

## **Efficacy and Safety of Re-treatment with Rituximab (RTX) in Patients with Rheumatoid Arthritis (RA). Our Experience.**

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### **Background**

B cells are involved in the pathogenesis of rheumatoid arthritis (RA). Rituximab (RTX), a monoclonal anti-CD20 antibody, has proven efficacious in the treatment of rheumatoid arthritis (RA) due to B-cell depletion.

### **Aim**

To determine the efficacy and safety of re-treatment with RTX in an open label extension of a previously reported controlled randomized trial.

The extension study is international and multi-center, and still goes on. We report here the interim results of the patients in our center.

### **Methods**

Eight patients who relapsed (mean DAS 28 >5.1) after a single course of RTX (alone or in combination with MTX or/and cyclophosphamide) were re-treated with RTX + MTX. All patients received IV 1g RTX on days 1 and 15, and 17-days course of corticosteroids. In all the patients there was a recovery of peripheral B cells after the first course of treatment.

### **Results**

The mean time to the first re-treatment, i.e. 1<sup>st</sup> relapse, was 18 months (range 15-24 months).

Among the 8 patients, 5 are still in remission (mean follow-up 64 weeks; range 24-96 weeks), 3 relapsed and the mean time for 2<sup>nd</sup> re-treatment was 53 weeks (compared to 74 weeks after the first treatment). The extent of the remission, judged by DAS 28 at the same point of time after treatment for each patient, was similar for both the primary treatment and the recurrent treatments.

Within this small group during the 3-4 years of RTX therapy one patient became pregnant, and had no exacerbation following abortion, another one underwent elective total hip replacement without any complications. A third patient had viral uveitis which had completely resolved, a fourth one had septic arthritis and the fifth had pheochromocytoma and solid renal tumor.

### **Conclusion**

A second short course of RTX in combination of MTX is well tolerated and effective as the first course. The long-term side effects of recurrent prolonged B cells depletion are unknown.

Synovial fluid levels of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis, psoriatic arthritis and osteoarthritis.

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**Background:** Serum antibodies to cyclic citrullinated peptide (CCP) are considered to be a specific marker of rheumatoid arthritis (RA). Cyclic citrullinated proteins are found in the synovial membrane of RA patients.

**Aim:** This study aimed to assess the levels of anti-CCP in synovial fluids (SF) of patients with RA, Psoriatic Arthritis (PsA), and Osteoarthritis (OA) and to evaluate their diagnostic value in distinguishing between these entities.

**Methods:** Knee effusions of 27 RA patients (21 women/6 men, mean age 59.2±11, mean disease duration 11.7±8.8 years), 20 PsA patients (6women/14men, mean age 48.9±9.4, mean disease duration 5.8±4.2 years) and 19 OA patients (9 women/10 men, mean age 71±11.8, mean disease duration 9±7.8) was aspirated, centrifuged and the supernatant frozen at -20°. Serum (S) of 22 of the RA patients, 11 of PsA and 12 of OA patients were frozen at -20°C. IgG anti-CCP and IgA-RF were detected by ELISA (Quanta lite™, Inova diagnostics, San Diego CA, USA). Serial dilutions of the SF and S were performed at titers of 1/10 and 1/100 respectively.

**Results:** The mean levels of SF anti-CCP and IgA-RF were significantly increased in RA joint effusions. For anti-CCP: 139±129 Units vs. 33±26 Units in PsA and 25±19.7 Units in OA (p<0.003). For IgA-RF: 69±73 Units vs. 16±10 in PsA and 17±19 in OA. No significant difference was noted between OA and PsA patients. A significant correlation was found between synovial fluid and serum levels of anti-CCP and IgA-RF.

**Conclusions:** Anti-CCP and IgA-RF were significantly increased in synovial fluids of RA patients in comparison with PsA and OA patients. Levels of anti-CCP and Ig-A in synovial fluids may help in discriminating RA from other arthritides.

### **Impact of treatment with infliximab on serum cytokine concentration in patients with rheumatoid arthritis (RA)**

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**Background:** Anti-tumor necrosis factor alpha (TNF $\alpha$ ) is known to induce considerable clinical improvement in RA patients who are regarded to be refractory to conventional therapy with disease modifying anti-rheumatic drugs (DMARDs). Although the clinical impact of anti-TNF $\alpha$  has thoroughly been investigated, the exact immunological mechanisms by which this outcome is mediated still remains partly obscure.

**Aim:** To analyze the serum cytokine profile of a non randomized group of RA patients who were destined to be treated with infliximab following failure after failure of at least three different DMARDs, according to the regulations of the Israeli health ministry.

