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ABSTRACTS

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INFLIXIMAB TREATMENT IN RHEUMATOID ARTHRITIS AND SPONDYLOARTHROPATHIES: DRUG SURVIVAL AND REASONS FOR DISCONTINUATION DURING 2 YEARS OF FOLLOW-UP

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Objective: The aim of the present study was to determine the treatment continuation, discontinuation, and reasons leading to discontinuation during 2 years of follow-up in patients with active rheumatoid arthritis (RA) or spondyloarthropathies (SpA) who were treated with infliximab as their first biological anti-rheumatic drug in a single rheumatological centre (Tampere University Hospital, Tampere, Finland).

Methods: The present survey included 104 patients with active RA or SpA who were treated with infliximab as their first biological treatment according to national

guidelines during 1999–2005 in the Centre for Rheumatic Diseases, Tampere University Hospital. Patient data were analysed at baseline and after 2 years of follow-up. The response to treatment was determined as inadequate if the response was lower than 50% improvement in symptoms according to the American College of Rheumatology criteria (ACR50) in RA or the reduction in the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score was < 50% or < 2 cm in SpA.

Results: Forty-one patients (39%) continued the infliximab treatment successfully for at least 2 years, and prednisolone dose was decreased by 52% among these patients. Sixty-two patients (60%) had discontinued the infliximab treatment during the 2 years of follow-up. Discontinuation rate due to remission was 7%, and 22% of the patients had discontinued due to poor response. Twenty-five patients (24%) discontinued the treatment because of adverse events, which in most cases were infections and hypersensitivity reactions, but also two cases of drug-related leucopaenia were diagnosed.

Conclusions: In the present study, infliximab was added to previous disease-modifying anti-rheumatic drug (DMARD) treatment in 104 patients who had active RA or SpA despite the ongoing treatment with combinations of DMARDs. After 2 years, 39% of the patients had achieved at least 50% response and continued the treatment. In 22% of the patients, dissipating efficacy caused them to discontinue the infliximab treatment during the 2 years of follow-up. Similar adverse events (mainly infections and hypersensitivity reactions, but also two cases of leucopaenia) appeared, as in previous reports.

LEFLUNOMIDE AND BIOLOGICAL THERAPY IN CLINICAL PRACTICE WHEN TREATING PATIENTS WITH RHEUMATOID ARTHRITIS: LESSONS FROM THE ROB-FIN REGISTRY

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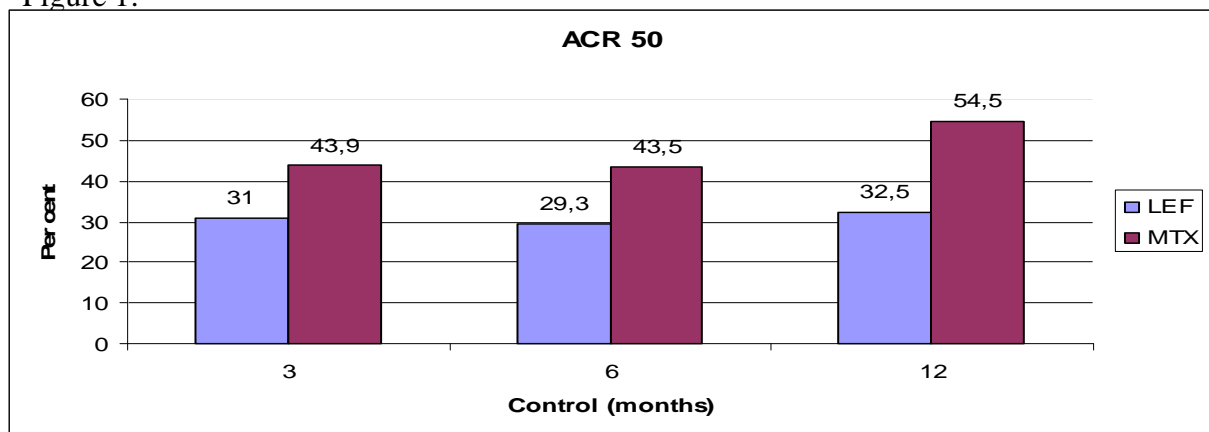
Objective: In Finland, variable combination therapy is customary in the context of biological therapy when treating rheumatic diseases. This also holds true for leflunomide (Lef), which is not generally recommended to be combined with other haemato- or hepatotoxic disease-modifying anti-rheumatic drugs (DMARDs). We used the registry of biological therapy in Finland (ROB-FIN) to identify patients also using Lef, compared to methotrexate (MTX), as the anchor drug in biological combinations.

Methods: Ninety-five RA patients receiving Lef in combination with biological therapy were identified from the ROB-FIN registry. The performance of Lef with biological therapy and also in combination with various other DMARDs was assessed at 3, 6, and 12 months, in comparison with MTX. Outcome measures were followed up for 12 months and compared with those achieved with a biological combination therapy including MTX.

