
-Anti-HU, 3 patients (37,5%): one with PNS associated with small cell lung carcer (SCLC), another had a limbic encephalitis and possible testicular tumor pathology in study; and other, peripheral neuropathy without tumor pathology so far;
-Anti-CV2, 3 (37,5%): one patient diagnosed of SCLC and carcinoma of prostate with associated sensory neuropathy; another with sensory neuropathy and one with dementia and psychosis.
-Anti-Ma2, 1 (12,5%) with ataxic cerebellar paraneoplastic syndrome and diagnosed of gastric carcinoma.
-Anti-amphiphysin and anti-HU, 1 (12,5%) with SCLC and peripheral neuropathy including quadriplegia and quadriparesis.

CONCLUSION

In our study all positive OAB had a SPN, most with antibodies characteristic associated primary tumor, and others in monitoring and study of a possible neoplasia associated to early diagnosis and treatment. The presence of neurological syndromes should alert to the possibility of a PNS where the determination of OAB is useful since they´re oriented towards the search for a hidden neoplasm with the subsequent benefit for the patient.

Autoimmune diseases, autoimmunity, allergy

M169

AUTOANTIBODIES PROFILE IN SYSTEMIC SCLEROSIS USING IMMUNOBLOTTING.

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BACKGROUND-AIM

One of the hallmarks of systemic sclerosis is the presence of serum autoantibodies against a variety of nuclear and cytoplasmic antigens. They are produced prior to the onset of clinical manifestation and are of predictive clinical value. The primary purpose of this study was to identify the autoantibodies profile in the scleroderma sera and the secondary goal was to determine the sensibility and specificity of those antibodies using a control group.

METHODS

A descriptive analysis was conducted in the period 2012-2014. Patients were divided in two groups. The study group was represented by those satisfying the ARA criteria for Systemic Sclerosis and the control group included patients with systemic lupus, rheumatoid arthritis, Jogren syndrome and inflammatory myopathies. Immunoblotting was used to determine the antibody profile which includes: Anti-centromere (ACA), Anti-topoisomerase 1 (Scl 70), Anti-RNA polymerase III, Anti-fibrillarin (anti-U3RNP), Anti-PM-Scl75 and Anti-PM-Scl100, Anti-U1RNP (anti-nRNP), NOR 90, Th/To, Ro52 and PDGFR. The control group was used to determine the sensibility and specificity of the antibodies. Data was analyzed by SPSS software (version 11.5 for windows).

RESULTS

A total of 29 patients with systemic sclerosis and 37 with other rheumatic diseases were included. 11.6% SLE, 11.6% rheumatoid arthritis and inflammatory myopathies respectively, 2% Jogren syndrome. Mean age was 55.7 years in the study group versus 54.6 years in the control group, 93.1% were women. Sensibility and specificity were Scl70 S=48.3%, E=51.7%, ACA S= 13.8%, E=86.2%, Nor90 S=3.4%, E=96.6%, PR155 S=3.4%, E=96.6%, Th/To S= 24.15, E=75.9%, PM-Scl75 S=6.9%, E= 93.1%, Ro-52 S=3.4%, E=96.6%, Ku S=6.9%, E=93.1, RP11 S= 20.7%, E=79.3%, fibrillarin S=3.4%, E=96.6%.

CONCLUSION

The immunoblotting may help in the evaluation of antibodies in systemic sclerosis. The antibodies Nor90, PR155, PM-Scl75, Ro52 and Ku showed a low sensibility but a high specificity in patients with systemic sclerosis.

Autoimmune diseases, autoimmunity, allergy

M170

PREVALENCE OF ANTI-TH/TO ANTIBODIES IN A SPANISH POPULATION AND THEIR CLINICAL FEATURES

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BACKGROUND-AIM

Anti-Th/To antibodies are anti-nucleolar antibodies that have been known for more than 25 years. Despite their clinical importance, these SSc autoantibodies have not been utilized clinically because of the unavailability of antibody testing. Therefore, whether anti-Th/To were also detected in other rheumatic diseases, their clinical significance remained unclear. This study was aiming at determining the prevalence and clinical relevance of the patients with anti-Th/To in various connective diseases (CTDs).

METHODS

Clinical data and serum samples of patients with CTDs were evaluated in the period January 2013 to January 2015. Anti-Th/To Abs were screened using the RNA immunoprecipitation assay and determined as positive if 7-2 and 8-2 RNA were immunoprecipitated. Data was analyzed by SPSS software (version 11.5 for windows).

RESULTS

A total of 100 samples were analyzed and anti Th/to were found in 11 patients (11%). Mean age was 55±13.4 years old. 7(63.3%) were detected scleroderma (SSc), 2 (18.1%) systemic lupus and 1(9.09%) rheumatoid arthritis and MCTD. 6(54.5) were detected interstitial pneumonia ($p<0.01$), 4(36.3%) were detected pulmonary fibrosis ($p<0.01$) and 1(9.09%) were detected primary pulmonary hypertension ($p>0.01$). Positive antinuclear antibodies were found in 10 patients (90%) with significant higher skin score and Scl70 antibodies were the most often associated with anti Th/To (81.8%). Raynaud´s syndrome and calcinosis were found in 7(63.3%) and 4(36.3%) patients and sclerodactyly, erythroderma and myositis in only one patient respectively.

CONCLUSION

Th/To antibodies has a low prevalence even though in our study they were specific for SSc. Anti-Th/To were related to the presence of interstitial pneumonia and pulmonary fibrosis but not with pulmonary hypertension

Autoimmune diseases, autoimmunity, allergy

M171

SERUM LEVEL OF MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF METALLOPROTEINASES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Background: Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play an important role in the remodeling of the joint tissues in rheumatoid arthritis (RA). We assessed serum level of MMP-2, MMP-3 and TIMP-1, TIMP-2 in relation to other laboratory parameters.