**Methods:** Serial serum samples were collected from thirteen patients with refractory RA, all were treated with the anti-TNF $\alpha$  agent, infliximab (Remicade) after failing to sustain a clinical remission with at least three DMARDs. All of the patients were concomitantly treated with methotrexate. Blood samples were obtained at different phases of their therapy, namely samples were taken prior to the administration of infliximab and about four weeks following infusion and in several cases by the same manner in future cycles. Serum concentrations of tumor necrosis factor (TNF) $\alpha$ , interferon (IFN) $\gamma$ , interleukin (IL)-1 $\beta$ , IL-6, IL-10, IL-1 receptor antagonist (IL-1RA) were determined by commercial ELISA kits.

**Results:** All the thirteen patients included in this study suffered from refractory RA that was unresponsive to conventional DMARD therapy. Interestingly, only 10 of them had elevated TNF $\alpha$  serum concentrations (at least once in their serial measurements). Of the thirteen patients only one was unresponsive to therapy

and her serum concentrations of TNF $\alpha$  remained extremely high. Three patients who responded to therapy had a paradoxical increase of serum concentrations of TNF $\alpha$  following infliximab administration.

Two of the three patients who had normal TNF $\alpha$  concentrations throughout the study responded to therapy. In one of these responders we detected a parallel increase in the concentration IL-1RA and in another a mild increase in the concentration of IL-6 was detected.

Eleven patients had high concentrations of IL-2R at least once during the study; in five IL-2R concentrations increased as therapy was continued and as a clinical response was obtained. In five patients who had elevated concentrations of IFN $\gamma$  at the beginning of infliximab therapy a subsequent decrease was measured as additional infliximab cycles were given.

**Conclusions** In an unselected population of RA patients who were entitled to receive therapy with infliximab we noticed diverse patterns of serum cytokine profiles. Even though most of the patients clinically responded in our group, we could not identify an expected valid trend of serum cytokine concentrations.

### **Modification of neutrophil function by plasma of Rheumatoid arthritis patients treated with Infliximab.**

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**Background:** One of the factors that plays a key role in the pathogenesis of Rheumatoid arthritis (RA) is the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF $\alpha$ ). Inhibiting the actions of TNF- $\alpha$  has proved very effective in the management of early and chronic rheumatoid arthritis. In addition to soluble mediators such as cytokines and growth factors, as well as mechanical stimuli, an excessive and/or sustained increase in neutrophil production of free oxygen radicals including superoxide anion release (SOR) has been implicated in the pathogenesis of rheumatoid arthritis and in other diseases such as atherosclerosis.

**Objective:** To examine whether the release of superoxide anions from polymorphonuclear leukocytes (PMN) of healthy donors was affected when incubated with plasma from Infliximab-treated patients.

**Methods:** Fifteen consecutive seropositive RA patients were treated with infusion of 3mg/kg Infliximab on weeks 0, 2, 6, and 14. Clinical assessment and blood withdrawal were made before each treatment, i.e. at the minimal concentration of the drug. Disease activity was assessed by DAS28 score and by IL-6 level. Neutrophils from healthy donors were incubated with plasma drawn before each Infliximab treatment, and the SOR was measured by the ferricytochrome C reduction method.

**Results:** 53% of the patients were found to have a favorable clinical response, and the  $\Delta$ DAS28 score was -0.96, which is not significant. IL-6 levels showed a significant decline at week two, with a gradual increase thereafter. Treatment with Infliximab did not change the release of superoxide anion. However, when the group was divided retrospectively on clinical ground to responders ( $\Delta$ DAS28 > -1.2) and non-responders ( $\Delta$ DAS28 < -1.2), two different patterns were seen, although the initial pre treatment levels were similar: Among the responders IL-6 remained low at its 2 weeks level till week 14, while in the non responders IL-6 increased 3 times ( $P < 0.03$ ) from week 2 to 14. The responders showed mild, but continuous, reduction of SOR, while in the non-responders SOR took an opposite direction and increased significantly from week 2 on.

### **Conclusions:**

The high neutrophil superoxide release stimulating activity of the plasma of RA patients might contribute to the extra-articular manifestations of the disease and to the known early development of atherosclerosis in these patients. The minimal change in SOR in spite of marked reduction in IL-6 implies that SOR is stimulated by non-TNF $\alpha$  dependent mechanisms. The existence of such pathways might explain some of the failures of anti-TNF $\alpha$  therapy. The increasing level of IL-6 after initial dramatic decline among the non-responders might represent an escape phenomenon.

This finding raises the question, whether a more frequent administration of Infliximab would increase the percentage of responders. Moreover, different patterns of IL-6 and to a lesser extent SOR between

responders and non-responders might be used as clinical tool for early identification of these two subpopulations.

#### Duplex Study of The Carotid and Femoral arteries of patients with RA: A Controlled Study

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**Background:** “Ultrasonic Biopsy” (U-B) is a non-invasive screening technique to detect early atherosclerotic (AS) plaques and arterial wall changes.

**Aim:** To identify AS in the common carotid (CCA) and common femoral arteries (CFA) of patients with rheumatoid arthritis (RA) and their matched controls.

