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Abstracts der Posterpräsentationen

A. Klinische Studien

Excellent Response of Lupus Hepatitis to Mycophenolate Mofetil: A Case Report 01

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Aim To show the efficacy of mycophenolate mofetil in lupus hepatitis.

Methods Single case report.

Results We report on a 63-year-old male patient with systemic lupus erythematodes (SLE), primarily treated for cutaneous manifestation 11 years ago. He received local corticosteroids and following systemic immunosuppressants: methylprednisolone (MP), chloroquine (removed due to retinal haemorrhage), cyclosporine A, and azathioprine (both removed due to non-response). In 2008, the patient developed polyarthralgia, and methotrexate as well as golimumab were started. His cutaneous and articular manifestations responded well, but both drugs had to be stopped due to marked increase in liver enzymes (with the most pronounced increase of GGT up to max. 42-fold). The liver biopsy showed hepatitis that was interpreted as lupus hepatitis, since virus serological tests were negative. Neither anti-DNA-antibodies nor complement activation were detected. At the beginning of 2013, the patient was referred to our department with above-mentioned findings. A combination treatment with 1 g mycophenolate mofetil (MMF) per day and 10 mg/kg belimumab at weeks 0, 2, 4, followed by every 4 weeks (due to extensive cutaneous manifestation on the whole body), was introduced. Additionally, methylprednisolone was administered at a dose of 10–20 mg per day (there has been no interruption in methylprednisolone therapy since 2003). A marked decrease in liver enzymes could be detected, reaching normal values for transaminases and a GGT-level of only 3-fold above the upper normal value within 7 months after onset of the combination therapy with MMF and belimumab. Due to insufficient response of lupus exanthema, hydroxychloroquine (HCQ) at a dose of 200 mg per day was added. This led to a better control of the polyarthralgia, which made it possible to reduce the dose of MP and discard NSAIDs. Parallel to this combination treatment consisting of 4 immunosuppressants, a pneumocystis jirovecii prophylaxis with cotrimoxazole 3 times a week was introduced. After 9 months of belimumab administration, the biologic therapy was switched to rituximab in order to achieve a better response of cutaneous manifestation. At this time point, MMF was discarded and the dose of HCQ was increased to 400 mg per day. Rituximab was administered at a dose of 1000 mg i.v. at weeks 0 and 2. Surprisingly, after the first infusion a rapid and marked improvement of lupus exanthema was noticed. Such an improvement has not been experienced since 2003.

Summary/Conclusion Mycophenolate mofetil shows high efficacy in lupus hepatitis and thus may be a drug of choice in liver manifestation of SLE.

Therapie von Osteoporose-Patienten auf einer Akutgeriatrie – von der Aufnahme zur Entlassung 02

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Ziel Wie vielfach berichtet, lässt die Versorgung von Osteoporosepatienten mit entsprechenden Therapien sowohl in Bezug auf die Basistherapie als auch auf die spezifische Therapie im Allgemeinen sehr zu wünschen übrig. Selbst nach unfallchirurgischer Versorgung kann nicht mit Sicherheit damit gerechnet werden, dass die Patienten eine adäquate Therapie erhalten. Ziel der Untersuchung war, den Anteil der mit osteoporotischer Basis- bzw. Spezialtherapie versorgten Patienten auf einer akutgeriatrischen Abteilung zu den Zeitpunkten der Aufnahme bzw. der Entlassung zu erfassen.

Methoden Untersucht wurde über einen Zeitraum von knapp 3 Jahren eine konsekutiv erfasste Patientenpopulation auf einer akutgeriatrischen Abteilung hinsichtlich einer anti-osteoporotischen Therapie zu den Zeitpunkten der Aufnahme bzw. der Entlassung nach durchschnittlich 3 Wochen. In dieser retrospektiven Datenanalyse wurden sowohl die Gabe von Vitamin D und Kalzium als Basistherapie der Osteoporose als auch die Gabe einer spezifischen Therapie im Sinne von oralen bzw. parenteralen Bisphosphonaten erfasst.

Ergebnisse Insgesamt handelt es sich um Daten von 679 Patienten. Bei 497 Patienten (73,2 % des Kollektivs) lag eine Osteoporose vor. Davon hatten 25 % bereits eine Schenkelhalsfraktur erlitten, 16 % eine Wirbelkörperfraktur. Durchgeführte Röntgenaufnahmen im Rahmen der Erstuntersuchung zeigten Wirbelkörperdeformationen bei 39 %. Zum Zeitpunkt der Aufnahme betrug der Anteil aller mit Vitamin D versorgten Patienten lediglich 30 %. 92,7 % aller Patienten wiesen einen inadäquaten Vitamin-D-Spiegel (< 75 mmol/l, als Grenzwert laut ÖGKM-Leitlinien 2012) auf, bei 71,2 % lag ein Vitamin-D-Defizit (< 50 mmol/l als Grenzwert des Entwurfs der DVO-Leitlinien 2014) vor. Von den Patienten mit diagnostizierter Osteoporose erhielten 37 % zum Zeitpunkt der Aufnahme Vitamin D in nicht berichteter Dosis. Bis zum Zeitpunkt der Entlassung konnte dieser Prozentsatz auf 91 % gesteigert werden. Im Gesamtkollektiv konnten wir eine Zunahme von 30 % auf 91 % erreichen. Es darf dabei nicht vergessen werden, dass Vitamin D nicht nur bei der eindeutigen Diagnose einer Osteoporose mit der direkten Wirkung am Knochen eine tragende Rolle spielt, da Vitamin D nicht nur die Kalziumaufnahme fördert, sondern ebenfalls die Muskelkoordination verbessert, wodurch unter anderem die Sturzneigung und somit eventuell nachfolgende Frakturen vermindert werden könnten. Aus diesem Grund sollte generell bei alten, multimorbiden Patienten eine Vitamin-D-Gabe angedacht werden, auch wenn keine Osteoporose vorliegt. Ebenfalls gesteigert werden konnte die parallele Gabe von Kalzium. Waren es zum Zeitpunkt der Aufnahme lediglich 30 % der Patienten, die mit einem Kalziumpräparat versorgt waren, konnte dies zum Zeitpunkt der Entlassung auf 70 % gesteigert werden. Bezüglich der spezifischen Osteoporosetherapie wurde Hauptaugenmerk auf die Bisphosphonate gelegt, da der Anteil an anderen spezifischen Therapeutika eher gering ausfiel. Hier-

bei kam es bei Patienten mit einer Osteoporose-Diagnose zu einem Anstieg von 25 % bei Aufnahme auf 59 % bei Entlassung.

Zusammenfassung/Schlussfolgerung Zusammenfassend ist zu sagen, dass die Versorgung mit einer Osteoporosetherapie bei einem durchschnittlichen Patientengut vor der stationären Aufnahme auf einer Spezialabteilung eher zu wünschen übrig ließ, sowohl bei Patienten mit bereits im Vorfeld bekannter Osteoporose als auch bei denen, die nach einer rezenten Fraktur im Rahmen einer neu diagnostizierten Osteoporose zur Remobilisation an unsere Abteilung kamen. Dabei ist zu erwähnen, dass eine adäquate Therapie von entscheidender Bedeutung ist, um in weiterer Folge Stürze mit eventuellen Frakturen langfristig zu verhindern.

Osteoporose und Komorbiditäten innerhalb eines akutgeriatrischen Patientenkollektivs 03

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Ziel Erfassung des Anteils an Patienten mit Osteoporose sowie der neben einer möglichen Osteoporose vorliegenden Begleiterkrankungen an einem repräsentativen Patientenkollektiv an einer akutgeriatrischen Abteilung mit den Schwerpunkten Rheumatologie und Osteologie. Evaluierung allfälliger Korrelationen im Sinne zuordenbarer Risikofaktoren.

Methoden Im Rahmen einer Querschnittstudie wurden konsekutiv erfasste Patientendaten retrospektiv ausgewertet. Im Rahmen der Aufnahme an die Abteilung wurden anamnestisch erhobene Daten und Ergebnisse von Untersuchungen entsprechenden Diagnosen zugeordnet. Die Daten wurden prinzipiell von allen Patienten registriert, ausgeschlossen waren lediglich diejenigen mit seniler Demenz sowie mit einer Tumorerkrankung.

Ergebnisse Insgesamt standen die Daten von 679 Patienten zur Verfügung, davon 172 Männer (25,3 %) sowie 507 Frauen (74,7 %) mit einem durchschnittlichen Alter von 78 Jahren, wobei die Männer im Mittelwert jünger waren als die Frauen, dafür mit einem durchschnittlich höheren BMI (Mittelwert 25). Osteoporose konnte bei insgesamt 497 Patienten (73,2 %) nachgewiesen werden, wobei es sich sowohl um anamnestisch oder klinisch vorbestehende als auch um erstmals diagnostizierte Fälle handelte. Bei 202 Patienten (29,7 %) lag eine rezente osteoporotische Fraktur als Aufnahmegrund vor. Zu erwähnen wäre, dass lediglich 66 % der Patienten mit vorbestehender Osteoporose bei Aufnahme auch über ihre Diagnose Bescheid wussten. Die Analyse der erfassten Begleiterkrankungen ergab im Gesamtkollektiv ein weitgehend gleichmäßig abgestuftes Verteilungsmuster. Lediglich die arterielle Hypertonie zeigte mit einem Auftreten in 72,6 % (493 Patienten) einen Spitzenwert, während sich die nachfolgenden Erkrankungen in ca. 20 % zeigten: Diabetes mellitus war bei 22,5 % (153 Patienten) nachweisbar, eine KHK hatten 23,6 % (160 Patienten). An einer Depression litten 22,2 % (150 Patienten). Im Bereich von 10 % lagen die Häufigkeit von pAVK sowie eines stattgehabten Insults. Dieses Verteilungsmuster zeigte sich sowohl bei Patienten mit als auch ohne Osteoporose. Auch die Anteile an Patienten mit vorangegangener oder bestehender Kortisontherapie, rheumatoider Arthritis oder positiver Familienanamnese in Bezug auf Frakturen lagen annähernd gleich häufig in diesen beiden Gruppen vor.

Zusammenfassung/Schlussfolgerung Zur Bewertung des Frakturrisikos bei Patienten mit Osteoporose oder Osteopenie werden verschiedene Parameter und Begleiterkrankungen herangezogen. Einen dominanten Faktor stellt das Alter der Patienten dar. Mit fortschreitendem Alter und wachsender Multimorbidität scheinen weitere Risikofaktoren an Stellenwert zu verlieren. Die wichtigsten Begleiterkrankungen wie arterielle Hypertonie, Diabetes mellitus, pAVK oder Depression finden sich in unserem Kollektiv in ähnlicher Verteilung sowohl bei Patienten mit Osteoporose als auch bei jenen ohne entsprechende Diagnose. Es konnten keine zuordenbaren Korrelationen mit dem Auftreten einer Osteoporose detektiert werden.

Behandlung rheumatoider Arthritis mit Baricitinib, einem oralen Januskinase-Inhibitor: Wirksamkeit und Sicherheit aus einer offenen Langzeit-Verlängerungsstudie [1] 04

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Ziel Baricitinib (ehemals LY3009104/INCB028050) ist ein neuartiger oraler JAK1/JAK2-Inhibitor des JAK-STAT-Signalwegs zur Behandlung der rheumatoiden Arthritis (RA). Ziel ist es, die Wirksamkeits- und Sicherheitsergebnisse nach 52 Wochen einer offenen Verlängerungsstudie der Phase IIb zu berichten.

Methoden Patienten wurden initial für den Erhalt von Placebo (PBO) oder 1 von 4 Tagesdosen (QD) Baricitinib (1, 2, 4 oder 8 mg) für 12 Wochen randomisiert. Patienten, die 2 mg, 4 mg oder 8 mg erhielten, behielten die Behandlung bei, während die mit PBO oder 1 mg behandelten Patienten für weitere 12 Wochen verblindeter Behandlung mit 4 mg QD oder 2 mg BID neu eingeteilt wurden. Im offenen Teil der Studie erhielten Patienten der 8-mg-Gruppe weiterhin 8 mg QD und alle anderen 4 mg QD. Die Dosierungen konnten bis 8 mg QD in Woche 28 oder 32 erhöht werden, je nach Ermessen des Prüferarztes, wenn > 6 schmerzempfindliche und geschwollene Gelenke vorhanden waren. Die hier vorgestellte Auswertung beinhaltet Daten bis Woche 52 für Patienten, die in der offenen Verlängerung behandelt wurden (Patienten mit vorzeitiger Beendigung wurden als Non-Responder bewertet).

Ergebnisse Von 212 teilnahmeberechtigten Patienten traten 201 (95 %) Patienten in die offene Verlängerungsstudie ein, 184 schlossen 52 Behandlungswochen ab, 15 verließen die Studie und 2 Patienten hatten die 52 Wochen noch nicht beendet. Bei Patienten, die 4 mg erhalten hatten (n = 108), gab es 57 (53 %) TEAE (behandlungsassoziierte unerwünschte Ereignisse), 11 (10 %) SAE (schwerwiegende unerwünschte Ereignisse), 34 (31 %) Infektionen und 4 (4 %) schwerwiegende Infektionen. Bei Patienten, die zu irgendeinem Zeitpunkt 8 mg erhalten hatten (n = 93), gab es 59 (63 %) TEAE, 8 (9 %) SAE, 37 (40 %) Infektionen und 2 (2 %) schwerwiegende Infektionen. Es wurden keine opportunistischen Infektionen oder Fälle von TB beobachtet. Es gab einen herzinfarktbedingten Todesfall in der 8-mg-Gruppe. Von allen zusammengenommenen Patienten der offenen Verlängerung war der Anteil der Patienten, die ACR20, ACR50, ACR70, CDAI-Remission, SDAI-Remission, DAS28CRP ≤ 3,2, DAS28CRP < 2,6, DAS28ESR ≤ 3,2, DAS28CRP < 2,6 oder ACR/EULAR-Boolean-Remission zu Beginn der offenen Verlängerung (Woche 24) erreichten, in Woche 52 ähnlich oder höher.

Schlussfolgerungen Bei Patienten, die 52 Wochen der Phase-IIb-Studie beendet hatten, wurden die in Woche 24 beobachteten klinischen Verbesserungen während der offenen Verlängerung beibehalten oder gesteigert. Die während der offenen Verlängerung beobachteten Sicherheitsmerkmale stimmten mit den bereits berichteten Ergebnissen von Baricitinib überein.

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Patientin mit adulter Form des Morbus Still und sekundärem Makrophagenaktivierungssyndrom – ein Fallbericht 05

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Einleitung Das Makrophagenaktivierungssyndrom (MAS) ist eine bekannte und potenziell lebensbedrohliche Komplikation der adulten Form des Morbus Still. Es resultiert aus einer exzessiven Aktivierung von Makrophagen mit Ausschüttung von Zytokinen. Typisch sind das Auftreten von Fieber, Panzytopenie, Hyperferritinämie, neurologische Symptome, Transaminasenerhöhung, Hepatosplenomegalie und Lymphadenopathie. Die Diagnose wird durch den Nachweis einer Hämophagozytose im Knochenmarkaspirat gestellt. Derzeit gibt es noch keine etablierte Therapie des MAS bei der adulten Form des Morbus Still. Bei systemischer juveniler idiopathischer Arthritis erfolgt die Behandlung des MAS meist mit Cyclosporin A und Glukokortikoiden.

Fallbericht Die 64-jährige Patientin berichtete über rezidivierende Gelenksschmerzen seit Herbst 2012. Im März 2013 stellte sich die Patientin mit intermittierenden Fieberschüben bis 38,4 °C (unter NSAR-Therapie), Leukozytose, erhöhten Transaminasen, LDH-Erhöhung, Hyperferritinämie (6200 µg/l) sowie negativem Rheumafaktor und negativen ANA vor. Zu diesem Zeitpunkt wurde die Diagnose einer adulten Form des Morbus Still gestellt und mit Kortison und ab Mai 2013 mit Anakinra 100 mg 1× tägl. s.c. therapiert. Die Gabe von Ebetrexat war zu diesem Zeitpunkt aufgrund von Transaminasenerhöhung kontraindiziert. Unter dieser Therapie war die Patientin oligosymptomatisch, eine Prednisolonreduktion unter 12,5 mg/Tag war jedoch mit Wiederauftreten von Krankheitssymptomen verbunden. Im November 2013 wurde aufgrund eines fieberhaften bronchopulmonalen Infekts Anakinra pausiert, worunter es zu einer schwersten Neutropenie (absolute Neutrophile 0,1 G/l) und einem deutlichen Anstieg von Ferritin (16.200 µg/l) kam. Es wurde damals schon neben einer Medikamentennebenwirkung (Metamizol) bzw. infektgetriggerten Neutropenie differenzialdiagnostisch an ein MAS gedacht. Nach G-CSF-Gabe (Ratiograstim 48 IE s.c. für 3 Tage), Breitbandantibiose und antimykotischer Therapie sowie Kortisonsteigerung (Urbason 48 mg/Tag) kam es zur Normalisierung der Leukozyten am 4. Tag. Aufgrund eines Ulcus recti sowie mehrerer rezidivierend inflammierter kutaner Lipoidnekrosen wurde eine Monotherapie mit Glukokortikoiden gegeben und mit der Wiedereinleitung einer immunsuppressiven Therapie zugewartet. Im Februar 2014 erfolgte schließlich wiederum die stationäre Aufnahme mit Fieber > 39 °C und exorbitant hohem Ferritin (48.700 µg/ml). Trotz Steigerung der Kortisondosis (Urbason 48 mg i.v. tägl.) kam es jedoch zu einem Abfall der Leukozyten (6,9 G/l), des Hämoglobins (6,9 G/l) und der Thrombozyten (107 G/l) und zu einem deutlichen Anstieg der LDH (> 1000 U/l). Der Verdacht auf ein MAS wurde mittels Knochenmarksbiopsie bestätigt. Nachdem die Patientin zu diesem Zeitpunkt auch ein akutes Nierenversagen (Kreatinin 4,9 mg/dl) hatte, wurde zusätzlich kein Cyclosporin A verabreicht. Insgesamt wurde 14 Tage Urbason 48 mg i.v. tägl. (aufgeteilt auf 2 Tagesdosen) verabreicht. Unter dieser Therapie kam es zum raschen Rückgang von Ferritin auf 1120 µg/ml und zur Normalisierung des Blutbildes. Es wurde im Anschluss daran im März 2014 eine Therapie mit Tocilizumab begonnen. Wegen der rezidivierenden Infekte und um das Risiko einer möglichen Zytopenie zu minimieren, wurde Tocilizumab in reduzierter Dosierung (4 mg/kg KG) alle 2 Wochen verabreicht. Aufgrund von zunehmender Krankheitsaktivität und einer Kortisondosis über der Cushing-Schwelle wurde im Mai 2014 zusätzlich eine Basistherapie mit Ebetrexat 15 mg 1×/Woche begonnen. Bei laufender Kombinationstherapie war eine Reduktion von Kortison auf 7,5 mg tägl. möglich. Unter dieser Therapie bestanden keine Krankheitssymptome, es kam aber zum neuerlichen Auftreten größerer Hautabszesse, was eine Pausierung von Ebetrexat und Tocilizumab notwendig machte.