Results: Biological drugs used among Lef- vs. MTX-treated patients were: adalimumab 37/35, infliximab 30/37, and etanercept 24/20. The most frequently used other DMARDs among the Lef/MTX groups included: sulfasalazine (n = 19), hydroxychloroquine (12), aurothiomalate (8) vs. hydroxychloroquine (26), sulfasalazine (22), and aurothiomalate and podophyllotoxin (7). During follow-up, per oral corticosteroid intake was reduced in both groups. American College of Rheumatology (ACR) 50 responses in both groups at 3, 6, and 12 months are shown in **Figure 1**. When evaluating European League Against Rheumatism (EULAR) responses, both groups showed significant reductions in disease activity ≥ 1.2 .

Discussion: It seems possible to combine Lef with other DMARDs, including biological drugs, with considerable success. The performance of Lef compared to MTX in this setting was somewhat more modest, perhaps due to a more resistant disease including failure to respond to initial MTX, as Lef is usually added after MTX exposure.

Figure 1.



ADVERSE EVENTS DURING ANTI-TNF THERAPY IN 292 PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS

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Background: Patients with juvenile idiopathic arthritis (JIA), non-responsive to disease-modifying anti-rheumatic drugs (DMARDs), are treated with anti-tumour necrosis factor (TNF) agents. The aim of this study was to evaluate the occurrence of adverse events (AEs) in these refractory patients.

Methods: In three tertiary centres, patient charts were reviewed, and for each anti-TNF drug the severity and type of AEs were specified. Of 292 patients, 97% were on concomitant DMARDs at anti-TNF onset. Their mean age was 5.4 years (SD 3.8) at disease onset and 10.5 years (SD 3.9) at anti-TNF onset.

Results: The total drug exposure was 781 years; etanercept exposure was 403 years, infliximab 337, and adalimumab 41. Of altogether 1418 AEs, 66 (5%) were considered serious (Table 1). A total of 627 (44%) infections occurred, of which 313 (50%) were upper respiratory tract infections. Dermatological problems were documented in 69 (24%) patients, and hypersensitivity reactions (including infusion and injection site reactions) in 70. Three patients developed an inflammatory bowel disease during anti-TNF treatment and one had colitis ulcerosa at arthritis onset. Neither malignancies nor tuberculosis appeared, although one patient had a *mycobacterium avium pneumonia* during adalimumab treatment.

Conclusions: Infections were the most common AEs in patients with JIA receiving anti-TNF therapy, but the rate of serious infections seemed to be low.

Table 1. Adverse events (AEs) and serious adverse events (SAEs) per patient-year during anti-TNF therapy.

	Etanercept	Infliximab	Adalimumab	All
AEs	1.70	1.87	2.52	1.81
SAEs	0.09	0.08	0.05	0.08
All infections	0.79	0.81	0.82	0.80
Upper respiratory	0.41	0.40	0.34	0.40
Serious infections	0.03	0.02	0.02	0.03
Hypersensitivity reactions	0.04	0.29	0.19	0.14

STEEP DECLINE IN THE INCIDENCE OF RENAL REPLACEMENT THERAPY FOR AMYLOIDOSIS ASSOCIATED WITH INFLAMMATORY RHEUMATIC DISEASES

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Objective: To assess possible changes in the incidence of end-stage renal disorder due to amyloidosis associated with rheumatic diseases on a nationwide basis.

Methods: We examined the files of the Finnish Registry for Kidney Diseases for patients suffering from amyloidosis associated with rheumatoid arthritis (RA), ankylosing spondylitis (AS), or juvenile idiopathic arthritis (JIA) over the period 1987–2006. The registry has an estimated 97–99% coverage of all patients accepted for renal replacement therapy (RRT; i.e. dialysis or kidney transplantation) in the country.

Results: A total of 435 patients with amyloidosis were identified. There was a stable number of new admissions around 100 (range 97–106 and more than 75% with RA) per 4-year period until 2002. By contrast, during the last 4-year period (2003–2006), the corresponding figure was only 34 (Figure 1).

Discussion: The present series, which is based on nationwide data, shows that the occurrence of end-stage renal disease due to rheumatic diseases associated amyloidosis is decreasing. An obvious reason for the change is successful

suppression of the acute phase reaction, including the circulating amyloid precursor serum amyloid A. It seems that active drug policy to control inflammation now has a lag of 10–15 years reflected in the lower number of new patients with amyloidosis admitted to RRT.

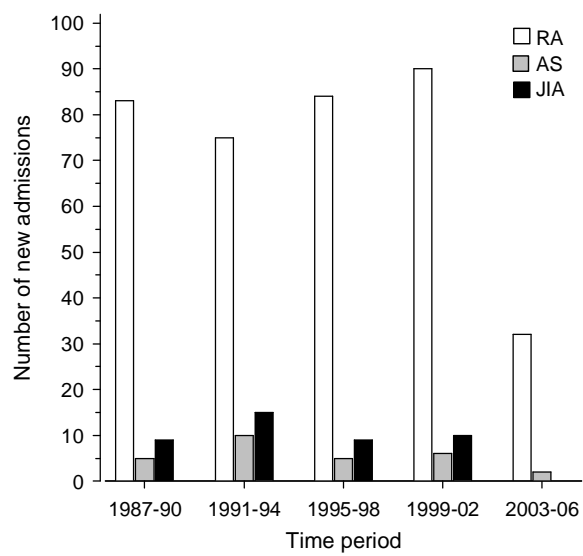


Figure 1.