**Methods:** 57 consecutive RA patients were enrolled in the study. Controls were matched by age, sex, ethnicity, and AS risk factors. All patients and controls underwent U-B study of the CCA and CFA. The U-B features were classified and scored as follows: Class A- normal (score 0), Class B –interface disruption (score 2), class C- intima-media (I-M) granulation (score 4), Class D- plaque without hemodynamic disturbance (score 6), Class E-stenotic plaque (score 8) and Class F –plaque with symptoms (score 10). Total score per patient was calculated. Classes A-B indicate an intact media or minimal interphase changes, classes D-F point to a significant medial involvement. Class C signifies a borderline lesion, with a potential for regression to normal, being unchanged or progression to a plaque.

**Results:** Mean ages were 52.1 yrs for RA and 51.4 yrs for controls (P=0.81). Eighty-six percent of the patients and 85% of controls were women. The mean disease duration of RA was 12.8 years. Frequencies of risk factors among the RA patients compared to controls were: Hypertension (28% vs. 32%), Smoking (37% vs. 29%), Dyslipidemia (23% vs. 25%), diabetes mellitus (DM) (14% vs. 13.7%) and family history of cardiovascular disease (CVD) (4% vs. 7%).

Forty-five percent of the RA patients had at least a single Classes D-F lesion (plaque) in one of the 4 vessels tested, compared with 40% in the control group (P=0.19). The mean total U-B scores of the RA patients and controls were not significantly different (8.87 vs. 8.99, P= 0.7).

Univariate analyses have shown that the development of plaques in RA patients was associated with age > 50 yrs, disease duration, hypertension, dyslipidemia, and smoking. Multivariate analysis found plaques to be strongly associated with age above 50 yrs and dyslipidemia.

**Conclusion:** In unselected RA patients, besides classic AS risk factors, an older age in longstanding disease can predict the development of a severe morphological expression of AS disease.

#### Prevalence of Antiphospholipid Antibodies in Rheumatoid Arthritis

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**Background:** Antiphospholipid antibodies characterize the antiphospholipid syndrome, but can also be found in various autoimmune, infectious and malignant conditions.

**Objectives:** To detect the prevalence of antiphospholipid antibodies among patients having rheumatoid arthritis who do not have clinical manifestations of the antiphospholipid syndrome.

**Methods:** The levels of IgG and IgM anti-cardiolipin, IgG and IgM anti-beta-2-glycoprotein-I and anti-oxidized LDL autoantibodies has been evaluated in 82 patients having rheumatoid arthritis, using standard ELISA. The cut-off levels for detection of these autoantibodies were 15 GPL, 15 MPL and 25 EU/ml, respectively.

**Results:** Elevated levels of IgG anti-cardiolipin antibodies were detected in 17 of 82 (21%) rheumatoid arthritis patients. These 17 patients included 10 with low levels of IgG anti-cardiolipin, and 7 with medium

to high levels of anti-cardiolipin autoantibodies. IgM anti-cardiolipin was found in only 1 (1%) patient, and both IgG and IgM anti-beta-2-glycoprotein-I were found in 3 (4%) patients having rheumatoid arthritis. Elevated levels of anti-oxidized LDL antibodies were found in 8 (10%) patients, 4 of whom had also elevated levels of IgG anti-cardiolipin.

**Conclusions:** IgG anti-cardiolipin autoantibodies can be found in about one fifth of rheumatoid arthritis patients who do not have clinical manifestations of the antiphospholipid syndrome. Whether anti-cardiolipin presence signifies increased risk for thrombosis in these patients should be further studied.

### **Intravenous Immunoglobulin Treatment of 100 Systemic Lupus Erythematosus Patients**

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**Background:** Intravenous immunoglobulin (IVIg) is used for the treatment of several autoimmune diseases, and there are several reports suggesting a beneficial role of IVIg in systemic lupus erythematosus (SLE). As IVIg is very expensive, the appropriate treatment dosage should be well defined.

**Objectives:** To compare the clinical outcome of treatment with different dosages of IVIg (low-dose versus high-dose).

**Methods:** The clinical data of 100 SLE patients treated with IVIg were retrospectively analyzed (the minority of them have been previously reported)<sup>1,2</sup>. 68 patients were treated with approximately 0.5 g/kg body weight in every treatment course (low-dose) for a mean number of 6 courses at a mean interval of 5 weeks between subsequent therapeutic IVIg course. 32 other patients were treated with 2 g/kg body weight in every treatment course (high-dose) for a mean number of 6 courses at a mean interval of 4-5 weeks between subsequent therapeutic IVIg course. There were various indications for IVIg administration, but generally IVIg was given as an adjunct for the regular therapy. Disease activity scores and specific clinical manifestations were followed-up.