METHODS

Materials and Methods: 50 patients (41 women and 9 men) aged 25-79 years treated with non-steroidal anti-inflammatory (NSAIDs) and disease modifying anti-rheumatoid drugs (DMARDs), that fulfilled ACR-criteria for RA, were included. Patients were divided into four groups according to Steinbrocker scale: I°- 19 patients, II°- 18, III°- 5 and IV°- 4 patients. 30 healthy subjects (19 women and 11 men) aged 29-68 years were included as controls. Serum MMP-2, MMP-3, TIMP-1, TIMP-2 (R&DSystems), anti-CCP (Euroimmun) were determined by ELISA and CRP (Horiba, ABX Pentra 400) by latex-enhanced immunoturbidimetric assay. Statistical analysis was performed using Statistica 10.0 for Windows.

RESULTS

Results: In RA patients significantly higher levels of MMP-2 (257,03 ng/mL), MMP-3 (9,91 ng/mL), TIMP-2 (100 ng/mL), anti-CCP (1,07 RU/mL) and CRP (1,36 mg/L) were observed compared to controls (208 ng/mL; 7,48 ng/ml; 94,39 ng/mL; 0,68 RU/mL and 0,40 mg/L). In anti-CCP positive patients significantly higher values of anti-CCP (73,54 RU/mL) and MMP-3 (27,65 ng/mL) were found comparing to anti-CCP(-) (0,75 RU/mL and 9,40 ng/mL, respectively). MMP-3 (22,60 ng/mL), TIMP-1 (180,85 ng/mL), anti-CCP (1,90 RU/mL) and CRP (3,10 mg/L) levels were higher in patients with advanced disease compared with early and moderate stages (9,91 ng/mL, 152,2 ng/mL, 0,98 RU/mL, 1,31 mg/L). Significant differences between RA patients and controls were observed for the ratios MMP-2/TIMP-1 and MMP-2/TIMP-2 (p=0,04 and p=0,000001, respectively). Positive correlations were found between MMP-2 and TIMP-2 (R=0,86; p=0,000001), MMP-3 and anti-CCP (R=0,58, p=0,000009). MMP-2 showed a very good diagnostic utility (AUC 0,82; 95% CI: 0,73-0,92).

CONCLUSION

Conclusion: Measurement of MMP-2, MMP-3 and TIMP-1, TIMP-2 together with currently used laboratory biomarkers may have an essential diagnostic value for assessment of disease progression in patients with rheumatoid arthritis.

Autoimmune diseases, autoimmunity, allergy

M172

ASSESSMENT OF SPECIFIC ALLERGENS USING A 30 IGE SPECIFIC ANTIBODY PANEL IN A GROUP OF PATIENTS WITH FOOD AND RESPIRATORY ALLERGIES IN ALBANIA

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BACKGROUND-AIM

Allergies are a common cause of disease in Albania. The aim of this study was to identify the most common specific aeroallergens and food allergens that cause allergy symptoms in this population.

METHODS

We studied 331 patients who were referred by the physician for the determination of specific aeroallergens and 117 patients referred for the determination of food allergens. For each patient we determined serum total IgE with Architect ci8200 system. Serum specific IgE for aeroallergens and food allergens were determined using AlleisaScreen Panel 30 RespA and 30 FoodA by MEDIWISS Analytic GmBH: an immunoblot assay for the quantitative determination of circulating allergen specific IgE in human serum.

RESULTS

The results were divided into 3 groups: Negative: Class 0-I; Positive: Class II; High sensitivity: Class III-VI. In 331 patients tested with RespA we found that Acarus Siro had the highest prevalence of sensitization: 156 (47.1%), followed by Dermatophagus Pteronyssinus: 105 (31.7%), Dermatophagus Farinae: 77 (23.3%); Mixed Grasses: 76(23%) and Rye Pollen: 74 (22.4%). Dermatophagus Pteronyssinus had the highest rate of significant (Class III-VI) sensitization: 66 (20%), followed by Dermatophagus Farinae: 57 (17.2%), Acarus Siro: 51(15.4%), Mixed Grasses: 48 (14.5%) and Rye Pollen: 43 (13%). In 117 patients tested with FoodA we found that Horseradish Peroxidase had the highest rate of sensitization: 26 (22.2%), followed by Bromelain: 20 (17%), Pepper: 16 (13.7%), Ascorbat Oxidase: 15 (11.7%) and Rye Flour: 14 (12%). Horseradish Peroxidase was also the allergen that caused the highest rates of significant (Class III-VI) sensitization: 15 (12.8%) followed by Bromelain: 13 (11.1%), Pepper and Ascorbat Oxidase: 3 (2.6%).

CONCLUSION

The results of this study show that house dust mites and processed food mites like Acarus Siro, Dermatophagus Pteronyssinus and Dermatophagus Farinae helped by indoor damp conditions are the major causes that trigger respiratory allergies while common food additives and preservatives like Horseradish Peroxidase and Bromelain are the mayor causes that trigger food allergies in Albania.

Autoimmune diseases, autoimmunity, allergy

M173

25-HYDROXYVITAMIN D INSUFFICIENCY AND BIOMARKERS OF EOSINOPHILIC INFLAMMATION AT ASTHMA INCIDENCE IN CHILDREN

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BACKGROUND-AIM

Bronchial asthma is a chronic inflammatory disorder of the airways that is related to their hyperresponsiveness and remodeling, both contributing to variable degrees of airflow obstruction. The common host and environmental factors that affect the development and expression of asthma in children include the exposure to allergens and respiratory infections, chemical irritants and drugs, changes in lifestyle conditions, e.g. dietary habits, high prevalence of vitamin D insufficiency, decreased outdoor and indoor physical activity leading to excessive body weight. Evaluation of the relationship between asthma and 25-hydroxyvitamin D insuficiency must consider the association with biomarkers of asthma pathogenesis: airway remodeling, serum immunoglobulin E (IgE) and eosinophil count. We assessed the association of 25(OH)D with peripheral blood eosinophil counts, serum IgE and periostin, in children at asthma diagnosis.

METHODS

The study included 160 children aged 2-12 yrs. Atopic asthma was diagnosed in 110 children, non-atopic in 10; in 40 children asthma was excluded (reference group). Fasting blood was collected for cell counts, serum was obtained to measure C-reactive protein (hsCRP), 25(OH)D, periostin, total IgE, lipid profile.

