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26. und 27. November 2004, Abstracts der Vorträge*

Patient Satisfaction and Disease Activity Indices in Rheumatoid Arthritis Patients

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Background: Disease activity indices, such as the DAS28 or the SDAI, become increasingly important in daily rheumatological practice for documentation purposes.

Objective: To assess whether, and if how, the DAS28, the SDAI, disease activity categorizing according to both, and the MHAQ are related to patient satisfaction or the patient's wish to change the therapeutic regime.

Patients and Methods: 94 RA patients (37 to 77a; 73 female, 21 male) on DMARD and low-dose corticoids were enrolled into this cross-sectional evaluation. At the occasion of a regular control visit, the DAS28, the SDAI, the MHAQ were determined. Simultaneously patient satisfaction (PATSAT) from 1 (excellent) to 5 (completely unsatisfied), according to the school mark system, and the wish to change the therapeutic regime (WtC; yes or no) were assessed. Patients were classified with respect to their disease activity according to the EULAR response criteria and the SDAI activity categories. Statistical analyses comprised tests for normal distribution, regression and correlation analyses (Pearson's r), t-tests, where applicable, the Wilcoxon rank-test, as well as the Friedman test.

Results: The mean DAS28 amounted to 3.84 (0.77–7.1), the mean SDAI to 15.16 (0–45.5) and the mean MHAQ to 0.7 (0–2.6). Average satisfaction was 2.5; 47 patients expressed their wish for a therapeutic change (23 less medication, 24 more medication). DAS28, SDAI and MHAQ were significantly correlated to one another (r between 0.906 and 0.473; all $p < 0.001$). PATSAT was significantly correlated to the DAS28 ($r = 0.611$; $p < 0.0001$), to the SDAI ($r = 0.611$; $p < 0.0001$) as well as to the MHAQ ($r = 0.393$; $p < 0.001$) and to the WtC ($p < 0.0001$). A significant relationship was also seen between PATSAT and disease activity categorizing according to the DAS28 ($r = 0.541$; $p < 0.0001$) and to the SDAI ($r = 0.491$; $p < 0.001$), although the distribution of disease activity categories according to the DAS28 on the one hand and to the SDAI on the other was statistically significant different ($p < 0.0001$). PATSAT showed an impressive relationship to the WtC ($p < 0.0001$), which either showed a statistically significant relationship to all activity indices as well as to disease activity categorizing. The mean DAS28 in pts who wanted to reduce their medication amounted to 3.26 (1.31–6.09), in pats longing for an increase of therapy to 4.68 (2.3–7.1) ($p-< 0.0001$). Linear regression analysis revealed PATSAT to be not DAS28, SDAI, or MHAQ dependent, but highly related to patients global self assessment ($p < 0.003$).

Conclusion: Patients' therapeutic attitude is somewhat in line with their satisfaction, which mirrors disease activity to a great extent, however not with the common therapeutic recommendations. The disease activity indices were highly correlated to one another with the exception of disease activity categorizing. These results may provide guidance in considerations about patient care and education as well as therapeutic strategies.

Reference:
Leeb BF, Andel I, Leder S, Leeb BA, Rintelen B. The Patients' perspective and rheumatoid arthritis disease activity indexes. Rheumatology 2004; in press.

COMP: Erfahrungen in der rheumatologischen Routine-Diagnostik

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Einleitung: Cartilage oligomeric matrix protein (COMP) ist ein nichtkollagenes Knorpelmatrixprotein, das bei Knorpel-Um- und Abbauprozessen an die Synovialflüssigkeit abgegeben wird und auch im Serum in meßbaren Konzentrationen nachweisbar ist. Hohe Serumkonzentrationen von COMP werden als Zeichen eines vermehrten Knorpelabbaus interpretiert. Im Vergleich zu gesunden und bezüglich Gelenke beschwerdefreien Personen zeigen Patienten mit klinisch manifester Arthrose ebenso wie Patienten mit chronischer Polyarthritis signifikant höhere COMP-Werte.

Methode: Sera von insgesamt 1.174 ambulant gesehenen Patienten (1.110 Frauen, 64 Männer) mit Schmerzen am Bewegungsapparat wurden routinemäßig auf COMP mit einem kommerziell erhältlichen ELISA (AnaMar Medical AB, Uppsala, Schweden) getestet.

