Day of Immunology 2019

Abstracts of the PanHellenic Multidiscipline Congress of Autoimmune Diseases, Rheumatology and Clinical Immunology
ΕΣΩΤΕΡΙΚΗ ΠΑΘΟΛΟΓΙΑ
Ε’ έκδοση
Ιατρική Σχολή Α.Π.Θ.
Τομέας Παθολογίας
Επιμέλεια: Αστέριος Καραγάνης
σελ. 964, τιμή: 175,00 €

Η πέμπτη έκδοση του βιβλίου «Εσωτερική Παθολογία» έχει ανανεωθεί και έχει συμπληρωθεί με την παράδοση νέων γνώσεων της Ιατρικής επιστήμης. Το βιβλίο είναι εύχρηστο και περιλαμβάνει όλα εκείνα τα στοιχεία της Παθολογίας που οφείλει να γνωρίζει ο φοιτητής και ο νέος ιατρός.

Συντονιστής της έκδοσης είναι ο Καθηγητής κ. Αστέριος Καραγάνης και οι συγγραφείς είναι όλοι μέλη ΔΕΠ του Τομέα Παθολογίας του ΑΠΘ.

ΒΑΣΙΚΕΣ ΚΛΙΝΙΚΕΣ ΔΕΞΙΟΤΗΤΕΣ
Β’ έκδοση
Επιμ.: Ε. Σμυρνάκης, Μ. Μπατσαγεντή, Κ. Τούφας, Β. Γροσσομανίδης, Α. Μπένος
σελ. 528, τιμή: 60,00 €

ΚΛΙΝΙΚΗ ΑΝΟΣΟΛΟΓΙΑ
Γ’ έκδοση
Π. Μπούρα
Ε. Βλαχάκη, Α. Γαρύφαλλος, Γ. Γκιούλα, Μ. Δανιλίδης, Γ. Κυριακίδης, Ε. Μπεκιάρη, Δ. Παπακώστα, Μ. Χατζησταυλανός
σελ. 334, τιμή: 37,00 €
Inhibitory effect of a fatty-acid-based oral formula on peripheral blood NK cells of subfertile women
B. Geladakis, Ch. Mpalamoti, Ch. Tsekoura, Th. Keramitsoglou, Ch. Tsarmakis, M. Varla-Leftherioti ........................................ 3

DAY OF IMMUNOLOGY 2019 .................................................. 10

Abstracts of the PanHellenic Multidiscipline Congress of Autoimmune Diseases, Rheumatology and Clinical Immunology ..................... 15
INTRODUCTION

Subfertility is the inability for childbearing, which can possibly be encountered by proper treatment. It is estimated that it affects 10-15% of couples at reproductive age and may be due to various male- and/or female-derived factors. In some cases, it is associated to immunological disturbances of the women, resulting either to no conception or to inability in maintaining a pregnancy. Clinically, these cases appear as repeated failures of embryo implantation (repeated implantation failures – RIF) or as repeated spontaneous miscarriages (recurrent spontaneous abortions – RSA).

Immune-mediated female subfertility is characterized by either autoimmune or alloimmune disturbances, which represent a broad immunological imbalance that leads to pregnancy loss (reproductive immune failure syndrome – RIFS). In autoimmune-mediated subfertility, which is mainly associated with antiphospholipid antibodies targeting decidual and trophoblastic molecules and affecting the development of the placenta and the embryo, the immunopathology is better defined, the diagnosis is relatively simple and the therapy has been widely described to be beneficial.

ABSTRACT

Increased peripheral blood NK subsets and NK cell activity have been associated to abortions of chromosomally normal embryos and repeated implantation failures after IVF. We have previously presented that the use of a fatty-acid-based diet appeared to have suppressive effect on NK cell number and activity. Based on these preliminary data, we derived a normalized formula, the effect of which is investigated in the present study.

The study included 42 women with sub-fertility problems (RIF and/or RSA). Repeated PB immunophenotyping in all women had revealed persistent increase of the percentage of NK cells (>12%). The women were advised to include in their normal diet a daily dose of a rich in long chain fatty acids supplement for a period of 40 days.

The percentage of NK cells were measured by flow cytometry immediately before and after treatment. In 12 out of the 42 women the NK cell cytotoxicity was also measured by a flow cytometry-based cytotoxicity assay.

pNK disturbances of women were modulated by the intake of the designed fatty-acid-based formula. The mean value of pNK cells percentage decreased from 19.5±7.36% to 15.24±5.05% (p<0.0001). Additionally, the NK cytotoxicity in the 12.5/1 dilution was initially found increased in 12 out of 14 women tested and in all of them (100%) it was normalized after the 40 days consumption of the formula.

The results of the present study indicate that a planned consumption of an innovated fatty-acid-based formula offers benefit for the downregulation of NK cells in sub-fertile women, eliminating a serious risk factor possibly associated with reproductive failure.

Inhibitory effect of a fatty-acid-based oral formula on peripheral blood NK cells of subfertile women

B. Geladakis¹, Ch. Mpalamoti², Ch. Tsekoura³, Th. Keramitsoglou³, Ch. Tsarmaklis¹, M. Varla-Leftherioti³

¹ Rodi ®Pharmaceuticals US LLC
² Dept. of Nutrition “Helena Venizelou” Hospital, Athens Greece
³ Dept. of Immunology and Histocompatibility,”Helena Venizelou” Hospital, Athens, Greece
the maternal immune system reacts against the “semi-allogeneic” embryo and damages the trophoblast through allogenic, rejection-type reactions, the underlying mechanisms are not fully defined, and reliable diagnostic markers and appropriate therapeutic interventions are still debatable.

It is accepted that, alloimmune-mediated subfertility is characterized by the predominance of a Th1 response or the defective production of Th2-type cytokines needed for successful pregnancy. In RIF cases, impaired expression of cytokines and Th1 shift, failure of expression of specific integrins in the endometrium, and disturbed expression and function of NK cells may prevent blastocyst implantation and affect the receptivity of the endometrium. In RSA cases, decidual lymphocytes respond to the conceptus or to other antigens, and there is a T regulatory cells /Th17 cells imbalance in favor of Th17 cells. The secretion of Th1 (IL-2, IFN-γ, TNF-α) and Th17 (IL-17A, IL-17F, IL-6, IL-21, IL-23) cytokines adversely affect the development of the embryo. Fetal rejection occurs through inflammation and lymphocyte infiltration of the trophoblast, trophoblast damage by NK cells and cytotoxic antibodies, and vasculitis affecting the maternal blood supply to the implanted embryo. Natural Killer cells of the uterus (uNK) play an important role in the cytotoxic reaction developed in the context of Th1 response in RSA and RIF. CD3-CD16bright CD56dim phenotype in women with spontaneous early pregnancy loss as well as in women with RIF. Furthermore, it has been demonstrated that RSA and RIF women have increased peripheral blood NK subsets (pNK) and NK cell activity preconceptionally, which have been associated with loss of chromosomally normal embryos.

So far, there are no specific tests recommended for the alloimmune investigation in RSA and/or RIF and the prognostic value of measuring uNK or pNK cell parameters remains uncertain. Nevertheless, peripheral blood immunophenotyping for the detection of NK cell disturbances (increase of CD3-CD16+ CD56+ cells) is promoted as the diagnostic test to be routinely used for the identification of women with an alloimmune aetiology of their subfertility. In addition, increased pNK cells are considered to be a useful diagnostic tool for the selection of women who may benefit from immunotherapy, and monitoring of NK cells is used for the estimation of its effect.

The main forms of preventive therapy used for the reduction of NK cells include passive immunization with intravenous administration of Immunoglobulin G (IvIg) and soybean-oil-based lipid infusions (Intralipid formulations) and possibly active immunization with allogeneic lymphocytes (Lymphocyte Immunization Therapy - LIT). These treatments have a number of drawbacks and disadvantages, including required repeated hospitalizations of the patients and permanent doctor’s surveillance, possibly side effects and significant associated cost implications. Single and multicenter studies have shown that they are effective when applied to selected cases after correct verification of immunological factors and careful evaluation of results. Current guidelines recommend applying them only as part of research protocols and well-designed studies.

Considering the aforementioned data and the need to seek alternative safe, effective and cheap remedies, our group has proposed the suppression of NK cells by a personalized diet rich in long-chain fatty acids derived from the everyday food. The diet was showed to be effective in reducing both the number and the toxicity of NK cells in women with RIF and/or RSA and corresponding persistent NK cell distur-
bances. Moreover, it seemed that the suppressive effect was improved by the concomitant intake of a nutritional fatty-acid-based supplement. Following this observation and intending to release the women from the personalized diet, an oral formula was designed, the dosage of which does not require other interventions on the women’s dietary habits. The results of the formula’s effect on the NK cells of sub-fertile women are presented here.

MATERIAL AND METHODS

This observational study was conducted between January 2016 and September 2018. All women agreed to be included in the study after they had been informed about its aim and the procedure to be followed.

The cohort of sub-fertile women

The study included 42 women (mean age 36.7 years old, range 22-49) with well-defined sub-fertility problems, RIF and/or RSA. They derived from a population of women who were addressed to our clinics because alloimmune etiology was suspected for their reproductive failures. Inclusion criteria were: a) Unexplained RIF (no pregnancy despite multiple embryo transfers and a cumulative total of at least six embryos transferred during the IVF/ICSI cycles) and/or RSA (at least two unexplained spontaneous miscarriages before the 20th week of gestation). b) Persistent increase of the percentage of pNK cells (>12%) in repeated (at least two) tests. c) Not any treatment received for the suspected alloimmune etiology of the reproductive failures.

Study Design

The women were advised to use a daily dose of an innovated (patent pending) oral formula for a period of 40 days.

The percentage of pNK cells were measured immediately before and after treatment. In 14 out of the 42 women the quantitative determination of the cytotoxic activity of NK cells was also measured before and after treatment. The analyses were performed in accredited laboratories with established, validated and well-known protocols, routinely used.

The formula

The formula was composed of long-chain polyunsaturated fatty acids (LC-PUFA), long-chain monounsaturated fatty acids (LC-MUFA), LC-saturated fatty acids (SFA), medium-chain fatty acids (MCFA), vitamins and minerals. It was prepared in accordance to the medical prescription in a lab meeting the legally provided prerequisites for the safe manufacture of Galenic formulations (magistral formula), according to the GPP (good preparation practices) European rules.

Flow-Cytometry analyses:

Detection of the percentage of pNK cells: Peripheral blood samples were used for immunophenotypic analysis by Flow-Cytometry, with the use of specific moAb (CD3, CD2, CD4, CD8, CD16, CD56) conjugated with either fluorescein-isothiocyanate (FITC) or phycoerithrin (PE). The analysis of cell populations was performed on Epics XL-MCL flow cytometer (Beckman-Coulter) using a two-color analysis protocol. NK cells were reported as the percent of CD3-16+56+ cells among the lymphocyte population.

NK cell toxicity measurement: NK cells (effectors) were co-cultured with K562 cells (targets) pre-labeled with CFSE [5-(and 6)-carboxyfluorescein diacetate succinimidyl ester] at different ratios (50:1, 25:1 and 12.5:1). Then, cells were harvested and incubated with 7-AAD (7-amino actinomycin d), a fluorescent cell viability dye which is excluded from live cells but penetrates dead or damaged cells. The samples were analyzed by multicolor flow cytometry immediately after the end of the incubation period. NK cytotoxicity was reported as the percentage of K562 cells positive for 7-AAD (dead target cells). Reference values derived from measurements in fertile women of reproductive age (26-56% in 50:1 ratio, 15-39% in 25:1 and 12-26% in 12.5:1 ratio).

Statistical Analysis

Statistical analyses were performed with SPSS V16.0 for windows. Assumptions were met for the use of paired t test to analyze the effect of the intake of the formula on NK cells. The level of statistical significance was set to <0.05.

RESULTS

After the use of the formula for 40 days, decrease of the NK cell percentages occurred in 37 out of 42 women (88.1%), in 4 women the NK percentages slightly increased (mean increase 1.5%), whereas in one case NK were found stable. In 18 out of 42 women (42.9%), the NK percentage reached levels ≤ 16%.

The mean value of pNK cells percentage before treatment was 19.52±7.36% (range 13.38-33.60%), and...
was reduced to 15.24±5.05 (range 8.05-30.22%) (Figure I). The difference was highly statistically significant (p= 0.0001).

NK cytotoxicity in the 12.5/1 dilution was initially found increased in 13/14 women (average percentage 32.8%±6.9). In all of them the cytotoxic activity was normalized after the 40 days consumption of the formula (average percentage 19.2%±6.0). (Figure II). The difference was highly statistically significant (p= 0.00001).

DISCUSSION

According to the results of the study, pNK disturbances of women experiencing RIF and/or RSA were modulated by the intake of the designed fatty-acid-based formula. In the majority (88.1%) of the 42 subjects with persistent increased NK cells (>12%), the mean NK percentage decreased from 19.5±7.36% to 15.24±5.05%, signifying a highly statistical difference (p< 0.0001). Additionally, 100% reduction to normal levels after treatment was observed in the pNK cell cytotoxic activity of those women who had been relatively tested. In 18 out of 42 women (42.9%), the NK percentage reached levels ≤ 16%, a percentage shown in a meta-analysis to be the highest percentage of pNK cells observed in fertile women37.

The results follow up with our previous observations and strengthen our suggestion for downregulation of NK cells by fatty-acid-based regimen35,36. The choice of fatty acids to constitute the basic ingredient of the formula was made because of the known modulatory effect of fatty acids on the immune system in general and on NK cells in specific. There is well-documented evidence that essential fatty acids (EFA) act as both intracellular and extracellular mediators and interfere with many steps of the immune response. They can control inflammation by affecting extravasation, phagocytic response, generation of cytokines, inflammatory mediators and adhesion molecules, and can enhance or suppress humoral and cell-mediated lymphocyte responses at the same time that they do not affect protective immunity38-41. Suggested molecular mechanisms through which EFA act, include alteration of eicosanoid (prostaglandin, leukotriene) synthesis, orphan nuclear receptor activation (i.e. peroxisome proliferator-activated receptors -PPAR, liver X receptors) and T lymphocyte signaling by changing the molecular composition of lipid rafts42. Since the effects of fatty acids can positively or negatively regulate physiological and pathological conditions, fatty acids and specifically long-chain polyunsaturated ones (LC-PUFA, i.e. ω-3, ω-6) are considered as modulators of the immune system in health and diseases43,44. Actually, during the last years, PUFA supplementations have been shown to exert clinical benefits and possibly changes in disease activity when they are used in the treatment of a variety of disorders related to inflammation45,46, allergy47 and autoimmunity48,49. Furthermore, LC-PUFA intake during pregnancy has effects upon fetal acid composition and alter fetal immune parameters (such as the expression of CD3 and CD8 on fetal thymic cells), so that may influence fetal and child health50,51. Decreases in maternal and cord blood erythrocyte fatty acids have been observed in cases of preeclampsia52.

In regard to NK cells, several studies in both animals and humans have demonstrated that PUFA exert inhibitory effect on their function. In mice, dietary ω-3 PUFA were found to attenuate splenic NK cell...
and lymphokine activated killer cell (LAK) activities\textsuperscript{53}. In rats, the consumption of ω-3 PUFA reduces NK cell activity and ameliorates inflammatory disorders which are affected by the reduction of proinflammatory cytokines\textsuperscript{54}. In humans, in vitro and in vivo studies have shown that high fat diets (consumption of PUFA and also MUFA enriched foods) can selectively lower NK cell activity, as well as the number of circulating cells\textsuperscript{55,56,57}. The inhibitory effect of fatty acids on NK cells cytotoxicity may be of benefit in diseases with increased natural cytotoxicity. Almallah et al have demonstrated suppression of natural cytotoxicity and concurrent reduction in disease activity in patients with proctocolitis receiving ω-3 PUFA supplementation\textsuperscript{58}. For the suppression of NK cell toxicity in RSA and RIF women, fatty-acid-based treatment is used in the form of intravenous Intralipid infusions. Intralipids are soybean-oil-based emulsions providing principally PUFA but also MUFA and SFA. Several studies have shown the in vitro and in vivo suppressive effect of these emulsions on the NK cell number and functional activity and suggest intralipid as a therapeutic option to modulate NK abnormalities and possibly to promote trophoblast invasion in women with reproductive failure seeking to achieve successful conception/ pregnancy\textsuperscript{33,59,60}.

Because of the different effect that the various fatty acids have on the immune system and specifically on NK cells, our main concern in the design of the formula was the cautious determination of its composition in terms of the types and proportions of fatty acids and other active substances. After evaluation of different combinations of fatty acids, long-chain PUFA provided at ester or free forms composed the basis of the formula. Other fatty acids and/or esters, such as LC-MUFA, LC-SFA, and MCFA, were also included. Vitamins and minerals contained in the formula were expected to have a synergistic effect because of their role in regulating immune responses, and possibly the negative effect of their deficiency in pregnancy. They may not directly affect NK cell number and function, but contribute to the body’s immune defenses by supporting physical barriers, cellular immunity and antibody production, exhibiting an immune modulating and homeostatic effect to the environment where the NK cells are found. Therapeutic application of some of them may be useful for dysregulated NK-cell immunity\textsuperscript{61}. For example, supplementation of Vitamin D, a vitamin which has a pivotal role in regulating immune responses by promoting Th2 immune responses and suppressing Th1 responses, and whose deficiency has been shown to associate with increased auto- and cellular immune abnormalities in women with RSA\textsuperscript{62}, may decrease the expression of activating receptors on NK cells\textsuperscript{63}. Folic acid is also suggested to have an indirect effect on NK cell activity. Its intake beyond the metabolic capacity of the body (unmetabolized folic acid) by women >50 y has been shown to associate with reduced NK cell cytotoxicity\textsuperscript{64}, while high folic intake reduces NK cell toxicity in aged mice\textsuperscript{65}. The precise analogy of the active substances and the precise dosage used resulted in the uniqueness of the formula, which significantly differs from other fatty-acid-based dietary combinations used so far.

Dietary supplementation usage is a popular practice nowadays. In contrast to the treatments, in which intravenous or subcutaneous infusions are applied, dietary regimes are administered orally, and their components follow the normal process of food consumption and absorption. So, they are more patient friendly and of negligible risk, at the same time that their intake may result in achieving desirable effects. The results of the present study indicate that a planned consumption of an innovated fatty-acid-based formula offers benefit for the downregulation of NK cells in sub-fertile women. Following this result, and given the advantages deriving from its nutraceutical nature (no need for hospitalization or permanent surveillance by doctors, no risks and side-effects), the formula can be suggested as standalone or adjunctive to other treatment, in order to contribute to the maintenance of normal NK cell level and activity in sub-fertile women. Because NK cell abnormalities is the most common finding in women with alloimmune-mediated RSA and RIF, the reduction of NK cell number and activity would be expected to eliminate a serious risk factor possibly associated with reproductive failure. Whether, by decreasing NK cells, the formula increases the possibility of successful embryo implantation and pregnancy, was not investigated here. Further controlled studies will address this question, while ongoing studies investigate the effect of the formula on other immune factors, so that its use could be suggested for immunomodulation of additional immunological disturbances in sub-fertile women, and could also be considered for “treatment” in other clinical situations.
Acknowledgments

The authors thank the patients who agreed to participate in the study, followed the instructions for the proper intake of formula and underwent timely the tests for the assessment of its impact. They also particularly thank all people from the Research and Development Department of RODiPharmaceuticals US LLC, the company that obtained the IP rights of the formula and prepares its commercial production.

Acknowledgments

The authors particularly thank all people from the Research and Development Department of RODiPharmaceuticals US LLC, the company that obtained the IP rights of the formula and prepares its commercial production.

**M. Varla-Leftherioti**
e-mail: grlbfmdr@hol.gr

**References**


“DAY OF IMMUNOLOGY 2019”

WWW.HELSIM.GR
Day of immunology 2019 - Education on Immunology

This year's Day of Immunology (DOI) theme is "Cancer checkpoint blockade". A recently Nobel awarded therapeutic approach of treating cancer by stimulating effector immune cells. These novel Immunotherapies are the result of applied Immunology at the forefront of experimental medicine and vouch that Immunology is the science that will provide novel therapies to diseases such as infections, cancer, autoimmunity or immunodeficiencies.

In this new era of intense scientific progress and knowledge acquisition, it is essential that all scientists around the world communicate in a homogeneous language regarding Immunology. The first step of conquering this universal vocabulary is the introduction in universities of a consortium-based academic plan of Immunology teaching so that students from all around the world become familiar with the same principles of Immunology, rendering much more easy, rapid and efficient next generation of immunologists' inter-communication.

On this regard, our society has performed a National survey among medical students depicting both today's state of teaching Immunology across different academic units in Greece, as well as the students' opinion on the importance and teaching methods regarding Immunology. Part of the results were presented in our recent Annual Seminar in Thessaloniki, and were enthusiastically discussed by the local university authorities. Furthermore, our idea of expanding this project to a European Level, was also warmly accepted by the European Federation of Immunological Societies (EFIS), at the recent EFIS Strategic Retreat meeting.

In order to promote this initiative, the Hellenic Society of Immunology has decided to dedicate this year's DOI to education and incorporate the related scientific action to the PanHellenic Multidiscipline Congress of Autoimmune Diseases, Rheumatology and Clinical Immunology. The congress is organized by the National Institute of Rheumatic Diseases and will be held between 05-07 April 2019, in Portaria, Pelion. We are optimistic that our initiative will be welcomed from related scientific partners, strengthening immunologists' voice on a National and eventually a European level.

On behalf of the HIS's Board

Alexandra Tsiroglanii
HIS President

Alexandros Sarantopoulos
HIS Secretary General
PanHellenic Multidiscipline Congress on Autoimmune Diseases, Rheumatology and Clinical Immunology

Day of immunology 2019
Education on Immunology

Chair: Tsirogianni A, Sarantopoulos A, Trontzas P.

- Medical Students’ Education on Immunology; Survey - Results
  Gkantaras A, Kontaxi O.

- Medical Specialization of Immunology in Greece – An Update
  Nikolaou Ch, Sarantopoulos A, Chatzistylianou M.

Chatzistylianou M.  Professor Em. AUTH

Gkantaras A.  Medical Student – HelMSIC

Kontaxi O.  Medical Student – HelMSIC (Head of Workgroup on Education)

Nikolaou Ch.  Professor of Neuroimmunology – EKPA
  Board Member of HSI

Sarantopoulos A.  Internist – Immunologist.
  Secr. General of HSI

Trontzes P.  Rheumatologist, Past President of the Hellenic Society of
  Rheumatology

Tsirogianni A.  Biopathologist, Director of Immunology-Histocompatibility Lab,
  Hospital “Evangelismos”.
  President of the HSI
1η Νοσηλευτική Ημέρα Ρευματολογίας Κέντρικης Ελλάδος & Πανελλήνιο Πολυθεματικό Συνέδριο Αυτοάνοσων Παθήσεων, Ρευματολογίας και Κλινικής Ανουσολογίας

Κοινές Συναντήσεις Ρευματολογίας, Δερματολογίας, Νευρολογίας και Γαστρεντερολογίας

05-07 Απριλίου 2019
Portaria Hotel, Πήλιο

Σε συνεργασία με την
Κλινική Ρευματολογίας και Κλινικής Ανουσολογίας
tου Πανεπιστημίου Θεσσαλίας,
Πανεπιστημιακό Γενικό Νοσοκομείο Λάρισας

Υπό την αιγίδα
tου Τμήματος Ιατρικής Πανεπιστημίου Θεσσαλίας
tου Πανελληνίου Ιατρικού Συλλόγου
tης Ελληνικής Ρευματολογικής Εταιρείας & Επαγγελματικής
Ένωσης Ρευματολόγων Ελλάδος [Ε.Ρ.Ε.-Ε.Π.Ε.]
tης Ελληνικής Δερματολογικής & Αφροδισιακής Εταιρείας
tης Ελληνικής Εταιρείας Ανουσολογίας
&tου International Chamber Of Commerce

Βράβευση 3 καλύτερων εργασιών στην τελετή λήξης του Συνεδρίου

Χαρίζονται Πιστοποιητικοί Παρακολούθησης με 22 Μέρια Συνεκπαιδέυσης Ιατρικής Εκπαίδευσης (CME - CPD), από τον Πανελλήνιο Ιατρικό Σύλλογο

Οργάνωση - Γραμματεία
The MASTERMIND Group
Organizing your success
Greetings

Dear Colleagues,

We are delighted to invite you to our Congress on 5-7 April 2019 at the Portaria Hotel Conference Center in Pelion.

The themes of our Conference will focus exclusively on recent developments in Autoimmune Diseases of five specialties, namely Rheumatology, Gastroenterology, Neurology, Dermatology and Clinical Immunology. Particular emphasis will be placed on the most recent diagnostic problems, clinical dilemmas and therapeutic options as they have emerged from clinical experience and published work over the last two years.