RESULTS

Children with atopic asthma had lower 25(OH)D ($p<0.0001$). Significantly elevated IgE concentration, eosinophil counts and a trend to higher periostin ($p=0.06$) were found in asthmatics. Periostin and CRP were significanly higher in 25(OH)D-deficient children with atopic asthma ($P=0.018$; $P=0.032$); moreover, periostin and IgE concentrations were significantly higher in the eosinophil-high subgroup, whereas a tendency to lower 25(OH)D was observed. 25(OH)D insufficiency and IgE concentration were significant predictors (OR=3.0; OR=9.04) of atopic asthma.

CONCLUSION

25(OH)D monitoring is essential in prevention of pediatric asthma as there is evidence of the association between 25(OH)D insufficiency, the risk of eosinophilic inflammation and atopy.

Autoimmune diseases, autoimmunity, allergy

M174

HASHIMOTO`S THYROIDITIS-RELATIONSHIP BETWEEN SERUM TSH,FT4, (TGAB), (TPOAB) AND IMUNOGLOBULIN G

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BACKGROUND-AIM

Hashimoto´s thyreoiditis (HT) is one of the autoimmune diseases of the thyreoid gland. It is characterized by diffuse lymphocytic infiltration of the thyroid gland and the elevated levels of the serum thyreoglobulin antibody (TgAb) and thyroid peroxidasa (TPOAb). We aimed to evaluate the relationship between serum TSH,FT4, (TgAb), (TPOAb) and imunoglobulin G in our female population.

METHODS

From 01.January 2014 to 30.June 2014, 44 women's were grouped into two age groups:
I group 20-40 years old (n=22) and II group 40-60 years old (n=22). Serum levels of TSH, FT4, (TgAb) and (TPOAb) were measured by immunochemiluminesce using Cobas e 411 Roche . Imunoglobulin G was measured by immunoturbidimetric method using Beckman Coulter AU-680.
The diagnostic assessment of the thyroid gland done by thyreodologist was consisted of physical exam thorough anamnesis of the patients. Thyroid ultrasound exam was done on every each of the patients.

RESULTS

Median relative quantification values were: TSH was positively correlated with IgG 63% and FT4 was negatively correlated with IgG 91%.TgAb and TPOAb were positively correlated 87% with IgG.
I group: TSH 2,4 ± 0,8 μIU/ml, FT4 17,8 ± 3,2 pmol/l,
TgAb160 ± 16 IU/ml,TPOAb 310± 47 IU/ ml IgG 10± 3g/l
II group: TSH 3,6 ± 0,9 μIU/ml, FT4 18 ± 4,1 pmol/l,
TgAb234 ± 36 IU/ml,TPOAb 48o ± 43 IU/ ml IgG12,8 ± 4g/l.

CONCLUSION

In a cross-sectional analysis, intensity of autoimmunity as indexed by (TgAb), (TPOAb) was more closely correlated with the elevation of IgG than the degree of hypothyroidism as indexed by serum TSH concentration. Women's in the group between 40-60 years had statistically significant higher values (TgAb), (TPOAb) and correlation with IgG.

Autoimmune diseases, autoimmunity, allergy

M175

IL-1β AND IL–6 ARE DOMINANT CYTOKINES IN SLE SUBTYPE BASED ON PEDIGREE STUDY

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BACKGROUND-AIM

Systemic lupus erythematous(SLE) is a typical autoimmune disease involving multiple organs. SLE patients with severe heterogeneous themselves have quite different pathogenic factors. In order to reduce the otherness of SLE patients, some scholars are committed to stratification analyses on gene type, laboratory tests or clinical characteristic. That means SLE patients should be divided into different subtypes to search the main pathogenic factors easily. The patients from a family maybe represent a subtype of SLE according to the gene factor. The dominant cytokines and pathogenic characteristics were analyzed for the SLE subtype based on pedigree study.

METHODS

Ten cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17, IFN–γ, IP-10, MCP-1, MIP-1β and RANTES) were detected for SLE patients (3 in a family and 108 sporadic patients) and 80 healthy controls. The dominant cytokines were filtered form patients in SLE family and validated in the screened SLE patients with the same tests model as patients in the family (called as screened patients). The association was analyzed between dominant cytokines and laboratory tests. Mann-Whitney test was used between two groups and Kruskal-Wallis H test were compared among multiple groups for non-normal distribution data. Pearson correlation analysis was used for correlation analysis. Probability value (P) less than 0.05 was considered to be statistically significant. All data were analyzed by SPSS 19.0.

RESULTS

The rheumatoid factor (RF), IgE and antinuclear antibody (ANA) are positive in SLE family patients and 5 SLE cases were screened from 108 sporadic SLE patients(4.63%)according to the model. Both IL-1β and IL-6 are significantly higher in patients of SLE family and screened patients($P<0.05$)than other patients and healthy controls. The IL-1β is significantly higher in anti-dsDNA positive patients than negative cases and increase along with SLEDAI score($P<0.05$). The IL-6 is correlated with IgE and IL-1β in SLE patients($P<0.05$). No correlativity was found among IL-1β, IL-6 and ANA, anti-Sm etc.

CONCLUSION

IL-1β and IL-6 were both dominant cytokines of SLE subtype with positive ANA, RF and IgE. IL-1β was related to anti-dsDNA and the SLEDAI score, IL-6 and IgE or IL-1β were same. IL-1β and IL-6 may had interdependent and cooperative relations in pathogenesis of SLE subtype and played an important pathogenic role in similar family of SLE subtype so that intervening IL-1β and IL-6 maybe become an effective method to treat this kind of SLE subtype.