Ergebnisse: Nur bei 13 % (156/1.174) der gemessenen Serumproben zeigten sich COMP-Werte über dem Normbereich (Männer 10/64, Frauen 146/1.110). Die Verteilung der individuellen Serumkonzentrationen waren in den einzelnen Krankheitsgruppen (chronische Polyarthritis, Arthrose, weichteilrheumatische Erkrankungen) ähnlich, die Werte im Gruppenvergleich nicht wesentlich verschieden. Einzelne Patienten zeigten im Krankheitsverlauf zum Teil auffallende Schwankungen und bei erfolgreicher medikamentöser Therapie mit der klinischen Besserung einen Abfall im Serum-COMP-Wert.

Schlußfolgerung: Trotz der beschriebenen statistisch signifikanten Unterschiede der Serum-COMP-Werte zwischen verschiedenen Personen- und Patientengruppen ist der Unterschied gering und im klinischen Alltag für eine Differentialdiagnose der Gelenkserkrankung nicht verwertbar. Die Streubreite der individuellen Werte ist relativ hoch. Auch die Interpretation der Serumkonzentrationen von COMP in bezug auf die angegebenen Normalbereiche ist von untergeordneter Bedeutung. Eher zielführend scheint der Vergleich einzelner Werte einer Person im Krankheitsverlauf. Fallende COMP-Serumwerte lassen auf einen verminderten Knorpelabbau und damit auf einen Erfolg der eingeschlagenen Therapie schließen.

Rheumatoid Arthritis-Specific Autoantibodies in Chronic Lymphatic B-Cell Leukemia (B-CLL)

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Purpose: B-CLL, the most common leukemia in the Western world, is well known for the production of polyreactive natural autoantibodies. Among those, IgM-rheumatoid factor (RF) is most frequently expressed.

* Reihung alphabetisch nach Erstautor

Interestingly, nothing is known about the occurrence of IgG- or IgA-RF as well as antibodies against cyclic citrullinated polypeptides (aCCP) in patients suffering from this disease. The aim of this study was to determine the frequency of RA-specific autoantibodies in sera of patients with B-CLL.

Methods: Peripheral blood samples of 70 patients with B-CLL (39 male, 31 female; mean age 66.2 years) were analyzed by commercial ELISA-kits for the presence of aCCP, IgA-, IgM- and IgG-RF. None of the patients showed any signs of arthritis. As a control sera from 20 patients (2 male, 18 female, mean age 52.7 years) fulfilling the ACR-criteria for RA were used.

Results: RF-IgM antibodies were detected in 12/70 B-CLL patients (17.1 %). RF-IgG was found in 8/70 (11.4 %) patients, whereas RF-IgA was only present in one patient with B-CLL (1.4 %). Both, RF-IgG and -IgM were found in 3/70 CLL-patients (4.2 %). None of the B-CLL patients had detectable aCCP levels. Thus, the highest specificities for RA were observed for aCCP (100 %) and RF-IgA (98.6 %). The results obtained from the RA-patients were comparable to those reported in other studies. IgM-RF and aCCP were found in 70 %, IgG- and IgA-RF in 50 % of the tested samples.

Conclusion: Our results indicate that aCCP and to a lesser extent IgA-RF are limited to RA and therefore highly valuable diagnostic markers for this disease. Thus, the importance of IgM-RF as a criterium for RA should be reconsidered.

The relationship of Lipoprotein (a) and homocysteine with markers of inflammation and disease activity in patients with rheumatoid arthritis

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Background: Patients with rheumatoid arthritis are at increased risk for coronary artery disease. Lipoprotein(a) (Lp(a)) and homocysteine (Hcy) are emerging risk factors for coronary artery disease. In the present study we investigated whether Lp(a) and Hcy are elevated in patients with RA and whether they are correlated to markers of inflammation or disease activity in these patients.

Methods: 33 patients with RA were included in the study and compared to healthy controls. Lp(a) was measured enzymatically and Hcy by HPLC. Markers of inflammation (CRP, ESR) and RF were determined by standard assays.

Results: Lp(a) and homocysteine plasma concentrations were significantly higher in RA patients than controls [31.4 ± 37.5 mg/dl (median 14.9) versus 15.4 ± 21.7 mg/dl (median 12.1), $p < 0.05$, and 15.6 ± 7.1 µmol/l (median: 14.7) versus 7.9 ± 7.3 (median 7.8), $p < 0.03$, respectively]. Lp(a) was significantly correlated to markers of inflammation (CRP: $p < 0.03$ and ESR: $p < 0.004$) and RF ($p < 0.004$) in RA patients. Homocysteine was significantly correlated to RF ($p < 0.03$) but not to markers of inflammation or disease activity in RA patients. There was also no significant correlation between Lp(a) and homocysteine.