Schlussfolgerung Das MAS ist eine seltene, aber schwerwiegende und potenziell lebensbedrohliche Komplikation bei adulter Form des Morbus Still und eine Herausforderung in der Diagnostik und Therapie. Derzeit gibt es keine etablierte Therapie bei MAS. Die Nebenwirkungen von Tocilizumab (Zytopenie, erhöhte Leberwerte) sind ähnlich wie die Laborveränderungen bei MAS. Ferritin korrelierte bei dieser Patientin als Laborparameter gut mit dem Therapieansprechen und kann zur Differenzierung helfen. Mit diesem Fallbericht konnte gezeigt werden, dass der Interleukin-6-Rezeptor-Antikörper Tocilizumab eine Therapieoption bei einem MAS sein kann.

Prevalence of Anaemia in a Cohort of Rheumatoid Arthritis Patients – an Interim Analysis 06

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Purpose According to older literature, the prevalence of anaemia in RA patients is between 30 and 66 %. Our purpose was to evaluate the actual prevalence of anaemia in outpatients with rheumatoid arthritis (RA) at a tertiary centre, to characterize the type of anaemia, and furthermore to study the association between anaemia, course of disease, and specific therapies.

Methods We retrospectively analysed RA outpatients from a study cohort which was designed to evaluate iron metabolism in chronic inflammation. As far as available, blood count, iron parameters (iron, ferritin, transferrin, and transferrin saturation), renal function, vitamin status, disease activity (CDAI, DAS28), and drug therapy were collected at the time of initial diagnosis and at a routine follow-up. Wilcoxon or Mann-Whitney-U test was performed to compare anaemic patients with those with normal haemoglobin levels (anaemia was defined as haemoglobin < 120 g/l in women and < 130 g/l in men). Spearman-Rank Analysis was applied to analyse correlations with haemoglobin levels, iron parameters, disease activity, and medical treatment.

Results RA patients, aged between 44 and 83 years, with mean disease duration of 17.4 years, were analysed so far. Data of patients at initial diagnosis were only available after 2000. Prevalence of anaemia at initial diagnosis was 25 % and declined upon treatment and during follow-up in 2014 to 18.6 %. Anaemia was significantly linked to more advanced inflammation, as reflected by a significant correlation between ESR, CRP, and haemoglobin levels at these time points. The risk for persisting anaemic at follow-up was highest if patients were anaemic at initial diagnosis of RA. DMARDs (disease-modifying anti-rheumatic drugs) were not inferior to biological treatment regarding the prevalence of anaemia.

Conclusion The prevalence of anaemia in RA patients appears nowadays to be significantly lower than previously described. This may be due to an earlier diagnosis of RA along with an overall better disease control with modern therapy strategies. DMARDs are, in regard to the prevalence of anaemia, not inferior to biologicals. This is an interims report of a preselected study cohort. A registry to systemically study and characterize anaemia in Austria along with the evaluation of the impact of anaemia on the course of RA has been already started to avoid a possible bias. We have also the aim to encourage other centres to participate in our study.

Frequency of Various Symptoms in Patients with Rheumatoid Arthritis and Their Impact on Quality of Life 07

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Aim The treatment of rheumatoid arthritis (RA) aims at clinical remission or low disease activity by the means of early diagnosis, aggressive treatment, and frequent monitoring. The updated EULAR and DGRh guidelines recommend individual assessment of health-related quality of life (QoL) in patients with RA.

Methods A total of 120 community-dwelling out-patients with RA, aged 22–83 years, 82.5 % female, who were drug-treated at least

for 3 months, were included in the study. Various symptoms were recorded with means of the German version of the revised Illness Perception Questionnaire (IPQ-R). For each symptom, patients were asked if the symptom was related to their illness. QoL was measured with the Short Form (36) Health Survey (SF-36) questionnaire. For this analysis, means for the summary scores for physical and mental QoL were used and adjusted for age, sex, family situation, level of education, and presence of other diseases.

Results The most common reported symptoms related to rheumatoid arthritis were pain (97.5 %) and stiff joints (95.8 %), followed by fatigue (60.8 %), loss of strength (59.2 %), exhaustion (49.2 %), and sleep difficulties (35.8 %). All symptoms were associated with lower estimates in the summary scores in physical and mental quality of life. Regarding physical QoL, the highest differences were found in patients with or without pain (38.1 vs 49.4; P = 0.048) and with or without breathlessness (29.9 vs 39.3; P = 0.001). Regarding mental QoL, the highest differences were found in patients with or without nausea (38.0 vs 48.7; P < 0.001), with and without breathlessness (38.8 vs 47.9; P = 0.012), and with or without dizziness (39.0 vs 48.0; P = 0.013).

Summary/Conclusion Besides the typical RA-associated symptoms pain and stiff joints, symptoms related to muscle mass or muscle function are very common in RA patients. Symptoms not usually attributed to RA are associated with deteriorated QoL in the same or even higher amount as typical RA symptoms. This can either mean that RA-typical symptoms are quite well controlled or that more attention should be drawn to non-typical symptoms in RA patients, however, further research is required.

Discrepancy Between Patients' and Evaluator Global Assessment of Disease Activity in Psoriatic Arthritis 08

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Background/Purpose Psoriatic arthritis (PsA) is a chronic inflammatory disease characterized by musculoskeletal symptoms such as arthritis, enthesitis, spondylitis, and/or dactylitis as well as psoriatic skin and/or nail manifestations. Due to the heterogeneous nature of the disease, the assessment of disease activity may be challenging. The purpose of this study was the analysis of factors explaining the discrepancy between patients' (PGA) and evaluator global assessment (EGA) of disease activity.

Methods We performed a prospective study on 83 consecutive PsA patients with study visits at baseline and after 6 months. All patients fulfilled the ClASsification for Psoriatic ARthritis criteria and had peripheral articular manifestations. The patients underwent the following assessments: physical examination including tender and swollen joint count, skin and nail involvement, dactylitis score, and the Leeds Enthesitis Score. Blood samples were routinely tested for erythrocyte sedimentation rate (ESR, range 0–10 mm/first hour) and C-reactive protein levels (CRP, range 0–5 mg/L). The Psoriasis Area Severity Index (PASI), Dermatology Life Quality Index (DLQI), Health Assessment Questionnaire (HAQ), Bath Ankylosing Spondylitis Disease Activity Index, Ankylosing Spondylitis Quality of Life, PGA, patients' pain assessment, as well as EGA determined on Visual Analogue Scales (VAS; 0–100 mm) were recorded.

Results At visit 1 females had higher EGA values than males (30 mm vs 19 mm; P < 0.001), whereas no difference was found regarding PGA (31 vs 39 mm, n.s.). According to multivariate regression, half of the variability of PGA results could be explained by pain VAS (30.5 %), swollen joints (15 %), and tender joints (6.5 %). Besides, the model revealed pain VAS (B-coeff = 0.534; P < 0.001) and HAQ (B-coeff = 6.266; P < 0.05) as significant predictors of PGA. The multivariate analysis displayed that nearly half of the variability of EGA results could be explained by swollen joint count (48.5 %). The model displayed that swollen joint count (B-coeff = 3.098; P < 0.001) is a significant predictor of EGA. Disease activity was differently evaluated by patients and physicians in 65 % of cases: in 53 % (n = 44) of patients PGA scores were higher than EGA and vice versa, 12 % (n = 10) of cases had higher EGA scores. Correlating the difference between PGA and EGA (ΔPGA-EGA) with other clinical factors, we observed a moderate association with pain VAS (r = 0.433; P < 0.01) and swollen joint count (r = 0.235; P < 0.05).

Conclusion In the present study we identified pain and the HAQ score as the most important determinants of PGA, whereas swollen joint was the most important predictor of EGA. Patients tended to score their disease activity higher than physicians and the difference between PGA and EGA was linked with pain and swollen joints.

Adherence with Medication in Patients with Rheumatoid Arthritis and Association of Socioeconomic Variables with Adherence 09

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Introduction Rheumatoid arthritis (RA) is a chronic disease which, despite the progress made in recent years, still requires regular intake of medication by the patients. Medication adherence is, therefore, an important factor in disease management, but dependent on patient reliability.

Methods 120 consecutive patients with RA were examined in a hospital-based outpatient clinic specialised on rheumatology. Age ranged between 22 and 83 years, 82.5 % were female. For the assessment of adherence the Medication Adherence Report Scale (MARS) was used, which contains 5 questions regarding forgetting, changing dosage, pausing, skipping, or reducing intake of prescribed medication. Each item was valued by the patients in the categories "always", "often", "sometimes", "seldom", and "never". For this analysis the items were dichotomised, and patients who ticked of "never" in all items were classified as adherent, and all other patients were classified as non-adherent. Cross tabs with various socio-economic and socio-demographic variables were undertaken and the Chi² Test was applied.

Results 52.5 % of the patients were classified as adherent. The proportion of adherent patients was significantly higher in women (57.6 % vs 28.6 %; P = 0.016) and in subjects > 55 years (43.3 % vs 64.2 %; P = 0.023). The proportion of adherence in patients with primary, secondary, and tertiary education was 52.0 %, 51.2 %, and 66.7 %, respectively (P = 0.674). Patients with an income of less or more than 2000 Euro per month showed an adherence proportion of 57.1 % and 37.9 %, respectively (P = 0.071). 42.9 % of currently gainfully employed and 59.2 % of currently not gainfully employed patients were adherent (P = 0.079).

Conclusions There is room for improvement of adherence with prescribed medications in RA patients. A striking difference exists between men and women, with less than one third of men being classified as adherent. In addition, age also plays an important role as older patients are significantly more adherent than younger ones. Additionally, there are hints for socio-economic gradients in drug adherence with an increase of adherence in higher education. Consideration of these variations in future patient management is advised.

Table 1: Studenic P, et al. Summary of the multivariate logistic regression model for the outcome PGA ≤ 1 cm.

		Regression coefficient	Standard error	p	95-% confidence interval for odds ratio (OR)		
					Lower	OR	Upper
MTX	Baseline HAQ	–0.77	0.36	0.034	0.23	0.46	0.94
	Baseline pain	–0.02	0.01	0.026	0.96	0.98	1.00
	Constant	0.18	0.35	0.611		1.19	
TNFi	Baseline pain	–0.03	0.01	0.022	0.95	0.98	0.99
	Baseline HAQ	–0.16	0.41	0.004	0.14	0.31	0.70
	Constant	0.87	0.41	0.036		2.39	

Low HAQ and Pain Predict Patient-Perceived Remission in Rheumatoid Arthritis Patients Receiving MTX or Anti-TNF-Alpha Treatment 10

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Aim The induction of remission is the primary target of RA therapy. Failing to achieve the patient global estimate of disease activity criterion (PGA ≤ 1 cm) has been shown to be the primary cause hindering patients to be classified as being in remission. Here, we aimed to determine factors that predict achieving the PGA criterion of remission in RA patients in usual clinical care.

Methods We selected patients from a longitudinal RA database, who started MTX monotherapy or TNFi treatment in combination with MTX or leflunomide and had at least 6 months of follow-up. In univariate analysis, we tested core-set variables to identify candidate predictors of patient-perceived remission (PGA ≤ 1 cm), which we then subjected to multivariate logistic regression analysis for the outcome: PGA ≤ 1 cm.

Results Data of 172 patients receiving MTX (82 % female, 65 % rheumatoid factor positive, mean SDAI: 18.5 ± 12.3) and of 112 patients on TNFi (82 % female, 62 % rheumatoid factor positive, mean SDAI: 19.7 ± 13.3) were used for analysis. After 6 month of treatment, 70 % of those receiving MTX and 55 % of the TNFi group were in a state of remission or low disease activity, based on the SDAI. 47 % of MTX and 34 % of TNFi patients evaluated their PGA as being ≤ 1 cm. 74 % of MTX and 58 % of TNFi-treated patients who had PGA ≤ 1 cm had also an evaluator global assessment of ≤ 1 cm. The overlap of patient-perceived remission and remission by SDAI was 49 % in the MTX group and 46 % in the TNFi group. Univariate analyses in MTX-treated patients showed an association of pain, HAQ scores, and TJC with the outcome PGA ≤ 1 cm. In the multivariate logistic regression model, the odds ratio (OR) was 0.46 for baseline HAQ **(Table 1)** and 0.98 for pain as predictors for achieving remission after 6 months of treatment. Higher HAQ and pain scores therefore lead to a lower odds for achieving PGA ≤ 1 cm. Patients with an improvement in HAQ within the first 3 months have a 3.5 (CI: 1–13) times higher odds to achieve PGA ≤ 1 cm after 6 months of treatment than patients who report a worsening in HAQ, which corresponds to a probability of 23 % versus 8 % to achieve PGA ≤ 1 cm. In patients receiving TNFi therapy, baseline HAQ (OR: 0.31) and pain (OR: 0.98) were shown to be predictive, i.e. a patient with a baseline HAQ of 1.25 or a pain score of 50 mm has a 20 % probability of achieving PGA remission, whereas a baseline HAQ of 0.5 or a pain score of 20 mm coincides with a 40 % probability.

Summary/Conclusion We demonstrated here that patients with poor function or high pain levels are likely to fail patient-reported remission, as shown here for the patient global variable, which is part of the established remission criteria. These findings were independent of the treatment regimen. Improvement in function enhances the chances for achieving patient-perceived remission after 6 months of DMARD treatment.

Resochin-induzierte Veränderungen bei einer Patientin mit Lupusnephritis … oder doch ein Morbus Fabry? 11

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Ziel Chloroquin und Hydroxychloroquin finden in der Rheumatologie seit Langem eine verbreitete Verwendung, doch mögliche potenziell schwere und unerwünschte Nebenwirkungen erfordern eine sorgfältige Überwachung.

Fallbericht Es handelt sich um eine 47-jährige schwarzafrikanische Patientin, bei der seit 2002 ein SLE mit initial normalen Nierenwerten bekannt ist. Es bestand seit dieser Zeit über Jahre hinweg eine Therapie mit Resochin. Zusätzlich klagte die Patientin über Doppelbilder sowie eine Visusverschlechterung im Rahmen eines Ermüdungssymptoms, welches als okuläre Symptomatik einer Myasthenia gravis ohne weitere Progredienz zu werten ist. Über Jahre hinweg erhielt die Patientin diesbezüglich eine Therapie mit einem Cholinesterase-Hemmer (Mestinon). Im Sommer 2013 kam es zu einem Anstieg der Nierenretentionsparameter sowie einer Proteinurie von > 0,5 g/d, weshalb auch eine Nierenbiopsie durchgeführt wurde. Histologisch zeigten sich Veränderungen im Sinne einer Lupusnephritis Klasse III. Als Nebenbefund fand man jedoch auch podozytäre Veränderungen mit einer deutlichen Verbreiterung der Zytoplasmen mit feinvakuoliger Veränderung, dies war fokal auch in den Tubuli erkennbar. Ein solches Bild sieht man typischerweise bei Speichererkankungen wie zum Beispiel dem Morbus Fabry, als Differenzialdiagnose stand auch eine Chloroquin-induzierte Lipoidose im Raum. Wegen Verdachts auf Morbus Fabry wurden diesbezüglich weitere Untersuchungen eingeleitet: Die Alpha-Galaktosidase A war im Normbereich, Globotriasylceramid im 24-h-Harn war nicht nachweisbar. Die genetische Untersuchung ergab keine Fabry-typische Mutation im GLA-Gen. Größere Deletionen oder Insertionen wurden mittels MLPA ausgeschlossen. Zusammenfassend konnte somit die Verdachtsdiagnose Morbus Fabry ausgeschlossen werden. Insgesamt wurden die oben beschriebenen morphologischen Veränderungen auf die im Rahmen sehr selten zu findenden, medikamentös-toxischen Resochin-Ablagerungen zurückgeführt. Weiters kam es bei fraglich regelmäßigen augenärztlichen Kontrollen zu einer zentralen Makulopathie. Nach Absetzen des Resochins und Therapieumstellung besserte sich neben der Proteinurie auch die okuläre Myopathie, sodass auch die Mestinontherapie beendet werden konnte. Bereits niedrige Dosierungen an Resochin können neben Störungen der neuromuskulären Übertragung auch zu Neuro- oder Myopathien führen.

Ergebnisse Zusammenfassend zeigt die Patientin nicht nur die uns eher bekannten Resochin-Nebenwirkungen an den Augen, sondern auch sowohl eine okuläre Myopathie als auch eine seltene Form der renalen Mitbeteiligung im Sinne einer Chloroquin-induzierten Lipoidose. Dieser Fallbericht zeigt, dass eine Reevaluierung der Therapie mit Resochin in regelmäßigen Abständen wichtig ist, da Nebenwirkungen bereits nach kürzerer Zeit und dosisunabhängig auftreten können.

Table 2: Nell-Duxneuner V, et al. The number of patients still on the prescribed drug (drug survival) after 1, 2, and 3 years is demonstrated. Abatacept showed the highest drug survival, followed by tocilizumab, adalimumab, golimumab, and etanercept. Three-year drug survival was again highest for abatacept, followed by etanercept and adalimumab.

Number of first prescriptions				Drug survival								
				1 year			2 years			3 years		
	f (n)	m (n)	Total	f	m	Total	f	m	Total	f	m	Total
Abatacept	51	16	67	69 %	86 %	73 %	63 %	78 %	67 %	61 %		61 %
Adalimumab	695	218	913	70 %	75 %	71 %	59 %	66 %	60 %	53 %	56 %	54 %
Certolizumab	114	19	133	62 %	56 %	62 %						
Etanercept	704	199	903	70 %	70 %	70 %	62 %	63 %	62 %	57 %	49 %	55 %
Golimumab	160	58	218	70 %	72 %	71 %						
Infliximab	167	55	222	62 %	63 %	62 %	54 %	53 %	54 %	49 %	51 %	50 %
Tocilizumab	113	18	131	73 %	70 %	72 %	62 %	63 %	62 %			

f: female; m: male.

Table 3: Nell-Duxneuner V, et al. The number of patients on monotherapy of a bDMARD is very similar with the exception of tocilizumab showing higher rates of monotherapy (46 % vs 33–38 %).

	Co-medication with a conventional DMARD	
	Yes	No
Abatacept	63 %	37 %
Adalimumab	67 %	33 %
Certolizumab	62 %	38 %
Etanercept	64 %	36 %
Golimumab	66 %	34 %
Infliximab	67 %	33 %
Tocilizumab	54 %	46 %

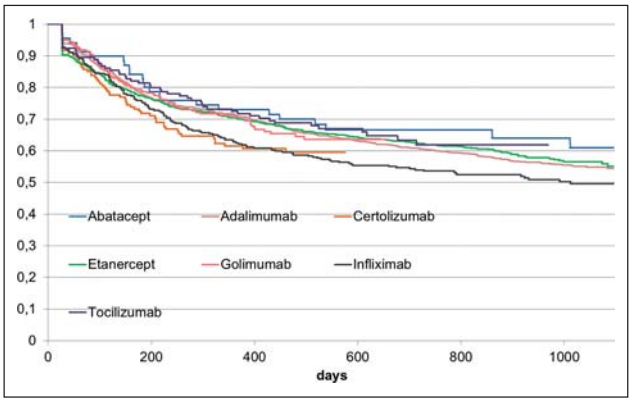

Figure 1: Nell-Duxneuner V, et al. Drug survival of all drugs in comparison.