Autoimmune diseases, autoimmunity, allergy

M176

ANTINUCLEAR AUTOANTIBODIES IN SERUM OF PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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BACKGROUND-AIM

Primary biliary cirrhosis (PBC) is a slowly progressing cholestatic, autoimmune liver disease characterized by the presence of antimitochondrial (AMA) and antinuclear antibodies (ANA) in the serum. PBC-specific ANA can be used to confirm the diagnosis of PBC, especially in AMA-negative cases. Some of ANAs targets promyelocytic leukemia protein (PML) nuclear body components such as Sp100, PML and Sp140, part of them targets the nuclear envelope (NE) proteins. We detected the autoantibodies reactive against NE proteins (anti-gp210, anti-p62, anti-LBR), against components of PML nuclear body and antibodies characteristic for collagen diseases - anti-Ro52 and anti-centromere (ACA) in a well characterized group of polish PBC patients.

METHODS

Material - 160 PBC patients, 60 pathological controls - primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH), 30 healthy blood donors. AMA, anti-Sp100, anti-PML, anti-gp210, anti-Ro52 antibodies and ACA were detected by commercially available kits (IMTEC-Human, Euroimmun; Germany and Inova Diagnostics; USA). The ELISA “in-house” test was established for the detection of anti- Sp140 and anti-p62 antibodies.

RESULTS

Anti-Sp140, anti-Sp100 and anti-PML antibodies were present in 28%, 34% and 35% respectively in PBC patients, also in AMA negative cases. Anti-Sp140 antibodies were found together with anti-Sp100 and anti-PML antibodies in 16 cases. Anti-gp210 antibodies were detected in 45% of PBC patients, in AMA –negative group at a frequency over 55% . Anti-p62, anti-LBR antibodies were detected in 25% and 9% respectively in PBC sera. The specificity of these tests was about 98-99%. Positive results of anti-Ro52 were obtained in 42% PBC subject. ACA were found in 9% patients. Some of patients showed multiple specificities.

CONCLUSION

PBC sera contain antibodies which recognize various nuclear protein, particularly antibodies against gp210 and p62 are highly specific for PBC. They can aid in the serologic diagnosis, especially in cases in which AMA are not detectable. The very frequent coexistence of different antibodies suggests an autoimmune reaction against multiple nuclear components in some of PBC patients. Part of PBC individuals with positive anti-Ro52 antibodies and ACA did not show any symptoms indicating collagen diseases.

Autoimmune diseases, autoimmunity, allergy

M177

EPITOPES OF HUMAN AND MICROBIAL TRANSGLUTAMINASES ARE SIMILARLY RECOGNIZED BY CELIAC DISEASE SERA

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BACKGROUND-AIM

The use of microbial transglutaminase (mTg) in the food industry is expanding alongside its ingestion in Western diet. Being a member of the human endogenous tissue transglutaminase (tTg), the mTg shares multiple functional similarities. However, immunogenic comparison of the two enzymes in celiac disease (CD) is lacking.

METHODS

Complexing mTg and gliadin results in mTg neo-epitopes (mTg-neo). These complexes were purified by asymmetric field flow field fractionation and confirmed by multi angle light scattering and SDS-PAGE. Sera of 81 CD patients (mean age 30 ± 17) and 81 healthy controls (mean age 29 ± 21) were analysed using the following ELISAs: AESKULISA® tTg New generation (tTg neo-epitopes) IgA and IgG, AESKULISA® Gliadin IgA and IgG, AESKULISA® DGP IgA and IgG and AESKULISA®s against mTg and mTg neo-epitopes (Research use only (RUO) kits as IgA and IgG).

RESULTS

Purified mTg-neo IgG and IgA (AUC=0.92, 0.93, respectively) showed an increased immunoreactivity compared to single mTg and gliadin ($p<0.001$) but similar immunoreactivity to the tTg-neo IgG and IgA ELISA (AUC=0.94, 0.95, respectively). Using a competition ELISA, the mTg neo-epitopes and tTg neo-epitopes have identical outcomes in CD sera both showing a decrease in optical density of 55±6%, ($p<0.0002$). Sera with high antibody titre [U/ml] against the tTg neo-epitope show also high antibody activities of the mTg neo-epitope and vice versa indicating the presence of similar epitopes within the transglutaminase-gliadin complexes.

CONCLUSION

mTg and tTg display a comparable immunopotent epitope. mTg neo-epitope IgA and IgG antibodies are immunogenic in CD. If substantiated, it will impact the food industry additive policy and regulation.

Autoimmune diseases, autoimmunity, allergy

M178

ANTI-NEO-EPITOPE TTG COMPLEXED TO GLIADIN ARE MORE RELIABLE THEN TTG FOR CELIAC DISEASE DIAGNOSIS

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BACKGROUND-AIM

The guidelines of ESPGHAN for the diagnosis of pediatric celiac disease (PCD) rely on anti-human tissue transglutaminase (tTg) as the prime and unique antibody for screening PCD population. None of the CD-associated antibodies has challenged tTg premiership. tTg complexed to gliadin presents neo-epitopes and antibodies against the complex are called tTg neo-epitopes (tTg-neo). Reliability of anti-tTg and tTg-neo antibodies in diagnosis of PCD was compared.

METHODS

95 pediatric CD patients (mean 8.3y), 99 normal children (NC) (mean 8.5y) and 79 normal adults (NA) (mean 28y) were tested using the following ELISAs detecting IgA, IgG or both IgA and IgG: AESKULISA® tTg (tTg; RUO) and AESKULISA® tTg New Generation (neo-epitope: tTg complexed to gliadin). The results were compared to the degree of intestinal injury, using revised MARSH criteria.

RESULTS

A significantly higher OD activity was detected for tTg-neo IgA, IgG and IgA+ IgG than for tTg ($p<0.0001$, $p<0.0001$, $p<0.001$, respectively). tTg-neo IgA, IgG correlated better with intestinal damage than tTg ($r2=0.968$, 0.989 compared to 0.909, 0.488 ($p<0.001$), respectively).

CONCLUSION

The tTg-neo IgA, IgG and IgA+IgG isotypes exhibited a higher OD activity and better reflected intestinal damage in PCD, compared to tTg isotypes. The tTg-neo IgA+IgG ELISA kit had higher sensitivity and a comparable specificity for the diagnosis of childhood CD. tTg-neo should be included in the ESPGHAN diagnostic flow chart.