Conclusions: Both Lp(a) and homocysteine are elevated in patients with RA. In our study, there was no correlation between Lp(a) and homocysteine and only Lp(a) was significantly correlated to markers of inflammation.

Increased peripheral levels of CD28-T-cells in small sized abdominal aortic aneurysms

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Introduction: Abdominal aortic aneurysm (AAA) is a common and potentially lethal disorder. Studies of human tissue and animal studies have led to a paradigm shift in AAA. Rather than a simple degenerative process, the majority of AAAs has proven to be a dynamic remodeling process. Inflammation which is associated with disruption of the orderly lamellar structure of the aortic media, appears to play a fundamental role in AAA development and progression. In other autoimmune diseases including rheumatoid arthritis, ankylosing spondylitis, Wegener's granulomatosis and multiple sclerosis, unusual subsets of pro-inflammatory, cytotoxic CD4+ and CD8+ T-cells were described, which lack the CD28 co-stimulatory molecule on their surface, and may perpetuate the inflammatory process.

Methods: 101 patients (age 69.4 ± 7.4 years) with AAA and 38 healthy volunteers (age 60.7 ± 8.9) were enrolled in the study. Three colour FACS analyses for CD4, CD8, CD28 and CD3 of PBMCs were performed. After stimulation with PMA/ionomycin, intra-cellular production of interferon-gamma perforin, IL-2 and IL-4 were examined. Cryofrozen sections from 8 patients, who underwent elective surgery, were used for immunohistological studies. Maximal diameters of AAA were routinely measured by sonography.

Results: Circulating levels of CD3+CD4+ CD28- and CD3+CD8+CD28- T-cells were enriched to 7.8 ± 8.8 % and 41.9 ± 15.7 % in AAA patients compared to healthy controls (2.2 ± 6.1 % and 24.9 ± 15.5 %; $P = 0.002$ and $P \leq 0.001$, respectively). During follow-up between 6 and 12 months the levels of CD3+CD4+CD28- T-cells increased to 11.1 ± 9.2 % ($p = 0.006$) and the levels of CD3+CD8+CD28- T-cells to 46.4 ± 16.6 % (n.s.). Both CD4+CD28- and CD8+CD28-T-cells showed reduced apoptosis, and produced interferon-γ and perforin, whereas they lack IL-2 and IL-4 production. Increased peripheral levels of CD28-T-cells were independent of co-existing peripheral arterial occlusive disease or coronary heart disease. Patients with an aortic diameter < 4 cm showed higher levels of CD4+CD28- T-cells than those patients with an aortic diameter > 4 cm ($p = 0.025$). Immunohistological examinations revealed the local presence of 39.1 ± 17.2 % CD28- out of all CD4+ and 44.0 ± 13.8 % CD28- out of all CD8+T-cells within lymphocytic aggregations in the adventitia and outer part of the media of AAA tissue specimens.

Conclusions: Peripheral pro-inflammatory, cytotoxic CD4+ and CD8+CD28-T-cells are enriched in the peripheral blood of AAA patients compared to healthy controls. In AAA specimens a high proportion of CD4+ and CD8+ T-cells lack the important co-stimulatory molecule CD28. Increased peripheral levels of CD28-T-cells are independent of co-existing diseases. It appears that immune-mediated processes play a more important role in smaller AAAs (< 4 cm) than in larger AAAs (> 4 cm). The peripheral and local presence of CD28- T-cells in AAA supports the hypothesis of a role of these T-cells in the pathogenesis of AAAs.

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Antipospholipid antibodies influence progression of abdominal aortic aneurysm disease

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Introduction: During the last decade, studies of human tissue and animal studies have led to a paradigm shift in the pathogenesis of abdominal aortic aneurysm (AAA). Inflammation which is associated with disruption of the orderly lamellar structure of the aortic media, appears to play a fundamental role in development and progression of the majority of AAAs. Antiphospholipid antibodies (aPLs) are a group of heterogenous autoantibodies, which have been reported in many autoimmune diseases and are related with the formation of thrombi in the antiphospholipid syndrome. In abdominal aortic aneurysms, intra-luminal thrombi have an influence on vessel wall dilatation and stress, and mural thrombi are considered as a site of protease release and activation in aneurysms.

Patients and Methods: 105 patients (age 70.6 ± 9.4 years) with AAA and without a medical history of malignancy or another immune-mediated disease and 39 healthy volunteers (age 47.4 ± 8.5 years) were enrolled in the study. Computerized tomography was used for the assessment of the aortic aneurysm diameter and volume of the intra-mural thrombi. ELISAs were routinely performed to detect aPLs in frozen serum samples. The percentage of CD28- out of the CD3+CD4+ peripheral blood mononuclear cells was determined by FACS analysis.