The Use of Biological DMARDs in Rheumatoid Arthritis in Austria with Special Attention to Gender Differences 12

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Objective The introduction of biological disease-modifying antirheumatic drugs (bDMARDs) offered new dimensions of symptom palliation and alteration of disease progress for patients with rheumatoid arthritis (RA). Drug expenditure data of 2008–2011 were retrieved to evaluate frequency of prescription, preferred substance in total and in relation to gender, and data on combination therapy with a conventional DMARD.

Methods Data from 9 sickness funds covering 6.1 million insured people, which correspond to 72 % of the population, were analyzed linking 2 databases, combining data on therapy of individual patients and their diagnosis. Well before 2008, infliximab, etanercept, and adalimumab were approved and reimbursed for outpatient care in Austria. Abatacept was reimbursed from 05/2008 on, tocilizumab from 03/2009, and certolizumab as well as golimumab from 06/2010.

Results 6694 patients with RA on bDMARDs were retrieved for data analysis in total, 2587 RA patients received a bDMARD for the first time. Choice in drug for first time prescription of a bDMARD from 2008–2011 **(Table 2)**: Adalimumab, followed almost equally by etanercept, were prescribed most often in 35.3 % and 34.9 % of patients, respectively. Infliximab and golimumab were prescribed almost equally by 8.6 % and 8.4 %, followed by certolizumab and tocilizumab with 5.1 % and abatacept with 2.6 %. We analyzed the proportion of female/male and found no relevant preference for any bDMARD by gender. Drug survival **(Table 2, Figure 1)**: Of note is that abatacept and tocilizumab as well as infliximab might be underrepresented in our data due to inpatient coordination of the infusion, which would not be included in our present data on outpatient prescription. The number of patients still on the prescribed drug (drug survival) after 1, 2, and 3 years is demonstrated. Abatacept showed the highest drug survival after one year followed by tocilizumab, adalimumab, golimumab, and etanercept. Three-year drug survival was again highest for abatacept, followed by etanercept and adalimumab. While all other drugs showed no consistent relation to gender, adherence to abatacept was very high in male patients. Mono- vs combination therapy **(Table 3)**: The number of patients on monotherapy of a bDMARD is very similar with the exception of tocilizumab showing higher rates of monotherapy (46 % vs 33–38 %).

Conclusions Patients with rheumatoid arthritis starting on first time bDMARD in Austria in 2010–2011 were treated most often with adalimumab and etanercept. There was no relevant gender difference in the initial choice of drug in our data. Three-year drug survival was highest for abatacept, followed by etanercept and adalimumab.

BioReg – an Austrian Biologics Register 13

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Aim To investigate the long-term outcome of patients with chronic inflammatory rheumatic diseases treated with biological agents.

Methods BioReg, a biologics register for inflammatory rheumatic diseases (www.bioreg.at) was launched 2009 to document patients with inflammatory rheumatic diseases and treated with biological agents. Starting with the documentation of patients with rheumatoid arthritis (RA), spondylarthritis (SpA), and psoriatic arthritis (PsA) who are treated with a biological disease-modifying antirheumatic drug (DMARD), the documentation was extended to patients who are treated out of label with a biological DMARD. In 2010 the cooperation of BioReg and the Austrian Society of Rheumatology (ÖGR) was decided. At last twice a year patient's disease activity and qual-

ity of life are documented with validated instruments, side effects are recorded as soon as possible.

Results 4840 patients were registered until September 15th 2014 (2842 RA, 1205 SpA, 714 PsA, 79 other chronic inflammatory rheumatic diseases). Some patients meanwhile have follow-up visit 9, single ones even visit 10. All 9 biologics labelled in Europe are used. The distribution of their use is comparable to the distribution seen in other registers (e.g. RABBIT in Germany). With increasing number of control visits the number of RA patients with biological monotherapy is increasing (36 % at baseline, 59 % at visit 4).

Summary/Conclusion Since 2009 the number of doctors joining BioReg as well as the number of patients included in BioReg is rapidly increased, showing the easy use and the broad acceptance of a documentation of disease activity and treatment outcome with validated scores.

Characterization of In Vitro-Generated Senescent Regulatory T Cells 14

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Background/Purpose To investigate the role of pro-inflammatory tumour necrosis factor alpha (TNF-α) and interleukin-15 (IL-15) on the phenotype and function of regulatory T cells (Tregs).

Methods Regulatory T cells ($CD4^+CD25^+CD127low$) from healthy individuals were gained via isolation with magnetic beads. The cells were stimulated with anti-CD3/CD28 beads, interleukin (IL)-2 with or without TNF-α (100 ng/ml), or interleukin-15 (IL-15; 100 ng/ml) for 6 or 14 days. Phenotypic description of Tregs was performed with surface markers anti-CD3, anti-CD4, anti-CD8, anti-CD28, anti-CD25, and anti-CD127 via flow cytometry. The suppressive activity of cultured Tregs was characterized with a CFSE-based assay. Briefly, CFSE-labelled autologous $CD4^+CD25^-CD127^+$ cells were co-incubated with Tregs at a 1:1 ratio. To induce proliferation, the cells were stimulated with anti-CD3/CD28 beads at a 1:1 ratio for 3 d. Thereafter, cells were harvested and analyzed by flow cytometry. Cytokine profile of senescent Tregs was determined via intracellular staining. Tregs were treated with Brefeldin A (10 ng/ml) for 4 hours before cytokine staining. Afterwards the cells were harvested and stained for IL-2, IL-4, IL-10, IL-17, TNF-α, and IFN-γ in addition to surface markers.

Results After exposure to TNF-α for 14 days, $CD4^+CD28^+$ Tregs showed a downregulation of CD28 *in vitro* (median MFI: 3295 [range: 1293–16,853] vs 7423 [3986–132,529]; p = 0.05). In addition, an upregulation of CD25 (27,649 [15,085–43,991] vs 14,779.5 [10,119–28,332]; p = 0.025) and CD127 (996.5 [–35 to 1480] vs 723.5 [–492 to 828]; p = 0,028) on TNF-α-treated Tregs in contrast to unstimulated Tregs could be detected. The expression of FoxP3, however, was similar in all groups. In general, stimulation with IL-15 showed no effect. Notably, suppressive activity of TNF-α-treated Tregs was significantly increased compared to untreated Tregs (12.8 % [–50.8 to 48.3] vs –26 % [– 58 to 25]; p = 0.28). Stimulation with IL-15 did not influence suppressive activity. Although, the presence of IL-15 caused an increased production of IL-4 (637 [414–1247] vs 461.5 [297–725]; p = 0.028), IFN-γ (912.5 [644–1120] vs 828 [566–1075]; p = 0.028), as well as IL-17 (781.5 [515–1039] vs 475.5 [356–792]; p = 0.028). Stimulation with TNF-α did not lead to changes in cytokine profile of Tregs.

Conclusion A T cell subset that combines senescent as well as regulatory properties can be generated *in vitro* by TNF-α and indicates an altered phenotype and function compared to Tregs.

B. Bildgebung

How Long Does Sonographic Joint Activity Continue in Clinically Remittive Joints of Patients with Rheumatoid Arthritis? 15

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Aim Ultrasound (US) assessment was shown to be a sensitive tool for the evaluation of inflammatory joint activity in patients with rheumatoid arthritis (RA). Synovial effusion and hypertrophy are evaluated by gray scale (GS), while hypervascularisation can be measured using Power Doppler (PD) signals. Both types of signals are highly sensitive and may persist even in clinical inactivity, i.e. when swelling or tenderness is absent. It is conceivable that such subclinical US signals may resolve if clinical inactivity of the respective joint is sustained, but this has not yet been shown during long-term follow-up. It was the objective of this study to evaluate the persistence of subclinical US signals in previously clinically active joints which have reached a state of continuous clinical inactivity.

Methods We performed US imaging of 22 joints of the hands of RA patients, including GS and PD, each graded on a 4-point scale (0 = no, 1 = mild, 2 = moderate, and 3 = marked). All joints with no activity by clinical assessment at the same time of the US examination were selected, and we identified the last time point of clinical joint activity (by swelling, tenderness, or both). The time between the last clinical joint activity and the current sonographic assessment in that joint was determined, and persistence of subclinical US activity was estimated for all patients and all joints using time-to-event analysis.

Results A total of 90 RA patients with 1980 assessed joints were included in this study: 67.1 % (1329) of the joints were positive on GS and 20.7 % (410) showed PD signals. The mean ± SD number of joints showing signs of sonographic activity was: 15 ± 5 for GS, 5 ± 3.8 for PD. The median (IQR) time between the last visit exhibiting clinical activity in a single joint and the US assessment in the same joint was 3.6 (1.2; 6.3) years for joints with PD signals, and 3.5 (1.3; 5.6) years for joints with GS signals. If GS signals were ≥ 2, we found a significantly shorter time to the last visit with clinical activity compared to joints with GS = 1 (median [IQR] 2.6 [0.6; 2.6] vs 3.9 [1.9; 6.6]; p < 0.001); for PD signals we saw the same trend (median [IQR] of 2.4 [0.5; 5.3] for PD ≥ 2 vs 4.3 [1.0; 6.2] for PD = 1; p = 0.066). In joints showing both highly positive GS and PD signals (both ≥ 2), the time to the last clinical activity was even shorter, with a median of 1.4 years.

Summary/Conclusion We conclude that subclinical joint activity is long-lasting in RA joints in clinical remission, but resolves over time. The latter is indicated by a shorter period from last clinical activity for strong signals (PD ≥ 2, GS ≥ 2) as compared to weak signals (PD ≤ 1, GS ≤ 1).

Fluoreszenzoptische Bildgebung als neues Verfahren zum Nachweis von Durchblutungsstörungen der Hände von Patienten mit Systemischer Sklerose 16

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Ziel Die fluoreszenzoptische Bildgebung (FOI) wird derzeit vor allem zur Detektion inflammatorischer Aktivität in den Händen von Patienten mit entzündlichen Gelenkerkrankungen eingesetzt. Die lokal erhöhte Durchblutung in den entzündeten Gelenken führt durch Anreicherung des Fluoreszenzfarbstoffs Indocyaningrün (ICG) zu einer gesteigerten Signalintensität. Patienten mit Systemischer Sklerose (SSc) leiden häufig an einer verminderten akralen Durchblutung (Raynaud-Phänomen), welche das Risiko digitaler Ulzerationen birgt. Das Ziel der Studie ist die Analyse der ICG-Verteilungsmuster bei Patienten mit SSc zum Nachweis von Mikrozirkulationsstörungen im Vergleich zu Gesunden.

Methoden Es wurden 79 Probanden (53 SSc-Patienten, 26 Gesunde) eingeschlossen, die eine FOI-Untersuchung nach den Xiralite-System-Guidelines (ICG 0,1 mg/kg KG i.v.; Untersuchungsdauer: 6 Minuten) erhielten. Zur Analyse dienten der PrimaVista-Mode, welcher die summierte ICG-Verteilung über den gesamten Untersuchungszeitraum anzeigt, sowie die 360 Einzelbilder, mit deren Hilfe sich der zeitliche Verlauf nachverfolgen lässt.

Ergebnisse Nach einer initialen Signalanreicherung in den Fingerspitzen (Anflutungsphase) kommt es bei den gesunden Probanden zu einer physiologischen Abflutung des Farbstoffs in die proximalen Anteile der Hände. In dieser Studie konnten in der Anflutungsphase umschriebene, ICG-reiche Areale („Inseln") beobachtet werden, die keinen Bezug zu bestimmten Gelenken hatten. Diese „Inseln" fanden sich signifikant häufiger bei SSc-Patienten (58 %) als bei Gesunden (23 %; p = 0,0004). Zum Nachweis einer Durchblutungsstörung wurde untersucht, ob alle Fingerspitzen in der Anflutungsphase ICG anreichern. Dies war signifikant häufiger bei der gesunden Kontrollgruppe (73 %) der Fall als bei den SSc-Patienten (33 %; p = 0,0016). 19 % der SSc-Patienten (0 % der Gesunden) zeigten einen kompletten Abbruch der Fingerdurchblutung, definiert als ein unzureichendes Signal in mindestens einem Fingerendglied während des gesamten Untersuchungszeitraums (p = 0,026).

Zusammenfassung/Schlussfolgerung Mithilfe des FOI-Verfahrens konnten in dieser Studie SSc-typische Merkmale als umschriebene ICG-Anreicherungen („Inseln") beobachtet werden, die Ausdruck entzündlicher Prozesse in der Haut sein können. Zudem ließ sich die Mikrozirkulationsstörung bei SSc-Patienten durch eine verzögerte bzw. ausbleibende periphere ICG-Verteilung nachweisen.

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Assessment of Microstructure, Bone Mineral Density, and Bone Erosions by HR-pQCT in Psoriatic Arthritis and Psoriasis 17

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Aim Psoriatic arthritis (PsA) is a chronic inflammatory joint disorder characterized by local bone loss. Additionally, a skin-bone axis in skin psoriasis was suggested recently. However, data on systemic bone loss and bone mineral density (BMD) in PsA as well as in psoriasis are inconsistent. Moreover, data on bone microarchitecture are missing. The aim of this study was to evaluate bone microstructure and volumetric bone mineral density (vBMD) and to detect local bone erosions by HR-pQCT (high-resolution peripheral quantitative computed tomography) in patients with PsA and psoriasis.

Methods HR-pQCT scans were performed at the periarticular and the non-periarticular radius in patients with PsA (n = 50), patients with psoriasis without any signs of arthritis (n = 30), and healthy, age- and sex-related controls (n = 70). We assessed vBMD and parameters of microstructure including trabecular bone volume (BV/TV), trabecular number (Tb.N), inhomogeneity of the trabecular network, cortical thickness (Ct.Th), and cortical porosity (Ct.Po). Moreover, HR-pQCT scans of the metacarpophalangeal joints (MCP) were performed to detect bone erosions.

Results At the non-periarticular radius trabecular BMD (p = 0.009), cortical BMD (p = 0.031), BV/TV (p = 0.009), and Tb.N (p = 0.0013) were significantly decreased in PsA patients compared to healthy controls. In addition, Tb.Sp (p = 0.014) and inhomogeneity of the network (p = 0.040) were increased in PsA. Similar results were found at the periarticular radius. No differences were found regarding cortical thickness and cortical porosity between the 3 subgroups. In contrast, patients with psoriasis without arthritis had a bone phenotype comparable to healthy controls. Nonetheless, duration of skin disease was found to be associated to low BV/TV and Tb.N in patients with PsA. Bone erosions were detected in 70 % of patients with PsA, 47 % of patients with psoriasis, and 30 % of healthy controls.

Summary/Conclusion Trabecular and cortical vBMD as well as trabecular bone microstructure are significantly decreased in PsA. Our data suggest a skin-bone axis linked to duration of psoriasis and a pre-PsA status in patients with psoriasis without arthritis. However, systemic bone loss seems to be triggered by additional factors linked to arthritis.

Osteophytes Increase the Ambiguity of Clinical Evaluation of Joint Swelling in Rheumatoid Arthritis 18

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Objective It is recommended that a joint be classified as clinically swollen if this swelling is beyond doubt. However, in clinical practice the evaluation of joint swelling in patients with rheumatoid arthritis (RA) is often hindered by joint deformity, secondary osteoarthritis (OA), or adiposity. The aim of this study was to evaluate the ambiguity and reliability of clinical swollen joint assessment in patients with RA.

Methods Clinical joint swelling was evaluated in 2 cohorts of consecutive RA patients with at least 1 swollen joint. In Cohort A (n = 20) a conventional 28 swollen joint count (SJC) was performed on the same day by 2 independent, blinded examiners. In Cohort B (n = 28) the same examiners performed a modified 28 SJC in which joints were graded as either definitely swollen, non-swollen, or doubtfully swollen (defined as a joint where swelling cannot be excluded or confirmed due to limited evaluation attributed to the physical characteristics of the joint). In addition, a standard grey-scale (GS) and Power Doppler (PD) ultrasonographic evaluation (US) was performed by a sonographer blinded to clinical data in Cohort B patients. Presence/absence of GS synovitis, PD signal, erosion, and osteophytes were recorded.

Results A total of 1316 joints were clinically evaluated in 48 RA patients (89 % women; mean [±]: age: 59.4 [12.1] years, disease duration: 12.5 [8.1] years, SDAI: 10.74 [8.9]) in 2 cohorts. 85 % (24 out of 28) of patients in Cohort B had at least 1 doubtfully swollen joint, with a maximum number of 4 doubtful joints/patient. The top joints with doubtful swelling were the wrist, knee, MCP3 and MCP1 joint. Interobserver reliability, evaluated by intraclass correlation coefficient in Cohort A for the conventional SJC and in Cohort B for the modified SJC, was 0.80 (95-% confidence interval [95-% CI] 0.7–0.83) and 0.83 (95-% CI: 0.81–0.85), respectively. Agreement between the 2 examiners for definitely swollen and doubtfully swollen joints was 65 % and 16 %, respectively. Doubtfully swollen joints were more often GS (p < 0.001) and PD positive (p < 0.001) as compared to non-swollen joints (80 % vs 54 % and 26 % vs 12 %, respectively) and had more frequently osteophytes on US than either swollen (p = 0.021) or non-swollen joints (p = 0.003; 11 % vs 4.5/4.5 %, respectively). Erosions were more commonly detected in swollen joints than in doubtfully swollen or non-swollen joints (4.8 % vs 1.8/2.2 %). No association was found between body mass index and the number of doubtfully swollen joints.

Conclusion A modified SJC including doubtfully swollen joints is characterized by similar interobserver reliability as the conventional SJC. Agreement between 2 blinded examiners was low for the evaluation of doubtful swelling. Doubtfully swollen joints had significantly more osteophytes on US suggesting a relationship between ambiguity of swelling and secondary OA in patients with RA.

Ultrasound Composite Scores for the Assessment of Inflammatory and Structural Pathologies in Psoriatic Arthritis (PsASon-Score) 19

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Purpose This study was performed to develop ultrasound composite scores for the assessment of inflammatory and structural lesions in psoriatic arthritis (PsA).

Methods Prospective study on 83 PsA patients undergoing 2 study visits scheduled 6 months apart. B-mode and Power Doppler (PD) findings were semiquantitatively scored at 68 joints (evaluating synovia, peritendinous tissue, tendons, and bone) and 14 entheses. We constructed bilateral and unilateral (focusing the dominant site) ultrasound composites selecting relevant sites by a hierarchical approach. Discriminatory, internal, and external validity, sensitivity, reliability, and feasibility of the scores were tested.