**Results:** Both low-dose and high-dose IVIg therapy were associated with beneficial clinical outcome in most SLE patients. Systemic Lupus Activity Measure score significantly decreased after high-dose IVIg, and SLE Disease Activity Index also significantly decreased after low-dose IVIg therapy, compared with their respective values before IVIg had been administered. In the high-dose group, the clinical manifestations which responded more to treatment were arthritis, fever, thrombocytopenia, and neuropsychiatric lupus. In the low-dose group, the clinical manifestations which responded more to treatment were mucosal ulcers, fever, urinary casts, new rash, pleurisy, pericarditis and hematuria.

**Conclusions:** In an open uncontrolled study, both low-dose and high-dose IVIg seems to have a beneficial role in the management of SLE. More studies are needed in order to determine the specific indications for IVIg use in SLE, as well as the most appropriate IVIg dose used.

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2. Levy Y, Sherer Y, George J, Rovensky J, Lukac J, Rauova L, Poprac P, Langevitz P, Fabbri F, Shoenfeld Y. Intravenous immunoglobulin treatment of lupus nephritis. *Semin Arthritis Rheum.* 2000;29:321-7.

## **Venous and Arterial Thromboembolism Following Administration of Intravenous Immunoglobulins**

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**Background:** Intravenous Immunoglobulins (IVIg) are widely used in clinical practice to attenuate immune mediated tissue damage or in humoral immune deficiency. IVIg has been considered extremely safe. In the last year there has been a marked increase in the reported cases of thrombosis following IVIG administration.

**Aim:** To define clinical characteristics, risk factors and outcome for venous as opposed to arterial thrombosis following IVIg administration.

**Methods:** Patients with post-IVIg thrombosis, defined as occurring within 30 days of IVIg infusion, were identified at our institution. The medical literature was searched for published reports of IVIg-associated thrombotic events. Time-course and comorbidities were compared between arterial and venous thrombotic events.

**Results:** We found six patients with post-IVIg venous thrombosis at our institution. In addition, review of the literature revealed 64 reported events. The incidence rate was estimated at 0.15% to 1.2% per treatment course, but the large increase in reported cases in 2003 suggests that the incidence may be significantly greater. Arterial thrombosis was three times more common than venous thrombosis. Arterial events occurred early after IVIg administration (50% within 4 hours) and were associated with advanced age and atherosclerotic vascular disease; venous thrombosis occurred later (54% percent more than 24 hours after IVIg administration) and was associated with factors contributing to venous stasis (obesity and immobility). Twelve patients reported in the literature died (mortality 19%); 11 of which occurred following arterial thrombosis.

**Conclusion:** IVIg-associated thrombosis is more common than previously recognized, and causes significant mortality. The different characteristics of arterial and venous events may reflect different pathophysiological mechanisms. A better understanding of these mechanisms should aid in defining a risk-benefit ratio for the individual patient.

## AORTITIS PRESENTING AS FEVER OF UNKNOWN ORIGIN – FDG PET FOR DIAGNOSIS

### AND FOLLOW UP.

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**Background:** Fever of unknown origin (FUO) is a challenging medical problem. <sup>18</sup>F-fluorine-2-deoxy-D-glucose positron emission tomography (FDG PET) has been recently assessed as useful diagnostic method. Furthermore, FDG PET appears to be a valuable imaging technique for evaluating the effect of treatment of infectious and inflammatory processes that cannot reliably be visualized by conventional techniques. Vasculitis of large vessels has a limited number of tools for diagnosis and follow-up.

**Aim:** To assess FDG-PET for diagnosis and follow-up of large vessels vasculitis we report two patients with FUO, finally diagnosed with vasculitis with predominant aortic involvement.

**Methods:** Two Sefaradic jewish females, 78-year-old (patient 1) and 57-year-old (patient 2), were referred to internal department with two months history of fever of unknown origin (FUO), night sweats, weight loss and markedly elevated ESR and CRP. Extended routine investigation found no infection, malignancy, hypersensitivity or autoimmune disorder. The patients did not suffer from claudication, systolic blood pressure difference between arms was 20 mm Hg. Temporal artery biopsies were negative. FDG PET scan imaging of both patients demonstrated intense FDG uptake along the aorta and in the brachio-cephalic and carotid arteries, consistent with arteritis.

**Results:** High dose of corticosteroid (CS) therapy (1mg/kg) was instituted in both women with further tapering. The therapy was followed by complete resolution of the symptoms and pathological FDG uptake on repeated FDG PET. During CS tapering the disease course of patient 2 was complicated with seronegative knee monoarthritis, which responded to arthrocentesis and appropriate dose of CS and methotrexate (MTX) therapy. Second line therapy was not added to patient 1 because of positive conversion of Mantou test followed by rifampicin prophylaxis.

**Conclusion:** FDG-PET should be a part of the workup of FUO when routine investigation fails. FDG PET is useful both for diagnosis and assessment of response to therapy of large vessels vasculitis, especially in the presence of aortitis. Aortitis may be extraarticular presentation of seronegative arthritis.