Autoimmune diseases, autoimmunity, allergy

M179

CLINICAL SIGNIFICANCE OF ONCONEURONAL ANTIBODIES IN PATIENTS WITH NEUROLOGICAL SYMPTOMS

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BACKGROUND-AIM

Onconeuronal antibodies (OA) are strongly associated with cancer and paraneoplastic neurological syndromes (PNS). PNS can be defined as remote effects of cancer and are seen <1% of patients with cancer. Most of these antibodies are well-characterized (antibodies against Hu, Yo, Ri, CRMP5, amphiphysin, Ma-2 and Tr) and are in common use for the diagnosis of definite PNS. The aim of our study is to determine the percentage of OA detected in our Laboratory of Autoimmunity during last two years (2013-2014) and the possible association with PNS and tumor pathology.

METHODS

OA were studied on 421 patients with neurological symptoms during a period of two years. OA were identified in serum sample by indirect immunofluorescence (IIF, Euroimmun AG) and recombinant immunoblot assay (Ravo Diagnostika) that detects Hu, Yo, Ri, CV-2, Ma-1, Ma-2 and amphiphysin autoantibodies. One result is considered positive when it is confirmed by the two techniques.

RESULTS

OA were positive in 7 patients only (2%). The OA detected were: anti-Hu in 5 samples (72%), anti-amphiphysin in one sample (14%) and anti-Ma-2 in one sample (14%). Three positive results of anti-Hu corresponded to the same patient with multiple sclerosis and suspected of tumor pathology in which the OA were measured periodically during the two years of the study without finding associated neoplastic pathology. The PNS and tumor associated to the other four positive results were:
Patient 1 (man, 67 years): anti-Hu positive
PNS: paraneoplastic encephalitis
Tumor: lung adenocarcinoma
Survival: 19 months, still alive
Patient 2 (woman, 50 years): anti-Hu positive
PNS: paraneoplastic encephalitis
Tumor: small cell lung cancer
Survival: 7 months, exitus
Patient 3 (woman, 65 years): anti-Ma-2
PNS: Acute cognitive impairment
Tumor: breast cancer
Survival: 2 months, exitus
Patient 4 (man, 79 years): anti-amphiphysin
PNS: limbic encephalitis
Tumor: squamous cell lung carcinoma
Survival: 2 months, exitus

CONCLUSION

In our study the percentage of OA detected is very low (2%). Except one patient with multiple sclerosis, positive anti-Hu antibodies and absence of tumor pathology, the rest of the OA were associated with tumors and poor prognostic outcome.

Autoimmune diseases, autoimmunity, allergy

M180

EVALUATION OF "HISCL-TARC", A BIOMARKER FOR ATOPIC DERMATITIS, MEASURED BY AUTOMATED IMMUNOASSAY SYSTEM " HISCL-5000 "

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BACKGROUND-AIM

Atopic dermatitis (AD) is an inflammatory skin disease in which the inflammation is characterized by the influx of lymphocytes into the dermis. Thymus and activation regulated chemokine (TARC, CCL17) is one of CC chemokines, and is expressed predominantly by keratinocytes in the atopic dermatitis skin. Serum TARC levels are associated with disease activity of AD, and its measurement is epected for medical care of AD. Recently, a novel reagent for TARC measurement has developed, and it can be automated analyzed by HISCL®-5000 (Sysmex Co., Japan). Here we investigated the usefulness of the reagent coupled with the automated analyzer, HISCL®-5000.

METHODS

The fully-automated random-access chemiluminescence enzyme immunoassay system, HISCL®-5000 is based on a solid phase two-site chemiluminescent enzyme immunoassay (CLEIA). Here we evaluated analytical performance of the HISCL®-5000 measurement system of TARC. We used serum samples collected from our inpatients/outpatients, as well as control samples commercially available. This study has been approved by the ethical committee in Hamamatsu University School of Medicine. We compared HISCL®-5000 Immunoassay System and its dedicated reagents (Sysmex Co., Japan) with Alaport TARC microplate Enzyme Immunoassay analyzer (Shionogi & Co. Ltd., Japan) .

RESULTS

The Within-run precision for clinical samples at three concentrations measured 20 times were from 2.5 % to 3.7 % as CV. Linearity observed in high concentration range was up to 25,994 pg/mL. The minimal detection limit was 10 pg/mL. No significant interferences were observed with coexisting materials used Interference Check A Plus and Interference Check RF Plus (Sysmex Co., Japan). Regression and correlation were y = 0.98x + 29.7 and r = 0.995 (n=45).

CONCLUSION

The basic performances of TARC measurement system by use of HISCL®-5000 automated analyzer were satisfactory. We assessed the device useful for routine tests. Especially the device has some excellent properties, for example, easy handling and maintenance, reducing measurement time for reporting (17 min). The properties will give us an improvement in patient care.

Autoimmune diseases, autoimmunity, allergy

M181

TRANSCRIPTOMICS REVEALS ASSOCIATION BETWEEN PSORIASIS AND RHEUMATOID ARTHRITIS

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BACKGROUND-AIM

Autoimmune diseases have a complex genetic basis; multiple genes contribute to disease risk, each with generally modest effects independently. There is enough evidence to indicate that common genes underlie multiple autoimmune diseases. Previous studies point to a greater frequency of autoimmune diseases among patients with psoriasis than in the general population and many inflammatory autoimmune diseases are a result of derangements in multiple cytokine pathways. This study examined the association between psoriasis and rheumatoid arthritis, both of which are declared inflammatory autoimmune diseases.

METHODS

Four independent transcriptome data associated with psoriasis and rheumatoid arthritis were analyzed comparatively. Each dataset was statistically analyzed in order to identify differentially expressed genes (DEGs). Proteins encoded by DEGs were determined and integrated with protein-protein interaction data for further analyses and hub proteins were identified. Enrichment analyses were performed to map the interconnectivities between diseases and biological pathways.

RESULTS

Comparative analyses indicated that psoriasis and rheumatoid arthritis have 20 common DEGs. 12 of these DEGS have previously been linked to RA and 9 have been linked to psoriasis. Related pathways of these DEGs are: chemokine signalling pathways and Cytokine-cytokine receptor interaction. Main hubs for the PPI network are STAT1, CEBPD, MMP1 and SERPINA1.