The conference is addressed to doctors of the relevant specialties, physicians, general practitioners, life science/biomedicine scientists, dietologists/nutritionists, nurses and students. More than 100 distinguished scientists, clinical and laboratory researchers from Greece and abroad will lecture and comment on the conference. Particular emphasis is placed on recent developments and controversies in the treatment of autoimmune rheumatic, gastrointestinal, neurological and skin disorders. The more recent data will be presented in immunopathogenesis, with direct applications in therapy.

The role of environmental factors and nutrition in the development of autoimmune diseases will be developed. Clinical problems, prevention measures, nursing needs, surgical options and rehabilitation treatments will also be discussed.

Emphasis is given to juniors through the Young Investigators Forum through oral and poster presentations and 3 Prizes to the best presentations. The program includes the 1st Nursing Rheumatology Seminar of Central Greece aiming at the holistic treatment of the diseases and the improvement of the provided health services.

All abstracts (oral and e-posters) will be published in Anosia, the official journal of the Hellenic Society of Immunology. Selected speeches / papers will be published in the Mediterranean Journal of Rheumatology.

We are delighted to welcome you to the enchanting Portaria.

Chairman of the Congress
Lazaros I. Sakkas

Co-Chairman of the Congress
Dimitrios P. Bogdanos
FEVER IN IMMUNOSUPPRESSED PATIENT, 
THE ROLE OF A NURSE – THERAPEUTIC ADDRESS

A. Mosiou
Emergency Department, University General Hospital of Larisa, Larisa, Greece

The spectrum of immunocompromised hosts has expanded with prolonged survival for transplant recipients, patients with immune deficiencies (especially HIV/AIDS), and autoimmune disorders, as well as the development of novel therapies including immunotherapies and checkpoint inhibitors. Although these drugs revolutionized the treatment autoimmune disorders, there is some potential safety issues. These patients present to primary care and general hospitals with fever. The most common reason is an infection, quite often severe or opportunistic. Based on the patient’s underlying disease or related therapy, it helps to determine what major component of the immune system has been damaged (i.e., T-cell, B-cell, phagocytosis, complement system, or splenic function). Knowledge of what immune defect has occurred, often aids in establishing a definitive diagnosis. In these patients, a standardised diagnostic investigation should be done immediately at admission before deciding whether to perform more invasive diagnostic procedures or to start empirical treatments. We also present data on the epidemiology and the nursing care plans for febrile immunosuppressed patients. Moreover, prevention and especially vaccination are important in immune deficiency. Doctors and nurses should have sufficient knowledge about efficiency, safety and contraindications of vaccines in individuals with immune deficiency and in people who live in the same house with these individuals.

PREVENTION OF OSTEOPOROSIS

A. Chatziefstratiou
RN, MSc, PhD, Cardiothoracic Intensity Unit, Pediatric Hospital of Athens, Agia Sophia Greece

Introduction: Osteoporosis is a common health condition mainly among women and especially after menopause. However, it is observed a significant increase in incidence of osteoporosis in younger women and men, which leads to high risk of fractures and major disability.

Materials-Methods: A research of international literature in the scientific data bases«PubMed» and «GoogleScholar» was conducted from November 2018 to February 2019, by the use of the following key-words: «prevention», «osteoporosis», «nurse», «intervention». Additionally, the research involved web-sites of international scientific organizations according to the prevention of osteoporosis.

Results: Prevention of osteoporosis is imperative for the improvement of persons’ quality of life and in order to reduce the rate of accidents, falls and broken bones. Nurses have a significant role in prevention of osteoporosis providing education and information to people. The prevention of osteoporosis included alcohol restriction, smoking cessation, food recommendations and physical activity. The benefits of prevention of osteoporosis is associated with reduction in broken bones, people’ disability, improvement of quality of life and reduction of health care cost.

Conclusions: Osteoporosis is a common health condition for many people worldwide. Prevention of osteoporosis is essential both for patients and Health System with nurses to play a significant role.
OSTEOPOROSIS AND PHYSIOTHERAPY
Th. Kostopoulou
Department of Physiotherapy, General University Hospital of Larisa

Introduction: Osteoporosis is a systemic skeletal disease characterized by a reduction in bone mass and qualitative skeletal changes that cause an increase in bone fragility and higher fracture risk. A “silent disease,” it is often unrecognized until a fracture. It predominantly affects postmenopausal women and older people although in individual cases could concern people in younger age. Prevention of Osteoporosis aims at obtaining peak bone density prior to the critical osteoporosis period and in correcting its modifiable factors. The treatment targets the subjects already suffering from osteoporosis, with or without a pre-existing osteoporotic fracture. The aim of this review was to evaluate current evidence regarding the use of physiotherapy, rehabilitation and exercise interventions to prevent and treat fractures in osteoporosis patients.

Materials-Methods: Revised articles and research papers published in English over the last five years were searched for in the electronic databases “Pubmed”, which concern the prevention and treatment of osteoporosis.

Results: Lifestyle modifications include lifelong participation in regular low or high impact aerobic activity. Muscle-strengthening activities, weight-bearing resistance exercise and balance-improving exercises minimize falls, decrease risk of fracture and prevent fractures in osteoporosis patients. Physiotherapy can provide personalized exercise, balance training and gait rehabilitation programs to prevent falls—especially in Elderly patients—and additionally rehabilitation after osteoporotic fractures. Personalized therapeutic plan with selective orthotic use may help reduce discomfort, prevent falls and fractures, and improve quality of life in patients with significant Kyphosis. Hip protectors do not reduce the risk of falling, but they should reduce the risk of fracture.

Conclusions: Osteoporosis is preventable and treatable disease, if diagnosed early. Physiotherapy can contribute not only to the prevention of Osteoporosis negative consequences, but it can also manage them.

NEWER DATA ON PAIN MANAGEMENT IN PATIENTS WITH RHEUMATIC DISEASES
K. Chatzidimitriou¹, E. Tsimitrea²
¹ 404 General Military Hospital, Larissa, Greece, ² University Hospital, Larissa, Greece

Introduction: Pain is a matter of primary importance and holds a central position in Rheumatology. It is the most common symptom of a visit to a rheumatologist and its reduction is the main goal of clinical care, but this is not always achieved. Therefore, it is a particular duty of the nurse to help alleviate the pain.

Materials-Methods: Search for articles over the last five years in the Pubmed, Cochrane electronic databases. Keywords: chronic pain, pain management, rheumatology, opioids.

Results: Clinical pain reliever includes both pharmaceutical and interventional therapies as well as non-pharmaceutical treatments. Acetaminophen is recommended due to its low side effects without being effective in back pain, a common symptom of rheumatic diseases. NSAIDs are a key factor in treatment, but their toxicity and inconsistency in pain relief in some cases limit their usefulness. Opioids are effective but only if the expected benefits outweigh the risks for patients. Selective serotonin and norepinephrine reuptake inhibitors have shown effectiveness in reducing pain in osteoarthritic joints but are also associated with increased toxicity. Non-pharmaceutical interventions include patient education, physiotherapy, psychotherapy, exercise, nutrition and weight control. The benefits of these interventions are surprising and in some cases may be greater than those of pharmaceuticals.

Conclusions: Pain and its treatment are important areas of research and clinical practice. Many treatments remain ineffective in their effectiveness in combination with their side effects. Nursing science contributes substantially to the management of pain and to improving the quality of life of patients.
EDUCATION OF IMMUNOSUPPRESSED PATIENT

N. Fotos

Department of Nursing, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Immunosuppression is a life-threatening condition and is found in many diseases (HIV-infection, hematological and other malignancies, etc.), and is also caused by certain therapeutic interventions such chemotherapy, administration of immunosuppressive agents and corticosteroids.

Materials-Methods: A research of international literature in the scientific data bases «PubMed» and «GoogleScholar» was conducted from December 2018 to February 2019, by the use of the following key-words: «education», «immunosuppression», «immunosuppressed patient», «infection», «prevention». Additionally, the research involved sites on the internet of international scientific organizations and nursing institutions according to the education of immunosuppressed patients.

Results: Education of immunosuppressed patient is one of the major obligations of health care providers, nurses among them. This education involves the following themes: information about the disease or the medical treatment that causes immunosuppression, symptoms and sights of infection, measures for the prevention of infections and nutrition. The benefits of education of immunosuppressed patient include the promotion of self-care, the reduction of anxiety, the increase of patient’s adherence to medication, the reduction of incidence and severity of infections and finally the reduction of hospitalizations and cost for Health System.

Conclusions: Immunosuppression is a life-threatening condition for many people worldwide. Education of immunosuppressed patients should be one of the main duties of healthcare providers and especially nurses, in order to be effective and produces the maximum benefits for the patients and the Health System.

THE ROLE OF SPECIALIZATION IN THE NURSING MANAGEMENT OF RHEUMATOLOGICAL DISEASES

P. Tsiamalou, M. Tegousi

Department of Reumatology and Clinical Immunology, General University Hospital of Larissa, Larissa, Greece

Introduction: Chronic disease, including rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, have a global impact on individual’s life, affecting not only physical functioning, but also, self-esteem, interpersonal relations and financial prosperity. The role of Nursing is crucial in the overall management of these patients, offering specialized care, psychological support, and education.

Materials-Methods: We performed a narrative review in the pertinent Medical and Nursing Literature, using as mesh terms the words “rheumatological patient”, “chronic disease”, “management”, “Nursing”, and “evidence-based care”. The search was limited in English, and in the period from December 2000 to December 2018. The data synthesis was categorised in five sections: “evidence-based nursing”, “critical assessment”, “rheumatological patient”, “nursing specialization”, and “nursing care”.

Results: Chronic disease is defined as an altered state of health, that is not improved with after surgical intervention or a short therapeutic regime. Patients with rheumatological are included in this category and suffer from a variable degree of physical disability, social isolation, and emotional frustration. Apart from therapeutic care, specialised nurses are capable to provide to the patient with evidence-based care, emotional support, and empowerment. Evidence-based nursing tailors the best available knowledge and practical experience to the patient’s idiosyncrasy (beliefs, knowledge and needs). Proper question formation, skilful literature digging, and critical appraisal of the gathered data form the cornerstone of theoretical knowledge. Things become more complicated in the modern era of the ageing population, shortage of time and resources, and diversity of treatments, including the biological factors.
Conclusions: The role of specialised nursing has evolved to provide therapeutic care, knowledge, guidance, empowerment, and emotional support to the chronic patient with rheumatological pathology.

RHEUMATIC DISEASES AND MENTAL DISORDERS

M.A. Karatziou
Nurse at MSc, Psychiatric Clinic, PGNL

Introduction: Rheumatic diseases are very common in the general population. They affect 27% of adults and more often women. They are among the major health problems in the working population.

The functional and aesthetic problems that cause to patient and the uncertainty about the course of the disease are related to the occurrence of mental disorders such as long-term anxiety, depression and, in the case, of Systemic Lupus Erythematosus (SLE), psychosis.

Purpose: The purpose of this review is to investigate mental disorders in patients suffering from rheumatic diseases.

Methods: Review of Greek and foreign bibliography.

Results: The prevalence of mental disorders between hospitalized and outpatient rheumatoid arthritis patients ranges between 14%-21%. More often, generalized anxiety disorder and major depression. A wide range of results is given for anxiety levels in patients with rheumatic diseases ranging from 20%-70%, depending on the study. Co-morbidity of depression in rheumatoid arthritis is high. Its prevalence is estimated at 15% - 40%. Significant deviations occur in the frequency of neuropsychiatric disorders in Systemic Lupus Erythematosus (SLE). The prevalence ranges between 57% -90%. Disorders of mood occurring at 17%, psychoses at 7.5%, anxiety disorders at 4%. Other neuropsychiatric disorders such as headache at 25% delirium 3.5%, vascular disease 15%, and so on.

Conclusions: Mental disturbances occur in patients suffering from rheumatic diseases. Anxiety and depression are more frequent. In SLE, besides anxiety and depression, psychoses, headaches, delirium and others are also present.

The treatment and of mental disorders involves both psychopharmaceutical and psychotherapeutic interventions.

Medication, psychological support as well as psycho-educational interventions help to treat the disease more effectively and improve the quality of life of the patient.

Key words: Anxiety, stress, depression, rheumatic diseases.

EMERGENCY CASES IN RHEUMATOLOGY

H. Papadaki
Nurse in the Department of Orthopaedic Surgery, University Hospital of Larissa, Larissa, Greece

Introduction: In comparison with all other group of diseases in the general adult population, rheumatic diseases are the most common cause of a chronic health problem. Therefore, nursing staff must be familiar with these diseases, so they can be in a position to deal with these problems.

Materials-Methods: The study was performed in electronical data bases and literature reviews of the last decade.

Results: Rheumatic diseases are common in our country, involving about 27% of the adult population. It means that one out of four adults presents some signs and symptoms of a rheumatic disease. In total, about 2,500,000 Greek adults suffer from rheumatic diseases. Current literature provides a broad spectrum of results for the levels of psychological stress in patients with rheumatic diseases, with the percentages varying from 20% to 70%. The most common emergency cases are related to gouty arthritis (4.7%), ASD (0.16 cases/100,000 people), Systemic lupus erythematosus (0.05%) and vasculitis.
**Conclusions:** Rheumatic diseases are common in the general population of our country, with implications for patients themselves and their families, in the health system, and for the national economy. Emergency cases, those for which nursing personnel are needed to deal with, require knowledge of all aspects of the disease. In this way, accurate diagnosis can be achieved followed by early and effective treatment.

---

**RHEYMATOID ARTHRITIS AND THE LUNGS**

V. Manolopoulou  
Pulmonary Clinic, General University Hospital of Larissa, Larissa, Greece

**Introduction:** RA is a systemic autoimmune disease which gradually manifests as chronic symmetrical destructive polyarthritis. The destruction is caused by the effect of the inflammatory tissue created progressively in the affected joints. Initially, the damage affects the cartilage and penetrates the bone and the tendons. The disease also causes extra-articular manifestations that affect the lungs, heart, eyes. This presentation concerns a case admitted to the pulmonary clinic as a transfer from a secondary hospital.

**Materials-Methods:** Male aged 49, smoker with a clear medical history, bilateral pleural effusion, fever, and hypoxia.

**Conclusions:** Patients with a chronic disease are under constant stress, and the psychological, social, and financial problems that arise during their treatment require medical intervention.

---

**NURSING CARE IN AUTOIMMUNE BULLOUS SKIN DISEASES**

A. Bairamoglou, A. Terlimpakou, E. Zafeiriou  
Department of Dermatology, University Hospital, Larissa, Greece

**Introduction:** Autoimmune bullous diseases are a common clinical entity in dermatology. These skin conditions are caused by a variety of triggers and affect normally elderly people. Usually in the initial phase, the extent of the disease requires hospitalization, becoming a diagnostic and therapeutic challenge for health professionals. Because of the high number of patients arriving and getting hospitalized at the University Dermatology Clinic, and the special treatment and care that these diseases require, research on methods and nursing care of these patients was considered appropriate. Nursing intervention, evaluation of patient's condition, and caring purposes are parameters helping to control and treat bullous diseases, providing an optimal quality of life.

**Materials-Methods:** Revised bibliography. Search engines, PUBMED, MEDILINE, CINAHL, IATRONET. Clinical practice and experience in dealing with patients at the University Dermatology Clinic.

**Results:** Autoimmune bullous diseases consist of serious dermatological diseases that often comorbidities with other serious. First-line therapy consists of corticosteroids, which have greatly increased survival expectation. Nurse's role in patient's healthcare is extremely useful on improving the integrity of the skin, offering at the same time relief of symptoms, such as pain and itching. This can be done through hygiene care and topical therapies, psychological support and treatment.

**Conclusions:** Care on patients with dermatological diseases has a wide range. Systematic medication and proper nursing care helps to make those diseases treatable. It is imperative and necessary to train nurses in hospitals. More research and studies are needed to establish protocols of care for patients with autoimmune bullous diseases.
NURSING APPROACH IN INTRAVENOUS ADMINISTRATION OF BIOLOGIC AGENTS

E. Sdoukou, P. Sofidou

Education Office of Larissa University General Hospital, Greece

Rheumatic diseases may cause severe pain and a significant decrease in patient’s function. Insufficiency of traditional drugs in treatment of rheumatic diseases revealed the need for specialized treatment modalities since 1999.

Biologic agents -protein molecules- inhibit function of a great number of molecules and cells and are involved in induction of autoimmunity and inflammation. They hold a prominent position as biotechnology products in the armamentarium of the scientific community.

The modifying drugs (infliximab, abatacept, ticilizumab, ritukimab, cyclophosphamide, belimumab) have a targeted function, in contrast with the classic drugs like methotrexate which had a general influence in the inflammatory process.

Frequently, patients change their treatment secondary to failure (primary or secondary) and not due to adverse effects.

As protein molecules, they are degraded fast in the stomach and therefore are administered parenterally, either intravenously or subcutaneously, in regular time intervals.

Health institutions have the responsibility to dispose protocols for safe intravenous administration of Biologic agents.

Nursing staff has a main role in educating, consulting and supporting patients and their families to adjust in diagnosis and subsequent necessary treatment. Clinically, the nursing staff can apply all necessary nursing process and practice based on evidence and indications. The nurse has to inform the patient for the process and purpose of drug administration and type of drug. His responsibilities are oriented in three directions: safety during administration, prevention and early recognition and treatment of complications.

Knowledge of the international literature and guidelines, continuous education and excellent co-operation among the scientific teams are the required factors for the effective treatment of patients for whom we stand responsible.

Key words: biological agents, intravenous administrations, nursing intervention.

PATIENT’S INFORMED CONSENT IN MEDICAL PRACTICE

A. Mavroforou

Medical Ethics and Deontology Department, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa

Aim:

Methods and Material: Search of the pertinent medical and legal Greek literature.

Results: The patient is the only one who can make decision about his own body and the therapeutic process to be followed-up. Nevertheless, in order patients to be able to reach to a decision should have been thoroughly informed about all related aspects such as, the nature of their disease, its natural history, the available therapeutic methods, the potential complications, the competency of the treating physician and the whole team and the quality of life after the course of each treatment. Minors and incompetent persons are needed additional care and in such cases, the family or the caring person should be included in the process.

Special attention is required on who obtains the consent, when it should be taken and in what form. Most malpractice claims in modern clinical practice are not consequences of technical faults but because of inadequate patient selection criteria and lack of adequate communication between patient and surgeon.

Conclusions: In the current litigious environment of daily medical practice, written patient's informed consent remains an integral part of the communication between physicians and patients, and plays an important role in the professional protection.
ETHICS AND MORAL ISSUES IN HEALTHCARE RESEARCH

M. Kalafati
National and Kapodistrian University of Athens, Faculty of Nursing

Introduction: Ethics is an understanding of the nature of conflicts arising from moral imperatives and how best we may deal with them. The word “ethics” is derived from the Greek word “ethos”, that means custom or character. The term “research” refers to a kind of activity designed to develop or contribute theories, principles, or relationships, or the accumulation of information that can be corroborated by accepted scientific methods of observation and inference. For clinical research, ethically justified criteria for the design, conduct, and review of clinical investigation can be identified by obligations to both the researcher and human subject.

Materials-Methods: A literature search of English and Greek language articles on Pubmed, Scopus, CINAHL and Google scholar, was performed by connecting the Mesh terms of “ethics”, “medical research”, research ethics”, “nursing research”, “healthcare research”, “research ethics principles” and “GDPR” up to the last five years.

Results: It was found 487 articles were reviewed for the relevancy of topic and analyzed in terms of application and validity. Out of these, 16 studies were found relevant as they concentrated principles of ethics in healthcare research, their practical applications, and suggested guidelines for future research. Informed consent, confidentiality, privacy, privileged communication, and respect and responsibility are key elements of ethics in healthcare research. The four principles of autonomy, non-maleficence, beneficence, and justice have been extremely influential in the field of healthcare research ethics.

Conclusions: Research ethics hospital committees must promote greater understanding of ethical issues on healthcare research and to GDPR regulation. Application, research protocol, patient information leaflet and informed consent form are thoroughly reviewed by research ethics hospital committees for legal and moral safety, integrity, and welfare of the research subjects.

UN USUAL CASE OF THROMBOCYTOPENIA IN A 17-YEARS OLD GIRL WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

K. Karagianni, Th. Simopoulou, D.P. Bogdanos, C.G. Katsiari
Department of Rheumatology, University of Thessaly Medical School, Larissa, Greece

Introduction: Thrombocytopenia is a common manifestation in patients with SLE. However, severe thrombocytopenia demands vigorous differential diagnosis and therapeutic intervention.

Materials-Methods: Case report

Results: A 17-year old female patient with SLE was admitted to our hospital with fever and thrombocytopenia. SLE diagnosis was coined the previous month on the basis of butterfly rash, arthritis, fever, anemia, leucopenia, mild thrombocytopenia, nephritis, ANA t:1280, direct Coombs (3+), Lac-test (+), antiCL-IgG(+)IgM(+), antiβ2GPI-IgG(+)IgM(+) and low C3/C4 levels. She was under treatment with high-dose steroids and cyclophosphamide according to the Euro-Lupus protocol. Although no thrombotic event had been documented, based on low serum albumin levels, triple-positive anti-phospholipid antibody profile at high titres and the possible thrombotic nature of nephropathy (renal biopsy result was pending) fondaparinux had also been added to her treatment. At admission, she had 39οC with no other signs or symptoms and a platelet- count of 35000/mm3 with no other remarkable laboratory abnormalities. Fever subsided the next day while platelet count remained stable and the patient received IVlg. However, the following day platelets dramatically dropped to 5000/mm3. Differential diagnosis included infection-induced thrombocytopenia, thrombotic thrombocytopenic purpura (TTP), refractory lupus thrombocytopenia and heparin-induced thrombocytopenia (HIT), since antibodies against heparin were found positive although the patient
was receiving heparin analogue and not heparin per se. Since the patient was afebrile and there was no evidence of infection, she received 5-day pulses of methylprednisolone, cyclophosphamide and she underwent a series of 10 plasmapheresis until platelet count normalized.

**Conclusion:** This case report provokes the discussion on two issues: the significance of anti-heparin antibodies in patients with SLE as well as the potential of fondaparinux to induce HIT.

---

**VASCULAR RASH: PRESENTING A CASE REPORT AND ITS DIAGNOSIS**


Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly

**Case:** We report a case of a 57-year-old male who presented vascular type lesions with necrotizing center on both lower limbs, starting 10 days ago and accompanied with pain. The patient had unremarkable medical history and did not report intake of any medication or any injury recently. Clinical examination did not reveal any other pathological signs. During hospitalization, urine test showed microscopic hematuria (6-8 RBC, >50% dysmorphic) and 24-hour urine collection revealed severe albuminuria (2,632mg / 24h), while blood tests yielded no pathologic findings except from positive titles of antinuclear and anti-citrullinated antibodies. At that point, differential diagnosis included: small vessel vasculitis, pyoderma gangrenosum, Kaposi’s sarcoma or paraneoplastic syndrome. Diagnostic work-up included skin lesions biopsy, which showed fibromatous necrosis in the arterial wall of the capillaries and nuclear abrasions, but no pathognomonic findings. Abdominal ultrasound indicated no pathological results. Subsequently, the patient underwent a computed tomography of the thorax and the abdomen to examine for possible neoplastic disease. A 3.2 cm indiameter, exophytic mass in the left kidney was found, with solid and cystic components. Following consultation with the Urology department partial nephrectomy of the left kidney was recommended. Surgery was performed twenty days after the first hospitalization and the mass was removed up to clean surgical margins. Histopathologic findings of the tumor revealed clear-cell renal cell carcinoma (Furhman Grade 3-4). Skin rash faded away and completely disappeared approximately one month after the surgery, while hematuria and proteinuria did not resolve completely and patient is still under close monitoring.

**Conclusion:** Vascular rash has a complex differential diagnosis, and paraneoplastic syndrome should always be considered as a possible cause as it may be one of the first manifestations of asymptomatic neoplastic diseases.

---

**THE IMPORTANCE OF TRADITIONAL SYSTEMIC THERAPIES FOR ERYTHRODERMIC PSORIASIS IN THE ERA OF BIOLOGICS**

N. Ntavari, E. Savopoulou, A-V. Roussaki-Schulze, E. Zafiriou

Department of Dermatology, University General Hospital of Larissa, Greece

**Introduction:** Erythrodermic psoriasis (EP) is a severe and disabling form of psoriasis in children and adults. The condition presents with distinct histopathologic and clinical findings, which include a generalized inflammatory erythema involving at least 75% of the body surface area. Although the etiology and pathogenesis of erythrodermic psoriasis are not yet fully understood, substantial evidence supports the role of T cells and cytokines. The major risk factors for erythrodermic psoriasis are a personal or family history of psoriasis, medication exposure and infections. Unfortunately, the management of EP is difficult and has not been well standardized. We report a case of the treatment of EP with retinoid.