## IN-VIVO CLEARANCE OF APOPTOTIC KERATINOCYTES BY LANGERHAN'S CELLS.

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It becomes now apparent that dendritic cells (DCs) are not only excellent immuno-stimulators of the immune response but rather potent immuno-inhibitors, as immature DCs (iDCs), continuously sample self-antigen to maintain T cell self-tolerance. We have recently shown (J Exp Med, December 2002) in vitro generation of tolerant DCs following exposure to apoptotic cells. We now present our human in-vivo model for uptake of apoptotic cells by DCs.

**Methods.** Healthy volunteers that signed an informed consent-approved by the institutional Ethics committee were exposed to 4 MED of UVB irradiation and epidermal suction blisters and biopsies were taken at 0, 4 and 24 hours. Apoptosis was confirmed H & E staining for sunburn cells as well as with annexin/PI staining and mRNA patterns using RT-PCR. Single cell suspensions were prepared by limiting trypsinization. Flow-cytometry, EM and confocal microscopy analysis was used for morphology and CD analysis. **Results.** 2.99%± 0.66 of cells were identified as DCs using double staining with CD1a and Langerin. Following UVB irradiation and apoptosis induction in keratinocytes, sharp reduction in DCs numbers was evident already in 4 hours (1.0 ±0.5) and 24 hours (1.5 ±0.4). Staining for CCR7 confirmed sharp decline in staining and that over 70% of the remaining cells did not express CCR7. Of the receptors reported to mediate clearance of apoptotic cells by DCs, CD11b and CD36 were expressed in less than 10%, αvβ3 and αvβ5 were expressed on <50% of DCs while CD11c was expressed on most DCs. No evidence for macrophage clearance was seen in 24 hours.

Conclusion. This observation would support the role of tolerant DCs in maintaining peripheral tolerance and that DCs are the first line of professional phagocytosing cells that interact with apoptotic cells.

## ARTHRITIS AS THE SOLE EPISODIC MANIFESTATION OF FMF

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**Objective:** Arthritis as the sole manifestation of FMF has been estimated to occur in 1% of patients. The present study clinically and genetically characterizes these patients, aiming to establish criteria that distinguish FMF arthritis from other recurrent arthritides.

**Patients:** The study population comprised 14 patients (9 female, 5 male) from 5,000 patients enrolled at the FMF clinic. All patients fulfilled the clinical criteria for the diagnosis of FMF. Patients who suffered from attacks attributable to FMF at any site other than a joint were excluded from the study. The control group comprised 28 patients with episodic mono/oligoarthritis due to various conditions including reactive, palindromic, IBD associated, as well as seronegative spondyloarthropathies, SLE, JCA, Behcet's disease, sarcoidosis and gout.

**Results:** Patients of the study group were more often of North African origin, had earlier onset of disease, family members with FMF and suffered typical features of episodic arthritis, with predominance of ankle joint involvement. Genetically, MEFV mutations were seen significantly more often in the study group, with dominance of M694V.

**Conclusion:** The study highlights the distinctive features of FMF, helping in the differential diagnosis of acute monoarthritis in young adults and allowing for earlier treatment of the joint attacks as well as prevention of FMF related amyloidosis that may develop unhindered if the arthritis is treated by alternative means.

## The Familial Mediterranean Fever gene as a modifier of Crohn's disease

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**Background:** Crohn's disease (CD) has been reported to be more frequent among patients suffering from familial Mediterranean fever (FMF). Interestingly, the CD susceptibility gene, *NOD2/CARD15* and the FMF gene *MEFV* both belong to the same super family of death domain proteins, which are important in the regulation of apoptosis, cytokine processing and inflammation.

**Aim & Methods:** In order investigate the prevalence of MEFV mutations in non-Ashkenazi CD patients, as well as its effect on CD behavior, DNA has been collected from 105 CD patients. Seventy patients were from non-Ashkenazi and 35 from mixed Ashkenazi-non-Ashkenazi background. Five patients had a concomitant diagnosis of FMF. Data obtained from each patient included: age of onset, ethnic background, disease location and behavior, and the presence of extra-intestinal manifestations. Patient's DNA was analyzed for three common *MEFV* mutations: M694V, V726A and E148Q.

**Results:** The overall prevalence of mutation carriers among non-FMF patients was 13% (13 out of 100), as compared to 14 to 29% in the general population. A stricturing disease pattern was observed in 56% (10 out of 18) of mutation carriers and in 25 % (22 out of 87) of non-carriers (OR=3.7, 95% CI 1.3-10.5, p=0.015), whereas the prevalence of fistulas was comparable in both groups. No differences were observed in disease location and age of onset or disease severity. Extra-intestinal manifestations were significantly more frequent among carriers than non-carriers (65% vs. 32%, OR 3.9, 95% CI 1.3-11.5, p=0.015).

