Results In patients with RA the significant increasing of SF immunoglobulins, especially IgG, circulating immune complexes, complement proteins, IL-1 beta and TNF-alpha was found. C-reactive protein was relatively low. There was no significant serum IgG, phagocyte activity, IL-1 beta and TNF-alpha elevation. RF at the RA onset was higher in SF, than in serum. In RA seronegative patients was determined exclusively in the SF in low quantity.

Conclusion We concluded that immunological investigation of SF is important tool for RA diagnosis, especially in activity processes estimation.

AB0012 URINE AND PLASMA INTERLEUKIN-6 (IL-6) LEVELS IN PATIENTS WITH GLOMERULAR DISEASE

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Background IL-6 is involved in the induction of acute phase protein synthesis in liver cells, as well as in other systemic changes associated with infection and tissue injury. IL-6 is probably produced by mesangial cells in patients with mesangial proliferative glomerulonephritis (MPG).

Objectives The aim of this study was to determine IL-6 levels in urine and plasma of patients with primary glomerular disease ? MPG and of patients with systemic lupus erythematosus who had MPG (II class of lupus nephritis according to WHO).

Methods The study included 23 patients and 10 healthy volunteers. 12 out 23 patients had MPG and 6 had SLE and MPG. 5 out 23 patients had membranoproliferative glomerulonephritis. Plasma and urine IL-6 levels were determined using sensitive bioassay.

Results Measurable quantities of IL-6 were detected in the urine (mean $\pm$ SEM = 24.75 ± 0.61 pg/ml; $n = 10$) as well as in the plasma (14.18 ± 0.43 pg/ml; $n = 10$) of healthy controls. Increased urine IL-6 levels were found in patients with primary and secondary (with SLE) MPG (29.75 ± 0.75 ; $n = 17$; $p < 0.01$), while in patients with membranoproliferative glomerulonephritis the IL-6 concentration did not differ from the control values (25.24 ± 1.4 ; $n = 5$; $p > 0.05$). In plasma the mean IL-6 concentration in patients with MPG and SLE was slightly but not significantly higher than in the healthy subjects.

Conclusion The data show that the presence of IL-6 in urine but not in plasma, may be a valuable indication of the disease in the majority of patients with primary MPG and patients with SLE who had MPG.

AB0013 SOME CYTOKINES AND BONE MARKERS IN OSTEOARTHRITIS

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Background Osteoarthritis (OA) is a dynamic process involving joint as an organ and causing successive loss of structural integrity of different joint compartment constituents. OA is not a single disease entity but it is a group of overlapping disorders in which articular cartilage and subchondral bone are disturbed. Synovitis and formation of granulation tissue in subchondral

bone take place in many patients with OA. Therefore at least some OA forms may be classified rather as inflammatory disease rather than degenerative disease. It is also a process where different biochemical factors besides mechanical take place.¹

Objectives To elucidate the participation of some cytokines in OA pathological processes and to compare their values with those of bone markers.

Methods In sera of 32 OA patients in early as well as late stages with levels of IL-1b, IL-6, IL-8 and TNFa were established in Immulite (DPL, Los Angeles, CA, USA). Because serum levels of IL-1b and IL-6 were normal in all patients, in extracts from tissues obtained in TEP installation, only IL-8 and TNFa levels concentration were determined. Urinary pyridinoline (UPD) and deoxypyridinoline (UDP) were determined with HPLC in the equipment of Spectra Physics (San Jose, CA, USA, type SP).² Chondrex (YKL-40) and bone alkaline phosphatase (BAP) were measured with ELISA test (Metra Biosystems, CA, USA). Values of IL-8 and TNFa don't correlate with Upyr and chondrex levels.

Results Concentration of cytokines in sera, synovial fluids and tissue extracts were increased only in some patients and mainly in synovial fluid. Markers of activity were increased in the both sera and tissue extracts.

Conclusion According to the results obtained IL-1b and IL-6 do not play any role in OA processes. On the other hand tissue concentrations of IL-8 and TNFa do not correlate with bone markers and therefore they not reflex activity of pathological process.

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AB0014 TUMOUR NECROSIS FACTOR-ALPHA IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background

Objectives

Methods To obtain information about the involvement of tumour necrosis factor-alpha (TNF-a) in the pathophysiology of SLE this cytokine was measured, by an enzyme linked immunosorbent assay, in sera from 109 patients (pts) with systemic lupus erythematosus (SLE) (44 men and 65 women). The results were compared with 20 healthy subjects. The mean level of TNF-alpha was 84.4 ± 43.2 pg/ml in males (0-859 pg/ml) and 11 ± 7.4 pg/ml in females (0-192.2 pg/ml). The difference between male and female cohorts was significant ($p < 0.002$). The mean level of TNF-alpha was 0.6 ± 0.8 (0-8.5 pg/ml) in donors. Patients of both groups (males and females) had a significantly higher serum TNF-alpha than control subjects ($p < 0.001$).

Results TNF-alpha was found positive (more than 3s.d. above the mean in normal controls) in 17 females (26%), 36 males (81.8%) in pts with SLE compared to 1 person (5%) of normal controls ($p < 0.05$). Data differed statistically between patients in male and female groups and controls. The raised level of TNF-alpha was defined as low, moderate and high positive. We have found the low-positive TNF-alpha in 2 pts (3%), the moderate-positive in 8 pts (12%) and the high-positive in 7 pts (10%) in females. We found significant positive correlation

between TNF-alpha and ESR ($p < 0,02$), arthritis ($p < 0,01$), capillaritis ($p < 0,01$), lymphadenopathy ($p < 0,001$); the negative correlation was observed with serum albumin ($p < 0,02$) in females. The level of TNF-alpha was the low-positive in 2 pts (4,5%), the moderate-positive in 8 pts (18,1%), the high-positive in 26 pts (59%) in males. There was a positive correlation between TNF-alpha and SLAM index ($p < 0,01$), SLEDAI ($p < 0,06$), antiphospholipid syndrome ($p < 0,01$), IgG-anticardiolipin antibodies ($p < 0,03$) in male group.