**Materials-Methods:** A 34-year-old man with generalized erythema and extensive scaling was admitted to our hospital. The involved body surface area was approximately 90% with a Psoriasis Area and Severity Index (PASI) score
The patient had psoriasis vulgaris for 20 years and was successfully treated with secukinumab in the last year. Laboratory tests revealed a clear finding of urinary tract infection with Pseudomonas aeruginosa.

**Results:** The initial management of our patient’s condition included a discontinuation of secukinumab, fluid resuscitation and antibiotics. Initiation of systematic treatment with acitretin 25mg per day was decided. At week 5, a clinical improvement was observed. Erythema, scaling and itching showed a rapid response, significantly reducing the mean PASI score from baseline to 16.1.

**Conclusions:** Erythrodermic psoriasis is a rare subtype of psoriasis characterized by generalized erythema of the entire body with scaling. The current management of EP is difficult, not standardized, and often unsatisfactory. Traditional systemic therapies for EP include methotrexate, cyclosporine, and oral retinoids and this case confirms the theory.

---

**THE KOEBNER PHENOMENON IN DERMATOLOGICAL AUTOIMMUNE DISEASES**

C. Kostopoulou, I. Chondrodimou, A. Gravani, P. Gidarokosta, A. Roussaki, E. Zafiriou

Department of Dermatology, University Hospital of Larissa, Greece

The Koebner phenomenon, one of the most well-known entities in dermatology, refers to the development of dermatologic lesions in otherwise healthy appearing skin at sites of cutaneous injury. It is known to occur in various skin diseases, especially in dermatological autoimmune diseases such as psoriasis, vitiligo, autoimmune bullous dermatosis and discoid lupus erythematosus.

A variety of cutaneous injuries have been reported to induce the Koebner phenomenon including: bites, burns, excoriation, freezing, lacerations, pressure, tattoos, mantoux test, drug reactions, dermatoses. The response to trauma is distinguished into four outcomes: 1. Maximum Koebner response, 2. Minimal Koebner response, 3. Abortive Koebner response, 4. No Koebner response.

The time from injury to lesion formation depends on the specific skin disease. For example, in psoriasis the timeline is between 10 and 20 days, but it can range from 3 days to as long as 2 years.

The pathogenesis of this phenomenon is still unknown. Multiple factors are postulated to play a role including immune response, vascular changes, dermal and epidermal involvement, various growth factors, genetic predisposition, hormonal status, infections and pharmacological agents. The role of chemical messengers such as nerve growth factor (NGF) may be important and is being investigated.

Patients with dermatosis that have been associated with Koebner phenomenon should be informed about the risk of appearance of new skin lesions after injury. Although it is not possible to prevent all cutaneous injuries, patients should avoid: sunburn, contact with irritants and scratching. Treatment for cutaneous lesions arising from the Koebner phenomenon depends on the associated skin condition.

---

**LINEAR IGA BULLOUS DERMATOSIS (LABD) INDUCED BY NAIL POLISH**


Department of Dermatology, University General Hospital of Larissa, Greece

**Introduction:** Linear IgA bullous dermatosis (LABD) is a rare autoimmune subepidermal blistering vesiculobullous disease that can occur in both adult and children. Lesions may appear as tense arciform bullae in a «cluster of jewels» configuration, as seen in bullous pemphigoid (BP), or less commonly as grouped papulovesicles, as seen in dermatitis herpetiformis (DH). It has been associated with certain drugs, mostly intravenous vancomycin, as well as with chemical exposure, autoimmune disorders and infections.
**Materials-Methods:** A 28-year old female appeared with a gradually spreading skin rash around the fingers of the hands. She mentioned use of new chemical materials at work (nail artist). On examination she presented with blisters, vesicles and hemorrhagic crusts with an arciform configuration, on her neck, trunk, upper and lower limbs. She had no mucosal lesions. She reported no recent use of medication. Two biopsies were obtained, one for routine hematoxylin and eosin (H and E) staining, from an actual skin lesion, and the other for direct immunofluorescence (DIF), from the perilesional skin.

**Results:** Histopathological examination demonstrated subepidermal bullous dermatosis with neutrophilic inflammatory infiltration (papillary microabscesses) and DIF showed moderate linear IgA deposition, along the basement membrane zone (BMZ). The patient was treated with corticosteroids, antibiotics, (doxycycline) antihistamines and colchicine.

**Conclusions:** Linear IgA dermatosis is a clinically heterogeneous disease which is due to various causes such as chemicals. We report for the first time in literature a case of LABD induced by nail polish products. Linear IgA dermatosis is a rare bullous disease, whose diagnosis is usually based on linear IgA without IgG deposition along BMZ and shares many characteristics with other subepidermal bullous diseases. Early diagnosis is essential to provide adequate treatment.

---

**AUTOANTIBODY PROFILES IN AUTOIMMUNE RHEUMATIC DISEASES**

N. Bizzaro

Laboratory of Clinical Pathology, San Antonio Hospital, Tolmezzo. Azienda Sanitaria Universitaria Integrata di Udine, Italy

A paradigmatic feature of autoimmune rheumatic diseases (ARD) is the presence of multiple autoantibodies. The use of antibody profiles in the study of ARD therefore should be the best strategy for both diagnostic and classification purposes. To this end, systems using micronized components (protein chips or arrays), consisting of solid phase-linked autoantigens capable of simultaneously detecting many autoantibodies at the same time, are particularly suitable for testing autoantibody profiles.

The reasons that support the use of antibody profiles in the diagnostic framework of ARD are many. Antibody profiling can be advantageous for (early) diagnosis because it increases overall clinical sensitivity; since, in the very early stages of disease, the signs and symptoms do not always point to a single high pre-test probability of disease. Also, antibody profiling is advantageous for several other purposes: for classification, because it allows the definition of disease subtypes with different clinical manifestations; for prediction, because it may direct the diagnosis towards an ARD even in the early asymptomatic phases of the disease; for prognostic evaluation, because the presence of certain antibodies is linked to the involvement of some organs and to the evolution of the disease; and, finally, for the purpose of determining a more personalized therapy, because the antibody profile could identify which subjects will be responsive or not responsive to a specific pharmacological treatment. Furthermore, it has to be considered that in recent years, due to the increased request for tests that were once almost exclusively requested by rheumatologists but that today are ordered by many other specialists and by family doctors, tests are often ordered when there is a low pre-test probability – to rule out underlying ARD rather than to confirm an ARD. As a consequence, the positive predictive value of autoantibody test results has been greatly reduced, while its negative predictive value remains high. This is why the laboratory has to cope with these changes by providing screening profiles with high sensitivity to quickly discriminate negative results (about 70% of all antibody tests) and highly specific disease profiles, to confirm the results of the screening tests and to identify the antibody specificity.

Given the available evidence, it is very likely that further technological progress will substantially change the diagnostic approach to autoimmune diseases in the near future. Autoantibody profiles consisting of dozens if not hundreds of autoantibodies will be able to define each patient’s autoantibody fingerprint and identify subclasses of patients with different prognostic characteristics and different therapeutic responses.
SECOND GENERATION OF AUTOANTIBODY TESTING BY DIGITAL FLUORESCENCE

M. Sowa1, R. Hiemann2, P. Schierack2, D. Reinhold3, K. Conrad4, D. Roggenbuck1,2

1 GA Generic Assays GmbH, Dahlewitz/Berlin, Germany
2 Institute of Biotechnology, Faculty Environment and Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg, Senftenberg, Germany
3 Institute of Molecular and Clinical Immunology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany
4 Institute of Immunology, Medical Faculty, Technical University Dresden, Dresden, Germany

Autoantibodies (autoAbs) are a hallmark of autoimmune illnesses and their assessment is considered an essential part in the serological work-up of patients with autoimmune organ-specific as well as systemic autoimmune rheumatic diseases (SARD). The latter are also referred to as connective tissue diseases (CTD) in a narrower context. Although triggering factors for the occurrence of autoAbs and their role in the pathogenesis of CTD are still not entirely understood, autoAbs are widely used as diagnostic markers in clinical routine. Due to the occurrence of several autoAbs leading to specific profiles in patients suffering from SARD and the complexity of the corresponding serological diagnosis in particular, different diagnostic strategies have been proposed for proper auto Ab testing.

In the history of autoAb detection, evolving assay techniques and the continuous discovery of novel autoantigenic targets have shaped the development of these diagnostic strategies. Thus, antinuclear antibody (ANA) testing by indirect immunofluorescence (IIF) on initially tissue and later on cellular substrates was one of the first assay techniques to be introduced into clinical routine and is still an indispensable tool. Today, the most frequently used serological work-up of sera from individuals with a suspicion of SARD involves the screening for ANA by IIF on HEp2 cells followed by confirmatory (reflex) testing of positive IIF findings by different assay techniques. Yet, due to the tremendous growth in the demand for autoAb testing over the years, IIF has been challenged as the standard method for ANA and other auto Ab testing due to lacking automation, standardization, modern data management and human bias in IIF pattern interpretation. Novel automated assay platforms employing in particular quantifiable solid-phase multiplex techniques with purified autoantigens have emerged addressing the increased workload instead.

A similar development has occurred regarding the serological work-up of patients with autoimmune vasculitides comprising the testing for antineutrophil cytoplasmic antibody (ANCA). Similar to SARD serology, a two-tier analysis of ANCA with IIF as screening technique and subsequent reflex testing by different assays for auto Abs to myeloperoxidase and proteinase 3 was recommended by the consensus guidelines. However, recently published novel consensus guidelines highlight the performance of immunometric assays for the detection of myeloperoxidase and proteinase 3 autoAbs and consider IIF as non-essential anymore. Nevertheless, the need for emergency testing in cases such as rapid progressive glomerulonephritis serology requires cost-effective comprehensive ANCA analysis and challenges this time-consuming and laborious two-tier approach.

To overcome limitations of autoAb profile testing, second generation autoAb assessment by the CytoBead technique has been introduced recently. This novel technique using digital fluorescence enables automated interpretation of cell-based IIF and quantitative autoAb multiplexing by addressable microbead immuno assays in one test. Thus, this technique allows the combination of autoAb screening and confirmatory testing for the first time.

COMPLEMENT AND AUTOIMMUNITY: LOST AND FOUND

Roberto Perricone, Paola Triggianese

Rheumatology, Allergology and Clinical Immunology, University of Rome Tor Vergata, Italy

The Complement System (CS) is a major component of the immune response and the host defense to infection bridging the innate immunity with the adaptive immunity. The link between the CS and autoimmunity is apparently paradoxical since both CS activation and CS deficiency contribute to autoimmune diseases. The inadequate clearance
Lectures

of immune-complexes (IC) in the presence of reduced levels of CS components along with an excess of IC and/or a high concentration of apoptotic cells result in inflammatory damage and release of autoantigens triggering autoimmune responses.

IC diseases can arise in complement deficiency disorders because of the unrestrained action of CS. In patients with Hereditary Angioedema (HAE), a rare genetic deficiency of Cl esterase inhibitor protein (Cl-INH), the reduction of the complement components could affect the immune-regulation and the IC clearance, and that may in turn predispose patients to autoimmunity. Occasional reports documented autoimmune disorders in HAE patients and only a few studies have been conducted on large patient populations with controversial results. In this view, the levels of complement components although reduced can result to be sufficient to avoid the IC precipitation and thus to increase the chance of autoimmunity in HAE. Acquired angioedema (AAE) occur in patients exhibiting low or undetectable CH50, C2, C4, and, frequently, decreased Clq, in addition to low levels of Cl-INH commonly in association with lymphoproliferative and/or autoimmune disorders. Circulating autoantibodies to Cl-INH are frequently encountered in AAE and they seem to prevent the inhibitory activity of the Cl-INH on target proteases and convert the inhibitor into a substrate that can be cleaved to an inactive form. Although lymphoproliferative diseases represent the main group encountered in AAE, evidence documented AAE in patients with Systemic Lupus Erythematosus (SLE) showing high anti-Cl-INH level correlating with duration and activity of the disease. Among complement proteins, a primary role in clearance activity is attributed to Clq. Autoantibodies directed to Clq have been described in the serum of patients with autoimmune diseases, mainly SLE and hypocomplementemic urticarial vasculitis syndrome, contributing to clinical manifestations. Complements C2 and C4 are HLA encoded components and their link with other HLA disease susceptibility genes may explain the relationship between complement deficiencies and IC diseases. The association between genetic deficiency of any early component of the classical pathway (Clq, C1r/s, C2, C4) with SLE-like syndromes has been explained by the failure of clearance of IC and apoptotic materials and impairment of normal humoral response.

Autoimmunity encompasses a mosaic of genetic susceptibility along with hormonal and environmental factors. In this context, CS play key roles in the network of immune dysregulation occurring in autoimmune diseases in a dual and apparently opposite way.

**SEX, GENES AND ENVIRONMENT IN SUSCEPTIBILITY AND SEVERITY OF AUTOIMMUNE DISEASE**

C. Selmi

Rheumatology and Clinical Immunology, Humanitas Clinical and Research Center, Milan BIOMETRA Department, University of Milan

Chronic autoimmune diseases affect the 5-10% of the population worldwide, being largely predominant in women. Sex hormone changes have been widely investigated based on changes in the clinical phenotypes observed during pregnancy and menopause. From a semantic point of view, the terms “gender” and “sex” should not be used as synonyms since the former refers to the social construction of men and women, while the latter indicates the biology associated with the anatomic reproductive system and the secondary sex characteristics, as established by the World Health Organization. The gender difference in the prevalence of these autoimmune diseases can be really striking as in particular for autoimmune thyroid diseases (including Hashimoto’s thyroiditis and Graves’ disease), Sjögren syndrome, Addison disease, systemic sclerosis, systemic lupus erythematosus and primary biliary cholangitis (PBC). In each of these conditions, more than 80% of the patients are women, and it is around ~60–75% for RA and inflammatory myositis. Similar to rheumatic diseases, multiple sclerosis also occurs more frequently in women than men (estimated ratio 2-3:1, increased in the last decades) and it is increasing in particular in younger patients (3.2:1). Beside the proven predisposition of women to autoimmune diseases, an important issue that characterizes sex differences in systemic autoimmune diseases is represented by the variable clinical expression between male and female affected by a paradigmatic chronic inflammatory rheumatic disease, such as psoriatic arthritis (PsA) and spondyloarthritis, in which the male to female ratio is around 1:1. It is intriguing to dissect the reasons of the differences between sexes in the onset and clinical manifestations of autoimmune diseases, to understand why over 80% of patients are women.
A considerable number of sex- and immune-related genes are located within the X chromosome, and major X chromosome abnormalities, such as Turner syndrome and premature ovarian failure, are associated with a higher incidence of autoimmunity. Sex chromosomes alterations such as male 47,XXY and female 47,XXX predispose to SLE and SjS but not RA or PBC, and X monosomy has not been identified in SLE patients. These aspects seem to indicate that more than pathway leads to autoimmunity sex bias, and this is supported by data on animal models that are relevant to the question of whether the number of X chromosomes influences the risk of autoimmune disease.

As suggested by changes in disease manifestations during pregnancy, there is a significant influence of sex hormones on the immunological balance of patients affected by rheumatic diseases, as women show a more active immune response compared to men, and men can show more severe manifestations of rheumatic diseases such as SLE, in which they tend to have more renal involvement, serositis, thrombocytopenia, and anti-dsDNA antibody levels. Immunological changes associated with pregnancy can lead to the first clinical manifestations of systemic autoimmune diseases, such as SLE or RA, in which different immune mechanisms are involved, but serum autoantibodies or other signs of autoimmunity preexisting to pregnancy can only be speculated. Menopause can affect morbidity and mortality in autoimmune disease patients directly or acting on cardiovascular and skeletal systems that are already impacted by autoimmune disease-related changes. Menopause has also been suggested to influence the onset or the activity of certain autoimmune diseases, through alterations in gonadal hormone levels or in the androgen/estrogen ratio.

In recent years, the importance of microbiota in the onset of inflammatory diseases has become increasingly important, and its connection with the immune system has been demonstrated to be bidirectional in conditions such as type I diabetes. Male puberty in mice leads to changes in the gut microbiota that reinforce testosterone production, which is protective against type I diabetes.

**ARE CHRONIC PAIN SYNDROMES BEHIND STATIN-ASSOCIATED MUSCLE PAIN?**

R. Sheinin1,2, A.R. Nogueira1, Nicola Luigi Bragazzi3, Abdulla Watad1,2, Shmuel Tiosano1,2, Yarden Yavne1,2, Kassem Sharif3, H. Cohen2,4, H. Amital2,4, D. Amital2,5

1 Department of Medicine ‘B’ & The Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel
2 Sackler Faculty of Medicine, Tel-Aviv University, Israel
3 Postgraduate School of Public Health, Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy
5 Ness Ziona Beer-Yaacov Mental Health Center

**Background:** Statin myalgia, defined as muscle pain or weakness without elevation of serum CPK levels, is a common complaint among statin users. Chronic pain syndromes affect high percentage of the population, therefore it is likely that such syndromes may confound reports of statin myalgia. Thus, we sought to compare the occurrence of chronic pain between patients taking statins who later developed myalgia with those who did not.

**Methods:** This study included 112 statin-treated patients that were followed at the Lipid Center in Tel-Hashomer Hospital - 56 of whom were diagnosed with Statin Associated Muscle Symptoms [SAMS] and 56 patients who were not. Questionnaires were used to assess diagnoses of fibromyalgia, pain intensity, functional impairment, anxiety and depression in the study population.

**Results:** Patients with statin myalgia were more likely to fulfill the diagnostic criteria for fibromyalgia than patients without stating myalgia (II vs. 0, respectively). Patients in the SAMS group also exhibited higher levels of anxiety and depression in comparison with the control group. Female sex, scoring in Brief Pain Inventory pain intensity, and a Hamilton level indicative of an anxiety disorder were found to be significant predictors for fibromyalgia in patients suffering from stating myalgia.

**Conclusion:** A significant percentage of patients who were thought to suffer from statin myalgia fulfill the diagnostic criteria for fibromyalgia, depression or anxiety disorder. Detection of these patients and treatment of their primary pain or psychiatric illness may prevent unnecessary cessation of effective statin therapy.
THREE SETS OF RECOMMENDATIONS FOR PSORIATIC ARTHRITIS IN 2 YEARS:
WHAT DIFFERENCES OR ADVANTAGES?

C. Selmi

Rheumatology and Clinical Immunology, Humanitas Clinical and Research Center, Milan. BIOMETRA Department,
University of Milan

The ultimate goal of modern medicine is a personalized approach being tailored on the single patient, i.e. tailored,
based on a finely tuned definition of the immunogenetics, epigenetics, microbiome, and biomarkers, to maximize
results and minimize risks particularly of new biologics and small molecules, as in rheumatoid arthritis. While this may
appear as an overambitious goal, we should note that our current genotyping capacity, the proteomic tools, and the
examples of rheumatoid arthritis and other autoimmune diseases are strongly supporting the likelihood of the
success of this hypothesis. Indeed, biomarkers are central to this pathway and the paradigm of anti-nuclear and anti-
citrullinated peptide antibodies should be discussed. Among individual factors around which to tailor the patient man-
gagement are sex and age, with gender-medicine finally becoming central to the research agenda. Over the past three
years there have been efforts from three scientific entities, i.e. the European League against Rheumatism (EULAR),
the group for research and assessment of psoriasis and psoriatic arthritis (GRAPPA) and the American College of
Rheumatology (ACR). These efforts have resulted in three sets of recommendations for the treatment of psoriatic
arthritis (PsA) which differ in several ways, particularly in the way to assess and treat this multifaceted condition.
Indeed, compared to rheumatoid arthritis, patients with PsA often manifest extra-articular affections which often in-
clude skin and nail psoriasis as well as comorbidities.

PsA and skin psoriasis represent a continuum in the spectrum of chronic inflammatory diseases. Mainly secondary
to chronic inflammation and to the increased prevalence of obesity, there is a strict link between these conditions
and the metabolic syndrome which obviously impacts the clinical phenotype, treatment response, and ultimately out-
comes, including survival. Most recently, the group for research and assessment of psoriasis and psoriatic arthritis
(GRAPPA) reported a systematic review on the comorbidities of psoriatic diseases and is producing specific recom-
mandation for the screening and management of patients.

While we believe that the ACR recommendations are of limited use in Europe due to the less stringent criteria
for drug prescription and reimbursement in the US, a comparison of the EULAR and GRAPPA recommendations is
of help in decision-making. In both recommendations, PsA is represented as a heterogeneous disease to be treated
with conventional synthetic DMARDs, such as methotrexate, and targeted therapies including biotechnological agents
targeting IL12/IL23, IL17, TNFalpha and the targeted synthetic small molecule against PDE4. Common grounds include
major over-arching principles and the role of physical therapy and NSAIDs as well as the recognition of an expedited
route to more advanced treatments based on negative prognostic factors. Differences between the two sets are
largely due to the different processes of the recommendation development, particularly the strictly rheumatological
viewpoints within EULAR and the inclusion of a large dermatological component with GRAPPA which also includes
members from Countries worldwide. In the case of GRAPPA, each patient with PsA is arrayed according to the
dominant domain and the 6 domains include skin and nail psoriasis, enthesitis, dactylitis, arthritis, and axial disease.
Updates of both sets of recommendations are awaited in the next months.

HARMONIZATION OF AUTOANTIBODY DIAGNOSTICS: INTRODUCING ICAP
AND EASI APPROACHES

N. Bizzaro

Laboratory of Clinical Pathology, San Antonio Hospital, Tolmezzo. Azienda Sanitaria Universitaria Integrata di Udine, Italy

Standardization and harmonization are complementary tools to achieve higher testing quality in laboratory med-
Lectures 29

icine. Both are of great relevance and are strongly needed in autoimmune diagnostics, due to the impressive advance in basic research and technological development observed in this diagnostic field in recent years that has led to the introduction of many new tests and new analytical methods. It is, therefore, essential that this strong innovativethrust is translated into clinical practice in a coordinated way, to avoid confusion and the risk of potentially harmful errors for the patient. Harmonization of procedures and behaviors should include all the phases of the testing process, such as appropriateness of test requests, autoantibody terminology and adoption of uniform nomenclature for laboratory tests, definition of test profiles and diagnostic algorithms and, harmonization of data reporting and criteria for interpreting immunoserological results.

The antinuclear antibody (ANA) harmonization process involves many factors including the starting dilution, the choice of the clinically relevant titer, and diagnostic algorithms (such as when and which confirmatory tests should be performed in the presence of a positive ANA result or even in the presence of a negative ANA test when an autoimmune rheumatic disease is strongly suspected).

Uniform terminology is needed also in the description of the ANA-IIF patterns. Toward that end, the initiative of the International Consensus on ANA Patterns (ICAP) looks very helpful especially because the consultation is readily and freely available online at the www.ANApatterns.org website, and can be done very quickly, even during the reading at the microscope stage. With this on-line program it is possible to get information on the different patterns, the target antigens corresponding to the autoantibodies that may produce that given pattern, and their clinical associations.

The European Autoimmunity Standardization Initiative (EASI), was founded in 2002 to facilitate communication between laboratory specialists and clinicians to get the best information out of the lab results. Currently, 19 European nations are joining this forum group. EASI has published a general practice guide to autoimmune diseases, has developed recommendations for the assessment of ANA in autoimmune rheumatic diseases and for ANCA measurement in ANCA-associated vasculitis. In addition, a recent document by EASI provides criteria and minimal requirements for accreditation of immunology laboratories.

These initiatives could be the starting steps to achieve a wider consensus and a closer interaction among stakeholders in the path of autoimmune diagnostics harmonization, to enhance clinical effectiveness and provide greater patient safety.

OPTIMIZED DIAGNOSTICS STRATEGIES FOR ANA DIAGNOSTICS – ICAP AND BEYOND

M. Mende
EUROIMMUN a Perkin Elmer company

The international consensus on standardized nomenclature of human epithelial cell (HEp-2 cell) patterns in indirect immunofluorescence (ICAP, www.anapatheory.org) defines fifteen nuclear patterns, nine cytoplasmic patterns and five mitotic patterns which are relevant for the diagnosis of various autoimmune diseases. Furthermore, the consensus stipulates that autoantibodies detected by indirect immunofluorescence on HEp-2 cells should be confirmed by additional monospecific tests. Two new immunoblots have been developed for multiparametric autoantibody characterization.

The EUROLINE ANA Profile 23 provides multiplex confirmation and differentiation of the most important ANA in systemic autoimmune diseases. This broad profile is the first confirmatory assay to cover all of the nuclear patterns defined in the ICAP consensus. The test antigens comprise dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B, RNP/Sm, Sm, Mi-2, Ku, CENP A, CENP B, Spi100, PML, Scl-70, PM-Scl100, PM-Scl70, RPII, RPII5, gp210, PCNA and DFS70.