Results: An intramural thrombus was present in 82.9 % of AAA patients and the volume of the thrombi ranged from 1.9 to 377.5 cm^3 (66.2 ± 69.9 cm^3). The volume of the thrombi correlated with maximal AAA size measured by computerized tomography (n = 57; r = 0.753; P < 0.001). Those patients with intramural thrombi showed increased diameters of AAA compared to those without a thrombus (P = 0.019). In the cohort of AAA patients, 13 patients (12.4 %) but no controls tested positive for aPLs. AAA patients positive for aPLs showed increased levels of CD4+CD28- T-cells in the peripheral blood compared to AAA patients without aPLs (11.4 ± 9.0 % vs. 7.2 ± 7.9 %, P = 0.038) and a more progressive disease (P = 0.038).

Conclusions: There is a dependency between the volume of the intraluminal thrombus and maximal diameter of AAA. In patients with aPLs, AAAs showed a more progressive disease than in patients without aPLs. Increased peripheral levels of CD4+CD28- T-cells are higher in AAA patients with aPLs than in those without aPLs. The peripheral and local presence of unusual CD28-T-cells in AAAs together with a role of aPLs for the progression of AAA disease suggest an activated B-cell status in AAAs. These findings further support the model of AAA an immune-mediated disease.

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Serum-Chemokine und Expression von Chemokinrezeptoren beim Morbus Bechterew

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Einleitung: Chemokine und Chemokinrezeptoren spielen bei der Extravasation von Lymphozyten in entzündetes Gewebe eine essentielle Rolle. Die Expression von Th1-assoziierten Chemokinrezeptoren auf Lymphozyten gilt als Aktivitätsmarker bei verschiedenen Autoimmunerkrankungen. Vor kurzem wurde eine Subgruppe von pro-inflammatorischen, zytotoxischen T-Zellen bei Mb. Bechterew (MB) beschrieben, die durch das Fehlen des ko-stimulatorischen Signals CD28 charakterisiert werden kann.

Methoden: 12 MB-Patienten (Alter 44,9 ± 14,7 Jahre) und 27 altersgleiche gesunde Kontrollen (Alter 46,4 ± 12,8 Jahre) wurden prospektiv in die Studie eingeschlossen. Von den 12 MB-Patienten erhielten 6 eine Therapie mit Remicade®. Dreifarben-FACS-Analysen wurden zur Detektion von CD4, CD8, CD28, CXCR3 und CCR4 auf der Oberfläche und die intrazelluläre Produktion von Zytokinen von frischen PBMCs durchgeführt. Mittels ELISAs wurden IP-10, CCL-5, CCL-3, CXCL-9 und CXCL-17 im Serum untersucht.

Ergebnisse: Die Oberflächenexpression von CXCR3 war vergleichbar zwischen CD4+ und CD4+CD28-T-Zellen (31,3 ± 7,4 % and 43,8 ± 22,9 %; n.s.), während CCR-4 mit 40,9 ± 11,3 % auf CD4+CD28+ und 7,4 ± 10,8 % auf CD4+CD28-T-Zellen (P = 0,012) exprimiert wurde. CXCR-3 war in 27,4 ± 20,8 % von CD8+CD28+ T-Zellen und in 13,5 ± 30,5 % der CD8+CD28- T-Zellen (P = 0,025) positiv. CCR-4 wurde auf 20,6 ± 10,7 % der CD8+CD28+ T-Zellen im Vergleich zu 1,7 ± 1,9 % der CD28-Gegenstücke (P = 0,018) exprimiert. Auf Grund der berechneten CXCR-3/CCR-4-Ratio können sowohl CD4+ als auch CD8+CD28-T-Zellen als Th1/Tc1-Lymphozyten angesehen werden (18,1 ± 24,6 und 11,0 ± 19,8), im Vergleich zu CD4+ und CD8+CD28+-T-Zellen mit 0,8 ± 0,3 und 1,4 ± 0,7 (P = 0,012 und P = 0,091). CD4+ und CD8+CD28-T-Zellen produzieren große Mengen an Interferon-γ, TNF-α, IL-10, hingegen fehlt ihnen die Produktion von IL-2 and IL-4. Die Serumwerte des CXCR3 Liganden CXCL9 waren bei MB-Patienten mit 133,7 ± 198,4 ng/ml erhöht, im Vergleich zu 42,5 ± 24,5 ng/ml bei gesunden Kontrollen (P = 0,016). Die Serumwerte von IP-10, CCL-5, CCL-3 und CCL17 zeigten keine Unterschiede zwischen MB-Patienten und gesunden Kontrollen. Unter Therapie mit monoklonalen anti-TNF-α-Antikörpern (Remicade®) fielen die Serumwerte von IP-10, CCL-5 und CCL-17 von 206,3 ± 121,7, 38,1 ± 27,1 und 138,0 ± 28,1 ng/ml auf 75,7 ± 14,3 (P = 0,004), 10,3 ± 4,3 (P = 0,002) und 70,9 ± 25,0 ng/ml (P = 0,002).