Results The bilateral score (termed PsASon22) included 22 joints (6 MCPs, 4 PIPs, 2 MTPs, 6 DIPs, 4 large joints) and 4 entheses, whereas the unilateral score (PsASon13) compromised 13 joints (2 MCPs, 4 PIPs, 2 MTPs, 3 DIPs, 2 large joints) and 2 entheses. Both composites revealed a moderate to high sensitivity (bilateral composite 42–100 %, unilateral 36–100 %) to detect inflammatory and structural lesions. Data from both scores correlated moderately with results from 68-joint/14-entheses scores (corrcoeffs 0.39–1.0) and weakly with clinical parameters (corrcoeffs 0–0.41). Patients with active disease achieving remission at follow-up yielded greater reductions of ultrasound scores than those with stable clinical activity. Reproducibility of bi- and unilateral composites was moderate to good with ICCs ranging from 0.42 to 0.96 and from 0.32 to 0.71, respectively, for global scores and subscores. The scores required 16–26 and 9–13 minutes, respectively, to be completed.

Conclusion Both new PsA ultrasound composites (PsASon22 and PsASon13) revealed sufficient discriminatory, internal, and external validity, reliability, and feasibility.

C. Kinderrheumatologie

Complement Diagnostic in Patients with Juvenile Idiopathic Arthritis (WIELISA, SC5b9-ELISA) 20

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Juvenile idiopathic arthritis is a well researched disease in the group of autoimmunopathies. Beside the deregulation of T cells and cytokines, also the complement system is involved in the pathogenesis of this group of diseases. This prospective longitudinal study investigated the contribution of the complement system in patients with juvenile idiopathic arthritis, using practicable ELISA techniques (Wieslab® screening kit; SC5b9 soluble terminal complement complex ELISA). Serum and plasma of the peripheral blood and the synovial fluid were investigated for the activity of the 3 complement pathways – classical (CP), mannose binding lectin (MBL), and the alternative pathway (AP) – and total complement activity by measuring SC5b9. Results where compared to published reference controls and 18 children without activation of inflammation as an age-matched control group. In total 57 samples of peripheral blood (PB) and 8 samples from synovial fluid (SF) from 28 children with JIA were investigated in a longitudinal observation during acute phase and remission. The screening of complement system showed debasement of the AP (8 of 10) and CP (7 of 10) in patients during acute phase (7 of 10). The SC5b9 measurement showed a significant ($p < 0.002$) higher amount in plasma (3,6 AU/ml in median) and serum (31,4 AU/ml) during acute phase compared to the control group (serum – 7,72 AU/ml, and plasma – 1,25 AU/ml in median). In conclusion, the study confirmed that the CP and AP of the complement system are main contributors in the pathogenesis of JIA. Because of significant elevation of SC5b9 in acute phase of JIA, complement blockade with Anti-C5 may be a therapeutically option in the future.

Interleukin 1 Blockade with Canakinumab for Hyper-IGD Syndrome (HIDS) 21

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Introduction Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS; MIM# 260920) is a rare autosomal recessive autoinflammatory condition caused by mutations in the MVK gene, which encodes for mevalonate kinase. There is no standard treatment for HIDS.

Case report We report on a 2-year-old Austrian boy with recurrent episodes of fever, febrile seizures, arthralgias, and splenomegaly. Rash and abdominal pain were also seen occasionally. During attacks an acute-phase response was detected. Clinical and laboratory improvement was seen between attacks. These findings led to the tentative diagnosis of HIDS. Sequencing of the MVK gene showed a homozygous c.1129G > A (p.Val377Ile, also known as V377I) mutation in the child, while the healthy non-consanguineous parents were heterozygous. The mutation is known to be associated with HIDS. Therapy with non-steroidal anti-inflammatory drugs during attacks had poor benefit. A further febrile episode resulted in a status epilepticus. Treatment with canakinumab was initiated and a final dose of 4 mg/kg every 4 weeks resulted in the disappearance of febrile attacks and a considerable improvement of the patient's quality of life during a 6-month follow-up period. The drug has been well tolerated, and no side effects were observed.

Conclusion Treatment with canakinumab is a therapeutical option for patients with HIDS.

The Toll-Like Receptor 4 Agonist MRP8/14 Protein Complex (Calprotectin) in Autoinflammation: Potential Biomarker in Chronic Nonbacterial Osteomyelitis – A Case Report 22

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Background The cytoplasmic S100 proteins derived from cells of myeloid origin. Calprotectin (MRP8/14 protein complex) might be a biomarker either for autoinflammation or autoimmunopathy. Since autoinflammatory diseases might be a diagnostic challenge, calprotectin may be helpful in the diagnosis of autoinflammatory diseases. Chronic nonbacterial osteomyelitis (CNO) is an autoinflammatory, non-infectious disease. CNO describes a wide spectrum from a monofocal bone lesion to the chronic recurring multifocal osteomyelitis (CRMO). Laboratory and histopathological findings are non-specific. In some patients systemic inflammatory signs such as elevated acute phase proteins cannot be found.

Objective To test the ability of Calprotectin (MRP8/14 protein complex) serum concentrations to monitor disease activity in patients with CNO.

Methods Serum concentrations of Calprotectin (MRP8/14 protein complex) in a patient with CNO were determined by a sandwich ELISA.

Results Calprotectin (MRP8/14) level were raised heralding active disease when acute phase proteins (CrP, erythrocyte sedimentation rate). The calprotectin level was 7872.7 ng/ml (normal range 0–3000 ng/ml).

Conclusion Calprotectin (MRP8/14) serum concentrations correlate closely with disease activity and may herald a flare before clinical manifestation. Therefore, MRP8/14 serum concentrations are a biomarker indicating disease activity in CNO patients.

Tryptophan as Biomarker of Inflammation in Juvenile Idiopathic Arthritis (JIA) 23

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Background Juvenile idiopathic arthritis (JIA) is a relevant autoimmune disease in children. T cells, B cells, and damage-associated molecular patterns (DAMPS) are involved in the pathogenesis of the disease. Biomarkers for JIA and its subtypes are not established. Pro-inflammatory pathways activate enzyme indoleamine 2,3-dioxygenase (IDO) which enhances tryptophan (Trp) conversion to kynurenine (Kyn). Thus, in conditions of chronic immune activation, reduced Trp availability and production of Kyn and its downstream metabolites may inhibit cell proliferation. In rheumatoid arthritis (RA), Trp concentrations are lower in patients than in controls and the Kyn/Trp ratios are higher and correlate with neopterin concentrations [1–3].

Objectives To evaluate Tryptophan as biomarker in JIA.

Methods In this study, Trp and Kyn metabolism was investigated in children with JIA and compared to serum neopterin concentrations. 54 sera of 25 JIA patients and 10 samples of synovial fluid were examined with HPLC (Trp and Kyn) and ELISA (Neopterin, BRAHMS, Hennigsdorf, Germany). 18 sera from 18 children with non-inflammatory diseases were used as controls.

Results Trp in the sera of patients was mean 57.2 ± SD 19.0 µmol/L and Kyn was mean 2.40 ± SD 0.81 µmol/L). Serum neopterin was 5.69 ± SD 1.72 nmol/L. In the synovial fluid, neopterin was mean 10.5 ± SD 7.41 nmol/L), Trp was 36.7 ± SD 17.4 µmol/L, and Kyn was 2.13 ± SD 0.75 µmol/L). In control patients, neopterin was 6.93 ± SD 3.10), Trp was 57.6 ± SD 14.8), and Kyn was 2.60 ± SD 1.60 µmol/L.

Conclusions Serum Trp concentrations showed no relevant difference in JIA patients vs controls. IDO activity reduces Trp primarily in the synovial fluid in JIA patients.

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Psoriasiform Eruption-Related Alopecia During Anti-TNF-α Therapy 24

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Background/Aim Tumour necrosis factor-alpha inhibition is known to be a highly effective treatment of psoriasis. Conversely, few cases of anti-TNF-α-induced psoriasiform lesions with scalp involvement have been reported in the literature. We describe a 10-year-old girl with development of inflammatory alopecia areata and psoriasiform eruptions during treatment with adalimumab.

Methods Case report.

Results In our patient, adalimumab therapy was started due to severe uveitis with band keratopathy and cataracta complicata, after failure of treatment with methotrexate. 18 months later she concurrently developed inflammatory alopecia areata and circumscribed erythematosquamous lesions on the limbs. Apart from a discreet hemangioma on the left shoulder, there were neither previous skin lesions nor was there a family history of psoriasis. Infections were excluded. A skin biopsy confirmed clinical diagnosis, showing parakeratosis with neutrophils in the epidermis and predominantly lymphocytic infiltrates in the dermis, as well as early fibrosis. Under topical therapy, the psoriasiform skin lesions promptly resolved on the limbs; however, no improvement of the alopecia was observed. Finally, adalimumab was discontinued, resulting in continuing recovery of the scalp lesions followed by hair re-growth. Currently, the patient is treated with oral prednisolone and shows normal ophthalmologic findings.

Conclusion Psoriasiform eruption-induced alopecia has been reported in a few patients with adalimumab therapy in the literature and may be a rare side effect of anti-TNF-α treatment.

Fieberschübe, Schulterschmerzen und Hypertonie 25

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Ziel Vorstellung eines 16-jährigen Patienten mit Takayasu-Arteriitis.

Methoden Kasuistik. Die Langzeitanamnese des Patienten ist bis auf selten auftretende Pharyngitiden unauffällig, ebenso die Familienanamnese. 8 Wochen vor der Erstvorstellung wurde am Heimatkrankenhaus bei Tonsillitis und geringgradiger Aorten- und Mitralinsuffizienz die Diagnose „Rheumatisches Fieber" gestellt und eine Penicillin-Dauerprophylaxe eingeleitet. Darunter traten in unregelmäßigen Abständen neuerliche Fieberschübe (bis 38,7 °C) für 2–3 Tage sowie kurzfristige Thoraxschmerzen auf. Bei dabei erhöhten CRP-Werten (bis 69 mg/l) und radiologischen Bronchitiszeichen erhielt der Patient kurzfristige Umstellungen der antibiotischen Therapie auf Makrolide, was innerhalb weniger Tage zur Beschwerdefreiheit führte. Echokardiographische Verlaufskontrollen erbrachten unveränderte Befunde. Die Lungenfunktion war unauffällig, ebenso Blutbild, Serumroutineparameter und D-Dimer. Blutdruckmessungen erfolgten jeweils am rechten Oberarm und lagen im normotensiven Bereich. Zwischen diesen Episoden war der Patient in ausgezeichnetem Allgemeinzustand und spielte regelmäßig Fußball im Verein. Bei einer weiteren Vorstellung wegen Fieber und Thoraxschmerzen klagte der Patient zusätzlich über Arthralgien, weswegen eine genetische Untersuchung auf Vorliegen eines Familiären Mittelmeerfiebers erfolgte, die negativ war. 3 Monate später hatte der Patient starke Schulterschmerzen und neuerlich Fieber bei erhöhten Entzündungswerten (CRP: 95 mg/l, BSG: 106 mm/h). Der Blutdruck war normotensiv. Ein MRT der Schulter zeigte eine langstreckige Verdickung des N. brachialis sowie den V. a. Gefäßabbrüche, weswegen eine Duplexsonographie der Armgefäße angeschlossen wurde, die jedoch keinen Hinweis auf Arteriitis erbrachte. Immunologische Untersuchungen (ANA, ds-DNA-AK und ANCA) waren negativ. 2 Monate später erhielt der Patient beim Fußballspiel einen Schlag gegen den Hals. Die Sonographie der Halsregion erbrachte eine deutliche Wandverdickung der A. carotis bds. bei einer Blutdruckdifferenz zwischen der oberen und unteren Extremität von 90 mmHg. Bei V. a. Takayasu-Arteriitis erfolgten Gefäßdarstellungen mittels DSA, MRA und Duplexsonographie, bei denen sich neben der hochgradigen Intima-Media-Verbreiterung der supraaortalen Halsarterien eine längerstreckige Stenose der A. carotis communis dext. und der A. subclavia sin. zeigte. Unter Remissionsinduktionstherapie mit Aprednislon 1 mg/kg und Thrombo-ASS mit nachfolgender immunmodulativer Therapie mit Methotrexat kam es zur raschen Besserung der klinischen, laborchemischen und radiologischen Parameter.

Zusammenfassung/Schlussfolgerung Bei Patienten mit rezidivierenden Fieberschüben und erhöhten Entzündungswerten sollte eine Takayasu-Arteriitis differenzialdiagnostisch erwogen werden, wenngleich diese eine Rarität in der pädiatrischen Rheumatologie darstellt.

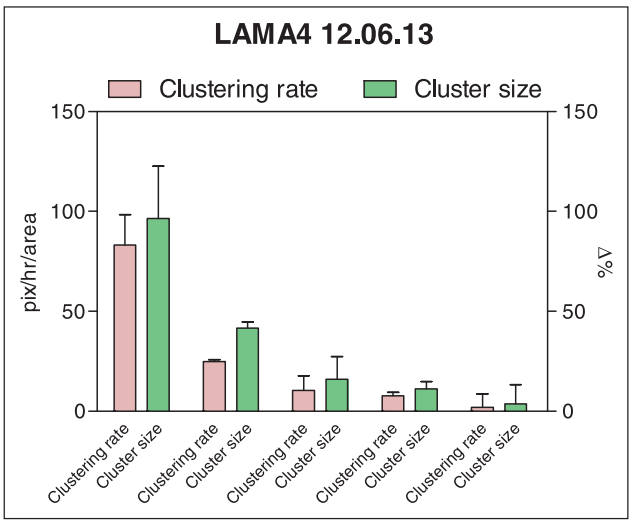

Figure 2: Moazedi-Fürst FC, et al. 2A3 (4 different concentrations) treatment results in a decrease of cluster rate and cluster size.

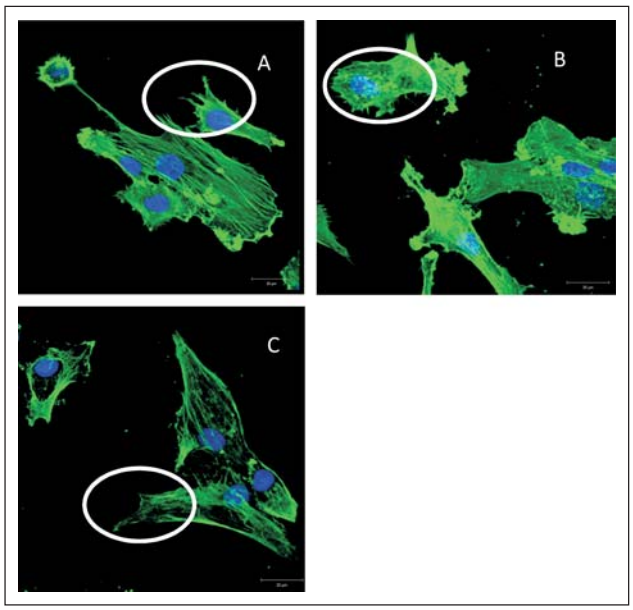

Figure 3: Moazedi-Fürst FC, et al. F-Actin staining of human chondrocytes: **(A)** untreated, **(B)** IgM-treated, and **(C)** treated with 2A3: little hairbrush disappeared, surface seems even.

S100-Proteine in der Kinderrheumatologie 26

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Ziel Laut Literatur korrelieren die S100-Proteine A8/9 und A12 mit der Krankheitsaktivität verschiedener rheumatischer Erkrankungen des Kindesalters. Vorstellung der Pathophysiologie der S100-Proteine A8/9 und A12. Überprüfung der Relevanz der erhöhten Werte im Vergleich zu CRP- und BSG-Voraussagekraft von niedrigen Werten im Hinblick auf Remission. Off-medication-Überprüfung des Cut-offs der Testergebnisse auf die klinische Relevanz.

Methoden Seit Juni 2014 bestimmen wir routinemäßig die S100-Proteine der Patienten der Kinderrheumaambulanz des SMZO. Die Ergebnisse der Untersuchungen von bisher etwa 60 Patienten mit verschiedenen Erkrankungen werden vorgestellt.

D. Pathophysiologie

Laminin-4 Blockade Reduces Cluster Formation of Chondrocytes in Osteoarthritis 27

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Cluster formation of chondrocytes is a morphological sign found in severe osteoarthritis additionally to cell hypertrophy and extracellular matrix degeneration. LAMA4 is an integrin involved in several biological processes including cell adhesion and migration; blocking LAMA4 has a suppressive effect on metalloproteinase expression. We investigated the effect of LAMA4 on the *in vitro* mobility of osteoarthritic chondrocytes.

Methods Cartilage was collected from patients undergoing total knee replacement (n = 15). All subjects signed an informed consent as approved by the local ethics committee. Chondrocyte cultures were treated with anti-LAMA4-antibody 2A3 (10 ug/ml, 20 ug/ml, 30 ug/ml, and 40 ug/ml) in a gel matrix. Cell migration was analyzed taking pictures every other second during 24 hours. Special software was programmed to measure changes over time in cell morphology and migration. In addition, confocal microscopy and filament staining were performed.

Results We documented the migration of chondrocytes towards each other to form clusters on the surface of a matrigel matrix over 24 hours. Cellular extensions (pseudopodia) were formed. This could be an attempt to communicate. Exposure to the 2A3 antibody decreased the formation of filopodia and the formation rate of cell clusters in a dose-dependent manner ($p < 0{,}001$). The unspecific immunoglobulin had no effect on cluster formation and cluster size **(Figures 2, 3)**.

Summary/Conclusion Active chondrocyte migration leads to formation of cell clusters *in vitro*. The integrin LAMA4 might play a role in the development of chondrocyte clusters in osteoarthritis.

Incidence and Functional Relevance of IL-23 Receptor Gene Polymorphisms in Ankylosing Spondylitis 28

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Background/Purpose Interleukin-23 (IL-23) signalling is involved in Th17 immune response and thus in the pathogenesis of ankylosing spondylitis (AS). The purpose of this study was to investigate the functional relevance of single nucleotide polymorphisms (SNP) in the IL-23 receptor (IL-23R).

Methods 105 patients with AS according to the modified New York criteria were prospectively enrolled into the study. Blood was drawn from all individuals and DNA was extracted to determine the SNPs rs 10889677 (SNP1), rs 11209026 (SNP2), rs 11465804 (SNP3), and rs 1343151 (SNP4) by polymerase chain reaction. To test functional relevance of the SNPs we performed intracellular flow cytometry and stained PBMCs with anti-CD3, CD4, CD8, IL-23R, pSTAT-3, and IL-17A. Phosphorylation of STAT-3 (pSTAT-3) following IL-23 stimulation (100 ng/ml) and the prevalence of Th17 cells were assessed.