**Conclusions:** *MEFV* mutations are not particularly frequent in CD patients, ruling out *MEFV* as a major susceptibility gene for CD. Analogous to *NOD2/CARD15*, *MEFV* mutations in CD patients appear to be associated with a stricturing disease pattern of CD. Extra-intestinal disease is more prevalent among carriers.

## **ELEVATED TITERS OF ANTI-RIBOSOMAL-P ANTIBODIES IN SLE**

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**Objective:** Anti-ribosomal P Ab are highly specific for SLE, and detected at a 15-20% frequency according to the literature. Elevated anti-ribosomal P titers correlate with disease activity and are specifically associated with neuropsychiatric disease such as psychosis/depression. and coexist with anti-dsDNA antibodies. The aim of our study was to evaluate the frequency of anti-ribosomal P antibody titers and the correlation with manifestations in SLE patients.

**Methods:** Sera samples from 176 individuals were evaluated for titers of anti-ribosomal P Abs: 77 samples from SLE patients, 22 patients with antiphospholipid syndrome (APS), 20 patients with familial Mediterranean fever (FMF), 12 patients with infections, and 43 healthy controls. Anti-ribosomal P Ab titers were tested by ELISA. Manifestations of SLE at time of serum sampling were determined by the SLEDAI score.

**Results:** Six SLE patients (11%) harbored elevated anti-ribosomal P Ab titers. Five SLE patients were females, mean age 44.3 yrs (range: 18-73 years old), the mean SLEDAI mean score was 7 (range: 3-10) indicating moderate disease. Elevated titers of anti-dsDNA were detected in 50% of SLE patients with elevated anti-ribosomal P Ab. One patient had secondary APS. One patient with elevated titers of anti-ribosomal had renal disease and psychosis. Three patients had a rash, while none of the patients had arthritis or leukopenia. Anti-ribosomal P titers were not elevated in patients with primary APS, FMF, infections and healthy controls.

**Conclusion:** The prevalence of elevated titers of anti-ribosomal P Abs was restricted to SLE patients. No correlation with a specific manifestation was found.

# Neuropsychiatric Manifestations in Pediatric Systemic Lupus Erythematosus and Association with Antiphospholipid Antibodies

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## Background:

Systemic lupus erythematosus (SLE) is an autoimmune multisystem disease with diverse clinical manifestations. Central nervous system (CNS) involvement is common in children with SLE, varying from 29 to 44% of patients in different studies and is a major cause of morbidity and mortality. However, its diagnosis is hampered by limitations in methods and criteria. Only a few large studies of pediatric SLE have included neuropsychiatric (NP) complications, and most of them did not use the newly developed American College of Rheumatology (ACR) criteria. Antiphospholipid antibodies (aPL) are found in approximately 65% of children with SLE. Although several cross-sectional cohort studies of adult SLE have noted a significant association of CNS disease with the presence of aPL, the data for children are sparse. The pediatric literature includes a few reports relating aPL with neurological signs such as seizures and chorea in children with no connective tissue disease. Studies of neurologic manifestations of pediatric SLE have focused on CNS thrombotic events, they have not addressed the role of aPL in other neurologic complications.

**Aim:** To determine the prevalence of NP complications in children SLE using the new ACR nomenclature and evaluate their association with aPL.

**Methods:** Clinical data of 106 SLE patients from two medical centers were obtained by chart review. Presence of aPL was tested with a series of assays. Pearson's chi-square test and Fisher's exact test were utilized to assess group differences.

**Results:** Twenty-five patients (24%) had NP manifestations, including seizures (9.4%), headaches (4.7%), mood disorders (4.7%), cognitive dysfunction (4.7%), cerebrovascular accident (CVA), psychosis and pseudotumor (2.8% each), aseptic meningitis (1.9%), acute confusional state (0.9%), anxiety (0.9%), and cranial neuropathy (0.9). The first event occurred within 6.4 years (mean 1.2) of the diagnosis of SLE; 50% of affected patients developed NP events within 7 months. NP events were not necessarily accompanied by an SLE flare. aPL were positive in 70%, including anticardiolipin antibodies (aCL) in 64%; aCL IgG in 56%; aCL IgM in 35%; rapid plasma reagin (RPR) in 13.5%; and lupus anticoagulant in 18%. The only significant association between NPSLE and aPL was between CVA and aCL IgM ( $p=0.03$ ).

**Conclusions:** NP manifestations occur in about one-fourth of children with SLE, are an early event in the course of the disease and not necessarily accompanied by an SLE flare. Seizures are the most frequent symptom. Although aPL are common, their association with NP events, unlike in adults, is not significant, except for CVA, suggesting a different pathogenetic mechanism in pediatric NPSLE.

Pediatric APLS and recurrent thrombosis: The role of anticoagulants is more important than the presence of hereditary thrombophilia

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**Background:** Whereas APLS in adults has been well characterized, there have been only few studies on APLS among children. Our aim was to analyze the clinical, laboratory manifestations and outcome in a pediatric APLS cohort.