Schlußfolgerungen: Chemokine sind in den Seren von MB-Patienten erhöht, und unter Therapie mit monoklonalen anti-TNF-α-Antikörpern kam es zu einem Abfall der Serumwerte von IP-10, CCL-5 und CCL-17. Das Expressionsprofil der Chemokinrezeptoren spiegelt den pro-inflammatorischen, Interferon-γ produzierenden Typ der CD28-T-Zellen wieder, was eine mögliche Rolle dieser T-Zellen in der Pathogenese des MB unterstreicht.

Projektförderung: Diese Studie wurde durch den Dr. Kolassa-Preis 2003 und den Verein zur Förderung der Hämatologie, Onkologie und Immunologie, Innsbruck unterstützt.

A NOVEL ELEMENT SIMILAR TO THE TRIIODOTHYRONINE (T3) RESPONSIVE ELEMENT (TRE) REGULATES T3 INDUCED ACTIVITY OF THE MOUSE MMP13 PROMOTER

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The Matrix Metalloproteinase MMP13 (collagenase 3) is involved in endochondral ossification and bone remodelling by degrading components of the extracellular matrix. It is also a key regulator of calcium homeostasis in adult bone by solubilizing calcium through bone resorption. MMP13 primarily degrades collagen II, but it is also involved in degradation of type I, III and X. Its expression is dependent on several growth factors like Interleukin1 (IL1) or tumor necrosis factor alpha (TNF-α) and on hormones like 1,25 dihydroxyvitamin-D3 and Triiodothyronine (T3). Despite the fact that most T3 regulated processes act via direct interaction of T3 bound T3-receptor (TR) with a distinct promoter element of the target gene, there is also evidence that T3 regulates MMP13 transcription via an indirect pathway but not through protein stability of the factor itself. The pronounced effect of T3 on MMP13 expression might reflect the general contribution of this hormone to bone remodelling. Although it has been clearly shown that T3 is involved in MMP13 gene expression, the mechanism of how T3 acts on the regulation of this gene still remains unclear. We were able to identify a sequence around 1100 bp upstream of the mouse MMP13 gene that shows a high degree of homology to the consensus sequence of TRE (TAAGGTCA) through computational analysis. To show that this sequence is a functional TRE we cloned a 2kb fragment of the 5-prime region of the MMP13 gene into a mammalian expression vector and assayed for reporter expression before and after T3 treatment in MC3T3 cell. N-terminally truncations of this construct revealed that its promoter activity significantly decreases whenever the region around the hypothetical TRE is deleted. Employing electrophoretic mobility shift assays (EMSA) we also showed that in vitro translated TR efficiently binds to an oligonucleotide representing the sequence of the hypothetical TRE. Moreover, oligonucleotides harbouring point mutations within the TRE showed a considerable decrease of the binding signal compared to the wild type sequence. In summary, these results suggest that there is a direct interaction of TR and a new TRE of the MMP13 promoter to regulate T3-induced MMp13 gene expression.

NEKROTISCHE UND NICHT-APOPTOTISCHE ZELLEN BINDEN ANTI-HISTON H1-ANTIKÖRPER, DIE AUSLÖSER DES LE-ZELL-PHÄNOMENS

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Einleitung: Das LE-Zell-Phänomen ist ein klassischer Lupus-Test. Es entsteht durch Granulozyten, die Kernmaterial phagozytieren. Die Herkunft dieses Materials ist bisher unbekannt. Sowohl isolierte Zellkerne nekrotischer Zellen als auch Zellen im Prozeß der Apoptose wurden bisher in der phagozytierten Masse vermutet. Der Anti-Histon H1-Antikörper ist für die Bildung der LE-Zelle ursächlich verantwortlich. Wir untersuchten daher die Bindung dieses Antikörpers auf der Suche nach den Auslösern des LE-Zell-Phänomens.