Results We detected homozygote carriers of SNP1 in 10 (9.5 %) and SNP4 in 4 (3.8 %) patients of our cohort. These patients were wild-type (WT) for the other SNPs tested. We found no homozygote carriers of the SNPs 2 and 3. 15 SpA patients were WT for all 4 SNP tested and served as control group. The frequency of IL-23R$^+$ CD4 T

cells was significantly increased in the SNP1 group (median 37.3 [27.9–52.7] vs 25.6 [16.1–38.1]; p = 0.05) but not in the SNP4 group (32.4 [29.3–34.9]; p = 0.248). IL-23 (100 ng/ml) stimulation of PBMCs-induced phosphorylation of STAT3 in the SNP1 group (2.2 [1.7–4.5] vs 1.3 [0.4–2.7]; p = 0.087) but not in the SNP4 group compared to WT (1.7 [0.7–2.6]; p = 0.715). In addition, patients with SNP1 (1.2 [0.8–1.4]; p = 0.015) but not with SNP4 (1.1 [0.6–1.2]; p = 0.162) exhibit significantly elevated levels of Th17 cells after stimulation with OKT-3 compared to WT (0.6 [0.5–0.9]).

Conclusion The SNP rs 10889677 increases IL-23R abundance in $CD4^+$ T cells and fosters Th17 response in patients with ankylosing spondylitis.

Altered Phenotype and Function of Senescent Regulatory T Cells in Rheumatoid Arthritis 29

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Background/Purpose Immunosenescence accompanied by accumulation of senescent T cells in rheumatoid arthritis (RA) is a hallmark feature in the pathogenesis of RA. Here we characterize a novel senescent regulatory T cell (Tregs, $CD4^+CD28^-FoxP3^+$) subset in RA patients.

Methods Prospective, cross-sectional study on 35 patients with RA (mean age 58 [± SD 9.5], 71.4 % female, SDAI 8.15 [± 1.2]) and 25 healthy controls (HC, mean age 56.4 [± 6.7], 60 % female). We used flow cytometry to determine the prevalence of senescent $CD4^+CD28^-FoxP3^+$ T cells and to characterize their phenotype, proliferation, cytokine production, and apoptosis. T cell receptor diversity was determined by RT-PCR.

Results 2 % (± 2.8) of $CD4^+$ T cells were $CD28^-FoxP3^+$ in RA patients whereas this subset was almost absent in HC (0.6 [± 0.8]; p = 0.077). The number of $CD4^+CD28^+FoxP3^+$ Tregs was comparable in both groups (28.6 [± 18.5] vs 32.7 [± 18]; p = 0.480). Surface receptor expression analysis of $CD28^-FoxP3^+$ and $CD28^+FoxP3^+$ Tregs demonstrated that $CD28^-FoxP3^+$ cells expressed higher levels of the regulatory protein PD-1 (17.45 % [0–36.4] vs 5.45 % [1.8–13.5]; p = 0.034), whereas CTLA-4 expression was similar in both subsets. Production of various cytokines including IL-2, IL-4, IL-10, IL-17, TNF-α, and IFN-γ was increased in $CD28^-FoxP3^+$ compared to $CD28^+FoxP3^+$ Tregs (all $p < 0.05$), whereas proliferation rate was lower than in the $CD28^+$ counterparts (50 % [0–93.6] non-proliferating cells vs 4.6 % [0–30.6]; p = 0.001). In contrast, apoptosis induction was higher in $CD28^-FoxP3^+$ than in $CD28^-FoxP3^+$ Tregs (22.1 % [0–30.8] vs 4.4 % [0–7.8]; $p < 0.001$). TCR diversity was also reduced in $CD28^-FoxP3^+$ Tregs compared to their $CD28^+$ counterparts (median TCR diversity score: 84 [36–104] vs 115 [109–125]; p = 0.037).

Conclusion We discovered a novel T cell subset which combines both senescent as well as regulatory properties. This subset favours the pro-inflammatory milieu and shows altered phenotype and function compared to normal (non-senescent) Tregs.

The Role of microRNA-146a in Inflammatory Arthritis 30

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Aim MicroRNA (MiR-) 146a is a key regulator of the innate immune response and has also been shown to suppress cancer development in myeloid cells. Elevated expression of miR-146a has been detected in synovial tissue of rheumatoid arthritis patients, but its role in the development of inflammatory arthritis is yet unknown.

Methods We induced K/BxN serum transfer arthritis in wild-type and miR-146a$^{-/-}$ mice. As a second inflammatory arthritis model we crossed miR-146a-deficient into hTNFtg mice. Disease severity was assessed clinically and histologically in both arthritis models. Blood of arthritis animals was analyzed by flow cytometry. Serum cytokine levels were measured by ELISA.

Results Absence of miR-146a leads to increased clinical signs of the induced serum transfer arthritis. In line, higher serum levels of the proinflammatory cytokines IL-12 and TNF were measured in miR146a-deficient mice compared to wt mice. When we crossed miR-146a$^{-/-}$ mice into hTNFtg mice, while detecting no clinical difference between hTNFtg and miR-146a/hTNFtg mice, we found a significant increase in circulating $CD11b^+$ myeloid cells as well as $CD11c^+$ dendritic cells in blood of miR-146a$^{-/-}$/hTNFtg mice compared to hTNFtg mice. Histological examination revealed a significant increase in synovial inflammation miR-146a$^{-/-}$/hTNFtg mice compared to hTNFtg mice. Even more striking, miR-146a$^{-/-}$/hTNFtg mice displayed a more than 2-fold increase in local bone destruction which was due to increased generation of osteoclasts in the tarsal joints of the mice. Measuring cytokine levels in serum, we show that IL-1β levels are increased in mice lacking miR-146a.

Summary/Conclusion These data clearly demonstrate a negative regulatory role of the miR-146a in inflammatory arthritis. During arthritis, miR-146a is centrally involved in the regulation of proinflammatory cytokines as well as local bone destruction. These results identify an important anti-inflammatory role of miR-146a, which might possibly be exploited for therapeutic purposes.

Phlebotomy Reduces Systemic Inflammation and Joint Destruction in a Rat Model of Rheumatoid Arthritis via Modulation of Iron Homeostasis 31

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Background Rheumatoid arthritis (RA) is a chronic inflammatory disorder that typically affects the small joints of hands and feet. Pro-inflammatory cytokines such as TNF-α, IL-6, or IL-17 are central in the immunopathology of RA. These cytokines also promote macrophage iron retention by increasing erythrophagocytosis and cellular iron uptake. Iron induces oxidative stress which upregulates cytoprotective mechanisms that may involve Nrf2. We aimed to investigate the modulating iron homeostasis via phlebotomy in a rat model of chronic arthritis.

Design and Methods To elucidate the underlying pathways regulating the expression of inflammatory cytokines and clinical outcome of phlebotomy *in vivo*, we used a rat model of rheumatoid arthritis. Female Lewis rats were inoculated with a single intraperitoneal injection of group A streptococcal-peptidoglycan-polysaccharide (PG-APS). Three weeks after inoculation, one group was phlebotomized every second day. In an additional experiment we treated rats with DFO (desferrioxamine), an iron chelator, or SFN (sulforaphane), an Nrf2 inhibitor.

Results Phlebotomy significantly reduced systemic inflammation which was paralleled by reduced expression of IL-6 and IL-17 in the liver and spleen. A similar effect could be observed upon treatment with the iron chelator DFO. Of interest, application of SFN, which activates Nrf2 but also promotes iron cellular export, resulted in a similar effect. The reduced inflammatory activity in phlebotomized animals was associated with a reduction of joint swelling along with reduced erosion and cartilage destruction in histological examination.

Conclusion This work demonstrates that phlebotomy improved clinical signs of arthritis and suppressed local and systemic bone destruction. In part, this effect is caused by iron-mediated modulation of innate immune function and iron mobilization from macrophages.

Clec4n Is Essentially Involved in Innate Immune Response of Experimental Arthritis 32

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Background C-type lectin receptors, like Toll-like receptors, belong to the pathogen pattern recognition family (PPR) and are essentially involved in immune responses against microbial infections. Clec4n (also known as dectin-2) is mainly expressed on myeloid cells and has been shown to interact with mannan ligands of candida albicans, mycobacteria, and house dust mite. The role of Clec4n in autoimmune diseases has been less elucidated.

Purpose To investigate the role of Clec4n deficiency in the development and severity of experimental arthritis using the K/BxN serum transfer model.

Methods We administered 100 µl serum from K/BxN mice into Clec4n$^{+/+}$ and Clec4n$^{-/-}$ mice on day 1 and 3. We assessed daily clinical signs of arthritis including increase in paw swelling and loss of grip strength on a scale from 0 to 3 (no to severe swelling) or 0 to –3 (no to severe loss of grip strength), respectively. On day 11 we analyzed gait profiles using the CatWalk gait analysis system. One day later, we sacrificed the animals, collected serum for cytokine analysis, and isolated hind paws for subsequent histological analysis. We assessed quantitatively histopathological extent of synovial inflammation (on H&E-stained sections), number of osteoclasts and subchondral bone erosions (on TRAP-stained sections), and cartilage damage (on TB-stained sections) with Osteomeasure software. Immunohistochemical stainings were used to identify neutrophil granulocytes (7/4 clone), macrophages (F4/80 clone), T cells (CD3ab), and B cells (CD45R) within inflammatory synovial tissue using TissueQuest software.

Results We found a significant reduction in clinical signs of arthritis such as paw swelling and loss of grip strength as well as a significant prevention of loss of body weight in the K/BxN serum transfer model in Clec4n$^{-/-}$ mice compared to wt littermates (Clec4n$^{+/+}$). Furthermore, we found a significant protection from functional impairment indicated by increased gait parameters such as maximal intensity and print area in Clec4n$^{-/-}$ mice. Lack of Clec4n revealed a significant protection from synovial inflammation, generation of synovial bone-resorbing osteoclasts, and formation of subchondral bone erosion as quantitatively assessed in hind paw sections. In line, we observed a significant protection from proteoglycan loss in articular cartilage in Clec4n$^{-/-}$ mice compared to Clec4n$^{+/+}$ mice. Interestingly, we found a significant reduction of infiltrating neutrophil granulocytes into the inflamed joints in mice lacking Clec4n.

Conclusion C-type lectin receptor 4n is essentially involved in the regulation of the innate immune response in autoantibody-driven K/BxN serum transfer model. Lack of Clec4n significantly diminished the infiltration of inflammatory cells, particularly of neutrophil granulocytes, responsible in driving inflammatory responses and subsequent structural bone and cartilage damage.

Treatment with BGP-15, a Novel Insulin Sensitizer, Attenuates Collagen-Induced Arthritis in DBA/1 Mice 33

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Objective BGP-15, a small synthetic hydroxylamine derivative, is a member of a new class of insulin-sensitizing medications also known as chaperone-inducers. Chaperones have been suggested to play a role in the regulation of inflammation. Our objective was to evaluate the *in vivo* effects of BGP-15 on collagen-induced arthritis (CIA) in DBA/1 mice.

Materials and Methods Arthritis was induced by intradermal injection of bovine type-II collagen (bCII) and incomplete Freund's adjuvant (CFA) in male DBA/1 mice. BGP-15 was administered either one week prior to the first immunization (prophylactic experiment, n = 14 in both groups) or upon the appearance of symptoms (therapeutic experiment, n = 12 in both groups) in drinking water. Arthritis incidence and severity were assessed for 28 days following the second immunization (boost) with bCII and CFA on day 21. Histological evaluation was carried out on hind paws using Osteomeasure® software. Anticollagen antibodies were measured by enzyme-linked immunosorbent assay. The cellular composition of the draining lymph nodes was measured by flow cytometry.

Results BGP-15 significantly reduced the incidence of CIA by 28 % and also reduced both paw swelling ($p \leq 0.01$) and grip strength ($p \leq 0.05$) in the prophylactic experiment. In the therapeutic experiment BGP-15 significantly attenuated both paw swelling ($p \leq 0.01$) and grip strength ($p \leq 0.05$). Histological evaluation of the hind paws demonstrated reduced area of inflammation ($p \leq 0.05$), area of erosion ($p \leq 0.01$), and number of osteoclasts ($p \leq 0.05$) in the BGP-15-treated group when compared to the control group. No significant differences were revealed between anti-collagen antibody levels or in the distribution of T cells, B cells, dendritic cells, and monocyte/macrophages harvested from draining lymph nodes, suggesting an effect predominantly involving the innate immune system.

Conclusions Our results demonstrate that the novel chaperone-inducer BGP-15 has a profound prophylactic and therapeutic effect on autoimmune arthritis, likely due to an effect on the effector phase.

Nicotinic Acetylcholine Receptor Ligands Inhibit Osteoclastogenesis by Blocking RANKL-Induced Calcium Oscillation and Induction of NFATc1 and c-fos 34

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Objectives We have previously shown that nicotinic acetylcholine receptors (nAChRs) are key regulators of osteoclastogenesis [1]. nAChRs are ion-channel receptors, permitting the movement of $Ca2^{+}$, the intracellular oscillation of which is known to play an essential role in mediating the effect of RANKL on osteoclastogenesis. We therefore elucidated the potential effects of nAChR ligands on RANKL-induced $Ca2^{+}$ oscillation. To assess downstream elements of the RANK pathway, we also evaluated their effect on the transcription factors NFATc1 and c-fos.

Methods For intracellular $Ca2^{+}$ measurement, murine osteoclast precursors (pOCs) were incubated with 5 µM Fura-2-AM and 0.05 % pluronic F-127 for 30 minutes at 37 °C, then post-incubated in HBSS media containing $Ca2^{+}$. The intracellular $Ca2^{+}$ levels were detected with a fluorescent microscope (Visitron Systems). The images were scanned and plotted with an interval of 5 seconds at 340 and 380 nm wavelengths. Evaluation of the images was carried out with MetaFluor software. Quantitative RT-PCR was performed using mRNA extracted from osteoclasts and the amount of dsDNA was quantified using SYBR Green I and its detection by LightCycler (Roche Molecular Biochemicals). For Western blot, BMMs were incubated with M-CSF for 1–7 days and treated with RANKL and/or nicotine. Protein extracts were separated by electrophoresis on acrylamide gel, followed by electrotransfer onto nitrocellulose membrane and incubation with monoclonal antibodies against NFATc1, c-fos, and actin.

Results POCs show characteristic $Ca2^{+}$ oscillations after 72-h treatment with RANKL. Treatment with the non-specific nAChR agonist nicotine, its physiological ligand acetylcholine (ACh), and the α7 nAChR agonist PNU-282987 led to marked, immediate abrogation of RANKL-induced $Ca2^{+}$ oscillation. To evaluate the nature of this inhibition, we added the α7 nAChR antagonist alpha-BTX, which caused a similar effect. We next added the non-specific nAChR antagonist mecamylamine shortly before treating oscillating BMMs

with ACh, and found that in such conditions ACh failed to inhibit $Ca2^+$ oscillation. In BMMs subjected to prolonged exposure (72 h) with high-dose nicotine, concomitant treatment with RANKL failed to induce characteristic $Ca2^+$ oscillations. In qPCR and Western blot experiments, nicotine inhibited the induction of c-fos and NFATc1 caused by RANKL.

Conclusions We have shown that nAChR ligands inhibit $Ca2^+$ oscillation in osteoclast precursors which blocks the RANKL-induced expression and accumulation of c-fos and NFATc1. These results elucidate our earlier findings that nAChRs play a key role in the regulation of osteoclastogenesis in mice.

References:
1. Mandl P, Hayer S, Sykoutri D, et al. Nicotinic acetylcholine receptor are key regulators of osteoclastogenesis. Ann Rheum Dis 2011; 70 (Suppl 3): 99.

Regulatory T Cells Ameliorate Severity of Lupus Arthritis 35

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Background/Purpose Arthritis is often reported as a first symptom of systemic lupus erythematosus (SLE) and is seen in the majority of patients over the course of the disease. Even though joint involvement is a non-life-threatening condition, patient surveys reveal arthritis as a major burden of SLE patients leading to impairment and daily life hurdles. We herein investigate the therapeutic effects of regulatory T cells (Treg) in the murine model of pristane-induced lupus in regard to joint involvement.

Methods Mice were injected i.p. with 0.5 ml of pristane or PBS as control and killed after 8 months. Naïve $CD4^+$ thymocytes were cultured under Treg-inducing conditions and tested for $CD4^+Foxp3^+$ expression by FACS. Cell suspensions with > 80 % purity for $CD4^+FoxP3^+$ iTreg were injected intravenously either once at start of experiments (iTreg-boost) or monthly (iTreg-rep) over the course of the experiment. Animals were monitored for clinical signs of arthritis: paw swelling and grip strength were assessed every 2 weeks using semiquantitative scores. Changes in locomotors behaviour were evaluated after disease induction and at the end of the experimental period utilizing CatWalk system. Further, histological features of arthritis were quantified by Osteomeasure Software, an image analysis system.

Results Regarding the onset and course of the disease, mice only receiving pristane (PIL) were affected the most: compared to iTreg-rep, PIL showed an earlier onset and a more severe course with a constant increase in paw swelling and decrease in grip strengths **(Figure 4)**. Continuous monthly injections of Treg significantly decreased clinical signs of arthritis (mean paw swelling 0.3492 ± 0.07093 vs 0.05556 ± 0.03063; $p < 0.01$; mean loss of grip strength 2.732 ± 0.06287 vs 2.964 ± 0.02373; $p < 0.01$) and the Arthritis Severity Score (ASS; 4.762 ± 0.8133 vs 1.667 ± 0.7601; $p < 0.01$; **Figure 5**). Further, iTreg-rep showed a significant reduction of all histological parameters compared to PIL (inflammatory area 0.7007 ± 0.1120 vs 0.1882 ± 0.05742 mm^2; $p < 0.01$; erosive area 0.06930 ± 0.01707 vs 0.01083 ± 0.008950 mm^2; $p < 0.01$; number of osteoclasts 9.143 ± 1.999 vs 2.000 ± 1.125; $p < 0.01$; cartilage degradation 0.1871 ± 0.03337 vs 0.05857 ± 0.004209; $p < 0.01$; **Figure 6**). The single Treg boost had a retarding effect on clinical symptoms and led to a significantly decreased erosive area at the end of observation; the inflamed area, however, was similar to that in PIL group. Catwalk parameters correlated with histological parameters, with the erosive area as a sign of definitive joint destruction showing the best correlation with changes in locomotor behaviour.

Conclusion Repeated injections of *in vitro*-induced regulatory T cells ameliorate the clinical and histological severity of arthritis. A single injection of iTreg is not effective, but appears to retard the onset of symptoms. Catwalk results correlated with the conventional clinical scoring and can be used as an objective tool for analyzing pain-related loss of joint function.

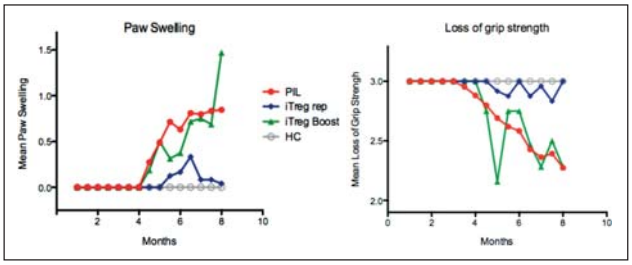

Figure 4: Schwarzecker B, et al. Progression of paw swelling and loss of grip strength.