Conclusion There are differences between the level, the clinical value of TNF-alpha in male and female cohorts of pts with SLE. The measurement of TNF-alpha maybe a useful tool for monitoring disease activity, in the male cohort especially.

AB0015 SYNOVIAL FLUID ANALYSIS: CORRELATION BETWEEN REAGENT STRIPS ANALYSIS AND WHITE BLOOD CELL COUNT

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Background

Objectives To determine if reagent test strips used to analyse inflammatory synovial fluid (SF) is as performing as white blood cell (WBC) count.

Methods 100 synovial fluid specimens obtained were tested using two techniques:

- reagent strips used to test urine (multistix 8SG Bayerdiagnostics) to check the presence of leucocytes. An inflammatory SF was defined as a strip showing more than a trace for leucocytes. The analysis was performed by an investigator unaware of WBC count and of diagnosis.
- White blood cell count. An inflammatory SF was defined as $WBC \geq 2000 \text{ cells/mm}^3$.

Specificity, sensitivity and like hood ratio (LR) of the reagent strip in diagnosing inflammatory SF were calculated.

Results 100 samples SF were tested. The performance of reagent strip were: sensitivity: 82% (IC: 95%); specificity was 88% (IC: 95%) LR: 5.6.

Conclusion Our study suggest that an evaluation of SF by reagent strips could be of interest to guide the diagnosis.

OP0030 CARTILAGE DEGRADATION BY IL-18 IS PREDOMINANTLY MEDIATED BY IL-1 GENERATION

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Background Interleukin-18 (IL-18) is a member of the IL-1 family of proteins that exerts proinflammatory effects.¹ It was formally known as IGIF and is a pivotal cytokine for the development of Th1 responses.² IL-18 is structurally related to IL-1b both cytokines need ICE or caspase-1 for cleavage of the precursor to release the bioactive molecules. Apart from immune-stimulatory activity, IL-18 induces production of TNF and IL-1 *in vitro*. IL-18 synthesis is found in both articular chondrocytes and osteoblasts and, with respect to cartilage, IL-18 promotes gene expression of nitric oxide synthase, inducible cyclooxygenase, IL-6 and stromelysin.

Objectives The goal of the present study was to investigate whether IL-18 mediates cartilage degradation directly or via induction of other cytokines, such as IL-1 and IFN-g.

Methods Patellae of C57Bl/6, IL-1b deficient, Balb/C and IFN-g deficient mice were used for *in vitro* cartilage degradation experiments with either murine IL-18 or IL-1b. Patellar cartilage was pre-labelled with 35S-sulphate. The explants were cultured for 24 h, 48 h or 72 h in either IGF-1 (0.25 ug/ml), IGF-1/IL-18 (10 and 100 ng/ml) or IGF-1/IL-1b (10 ng/ml). Patellae were washed in saline, fixed in 4% formaldehyde and subsequently decalcified in 5% formic acid. Patellae were punched out of the adjacent tissue, dissolved and the 35S content was measured by liquid scintillation counting. For IL-1 inhibition we added IL-1Ra (10 ug/ml) or an ICE inhibitor (2.5 uM) to the culture system.

Results IL-18 exposure for 24 or 48 h did not induce cartilage degradation, determined as release of prelabelled proteoglycans. Cartilage degradation by IL-18 was only found after a 72 h culture period. In contrast, IL-1b did already induce marked cartilage degradation after 24 h. To investigate whether generation of another destructive cytokine was responsible for the delay of cartilage degradation we added IL-1Ra or ICE inhibitors to the culture system. Blocking of IL-1 resulted in almost complete protection against IL-18 mediated cartilage degradation. Additionally, studies in IL-1b -/- mice showed that IL-1b was pivotal in IL-18-induced cartilage degradation. Since IL-18 is a potent inducer of IFN-g, we investigated whether IFN-g induction by IL-18 was involved. It was revealed that IL-18 mediated cartilage destruction was partly dependent on IFN-g induction.

Conclusion The present study demonstrated that IL-18 induces cartilage destruction *in vitro*. Here we showed that IL-1b generation, due to IL-18 exposure was essential for marked cartilage degradation. In addition, it was shown that induction of IFN-g was also involved in IL-18-induced cartilage degradation. These findings implicated that IL-18 contributed to cartilage destruction by induction of IL-1b and IFN-g. Inhibition of both IL-18 and IL-1b generation by ICE inhibitors might be a protective therapy in RA.

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OP0031 PROLONGED EXPOSURE OF T CELLS TO TNF DOWNREGULATES TCRZ AND EXPRESSION OF THE TCR/CD3 COMPLEX

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Background A role for tumour necrosis factor α (TNF) in the pathogenesis of chronic inflammatory is now firmly established. Paradoxically, TNF also has potent immuno-modulatory effects on CD4⁺ T lymphocytes, since antigen specific proliferative and cytokine responses are suppressed following prolonged exposure to TNF. We studied whether TNF attenuated T cell activation by uncoupling proximal TCR signal transduction pathways using a mouse T cell hybridoma model. In this model, IL-2 production following peptide stimulation was suppressed by up to 90% by chronic TNF exposure, and was restored to control levels within