CHANGE OF DIAGNOSES AMONG PATIENTS WITH UNDIFFERENTIATED ARTHRITIS DURING A 6-YEAR FOLLOW-UP

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Objective: To assess change of diagnoses and their predictive factors in patients with early inflammatory joint diseases during a 6-year follow-up.

Methods: At year 2000, patients living in Kuopio (adult population 76 000) with earlier undiagnosed arthritis were included in the Kuopio 2000 Arthritis Survey. Patients with inflammatory arthritis were classified as having rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, reactive arthritis, undifferentiated spondylarthritis, or undifferentiated arthritis (UA). The patients were followed up at 1 and 6 years. Their diagnosis was changed if the disease met the criteria for any specific syndrome.

Results: A total of 96/173 subjects had UA at baseline. Of these, 85% were female, 14% rheumatoid factor (RF) positive, 11% had anti-cyclic citrullinated peptide (anti-CCP) antibodies, and 23% showed human leucocyte antigen (HLA)-B27. Their mean age at diagnosis was 48.5 ± 15.3 years. Twenty-six patients (27%) changed diagnosis to a more specific one. Of all patients, 17% were classified as having RA, 6% spondylarthritis, 7% osteoarthritis, and 1% other rheumatic syndrome. Constancy of UA diagnosis was 69% [95% confidence interval (CI) 57–78]. In multivariate analysis age was the only predicting factor for change of diagnosis. In a

univariate model, age, RF, anti-CCP antibodies, HLA-B27, and polyarthritis predicted the change from UA to RA.

Conclusion: UA is a prolonged disorder. Age, marker antibodies such as RF and anti-CCP antibodies, HLA-B27, and polyarthritis predicted the development of RA.

RASBURICASE IN TOPHACEOUS GOUT: A CASE REPORT

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Severe rheumatoid arthritis (RA) started in 1969; hip and knee arthroplasty had been performed. Aurothiomalate was stopped in 1989 because of immunoglobulin A (IgA) nephropathy, which had not progressed. In kidney, amyloidosis was not found, but in 1990 gastroscopy did reveal amyloidosis. In 2002 treatment of RA was prednisolone 10 mg and of nephropathy furosemide 80 mg daily. S-urate was 503 $\mu\text{mol/L}$, but allopurinol caused severe dermatitis and losartan low blood pressure. The nephrologist did not accept probenecid. In 2005, several small tophi were seen in the hands and urate crystals in proximal interphalangeal (PIP) joint fluid. In 2006 the tophi had grown and S-urate was 631 $\mu\text{mol/L}$, S-creatinine 135 $\mu\text{mol/L}$, and creatinine clearance 0.63 mL/s/L. Probenecid was started with care. After 8 months S-urate was 548 $\mu\text{mol/L}$, and abundant tophi were painful and bleeding in the hands and feet. The 56-year-old female patient was admitted to Turku University Hospital, where rasburicase (Fasturtec) infusions 15 mg (0.2 mg/kg) per month were started. Furosemide dose was 30 mg and probenecid 1500 mg; the patient reduced the dose of probenecid to 1000 mg daily. Rasburicase probably lowered S-urate rapidly (before the infusion S-urate was 394–582 $\mu\text{mol/L}$; mean 450, SD 58; postinfusion values not available) as tophi decreased in size and disappeared after 1 year of treatment. In June 2008 probenecid was stopped. Thereafter, the S-urate level has been 449–607 $\mu\text{mol/L}$ (mean 533, SD 57) before infusions.

Conclusion: Rasburicase is one possibility for the treatment of severe gout complicated by moderate renal insufficiency.

Reference

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THE OCCURRENCE AND TRIGGERING INFECTIONS OF REACTIVE ARTHRITIS IN A POPULATION EXPOSED BY A LARGE WATERBORNE GASTROENTERITIS OUTBREAK CAUSED BY SEWAGE WATER POLLUTION IN PIRKANMAA, FINLAND

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Background: In November 2007, faecal contamination of the water supply system resulted in a large gastroenteritis outbreak in the town of Nokia in Pirkanmaa, Finland. Almost 8500 residents of the 30 000 inhabitants of Nokia fell ill. A large spectrum of microbial findings was confirmed in both water and stool samples. *Campylobacter jejuni* and other *Campylobacter* strains together with Noroviruses were considered to be the most prevalent pathogens at the beginning of the gastroenteritis outbreak, and later on numerous *Giardia lamblia* findings were made. The aim of this study was to estimate the occurrence and triggering infections of reactive arthritis associated with this outbreak.

Methods: The general practitioners and personnel working in the Nokia Health Centre and local occupational physicians were advised to refer any new patients (from December 2007 to 31 May 2008) with suspicion of reactive arthritis (e.g. swollen joints or sacroiliitis) to the Centre for Rheumatic Diseases in Tampere University Hospital. Clinical rheumatological examination included structured

physical examination focused on evaluation of spondyloarthropathies. Human leucocyte antigen (HLA)-B27 antigen was determined as well as stool culture and antibody tests for *Campylobacter*, *Salmonella*, and *Yersinia* infections.