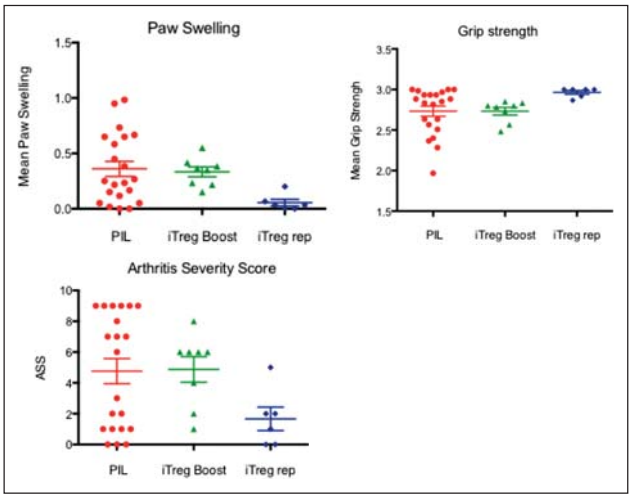

Figure 5: Schwarzecker B, et al. Clinical signs of arthritis and Arthritis Severity Score.

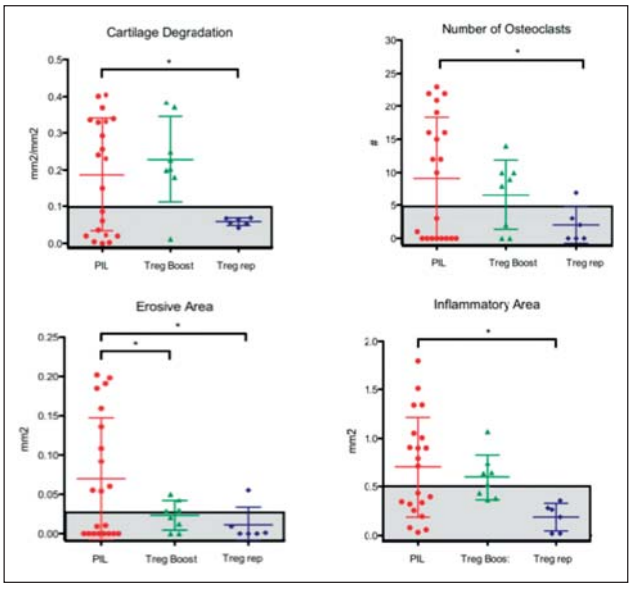

Figure 6: Schwarzecker B, et al. Histological parameters of arthritis.

Lymphocyte Infiltrate in Skin Biopsies of Systemic Sclerosis Patients After Successful Rituximab Therapy 36

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Aim B cell depletion has been proposed as a treatment option in severe systemic sclerosis. We previously reported about the beneficial clinical effect of rituximab (RTX) on skin and lung changes in a case series of systemic sclerosis patients. Even capillary changes

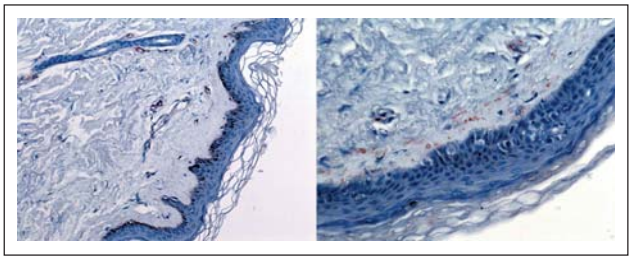

Figure 7: Kielhauser SM, et al. Staining of LAMA4 in the endothelial cells of SKL6 before and after rituximab therapy.

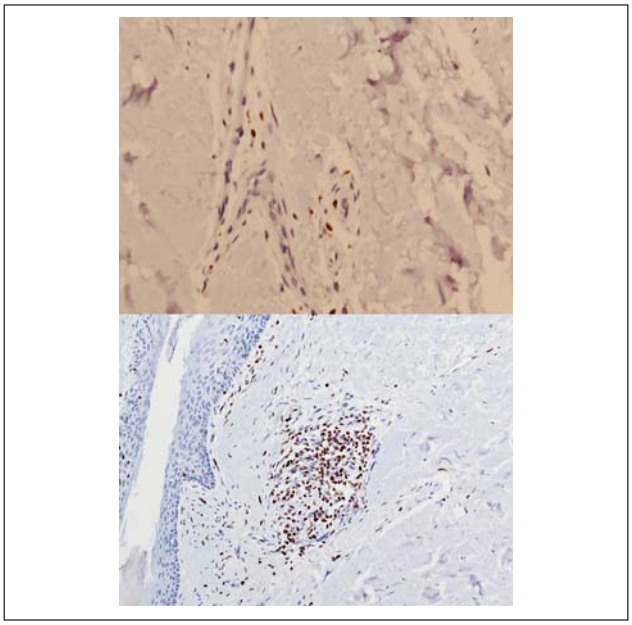

Figure 8: Kielhauser SM, et al. CD3+ lymphocytes in scleroderma skin before and after rituximab therapy.

are documented to be normal after rituximab therapy. We could show that the clinical improvement of the patient is not associated with the absence of $CD20^+$ cells in the peripheral blood. We investigated the effect of RTX on the phenotype of skin lymphocytes and endothelial cells during rituximab therapy.

Methods Five female patients fulfilling the ACR criteria for diffuse systemic sclerosis were biopsied before and after 3 cycles of rituximab therapy. All patients were on a stable therapy with mycophenolat mofetil and rituximab 500 mg twice every 3 months. All patients signed an informed consent proven by the local ethics committee. The biopsies were taken from affected areas of the forearm. Paraffin sections were stained for LAMA4, $CD3^+$ (T cells), $CD68^+$ (macrophages), and $CD20^+$ (B cells). The stained sections were blinded and semiquantitatively (0, 1, 2) investigated from an experienced pathologist.

Results 5/5 patients showed an increased LAMA4 staining before rituximab therapy and a decreased signal after therapy **(Figure 7)**. 4/5 patients showed an increased $CD3^+$ perivascular infiltrate after rituximab therapy compared to the rituximab-naïve samples **(Figure 8)**. 1/5 patient showed no difference of $CD3^+$ cells. Interestingly there was an increased number of $CD68^+$ cells perivascular in the post rituximab samples. CD 20^+ cells vanished in all specimens of treated scleroderma patients.

Summary/Conclusion Further characterization of perivascular lymphocytes is needed in order to clarify a possible "anti-fibrotic, anti-inflammatory" effect of RTX. The increase of LAMA4 expression in the endothelium maybe indicating a higher recruitment of "inflammatory" leukocytes in systemic sclerosis.

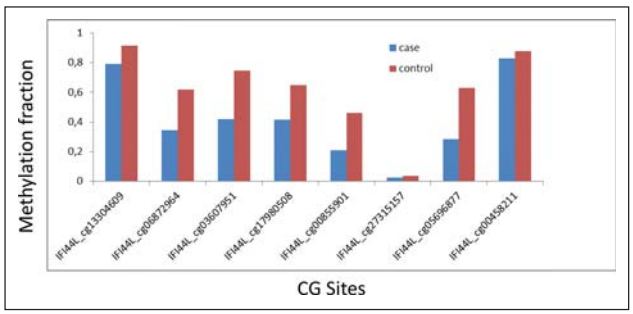

Figure 9: Ficjan A, et al. IFI44L gene DNA methylation in iNKT cells from lupus patients and controls. DNA methylation fractions across the CG sites evaluated in the IFI44L gene.

Hypomethylation of Interferon-Regulated Genes in Invariant NKT Cells from Lupus Patients 37

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Purpose To characterize the DNA methylome of invariant natural killer T (iNKT) cells in patients suffering from systemic lupus erythematosus.

Methods We performed a genome-wide DNA methylation study in 12 lupus patients and 12 age-matched healthy controls. Lupus patients fulfilling the ACR criteria were recruited consecutively; clinical data (including symptoms, laboratory findings, medication, and disease activity scores) and blood were collected at the same visit. Invariant NKT cells were separated from PBMCs at purities > 90 % (confirmed by flow cytometry using antibodies against CD3 and 6B11) using anti-iNKT Micro Beads (Miltenyi Biotec). DNA of iNKT cells was isolated using QIAamp DNA micro Kit (Qiagen). DNA methylation was quantified for > 485,000 methylation sites across the genome using the Illumina Infinium HumanMethylation450 Bead chip array. Differentially methylated sites were defined as CG sites with an average level of at least 1.2-fold after adjusting for multiple testing (FDR < 5 %).

Results Analysing the average level of DNA methylation of the whole genome revealed that iNKT cells of lupus patients exhibit globally hypermethylated DNA, with an average methylation level of 0.522 (CI: 0.5185–0.5245) in lupus patients and 0.515 (CI: 0.5119–0.5186) in controls (p = 0.014, Mann-Whitney-U Test). The average methylation level of the whole genome does not correlate with disease activity measured by SLEDAI and ECLAM and is not influenced by immunosuppressive medication. We identified 19 differentially methylated CG sites between patients and controls in 12 genes, with the majority (14) being hypomethylated. The entirety of hypomethylated genes is interferon-regulated. Significantly hypomethylated genes include IFI44L, MX1, and PLSCR1. We show consistent hypomethylation across multiple CG sites in the promoter region of IFI44L in iNKT cells. Regression analysis revealed correlation of the methylation level of cg00855901 (IFI44L) and ECLAM/SLEDAI ($r^2 = 0.334$, B coefficient = –8.577), suggesting that hypomethylation of this position is associated with disease activity. To ensure differential methylation of the CG sites affiliated to IFI44L between lupus patients and controls not being influenced by Chloroquin, Mycophenolate-mofetil (MMF), and prednisolon treatment, we performed a subset analysis in Chloroquin-, MMF-, and prednisolon-treated and non-treated patients. One out of the 4 differentially methylated CG loci of IFI44L (cg06872964) between lupus patients and controls was differentially methylated when comparing prednisolon-treated versus non-prednisolon-treated patients (Mann-Whitney-U Test; p = 0.026). Chloroquin and MMF treatment did not interfere with the methylation level of the differentially methylated CG loci of IFI44L (Kruskal-Wallis Test; **Figure 9**).

Conclusion We identified DNA methylation changes in iNKT cells from lupus patients for the first time. In contrast to CD4 T cell DNA being hypomethylated in lupus patients, iNKT cells of lupus patients exhibit globally hypermethylated DNA. Consistent with results

of previous genome-wide methylation studies on total CD4 T cells and naïve CD4 T cells, we detected hypomethylation of interferon-regulated genes in iNKT cells of lupus patients. To determine the functional consequences of the methylation changes, further experiments including gene expression analysis have to be performed.

mTOR Plays a Decisive Role in the Rheumatoid Mesenchymal Tissue Response to Inflammation 38

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Accumulating evidence supports the concept that mesenchymal stroma cells, namely fibroblast-like synoviocytes (FLS), actively participate in the destructive, inflammatory process of rheumatoid arthritis (RA). FLS maintain a synovial microenvironment that helps to recruit, retain, and activate immune cells, resulting in chronic inflammation with attendant joint destruction. Here we provide evidence that the mechanistic target of rapamycin (mTOR), which overall has evolved as a critical determinant for the maintenance of tissue homeostasis and function, is also a critical mediator in this regard. By using western blot or advanced cell biological methods, such as a recently described synovial tissue culture system, we show that TNF activates the mTOR pathway in FLS. Loss-of-function experiments using the specific mTOR-inhibitor, Torin-1, in combination with genome-wide transcriptome analysis, further reveal that mTOR decisively shapes the gene expression programmes induced by this TNF. Thus, inhibition of mTOR by Torin-1 increased the TNF-induced expression of genes known to be regulated by NFκB (e.g. PTGS2, IL6, IL8). On the other side, mTOR inhibition suppressed the TNF-mediated induction of interferon-regulated genes (IRG), including TNFSF10, CXCL11, and TNFSF13B. Overall, these studies provide insight into new determinants of the synovial tissue response to inflammation and suggest a multifaceted regulatory role for mTOR in synovial inflammatory processes.

Effects of Sodium Hydrogen Sulfide on Two Models of Experimental Arthritis 39

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Aim Rheumatic and musculoskeletal diseases (RMDs) are the major cause of disability world-wide. Hydrogen sulfide (H_2S) is a member of the gasotransmitter family and has emerged as a promising agent for resolution of inflammation in different diseases. H_2S is applied to patients suffering from RMDs in form of sulfur bath therapies, but information about its effectiveness is still poor. It was the objective of this study to investigate the *in vivo* effects of H_2S in 2 murine models of arthritis.

Methods For toxicological evaluation, mice were injected 3 times a week intraperitoneally (i.p.) with different concentrations of sodium hydrogen sulfide (NaHS). Two hours before the end of the experiment, 50 % of mice were challenged with LPS. Collagen-induced arthritis (CIA) is a well-established mouse model for rheumatoid arthritis (RA) in which collagen type II is injected twice subcutaneously, primary immunization and boost, respectively. 12–16 days later the development of disease is noticeable with an incidence between 60 % and 80 %. NaHS was applied 2 times before the first collagen injection and 6 times between the first collagen injection and the boost. On day 57 the mice were sacrificed and blood, spleen, lymph nodes, and paws were collected for detailed analysis. Serum transfer from K/BxN mice, which spontaneously develop severe arthritis, to C57BL/6 mice rapidly induces the development of arthritis with 100 % incidence. 150 µl serum were injected i.p. on day 0 and 2. NaHS was applied on days –1, 1, 3, 6, and 8. The experiment was terminated on day 9 and blood, spleen, and paws were collected.

Results Mice that received a 0.4 mM NaHS solution did not show signs of intoxication. Remarkably, in LPS-challenged mice treated with 0.4 mM NaHS serum levels of several cytokines were reduced compared to placebo-treated animals. However, NaHS treatment did not influence clinical outcome nor histological parameters in mice with CIA. In contrast, in mice with serum transfer arthritis the destruction of cartilage and bone as well as the number of osteoclasts were significantly reduced after NaHS treatment while the effects on inflammation and clinical scores were much less pronounced.

Summary/Conclusion Since prophylactic NaHS treatment starting before disease induction had no influence on severity of disease nor outcome of CIA, we assumed that sulfur treatment does not affect B and T cells which was supported by flow cytometric analysis of splenocytes and lymphocytes and the lack of effect on production of anti-collagen antibodies, which are the pathogenetic key players in this model. Therefore, we decided to continue our investigations in the serum transfer arthritis model which largely reflects the effector phase of arthritis in which mainly the innate immune system is involved. Although NaHS treatment did not influence clinical arthritis scores and had little effect on inflammation, bone erosion as well as the number of osteoclasts and cartilage loss were significantly reduced. These findings are in line with recently published data that NaHS treatment inhibits osteoclastogenesis, both in murine bone marrow cells and human $CD11b^+$ cells, and may encourage the development of a new class of sulfur-releasing drugs for treatment of bone diseases such as RA or osteoporosis.

The Role of Dendritic Cells During Inflammatory Arthritis 40

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Introduction Dendritic cells (DCs) play an important role in bridging the innate and the adaptive immune response by serving as antigen-presenting cells and are therefore implicated in the initiation of chronic autoimmune diseases, including rheumatoid arthritis. Using 2 different models of inflammatory arthritis, K/BxN serum transfer arthritis as well as hTNFtg arthritis, both depending only on the innate immune system, we investigated the innate role of dendritic cells in inflammatory arthritis.

Methods We analyzed histological sections of K/BxN serum transfer arthritis as well as hTNFtg arthritis for the presence of $CD11c^+$ cells by immunohistochemistry. We also performed synovial biopsies and analyzed the cellular composition of the inflammatory infiltrate with respect to DCs. We used CD11c-diphteria toxin receptor (DTR) transgenic mice, which express the human diphtheria-toxin receptor under the CD11c promoter, allowing for specific depletion of $CD11c^+$ cells by administration of diphtheria toxin (DT). K/BxN serum transfer arthritis was induced, and mice were given either DT or PBS. In addition, CD11c DTR mice were crossed into hTNFtg animals and also received either DT or PBS. The severity of arthritis was determined clinically and histologically.

Results We show that $Cd11c^+$ cells are present in significant numbers in the synovia of K/BxN- and TNF-driven arthritis. Both myeloid dendritic subsets, $CD8^+$ $CD11c^+$ and $CD11b^+$ $CD11c^+$, can be found in synovial tissue. In K/BxN serum transfer arthritis, clinical scores showed that CD11c-DTR transgenic mice that received DT had significantly reduced paw swelling and loss of grip strength compared to PBS-treated animals. Histological analysis found reduced inflammation after the depletion of $CD11c^+$ cells in K/BxN arthritis. In addition local bone destruction and the number of osteoclasts were significantly reduced. Also in TNF-driven arthritis in CD11c-DTR/hTNFtg mice, depletion of $CD11c^+$ cells led to a significant reduction of synovial inflammation as well as local bone erosions. To exclude unspecific effects of DT in mice, wild-type ani-

mals who received DT showed identical clinical and histological signs of arthritis as PBS-treated animals.

Conclusion These data show that CD11c$^+$ cells are involved in innate reactions leading to inflammatory arthritis and suggest that dendritic cells could be an important therapeutic target for patients suffering from rheumatoid arthritis.

Ly6C-Resident Monocytes with Osteoclastogenic Potential Arise Before Clinical Onset of Arthritis 41

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Introduction Bone erosions and systemic bone loss in rheumatoid arthritis patients results from an increased activity of osteoclasts which are derived from precursor cells of the myeloid lineage. Although there is much known about the mechanisms regulating the formation and activation of mature osteoclasts, the identity of an osteoclast precursor population in and its regulation by inflammatory cytokines during arthritis is poorly understood.

Methods HTNFtg mice were clinically scored once per week for grip strength and swelling. In addition, blood was collected every week starting on week 4. Mice were sacrificed at week 10, and blood, spleen, and bone marrow were collected for flow cytometry analysis. CCR2$^{-/-}$ mice were crossed into hTNFtg mice and histological analysis was performed. Different monocyte subsets were Facs-sorted and cultured in the presence of RANKL and MCSF to induce osteoclasts.

Results We show that during TNF-driven arthritis CD11b$^+$ CD115$^+$ cells are elevated in blood before the onset of clinical symptoms and remain elevated throughout. These cells are also elevated in spleen and bone marrow during arthritis. In blood, these cells can be separated by their expression of Ly6C into inflammatory monocytes (CD115$^+$Ly6Chigh) and resident monocytes (CD115$^+$Ly6Clow). Interestingly, especially resident monocytes are elevated preclinically. Upon sorting resident and inflammatory monocytes from blood, we demonstrate that only resident monocytes are able to form multinucleated TRAP$^+$ osteoclasts, but inflammatory monocytes do not. In order to further investigate the role of these monocyte subsets in the development of arthritic bone destruction and osteoclast formation, we used CCR2-deficient mice, which lack circulating inflammatory monocytes, and crossed them into hTNFtg animals. In line with our *in vitro* data, hTNFtg mice lacking CCR2 (i.e. inflammatory monocytes) showed no reduction in the amount of joint destruction but even enhanced local bone erosion and osteoclast generation.