**Methods:** This was a multicenter study of 26 children with APLS without previous systemic autoimmune disease. We reviewed retrospectively their clinical and laboratory data, including hereditary thrombophilic deficits and the outcome.

**Results:** Mean age at onset was  $11.0 \pm 5.8$  years, 16 were females. The most common initial APLS manifestations were deep and superficial vein thrombosis (12 and 2), CVA (5), thrombocytopenia (5), hemolytic anemia (3), chorea (2), and visual disturbances (2). Two patients presented with a thrombosis during SLE onset. Lupus anticoagulant was detected in 96% of tested, elevated ACL antibodies in 72% and anti- $\beta 2\text{gp}1$  in 75%. During follow-up of  $6.0 \pm 4.9$  years, CNS involvement appeared in 6 and hematological in 4 children. SLE developed in 3 more cases. Seven of 22 patients with vascular thrombotic events (venous 13 and arterial 9) had recurrences. Hereditary thrombophilia was detected in 54% of children with a single episode compared with 29% of those with recurrences. However, only 2 of the latter received anticoagulants after first manifestation compared with 11 of 15 patients without recurrences.

**Conclusions:** APLS in children has clinical features similar to adults. A significant percent of children presenting with APLS will develop SLE. Hereditary thrombophilia did not predict recurrent thrombosis and anticoagulant treatment following first thrombotic event was effective in preventing recurrences.

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### **Procalcitonin is a promising marker in diagnosing pediatric musculoskeletal infections**

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**Objective:** To evaluate the clinical informative value of Procalcitonin (PCT) in the diagnosis of osteomyelitis (OM) and septic arthritis (SA) in children.

**Methods:** PCT, C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell count (WBC) were measured in children who were admitted to our pediatric ward with fever, and working diagnosis of suspected OM or SA. A positive blood culture, synovial culture, or imaging study confirmed the diagnosis.

We used immunochromatography for the PCT assay. The results were divided into four categories; normal (under 0.5 nn/ml), mildly elevated (0.5-2nn/ml) highly elevated (2-10nn/ml) and very highly elevated value (>10nn/ml).

**Results:** 44 children; 30 boys and 14 girls were evaluated. Their mean age was 5.4 years. 12 (27.2%) had diagnosis of OM, and 11 (25%) had diagnosis of SA. 21 children had other diagnosis; (6- soft tissue infection, 6-transient synovitis, 3 juvenile idiopathic arthritis, 6 others).

PCT value was mildly elevated in 7/12 children with OM, and in 3/11 children with SA. All 21 children with the other diagnosis had normal values. The differences between the two groups was significant ( $p < 0.001$ ). The sensitivity of PCT to diagnose OM and SA were 58.3% and 27.2% respectively. The specificity and positive predictive value for diagnosis of musculoskeletal infection were 100%. WBC, CRP, ESR values were higher in the musculoskeletal infection group compared with the other group, but the differences were not significant ( $p = 0.186$ ,  $p = 0.391$ ,  $p = 0.485$  respectively).

**Conclusion:** PCT might be a promising marker for the diagnosis of OM and possibly for SA. It is sensitive and particularly specific. In most cases it is a better marker than the other common acute phase reactants, such as CRP and ESR to distinguish between deep and local musculoskeletal infections.

Living with children with growing pains: how does it affect the parents?

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**Objectives:** To assess the quality of life (QOL) and the psychological status of parents of children with growing pains (GP) in Jewish and Arab populations.

**Methods:** 33 mothers and 28 fathers of children with GP, and 20 mothers and 19 fathers of age and gender matched control children were studied. QOL was measured using a QOL Scale developed by Falangan. Included were 16 questions measuring different domains of life including health, vocation, independence, and relations with family and friends. Psychological status was assessed by the anxiety and depression subscales of the Arthritis Impact Measurement Scales (AIMS2). A third questionnaire, also assessing anxiety, was based on the SCL-90 psychiatric instrument and included 10 questions, on a 5-point scale. Questionnaires were translated and validated in Hebrew. Student's t-test was used to compare the groups, and fathers and mothers within groups.

**Results:** Parental age, education, and family size were similar in both groups. QOL scores were similar in both groups. Anxiety levels were higher, but not significantly, among mothers of children with GP.

Depression levels were significantly higher among mothers of children with GP ( $2.3 \pm 1.0$  vs.  $1.9 \pm 0.5$ ,  $P < 0.03$ ), but not in fathers. Arab mothers had similar QOL scores as Jewish mothers but anxiety (AIMS and SCL90) and depression (AIMS) scores were significantly higher ( $P < 0.02$ ,  $P < 0.01$ ,  $P < 0.05$ , respectively).

**Conclusions:** The QOL of parents of children with GP does not appear to be affected. However, depression levels and anxiety levels (especially among Arabs) were mildly increased among mothers of these children. Low- Dose MTX treatment for Pauciarticular JIA unresponsive to Intraarticular Cortocosteroids injections. Riva Brik, Vardit Gepstein, Drora Berkovitz.