Methoden: Neutrophile Granulozyten wurden durch Zentrifugation zwischen Percoll-Schichten unterschiedlicher Dichte isoliert, mononukleäre Zellen aus dem peripheren Blut (PBMC) wurden über Ficoll-Paque gereinigt. Die Zellen wurden vital sowie nach Induktion von Apoptose (mittels Gliotoxin für Granulozyten bzw. Actinomycin D für PBMC und Inkubation über Nacht) sowie Nekrose (durch Erhitzen auf 70 °C) untersucht. Die Quantifizierung von Apoptose und Nekrose erfolgte durchflußzytometrisch (FACS) sowohl durch Annexin/Propidium-Iodid-Färbung als auch durch TUNEL-Färbung. Die Zugänglichkeit von Histon H1 wurde einerseits mittels monoklonaler Anti-Histon H1-Antikörper und einem FITC markiertem F(ab')- Fragment, andererseits mittels LE-Zell-positiver (daher Histon H1-bindender) Patientensera getestet. Als Negativkontrollen fungierten neben Isotyp-gematchten Kontrollantikörpern Seren von gesunden Kontrollpersonen.

Resultate: Apoptose und Nekrose konnte verläßlich in PMNC und auch Lymphozyten induziert werden. Monoklonale Anti-Histon H1-Antikörper binden an nekrotische, nicht jedoch an apoptotische oder frische Granulozyten oder PBMC. Auch Seren von LE-Zell-positiven (und damit auch Anti-Histon H1-positiven) Patienten binden an nekrotische und nicht an apoptotische oder frische Zellen. Seren von Gesunden binden an keinerlei Zellen.

Schlußfolgerung: Im Gegensatz zu nekrotischen Zellen haben Zellen im Prozeß der Apoptose keine Bindungsmöglichkeit für den LE-Zell-auslösenden Antikörper gegen Histon H1. Es müssen daher nekrotische Zellen oder isolierte Kerne im Rahmen des LE-Zell-Phänomens phagozytiert werden.

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Endothelial Progenitor Cells are decreased in the Peripheral Blood of Patients suffering from Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is associated with increased cardiovascular morbidity and mortality [1] that cannot only be explained by traditional cardiovascular risk (CVR) factors [2]. CVR is related to disease activity and can be normalized by effective therapy. Since the quantity of endothelial progenitor cells (EPC) [3] in the peripheral blood correlates inversely with cardiovascular risk [4] we studied if EPC alterations could also be observed in patients with RA.

Methods: EPC were quantified in 52 RA patients and in 16 healthy referents (HR) by FACS analysis. Patients were divided into groups with active disease (n = 36) and low disease activity (n = 16). Cells positive by flow cytometry for CD34/KDR/AC133 within the lymphocyte population were characterized as EPC. Furthermore, in subgroups of patients circulating EPC were also quantified using a colony forming unit (CFU) and a circulating angiogenic cell (CAC) assay.

Results: EPC were significantly decreased in RA patients suffering from active disease compared to HR as measured by fluorescence activated cell sorting analysis (0.026 ± 0.002 % vs. 0.045 ± 0.008 % respectively, $p < 0.01$), CFU assay (mean of 5 ± 2 cells/well vs.14 ± 4 in HR, $p < 0.05$) and CAC assay (mean of 7 ± 2 vs. 52 ± 16 positive cells/high power field, $p < 0.005$). In contrast, the frequency of circulating EPC of patients with low disease activity was comparable to that of healthy individuals (0.052 ± 0.006 % by fluorescence activated cell sorting analysis), by CFU assay (10 ± 5 CFU/well) and by CAC (mean of 25 ± 5 positive cells). Moreover, EPC levels in peripheral blood correlated inversely with disease activity assessed by the disease activity score ($r = -0.38$, $p < 0.01$).

Conclusions: Our observations indicate that active RA is associated with impaired circulating EPC. We hypothesize that the increased cardiovascular risk of RA might be related to the depletion of EPC that is a consequence of the activity of the disease.

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Interleukin-1 is induced in articular cartilage after mechanical injury

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Introduction: Interleukin-1 (IL-1) may play an important role in the development of both osteoarthritis (OA) and rheumatoid arthritis (RA). It triggers synovial cell proliferation, induces matrix metalloproteases, causes cartilage and bone resorption and inhibits resynthesis of proteoglycans. To further elucidate the role of IL-1 in OA we looked for presence of this cytokine in cartilage explants and in chondrocytes. The aim of our study was to investigate the effect of mechanical stress upon intracellular inflammatory signalling pathways and expression of IL-1.