Conclusion CD115$^+$ CD11b$^+$ cells, especially Ly6C-resident monocytes with osteoclastogenic potential, increase during inflammatory arthritis. Elevated numbers of these cells can be detected before clinical onset of disease and therefore may provide a biomarker for erosive inflammatory arthritis and even a possible target for therapeutically intervention.

Fibroblast Network Serves as Guiding Structure for Directed Monocyte Migration in 3D Synovial Culture 42

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Aim The synovial lining tissue consists of fibroblast-like synoviocytes (FLS) and monocyte-derived macrophage-like synoviocytes (MLS) within a self-built meshwork of dense extracellular matrix (ECM) components. FLS are thought to direct ECM synthesis, assembly, and degradation. Whether FLS themselves or the ECM network serve as guiding structures for MLS migration is incompletely understood.

Methods We studied the dynamics of synovial tissue modelling as well as MLS migratory behaviour using a 3D synovial tissue *in vitro* model. Human FLS were prepared from synovial tissues obtained as discarded specimens following joint arthroplasty. CD14$^+$ monocytes (MO) were isolated from peripheral blood. FLS and MO were labelled with fluorescent membrane dyes and cultured in spherical extracellular matrix micromasses with an average size of 1.5 mm for up to 2 weeks. Second harmonic generation was used for the visualization of collagen fibers (ECM). Cell migration was monitored in individual micromasses by real-time confocal/multi-photon microscopy.

Results The formation of a synovial lining-like layer on the outside of the 3D cell culture takes 2–3 days. The first signs of collagen fibers co-localized with FLS clusters and appear as early as day 1. They increase density alongside the establishment of the lining layer. The majority of MO was found to be in close contact with the FLS network with low tendency for migration. A minor fraction of MO displayed directed cell movement with an impressive maximum speed of up to 15 mcm/min. MO migration occurred in intimate contact with FLS, however, did not necessarily follow FLS network boundaries, but rather the cell cultures surface. For sitting as well as migrating MO, contact with FLS seems to be more important than contact with ECM.

Summary/Conclusion The 3D synovial tissue culture system allows for monitoring and analyzing the dynamics of synovial lining modelling. Both, FLS and MO, appear to cooperate in the organization of the synovial lining tissue with subtle migration patterns of MO in relation to the organized synovial lining architecture. Ongoing experiments address molecular mechanism(s) of MO-FLS interaction in order to identify potential targets for future therapeutic intervention in arthritis.

CD4$^+$CD25$^-$Foxp3$^+$ T Cells: A Marker for Lupus Nephritis? 43

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Introduction Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease, which can affect different organs. Increased proportions of CD4$^+$CD25$^-$Foxp3$^+$ T cells have been described in SLE patients. The exact role of this cell population in SLE patients still remains unclear. We therefore analyzed this T cell subset in a large cohort of SLE patients with different organ manifestations.

Methods Phenotypic analyses, proportions, and absolute cell numbers of CD4$^+$CD25$^-$Foxp3$^+$ T cells were determined by flow cytometry (FACS) in healthy controls (HC; n = 36) and SLE patients (n = 61) with different organ manifestations. CD4$^+$CD25$^-$Foxp3$^+$ T cells were correlated with clinical data, the immunosuppressive therapy, and different disease activity indices. In patients with active glomerulonephritis, CD4$^+$CD25$^-$Foxp3$^+$ T cells were analyzed in urine sediment samples. Time course analyses of CD4$^+$CD25$^-$Foxp3$^+$ T cells were performed in patients with active disease activity before and after treatment with cyclophosphamide and prednisone.

Results CD4$^+$CD25$^-$Foxp3$^+$ T cells were significantly increased in active SLE patients and the majority expressed Helios. Detailed analysis of this patient cohort revealed increased proportions of CD4$^+$CD25$^-$Foxp3$^+$ T cells in SLE patients with renal involvement. CD4$^+$CD25$^-$Foxp3$^+$ T cells were also detected in urine sediment samples of patients with active glomerulonephritis and correlated with the extent of proteinuria.

Conclusion CD4$^+$CD25$^-$Foxp3$^+$ T cells resemble regulatory rather than activated T cells. Comparative analysis of CD4$^+$CD25$^-$Foxp3$^+$ T cells in SLE patients revealed a significant association of this newly described cell population with active nephritis. Therefore CD4$^+$CD25$^-$Foxp3$^+$ T cells might serve as an important tool to recognize and monitor SLE patients with renal involvement.

Table 4: Dragoi R, et al. Comparison between treatment of corticosteroid injection and platelet-rich plasma (PRP). ns: not significant.

Knee osteoarthritis	WOMAC initial assessment				WOMAC 3-month follow-up				HAQ initial assessment	HAQ 3-month follow-up
Corticosteroid injection (n = 20)	10.04	3.12	36.56	47.86	5.30	2.10	20.08	27.18	2.60	1.60
PRP (n = 20)	9.74	3.18	36.67	46.34	5.78	2.32	21.1	28.34	2.80	1.65
Subgroup t test	p = ns	p = ns	p = ns	p = ns	p = ns	p = ns	p = ns	p = ns	p = ns	p = ns

E. Physikalische Medizin und Rehabilitation

Comparison of Functional Improvement and Quality of Life in Knee Osteoarthritis Between Treatment of Corticosteroid Injection and PRP (Platelet-Rich Plasma) 44

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Aim To evaluate effectiveness of corticosteroid intra-articular injection versus PRP on functional and quality-of-life changes in knee OA patients, keeping in mind that PRP is a new treatment used in repairing of musculoskeletal disorders, some studies showing short-term efficacy of this treatment.

Method 40 patients with mild and moderate knee OA (Kelgren Lawrence grades I–III) and symptoms of > 3 months duration were included in the study. Patients were evaluated using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) for assessing joint stiffness and the Health Assessment Questionnaire (HAQ) for the quality of life at baseline, before the procedure, and at 3 months follow-up. Patients were randomized into 2 equal (n = 20 patients) subgroups with similar characteristics (sex, age equally distributed).

Results There were no adverse effects reported at 3 months follow-up for either of the method of treatment chosen. Mean age of patients is 62.96 ± 3 in both subgroups. Follow-up at 3 months demonstrated improved WOMAC and HAQ compared to baseline in both subgroups independently of the treatment methods chosen without any statistical significance difference observed between subgroups using the student t test **(Table 4)**.

Conclusions Both groups treated had better results than at baseline. There was no statistically significant difference between the subgroups. PRP showed to be a viable alternative to corticosteroid injection, but taking into consideration that PRP is a more laborious technique, a clinician may prefer using a corticosteroid injection. Studies on larger cohorts and with a longer follow-up period are needed in order to establish the effect of PRP in knee osteoarthritis.

F. Sonstiges

Österreichische aktualisierte Ernährungsempfehlungen bei Gicht und Hyperurikämie 2014 45

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Ziel Gicht ist bei Männern mittlerweile die häufigste entzündliche Gelenkserkrankung in der westlichen Welt. Der Zusammenhang mit diversen Komorbiditäten wie KHK, metabolischem Syndrom und chronisch renaler Insuffizienz ist bewiesen. Eine Hyperurikämie tritt in unseren Breiten bei bis zu 20 % sowohl bei Männern als auch bei Frauen auf. Es ist hinlänglich bekannt, dass Diätfehler, übermäßige Alkoholzufuhr sowie manche purinreiche Nahrungsmittel einen nachgewiesenen negativen Effekt auf die Erkrankung haben. In den letzten Jahren haben Untersuchungen betreffend Ernährung, Lebensstil und Gewichtsverlauf vor allem an Männern aber wesentliche neue Erkenntnisse erbracht, was eine Überarbeitung früherer Ernährungsempfehlungen notwendig gemacht hat.

Methoden Der Arbeitskreis für Arthrose und Kristallarthropathien der ÖGR (Österreichische Gesellschaft für Rheumatologie und Rehabilitation) hat eine Literaturrecherche zu Ernährung und Lebensstil für Gicht- und Hyperurikämie-Patienten durchgeführt. Unter Heranziehung dieser aktuellen Literatur wurden – unter Anwendung eines Delphi-Prozesses – insgesamt 9 Ernährungs- und Lebensstilempfehlungen (4 Don'ts bzw. 3 Do's zu Nahrungsmitteln und 2 allgemeine Lebensstilempfehlungen) in Form eines Folders erstellt.

Ergebnisse Der Folder ist 2-seitig aufgebaut, wobei auf einer Seite die 9 Empfehlungen, versehen mit der jeweiligen Evidenz und den relevanten Literaturzitaten (für Ärzte), aufgelistet sind. Auf der 2. Seite sind die Empfehlungen in einem mehrfärbigen Kreis bildlich als Icons dargestellt, was auch eine nonverbale Informationsübermittlung (z. B. bei bestehender Sprachbarriere) möglich macht.

Zusammenfassung/Schlussfolgerung Es ist gelungen, 9 aktuelle Ernährungs- und Lebensstilempfehlungen für Gicht und Hyperurikämie sowohl für Patienten als auch für Ärzte zu erstellen. Dies trägt sowohl dem Interesse der betroffenen Patienten, ihre Erkrankung mittels Ernährungs- und Lebensstilmodifikation positiv zu beeinflussen, als auch dem Bedarf an anschaulichem Informationsmaterial für niedergelassene Kollegen Rechnung.

Osteomyelitis oder doch Daktylitis? Wie eine geplante Großzehenamputation verhindert werden konnte 46

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Ziel Die Notwendigkeit der interdisziplinären Zusammenarbeit zur Diagnosefindung bei zunächst klarer Befundlage, die durch das Auftreten zusätzlicher Symptome eine Reevaluierung der Diagnose und Therapie notwendig macht, wie in unserem Fall durch ein rheumatologisches und dermatologisches Konsilium.

Methoden Bei einer 64-jährigen Patientin trat zunächst eine schmerzhafte Rötung und Schwellung der gesamten 1. und 2. Zehe rechts auf. Trotz mehrfacher lokaler und systemischer Therapien zeigte sich keine Besserung der Beschwerden. Bildgebend wurde in der MRT-Untersuchung die Diagnose einer Osteomyelitis mit bereits osteodestruktiven Prozessen beschrieben. Eine Antibiose mit verschiedenen Regimen brachte jedoch nicht die erhofften therapeutischen Erfolge. Laborchemisch erwähnenswert war nur eine leichtgradig beschleunigte BSG (22/36 mm). Aufgrund der frustranen Therapieversuche bei gleichzeitig deutlicher Zunahme der Beschwerden und ausgehend von der Diagnose einer therapierefraktären Osteomyelitis mit bereits osteodestruktivem Verlauf wurde eine Amputation der Großzehe als noch verbleibende Therapieoption erwogen. Zusätzlich zu den oben erwähnten Symptomen zeigten sich

nun nach zirka einem Jahr Krankheitsdauer noch psoriasiforme Hautveränderungen an Handflächen und Fußsohlen. Nach Bestätigung der Diagnose einer Psoriasis palmoplantaris durch die Dermatologin erfolgte die Zuweisung zum Rheumatologen mit dem Ziel einer Reevaluierung der bisherigen Diagnose einer Osteomyelitis. Die nunmehr durchgeführte konventionelle Röntgenuntersuchung beschrieb dann auch die typischen Veränderungen einer Psoriasisarthropathie. Diese fächerübergreifende Evaluierung der Patientin durch den Rheumatologen und Dermatologen führte nun zu einer Änderung der Diagnose, nämlich von einer ursprünglich angenommenen Osteomyelitis zu einer Daktylitis und somit auch zur dringenden Indikation einer TNF-α-AK-Therapie. Wir entschieden uns für eine Therapie mit Adalimumab (40 mg s.c. alle 2 Wochen).

Ergebnisse Bereits nach wenigen Wochen zeichneten sich die ersten Therapieerfolge ab und die geplante Amputation konnte damit glücklicherweise verhindert werden. 4 Monate nach Therapiebeginn mit Adalimumab zeigte sich im Verlauf eine deutliche Befundbesserung. Derzeit spritzt die Patientin die Adalimumabtherapie alle 3 Wochen bei anhaltendem Therapieerfolg.

Zusammenfassung/Schlussfolgerung Im hier gezeigten Fallbericht präsentieren wir eine Patientin mit einer anfangs vermuteten therapierefraktären Osteomyelitis der 1. und 2. Zehe rechts. Aufgrund der vielfachen frustranen Vortherapien wurde bereits die Indikation zur Amputation gestellt. Nach einer im Verlauf neudiagnostizierten Psoriasis palmoplantaris wurde vom Rheumatologen die Diagnose einer Daktylitis vermutet und eine Therapie mit Adalimumab begonnen. Diese Therapie zeigte dann im Verlauf ein rasches und anhaltendes Ansprechen. Die Amputation konnte somit glücklicherweise verhindert werden. Dieser Fallbericht unterstreicht einmal mehr die immens wichtige interdisziplinäre Zusammenarbeit zwischen den Fachärzten für Dermatologie und Rheumatologie.

Modulation of IL-1β-Stimulated Chondrocytes by Mild Mechanical Stimulation Is Tuned by the Disease-Modifying Osteoarthritis Drug Diacerein 47

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Background Disease-modifying osteoarthritis drugs (DMOADs) in OA therapy emerged to preserve normal joint function and reduce disease intensity and symptoms whereby progression rate of OA can be restrained [1]. It has been demonstrated that the DMOAD diacerein reduces the severity of OA and downregulates the interleukin-1β (IL-1β)-induced inflammatory pathways [2]. Alternatively, dynamic loading of joints also discussed as mild mechanical stimulation as a non-drug treatment modality in OA patients has demonstrated its effectiveness on the reduction of pain and improvement of function [3, 4]. *In vitro* studies implicated a direct mechanosensitive, anti-inflammatory effect in joint tissues exposed to moderate levels of cyclic hydrostatic pressure. Furthermore, we presume that diacerein-induced effects might be due to changes in intracellular calcium.

Objective To investigate the combination of mild mechanical stimulation and diacerein treatment in inflammatory-activated chondrocytes and to demonstrate at the cellular level if the combination of drug and exercise therapy might function as a model for an integrated biophysical approach for osteoarthritis (OA) treatments.

Methods Interleukin-1beta (IL-1β)-stimulated C28/I2 cells underwent mild mechanical treatment while cultured in the presence of diacerein. The pharmacological input of diacerein was evaluated by cell viability and cell proliferation measurements. Inflammation and treatment-induced changes in key regulatory proteins and components of the extracellular matrix were characterized by quantitative real-time PCR (qPCR). In addition, the effects on metalloproteinase-1 (MMP-1) activity and glycosaminoglycan (GAG) concentration in cell supernatants of treated cells were investigated. Calcium imaging on Fura-2-loaded chondrocytes were performed.

Results C28/I2 cells reacted to IL-1β stimulation by significant changes in the expression of inflammatory and cartilage destructive. Diacerein in mechanically stimulated cells caused a decrease in interleukin-8 (IL-8), fibronectin-1 (FN-1), collagen type-I (Col 1), and MMP-1 expression levels, respectively. Increase in interleukin-6 receptor (IL-6R) and the fibroblast growth factor receptor (FGFR) expression by diacerein was not abolished by mechanical treatment of the cells [4]. The observed effects were accompanied by a reduced cell proliferation rate, attenuated cell viability, and extenuated MMP-1 activity. Studying the histamine-induced calcium release under diacerein, we were able to observe a significant reduction of the calcium transient.

Conclusion Diacerein obviously influences the expression of several main regulatory proteins involved in signal transduction as well as factors necessary for regeneration and setup of the extracellular matrix. Application of mild mechanical stimuli did not reversely influence the chondroprotective effect induced by diacerein treatment in immortalized human C28/I2 chondrocytes. Measuring the modulation of intracellular calcium by diacerin might give us new insights of how diacerein exerts its role within OA.

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Decreased Pristane-Induced Lupus Severity in Long-Term MiR155-Deficient Mice 48

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Background/Purpose MicroRNAs (miRs) are an important class of regulators of gene expression that are associated with a variety of biological functions. Deregulation of endogenous miR155 was observed in many autoimmune conditions, including SLE. We herein examine the role of miR155 in the development of systemic manifestations in murine pristane-induced lupus by evaluating the severity of organ involvement and assessing serum antibody levels and T helper cell homeostasis.

Methods MiR155-deficient (miR155-PIL) and C57/Bl6 (WT-PIL) mice were injected i.p. with 0.5 ml of pristane or PBS as control (WT-PBS). In order to observe the effects of miR155 deficiency in fully developed SLE, we analyzed these mice 8 months after induction. A blinded specialist appraised histological features of GN using the composite kidney biopsy score (KBS). Histological features of pneumonitis were quantified by an image analysis system: lungs were scored for the severity of perivascular inflammation by analyzing the numbers of affected vessels and the area of the inflammatory infiltrate. In order to assess the composition of these infiltrates, specimens were stained with B220 (B), CD3 (T), Neu7/4 (neutrophils), and F4/80 (macrophages) and analyzed by cell-identification algorithms for nuclear segmentation (HistoQuest®). Lymphocytes were isolated from spleens and analyzed separately for each mouse by standard FACS procedures. For analysis of the Th1, 2, or 17 subsets, respectively, cells were re-stimulated *in vivo* with anti-CD3 and anti-CD28abs.

Results Lungs were affected in both pristane-treated groups, but not in controls. MiR155-PIL had reduced lupus severity as indicated by significantly decreased perivascular inflammatory area with B cells being the most prominent inflammatory cell type in the HistoQuest analysis. Without showing clinical abnormalities, WT-PIL had a more severe renal involvement in the kidney biopsy score than miR-PIL. Corresponding with reduced severity in organ involvement, miR155-PIL had lower serum levels of anti-dsDNA-abs, decreased frequencies of $CD4^+$ cells (14.24 ± 0.7587 vs 18.04 ± 1.075; p = 0.01), and slightly lower frequencies of activated $CD4^+CD25^+$ $Foxp3^-$ cells (1.539 ± 0.1279 vs 1.838 ± 0.2259; p = ns.). Interestingly, also frequencies of $CD4^+CD25^+Foxp3^+$ regulatory T cells were lower in Mir155-PIL (1.689 ± 0.1388 vs 2.375 ± 0.2320; p = 0.03). Upon restimulation, $CD4^+$ cells showed a more pronounced Th2 and Th17 response in WT-PIL, but no significant differences in Th1 phenotype.

Conclusion MiR155 deficiency in PIL mice did not prevent the development of disease, but was associated with less severe lung and kidney involvement, lower serum auto-abs levels, and lower Th17 and Th2 frequencies when analyzed in fully established PIL after 8 months. Thus, antagonisation of miR155 might be a beneficial future approach in treating SLE.