CONCLUSION

This study provides additional insight into the molecular mechanism of autoimmune diseases: psoriasis and rheumatoid arthritis. Results indicate that psoriasis has a strong association with rheumatoid arthritis thus suggesting a common genetic cause between them. Further evaluation of other autoimmune diseases may lead to a common mechanism between these diseases.

Autoimmune diseases, autoimmunity, allergy

M182

EVALUATION OF ANA AND ANCA TESTING BY THE FULLY AUTOMATED IIF SYSTEM HELIOS®

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BACKGROUND-AIM

Indirect immunofluorescence (IIF) is the main technique for the detection of antinuclear antibodies (ANA) and antineutrophil cytoplasmic antibodies (ANCA). HELIOS® is the first fully automated IIF processor that is able to automatically prepare slides and perform automatic reading. Aim of the study was to determine the diagnostic performance of the HELIOS® in comparison to visual IIF for ANA and ANCA testing using positive/negative discrimination.

METHODS

425 samples including 218 routine samples, 70 ANA/ENA positives, and 137 healthy subjects were evaluated for ANA. 150 samples comprising 90 healthy subjects, 40 anti-PR3 or anti-MPO positive samples and 40 routine samples were evaluated for ANCA. Both evaluations were performed utilizing the HELIOS® system as well as manual microscopic evaluation by two different expert observers.

RESULTS

A good correlation was found for the IIF ANA interpretation by observers and the HELIOS® that was kappa=0.633 for ANA positive samples and kappa=0.657 for ANA negative samples. For the ANCA evaluation a 100% agreement was found for the healthy subjects and the PR3/MPO positive samples. For the routine samples a 95% agreement was observed between automated and visual IIF discrimination.

CONCLUSION

Thus, HELIOS® system has proved to be able to discriminate correctly positive/negative samples for ANA and ANCA compared to manual microscopic IIF performed independently by two experts, and its introduction in clinical practice may reduce inter-laboratory variability and time required to perform this test especially in high throughput laboratories.

Autoimmune diseases, autoimmunity, allergy

M183

ASSESSMENT OF FREE LIGHT CHAINS IN HCV POSITIVE PATIENTS WITH MIXED CRYOGLOBULINEMIA VASCULITIS UNDERGOING RITUXIMAB TREATMENT.

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BACKGROUND-AIM

Mixed Cryoglobulinemia (MC) is an HCV-related lymphoproliferative disorder, secondary to a systemic vasculitis of small vessels. Treatment with anti-CD20 monoclonal antibodies Rituximab (RTX) is proved to be very useful. Free light chain (FLC) κ/λ ratio and FLC patterns were associated with MC and/or B-non Hodgkin's lymphoma.
The aim of this study was to evaluate changes in serum free light chains (FLC) in HCV positive patients with related Mixed Cryoglobulinemia (MC) undergoing anti-CD20 monoclonal antibody Rituximab (RTX) therapy. Furthermore, we attempted to correlate FLC values with therapy response.

METHODS

We retrospectively enrolled 46 patients with HCV infection (26 females, 20 males), including 10 patients without signs/symptoms of MC-related vasculitis, 36 with MC-vasculitis. Clinical and biological data were recorded at baseline and six months after RTX treatment. Nephelometric measurement of serum FLCs was performed using a serum FLC assay.

RESULTS

The mean serum FLC-κ level was significantly higher in MC patients, compared to HCV patients without MC and to blood donors ($p=0.05$ and $p<0.0001$, respectively); the mean serum FLC-ratio was significantly higher in MC patients, compared to HCV patients without MC and to blood donors ($p=0.0023$ and $p=0.0008$, respectively). An abnormal FLC-ratio at baseline correlated with presence of cryoglobulins, C4 consumption, higher RF level and higher vasculitis rate ($p<0.005$ for each parameter).
In order to evaluate the predictive value of FLC patterns, MC patients were divided into two groups according to RTX therapy outcome (responders and no/partial responders).
Abnormal baseline FLC-ratio was significantly associated with no/partial response (OR 4.86 – 95% C.I. 0.89-28.72, $p=0.0314$).

CONCLUSION

RTX-treatment in HCV-related MC induces a reduction of FLC-κ and RF levels. Moreover, pre-treatment FLC-ratio, which can be easily assessed by a routine test, may be useful to predict response to this expensive treatment for HCV-related MC patients ineligible to IFN-based therapy.

Autoimmune diseases, autoimmunity, allergy

M184

ELEVATED PLASMA LEVEL OF PENTRAXIN-3 (PTX3) IN PEDIATRIC PATIENTS WITH CELIAC DISEASE (CD)

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BACKGROUND-AIM

BACKGROUND: PTX3 has a large number of multiple functions in different contexts.This protein plays an important role in innate immunity, inflammation, angiogenesis, fertility and it is also involved in the development of autoimmune phenomena. PTX3 behaves as an acute phase response protein: the blood levels (normally <2ng/ml) increase rapidly during sepsis and other inflammatory and infectious conditions. Plasma PTX-3 concentration is also elevated in patients with active inflammatory bowel diseases and in some autoimmune conditions linked to celiac disease. AIM: to investigate the plasma PTX3 concentration in patients with celiac disease and to elucidate the usefulness of plasma PTX3 levels as an inflammation marker compared to other well-known markers (C-reactive protein (sCRP), plasma calprotectin (pCP) and fecal calprotectin (fCP).

METHODS

METHODS: PTX3 level was measured in 28 symptomatic pediatric patients and in 20 healthy children (age range: 2-14 years) by an ELISA test. All the celiac patients had serum anti-transglutaminase antibodies IgA>100U/ml, anti-endomysium antibodies and HLA DQ2 and/or DQ8 (Eurospital, Italy). pCP and fCP were measured by the ELISA test (Eurospital); sPCR by an automated analyzer.