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**Objective:** to study the efficacy of low-dose (0.2mg/kg) Methotrexate (MTX) in the treatment of children with pauciarticular Juvenile Idiopathic Arthritis (JIA) who do not response to repeated Intraarticular Corticosteroid (IA) injections.

**Methods:** Nineteen consecutive patients with pauciarticular JIA were studied prospectively. Sixteen had persistent course and 3 had extended course of the disease. Forty-eight injections were given to 17 patients. Patients were defined as non-responders to IA corticosteroids injections if the duration of improvement lasted less than 4 weeks for 2 consecutive injections. All of these patients were offered low dose MTX, orally, once a week for at least 6 months.

**Results:** Nine of the 11 patients who were non-responders to repeated IA injections were treated with low dose MTX for a median duration of 23.6 months. All but 1 patient with an extended course, responded very well to treatment and went into remission after a median duration of 9.2 (range 3-12months) of treatment. None of them needed more injections after initiation of the MTX treatment.

**Conclusion.** Low dose MTX is a very effective treatment for children with pauciarticular JIA, who are unresponsive to IA corticosteroids.

**Six month humoral response to vaccination against streptococcus pneumonia in patients with rheumatoid arthritis and systemic lupus erythematosus.**

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**Background:** Pneumococcal vaccination is recommended to prevent the morbidity and mortality associated with streptococcal pneumonia infection in Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) patients. We have shown that after 1 month, pneumococcal vaccination was safe in RA and SLE patients and produced significant increases in mean antibody levels in these groups of patients. However, a substantial proportion of patients, especially among RA patients, failed to respond to pneumococcal vaccination. The long-term immunogenicity of the vaccine in this group of patients is not known.

**Aim:** To assess the 1 and 6 month antibody response after immunization with a 23- valent pneumococcal vaccine in RA and SLE patients in comparison with healthy controls.

**Methods:** Forty four RA patients, 24 SLE patients and 20 healthy controls received intradeltoid injection with 0.5 mL of pneumococcal vaccine. Pneumococcal polysaccharide (PPS)-specific IgG to 7 vaccine PPS (representing high and low prevalence serotypes) was measured by ELISA in sera obtained before, at one

month and 6 months after pneumococcal immunization.

**Results:** At one month, when assessed as groups, both RA and SLE patients had significant rises in geometric mean concentrations of pneumococcal polysaccharide specific IgG to all seven serotypes tested following vaccination similar to the control group. However, 20.8% of SLE patients and 33,3 % of RA patients responded either to none or to only one of the 7 polysaccharides in comparison with the controls subjects of whom none failed to respond. At 6 months, a decrease in the antibody levels was found in the 3 groups, much less pronounced in the control group. In comparison to controls, the decrease achieved statistical significance for serotypes 9N, 8 and 14 with regard to RA patients and for serotype 14 concerning SLE patients. At 6 months, 56 % of RA patients and 55 % of SLE patients responded to less than 2 serotypes in comparison with 25% of controls (p=0,01)

**Conclusion:** Pneumococcal vaccination produced significant increases in mean antibody levels after one month in RA, SLE and controls. However, a substantial decrease in the antibody response and levels was observed after 6 months in RA and SLE patients in comparison with controls.

Pneumococcal vaccination of patients with SLE: effects on generation of autoantibodies.

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**Background :** the effect of vaccination on generation of autoantibodies in SLE patients is not clear.

**Aim:** to assess the effect of vaccination against streptococcus pneumonia on the generation of autoantibodies in patients with SLE;

**Methods:** 24 consecutive patients with SLE were vaccinated against streptococcus pneumonia. Assessment was performed the day of vaccination and 2 months later and included evaluation of disease activity using the SLEDAI, serum levels of ESR, CRP, C3 and C4. The sera were tested by ELISA for anti-DNA, anticardiolipin (IgG and IgM), anti-Sm, anti-Sm/RNP, anti-Ro, and anti-La.

**Results:** The mean age at enrollment into the study was 39, mean disease duration 6.9 years. The SLEDAI score was 4.41±2.92 at vaccination and 4.47±3.11 after 2 months. At the time of vaccination, 10 patients had anti-dsDNA, 2 patients had anti-Sm, 5 had anti-RNP, 9 had anti Ro, 4 had anti La, 4 had anti-cardiolipin IgG and IgM. Two months after vaccination, no change was observed in the proportion of patients with anti-Sm, anti-dsDNA, anti-RNP, anti-Ro and anti-cardiolipin IgM. A single patient developed anti-cardiolipin IgG and another one turned anti-RNP negative.

**Conclusions:** Vaccination against streptococcus pneumonia did not trigger the generation of autoantibodies or a significant change of SLEDAI, confirming the clinical safety of this vaccine in SLE patients.