The Role of MicroRNA-155 in Autoimmune Arthritis 49

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MicroRNAs are a novel class of regulatory RNAs that have been demonstrated to control several important biological processes. They have been implicated in the pathogenesis of auto-immunity; however, their true contribution in these diseases is not truly clear. MicroRNA-155 (miR-155) has been demonstrated to be present in inflamed joints of patients suffering from rheumatoid arthritis (RA). We have previously shown that miR-$155^{-/-}$ mice are completely protected from collagen-induced arthritis (CIA) due to the absence of pathogenic autoreactive B and T cells and low auto-anti-collagen antibodies. Our main goal is to understand the role that innate and adaptive immunity play in the resistance to experimental autoimmunity of miR-$55^{-/-}$ mice. In the hTNF innate immunity-dependent arthritis model we saw no difference in the development of the disease between miR-$155^{-/-}$ and wild-type (wt) mice. Analysis of antigen-presenting cells (APC) lacking miR-155 showed no alteration in the expression of co-stimulatory molecules or MHCII after *in vivo* and *in vitro* stimulation. Pro-inflammatory cytokine production (such as IL-12p40 and IL-6) *in vivo* was similar in serum of wt and miR-$155^{-/-}$ mice after induction of CIA or after *in vivo* stimulation with LPS. In addition, the T cell stimulatory capacity of wt and miR-$155^{-/-}$ was identical *in vitro* and *in vivo*. In contrast, miR-155-deficient OTII T cells (which have a transgenic expression of MHC class II-ovalbumin specific) showed severely impaired proliferation upon stimulation with antigen-presenting cells *in vitro* as well as *in vivo*. However, *in vitro* stimulation with anti-CD and anti-CD28 antibodies of wt or miR-$155^{-/-}$ naïve T cells resulted in similar proliferation. In conclusion, contrasting with its limited influence in innate immunity-dependent arthritis, miR-155 plays a central role in adaptive autoimmune arthritis. Therefore, miR-155 might represent an interesting therapy target for autoimmune diseases such as rheumatoid arthritis.

The Synthetic Curcumin Derivative HP102 Shows Potent Anti-Inflammatory and Pro-Apoptotic Effects in Jurkat T Cells 50

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Aim Curcumin is a naturally occurring polyphenol produced in the rhizome of Curcuma longa. The potential positive health effects of curcumin (anti-inflammatory, anti-carcinogenic, and antioxidative properties) have long been known. Currently, many groups are working on curcumin derivatives to improve the stability, solubility, and activity of the natural compound. In this study, we compared the anti-inflammatory and pro-apoptotic potential of curcumin with 2 chemically synthesized curcumin derivatives in Jurkat T cells.

Methods Jurkat T cells (containing a luciferase reporter gene under the control of the IL-2 promoter) were stimulated with PMA/PHA in the absence or presence of different concentrations of curcumin, HP102, or HP109. Luciferase activity was measured 6 h after stimulation. IL-2 release was quantified by enzyme-linked immunosorbent assay (ELISA). Cell viability, proliferation, and apoptotic processes were monitored by XTT assay, Annexin-V/7-AAD staining, and Western blot.

Results Data show that treatment of Jurkat cells with curcumin or HP102 decreased IL-2 promoter activity and IL-2 expression in a concentration-dependent manner. However, compared to curcumin, HP102 was about 10 times more effective than curcumin in inhibiting IL-2 synthesis. Similar effects were observed by monitoring apoptosis via annexin-V staining and caspase activation. In contrast to HP102, cells treated with HP109 showed only a slight decrease in IL-2 production and did not induce apoptosis.

Summary/Conclusion HP102 strongly improves the anti-inflammatory and apoptotic potential of curcumin in Jurkat T cells and might be a useful tool for the supportive care in leukaemia or other forms of cancer in the future.

„Leben mit Lupus“ 51

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Ziel Der systemische Lupus erythematodes (SLE) ist eine systemische Autoimmunerkrankung mit vielen Gesichtern; die Symptome reichen von milden muskuloskelettalen Beschwerden bis zur lebensbedrohlichen Organmanifestation. Eine rasche Diagnosestellung und Therapieeinleitung ist bei dieser Erkrankung besonders wünschenswert, allerdings gibt es nur wenige verlässliche Zahlen über den Weg der Patienten bis zur richtigen Diagnose. Die Aktion „Leben mit Lupus“ soll hier neue Information liefern, Patientenwünsche aufzeigen und so zukünftige Verbesserungen ermöglichen.

Methoden Patienten wurden im Internet und mittels Schriftstücken und Postern in Ordinationen und Ambulanzen aufgefordert, anonym Fragen zu ihrer Krankengeschichte zu beantworten. Insgesamt wurden 118 Fragebögen ausgefüllt. 107 (91 %) Patienten waren weiblich, 11 männlich; 56 % waren < 40 Jahre alt, weitere 27 % zwischen 40 und 50, lediglich 18 % > 50.

Resultate Der erste Kontakt nach der Erstmanifestation von Lupussymptomen erfolgte meist über den Allgemeinmediziner (67 %). In den meisten Fällen wurde die Diagnose durch einen Rheumatologen gestellt (62 %). In 42 % erfolgte die Diagnose innerhalb eines Jahres, in weiteren 21 % innerhalb von 3 Jahren, bei 37 % dauerte es bis zur Erstdiagnose > 3 Jahre (3–12 Jahre). Die häufigsten Erstsymptome waren dabei durchaus typisch, wenn auch wenig spezifisch: extreme Müdigkeit (von 73 % genannt), Arthralgien (71 %), Fieber (49 %), Hautausschlag/Haarausfall (44 %), Sonnenunverträglichkeit (33 %). In 57 % der Patienten waren > 3, bei 14 % > 9 Arztbesu-

che einer Diagnose vorausgegangen. Unter Therapie besserten sich in der Regel die Symptome der Patienten: Fanden vor Therapie 89 % ihren körperlichen Zustand „schlecht" oder „sehr schlecht" (insgesamt 55 % „sehr schlecht", 43 % „schlecht", 10 % „mittel", 1 % „gut"), waren es unter Therapie nur mehr 40 %. Für jeweils > 60 % der Patienten ergaben sich durch die Erkrankung Probleme im Beruf, in der Freizeit und auf Reisen, interessanterweise gaben die meisten Patienten keine Probleme in Partnerschaft oder sozialem Umfeld an. Von 82 % der Patienten wurde der Wunsch geäußert, dass die Bevölkerung besser über SLE informiert werden sollte.

Schlussfolgerungen SLE kann meist gut behandelt werden, sobald die richtige Diagnose gestellt ist. Aufgrund der Vielgestaltigkeit der Erkrankung dauert es aber in > $^1/_3$ der Patienten > 3 Jahre bis zur Erstdiagnose. Die meisten Patienten im berufsfähigen Alter haben Probleme im Beruf durch ihre Erkrankung, im gut aufgeklärten näheren Umfeld (Partnerschaft, Familie) jedoch nicht. Es besteht Bedarf hinsichtlich einer Steigerung des Wissens um die Krankheit SLE, sowohl bei der (erstversorgenden) Ärzteschaft als auch in der allgemeinen Bevölkerung.

Thiol-Activated Hydrogen Sulfide Donors and Their Anti-Inflammatory Potential in the Mouse Macrophage Cell Line RAW 264 52

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Background Hydrogen sulfide (H_2S) is an important signalling molecule that regulates many physiological and pathophysiological processes in organs and cellular systems. Inorganic H_2S donors like sodium hydrogen sulfide (NaHS) rapidly enhance H_2S concentration. However, such fast generation does not reflect the situation *in vivo*. Therefore, synthetic donors have recently emerged as useful tools. In this study we report about the chemical and biological evaluation of a new class of organic H_2S donors (benzamide and penicillame derivatives) which release H_2S upon activation by thiols (cysteine or reduced glutathione).

Materials and Methods The H_2S-releasing compounds 5a, 8a, and yz-5-093 were synthesized and kindly provided by M Xian's group (Washington, USA). H_2S release was measured with a H_2S-selective microelectrode. In intact cells, H_2S production was monitored by the fluorescent probe WSP-1. To evaluate the anti-inflammatory potential of the H_2S donors *in vitro*, mouse macrophages (RAW 264 cell line) were incubated for 1 h with different concentrations of compounds 5a, 8a, and yz-5-093 (10–100 µM) before the cells were stimulated for 24 h with LPS. IL-6 and TNF-α levels in cell culture supernatants were quantified by enzyme-linked immunosorbent assays (ELISAs).

Results All compounds significantly downregulated IL-6 expression in RAW 264 cells. TNF-α expression, however, was largely unaffected seen by the fact that only the highest concentration used in the experiment (100 µM) was sufficient to obtain a significant reduction.

Summary We conclude that thiol-inducible H_2S donors represent a new scientific tool which allows to mimic the slow and continuous H_2S generation process *in vivo*. These donors will not only be useful for H_2S research but could also have therapeutic benefits in the treatment of chronic inflammatory diseases.

Effects of Free Curcumin and a Novel Nanocarrier System Called CurcuEmulsomes on Different Cell Types: A Comparative Study 53

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Aims The polyphenolic compound curcumin, which occurs naturally in the rhizome of the plant Curcuma Longa, has been shown to have potent anti-inflammatory and anti-cancer properties. Its medical use remains limited due to its extremely low water solubility and bioavailability. To bypass these problems, a novel nanocarrier system, so-called CurcuEmulsomes, where curcumin is encapsulated inside the solid core of emulsomes, was developed at the Department of Nanotechnology (BOKU, Vienna). The present study compares their effects with free curcumin on pro-inflammatory cytokine expression and proliferation in different cell types.

Methods Human synovial fibroblasts (SW982) were treated with different concentrations of free curcumin and CurcuEmulsomes for 24 or 48 hours, followed by stimulation with IL-1β to induce IL-6 expression. In addition, the effects of free curcumin and CurcuEmulsomes on their proliferation were investigated. Furthermore, murine macrophages (RAW264) were treated for 24 hours with different concentrations of free curcumin and CurcuEmulsomes and were then stimulated with LPS to induce TNF-α and IL-6 expression. IL-6 and TNF-α release was quantified by ELISA and the proliferation rate was determined by XTT-assay.

Results In SW982 cells, pre-treatment with free curcumin and CurcuEmulsomes for 48 hours significantly downregulated IL-1β-induced IL-6 expression. Similar effects were observed for the expression of IL-6 and TNF-α in RAW264 cells. Free curcumin led to a significant reduction of proliferation rate whereas CurcuEmulsomes did not influence the viability of SW982 cells.

Conclusion Our study demonstrated that CurcuEmulsomes have similar effects as free curcumin on the expression of pro-inflammatory cytokines in SW982 and RAW264 cells. In contrast to curcumin, CurcuEmulsomes did not reduce the viability of SW982 cells. In the future, we will further compare the effects of CurcuEmulsomes with free curcumin on viability, apoptosis, and cell cycle. In addition, we plan to use primary human synovial fibroblasts from healthy tissue and from patients suffering from rheumatoid arthritis.

Improving Patient Flow of People with Rheumatoid Arthritis Has the Potential to Simultaneously Improve Health Outcomes and Reduce Direct Costs 54

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Objective The purpose of the present study was to assess whether an improvement in the interdisciplinary management of RA patients has the potential to simultaneously improve health outcomes and reduce costs. We chose to model for joint destruction, i.e. radiographic progression, as the measure for health outcome and to use costs for physician visits and diagnostic tests as the indicator of direct costs.

Methods We used a health economic model to assess the effects of patient management on total costs. In a first step, we modelled the different ways which lead patients with RA to the correct diagnosis and the relevant specialist, respectively. We reconstructed the patient flow up to diagnosis in Austria, from onset of symptoms, presentation to GP or rheumatologist, GP referral to specialists, until, ultimately, the patient is diagnosed. After onset of symptoms, a patient has the option to consult with his/her GP, to see a specialist, i.e. a rheumatologist, directly, or to take advantage of the existing "Immediate Access Rheumatology Clinics" or office-based "Rapid Assessment Consultations" (only in Vienna and Upper Austria; not established nationwide). Data on practice patterns and diagnostic

steps taken at the different points of care were taken from a published study [Puchner et al.] and verified with practicing rheumatologists [Puchner et al.]. Patients with musculoskeletal disorders usually consult with their primary care physician first. On average, an RA patient experiences 3 GP visits before referral to a specialist. This, in turn, could have an effect not only on costs associated with diagnosis, but also on radiographic progression. To compare the current situation to a new approach, we first modelled the number of people with RA diagnosed within 3 months of symptom onset under current practice and the costs associated with diagnosis. We then compared this current situation against a reconfiguration of current practice towards a more proactive identification and referral method between GPs and specialists. We evaluated the impact of this reconfiguration on the number of RA patients diagnosed as well as the costs associated with the diagnosis. In addition to the cost impact, we incorporated radiographic progression and used estimated rates of progression and change in Larsen score before start of treatment as observed in a study by Wick et al.

Results Using data on epidemiology and Austrian practice patterns, we estimate a total of 13.767 people with undifferentiated arthritis (defined as people with symptoms similar to inflammatory arthritis which could be inflammatory, but also non-inflammatory arthritis) in Austria, of which 1645 suffer from inflammatory arthritis (IA). Modelling for diagnostic accuracy, we found that 1145 of these patients are misdiagnosed in a primary care setting. As inflammatory arthropathies are not straightforward to diagnose, even when consulting a specialist, an expected 91 of IA patients are not correctly diagnosed. However, there is a marked increase of diagnostic accuracy at the specialist level vs the GP level, as is expected. Our model found that a reconfiguration of current practice towards an approach of more integrated care with ports of call for each patient with suspected RA has the potential to be not only cost-effective, but cost-saving: EUR 95,596 could be saved yearly by exclusively adopting the new integrated approach in Austria.

Conclusions Our results show that by better directing the flow of people with RA, simultaneous benefits may be reaped: clinical and economic benefits. Initiation of care by a rheumatologist early on in the disease offers the potential for dramatically improving radiographic and clinical outcomes in patients with RA [Van der Linden et al.]. Our work further underscores the importance of Rapid Assessment Consultations, also in remote settings, as early diagnosis is crucial for patient prognosis. Institutionalizing early arthritis clinics benefits both colleagues in primary care and patients, while saving costs when compared to the current situation.

B-Cell Depletion Therapy in a Patient with Systemic Sclerosis Improves Parameters of Skin, Lung, and Heart Involvement 55

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Aim Recently, we reported on a beneficial effect of B-cell depletion therapy with rituximab in a case series of systemic sclerosis patients. Skin involvement as documented by the modified Rodnan skin score (mRSS) and the lung function test measured by DLCO and FVC improved by our therapy regimen. A 45-year-old female presented with diffuse scleroderma, alveolitis, and pathologic diastolic function at onset within 4 months after developing Raynaud syndrome. The patient had an antinuclear antibody titer of 1:5120 and pathologic capillary microscopy. Anti-centromer antibodies and anti-topoisomerase I antibodies were negative. Cardiac MRI was without any pathology but echocardiography showed impaired diastolic function.

Methods The patient fulfilled the ACR criteria for systemic sclerosis. Due to the rapid progression of skin and organ involvement, we administered rituximab 500 mg twice every 3 months. Before immunomodulatory treatment the patient received a vaccination against pneumococcal infection and influenza infection. Every 3 months the patient was seen by a rheumatologist and a pulmonologist. Echocardiography was performed at baseline and after 6 months by an experienced cardiologist.

Results During the course of rituximab therapy, the mRSS decreased from 29 to 4, the DLCO increased from 67 % to over 80 %, and diastolic function as assessed by E/e' became normal (from 16.33 to 10.36). The laboratory values were all within the normal range.

Summary/Conclusion In this patient, B-cell depletion was an effective therapy for systemic sclerosis regarding the skin and inner organs. In order to obtain a sustainable reversion of organ damage, it might be beneficial to start treatment early while the disease process is still at an inflammatory stage.

Ermittlung des Cut-off-Titers zur Bestimmung von antinukleären Antikörpern (ANA) mit indirekter Immunfluoreszenz auf HEp-2-Zellen 56

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Ziel Die indirekte Immunfluoreszenz (iIF) ist die Methode der Wahl zum Nachweis antinukleärer Antikörper (ANA) bei Patienten mit systemischen Autoimmunerkrankungen (SAE). Seit 1997 wird ein Cut-off-Titer von 1:100 empfohlen, um Patienten mit systemischem Lupus Erythematodes (SLE) von gesunden Personen zu unterscheiden [1]. In einem aktuell veröffentlichen internationalen Konsens [2] wird empfohlen, dass jedes Labor seinen eigenen Cut-off-Titer ermitteln sollte, um Unterschieden zwischen den Reagenzien verschiedener Hersteller, den verwendeten Mikroskopen und anderen lokalen Faktoren Rechnung zu tragen.

Methoden Eine Kontrollpopulation ohne Anzeichen einer SAE bestehend aus Blutspendern mit normaler Blutsenkungsgeschwindigkeit (BSG) und normalen CRP-Werten (A; n = 29), Patienten der Rheumaambulanz der Univ.-Klinik in Innsbruck ohne Hinweis auf eine SAE mit normaler BSG und normalem CRP (B; n = 22) sowie einer Vergleichsgruppe mit älteren Patienten (Alter 50–101, Median 77 Jahre) einer geriatrischen Abteilung ohne klinischen Hinweis auf eine Autoimmunerkrankung (C; n = 340) wurden mit iIF auf HEp-2-Zellen (INOVA Diagnostics, Sacramento) beginnend mit einem Screeningtiter von 1:40 und stufenweiser Verdünnung positiver Proben bis zum Endtiter auf das Vorliegen von ANA getestet. Der optimale Cut-off-Titer wurde als 95-%-Perzentile und mittels graphischer Darstellung gegen die ANA-Titer von 114 ambulant behandelten Patienten mit SLE ermittelt.

Ergebnisse Bei Verwendung der Kontrollpopulationen A und B liegt die 95-%-Perzentile für den optimalen Cut-off bei einer Verdünnung von 1:80. Wird eine größere Anzahl älterer Patienten (Kontrollpopulation C) inkludiert, steigt die optimale Cut-off-Verdünnung auf 1:160. Die visuelle Auswertung der graphischen Darstellung ergibt ebenfalls einen optimalen Cut-off-Wert bei ungefähr 1:100.

Zusammenfassung/Schlussfolgerung In Übereinstimmung mit den Empfehlungen aus dem Jahr 1997 konnte auch bei unseren Populationen ein Cut-off-Titer von 1:100 als optimal bezeichnet werden, um Patienten mit SLE von Personen ohne Autoimmunerkrankung zu unterscheiden. Nahezu 20 Jahre nach der ersten Empfehlung eines Cut-off-Wertes wurden die Ergebnisse von uns bestätigt, obwohl sich in der Zwischenzeit die gesamte technische Ausrüstung zur ANA-Bestimmung änderte und vermutlich verbesserte. Basierend auf unserer Erfahrung scheint es nicht notwendig zu sein, dass jedes Labor seinen eigenen Cut-off-Wert ermittelt.

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