The EUROLINE Cytoplasm Profile allows parallel detection of autoantibodies against 10 cytoplasmic and mitochondrial antigens that give rise to some of the cytoplasmic patterns described in the ICAP consensus. The antigens comprise AMA-M2, M2-3E, ribosomal P-proteins, Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52. This profile is the most comprehensive assay for detection of cytoplasmic and mitochondrial autoantibodies. Since cytoplasmic antibodies are difficult to recognize in IIFT in some cases, their monospecific detection is of particular importance. The immunoblot is optimally performed in parallel to the IIFT HEp-2 screening to avoid overlooking any cytoplasmic antibodies present.

The EUROLINE system is highly suited for monospecific differentiation and characterization of autoantibodies, offering a multiplex format, ease of use and automatability. As the classification system matures, the profiles can be easily adapted, for example by supplementing them with important new antigens. Notably, the EUROLINE is the
most frequently used ANA confirmatory test in various national quality assessment schemes, reflecting its exceptional quality and successful application in clinical diagnostic laboratories.

The ICAP statement is an on-going process which will be adapted in the future based on feedback from clinicians. The long-term goal is to develop it into a global standard for the reporting of autoantibodies on HEp-2 cells.

A major concern is the standardization of assays. It has been reported and has also become obvious from quality assessment schemes that the quantitative and qualitative agreement of results obtained by test systems based on different methods and provided by different manufactures is not optimal. Several standardization initiatives exist independently of each other but to find the best approach remains challenging.

Idiopathic inflammatory myopathies (IIM) are characterised by a diverse range of autoantibodies, many of which are themselves rare and typically occur in isolation. Diagnosis of myositis is challenging due to rarity of the diseases, the varying clinical presentation and the possibility of overlap syndromes. An IIM diagnosis, moreover, necessitates targeted screening for associated underlying tumours. The determination of myositis specific antibodies (MSA) and myositis associated antibodies (MAA) can significantly reduce the time to diagnosis, with multiparametric testing ensuring the highest serological detection rate.

In suspected cases of myositis, patient sera are serologically investigated using indirect immunofluorescence test (IIFT) on a substrate combination of HEp-2 cells and primate liver, with confirmation of results by monospecific tests. Since antibodies against the cytoplasmic antigens are sometimes not clearly detectable with IIFT, parallel performance of the screening and confirmatory test is recommended. Immunoblots are an ideal confirmatory method, as they enable many different antibodies to be monospecifically detected simultaneously. Line blots fitted with individual membrane chips allow antigens with widely differing properties to be combined on one test strip, enabling profiles to be assembled according to the disease application, regardless of the antigens involved. The EUROLENE Autoimmune Inflammatory Myopathies 16 Ag contains the antigens Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAEI, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, Oj and Ro-52 on one test strip. The autoimmune inflammatory myopathies profile described here is the most comprehensive line blot for myositis antibodies. It is anticipated that ongoing research will identify further novel autoantibodies in myositis, enabling the diagnostic net to be expanded further. The role of specific myositis autoantibodies as predictors of disease course and therapy responses is also being explored.

Sporadic inclusion body myositis (sIBM) is a rare form of IIM. It is a degenerative autoimmune disease of muscle, with inflammatory infiltrates and inclusion vacuoles. Clinical manifestations of sIBM are muscle weakness and atrophy, preferentially affecting the quadriceps femoris and the wrist and finger flexors. The disease is chronic and slowly progressive, leading to severe disability.

sIBM is difficult to distinguish from other IIM. Cases that are suspected on clinical grounds are currently confirmed by muscle biopsy. However, some of the typical histological features are not detectable in up to 30% of patients. Diagnosis may be assisted by determining autoantibodies against the skeletal muscle antigen cytosolic 5'-nucleotidase IA (cN-IA, also known as Mup44 or NT5C1A). Anti-cN-IA autoantibodies are currently the only known serum marker for sIBM. Due to their high specificity, their detection can in particular aid the differentiation of sIBM from other muscle diseases such as PM, DM, necrotising myopathy, muscular dystrophy or myasthenia gravis which is critical due to different treatment regimes. Anti-cN-IA testing can play a valuable role in securing an early diagnosis and reducing the number of muscle biopsies per person.
Many disorders of the central and peripheral nervous system are associated with autoantibodies directed against intracellular onconeuronal antigens or against neuronal cell surface antigens. The detection of these autoantibodies in serum or CSF is of major importance for diagnosis. Due to the connection with various malignant diseases their disclosure often initiates tumour search. Many autoantibodies against neuronal surface proteins have been discovered just recently. They can be easily identified by monospecific analysis using indirect immunofluorescence based on transfected human cell substrates. In laboratory practice these antibodies are found three times more often than antibodies against intracellular targets, which used to be investigated primarily. Among all antineuronal parameters analyzed in laboratory practice, reactivity against glutamate receptors (type NMDA) is detected most frequently. Since some anti-neuronal antibodies occur only relatively rarely, multiparameter testing is favoured over selective or sequential analysis to avoid diagnostic gaps. In laboratory practice (clinical immunological reference laboratory Prof. Stöcker, Lübeck) this strategy enhances the serological hit rate compared to targeted analysis and often provides a fast and reliable, sometimes even life-saving diagnosis. For the investigation of autoantibody profiles multi-parametric test systems (biochip mosaics and immunoblots) with native and recombinant antigenic substrates can be used.

Peripheral immune-mediated neuropathies are a diverse group of rare neurological illnesses characterized mainly by nerve damage. Leading morphological features are in most cases nerve fiber demyelination or a combination of axonal damage and demyelination.

There has been remarkable progress in the clinical and electrophysiological categorization of acute (fulminant, life-threatening) and chronic (progressive/ remitting relapsing) immune-mediated neuropathies recently. For acute immune-mediated neuropathies often referred to as Guillain-Barré syndromes, several serological markers including autoantibodies to gangliosides and sulfatide have been employed successfully in clinical routine. However, the serological diagnosis of chronic variants such as chronic inflammatory demyelinating polyneuropathy (CIDP) or multifocal motor neuropathy has not yet been evolved satisfactory. The common CIDP and various atypical variants thereof demonstrate a wide variety of clinical phenotype and response to treatment. Therefore, serological diagnostic markers could assist in the differential diagnosis of these variants and particularly in the stratification of patients regarding better treatment response. In general, a majority of patients respond well to causal therapy that includes intravenous immunoglobulins
and plasmapheresis. As second line therapy options biologicals (e.g., rituximab) and immunosuppressant or immunomodulatory drugs may be used when patients do not respond adequately.

Save for electrophysiological and morphological makers, autoantibodies against glycolipids or paranodal/nodal molecules have been recommended as candidate markers for immune-mediated polyneuropathies. The progress in autoantibody testing in immune-mediated polyneuropathies has significant implications on the stratification of such patients and their treatment response.

---

## NOVEL ASPECTS IN THE GENETICS OF SLE

C. Perricone, F. Ceccarelli, G. Valesini, F. Conti

Lupus Clinic, Reumatologia, Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma

Systemic Lupus Erythematosus (SLE) is a chronic multifactorial inflammatory condition, prototype of autoimmune diseases. The cause of SLE is unknown to date. However, it has been demonstrated a complex interaction between genetic, environmental, hormonal and immunological in disease pathogenesis. The genetic basis of lupus has not yet been fully clarified and only 15% of the genetic contribution to the disease has been identified so far. Mostly, these genes code for proteins potentially involved in important pathogenetic pathways and seem able to modulate the phenotypic expression of the disease. We evaluated the association of polymorphisms in genes of immunity with susceptibility for SLE and the possible association of these polymorphisms with the clinical course and disease phenotype. Notably, we have revealed for the first time an association with HCP5, TRAF3IP2 and MIR1279a polymorphisms. We also confirmed the role of other important genes in the pathogenesis of the disease, such as STAT4 and IL10, and through genotype-phenotype studies we built a risk model for pericarditis in SLE.

Our data improve the knowledge on the pathogenesis of the disease and may be critical in unravelling novel treatment strategies for patients with SLE.

---

## CURRENT TREATMENT ON PEMPHIGUS

P. Gidarokosta

Department of Dermatology and Venereology, University of Thessaly Medical School, Larissa Greece

The term of Pemphigus includes a number of life threading autoimmune blistering diseases whose the main characteristic is mucocutaneous blisters as a consequence of acantholysis. The treatment of Pemphigus includes the use of corticosteroids which remains the first line drugs on promoting healing of blisters and erosions. Corticosteroids have improved overall mortality, but the use must be patient-tailored in order to avoid adverse effects of therapy. As a second line therapy are intended the so-called Steroid-sparing immunosuppressant drugs. This group contains azathioprine, mycophenolate and cyclophosphamide and must be considered in all cases of serious adverse effects of corticosteroids. Always keep in mind that they also may have serious side effects, including life-threading infection. If first and second line drugs don’t promote healing of the skin a third group of heterogeneous drugs must be considered. Dapsone, intravenous immunoglobulin and rituximab can be a valid alternative in medical choises.
TREATMENT OF BULLOUS PEMPHIGOID: CURRENT THERAPEUTIC STRATEGIES
A. Gravani, E. Zafeirioy, P. Gidarokosta, P. Siomou, A.-V. Roussaki-Schulze
Department of Dermatology, University Hospital of Larissa, Larissa, Greece

Bullous pemphigoid (BP) is a serious acquired autoimmunesubepidermal blistering disease of the skin and mucous membranes.

Tense bullae leading to erosions, eczematosus urticarial lesions, itching, erythema, often not all of them coexisting, consist the main clinical features.

Its pathophysiology is mediated by autoantibodies targeting hemidesmosome structural proteins (mainly BP180, COL17 and BP 230) leading to an inflammatory reaction at the dermoepidermal junctionresulting in subepidermal blistering. Diagnosis is confirmed by findings from histopathological examination and direct immunofluorescence.

The disease has been significantly associated with neurological disorders but occasionally also withunderlying neoplasms, other autoimmune diseases and use of certain drugs (e.g. furosemide, gliptins).

Since it typically affects the elderly, its treatment can be challenging, taking into account possible comorbidities, along with the potential side effects related to the medication used for therapeutic purposes.

The disease was considered life threatening before the use of corticosteroids, due to loss of fluids, electrolyte imbalance or severe infections, especially in cases with extensive skin lesions.

As far as today, corticosteroids remain the cornerstone of BP treatment. Taking into consideration the potential side effects along with the possibility of improving contraindications of long-term corticosteroid use, other drugs (e.g. immunosuppressants such as azathioprine, methotrexate, mycophenolate mofetil) have been tried as corticosteroid-sparing agents but also as adjunctive treatment for refractory cases.

We make an attempt to evaluate current therapeutic strategies, on the basis of the consensus for the treatment of BP (European Dermatology Forum with EADV-2015), the recent updates concerning treatment regimens and the observations from our clinical experience.

UP TO DATE APPROACHES FOR OPTIMAL USE OF ANTINUCLEAR ANTIBODY ASSESSMENT AND THEIR ADDED CLINICAL VALUE IN CLINICAL PRACTICE
K. Tsalimalma
General Hospital “LAIKO” Athens, Department of Immunology, Athens, Greece

Antinuclear autoantibodies (ANA) are the most frequently prescribed autoantibody test used to assess the likelihood of a systemic autoimmune rheumatic disease (SARD) diagnosis. Therefore it is important to have an understanding of the proper use of ANA testing, its value and its limitations. ANA immune fluorescence (IIF) pattern and titer are considered to be of additional clinical value related to other methods of ANA detection. With the use of the HEp-2 cell substrate, well defined IIF patterns to nuclear, cytoplasmic and mitotic antigens are shown to be helpful in determining the probable autoantibodies present and give indication on the type of rheumatic disease. The major advantage of IIF lies in the insights the patterns provide for specific autoantibodies and their clinical associations, as well as being a multipurpose tool for discovery of novel biomarkers. Over the last couple of decades awareness has increased that the value of ANA detection is not limited to diagnosis but further extends to prediction, prognosis and prevention of autoimmune manifestations. A major challenge of ANA testing was the lack of consensus on ANA pattern nomenclature, which is now being resolved due to the efforts of the International Consensus on ANA Patterns (ICAP).

In the field of standardization and harmonization several international groups are very active to optimize the communication between clinicians and laboratory specialists, to establish reference material for worldwide distribution and to harmonize testing algorithms.
Novel technologies on automated immune fluorescence, multiplexed autoantibody arrays and point of care detection methods allow the rapid detection and autoantibody profiling of SARD patients for diagnostic and prognostic purposes. Applications including those techniques for risk assessment of disease manifestation or progression are likely to enter clinical practice in the next years.

---

**CLINICAL SIGNIFICANCE OF ANTIPHOSPHOLIPID ANTIBODIES**

A. Pavlitou-Tsiontsi
Medical Biopathologist, ex. Director of Immunology and Histocompatibility Dept.

Antiphospholipid antibodies (aPLs) represent a heterogeneous group of autoantibodies that directed against plasma proteins (predominantly beta-2 glycoprotein I) which are bound to negatively charged phospholipids-membrane components. Their persistent presence in serum is associated with antiphospholipid syndrome (APS), an acquired autoimmune syndrome characterized by a wide spectrum of clinical manifestations, although it is primarily related to thrombotic and/or obstetric adverse events.

The revised international classification criteria for “definite APS” established that APS diagnosis requires the combination of at least one clinical and one laboratory criterion. The clinical criteria include recurrent thrombosis (arterial, venous or small-vessel) occurring in any tissue or organ and/or pregnancy morbidity. Laboratory criteria require the presence of a lupus anticoagulant (LA) and/or IgG or IgM anticardiolipin antibodies (aCL) present in medium or high titer (>40 GPL or MPL or >99th percentile) and/or anti-β2 glycoprotein-I (anti-β2 GPI) IgG and/or IgM antibodies >99th percentile. These aPL should be present on two or more consecutive occasions at least 12 weeks apart.

The aPL profile, which refers to the type (LA, aCL, anti-β2 GPI) and number (single, double, or triple positive) of aPL, is the most extensively studied and validated risk stratification strategy in patients with aPL. Triple positivity correlates more strongly with both thrombotic and pregnancy morbidity than the presence of double or single positivity. Despite latest APS criteria, diagnosis of this syndrome remains challenging as clinical and laboratory questions are still under discussion. Prospective studies will be needed to design and validate layered approaches that combine standard diagnostic criteria with newer analytical biomarkers, which are necessary to improve APS diagnosis.

---

**AUTOANTIBODIES IN AUTOIMMUNE LIVER AND GASTROINTESTINAL DISORDERS**

C. Kaliouli-Antonopoulou
Immunology-Histocompatibility Dept. General Hospital Nikaia Piraeus “Agios Panteleimon”, Piraeus Greece

Autoimmune Liver Disorders include Autoimmune Hepatitis (AIH), Primary Biliary Cholangitis(PBC), Primary Sclerosing Cholangitis(PSC), Variants (AIH-PBC, AH-PSC, AIH-HCV), APECED and IgG4-related disease.

A. Autoimmune Hepatitis characterized by inflammatory liver histology, elevated transaminase levels, polyclonal hypergamma globulinemia, especially IgG, autoantibodies (aabs) and favorable response to treatment. Autoantibodies detection is the hallmark for AIH diagnosis and allows differentiation of two types AIH-1 and AIH-2.

The AIH-1 autoantibodies are: ANA (Anti-Nuclear Antibodies), SMA V,T,G (Smooth Muscle Antibodies Vascular, Tubular, Glomerular), Ig-anti-SLA/LPA, (Soluble Liver Antigens/Liver Pancreas). The AIH-2 autoantibodies are: anti-LKM-I/LKM-3 (Liver Kidney Microsomal), and anti-LC1 (Liver Cytosol). Detection of Atypical Perinuclear Anti-Neutrophil Cytoplasmic Antibodies ANCA/ANNA, IgG anti-F actin, anti-actinin–a, anti-Ro, anti-ASGP-R, support the diagnosis of patients have tested negative for other aabs.

B. Primary Biliary Cholangitis characterized by Alkaline Phosphatase above 1.5xUpper limit, florid bile duct histology and high titers of AMA (anti-Mitochondrial Antibodies). Autoantibodies detected are:AMA, hallmark for PBC diagnosis and disease specific ANA: anti-MNDs(Multiple Nuclear Dots)/anti-spl100, PML and anti-nuclear membrane/anti-gp210,
anti-p62. Other aabs especially for IFA AMA(-) patients, anti-Kelch , anti-hexokinase,anti-MIT3, have described.

C. Sclerosing Cholangitis characterized by inflammation, destruction and fibrosis of bile ducts. Diagnosis is relies upon clinical assessment histopathology, cholangiography. None of the multiple aabs detected has clinical utility. Atypical P-ANCA is the most prevalent aab.

Gastrointestinal Autoimmune Disorders include Autoimmune Gastritis, Celiac Disease (CD) and Inflammatory Bowel Disease (IBD, Ulcerative colitis and Crohn’s Disease).

A. Autoimmune Gastritis aabs are anti-PCA (Parental Cell Antibodies) and anti-InF (Intrinsic Factor).

B. Coeliac disease is an autoimmune disorder that occurs in genetically predisposed individuals who develop an immune reaction to gluten. Serum tests for CD diagnosis, monitoring and prognosis are: IgA- anti tTG (tissue transglutaminase antibodies) (sensitivity>95%, specificity>95%) detected by ELISA assay and recommended as first-level screening test for diagnosis and serological monitoring since persistence or recurrence usually indicates poor diet compliance. IgG-anti tTG antibodies detected by ELISA assay, are useful in patients with IgA deficiency. IgA- anti Endomysial antibodies (EmA), determined by indirect fluorescence method, are useful in patients with an uncertain diagnosis. Finally, IgG- anti DGP (deaminated gliadin peptides), tested by ELISA, are useful in patients with IgA deficiency and young children. HLA-DQ2 or HLA-DQ8 typing (NPV99%) assess family members of patients or seronegative CD.

C. Inflammatory Bowel Disease, IBD aabs Atypical p-ANCA (Ulcerative colitis 60-70% and 10-15% of Crohn’s Disease patients), have been identified. On the other hand, ASCA (anti-Saccharomyces Cerevisiae) in 60-70% of Crohn’s Disease but 10-15% of Ulcerative colitis patients, are detected.

Keywords: autoantibodies, autoimmune liver disease, coeliac disease.

IMMUNOGENETICS OF AUTOIMMUNE DISEASES

A. Fylaktou

National Peripheral Histocompatibility Centre-Immunology Department, Hippokration General Hospital of Thessaloniki, Greece

The specific causes of most autoimmune diseases are not known. Genetic and environmental factors can overcome tolerance mechanisms and result in disease. Genome-wide association studies (GWASs) and the advent of gene knockout technology in mice have expanded our knowledge of the pathways contributing to autoimmunity and revealed a wide spectrum of genetic risk factors.

Mutations or polymorphisms of many genes have been identified as predisposing to autoimmunity and can be classified as affecting one or more tolerance mechanisms like autoantigen availability and clearance, apoptosis, inhibitory receptors such as PD-1 and CTLA-4, cytokine expression or signaling, co-stimulatory molecules or their receptors and regulatory T cells.

Predisposition to most of the autoimmune diseases is due to the combined effects of multiple genes, but there are some monogenic autoimmune diseases eg immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) and autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), where the unique mutant allele (FOXP3 and AIRE, respectively) confers a very high risk of disease to the individual, but the overall impact on the population is low because these variants are rare. Among genetic loci MHC genes have an important role in controlling susceptibility to autoimmune disease, particularly MHC class II alleles, thus implicating CD4 T cells in their etiology and the ability of different allelic variants of MHC molecules to present autoantigenic peptides to autoreactive T cells.

Genetic variants that impair innate immune responses can also predispose to T cell mediated chronic inflammatory disease as in Crohn’s disease (CD). The growing number of susceptibility genes that confer increased risk for CD point to abnormal regulation of homeostatic innate and adaptive immune responses to the intestinal microbiota as a common factor.

However, many individuals with genetic variants do not get the disease. More research is needed to define specific contribution of environmental factors to autoimmune diseases.

Key words: Autoimmunity, immunogenetics, gene polymorphism, mutations, predisposing MHC genes.
MEASURE, MEASURE, MEASURE: THERE IS ALWAYS SOMETHING OUT THERE

A. Brotis
Department of Neurosurgery, General University Hospital of Larissa, Larissa, Greece

Introduction: Knowledge should flow and be disseminated so that it can be shared and later used in other research studies. The ability to develop a research project and write a scientific paper is a constantly ongoing and improved learning process, together with professional health-related care. Data recording is a crucial part of research methodology, should be very well structured, followed and described in detail.

Material-Methods: We performed a selective literature review in the pertinent Medical Literature, using as mesh terms the words “research methodology”, “data recording”, “study design”, “biostatistics”, and “bioinformatics”. The search was limited in English, and in the period from December 2010 to December 2018. The data synthesis was categorised in five sections: “evidence-based medicine”, “research ethics”, “aims and study design”, “types of error”, and “data recording”.

Results: Data acquisition and research methodology should be very well structured, followed, and properly described in every scientific work. That is particularly true in the era of evidence-based medical care. In every case, the primary investigator should assure the basic ethical background are pursued, including harmonization with the Good Clinical Practices Guidelines and Helsinki Declaration of Human Rights, approval from the Ethics in Research Committee/ Institutional Review Committee of the Institution, acquisition an informed consent. Data should be recorded having in mind clearly defined aims and objectives and feasible study design. Every effort should be paid to minimize systematic errors and bias. On the contrary random error should be limited by properly estimating the sample size of the study. The recorded data should always include the patient unique identifier, the dependent and independent variables, and a limited number of confounders. Finally, a few tips and tricks are provided to maintain high research quality.

Conclusions: Data recording is the systematic registration of scientific observations and forms the foundations of science.

WHAT CAN WE LEARN FROM LARGE DATA BASES?

H. Amital
Center of Autoimmune Diseases & Department of Medicine ‘B’, Sheba Medical Center, Tel-Hashomer, Israel

The increasing use of computerized medical records has turned the clinical data of the entire population available for epidemiological research. The increasing accessibility to this data mandates careful adaptions of ethical guidelines regarding handling of clinical data. But it also grants a unique opportunity to explore the clinical nature of health and disease in large populations that include all the stratum of society, of all socioeconomical levels, ethnicities and geographical locations regardless to their vicinity or distance to tertiary care centers.

Analysis of large data bases gives us the opportunity to learn the public’s behavior towards medical services and to investigate how medical interventions overtime affect outcomes. Interaction between different comorbidities can also be better understood by large population studies.

The huge numbers of patients involved in these studies sets a good model of multivariate analysis, a statistical tool that following proper population adjustments underlines the true independent associations between different conditions.

Nevertheless, the limitations of these studies should be remembered such as inbuilt imprecisions of diagnoses, incompleteness of the medical data and the basic fact that these databases were initially planned for clinical and not investigational use.
As medical use of cannabis is increasingly growing worldwide, a better understanding of the medical and hazardous effects of this drug is imperative. The pain associated with rheumatic diseases is considered a prevalent indication for medicinal cannabis in various countries. Thus far, preliminary clinical trials have explored the effects of cannabis on rheumatoid arthritis, osteoarthritis and fibromyalgia; preliminary evidence has also found an association between the cannabinoid system and other rheumatic conditions, including systemic sclerosis and juvenile idiopathic arthritis. The potential medicinal effects of cannabis could be attributable to its influence on the immune system, as it exerts an immunomodulatory effect on various immune cells, including T cells, B cells and macrophages. However, the available evidence is not yet sufficient to support the recommendation of cannabinoid treatment for rheumatic diseases.
THE ROLE OF IMMUNE RESPONSES AGAINST EPSTEIN-BARRVIRUS IN SYSTEMIC SCLEROSIS

G. Efthymiou¹, C. Liaskos¹, E. Maroul¹, V. Tsimourtou², T. Schep², W. Meyer³, G. Hadjigeorgiou², L. I. Sakkas¹, E. Dardiotis², D. P. Bogdanos¹

¹ Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece
² Department of Neurology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece
³ Institute of Experimental Immunology, EUROIMMUN AG, Lübeck, Germany

Introduction: Epstein-Barrvirus (EBV) infection has been considered trigger of various autoimmune diseases, including systemic sclerosis (SSc). The aim of the current study was to assess ab reactivity against EBV viral capsid antigens (VCA), early antigens (EA) and EBNA-1 in SSc, and investigate their clinical relevance.

Materials-Methods: Sera from 59 SSc patients, including 31 diffuse SSc (dcSSc) and 28 limited SSc (lcSSc), 43 matched multiple sclerosis (MS) as controls and 32 matched healthy controls (HC) were tested for IgG anti-EBV VCA, EA and EBNA-1 abs by immunoblotting, using EBV whole SDS extract as antigen substrate.