RESULTS

RESULTS: PTX3 concentration was significantly higher in celiac patients (3.161±1.781 ng/ml) that in normal controls (1.181±0.887ng/ml) (test t.Student,P<0.001). fCP levels were also significantly higher in CD group (67.5± 91.4 mg/kg vs 24.2±9.4 mg/kg; test t. Student,P<0.001); pCP was incremented (>6.25 ng/ml) only in 14% CD group, while sCRP did not differ significantly across the two groups (P=0.96; test t Student). Moreover there was not relationship among PTX3 concentration and the other inflammatory markers. Area under the ROC curve was 0.85 for PTX3 and 0.64 for fCP and pCP. Optimum diagnostic cut off value derived from the PTX3 ROC curve is 2 ng/ml: a concentration of PTX3 > 2 ng/ml had 75% sensitivity and 78.6% specificity. Positive predictive value was 71.4% and negative predictive value was 81.4%.

CONCLUSION

DISCUSSION: Due the limited sensitivity of the test, the dosage of PTX3 can not be used as a diagnostic test in celiac patients but it could be used as a complementary test to biohumoral inflammation markers already in use.

Autoimmune diseases, autoimmunity, allergy

M185

ANALYTICAL AND CLINICAL PERFORMANCE OF IMMULITE 2000 TSI ASSAY*

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BACKGROUND-AIM

Background: In Graves' disease (GD) hyperthyroidism, thyroid stimulating immunoglobulins (TSI) bind to the TSH receptor and mimic TSH stimulation of the thyroid gland. The TSH receptor contains a large extracellular domain that presents epitopes for a variety of autoantibodies, including TSI and thyroid blocking immunoglobulins (TBI). In contrast to TSI, TBI inhibit TSH stimulation of thyroid cells, leading to hypothyroidism. The IMMULITE® 2000 TSI assay is designed for the specific, quantitative detection of TSI in serum and plasma. The clinical utility of a TSI assay includes a determination of the autoimmune etiology of thyrotoxicosis, monitoring Graves' patient therapy, prediction of remission or relapse, confirmation of Graves' ophthalmopathy, and prediction of hyperthyroidism in neonates.

METHODS

Methods: The IMMULITE 2000 TSI assay is an automated chemiluminescent immunoassay with time to first result of 65 minutes. It employs a pair of recombinant human TSH receptor chimeras in a bridging format. The assay is traceable to WHO NIBSC 08/204.

RESULTS

Results: The detection limits of the assay were determined in accordance with CLSI EP17-A2 as follows: LoB = 0.03 IU/L; LoD = 0.06 IU/L; LoQ = 0.10 IU/L. A total of 842 serum samples from apparently healthy males and females were analyzed. The results suggest a nonparametric upper 97.5th percentile of 0.07 IU/L. The assay precision was evaluated according to CLSI EP5-A2. The repeatability %CV varied from 3.5% to 7.0% across the assay range. The IMMULITE 2000 TSI assay was compared to the Thyretain™ TSI Reporter BioAssay using 244 serum samples from GD and other thyroid or autoimmune disease patients with the following results: Positive Agreement: 100% (129/129); Negative Agreement: 92.2% (106/115); Overall Agreement: 96.3% (235/244). Serum samples from 236 treated and untreated GD patients, 138 individuals with other thyroid or autoimmune diseases and 200 apparently healthy individuals were evaluated. At 0.55 IU/L cut-off, the clinical sensitivity and specificity were 98.3% (232/236) and 99.7% (338/339), respectively.

CONCLUSION

Conclusions: The IMMULITE 2000 TSI assay is a sensitive quantitative immunoassay for the specific detection of TSI in the routine diagnosis and assessment of GD patients.
*Not available for sale.

Autoimmune diseases, autoimmunity, allergy

M186

SPECIFIC ACTIVITY OF MOLECULAR COMPONENTS IN ALLERGY: IMPLICATIONS FOR CLINICAL OUTCOME STRATEGIES

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BACKGROUND-AIM

The ratio of specific-IgE to total IgE (=specific activity, SA) is used as a prognostic parameter of clinical outcome in food allergy. Until now, this ratio was evaluated primarily for allergen extracts, but not for recombinant allergen proteins. The aim of this study was to investigate the value of SA for the recombinant proteins and compare it to the absolute values of specific-IgE measured on ImmunoCAP250 or ISAC, skin-prick tests (SPT) and clinical data. Furthermore, we evaluated whether the use of the SA ratio would be useful to make the two principal allergy providers more comparable.

METHODS

In 2013, from the pool of allergy patients (n=70) who visited Maasstad Hospital allergy policlinics and to whom ISAC was issued, 24 patients were included based solely on ISAC results. In all patients included, the specific-IgE (sIgE) of selected recombinant proteins and their extracts as well as total IgE were determined on the ImmunoCAP250 (Thermofisher Scientific) and Immulite 2000XPi (Siemens), and compared retrospectively to SPT and clinical data.

RESULTS

Comparison of recombinant proteins to their sIgE extracts showed that these extracts contained approximately 60-125% for ImmunoCAP250 and 25%-275% for Immulite2000XPi of the concerning proteins. The differences between manufacturers remained despite the use of SA. In food allergy, the SA for recombinant proteins (especially peanut) of approximately 10% appeared useful in detecting severe disease.

CONCLUSION

In early stages of IgE-mediated allergy disease (limited number of sensibilisations), the SA might be a more powerful parameter to determine the severity and prognosis of systemic allergy disease, rather than the use of absolute values of individual recombinant proteins measured by either ImmunoCAP250 or ISAC. Our results show that the relevant cut off values for SA appeared to be dependent on the recombinant protein used and remain to be established.