Methods: Cartilage from porcine metacarpophalangeal joints was cultured in serum-free medium. Tissue extracts were examined for ERK activation by phosphorylated-Western blotting, for JNK and p38 MAPK kinase activity by kinase assay, and for IκBα by Western blotting. IL-1α and IL-1β messenger RNA (mRNA) was measured by reverse transcriptase polymerase chain reaction. IL-1 induction was measured by the induction of serum amyloid A protein in cultured chondrocytes. Porcine chondrocytes in monolayer culture were stimulated for 15 hours with cell or tissue extracts or with IL-1. Medium was then changed and newly synthesized proteins were labelled by adding for 5 hours fresh medium containing L- [^{35}S] cysteine and methionine. Proteins in the culture medium were then separated on a 12.5 % SDS gel. Gels were silverstained and then dried and exposed to x-ray film. Protein bands were excised and identified by mass spectrometry.

Results: All 3 MAKPs (p38, JNK, and ERK) were rapidly activated upon dissection and explantation of the cartilage. IL-1α and IL-1β mRNA was also induced. The speed and magnitude of induction were increased if the explants had been finely cut. IL-1 activity that could be inhibited by IL-1 receptor antagonist or antibodies to IL-1α was found in extracts of explants cultured for 20 hours or lysates of cells isolated from them. This acitivity was likely due to intracellular pro-IL-1α that was not secreted. Pro-IL-1β could not be detected because it is biologically inactive. The mechanism of inflammatory signalling pathway activation underlying the induction of IL-1 is unknown.

Discussion: Mechanical stress i.e. explantation and cutting of articular cartilage activates intracellular inflammatory signalling pathways and induces expression of mRNA for IL-1α and IL-1β. Biologically active IL-1α protein was detected in cartilage explants and lysates of chondrocytes. Our study suggests that IL-1 could play an important role in the pathogenesis of OA and that blocking this cytokine and/or other mediators in a specific manner could be a useful means of preventing this very common disease.

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CD44 IS A DETERMINANT OF INFLAMMATORY BONE LOSS

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Chronic inflammation is a major trigger of local and systemic bone loss. Disintegration of cell-matrix interaction is a prerequisite for the invasion of inflammatory tissue into bone. CD44 is a type I transmembrane glycoprotein which connects a variety of extracellular matrix proteins to the cell surface. Tumor necrosis factor (TNF) is a major inducer of chronic inflammation and its overexpression leads to chronic inflammatory arthritis. By generating CD44$^{-/-}$human TNFtg mice, we show that destruction of joints and progressive crippling is far more severe in hTNFtg mice lacking CD44, which also develop severe generalized osteopenia. Mutant mice exhibit an increased bone resorption due to enhanced number, size and resorptive capacity of osteoclasts, whereas bone formation and osteoblast differentiation are not affected. Responsiveness of CD44-deficient osteoclasts towards TNF is enhanced and associated with increased activation of the p38MAPK kinase. These data identify CD44 as a critical inhibitor of TNF-driven joint destruction and inflammatory bone loss.

WIRKUNG VON ADALIMUMAB (HUMIRA®) SOWOHL IN KOMBINATION MIT METHOTREXAT ALS AUCH MIT ANDEREN BASISTHERAPEUTIKA IN DER BEHANDLUNG DER CHRONISCHEN POLYARTHRITIS (RHEUMATOIDEN ARTHRITIS): ERGEBNISSE DER REACT STUDIE

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Einleitung: Basistherapeutika, entweder als Monotherapie oder bei ausbleibendem Erfolg in Kombination gegeben, sind die Voraussetzung einer erfolgreichen Behandlung der chronischen Polyarthritis. Methotrexat (MTX) ist das weltweit am häufigsten verwendete Basistherapeutikum und gilt als Goldstandard. Biologika wurden bisher immer in Kombination mit Methotrexat getestet. Im Gegensatz dazu wurde in der ReAct (research in active rheumatoid arthritis) Studie die Wirkung von Adalimumab (Humira®) in Kombination mit allen gängigen Basistherapeutika geprüft.

Methode: Patienten mit langdauernder aktiver chronischer Polyarthritis erhielten Adalimumab in Ergänzung zu ihrem eingenommenen, aber zu wenig wirksamen Basistherapeutikum. In mehr als 430 Zentren in 11 Ländern Europas erhielten Patienten Adalimumab 40 mg alle 2 Wochen subkutan. Kontrollvisiten zur Beurteilung der Sicherheit und Effizienz waren in den Wochen 2, 6 und 12.