Results: A total of 45 patients (33 females, 12 men) aged 16–77 years (median 53 years) were referred and examined because of arthritic symptoms. Reactive arthritis was diagnosed in 21, postinfectious arthralgia in 13, and other musculoskeletal conditions in 11 patients. HLA-B27 antigen was positive in seven of 44 patients (15.9%) including two of 21 patients (9.5%) with reactive arthritis, which corresponds to the occurrence of HLA-B27 in the general Finnish population. Of the 21 patients with reactive arthritis, triggering infections were observed in six patients (28.6%), namely *Campylobacter* in four, *Yersinia* in three, and *Salmonella* in one patient who also had *Campylobacter* infection. Reactive arthritis was mild in character in all but one patient with recurrent *Salmonella* infections including *Salmonella* sepsis and infectious arthritis of the hip joint with osteonecrosis. Of the 21 patients in the reactive arthritis group, 18 were treated with non-steroidal anti-inflammatory drugs (NSAIDs), 15 with intra-articular glucocorticoid injections, nine with oral prednisolone (5–10 mg/day), and 10 with disease-modifying anti-rheumatic drugs (DMARDs) (nine with sulfasalazine, four with hydroxychloroquine, two with methotrexate).

Conclusions: Taking into account the large population polluted with potentially arthritogenic agents, the occurrence of reactive enteroarthritis was rare and clinically mild in character, except in one patient with septicaemia and infectious arthritis caused by *Salmonella enteritidis*.

COMBINATION OF REUMACON[®] (CPH82) AND SIMVASTATIN AS A CAUSE OF RHABDOMYOLYSIS OR MUSCLE WEAKNESS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The cytotoxic drug Reumacon[®] (CPH82), consisting of podophyllotoxin, is used in the treatment of rheumatoid arthritis (RA), and is metabolized through the CYP3A4 enzyme system. The most common adverse effects of CPH82 are diarrhoea, abdominal pain, and nausea in 15–30% of patients using the drug. Rhabdomyolysis is the rapid breakdown of skeletal muscle tissue in which the destruction of the muscle leads to the release of the breakdown products of damaged muscle cells into the bloodstream and may cause kidney damage. The most reliable test in the diagnosis of rhabdomyolysis is the level of creatinine kinase (CK) in the blood. About 25% of patients also have abnormal liver function tests. Muscle pain and rhabdomyolysis have been described as adverse effects of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), which also use the CYP3A4 enzyme system in their metabolism. The drugs that use the same route in their metabolism may increase the proportion of statins in serum. We describe four patients who suffered muscular adverse events while using Reumacon[®] and simvastatin. The main laboratory findings are shown in [Table 1](#). All patients had simvastatin 10–40 mg/day and Reumacon[®] 100–200 mg/day. Changes in simvastatin or Reumacon[®] doses had been made a few weeks or months earlier.

Patient 2 had muscular trauma a few weeks before he had muscle weakness. Patient 4 lost weight (5 kg in 5 days) and was admitted to hospital due to heart failure. At admission her CK was 153 U/L and creatinine 95 µmol/L. In 5 days she lost power in her muscles, which became tender.

Table 1. Main findings in laboratory tests (normal values in parentheses).

Patient, sex, age in years	S-CK (F 35–210 U/L, M 40–280 U/L)	S-myoglobin (19–55 µg/L)	S-ALT (10–45 U/L)	S/P-creatinine (F 50–90 µmol/L, M 60–100 µmol/L)	S-albumin (36–45 g/L)
1; F, 75	11 455	6248	681	44	27
2; M, 76	2401	–	–	94	Hypoalbuminaemia in serum electrophoresis
3; M, 55	15 864	8554	473	200	31
4; F, 85	6816	17 940	352	98	27

F, female; M, female; CK, creatinine kinase (CK); ALT, alanine aminotransferase.

Patients 1, 3, and 4 were treated for rhabdomyolysis, Patient 2 had muscle weakness, which disappeared after stopping simvastatin and Reumacon®.

HOW IS METHOTREXATE TREATMENT OF PATIENTS WITH RHEUMATOID OR PSORIATIC ARTHRITIS CARRIED OUT IN ‘REAL LIFE’?

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Successive patients with rheumatoid arthritis (RA) or psoriatic arthritis (PsA) visiting the rheumatological clinic of RFH were asked whether they were undergoing methotrexate (MTX) treatment or had received it previously. A total of 298 patients (200 women and 98 men; 218 RA and 80 PsA) with a history of MTX treatment were included. They answered a questionnaire and MTX treatment histories were collected from the medical records.

MTX treatment was ongoing in 175/298 (59%) patients (61% RA and 51% PsA). The average dose was 15 mg/week. Parenteral MTX had been used by 16% patients and it was still being used in 14% of RA and 22% of PsA patients. MTX was combined with other drugs in 90% patients with RA and 64% with PsA. The median duration of MTX treatment was > 4 years.

Adverse effects (AEs) were common: 43% patients with RA and 60% with PsA ($p = 0.010$) reported them. The most common AE was hepatopathy, seen in 20% of patients (RA 17%, PsA 29%; $p = 0.019$). Gastrointestinal (GI) symptoms were reported by 18% of all patients. Hair loss was reported by 1.4% of RA and 5%

of PsA patients. MTX treatment had been stopped by 124 (42%) patients. AEs were the main reason for stopping the treatment in 64/85 (75%) of RA and 28/39 (72%) of PsA patients. Patients who had stopped the treatment also usually had active RA. However, the lack of efficacy was seldom the main reason for stopping the MTX treatment.