Results: Percentages of EA and EBNA-1 reactivities were significantly higher in SSc patients compared to HC (p=0.001 and p=0.012, respectively), but were comparable between SSc and MS. These differences remained when SSc was divided in dcSSc and lcSSc. VCA positivity was comparable between SSc or its two subgroups and MS or HCs. Also, triple positivity for all three antigen categories was observed more frequently in SSc, dcSSc and lcSSc compared to HCs (p=0.001, p=0.004 and p=0.001, respectively). Anti- EA was present more frequently in SSc patients with calcinosis compared to those without (p=0.014) and tended to be more frequent in patients with pulmonary fibrosis compared to those without (p=0.071).

Conclusions: Antibodies against EBV appear to be more frequent in SSc than in healthy controls, and equally prevalent with MS, a disease known to be associated with anti-EBV antibody responses and a known risk factor for MS. Whether an EBV-specific response is also an initiating trigger of SSc remains to be investigated.

ANTINUCLEAR ANTIBODIES BY IMMUNOFLUORESCENCE IN PATIENTS WITH PSORIASIS

E. Patrikiou¹, C. Liaskos¹, A. Gkoutzourelas¹, N. Ntavari², A. Roussaki-Schulze², E. Zafiriou², L. I. Sakkas¹, D. P. Bogdanos¹

¹ Department of Rheumatology and Clinical Immunology, and
² Dermatology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa

Introduction: Antinuclear antibodies (ANA) in patients with psoriasis (Ps) are well documented in the bibliography and most of them correlate their presence with the type of treatment with biologic agents. The aim of our study was to evaluate the presence and level of ANA in our series with Ps patients.

Material and Methods: Seventy three patients with Ps were tested for the presence of ANA by indirect immunofluorescence (IIF) on HEp-2 cells as substrate (Inova, USA). The cohort were considered by 47 men, aged 18-73 years, mainly with plaque type of Ps (64/73, 87.6%, guttate: 5/73, 6.8%, pustular/erythrodermic: 4/73, 5.6%). Dilutions of 1/40 and higher were initially considered as positive.
**Results:** Overall, ANA were present in 30/73 (41%) patients with Ps while their titers ranged from 1/40-1/1280 (median range 1/80). Amongst the 30 ANA positive Ps, 1/40 were present in 14, 1/80 in 8, 1/160 in 1, 1/320 in 4, 1/640 in 1, 1/1280 in 1 and 1/2560 in 1 patients. At 1/80 cut off, 21.9% Ps patients still would be considered positive. 15 (50%) had a fine speckled pattern, 8 (26.7%) had homogenous pattern and the remaining 7 (23.3%) had anucleolar staining pattern. No significant differences in age, gender, disease duration, PASI score, the presence of nails lesions, the treatment status (treatment or no treatment) or the type of treatment were found between ANA positive and negative Ps patients. ANA positivity was correlated with the type of Ps since patients with non-plaque psoriasis had higher prevalence of ANA compared with the patients with plaque psoriasis. No association was found between the titers of ANA’s and the type of pattern on HEp-2 cells.

**Conclusions:** ANA are frequently found in patients with psoriasis especially in patients with non-plaque type of disease even prior to treatment with biologic agents.

### IN ISOLATED HUMAN T-CELLS AND B-CELLS, CRYSTALLINE SILICA ACTIVATES THE T-CELL ANTIGEN RECEPTOR AND THE B-CELL ANTIGEN RECEPTOR AND INDUCES T-CELL AND B-CELL PROLIFERATION

T. Eleftheriadis¹, G. Pissas¹, S. Zarogiannis², V. Liakopoulos¹, I. Stefanidis¹

¹ Department of Nephrology and
² Department of Physiology, Faculty of Medicine, University of Thessaly, Larissa, Greece

**Background:** Silicosis is an occupational fibrotic lung disease, which is characterized by an increased incidence of autoimmune diseases. The effect of crystalline silica on the immune system is thought to be mediated by the antigen presenting cells. However, its direct effect on T-cells and B-cells has not been evaluated adequately.

**Methods:** CD4(+) T-cells and B-cells from 10 healthy individuals were isolated and cultured with or without Min-U-Sil 5. Cell proliferation was assessed with BrdU assay. The levels of phosphorylated zeta and phosphorylated Igα, which are indicative of the T-cell and B-cell antigen receptor activation respectively, and of the transcription factor c-Myc, which is required for cell proliferation, were assessed by western blotting.

**Results:** Crystalline silica triggered CD4(+) T-cell and B-cell proliferation. Also, it enhanced the level of phosphorylated zeta and phosphorylated Igα in CD4(+) T-cells and B-cells, respectively. In both cell types, treatment with silica increased c-Myc expression.

**Conclusions:** Crystalline silica induces T-cell and B-cell proliferation by activating T-cell and B-cell antigen receptors.

### DELPHINIDIN DECREASES PERIPHERAL IFN-γ (+) T CELL SUBSETS FROM PATIENTS WITH PSORIASIS AND PSORIATIC ARTHRITIS

S. Tsiogkas¹, A. Mavropoulos¹, D. Skyvalidas¹, E. Patrikiou¹, N. Ntavari², L. Sakka¹, E. Zafiriou², D.P. Bogdanos¹

¹ Department of Rheumatology and clinical Immunology,
² Department of Dermatology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

**Introduction:** In murine and reconstituted human skin models of psoriasis, delphinidin which is a member of the anthocyanidin family found in pigmented fruits and vegetables, reduced pathological markers on psoriasiform lesions and inhibited infiltration of inflammatory cells. We assessed the currently unknown in vitro effect of delphinidin in peripheral blood mononuclear cells (PBMCs) from patients with psoriasis and psoriatic arthritis.
**Materials- Methods**: We investigated the in vitro role of delphinidin supplementation in PBMCs isolated from 15 Ps patients, 9 PsA patients and 14 healthy controls (HCs). Cells were cultured in the presence or absence of delphinidin added at a final concentration of 20μg/ml for 1 hour prior stimulation. Lymphocyte subsets were identified by flow cytometry using fluorochrome-conjugated monoclonal antibodies against surface CD56, CD3, CD4 and CD8 epitopes. Intracellular expression of IFN-γ following PMA/ionomycin stimulation for 5 hours was also examined using standard cell permeabilization protocols.

**Results**: Co-culture of PBMCs with delphinidin significantly reduced IFN-γ producing T cell subsets from HCs, Ps and PsA patients. Specifically, the magnitude of IFN-γ inhibition in CD4+ T cells was 46% for HCs (p<0.001), 43% for Ps patients (p<0.001) and 57% for PsA patients (p=0.016). Similarly, IFN-γ reduction in CD8+ T cells was 44% for HCs (p<0.001), 29% for Ps patients (p<0.001) and 50% for PsA patients (p=0.015). Reduction of the IFN-γ producing NK and NKT cells was also observed in all three study groups (p values<0.05).

**Conclusions**: Delphinidin acts by directly inhibiting IFN-γ (+) peripheral blood cell subsets from Ps and PsA patients. It remains to be seen whether delphinidin can be used as a natural anti-inflammatory agent for Ps and PsA.

---

**CURCUMIN DOWN-REGULATES IFN-γ AND IL-17 EXPRESSION IN PATIENTS WITH PSORIASIS AND PSORIATIC ARTHRITIS**

D. Skyvalidas1, A. Mavropoulos1, S. Tsiogkas1, E. Patrikiou1, N. Ntavari2, D.P. Bogdanos1, L.I. Sakkas1, E. Zafiriou2

1 Department of Rheumatology and clinical Immunology,
2 Department of Dermatology, School of Health Sciences, University of Thessaly, Larissa, Greece

**Introduction**: Curcumin is widely used as a natural therapeutic agent for the treatment of chronic inflammatory conditions, as anti-oxidant, anti-inflammatory and immunosuppressant. Herein we explored the in vitro effect of curcumin on the production of interferon (IFN)-γ and interleukin (IL)-17 by peripheral blood mononuclear cells (PBMCs) from patients with psoriasis (Ps) and psoriatic arthritis (PsA).

**Materials- Methods**: PBMCs obtained from 20 Ps, 12 PsA patients and 12 healthy controls (HCs) were cultured in the presence or absence of curcumin supplemented at a final concentration of 10μg/ml 2 hours prior stimulation. Peripheral cell subsets were identified by flow cytometry using fluorochrome-conjugated monoclonal antibodies against surface CD56, CD16, CD3, CD4 and CD8 epitopes. Intracellular expression of IFN-γ and IL-17 following PMA/ionomycin stimulation for 5 hours was also examined using standard cell permeabilization protocols.

**Results**: Curcumin pre-treatment of activated PBMCs significantly inhibited IFN-γ and IL-17 expressing cells from HCs as well as Ps and PsA patients (p<0.001 for all). Amongst IFN-γ-producing cells, curcumin mostly inhibited CD4+ T and NKT cells. The mean percentages of IFN-γ inhibition for both subsets was 61% for HCs, 48% for Ps and 45% for PsA patients. Amongst IL-17-producing cells curcumin significantly decreased CD4+ T cells. The mean percentages of IL-17 inhibition were 64% for HCs, 55% for Ps and 48% for PsA patients.

**Conclusions**: Curcumin inhibits in vitro CD4+ (+) IFN-γ (+) and IL-17 (+) peripheral blood subsets from Ps and PsA patients, suggesting its potential role as a natural anti-inflammatory agent for Ps and PsA.
A-ENOLASE AS A TARGET OF AUTOIMMUNITY IN PATIENTS WITH BILIARY ATRESIA

M.G. Mytilinaiou1,2, T. Grammatikopoulos1,3, O. Romanidou1, M. Davenport4, G. Mieli-Vergani1,3, D. Vergani1, D.P. Bogdanos5

1 Liver Immunopathology, Institute of Liver Studies, King’s College London School of Medicine at King’s College Hospital, London, United Kingdom
2 Department of Rheumatology, Athens General Hospital GNA Gennimatas, Athens, Greece
3 Paediatric Liver Centre, King’s College London School of Medicine at King’s College Hospital, London, UK
4 Department of Paediatric Surgery, King’s College Hospital, London, UK
5 Department of Rheumatology, University of Thessaly, Medical School, Larissa, Greece

Introduction: A study by C. Mack et al shows anti-rabbit-α-enolase reactivity in a murine model of biliary atresia (BA) and in children with BA. We have investigated whether human α-enolase is a target of immune response in children with biliary atresia and therefore the prevalence and specificity of anti-human-α-enolase (anti-Hu-α-enolase) antibodies in a large cohort of BA patients and controls.

Materials-Methods: A proteomic analysis of human liver extract was used to identify possible targets of autoimmunity in children with biliary atresia. A total 587 serum samples were tested: 304 from 80 patients with BA, comprising 38 tested at diagnosis, before and after liver transplant (LT) (median follow-up 12 years, range 6-17; median 6 samples/patient, range 2-6) and 42 non transplanted tested at diagnosis and at last follow-up (7 years, 2-15; 2 samples/patient); 99 samples from 29 age matched children with α1-antitrypsin deficiency (α1-ATD, all PiZZ) tested over time at diagnosis, before and after LT (follow-up 9 years, 5-14; 3 samples/patient, 2-5); 165 from patients with other liver diseases at presentation, comprising 31 progressive familial intrahepatic cholestasis (PFIC), 29 Alagille syndrome (AGS), 16 idiopathic giant cell hepatitis (GCH), 41 autoimmune hepatitis type 1 (AIH-1), 33 autoimmune hepatitis type 2 (AIH-2), 15 autoimmune sclerosing cholangitis (ASC); and 19 healthy controls. Anti-Hu-α-enolase reactivity was investigated with an in house ELISA using full-length recombinant human α-enolase (Abcam) as target. A rabbit polyclonal anti-α-enolase antibody (Abcam) was used as positive control.

Results: Using a proteomic analysis, α-enolase was identified as the most likely target of immune responses in 30 children with biliary atresia tested using human liver tissue as substrate.

Overall, anti-Hu-α-enolase reactivity on at least one occasion was observed in 51% (41/80) BA patients, but only in 6% pathological controls [13/194, (3 α1-ATD, 3 PFIC, 3 AGS, 2 GCH, 1 AIH-1 and 1 AIH-2] and in none of 19 healthy controls (p<0.05 for both). Prevalence of anti-Hu-α-enolase was similar in transplanted (52%) and non transplanted BA patients (50%).

Of the 38 BA patients who required LT, 12 (31%) had anti-Hu-α-enolase antibodies at diagnosis, of whom 5 remained persistently positive during follow up and 7 lost reactivity after LT. Of the 26 negative at diagnosis, 8 developed anti-Hu-α-enolase antibodies after LT, while 20 remained negative.

Of the 42 non-transplanted patients, 18 (42%) had anti-Hu-α-enolase antibodies at diagnosis, of whom 4 remained persistently positive during follow up and 14 became negative. Of the 24 negative at presentation, 3 developed anti-Hu-α-enolase reactivity over time and 21 remained negative.

Conclusions: This study shows α-enolase is a target of autoimmunity in children with biliary atresia. Anti-human-α-enolase antibodies are highly prevalent in and specific for biliary atresia, supporting the notion of an autoimmune component in the pathogenesis of this condition. Positivity for anti-Hu-α-enolase does not predict early LT requirement.
INTERLEUKIN 10 AND SYSTEMIC SCLEROSIS – A SNP ASSOCIATION STUDY

D. Plageras¹, D.P. Bogdanos², Z. Mamuris¹, L.I. Sakkas²

¹ Laboratory of Genetics Evolutionary & Comparative Biology, University of Thessaly, Department of Biochemistry and Biotechnology, Larissa, Greece
² Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa, Greece

Introduction: Interleukin 10 (IL-10) is an immunoregulatory cytokine that takes part in both inflammatory and anti-inflammatory responses. It is secreted mainly from B cells and TH2 cells and downregulates the expression of TH1 cytokines while also suppressing macrophages’ functions. There is a subtype of B-cells, known as regulatory B-cells, which are responsible for the production of regulatory cytokines, such as IL-10 and TGF-β, and they have the ability to express inhibitory molecules that suppress pathogenic T cells and autoreactive B cells in a cell-to-cell contact-dependent manner. Various genetic studies have shown that 5’ regions flanking IL-10 gene are highly polymorphic and there are many single nucleotide polymorphisms (SNPs) in the promoter region, some of which have been associated with low IL-10 expression and prevalence of various infectious or autoimmune diseases, such as Systemic Sclerosis (SSc).

Materials – Methods: The aim of this study was to examine the IL-10 gene, as well as other IL-10 related genes, for SNPs that might influence the levels of IL-10 on SSc patients, compared with healthy controls. For this purpose 66 patients and 120 healthy controls were genotyped using a SNP-array. The minor allele frequencies of the genotyped SNPs were compared between cases and controls via Plink v1.07 software.

Results: Minor allele frequencies of 8 SNPs upstream and downstream of the IL-10 gene were found to be significantly different between controls and healthy patients. One of those SNPs has been shown to result on decreased levels of IL-10.

Conclusions: Minor allele frequencies of 8 SNPs upstream and downstream of the IL-10 gene were found to be significantly different between controls and healthy patients. One of those SNPs has been shown to result on decreased levels of IL-10.

HUMAN CYTOMEGALOVIRUS AS A VIRAL TRIGGER OF ANTI-RO52 ANTIBODIES IN PATIENTS WITH AUTOIMMUNE RHEUMATIC DISEASES

A. Gkoutzourelas¹, C. Liakos¹, M.G. Mytilinaiou¹, G. Fthymiou¹, T. Scheper², W. Meyer², C. Katsiari¹, D.P. Bogdanos¹, L.I. Sakkas¹

¹ Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa Greece
² Institute of Immunology affiliated to Euroimmun AG, Lubeck, Germany

Introduction: Human cytomegalovirus (HCMV) has been considered important for the development of systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) and a likely trigger for the induction of anti-Ro52 in SSc but not in SLE (Zhu J ClinExpImmunol 1996). Following these early studies, more recent data demonstrate an association of anti-HCMV with SS-A autoantibodies but remains unclear whether this association relates to Ro-60, Ro52 or both SS-A antigens (Agmon-Levin N et al ClinExpRheumatol 2017). The aim of this study was to assess anti-viral specific antibody reactivity to individual HCMV antigens and to study their association with anti-Ro52 antibody positivity in patients with autoimmune rheumatic diseases.
Oral presentation

Materials-Methods: Assessments were performed in 59 anti-Ro52 antibody positive and anti-HCMV antibody positive patients including 29 with SLE and 30 with SSc. Thirty two healthy individuals, all anti-Ro52 antibody negative but anti-HCMV antibody positive, were tested as normal controls (NCs).Antigen specific antibody responses against HCMV were tested by immunoblot.

Results: Antibody responses against the HCMV antigens UL57, UL83, UL55, UL44, p38 and UL99 antigens were present in 29 (96.6%), 30 (100%), 20 (66.7%), 26 (86.7%) and 27 (90%) patients with SLE, respectively compared to 29 (100%), 23 (79.3%, p=0.01), 19 (65.5%), 14 (48.3%, p=0.002), 15 (51.8%, p=0.005) and 25 (86.2%) patients with SSc, respectively.

Conclusions: Antibody responses against specific HCMV antigens differ amongst healthy individuals and patients with systemic lupus erythematosus or systemic sclerosis and this finding needs further investigation. In patients with anti-Ro52 antibodies, the extent of antibody reactivity to HCMV specific antigens relates to the nature of the autoimmune rheumatic diseases, largely differentiating systemic lupus erythematosus from systemic sclerosis.

---

**EFFECT OF A PATENTED FATTY ACID-BASED ORAL FORMULA ON PERSISTENTLY HIGH BLOOD NK CELL LEVELS OF SUB-FERTILE WOMEN EXPERIENCING REPEATED REPRODUCTION FAILURES**

B. Geladakis¹, Ch. Mpalamoti², Ch. Tsekoura³, Th. Keramitsoglou³, Ch. Tsarmaklis⁴, M. Varla-Leftherioti¹

¹ Rodi Pharmaceuticals, Research and Development, Dover, U.S.A.
² Helena Venizelou, Immunology and Histocompatibility, Athens, Greece

Introduction: NK cell abnormalities is the most common finding in women with alloimmune-mediated repeated implantation failures after IVF (RIF) and/or recurrent spontaneous abortions (RSA). Because of the need to seek safe, effective and cheap remedies for the reduction of NK cells, we have designed and patented a fatty acid-based oral formula, the intake of which was found to highly decrease NK cell number and toxicity in women with PB NK >12%. In the present study, we retrospectively analyzed the effectiveness of the formula in cases with highly increased NK percentages (>18%), which are particularly associated with reproductive failure.

Materials-Methods: The study included 26 RIF and/or RSA women (mean age 36.4±9.2 years). Repeated PB immunophenotyping in all of them had shown persisted highly increased percentages of NK cells (>18%). The women had used a daily dose of the patented formula for a period of 40 days, and the percentage of PB NK cells (CD3-CD16+CD56+) had been measured by flow cytometry immediately before (day 0) and after the use of the formula (day 40).

Results: The mean value of PB NK cells percentage on day 0 was 23.2±4.6% (range 18.0-33.6%). After the use of the formula for 40 days, a 29.9% decrease was observed in 92.3% of the women (24/26). In 19 of them (79.2%) the percentage of NK cells decreased <18%, with the mean value to be reduced to 13.9±2.1 (range 8.4-17.1%). The differences were highly statistically significant (p<0.0001).

Conclusion: According to the above analysis, the formula drastically decreases PB NK cells even in cases of sub-fertile women where these cells are highly elevated. Thus, its use, as standalone or adjunctive to other treatment, is expected to eliminate a serious risk factor for reproductive failure.
TREM2 R47H (RS75932628) VARIANT IS UNLIKELY TO CONTRIBUTE TO MULTIPLE SCLEROSIS SUSCEPTIBILITY AND SEVERITY IN A LARGE GREEK MS COHORT

D. Rikos1*, V. Siokas1*, A-M. Aloizou1, Z. Tsouris1, P. Aslanidou1, G. Koutsis2, M. Anagnostouli3, D. Bogdanos4, N. Grigoriadis5, G.M. Hadjigeorgiou1,6, E. Dardiotis1

1 Department of Neurology, Laboratory of Neurogenetics, University Hospital of Larissa, University of Thessaly, Larissa, Greece
2 Neurogenetics Unit, 1st Department of Neurology, University of Athens, Medical School, Eginition Hospital, Athens, Greece
3 Demyelinating Diseases Unit, 1st Department of Neurology, School of Medicine, Eginition Hospital, National and Kapodistrian University of Athens, Athens, Greece
4 Cellular Immunotherapy & Molecular Immunodiagnostics, Biomedical Section, Centre for Research and Technology-Hellas (CERTH), Institute for Research and Technology-Thessaly (IRETETH), Larissa, Greece
5 Laboratory of Experimental Neurology and Neuroimmunology, B’ Department of Neurology, AHEPA University Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
6 Department of Neurology, Medical School, University of Cyprus, Nicosia, Cyprus
*equal contribution

Introduction: Multiple Sclerosis is a multifactorial autoimmune disease of the central nervous system, characterized by focal inflammation, demyelination and secondary axonal injury. TREM2 is a signaling protein which participates in the innate immune system by implication to inflammation, proliferation and phagocytosis. The R47H (rs75392628) rare variant of the TREM2 gene has been related to various neurological diseases and leads to impaired signaling, lipoprotein binding, lipoprotein uptake and surface uptake. The aim of our study was to assess the role of TREM2 rs75932628 on MS risk through a genetic candidate gene association case-control study in a Greek population.

Methods: 1246 MS cases and 398 controls were genotyped for this variant.

Results: No MS or healthy subjects carried the variant.

Conclusions: This variant does not seem to play a determining role in the pathogenesis of MS, although further studies examining the presence of TREM2 mutations in other, phylogenetically different populations and the epigenetic regulation of this gene are needed in order to thoroughly investigate its role in MS.
THE IMPACT OF SYSTEMIC SCLEROSIS ON HEALTH-RELATED QUALITY OF LIFE: UNRESOLVED ISSUES
E. Georgiou, D.P. Bogdanos, C.G. Katsiari, L.I. Sakkas
Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly

EFFICACY OF CURCUMIN SUPPLEMENTS IN ULCERATIVE COLITIS: A SYSTEMATIC REVIEW AND META-ANALYSIS
K. Gkiouras1,3, M.G. Grammatikopoulou1,3, X. Theodoridis1,3, E. Asteriou1, A. Forbes4, D.P. Bogdanos1,5
1 Department of Rheumatology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Mezourlo, GR41110, Larissa, Greece
2 Department of Nutrition & Dietetics, Alexander Technological Educational Institute, Sindos, PO Box 141, GR57400, Thessaloniki, Greece
3 Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, University Campus, GR54124, Thessaloniki, Greece
4 Norwich Medical School, University of East Anglia, Bob Champion Building, James Watson Road, NR4 7UQ, Norwich, UK
5 Division of Transplantation Immunology and Mucosal Biology, MRC Centre for Transplantation, King’s College London Medical School, SE5 9RS, London, UK

IMMEDIATE AND LONG-TERM BENEFICIAL EFFECT OF IVIG ADMINISTRATION IN CASE OF A WOMAN WITH SLE EXPERIENCING SUB FERTILITY PROBLEMS
Ch Tsekoura1,2, Th. Keramitsoglou1, A. Troboukis2,3, G. Koliopoulos3, K. Mitromara, N. Papanisteidis, M. Varla-Leftherioti1,2
1 Dept of Immunology and Histocompatibility, Helena Venizelou Hospital, Athens Greece
2 RSA Clinic, Helena Venizelou Hospital, Athen Greece
3 B’ Ob/Gyn Clinic, Helena Venizelou Hospital, Athens Greece

POLYMYOSITIS OF UNKNOWN ORIGIN IN PATIENT WITH BILATERAL UPPER LIMB OEDEMA: A CASE REPORT
A. Anagnostara, F. Athanasiadis, M. Dimopoulou, K. Efthymiadou, S. Kalantzil, Th. Simopoulou, D.P. Bogdanos
Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa, Greece
MYOSITIS-RELATED PULMONARY FIBROSIS IN A PATIENT WITH CHOLECYSTOPATHY: A CASE REPORT

A. Anagnostara, F. Athanasiadis, M. Dimopoulou, K. Efthymiadou, S. Kalantzi, Th. Simopoulou, D.P. Bogdanos
Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa, Greece

EFFECTS OF YOGA ON PHYSICAL PAIN AND QUALITY OF LIFE AMONG PATIENTS WITH MULTIPLE SCLEROSIS

M. Gialama
Local Health Unit of Nea Ionia Volos

CASE REPORT: INTERSTITIAL LUNG DISEASES IN THE CONTEXT OF ANTI-SYNTHETASE SYNDROME IN A WOMAN PRESENTED WITH FEVER, ARTHRALGIAS AND DYSPNEA

E. Kourkouni1, T. Simopoulou1, G. Mitsogiannis1, C. Liaskos1, C. Katsiari1, Z. Danii2, K. Gourgoulianis2, D.P. Bogdanos1, L.I. Sakkas1
1 Department of Rheumatology and Clinical Immunology, and
2 Department of Respiratory Medicine, University General Hospital of Larissa, Faculty of Medicine, University of Thessaly, Larissa 41110, Greece

NURSING INTERVENTIONS IN MYASTHENIC CRISIS

K. Bogdanou
Emergency Department, University Hospital of Larissa

MEDICAL CANNABIS: ANOTHER PERSPECTIVE TO TREAT AUTOIMMUNE DISEASES?