Autoimmune diseases, autoimmunity, allergy

M187

COMPLETE REVERSION OF ANTI-INFLIXIMAB IMMUNIZATION BY METHOTREXATE COMBINATION IN A PATIENT WITH PSORIASIS

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BACKGROUND-AIM

Infliximab (IFX) is a chimeric monoclonal immunoglogulin G1 (IgG1) antibody that binds to and neutralizes tumour necrosis factor alpha (TNF-a) which is successfully used to treat moderate to severe psoriasis. Monotherapy with IFX is effective in most patients, but loss of therapeutic response after several cycles could be observed in some of cases. Auto-antibodies to infliximab (ATI) are induced during treatment with lower serum IFX concentrations, and are thought to be associated with loss of response (LOR) and a greater risk of infusions reactions. Currently, means of reducing the immunogenicity of biologics is becoming more widespread. The concomitant use of Methotrexate (MTX) has been shown to reduce the immunogenicity of IFX in patients with chronic inflammatory diseases such as rheumatoid arthritis, spondyloarthritis or Crohn's disease. However, very few data are available on the combination of MTX with IFX in psoriasis patients.

METHODS

Reliable detection methods for identifying patients who are at risk for LOR to IFX have been recently commercialized, but screening for ATI is still expensive and not routinely performed. Here we used a commercialized ELISA to detect the IFX serum concentrations and the anti-drugs antibodies (ADA) levels.

RESULTS

We reported one case of active arthritis psoriatic successfully controlled by IFX and MTX combination after the failure of IFX monotherapy due to ATI formation. After MTX induction, a complete disappearance of ATI was observed, with a rise of IFX at effective levels.

CONCLUSION

Algorithms have been proposed indicating that patients with ADA should be switched to another TNF inhibitor. The complete reversion of anti-IFX immunization we observed in our patient after addition of MTX suggests that in the case of a loss of response with IFX monotherapy, the introduction of an immunosuppressive drug such as MTX should be considered as it has been proposed for inflammatory bowel diseases.

Autoimmune diseases, autoimmunity, allergy

M188

A RARE CASE OF SELECTIVE KAPPA LIGHT CHAIN DEFICIENCY

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BACKGROUND-AIM

Immunoglobulin IgA deficiencies are fairly common. The selective deficiency of one type of light chain, however, is very rare. So far, there have been 5 reported cases of kappa deficiency; 3 partial and 2 complete. Of these, the genetic basis of one complete deficiency has been fully characterized. During immunofixation electrophoresis, we identified a patient with complete absence of kappa light chains. The 73 year old Caucasian female presented with the onset of a peripheral neuropathy that has undermined her sense of positioning but is healthy overall and has no history of immunodeficiency.

METHODS

Blood was collected for monoclonal protein serum studies. Serum protein electrophoresis (SPE), immunofixation electrophoresis (IFE), total immunoglobulin (nephelometry) as well free light chains (The Binding Site) quantitations were performed. Serum was also analyzed using Light Chain Monoclonal Immunoglobulin Rapid Accurate Mass Measurement (miRAMM) by electrospray-time-of-flight mass spectrometry (API 5600, AbSciex).

RESULTS

SPE was unremarkable with 6.7 g/dL of total protein, 1.2 g/dL in the gamma fraction, and no monoclonal protein detected. IFE was also normal; except that there was no kappa reactivity on the original and repeat gel. Total IgG was 879 mg/dL (reference range, RR: 767-1590), IgA 152 mg/dL (RR: 61-356) and total IgM 296 mg/dL (RR: 37-286). Free kappa light chain was undetectable (<0.11 mg/dL) and free lambda was 2.94 mg/dL (RR: 0.57-2.63 mg/dL). The distribution of kappa and lambda light chains identified by miRAMM mass spectrometry confirmed that the light chain repertoire of the patient was formed entirely by polyclonal lambda.

CONCLUSION

Previous reports of complete kappa deficiency were siblings who also suffered from cystic fibrosis, thereby complicating the clinical picture. So far, the absence of kappa light chains in this 73 year old woman has not been accompanied by any apparent manifestation of immunodeficiency, nor is it an obvious cause of her peripheral neuropathy. The molecular basis of this deficiency is under investigation, but it is certainly intriguing to have an apparently normal immune system with only a portion of the light chain repertoire.

Autoimmune diseases, autoimmunity, allergy

M189

MONOCLONAL ANTIBODY THERAPEUTICS AS POTENTIAL INTERFERENCES ON PROTEIN ELECTROPHORESIS AND IMMUNOFIXATION

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BACKGROUND-AIM

The use of therapeutic recombinant monoclonal antibodies (mAbs) has triggered concerns of confusion and misdiagnosis of a monoclonal gammopathy in treated patients. The purpose of this study is to determine if infliximab, adalimumab, eculizumab, vedolizumab, and rituximab are detected as monoclonal proteins by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE).

METHODS

Pooled normal sera were spiked with various concentrations (ranging from trough to peak) of infliximab, adalimumab, vedolizumab, eculizumab and rituximab. The peak concentration for each mAb was also added to samples (n=5) with known monoclonal gammopathies. All samples were analyzed by SPE (Helena Laboratories) and IFE (Sebia), and the ones with potential interferences were reflexed to electrospray-time-of-flight mass spectrometry (AbSciex Triple TOF 5600) for the intact light chain Monoclonal Immunoglobulin Rapid Accurate Mass Measurement (miRAMM). Intact light chains mass for these mAbs was calculated from the aminoacid sequence available at IMGT database and characterized using the pharmaceutical preparations.

RESULTS

For all mAbs tested, no quantifiable M-spikes were observed by PEL at any concentration used. Infliximab and adalimumab were not observed at 100 µg/mL, nor was eculizumab at 200µg/mL, on SPE or IFE. However, small gamma fraction abnormalities were noted in the SPE for vedolizumab at 300 µg/mL and rituximab at 400µg/mL, with identification of small IgG kappa proteins on IFE. The same small abnormalities were observed for the high concentrations of mAb therapeutics in sera with known IgG kappa M-spikes. All sera containing peak concentrations of mAbs, with and without M-spikes were reflexed to miRAMM. The therapeutic mAb light chain accurate masses were identified above the polyclonal background and distinct from any monoclonal gammopathy of each sample.

CONCLUSION

Biologics should not be easily confounded with monoclonal gammopathies in patients undergoing mAb therapy except when a SPE and IFE are performed within a couple of days from infusion (peak) for vedolizumab and rituximab. In ambiguous cases the use of the miRAMM technology will precisely identify the therapeutic mAb distinct from any endogenous monoclonal gammopathy.