Conclusion: MTX treatment is common and often long-lasting. However, it is often stopped because of AEs. If the treatment effect is good, some AEs are tolerated and MTX treatment continued.

THE EFFECTS OF *MTHFR* GENE VARIATIONS ON DRUG RESPONSE IN RHEUMATOID ARTHRITIS AND PSORIATIC ARTHRITIS PATIENTS TREATED WITH METHOTREXATE

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Methotrexate (MTX) is a folic acid antagonist widely used to treat immunosuppressive disorders such as rheumatoid arthritis (RA). The enzyme 5,10-methylenetetrahydrofolate reductase (*MTHFR*) is involved in maintaining folate and homocysteine homeostasis, and the common genetic variations c.677C>T and c.1298A>C in the *MTHFR* gene are associated with decreased enzyme activity and altered folate levels, which may predispose to increased susceptibility to the anti-folate effects of MTX therapy. We studied the effects of these variations on MTX response in 298 Finnish patients who had either RA (n = 218) or psoriatic arthritis (n = 80). Together with genotyping we also analysed folate, homocysteine, alanine amine transferase (ALAT), and vitamin B12 levels, as well as clinical patient data regarding the toxicity and efficiency of the MTX treatments. Statistical analyses were performed to evaluate possible associations between the *MTHFR* gene variations and MTX toxicity and efficiency. Our results show that the patients with two normal alleles (677CC/1298AA) are less likely to experience side-effects during MTX therapy although no statistically significant association between the variations and MTX toxicity was found. Of note, *MTHFR* 677TT was founded to be a risk

genotype for elevated ALAT as well as a serious risk factor for low folate and accordingly elevated homocysteine levels, especially when MTX and folic acid supplementation were not in use. In addition, the state of the disease stayed remarkably more often active in patients having the 677TT genotype and low folate levels, suggesting that *MTHFR* 677TT is a crucial genotype and should be taken into account in patient treatment strategies.

TWO SIMILAR ADVERSE EVENTS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AFTER RITUXIMAB THERAPY

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Introduction: Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disorder characterized by variable clinical and laboratory features. In SLE, immunological abnormalities lead to B-cell hyperactivity and the production of multiple autoantibodies. Rituximab is a chimeric monoclonal antibody directed against the CD20 molecule present in B lymphocytes. It is an approved biologic agent for rheumatoid arthritis and non-Hodgkin's B-cell lymphoma. Rituximab has non-SLE indication but is being used by rheumatologists for refractory lupus manifestations.

Case reports: We describe two patients with active SLE who developed a serum sickness-like reaction 1–1.5 weeks after their first infusion of the second cycle of rituximab (dosing of 1000 mg twice in biweekly infusions with background disease-modifying anti-rheumatic drugs). Both patients reported a good and long-lasting response after their first cycle of rituximab. They also had complement deficiency and anti-DNA antibodies during the active phases of the disease.

Patient 1 was a 32-year-old female and her SLE had been diagnosed in 1996 with neuropsychiatric and kidney manifestations and later there were skin lesions and oral ulcers. SLE was originally treated with intravenous (i.v.) cyclophosphamide and azathioprine and later with cyclosporin, methotrexate, and

corticosteroids. She had her first cycle of rituximab with clinical benefit towards the end of 2006. The second cycle of rituximab was given in January 2008.

Patient 2 was a 30-year-old female with SS-A antibody-positive SLE. She was diagnosed in 1993 with pericarditis and pleuritis. Initially she was treated with i.v. cyclophosphamide followed by hydroxychloroquine, azathioprine, and corticosteroids. She received her first cycle of rituximab in November 2006 with clinical improvement. She had a disease relapse in October 2008, 5 months after her first delivery.

Adverse events: Both patients developed fever, urticaria, arthralgia, and headache after retreatment with rituximab. Patient 1 also had alveolar haemorrhagia and neuropsychiatric symptoms.

Conclusions: Rituximab is an effective treatment in some cases of SLE. However, patients with SLE may experience serious adverse events compared to patients treated for oncological indications or rheumatoid arthritis due to different disease mechanisms. Biomarkers that can predict harmful immunological reactions are needed.

PRETREATMENT LEVELS OF ADIPOKINES ADIPSIN AND LEPTIN ARE ASSOCIATED WITH REMISSION RATES IN EARLY RHEUMATOID ARTHRITIS (NEO-RACO STUDY)

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Introduction: Obesity is a major public health problem in the Western world, and is associated with inflammation and arthritis. Adipokines (e.g. leptin, adiponectin, adipsin, and resistin) are hormones synthesized by adipose tissue that regulate energy metabolism and appetite, and recent findings support their role in inflammation and arthritis. NEO-RACo is a double-blind placebo-controlled study on early rheumatoid arthritis (RA), where either infliximab (INFL) or placebo (PL) was combined during the first 6 months of treatment with methotrexate, sulfasalazine, hydroxychloroquine, and prednisolone (= COMBI). We have previously shown that increasing body mass index (BMI) was associated with a reduced rate of remission in the COMBI + PL group but not in the COMBI + INFL group. In the present study, we report the predicting value of pretreatment adipokine levels for reaching remission by 6 months of treatment.