C. Alexoudi, M. Faki
Public Professional Training Institute of University General Hospital of Larisa
I0

ANALYSIS OF AUTOANTIBODY SPECIFICITY IN PATIENTS WITH DE NOVO AUTOIMMUNE HEPATITIS AFTER LIVER TRANSPLANTATION

M.G. Mytilinaiou1,2, A. Grammatikopoulos1,3, D.-P. Bogdanos4,5, G. Mieli-Vergani1,3, D. Vergani6

1 Liver Immunopathology, Institute of Liver Studies, King’s College London School of Medicine at King’s College Hospital, London, United Kingdom
2 Department of Rheumatology, Athens General Hospital GNA Gennimatas, Athens, Greece
3 Paediatric Liver Centre, King’s College London School of Medicine at King’s College Hospital, London, UK
4 Department of Rheumatology, University of Thessaly, Medical School, Larissa, Greece

II

IMMUNOFLOUORESCENCE PATTERNS OF ANA AND THEIR ASSOCIATION WITH MOLECULARLY DEFINED NUCLEAR TARGETS IN CHILDREN WITH TYPE I AUTOIMMUNE HEPATITIS

M.G. Mytilinaiou1,2, B. Teegen3, T. Grammatikopoulos1,4, E.T. Davies5, E.I. Rigopoulou6, L. Komorowski3, W. Meyer3, C. Dahnrich3, G. Mieli-Vergani1,4, K. Fechner3, D. Vergani1, D.P. Bogdanos1,7

1 Liver Immunopathology, Institute of Liver Studies, King’s College London School of Medicine at King’s College Hospital, London, United Kingdom
2 Department of Rheumatology, Athens General Hospital GNA Gennimatas, Athens, Greece
3 Department of Biochemical Research, Institute of Experimental Immunology, affiliated to Euroimmun AG, Lübeck, Germany
4 Paediatric Liver Centre, King’s College London School of Medicine at King’s College Hospital, London, UK
5 Department of Clinical Immunology and Allergy, King’s College Hospital, London, UK
6 Department of Medicine, Division of Internal Medicine and Research Laboratory of Internal Medicine, Larissa Medical School, University of Thessaly, Larissa, Greece
7 Department of Rheumatology, University of Thessaly, Medical School, Larissa, Greece

I2

TRIPTORELIN - INDUCED BLACKHAIKY TONGUE: A CASE REPORT

N. Ntavari, E. Savvopoulou, A. V. Roussaki, E. Zafeiriouy

Department of Dermatology, Larissa University Hospital, Larissa, Greece

I3

THE ROLE OF VITAMIN D IN SYSTEMIC SCLEROSIS


Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa Greece
MEDITERRANEAN DIET AS A CORNERSTONE IN THE MANAGEMENT OF RHEUMATOID ARTHRITIS

P. Vranou, A. Gkoutzourelas, C. Liaskos, D.P. Bogdanos, L.I. Sakkas
Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa Greece

A BIOINFORMATIC APPROACH REVEALS THAT HUMAN CYTOMEGALOVIRUS CAN GENERATE SYSTEMIC SCLEROSIS – SPECIFIC AUTOANTIBODIES

A. Gkoutzourelas, M. Barmakoudi, D.P. Bogdanos
Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa Greece

Introduction: Human cytomegalovirus (HCMV) has been considered a trigger of autoimmune diseases, mainly due to molecular mimicry involving viral and self autoantigens. The aim of the study was to review the literature and through a bioinformatics approach to identify viral/self mimics as potential targets of cross-reactive immune responses.

Materials-Methods: PUBMED library was used in search of studies investigating molecular mimicry involving HCMV antigens as cross-reactive targets of SSc-related antigens. BLAST2p was used to compare amino acid similarities between viral and self antigens.

Results: A comparison of the NH2-terminal portion of DNA polymerase I with DNAIS database revealed a 5-amino acid sequence homology (aa 121-126) with HCMV UL70 protein. The HCMV UL55 protein has a high aa homology with a large number of human proteins at the penta-, hexa- and hepta-peptide level.

Our Blast2p search revealed a significant homology between aa27 –RQVSLRSYDNIPPTS– aa41 part of the immunodominant–TTPGEPLKDALGRQVSLRSYDNIPPTSSSDEGEDDDC– epitope of UL99 with DNA topoisomerase (Scl-70) aa 311 RAVALYFIDKLALRA– aa324. The second most immunodominant UL99 epitope aa130– CETDDLDEEDTSYLSPPPVPVQVAKRPRPDTPRPT –aa160 also shares a significant degree of local homology between its core epitopic region aa132 –DDLDEEDTSYLS–144 and aa398– DDLFDRLTTTSLN–aa410 Scl-70.

Conclusions: Mimics between HCMV dominant antigens and SSc-specific autoantigens (Scl-70) have been identified and it will be of interest to investigate their extent of ab cross-reactivity. Of interest, no such similarities were found between HCMV epitopes and the lcSSc related centromere A autoantigen.

DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS SYNDROME SECONDARY TO ALLOPURINOL

Department of Dermatology, University General Hospital of Larissa, Greece
BRAIN ATROPHY IN MULTIPLE SCLEROSIS: MECHANISMS, CLINICAL RELEVANCE AND TREATMENT OPTIONS

A. Andravizou1*, E. Dardiotis1*, A. Artemiadis2, M. Sokratous13, V. Siokas1, Z. Tsouris1, A.-M. Aloizou1, I. Nikolaidis3, C. Bakirtzis4, G. Tsivgoulis2, G. Deretzi6, N. Grigoriadis4, D.P. Bogdanos3, G.M. Hadjigeorgiou17

*Shared first authorship

1 Department of Neurology, Laboratory of Neurogenetics, University of Thessaly, University Hospital of Larissa, Larissa, Greece
2 Immunogenetics Laboratory, 1st Department of Neurology, Medical School, National and Kapodistrian University of Athens, Aegionit Hospital, Vas. Sophias Ave. 72-74, Athens, GR 115-2
3 Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis 40500, Larissa, Greece
4 Multiple Sclerosis Center, 2nd Department of Neurology, AHEPA University Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
5 Department of Neurology, University of Athens, School of Medicine, “Attikon” University Hospital, Athens, Greece
6 Department of Neurology, Papageorgiou General Hospital, Thessaloniki, Greece
7 Department of Neurology, Medical School, University of Cyprus, Nicosia, Cyprus

ASSOCIATION OF MULTIPLE SCLEROSIS-LINKED AUTOANTIBODIES WITH ANTI-HELICOBACTER PYLORI SPECIFIC ANTIGEN ANTIBODIES IN PATIENTS WITH MULTIPLE SCLEROSIS


1 Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis 40500, Larissa, Greece
2 Institute of Experimental Immunology, EUROIMMUN AG, Lübeck, Germany
3 Department of Neurology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece

Introduction: Several antibodies against autoantigens have been linked with the pathogenesis of multiple sclerosis (MS)12. However, their association with antigen-specific anti-Helicobacter pylori (Hp) antibodies in patients with MS has not been investigated yet. The aim of the current study was to identify potential associations in patients with MS.

Materials-Methods: Sera from 60 Hp(+) MS patients were examined for IgG and IgM MS-related autoantibodies by specialized indirect immunofluorescence techniques (including transfected cells) and for IgG Hp antibodies against 15 dominant antigens3,4.

Results: When MS patients were grouped according to their reactivity to Hp antigens, autoantibody frequency was higher in the Hp(+) patients compared to the Hp(-). Anti-neurofilaments IgM antibodies were more frequent in anti-p41(+) (p=0.004) and anti-p29-UreA(+) patients (p=0.009), anti-neurofilaments IgG antibodies were more frequent in anti-p33(+) (p=0.047) and anti-p19-OMP(+) patients (p=0.038), anti-MAG IgM antibodies were more frequent in anti-VacA(+) patients (p=0.010), anti-GFAP IgM antibodies were more frequent in anti-p41(+) (p=0.042) and anti-p67-Flag(+) patients (p=0.002) and anti-myelin IgG antibodies were more frequent in anti-p67-Flag(+) patients (p=0.012).

Conclusions: MS-related autoantibodies are associated with the variable presence of antibodies against Hp antigens, a finding that requires further investigation in search of a potential pathogenic link.


---

LITERATURE REVIEW ON THE ROLE OF ANTI-HUMAN HSP60 ANTIBODIES IN AUTOIMMUNE AND INFLAMMATORY RHEUMATIC DISEASES

G. Efthymiou, L.I. Sakkas, D.P. Bogdanos

Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece

---

IMMUNE RESPONSES TO HELICOBACTER PYLORI-SPECIFIC ANTIGENS DIFFERENTIATE RELAPSING REMITTING FROM SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS


1 Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece

2 Department of Neurology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece

3 Institute of Experimental Immunology, EUROIMMUN AG, Lübeck, Germany

Introduction: Helicobacter pylori (Hp) has been considered an infectious trigger of MS, but detailed antigen specificity of anti-Hp antibodies (abs) in MS patients with RRMS or SPMS is still lacking. Our aim was to systematically investigate ab reactivity against individual Hp antigens and to assess their clinical relevance.

Materials-Methods: Sera from 139 MS (102 RRMS and 37 SPMS) and 63 matched healthy controls (HCs) were tested. IgG anti-Hp Abs were tested by ELISA. IgG abs to 14 individual Hp antigens were tested by immunoblotting.

Results: Anti-Hp abs were present in 43.2% MS patients compared to 48.5% HCs (p=ns).

Within anti-Hp positive MS, only CagA tended to be more frequently recognized in MS patients and HCs (p=0.06). In contrast, antibodies against p54-flagelin (p=0.046) and against p41 (p=0.037), were significantly less frequent in MS than HCs. More importantly, four of the 14 anti-Hp antibodies were less prevalent in RRMS patients than in SPMS, namely anti-p50 (p=0.002), anti-p41 (p=0.003), anti-p29-UreA (p=0.005), and anti-p54-flagellin (p=0.027).

The magnitude of anti-CagA was significantly higher in MS compared to HCs (p=0.005). In contrast, the responses against p66-UreB, p54-flagelin, p50 and p29-UreA were less strong in MS compared to HCs (p<0.05 for all).

Anti-UreA antibody responses were correlated with EDSS (p=0.01) and number of relapses (p=0.02); responses to p-30 OMP were correlated with age at onset (p=0.03) and the presence of anti-p-19 OMP antibodies tended to be correlated with EDSS (p=0.09).

Conclusions: Significant differences in the prevalence and magnitude of responses against Hp antigens is evident between MS and healthy individuals, as well as between RRMS and SPMS, indicating association with this bacterium. Clinical correlations involving responses to anti-UreA may suggest the pathophysiological importance of this antigen in MS.
ANTIBODIES AGAINST BORRELIA SPECIFIC ANTIGENS IN PATIENTS WITH SYSTEMIC SCLEROSIS

E. Marou1, C. Liaskos1, E. Patrikiou1, T. Scheper2, W. Meyer2, L.I. Sakkas1, D.P. Bogdanos1

1 Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece
2 Institute of Experimental Immunology, EUROIMMUN AG, Lübeck, Germany

Introduction: The aetiology of systemic sclerosis (SSc) is unknown but numerous infection agents have been considered potential triggers. There are a few cases in the literature were sclerosus skin lesions were associated with Borrelia infection but there are no studies associating Borrelia with the development of SSc. The aim of the study was to assess anti-Borrelia antibodies in patients with SSc.

Materials-Methods: Serum samples from 40 patients with SSc were tested for the presence of antibodies against various antigens of Borrelia. Seventeen patients with arthritis were tested as disease control. Reactivity to Borrelia-specific antigens were tested by western immunoblotting using blot strips with electrophoretically separated extract from Borrelia (Euroimmun AG, Lübeck, Germany). This assay can detect antibodies against various antigens, some of them are species specific (Category C) namely: p17, p19, p21, p25 (OspC), p30, p31 (OspA), p39 (BmpA), p83, VlsE. According to manufacture based on criteria published in the MiQ 12 Lyme-Borreliose the result of blot is positive (for past infection) if 2 or more bands of antigens Category C are present.

Results: Positivity against Borreliawere observed in 5 out of 40 patients and in 1 out of 17 controls. Antibodies against p31 (OspA) protein of Borrelia were found more frequently in patients with SSc compared to disease control (14/40, 35% vs 1/17, 5.9%). No patients with SSc neither control were found positive for antibodies against p17, p19, p39 (BmpA). We found 1 patient positive for anti-p25 (OspC), 3 positive for anti-p30, 3 positive for anti-p83 and 1 positive for anti-VlsE 1 in patients with SSc compared to 0 positive cases in controls (p=ns in all comparisons).

Conclusions: Anti-OspA (p31) Borreliaantibodies are frequently found in patients with SSc but their significance remains elusive.

ANTIBODIES AGAINST TRIM21 IN PATIENTS WITH MALIGNANT DISEASES

C. Liaskos1, V. Papadopoulos2, A. Goutzourelas1, C. Papandreou2, L.I. Sakkas1, D.P. Bogdanos1

1 Department of Rheumatology and Clinical Immunology and
2 Department of Oncology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Introduction: Monospecific anti-TRIM21 (Ro52) antibodies (Abs) are frequently detected in several autoimmune rheumatic diseases, as well as in a wide range of inflammatory disorders. Recent studies have described the presence of anti-TRIM21 Abs in the context of paraneoplastic syndromes, using limited numbers of patients with malignant diseases as pathological controls. The aim of the present study was to assess the presence of anti-TRIM21 Abs in a large cohort of cancerous patients of various types.

Materials-Methods: Testing of anti-TRIM21 autoAbs was performed by ELISA (Inova Diagnostics, San Diego, CA, USA) in a total of 484 serum samples from patients with various malignant diseases.

Results: Overall, 14.1% of patients were anti-TRIM21 Ab positive. Anti-TRIM21 Ab were presented in 39 (30.3%) patients with ovarian cancer, 11 (8.1%) patients with colorectal cancer, 6 (5.9%) patients with lung cancer, 3 (15.5%) patients with pancreatic cancer, 6 (11.5%) patients with breast cancer, 1 (7.1%) patient with urinary bladder cancer and 3 (10%) patients with head and neck cancer. Levels of anti-Ro52 Abs were higher in patients with ovarian cancer compared to patients with other cancerous diseases. The presence of anti-TRIM21 Abs in patients with ovarian cancer was not correlated with the duration of the disease, the age at diagnosis, the histological type and the stage of the
disease, the sensitivity to chemotherapy with platinum. On the contrary the presence of anti-TRIM21 abs was associated with OS of patients. In more details patients with anti-TRIM21 positivity had an estimated OS 116.3 ± 12.3 months compared to 70.4 ± 4.2 months of anti-TRIM21 negative patients (p=0.038).

Conclusions: Anti-TRIM21 abs are frequently found in patients with ovarian cancer and their presence can be used as prognostic marker of better overall survival.

INCREASED PROPORTIONS OF CD19(+)CD38(LOW) B CELLS ARE PRESENT IN SYSTEMIC SCLEROSIS PATIENTS WITH ASSOCIATED INTERSTITIAL LUNG DISEASE

Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Introduction: The precise function of CD38 epitope differentially expressed by B cells is currently unclear. Reports from CD38⁻/⁻ murine models have been contradictory as for its role in the induction or suppression of autoimmunity. In experimental lupus, the frequency of B regulatory cells (Bregs) was significantly increased in CD38⁻/⁻ splenocytes. Although CD38(hi) expression identifies Bregs, some investigators have targeted CD38⁺ plasma cells for monoclonal antibody mediated depletion in patients with autoimmune rheumatic diseases. The aim of present study we examined the levels of CD38 expression in B cells from patients with systemic sclerosis (SSc).

Materials-Methods: PBMCs were collected from 10 healthy volunteers and 40 SSc patients (10 early SSc, 15 established SSc without associated interstitial lung disease (ILD) and 15 established SSc with associated ILD. B lymphocyte subsets were phenotypically assessed by flow cytometry using fluorochrome conjugated antibodies against CD19, CD38, CD24 and CD27 surface epitopes (BD Biosciences).

Results: B cell subsets were classified based on low and high expression of both CD38 and CD24 epitopes. The percentages of CD19(+)CD38(low) B cells were significantly elevated in SSc patients compared to healthy controls (p<0.05). Amongst SSc patients, CD19(+)CD38(low) B cells were also significantly elevated in patients with associated ILD compared to both early and established SSc patients without ILD (p<0.05). In contrast, the percentages of CD19(+)CD38(high) B cells were decreased in SSc patients compared to healthy controls. CD19(+)CD38(low) B cells were also CD24(low) and CD27(-). Percentages of CD19(+)CD27(+) (memory B cells) negatively correlated with percentages of CD19(+)CD38(low) B cells (r² = 0.43 p=0.03).

Conclusions: Elevated frequency of CD19(+)CD38(low) B cells was detected in SSc patients with associated ILD. These findings suggest a role of dysregulated expression of the CD38 and occurrence of lung fibrosis in SSc.

ANTINUCLEAR ANTIBODIES AGAINST DFS70 IN PATIENTS WITH PSORIASIS AND PSORIATIC ARTHRITIS

E. Patrikiou¹, C. Liaskos¹, E. Zafiriou², A. Gkoutzourelas¹, T. Simopoulou¹, A. Mavropoulos¹, T. Scheper³, W. Meyer³, A. Roussaki-Schulze ², D.P. Bogdanos¹, L.I. Sakkas¹

¹ Department of Rheumatology and Clinical Immunology, and
² Dermatology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa 41 110, Greece; 3 Institute of Experimental Immunology, affiliated to EUROIMMUN AG, Lubeck, Germany

Introduction: It has been shown that autoantibodies directed against dense fine speckled-70 antigen (anti-DFS70) are common among ANA positive healthy individuals and are rarely occur in patients with autoimmune rheumatic diseases but further studies are needed to confirm these results while their clinical significance remains obscure. The
Antigens targets of anti-DFS70 is known as lens epithelium-derived growth factor p75 and a ectopic expression of this protein has been observed in keratinocytes suggesting that it might be involved in psoriatic diseases. The aim of our study was to investigate their presence in psoriatic arthritis (PsA) or in psoriasis (Ps).

**Materials- Methods:** Seventy patients (38 female, 54.3%, mean age 47.1±16.3 years) with Ps (n=36) or PsA (n=34), as well as 50 demographically matched healthy controls (HCs), were tested for the presence of anti-DFS70 abs by a line immunoassay.

**Results:** Positivity against DFS70 was more frequent in PsA patients (6/34, 17.6%) compared to Ps patients (1/36, 2.8%, p<0.05), and healthy controls (2/50, 4%, p=0.056). On the contrary, mean titres of anti-DSF70 abs in PsA, Ps and healthy controls were comparable. Anti-DFS70 antibodies were present not only in patients at baseline, but were also tested in patients with Ps/PsA following treatment with DMARDs or biologics.

**Conclusions:** The presence of anti-DFS70 was higher in PsA than in Ps and further studies needs to confirm if this detection bear a pathophysiological meaning.

**GENITAL ULCERS: AN AETIOLOGY BASED DIAGNOSTIC APPROACH**

E. Savvopoulou, N. Ntavari, A-V. Roussaki, E. Zafeirioy

Department of Dermatology, Larissa University Hospital, Larissa, Greece

**Introduction:** Genital ulcers represent common reason for examination at the outpatients department of the Dermatology clinic, with global incidence estimated to be more than 20 million cases annually. The multiple possible causative agents make diagnosis challenging and renders clinical and laboratory investigation necessary.

**Materials-Methods:** The electronically published literature related to genital ulcers was assessed in the basis of aetiology and diagnostic assessment. An index of the heterogeneity and variety of possible causative factors is the presence of 695 related case reports. The factors were categorized in infectious and non-infectious ones, as well as in isolated clinical manifestations or aspects of a systemic disease.

**Results:** Common genital ulcer related factors include sexually transmitted infections (herpes simplex virus, syphilis, chancroid, granuloma inguinale, lymphogranuloma venereum) and secondary bacterial or fungal infections. Noninfectious and more rare aetiologies include psoriasis, sexual trauma, Adamantiadis-Behçet disease, Sjögren syndrome, Wegener granulomatosis, Crohn disease, genital hidradenitis suppurativa, lichen planus, bullous diseases, pyoderma gangrenosum, Lipschütz acute genital ulceration and fixed drug eruptions. Diagnosing the specific cause of genital ulcers is based on patient’s history, physical examination, and laboratory findings.

**Conclusions:** The clinician, directly or indirectly implicated in the diagnosis and treatment of genital ulcers, should be aware of the wide spectrum of localized and systematic diseases of which they could be clinical manifestations, and perform a systematic diagnostic assessment.

**DETERMINATION OF ANA-SPECIFIC ANTIGENS AS PER ICAP IN PATIENTS WITH PSORIASIS AND PSORIATIC ARTHRITIS**

C. Liaskos1, E. Patrikiou1, E. Zafiriou2, A. Gkoutzourelas1, T. Scheper3, W. Meyer3, A. Roussaki-Schulze2, I. Alexiou1, L.I. Sakkas1, D.P. Bogdanos1

Department of 1 Rheumatology and Clinical Immunology, and
2 Dermatology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa 41 110, Greece;
3 Institute of Experimental Immunology, affiliated to EUROIMMUN AG, Lubeck, Germany

**Introduction:** Antinuclear antibodies are rarely found in patients with psoriasis (Ps) and psoriatic arthritis (PsA)
but their antigen-specificities has not been investigated in details. ICAP has reported a consensus of 14 anti-nuclear antibody immunofluorescence (IF) patterns and various commercial multiplex assays were developed to assist ICAP’s IF ANA testing. The aim of our study was to detect antibodies against nuclear antigen-targets in Ps and PsA patients using a recently developed multiplex immunoassay specifically designed for ANA testing as per ICAP.

Materials-Methods: A line immunoassay containing 23 different antigens (dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B, nRNP/Sm, Sm, Mi-2α, Mi-2β, Ku, CENP A, CENP B, Spt00, PM-L, Scl-70, PM-Scl100, PM-Scl75, RPL11, RPL155, gp210, PCNA and DFS70) was used for the detection of specific ANA. A total of 70 patients with Ps (n=36, 17 female) or PsA (n=34, 21 female) were tested. 50 healthy individuals are also tested as normal controls (NCs, demographically matched).

Results: Detection of at least one autoantibody against nuclear antigens was found in 23/70 patients (Ps and PsA) (32.9%) compared to 6/50 (12%) NCs. No statistically significant correlation for the presence of ICAP were found between Ps and PsA and between Ps/PsA and NCs. (p=NS; Ps vs PsA, p=NS). In more details, the ICAP-related abs in Ps/PsA patients were as follows: AC-1:4.3%; AC-2:10%; AC-3:0.7%; AC-4:11.4%; AC-5:0.7%; AC-6:0.7%; AC-8:2.8%, and AC-10: 2.1%, while in NCs ICAP-related abs were as follows: AC-2:4%; AC-4:4%, AC-3:2%; AC-8:2% and AC-11:2% pattern.

Conclusions: Although the frequency of ANA in patients with Ps and PsA are not intense and no sovereign target was found, ICAP’s ANA stratification is a useful tool for ANA reporting in patients with these diseases.

DETECTION OF ANTINUCLEAR ANTIBODIES RELATED TO RHEUMATIC DISEASES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES


1 King’s College London School of Medicine at King’s College Hospital, SE5 9RS London, UK
2 Department of Rheumatology, Faculty of Medicine, School of Health Sciences, University of Thessaly Viopolis 41110, Larissa, Greece
3 Department of Biochemistry and Biotechnology, University of Thessaly, 41221 Larissa, Greece
4 INOVA Diagnostics, San Diego, CA, USA
5 Norwich Medical School, Bob Champion Building, University of East Anglia Norwich Research Park, Norwich

Introduction: Crohn’s disease (CrD) and ulcerative colitis (UC) are immune-mediated diseases without clear evidence of antigen-driven loss of immunological tolerance such as that noted in various autoimmune rheumatic diseases (ARD), characterized by the detection of ARD-related autoantibodies against nuclear antigens and in particular those targeting SSA, SSB, Sm, RNP and Scl-70. The aim of our study was to detect the presence of ARD-related autoantibodies in patients with IBD.

Materials-Methods: A multiparametric assay (INOVA) to test 210 patients with CrD and 250 with UC for the presence of antibodies against SSA, SSB, Sm, RNP and Scl-70.