Results: At 6 months, 53% of the RA patients were in remission (47% in the COMBI + PL group and 58% in the COMBI + INFL group). Both COMBI + INFL ($p = 0.0001$) and COMBI + PL ($p = 0.031$) enhanced plasma concentrations of the anti-inflammatory adipokine adiponectin, but the concentrations of adipsin, leptin,

and resistin remained unaltered. Of note, serum adipsin and leptin levels at the start of the INFL/PL treatment were associated with the remission rates determined after 6 months of treatment. High adipsin levels at the start of INFL/PL treatment predicted high remission rates: 75% of the patients in the highest adipsin tertile were in remission as compared to 48% in the mid-tertile and 37% in the lowest adipsin tertile ($p = 0.004$ for linearity). The relationship between pretreatment adipsin levels and remission rates was also found in the COMBI + INFL group. By contrast, high leptin levels at the start of INFL/PL treatment predicted poor remission rates. However, that was found only in the COMBI + PL group, in which 81% of the patients in the lowest leptin tertile achieved remission as compared to 31% in the mid-tertile and 24% in the highest leptin tertile ($p = 0.026$ for linearity). Interestingly, infliximab treatment overcame the poor predictive value of high leptin levels.

Conclusions: High pretreatment adipsin levels in early RA predicted good remission rates in patients treated with a combination of disease-modifying anti-rheumatic drugs (DMARDs) with and without infliximab. High circulating leptin concentration in early RA may be a useful marker to distinguish the patients who respond better to a combination of DMARDs and tumour necrosis factor (TNF) α antagonists than to traditional DMARDs alone.

AUROTHIOMALATE INHIBITS COX-2 EXPRESSION AND PGE₂ PRODUCTION IN CHONDROCYTES BY INCREASING MKP-1 EXPRESSION AND DECREASING P38 PHOSPHORYLATION

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Objective: Disease-modifying anti-rheumatic drugs (DMARDs) suppress inflammation, and retard cartilage degradation and bone erosion in arthritis. The molecular mechanisms of action of many traditional DMARDs are not known in detail. Inducible prostaglandin synthase (cyclooxygenase-2, COX-2) is highly expressed in osteoarthritis (OA) and rheumatoid arthritis (RA) cartilage and it produces large amounts of proinflammatory prostanoids in the joint. Mitogen-activated protein kinases (MAPKs) are major signalling mechanisms involved in the upregulation of COX-2 expression in inflammation. In the present study we investigated the effects of DMARDs on MAPK pathways and MAPK kinase phosphatase-1 (MKP-1) in immortalized H4 chondrocytes.¹ MKP-1 is an endogenous regulator of MAP kinases p38 and JNK. Glucocorticoids have recently been shown to enhance MKP-1 expression, which partly explains their anti-inflammatory effects.

Results: We investigated the effects of traditional DMARDs on MKP-1 expression in chondrocytes. Unlike the other compounds tested (cyclosporin A, hydroxychloroquine, leflunomide, its active metabolite A771726, methotrexate, and sulfasalazine), aurothiomalate was found to enhance MKP-1 expression like

dexamethasone. Aurothiomalate inhibited interleukin (IL)-1 β -induced COX-2 expression and prostaglandin E₂ (PGE₂) production by destabilizing COX-2 mRNA, as did p38 MAPK inhibitor SB203580. Of note, aurothiomalate inhibited the phosphorylation of p38 kinase along with its enhancing effect on MKP-1 expression; and when MKP-1 was downregulated by siRNA, aurothiomalate's ability to inhibit p38 phosphorylation and COX-2 expression reduced significantly.

Conclusions: The results provide a novel mechanism for the anti-inflammatory action of aurothiomalate through increased MKP-1 expression and reduced p38 MAP-kinase activation, and COX-2 expression. The results suggest MKP-1 as a promising novel target for the development of disease-modifying drugs for RA and OA.

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PROTEIN KINASE C ISOENZYMES REGULATE THE EXPRESSION OF ARTHRITIS SUPPRESSOR GENE TTP IN ACTIVATED MACROPHAGES

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Objectives: Tristetraprolin (TTP), also known as Nup475, TIS11, G0S24, and Zfp36, is a factor that regulates mRNA stability by binding to the 3' untranslated region (UTR) of mRNA of many transiently expressed inflammatory genes. TTP has been implicated in the post-transcriptional regulation of, for example, tumour necrosis factor alpha (TNF α) and inducible nitric oxide synthase (iNOS). TTP-deficient mice develop a profound inflammatory syndrome with erosive arthritis, autoimmunity, and myeloid hyperplasia. This has been reported to be mainly due to excessive production of TNF α as treatment of TTP-deficient mice with antibodies to TNF α prevented the development of the phenotype. TTP seems to have a role as an anti-inflammatory or arthritis suppressor gene. The aim of the present study was to investigate the role of protein kinase C (PKC) isoenzymes in the regulation of TTP expression.

Methods: The expression of TTP in J774 macrophages was induced by a combination of lipopolysaccharides (LPS) and phorbol myristate acetate (PMA). The effects of PKC inhibitors, PKC activation and downregulation by PMA, and PKC δ downregulation by siRNA on TTP protein and mRNA expression were

determined by Western blotting and quantitative reverse transcription polymerase chain reaction (qRT-PCR), respectively. In addition, the effects of the PKC β II inhibitor CGP53353 and the PKC δ inhibitor Rottlerin on the activation of transcription factors important to TTP were assessed. The effects of PKC inhibitors CGP53353 and Rottlerin on TTP mRNA decay were also determined.

Results: Inhibitors of classical PKC isoenzymes RO318220 (inhibits PKC α , β , γ , and δ), Gö6976 (inhibits PKC α , β , and γ), LY333531 (inhibits PKC β I and β II), and CGP53353 (inhibits PKC β II), as well as the PKC δ inhibitor Rottlerin, inhibited LPS and PMA-induced expression of TTP protein and mRNA. Similar effects were obtained when PKC isoenzymes were downregulated by PMA or siRNA. The PKC β II inhibitor CGP53353 decreased the activation of the activator protein 2 (AP-2) transcription factor, whereas the PKC δ inhibitor Rottlerin had no significant effect on the transcription factors, but it decreased the TTP mRNA half-life.

Conclusions: The results show that the PKC isoenzymes PKC β II and PKC δ upregulate the expression of arthritis suppressor gene TTP in activated macrophages. PKC β II mediates its effects possibly through the activation of transcription factor AP-2, whereas PKC δ mediates its effects by stabilizing TTP mRNA.

LEPTIN ENHANCES MMP-1, MMP-3, AND MMP-13 PRODUCTION IN HUMAN OSTEOARTHRITIC CARTILAGE

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Objectives: Obesity is an important risk factor for osteoarthritis (OA) in weight-bearing joints, and also in hand joints, indicating an obesity-related metabolic factor that influences the susceptibility or pathogenesis of OA. Leptin is an adipokine regulating energy balance and appetite, and recently it has also been associated with arthritis and cartilage metabolism as a proinflammatory factor. In OA, proteolytic degradation of cartilage is mediated by matrix metalloproteinases (MMPs). In the present study, the effects of leptin on MMP-1, MMP-3, MMP-8, and MMP-13 production in human OA cartilage were investigated.

Methods: Cartilage tissue obtained from the leftover pieces of total knee replacement surgery from patients with OA was used in the experiments. MMP production in the culture medium was measured by immunoassay (multiplex bead array method, Luminex).

Results: Leptin alone and in combination with interleukin 1 (IL-1) enhanced production of collagenases MMP-1 and MMP-13, and stromelysin-1 (MMP-3) in human OA cartilage, while collagenase-2 (MMP-8) concentrations remained undetectable. The effects of leptin on MMP-1, MMP-3, and MMP-13 production

were mediated through transcription factor NF- κ B, and through protein kinase C (PKC) and MAP kinase JNK pathways. In addition, p38 was involved in the leptin-induced MMP-1 and MMP-13 production, the Erk 1/2 pathway in MMP-1 production, and the JAK3 pathway in MMP-3 and MMP-13 production.

Conclusions: In the present study we found, for the first time, that leptin enhanced the production of destructive enzymes MMP-1, MMP-3, and MMP-13 in human OA cartilage. The findings support the idea of adipocytokine leptin as a catabolic factor in the pathogenesis of OA, and as a possible link between obesity and OA.

ABNORMAL BASEMENT MEMBRANE TYPE IV COLLAGEN ALPHA CHAIN COMPOSITION IN LABIAL SALIVARY GLAND IN SJÖGREN'S SYNDROME

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Objective: Sjögren's syndrome (SS) is characterized by atrophy and malfunction of the acinar cells. It was therefore hypothesized that the type IV collagen α -chain composition of the acinar cell compartment might be abnormal in diseased glands.

Methods: mRNA from human submandibular gland (HSG) cells, cultured \pm growth factor-depleted Matrigel, was analysed using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Labial salivary glands (LSGs) were analysed using qRT-PCR and immunohistochemistry.

Results: HSG cells of both ductal and acinar phenotypes synthesized all α -chain mRNAs, in particular those of the $\alpha 1$ - and $\alpha 2$ -chains. LSGs contained $\alpha 1/2$ chains, but also mRNA of all the other α -chains although the $\alpha 3$ and $\alpha 4$ mRNAs were low and the corresponding proteins absent. Collagen type IV $\alpha 1/2$ -chains were found in all tubuloalveolar basement membranes (BMs). In healthy glands $\alpha 5$ - and $\alpha 6$ -chains were continuous around ducts but discontinuous around acini, and in SS absent or patchy around ducts and absent around acini.

Conclusions: Ductal and acinar epithelial cells are able to locally produce all six different α -chain mRNAs. Collagen type IV α 1/2-chains seem to form the backbone in the tubuloalveolar BM in salivary glands. Type IV α 3 and α 4 mRNAs were found in cultured salivary epithelial cells and LSG explants, but were not translated to the corresponding α -chains in LSGs. α 5 and α 6 mRNAs were found in salivary epithelial cells and glands. In healthy glands immunolabelling always disclosed the corresponding α -chains around ducts, but their synthesis and/or degradation seems to be locally regulated in acinar cells in healthy glands, where staining was patchy.