Results: None of the tested autoantibodies exceeded a threshold of approx 5% prevalence. The only significance difference comparing CrD and UC patients involved anti-RNP antibodies (II/210, 5.2% vs 3/250, 1.2% respectively, p<0.01). The frequency of all others autoantibodies in CrD and UC patients was as follows: SSA: 3/210, 1.4% vs 3/250, 1.2%; SSB: 1/210, 0.5% vs 1/250, 1.4%; Sm: 5/210, 2.4% vs 7/250, 2.8%; and Scl-70: 1/210, 0.5% vs 2/250, 0.8%.

Conclusions: Detection of ARD-related autoantibodies are very low discouraging autoantibody screening strategies in patients with MS.
LOW FREQUENCY OF ANTIBODIES SPECIFIC FOR AUTOIMMUNE LIVER DISEASES IN PATIENTS WITH MULTIPLE SCLEROSIS

Z. Tsouris\textsuperscript{1}, C. Liaskos\textsuperscript{2}, E. Dardiotis\textsuperscript{1}, T. Scheper\textsuperscript{2}, W. Meyer\textsuperscript{2}, V. Tsimourtou\textsuperscript{1}, G. Hadjigeorgiou\textsuperscript{1}, D.P. Bogdanos\textsuperscript{2}

\textsuperscript{1} Department of Neurology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa
\textsuperscript{2} Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly

\textbf{Introduction:} Elevate liver enzymes are frequently observed in patients with multiple sclerosis (MS) and their origin usually is attributed to the type of treatment. Since MS is considered to be an immune mediated disease, these abnormalities of liver function could represent a possible co-occurrence with autoimmune liver diseases (AILD, AIH-I and PBC). The aim of our study was to assess the presence of AILD-related autoantibodies in patients with MS.

\textbf{Materials-Methods:} Autoantibody testing was performed by indirect immunofluorescence (IF) using triple tissue and HEP-2, a multiparametric line immunoassay detecting anti-LKM1(anti-CYP2D6), anti-LCl(anti-FTCD), soluble liver antigen/liver-pancreas(anti-SLA/LP), AMA-M2, and AMA-MIT3, PBC-specific ANA (anti-gp210, anti-sp100 and anti-PML), and ELISA for anti-F-actin and anti-dsDNA antibodies. The presence of those antibodies were detected in 133 patients with MS (93 female, 102 RRMS/27 SPMS/ 5 PPMS, mean age 42.7±11.9SD years, mean duration of disease 11.2 ±7.2 years).

\textbf{Results:} Anti-F-actin antibodies (specific for AIH-I) were present in 21 (15.8\%) patients with MS, but at relatively low titres. In addition anti-dsDNA were detected in 3 (2.3\%), and anti-SLA/LP in none; AIH-2 related anti-LKM1 autoantibodies were found in 1 (0.8\%), and anti-LCl in none. PBC-specific AMA-M2 were found in 2 (1.5\%), and PBC-specific ANA anti-PML in 6 (4.5\%), anti-sp100 in 1 (0.8\%) and anti-gp210 in 1 (0.8\%). Overall, 30/133 (22.6\%) had at least one of the tested autoantibodies but only 4 (3\%) had overt AILD (2 AIH-I and 2 PBC). By IF, 24 patients were tested positive for SMA VG pattern but at low titres. No other reaction were noticed (all patients were negative for anti-LKM-1 and anti-LC-I).

\textbf{Conclusions:} There is no obvious co-occurrence of AILD in patients with MS despite the relatively frequent presence of related autoantibodies either by IF or molecular assays reducing the diagnostic necessity of AILD-related autoantibody screening in MS patients.

ASSOCIATION OF ANTI-PML ANTIBODY REACTIVITY AND DETECTION OF ANTIGENS AGAINST HSV1 IN PATIENTS WITH PRIMARY BILIARY CHOLANGITIS

M.G. Mytilinaiou\textsuperscript{1}, P. Pavlidis\textsuperscript{1}, A.L. Koutsoumpas\textsuperscript{1,3}, C. Liaskos\textsuperscript{2}, D. Vergani\textsuperscript{4}, D.P. Bogdanos\textsuperscript{1}

\textsuperscript{1} Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa 41 110, Greece;
\textsuperscript{2} Department of Experimental Immunobiology, MRC Centre for Transplantation, Guy’s Hospital, London, UK
\textsuperscript{3} Royal Free London NHS Foundation Trust, London, UK
\textsuperscript{4} Institute of Liver Studies, King’s College London, London, UK

\textbf{Introduction:} The most distinguished antinuclear antibodies in patients with primary biliary cholangitis (PBC) are those against nuclear body related PML and sp100 antigens but why these antigens become targets in PBC remains unclear. One of the most prominent viral triggers is herpes simplex virus (HSV1). Experimental data suggest that this virus specifically targets PML for degradation. We hypothesized that if HSV1 is responsible for the induction of anti-PML, serological evidence of HSV1 infection will be associated with the presence of anti-PML. So the aim of our study was to test patients with PBC for the presence of anti-PML and anti-HSV1 antibodies in order to reveal possible correlation.
Materials-Methods: Sixty six patients with PBC were tested for anti-PML and anti-HSV1 antibodies (against the gB1, gD1 and gC1 dominant HSV-1) by immunoblotting.

Results: Anti-PML antibody presence was associated with anti-HSV1 antibody seropositivity (p=0.002). Amongst 36 patients originally tested for anti-PML and anti-HSV antibodies, 10 (27.8%) were anti-HSV1/PML double positive, 7 (19.4%) were anti-HSV1pos/PMLneg, 2 (5.5%) were anti-HSV1neg/PMLpos and 17 (47.3%) were anti-HSV1/PML double negative. All anti-HSV1/PML double positive cases recognized HSV1 gC1 but this reactivity was not PML specific as all but one anti-PML antibody negative/HSV1 positive PBC patients also reacted with gC1.

Conclusions: An association between anti-PML antibody reactivity and anti-HSV1 seropositivity in patients with PBC was found but no specific viral antigen appears to be related to the loss of tolerance to this nuclear body autoantigen.

AUTOTIBODIES AGAINST NUCLEAR ANTIGENS ARE NOT CORRELATED WITH THE PRESENCE OF ANTIMITOCHONDRIAL ANTIBODIES IN PATIENTS WITH PRIMARY BILIARY CHOLANGITIS

M.G. Mytilinaiou1,2, C. Liaskos1, A. Gkoutzourelas1, A.L. Koutsoumpas3, G.L. Norman4, A. Pares5, D.P. Bogdanos1

1 Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece;
2 Institute of Liver Studies, King’s College London, London, UK
3 Royal Free London NHS Foundation Trust, London, UK
4 Inova Diagnostics, Research & Development, San Diego, CA, USA
5 Liver Unit, Hospital Clínic, IDIBAPS, CIBEREHD, University of Barcelona, Barcelona, Spain

Introduction: Although anti-mitochondrial antibodies (AMA) are the diagnostic hallmarks in patients with primary biliary cholangitis (PBC) and being present in the majority of patients, about 10% of PBC patients are AMA-negative. In these cases, detection of two disease-specific antinuclear autoantibodies being multiple nuclear dots sp100 and nuclear membrane gp210 antibodies are a very helpful diagnostic tool (approximately in to 25% of patients). Only around 10-25% of patients with PBC had all three reactivities. The aim of our study was to investigate possible relation between AMA and PBC-specific ANA against sp100 and gp210.

Materials-Methods: AMA-MIT3, anti-sp100 and anti-gp210 were testing using commercial and in house ELISAs in a total of 172 PBC patients. Antigenic preparations were based on a hybrid containing the major autoimmune regions of the 3 mitochondrial antigens (MIT3), the M2 mitochondrial preparation, the core epitopic regions or extended polypeptide sequences of sp100 or gp210 nuclear autoantigens.

Results: Anti-AMA-MIT3, anti-sp100 and anti-gp210 positivity were found in 158 (91.8%), 43 (25%) and 47 (27.3%) PBC patients respectively. 34 patients were tested AMA-MIT3/sp100 double positive (21.5%), 38 patients were tested AMA-MIT3/gp210 (24%) double positive while 9 patients were tested 9 AMA-MIT3/sp100/gp210 triple positive. No statistically significant correlation between AMA-MIT3 and anti-sp100, AMA-MIT3 and anti-gp210 or anti-sp100 and gp210 antibodies were found. Inhibition studies failed to absorbed anti-sp100 or anti-gp210 by MIT3 antigen or vice versa.

Conclusions: ANA specific for PBC are not targets of cross-reactive autoantibodies against mitochondrial antigens.
ANALYSIS OF IGG ANTIBODIES SUBCLASSES AGAINST ACTIN AND ASIALOGLYCOPROTEIN RECEPTOR IN PATIENTS WITH AUTOIMMUNE HEPATITIS TYPE I

M.G. Mytilinaiou¹, C. Liaskos¹, E.I. Rigopoulou¹, T. Grammatikopoulos², A. Mavropoulos¹, G. Mieli-Vergani, D. Vergani³, D.P. Bogdanos¹

¹ Department of Medicine, Medical School, University of Thessaly, Faculty of Health Sciences, Larissa, Greece,
² King’s College Hospital, Pediatric Liver, GI and Nutrition Centre,
³ Department of Liver Studies, King’s College London, Faculty of Life Sciences and Medicine, London, United Kingdom

Introduction: Autoimmune hepatitis type I (AIH-1) characterized by antibody responses of the IgG isotype against various antigens such as filamentous actin smooth muscle (F-actin) and asialoglycoprotein receptor (ASGPR). The aim of our study was to assess IgG subclasses specificity of anti-F-actin and anti-ASGPR in patients with AIH-1.

Materials-Methods: Serum samples from 28 patients with AIH-1 before initiation of immunosuppressive treatment were pre-selected in order to include sera with single anti-F-actin reactivity (n=10), single anti-ASGPR reactivity (n=7) double anti-F-actin/ASGPR reactivity (n=11). Commercial ELISAs specific for anti-F-actin (INOVA Diagnostics) and anti-ASGPR (Generic Assays) detection were modified using for secondary antibody Horseradish peroxidase-conjugated mouse anti-human IgG isotypes (Southern Biotechnology Associates, Inc., Birmingham, AL) for the detection of IgG subclass antibody reactivity.

Results: Values of absorbance significant for further analysis were obtained in 22/28 (79%) patients (6 double F-actin/ASGPR, 9 single F-actin and 7 single ASGPR). No anti-F-actin or ASGPR antibodies were IgG2. Anti F-actin autoantibodies in patients positive only for F-actin (n=9) belonged mostly to IgG1 (n=7) and to a lesser extent to IgG3 (n=2), while anti-ASGPR reactivity in 7 patients with single anti-ASGPR response was mainly IgG4 (4 IgG4 alone, 2 IgG4/IgG1, 1 IgG4/IgG3). In 6 double anti-F-actin/ASGPR positive cases, anti-F-actin belonged mainly to IgG1 (n=3), IgG4 (n=2) and IgG3 (n=1) while anti-ASGPR belonged exclusively to IgG4.

Conclusions: Anti-ASGPR antibodies are restricted to the IgG4 isotype (IgG subclass with no specific pathogenic connotation) while anti-F-actin reactivity belongs mainly to IgG1 (IgG subclass with the ability to react with complement and to induce antibody dependent cytotoxicity). This diverse antibody response in AIH-1 probably reflect different pathways leading to loss of tolerance.

B CELLS FROM PATIENTS WITH SYSTEMIC SCLEROSIS DISPLAY INCREASED LEVELS OF PHOSPHORYLATED SYK FOLLOWING BCR LIGATION


Department of Rheumatology and clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Introduction: Spleen tyrosine kinase (syk) is a fundamental signaling molecule lying downstream of B cell receptor (BCR) and elevated levels of phosphorylated syk, (p-syk) are found in human chronic graft versus host disease (cGVHD). In mice, inhibition of syk ameliorated sclerodermatous cGVHD, and prevented bleomycin-induced fibrosis. The role of B-cell syk activation in systemic sclerosis (SSc) is currently unknown. Our aim was to assess p-syk levels in B cells from SSc patients following activation through the BCR.

Materials-Methods: Levels of p-syk were assessed by phospho-specific flow cytometry in peripheral blood mononuclear cells isolated from 31 SSc patients (13 with lcSSc and 18 with dcSSc) and in 19 age- and sex-matched, healthy controls (HCS). Cells were stimulated with polyclonal IgM in the presence or absence of H2O2 in order to deactivate tyrosine phosphatases. B cells were stained with anti-CD20 and anti-CD27 fluorochrome-conjugated monoclonal antibodies (moAbs), p-syk was detected using p-syk (Y348)-PE moAb (BD Biosciences) following standard fixation and cell permeabilization protocols.
Results: Unstimulated B cells from SSc patients had significantly elevated levels of phosphorylated Syk kinase [p-syk (+) B cells] compared to HCs (p<0.05). Following BCR-specific stimulation, the percentages of p-syk (+) B cells were also significantly higher in SSc patients compared to HCs (p=0.007). In addition, the mean fluorescent intensity (MFI) levels of p-syk (+) B cells were significantly higher in SSc than in HCs (p<0.05) following activation of BCR in the presence of H$_2$O$_2$. Fluorescence intensity of syk phosphorylation was 2-fold higher in memory B cells than naïve B cells from SSc patients.

Conclusions: Basal and induced syk activation was detected in B cells from patients with SSc, which was significantly amplified compared to HCs. These findings suggest that syk is a main transducer of signals through the BCR and may be involved in the pathogenesis of SSc.

THE FREQUENCY OF PERIPHERAL CD19(+)CD24(HI)CD38(HI) IL-10(+) BREGS IS INDICATIVE OF REMISSION STATE IN RRMS PATIENTS

A. Mavropoulos1, G. Efthymiou1, E. Dardiotis2, Z. Tsouris2, V. Tsimourtou2, L.I. Sakkas1, G. Hadjigeorgiou2, D.P. Bogdanos1

1 Department of Rheumatology and clinical Immunology, 2 Department of Neurology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Introduction: Autoimmune diseases are characterized by deficiency and/or functional impairment of B regulatory cells (Bregs). However, the precise role of these cells in the induction and development of MS remains unclear. Previous studies have reported contradictory data describing either increases, decreases or no effect in Breg status under therapy. As Breg discrepancies were largely due to diverse treatment modalities, and heterogeneous patient cohorts, our aim was to investigate Breg homeostasis and functionality in a well-defined RRMS patient cohort on interferon-β medication.

Materials-Methods: PBMCs were collected from 24 RRMS patients on IFN-β therapy (12 remitting, 12 relapsing) and 12 healthy volunteers. Breg percentages and their functional capacity (ability to produce IL-10) were analyzed by flow cytometry using commercially available fluorochrome conjugated monoclonal antibodies. In order to minimize cross-signaling diversity in Breg-IL-10 expression, a single agonist for B cell specific IL-10 induction (ODN2006-TLR-9) was utilized.

Results: The frequencies of peripheral memory Bregs CD19(+)CD24(hi)CD27(+) were not significantly different in RRMS relapsing patients compared to HCs and RRMS remitting patients (p>0.05 for both). In contrast, the percentages of transitional Bregs CD19(+)CD24(hi)CD38(hi) were significantly increased in RRMS remitting patients compared to RRMS relapsing patients (p<0.001). The proportion of transitional Breg subset in RRMS remitting patients was comparable to the one detected in HCs. IL-10 producing Bregs were also significantly increased during interferon therapy-induced remission compared with RRMS relapse and were highly enriched within CD19(+)CD24(hi)CD38(hi) transitional cell compartment.

Conclusions: Interferon β-induced remission in MS is accompanied by significant increase of IL-10 expressing transitional B cells. Altered Breg status in terms of reduced proportions and compromised functionality, can predict disease relapses.

AUTOANTIBODIES AGAINST SS-A RO52 ARE POSITIVELY CORRELATED WITH B REGULATORY CELLS IN SYSTEMIC SCLEROSIS


Department of Rheumatology and clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Introduction: We have previously shown that IL-10-producing regulatory B cells (B10 cells) are decreased and
functionally defective in systemic sclerosis (SSc). However, we did not find any correlation of the proportions of Bregs and titers of SSc-specific autoantibodies against centromere, Scl-70 or RNA polymerase III. Since we detected anti-Ro52 SS-A antibodies in approx. 22% of patients with SSc, this being the 3rd most frequent autoantibody in this disease, we examined the number and function of Bregs in SSc in relation to anti-Ro52.

Materials-Methods: Serum samples and PBMCs were collected from 40 SSc patients (15 anti-Scl-70, 20 anti-CEN and 5 anti-RNA pol III) and were further subcategorized according to anti-Ro52-positivity into 22 anti-Ro52(+) and 18 anti-Ro52(-). All samples were tested for the presence of disease-specific autoantibodies against Scl-70, CENP, RNA-polymerase and against Ro52 using a line assay (EuroimmunGermany). The function of Bregs was assessed by the ability to produce IL-10 following activation. The proportions of transitional (CD19+CD24hiCD38hi) and memory (CD19+CD27+CD24hi) Bregs were analyzed by flow cytometry using fluorochrome conjugated antibodies (BD Biosciences).

Results: IL-10(+) Bregs were significantly elevated in SSc patients with high-titre antibodies against Ro52 (mean anti-Ro52 arbitrary units AU>100; positivity cut-off AU>20) compared to patients negative for Ro52 (mean AU<10) (p=0.03). IL-10(+) Bregs were enriched within transitional B cells which were also significantly increased in all SSc patients tested positive for anti-Ro52 autoantibodies compared to SSc patients negative for anti-Ro52 autoantibodies (p=0.02). Furthermore, transitional Bregs positively correlated with anti-Ro52 antibody levels (r² = 0.39 p=0.01). In contrast, memory Bregs were not significantly different between anti-Ro52-positive and -negative SSc patients (p>0.05).

Conclusions: Bregs proportions are elevated in SSc patients with high anti-Ro52 antibodies suggesting that this autoantibody could be a potential marker of Breg efficiency.

APREMILAST TREATMENT EXPANDS IL-10+ BREGS THAT ARE NEGATIVELY ASSOCIATED WITH IFN-γ (+) AND IL-17 (+) NKT CELLS IN PSORIATIC ARTHRITIS AND PSORIASIS

A. Mavropoulos1, E. Zafiriou2, A. Roussaki-Schulze1, D.P. Bogdanos1, L.I. Sakkas1

1 Department of Rheumatology and clinical Immunology,
2 Department of Dermatology, School of Health Sciences, University of Thessaly, Larissa, Greece

Introduction: IL-10-producing regulatory B cells (Bregs) are decreased and functionally defective in patients with psoriatic arthritis (PsA) and psoriasis (Ps). Our preliminary report showed that apremilast significantly increased transitional IL-10+ Bregs in patients with Ps and PsA. Herein we explored the relationship of Breg cells with innate subsets expressing the IFN-γ and IL-17 pro-inflammatory cytokines pre and post-Apremilast treatment.

Materials-Methods: PBMCs from 10 PsA and 15 Ps patients obtained pre and post Apremilast treatment (ie 0, 3 and 6 months) and 10 healthy controls (HCs) were studied. Peripheral cell subsets were identified by flow cytometry using MoAbs against surface CD56, CD16, CD3, CD7, CD19, CD24, CD27 and CD38 epitopes. Intracellular expression of IFN-γ, IL-17 and IL-10 following CpG (ODN2006) and PMA/ionomycin stimulation was also examined using standard cell permeabilization protocols.

Results: The baseline (month 0) percentages of CD3+CD56+ (NKT) cells from PsA patients were significantly increased compared to Ps patients and HCs (p<0.05 for PsA vs Ps and HC). In contrast, there was no significant difference in the percentages of CD56+CD7+CD3- (NK) cells between PsA, Ps and HCs. The proportions of NKT and NK cells did not alter post-Apremilast treatment (3 and 6 months). However, Apremilast significantly decreased IFNγ+ and IL-17+ NKT cells from PsA and Ps patients collectively, compared to the percentages at baseline (p<0.05 for both subsets). IFNγ+ and IL-17+ NKT cells from PsA and Ps patients inversely correlated with CD19+CD24hiCD38hi (transitional) Bregs that were also induced by Apremilast. Yet, there was no correlation between CD19+CD24hiCD27+ (memory Bregs) and cytokine producing NKT and NK cells.

Conclusions: Apremilast increased IL-10+ transitional Bregs, which were associated with a significant decline of IFN-γ- and IL-17-producing NKTs in Ps and PsA. These findings suggest an important role of apremilast in dampening pro-inflammatory innate immune responses by expanding IL-10+ Bregs.
DETERMINATION OF PULMONARY ARTERIAL HYPERTENSION IN PATIENTS WITH SYSTEMIC SCLEROSIS: A SINGLE CENTER EXPERIENCE


1 Department of Rheumatology and Clinical Immunology, and
2 Department of Cardiology, University General Hospital of Larissa, Faculty of Medicine, University of Thessaly, Larissa, Greece

Introduction: DETECT algorithm has been developed to determine patients with systemic sclerosis (SSc) with an increased risk of developing PAH (pulmonary arterial hypertension). The aim of our study was to record the experience of our center in the implementation of the DETECT algorithm in patients with SSc and to compare the results with the guidelines of the European Society of Cardiology / European Immunological Society / ERS 2015, for early detection of patients with PAH.

Methods: We have reviewed retrospectively the files of 64 patients (55 females / 9 males) with diagnosis of SS, according to ACR criteria, who had undergone heart ultrasound, and DETECT algorithm, annually, for the period 2015-2018. Right catheterization was performed in patients with score over 35 in step 2 of the DETECT algorithm.

Results: Using the DETECT risk calculation algorithm, 75% (48/64) of patients resulted a score up to 300 in the first step, so cardiac ultrasound was required. In 31.25% of them (15/48) there was a recommendation for right catheterization. PAH was found in 6 patients (6/64, 9.37%), 8 (8/64, 12.5%) had physiological pulmonary pressures, while one patient refused to undergo to right catheterization. Based on the ESC / ERS 2015 guidelines, patients who were considered to be at high risk for PAH and had evidence of right ventricular catheterization were only two (3.12%). These patients were also candidates for catheterization based on the DETECT algorithm. No patient, based on the DETECT algorithm, which does not meet the criteria for right catheterization, did not show ultrasound findings in the re-examinations to rank him in the high-risk group for the development of PAH.

Conclusions: The DETECT algorithm is a useful and sensitive method of detecting PAH at an early stage in patients with Systemic Sclerosis.

TARGETING SYK IN SYSTEMIC SCLEROSIS AND GRAFT-VERSUS-HOST DISEASE

E. Christoforidi, A. Mavropoulos, L.I. Sakkas, D.P. Bogdanos

Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Greece

THE ROLE OF CANNABINOID OIL IN FIBROMYALGIA: CLINICAL EXPERIENCE

A. Gkoutzourelas, G. Efthymiou, D.P. Bogdanos

Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, Larissa, Greece
OVEREXPRESSION OF MIR-21P IN THE PLASMA AND PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

M. Kourtiki, M. Socratous, C.G. Katsiari

Department of Rheumatology, University of Thessaly Medical School, Larissa, Greece

Introduction: Micro-RNAs (miRs) represent significant immune modulators engaging in both innate and adaptive immune response. Preliminary data implicate dysregulation of miR-21 in patients with Systemic Lupus Erythematosus (SLE). We aimed to study the expression of miR-21 in patients with active and inactive disease.

Materials-Methods: Plasma, peripheral blood mononuclear cells (PBMCs) and urine sample were collected at the same time from each patient. Every patient was matched for gender and age with a healthy control subject. Total RNA was isolated (NucleospinmiRNA-Macherey-Nagel), c-DNA synthesis was performed and quantification of mir-21-5p was made using real-time PCR (Qiagen). Levels of endogenous U6 were used for the normalization of mir-21-5p levels in PBMCs. Plasma and urine levels of mir-21-5p were normalized using synthetic mir-39. Statistical analysis employed the 2^-ddCt method.

Results: The expression of mir-21-5p was increased in plasma and PBMC from patients with active disease. Plasma expression in SLE patients was found 3.2 times (range 0.6-15.9) higher compared to normal controls. Patients with inactive disease presented similar levels compared to healthy individuals, in contrast to patients with active disease that had 5.9 times (range 2.4-15.9) higher levels (p<0.05). Mir-21-5p overexpression in patients with active disease was even more prominent in PBMCs, where a 27.7 folds increase (range 1.64-85.3) was documented, while PBMCs derived from patients with inactive disease showed levels similar to healthy controls.

Conclusions: Mir-21-5p is overexpressed in the plasma and PBMCs from patients with active SLE.

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Program «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project “Regulation of microRNA in Systemic Lupus Erythematosus: the role of miR-21 and miR-210” (MIS 5007258).