HEALTHY HUMAN SALIVARY GLANDS CONTAIN A DHEA-S PROCESSING INTRACRINE MACHINERY THAT IS DERANGED IN PRIMARY SJÖGREN'S SYNDROME

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Objective: Patients with Sjögren's syndrome (SS) have low salivary dehydroepiandrosterone (DHEA) and androgen biomarker levels, but high salivary oestrogen levels. The hypothesis was that the healthy glands contain DHEA-sulfate (DHEA-S) processing intracrine machinery; local androgen/oestrogen imbalance suggests that this is disarranged in SS.

Methods: Indirect immunofluorescence and polymerase chain reaction (PCR) of steroid sulfatase, sulfotransferase, 3 β - and 17 β -hydroxysteroid dehydrogenases (3 β - and 17 β -HSD), aromatase, and 5 α -reductase from SS-, and healthy salivary glands.

Results: In control acini, steroid sulfatase immunoreactivity was strong in the basolateral cell parts, whereas sulfotransferase was weaker. 3 β - and 17 β -HSD

formed strong, interrupted bands along the basal cell parts. 5 α -Reductase was mainly located in acinar cell nuclei and aromatase in the apical cell membrane. All enzymes were more widespread in ducts. PCR of acinar and ductal cell lines disclosed all corresponding enzyme mRNA molecules. In SS, steroid sulfatase was weak and deranged, 3 β - and 17 β -HSD had lost their strict basal acinar cell localization, and 5 α -reductase was mainly found in the cytoplasm of the acinar cells, whereas aromatase showed similar staining in SS and controls.

Conclusions: Healthy tubuloacinar epithelial cells contain complete intracrine machineries for DHEA processing. In healthy acini these enzymes have an organized architecture that corresponds with DHEA uptake from the circulation, the nuclear site of production of the active dihydrotestosterone (DHT) end product, and the production of oestrogens in saliva for export to ductal and oral epithelial cells. SS is characterized by low steroid sulfatase, which, together with impaired subcellular compartmentalization of HSDs and 5 α -reductase, may explain the low local DHT and androgen biomarker levels in SS.

TOLL-LIKE RECEPTORS IN THE SEPTIC LOOSENING OF TOTAL HIP ARTHROPLASTIES

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Objectives: Toll-like receptors (TLRs) recognize both endogenous necrotic cell-derived alarmins and exogenous microbe-derived pathogen-associate molecular patterns. These danger signals are crucial mediators in the activation of both innate and adaptive immunity. It was speculated that they might play a role in aseptic and septic loosening of totally replaced hip prostheses.

Methods: Bacterial culture-negative, aseptic (n = 5) and culture-positive, septic (n = 10) synovial-membrane-like interface tissues were compared to osteoarthritis synovial membrane (n = 5) for the presence of various inflammatory cells and two key TLRs, TLR4 and TLR9, by immunohistochemical staining using specific antibodies.

Results: Both TLR4 and TLR9 were expressed in aseptic and septic tissue samples and their expression was greatly increased when compared to osteoarthritis. Of note, septic cases also contained neutrophil and lymphocyte infiltrates in addition to monocyte/macrophage infiltrates, which dominated in foreign body synovitis in aseptic loosening. By contrast, in osteoarthritis, TLR-positive cells were mainly found in the vascular endothelium and synovial lining.

Conclusions: In aseptic cases a straightforward wear debris–macrophage interaction predominates, possibly related to a local release of intracellular alarmins upon cell death and tissue necrosis. In septic cases TLR4- and TLR9-positive neutrophils and lymphocytes are recruited and engaged, probably by pathogen-associated molecular patterns and antigens, respectively.

Clinical relevance: Biopsy samples may indicate implant-related infections even in the absence of neutrophils, when the interface membrane demonstrates accumulation of TLR immunoreactive cells and lymphocytes.

HIGH MOBILITY GROUP BOX-1 (HMGB-1) IN OSTEOARTHRITIC CARTILAGE

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Objective: Nucleosomal high mobility group box-1 (HMGB-1) is released from necrotic and activated cells as an endogenous danger signal (alarmin) and cytokine. It was hypothesized to play a role in osteoarthritis (OA) characterized by cellular activation, inflammation, and enchondral bone formation.

Methods: Bovine knee joint samples, collected from culled animals, were graded using histological/histochemical grading as mild (n = 7), moderate (n = 7), or severe (n = 7), and immunohistochemically stained for HMGB-1.

Results: In healthy-looking cartilage > 90% of chondrocyte nuclei were HMGB-1 negative, but in mild OA lesions HMGB-1 was seen in nuclei, in moderate lesions in the cytoplasm and often in the pericellular matrix, and in severe lesions often also in the intra- and interterritorial matrix. Linear deposits were seen at the tidemark in healthy areas and mild lesions, multiple wave-like deposits in moderate lesions, and heavy granular deposits in severe lesions.

Conclusions: In resting chondrocytes, tight nucleosomal HMGB-1 binding apparently causes steric hindrance of immunostaining. Advancing OA leads to nuclear, then cytoplasmic, and finally increasingly intense extracellular deposition of HMGB-1 alarmin, indicating local chondrocyte activation and/or necrosis. In particular, HMGB-1 at the tidemark might play a role in the normal remodelling and pathological thickening of subchondral bone plate and osteophyte formation.