VY9VΔ2 TCELL-MEDIATED ANTI-TROPHOBLAST ACTIVITY IN WOMEN WITH CHLAMYDIAL CERVICAL-VAGINAL INFECTION EXPERIENCING RECURRENT SPONTANEOUS ABORTIONS

E. Glynou1, I. Voskakis2, Ch. Tsekoura2, Th. Keramitsoglou2, D.P. Bogdanos3, M. Varla-Leftherioti2

1 Dept. of Microbiology “Helena Venizelou” Hospital, Athens, Greece
2 Dept. of Immunology and Histocompatibility “Helena Venizelou” Hospital, Athens, Greece
3 Faculty of Medicine. School of health Sciences. University of Thessaly, Larisa, Greece

Introduction: We have previously reported a significant association of Vγ9Vδ2T cells with cervical/vaginal chlamydial infection in women with RSA. This association raises questions on the chlamydial antigens that activate γ9δ2 T cells to develop an anti-trophoblast response as well as the factors that enhance this γ9δ2 mediated activity. In the present study we try to answer if the γ9δ2 mediated anti-trophoblast activity is related to the length of the infection by analysing the γ9δ2 T cells in aborters who have received or not anti-chlamydia treatment.

Materials-Methods: The percentage of γ9δ2 T cells in PB was analyzed by flow cytometry in 76 positive for Ct infections (Ct+) RSA women (A), within 10 days after a new miscarriage. 35 of them had received anti-Ct treatment.
upon diagnosis (T) and 41 were not treated (NT). Fertile women without Ct infection (C) served as controls (C=82).

**Results:** A highly statistically significant difference of mean percentages of γ9δ2 T cells was shown between T and C groups (58.95 vs 61.92, p<0.00001). The analysis performed in T and NT aborters revealed that the mean percentage was 58.95% and 83.29% respectively (p=0.0002), and that 53% of NT women had >80% γ9δ2 T cells (only 20% of T women, p=0.0017).

**Conclusions:** The increased levels of PB γ9δ2 T cells in untreated cases support the hypothesis that the persistence of Ct in the cervical/vaginal tract favors the recognition by γ9δ2 T cells Ct antigens sharing common epitopes with antigens expressed on the embryonic tissues, which results in the activation of anti-embryonic responses and possibly abortions. Knowing that chlamydial HSPs share common epitopes with human HSPs expressed on embryonic tissues, we currently investigate the hypothesis that the γ9δ2 mediated anti-trophoblast activity is triggered by CtHSPs.

---

**INVESTIGATION OF AUTOANTIBODY TITERS OVERTIME AS MARKERS OF DISEASE ACTIVITY IN PATIENTS WITH AUTOIMMUNE HEPATITIS TYPE 2**

M.G. Mytilinaiou1,2, A. Grammatikopoulos1,3, D.-P. Bogdanos1,4, G. Mieli-Vergani1,3, D. Vergani3

1 Liver Immunopathology, Institute of Liver Studies, King’s College London School of Medicine at King’s College Hospital, London, United Kingdom
2 Department of Rheumatology, Athens General Hospital GNA Gennimatas, Athens, Greece
3 Paediatric Liver Centre, King’s College London School of Medicine at King’s College Hospital, London, UK
4 Department of Rheumatology, University of Thessaly, Medical School, Larissa, Greece

**Introduction:** A small longitudinal study (Muratori et al. Gut 1998) has suggested that titres of anti-liver kidney microsomal autoantibody type 1 (anti-LKM1), the serological marker of type 2 autoimmune hepatitis (AIH-2), and IgG levels correlate overtime with biochemical evidence of disease activity, as assessed by serum AST and IgG levels.

**Aim and Methods:** To verify this, we investigated sequentially eleven anti-LKM1 positive AIH patients (10 female) on 202 occasions (median 16, range 10-45) at diagnosis and follow up (median 10 years, range 5-18). Correlations between AST or IgG and anti-LKM1 levels, tested by both indirect immunofluorescence (IFL) and an ELISA using recombinant cytochrome P4502D6 (CYP2D6), the target of anti-LKM1, were sought.

**Results:** The behaviour of anti-LKM1 antibodies by IFL paralleled that of AST in 9/11 cases. The decline of AST preceded that of anti-LKM1 antibodies during immunosuppressive treatment. AST flares in 9 patients who relapsed during a 7-18 year period were accompanied by an increase in anti-LKM1 titre. Anti-LKM1 antibody titres correlated positively with AST levels in all but one case and with IgG levels in 5/11 cases.

The behaviour of anti-CYP2D6 levels by ELISA was comparable to that of anti-LKM1 antibodies by IFL in 8/11 cases. At variance with anti-LKM1 antibodies, which became negative or decreased in titre by at least 50% from their highest level during immunosuppressive treatment in 10 cases, anti-CYP2D6 antibodies maintained levels exceeding 40 RU/ml (cut off: 20 RU/ml) in 8/11 cases. Anti-CYP2D6 antibodies correlated positively with AST levels in 7 and with IgG levels in 3 cases. A positive correlation was also observed between IgG and AST levels in 4 patients.

**Conclusions:** Our results indicate that measurement of anti-LKM1 autoantibodies by indirect immunofluorescence, and to a lesser extent of anti-CYP2D6 by ELISA, can be used to monitor disease activity in AIH-2.
ANALYSIS OF ADMISSIONS OF PATIENTS WITH AUTOIMMUNE RHEUMATIC DISEASES IN A REGIONAL UNIVERSITY HOSPITAL CLINIC OF RHEUMATOLOGY AND CLINICAL IMMUNOLOGY IN 2015 AND 2017: AN EPIDEMIOLOGICAL STUDY

Department of Rheumatology and Clinical Immunology, University of Thessaly Medical School, Larissa Greece

Introduction: The purpose of this study was to record all entries in a reference centre for rheumatic diseases in Central Greece, namely the Department of Rheumatology and Clinical Immunology (16 beds In-patient Clinic), University General Hospital of Larissa for the calendar year 2015 compared to that of two years after (2017), regarding five common autoimmune rheumatic diseases: Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Systemic Vasculitis (SV), Psoriatic Arthritis (PsA) and Systemic Sclerosis (SSc).

Materials-Methods: Submission were recorded and analyzed through "ASKLIPIOS" e-database. Recorded data included demographic data of the patients, the days of hospitalization and any case of infection as a cause of hospitalization.

Results: In 2015, 608 admissions (276 patients) (mean age= 60.17±17.1 years), 3364 total days of hospitalization were recorded in the Clinic. Of these, 262 (43%) entries were recorded for patients with RA, 114 (19%) with SLE, 109 (18%) with SV, 64 (10%) with PsA and 61 (10%) with SSc. Infection as the cause of entry was recorded in 59 patients (9.7%). The mean duration of hospitalization was 5.55 days, while the above five diseases accounted for 65.8% of the total occupancy of the Department. Respectively, for the year 2017, the total number of imports was 948 (431 patients) (mean age=58.66±21.56). 397 (42%) enrolled patients with RA, 214 (22%) with SLE, 180 (19%) with SV, 88 (10%) with PsA and 69 (7%) for patients with SSc. Infection as the cause of entry was recorded in 77 (8.1%) patients. The mean duration of hospitalization was calculated at 3.56 days, while the above five diseases accounted for 66.8% of the total occupancy of the department.

Conclusions: A considerable increase of admissions in a Rheumatology clinic of a University General Hospital has been noted. The reasons for this increase warrant further investigation. Approximately 8-10% of those are due to infectious causes and this must be taken into account for the proper management of patients with chronic conditions such as autoimmune rheumatic diseases.

AMELIORATION OF NECROBIOsis LIPOIDICA IN PATIENTS WITH PSORIASIS AND DIABETES MELLITUS WITH APREMILAST

N. Ntavari, E. Savopoulou, A-V. Roussaki-Schulze, E. Zafiriou
Department of Dermatology, University General Hospital of Larissa, Greece

Introduction: Psoriasis is a chronic inflammatory disease of the skin associated with a high prevalence of obesity and metabolic syndrome. In our study, two patients with plaque psoriasis and necrobiosis lipoidica on unregulated diabetes mellitus treated with methotrexate (MTX) are presented. Administration of apremilast resulted in improvement of both the glycemic index and the necrobiosis lipoidica.

Materials-Methods: Patient A. 68-year-old male, with severe plaque psoriasis (PASI: 24.4) by 15 years, coexisting necrobiosis lipoidica by a month and diabetes mellitus (type II) (baseline HbA1c: 8.0%) under anti-diabetic treatment. Patient B. 62-year-old male, with rheumatoid arthritis, under treatment with prednisolone, severe plaque psoriasis (PASI: 12.8) and necrobiosis lipoidica on the legs (baseline HbA1c 6.3%). At the time of attendance, patients received only local treatment for psoriasis. Initiation of systematic treatment with methotrexate 10-15mg / week and folic acid
5mg / week was decided. One year later, a deregulation of diabetes mellitus was observed. In patient A the HbA1c increased from 8.0% to 10.1% and in patient B from 6.3% to 7.3%. In addition, a significant clinical improvement of psoriasis (Patient A. PASI: 3.5 and Patient B. PASI: 7) was detected but the lesions of necrobiosis lipoidica on the legs remained stable.

**Results:** A discontinuation of methotrexate and administration of an immune response was then decided. Within 7 months, the following results were obtained. The HbA1c value was reduced to 6.1% in patient A and 6.2% in patient B, an improvement of necrobiosis lipoidica and a remission of psoriasis (PASI 0) were also noted.

**Conclusions:** Apremilast, an oral phosphodiesterase-4 inhibitor, is a safe and effective treatment option in patients with plaque psoriasis and diabetes mellitus as it is associated with an amelioration of necrobiosis lipoidica resulting from a decrease of HbA1c.

---

**A NOVEL NEUTROPHIL AUTOANTIGENIC TARGET IN INFLAMMATORY BOWEL DISEASE**

C. Deutschmann¹, M. Sowa¹², J. Murugaiyan³, K. Conrad⁴, M. Laass⁵, D.P. Bogdanos⁶, M. Papp⁷, S. Rödiger¹, D. Roggenbuck¹², P. Schierack¹,*

¹ Institute for Biotechnology, Faculty Environment and Natural Sciences, Brandenburg University of Technology, Senftenberg, Germany
² Medipan/ GA Generic Assays GmbH, Dahlewitz, Germany
³ Institute for Animal and Environmental Hygiene, Freie Universität Berlin, Berlin
⁴ Institute of Immunology, Medical Faculty Carl Gustav Carus, Technical University Dresden, Dresden, Germany
⁵ Children’s Hospital, Medical Faculty Carl Gustav Carus, Technical University Dresden, Dresden, Germany
⁶ Department of Rheumatology, School of Health Sciences, University of Thessaly, Larissa, Greece
⁷ Department of Internal Medicine, Division of Gastroenterology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
* Author whom to correspond to: Peter.Schierack@b-tu.de

**Introduction:** There is an increasing incidence of inflammatory bowel disease (IBD). Autoimmune responses partake in the pathophysiology of IBD, but underlying pathways and target antigens are not elucidated fully yet.

**Methods:** Autoantigenic targets in IBD were identified by immunoblotting of antineutrophil-cytoplasmic antibody (ANCA)-positive sera of IBD patients on neutrophil proteins and MALDI-TOF mass spectrometry. Enzyme-linked immunosorbent assays (ELISAs) using recombinant chitinase 3-like protein 1 (CHI3LI) was used to analyze the prevalence of IgG, IgA, and secretory IgA (sIgA) to CHI3LI in 110 patients with Crohn’s disease (CD), 95 with ulcerative colitis (UC), 126 with celiac disease (CeD) and 86 healthy controls (HCs).

**Results:** The 18-glycosylhydrolase family member Chitinase-3-like protein 1 (CHI3LI) was identified as neutrophilic autoantigenic target. CD patients displayed significantly higher levels of IgG, IgA, and sIgA to CHI3LI than patients with UC, CeD, and HCs (p< 0.05, respectively). sIgA to CHI3LI demonstrated the highest prevalence in CD (41.8 %; 46/110).

**Conclusions:** For the first time, we provide evidence that CHI3LI, overexpressed in enterocytes during inflammation of the large bowel and supporting the uptake of pathogenic intestinal bacteria, is a novel neutrophilicautoantigenic target in IBD. IgA and sIgA to CHI3LI may serve as novel markers for CD and may facilitate the serological diagnosis of IBD.
ANTI-HUMAN CYTOMEGALOVIRUS HUMORAL RESPONSES IN PATIENTS WITH SYSTEMIC SCLEROSIS


1 Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece
2 Department of Neurology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500, Larissa, Greece
3 Institute of Experimental Immunology, EUROIMMUN AG, Lubeck, Germany
4 Department of Obstetrics and Gynecology, Faculty of Medicine, School of Health Sciences, University of Thessaly, 41110 Larissa, Greece

Introduction: Human cytomegalovirus (HCMV) has been considered an infectious trigger of autoimmune diseases, including systemic sclerosis (SSc). The aim of the present study was to analyze a large number of antigen-specific anti-HCMV responses in a large cohort of patients with SSc.

Materials-Methods: Our study included 110 patients with SSc (59 with limited cutaneous [lcSSc] and 51 with diffuse cutaneous [dcSSc] disease), 60 demographically matched multiple sclerosis (MS) patients (35 relapse-remitting MS [RRMS] and 25 secondary progressive MS [SPMS]) as pathological controls, and 51 age- and sex-matched normal individuals as healthy controls (HC). Ab responses against HCMV-specific antigens were tested by Western immunoblotting.

Results: IgG anti-HCMV ab positivity was comparable between SSc and MS, but more prevalent compared to HCs. Anti-UL57 abs were more frequent in lcSSc compared to RRMS and SPMS (p<0.01 for all), while anti-UL55 abs were more frequent in both lcSSc and dcSSc compared to RRMS. The magnitude of ab response to UL57, UL83 and UL99 were stronger in SSc or its subtypes compared to HCs (p<0.001 for all). Anti-UL83 (+) SSc patients had higher anti-centromere and anti-RNApol1 ab levels and higher frequency of anti-RNApol55 abs compared to anti-UL83 (-) patients (p=0.040). Anti-UL44 (+) SSc patients had higher levels of CENPA and CENPB abs. Apart from a tendency of higher frequency of arthritis in anti-UL44 (+) patients no other clinical association was found.

Conclusions: Antigen-specific UL83, UL57, UL44 and UL99 HCMV ab responses are more prevalent and/or stronger in patients with SSc compared to HC or MS patients (or their phenotypes). These findings might imply a role for HCMV in SSc development.

DETECTION OF ANTI-GP2 AND ASCA ANTIBODIES IN RUMINANTS WITH PARATUBERCULOSIS

A. Goutzourelas1, V. Spyrou2, A.L. Koutsoumpas1, L.V. Athanasiou3, G.S. Amiridis4, D. Roggenbuck5, C. Billinis6, D.P. Bogdanos1, C. Liaskos1

1 Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece
2 Department of Animal Production, Technological Educational Institute of Thessaly, Larissa, Greece
3 Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece
4 Department of Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece
5 Research and Development Department, GA Generic Assays GmbH, Dahlewitz/Berlin, Germany; Faculty of Science, Brandenburg University of Technology Cottbus-Senftenberg, Senftenberg, Germany
6 Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece

Introduction: We have recently showed that ruminants (cattle and sheep) with Mycobacterium avium (MAP)-in-
duced paratuberculosis (ptb), the ruminant model of Crohn’s disease, exhibit pancreatic specific autoantibodies (PAB) against GP2. We have also found no evidence of anti-CUZD1 PABs in MAP-induced ptb. Since ASCA is a marker of Crohn’s, we tested ruminants with serological and molecular evidence of MAP infection as well as features of ptb for ASCA antibodies and compared them with ruminants lacking evidence of anti-MAP serology or with ruminants, which were positive for anti-GP2 antibodies.

**Materials-Methods:** A total of 98 samples from ruminants originating from cattle (48 samples), and from goats (50 samples) were included in the present study. IgG anti-MAP antibodies were tested using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (IDEXX Laboratories, Portland, ME). Testing of ruminants for ASCAs IgG antibodies has been performed using an in-house ELISA. Anti-GP2 antibodies has been also been tested using a similar in-house ELISA according to previous publication.

**Results:** Nine cattle (18.75%) and 20 goats (40%) were suffered by ptb. ASCA antibodies were present in 21/48 (43.75%) cattle and 10/50 (20%) goats while anti-GP2 antibodies were present in 14/48 (29.2%) cattle and 8/50 (16%) goats. ASCA antibodies were more prevalent in anti-MAP antibody positive (14/29, 48.3%) than in anti-MAP negative ruminants (17/69, 24.6%, p = 0.022) and also in anti-GP2 antibody positive (13/23, 56.5%) than in anti-GP2 negative ruminants (18/75, 24%, p = 0.003). No association between ASCA and anti-MAP antibody concentrations were found (r=0.159, p=0.117). A significant association between ASCA and anti-GP2 antibody concentration were observed (r=0.211 and p=0.037).

**Conclusions:** ASCA are present in a significant proportion of ruminants and correlate with antibody positivity, a finding further supporting the notion that Crohn's disease and ptb share common immunological mechanisms of antigen-driven loss of self-tolerance.

---

**TETRACYCLINES DIMINISH IFN-γ AND IL-17 PRODUCING CD4+ T AND NKT CELLS IN MULTIPLE SCLEROSIS**


1 Department of Rheumatology and clinical Immunology
2 Department of Neurology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

**Introduction:** Multiple Sclerosis (MS) is a complex central nervous system autoimmune disorder that currently lacks curative treatment. Tetracyclines are antibiotic compounds have been considered potentially therapeutic agents for the treatment of MS through their pleiotropic immunomodulatory actions, but experimental data are still lacking. The aim of the study was to investigate the in vitro efficacy of two tetracyclines, minocycline and doxycycline in the down-regulation of pro-inflammatory cell responses from MS patients.

**Materials-Methods:** PBMCs were obtained from MS patients without treatment (Naïve, n=5), patients with relapsing-remitting disease under standard treatment (RRMS, n=10) and healthy donors (n=10). Cells were cultured in the presence of50μg/ml minocycline or doxycycline and stimulated: a) by the combination of PMA+Ionomycin or b) physiologically by the combination of interleukins (IL)-12 and IL-18. Phenotypic characterization was assessed by flow cytometry using with MoAbs against surface CD56, CD16, CD3, CD4 and CD8 epitopes. Intracellular expression of IFN-γ and IL-17 was also examined using standard cell permeabilization protocols.

**Results:** Both tetracyclines significantly decreased IFN-γ expression (>50% inhibition) in three lymphocyte populations (NKT, CD4+ T and CD8+ T lymphocytes) following stimulation with IL-12 and IL-18 (p<0.05 for all). IFN-γ (+) NKT cells were mostly decreased following tetracycline administration. The reduction of IFN-γ (+) NKT cells was statistically significant between all patient groups as well as healthy controls. Tetracyclines had a greater suppressive effect in the NKT cells from naïve MS patients. Of the two tetracyclines, doxycycline was the one with the strongest effect on IFN-γ suppression. Neither of the two antibiotics, however, decreased IL-17 producing CD4+ T cells following PMA and lonomycin stimulation.

**Conclusions:** Tetracyclines can suppress in vitro IFN-γ producing cell subsets alone or in combination with standard MS medication. Doxycycline, with the advantage of fewer side effects and stronger pro-inflammatory action deserves to be further investigated as a future therapeutic agent for MS.
Το περιοδικό «Ανοσία» εκδίδεται από την Ελληνική Εταιρεία Ανοσολογίας και αποτελεί το μέσο προώθησης της ανοσολογίας στους χώρους διεξαγωγής ιατρικής έρευνας και κλινικής πράξης. Στο περιοδικό δημοσιεύονται άρθρα σύνταξης, ανασκοπικά άρθρα, ερευνητικές και άλλες πρωτότυπες εργασίες, ενδιαφέρουσες περιπτώσεις και γράμματα από και προς τη σύνταξη.

Υποβολή άρθρων: Τα άρθρα αποστέλλονται στον εκδότη στη διεύθυνση:
Α. Σαραντόπουλος
Για το περιοδικό ΑΝΟΣΙΑ
Τμήμα Κλινικής Ανοσολογίας
Β' Παθολογική Κλινική ΑΠΘ
Κωνσταντινούπολης 49, 546 42 Ιπποκράτειο Γ.Ν.Π.Θ.
Θεσσαλονίκη

Για κάθε τύπο άρθρου ισχύουν ιδιαίτερες οδηγίες:
1. Άρθρα σύνταξης: Συνοπτικά άρθρα που δεν υπερβαίνουν τις 2 σελίδες. Η έκτασή τους δεν πρέπει να υπερβαίνει τις 500 λέξεις.
2. Άρθρα ανασκοπικά: Πρόκειται για σύνθετη παρουσία του θέματος που περιλαμβάνει την εξέλιξή του μέχρι σήμερα αλλά κυρίως τις νεότερες απόψεις και προοπτικές. Το μέγεθος του άρθρου δεν πρέπει να υπερβαίνει τις 4.000 λέξεις.
3. Ερευνητικές, πρωτότυπες εργασίες: Έχουν κλινικό ή εργαστηριακό χαρακτήρα ή αποτελούν προϊόν βασικής έρευνας, ενώ η δομή τους πρέπει να περιλαμβάνει περίληψη, περιγραφή υλικού και μεθόδους, αποτελέσματα και συζήτηση. Δεν πρέπει να υπερβαίνουν τις 3.500-4.000 λέξεις.
4. Ενδιαφέρουσες περιπτώσεις: Παρουσίαση περιστατικών σπάνιων είτε ως προς την κλινικοεργαστηριακή τους πορεία είτε ως προς τη θεραπευτική τους αντιμετώπιση και εξέλιξη. Τα άρθρα πρέπει να είναι 1.000-1.500 λέξεων.
5. Γράμματα προς τη σύνταξη: Περιλαμβάνουν ανακοινώσεις πρόσφατων αποτελεσμάτων, είτε σχόλια που αφορούν δημοσιευμένα στο περιοδικό άρθρα. Δεν θα πρέπει να υπερβαίνουν τις 500 λέξεις.

Σύνταξη κειρογράφων: Τα άρθρα που αποστέλλονται στο περιοδικό πρέπει να είναι γραμμένα στη νεοελληνική δημοτική. Υποβάλλονται σε σελίδες τύπου A4, με διπλό διάστημα και περιθώρια, ενώ οι γραμματοσειρές που πρέπει να χρησιμοποιούνται είναι οι Times New Roman και Arial.

Περίπτωση εκτύπωσης έγχρωμων εικόνων: Ο συγγραφέας θα ενημερώνεται από τη Συντακτική Επιτροπή για τη διαφορά κόστους, την οποία και θα αναλαμβάνει να καλύψει.

Βιβλιογραφικές παραπομπές: Το περιοδικό «Ανοσία» ακολουθεί το σύστημα Vancouver σύμφωνα με το οποίο οι παραπομπές εμφανίζονται στο κείμενο με μορφή αριθμών.
θεί η σύντμηση: και συν. ή et al. Οι συντμήσεις των περιοδικών παρατίθενται σύμφωνα με το Abridged Index Medicus π.χ.


Όλα τα παραπάνω συγκροτούν το συνολικό κείμενο υποβολής και πρέπει αυτό να αποστέλλεται σε τρία αντίτυπα στον εκδότη του περιοδικού, μαζί με μια δισκέτα όπου θα περιέχεται η εργασία σε ηλεκτρονική μορφή.

Κάθε υποβολή πρέπει να συνοδεύεται από επιπόγραφη επιστολή του πρώτου συγγραφέα όπου θα δηλώνεται ότι η εργασία δεν βρίσκεται σε διαδικασία κρίσης και δεν έχει δημοσιευθεί από οποιοδήποτε άλλο περιοδικό και ότι όλοι οι συγγραφείς συμφωνούν στην πιθανή δημοσίευσή της.

Εφόσον η εργασία είναι τυπικά ολοκληρωμένη σύμφωνα με τις παραπάνω οδηγίες προωθείται από την επιτροπή επιμέλειας σύνταξης σε 2 μέλη της συμβουλευτικής επιτροπής. Οι κριτές κάνουν δεκτή ή απορρίπτουν την εργασία, ενώ διατυπώνουν τις παρατηρήσεις τους οι οποίες επιστρέφονται μαζί με την εργασία στους συγγραφείς. Στο στάδιο της τελικής φάσης της εκτύπωσης αποστέλλεται η εργασία το τελικό δοκίμιο το οποίο επιδέχεται πλέον μόνο ορθογραφικές διορθώσεις. Στο σημείο αυτό γίνεται και η παραγγελία των ανατύπωσης από τους συγγραφείς. Εργασίες που δημοσιεύονται στο περιοδικό «Ανοσία» αποτελούν πνευματική του ιδιοκτησία και η αναδημοσίευση μέρους ή ολόκληρου του κειμένου απαιτεί την έγγραφη συγκατάθεση του εκδότη.