Ergebnisse: Bis März 2004 konnten aus der noch laufenden Studie die Daten von 3.813 Patienten ausgewertet werden. Von 1.636 Patienten war die Begleittherapie bereits bekannt. Das Durchschnittsalter betrug 54 Jahre, die mittlere Krankheitsdauer war 11 Jahre. 28 % der Patienten waren unter Adalimumab-Monotherapie, 55 % nahmen zusätzlich 1 Basistherapeutikum, 13 % zwei Basistherapeutika und 4 % hatten begleitend zu Adalimumab 3 oder mehr Basistherapeutika. Die Effizienz von Adalimumab in Kombination mit Leflunomid (LEF), Sulfasalazin (SSZ) oder Chloroquin/Hydroxichloroquin (CQ/HCQ) war ähnlich wie in Kombination mit MTX (Tabelle 1).

Schlußfolgerung: Adalimumab 40 mg sc, alle 2 Wochen gegeben, führt zu statistisch signifikanter Besserung aller wesentlichen klinischen Parameter (Krankheitsaktivitätsindex DAS28, Fragebogen zur Lebensqualität HAQ, Zahl der druckschmerzhaften Gelenke TJC, Zahl der geschwollenen Gelenke SJC). Die Effizienz ist ähnlich bei Kombination von Adalimumab mit MTX, LEF, SSZ, CQ/HCQ oder mit Kombinationen dieser Basistherapeutika. Adalimumab ist eine wirkungsvolle Ergänzung in der Therapie der cP auch unter Bedingungen des normalen klinischen Alltags, in denen unterschiedliche Basistherapeutika eingesetzt und bei Versagen ergänzt werden.

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Tabelle 1: Herold M et al. Klinische Ansprechrate bei unterschiedlichen Basistherapeutika mit Adalimumab

Effizienz-Bewertung	MTX (n = 568)	LEF (n = 225)	SSZ (n = 38)	QC/HQC (n = 29)	MTX + LEF (n = 48)	MTX + SSZ (n = 40)
ACR20 (%)	70	64	70	75	69	78
ACR50 (%)	45	34	41	36	53	55
Moderater EULAR-Response (%)	84	79	75	85	92	93
Guter EULAR-Response (%)	39	31	39	25	38	48
DAS28*	−2,1	−1,9	−2,3	−2,0	−2,3	−2,6
HAQ*	−0,47	−0,48	−0,51	−0,50	−0,57	−0,50
TJC** (0–28)	−62	−60	−62	−51	−70	−72

* mittlere Änderung vom Ausgang, ** mittlere % Änderung vom Ausgang; p = 0,001 im Vergleich zum Ausgang für alle Subgruppen

ALENDRONAT-THERAPIE IN DER BEHANDLUNG DER HÜFTKOPFNEKROSE – EINE EXPERIMENTELLE TIERSTUDIE

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Einleitung: Osteonekrose des Femurkopfes kann zum Einbruch des Femurkopfes und zur Hüftgelenksarthrose führen. Die Nekrose an sich erklärt aber nicht Veränderungen

in der Knochenmikroarchitektur und erklärt auch nicht gänzlich den Einbruch des Femurkopfes. Es wird vermutet, daß die Resorption des nekrotischen Knochens, inbesondere der subchondralen Platte, zu einer Verringerung der biomechanischen Eigenschaften des Femurkopfes führt. Ziele dieser Studie waren: (1) Veränderungen in der 3D-Mikroarchitektur und Mineralisierung des trabekulären und kompakten Knochens im osteonekrotischen Femurkopf sowie im osteoarthrotischen Acetabulum zu beschreiben; (2) den Effekt von hochdosierter Alendronat-Therapie auf den Verlauf der Osteonekrose und auch auf die Entstehung der Hüftgelenksarthrose zu untersuchen.

Methoden: Ein experimentelles Osteonekrose-Modell am Hasen wurde entwickelt. Bei 60 NZW-Hasen wurde chirurgisch die Blutzufuhr zum linken Femurkopf unterbunden. Die Tiere wurden in vier Gruppen randomisiert; zwei Gruppen erhielten Alendronat (150 µg/kg/Tag, s.c., 3 x pro Woche), und zwei Gruppen erhielten 0,9 % NaCl-Injektionen als Kontrolle. Die Therapie wurde in der 4. postoperativen Woche begonnen. Nach 6 und 12 Monaten wurden die Tiere euthanasiert. Der osteonekrotische und der gesunde kontralaterale Femurkopf, sowie das arthrotische und das kontralaterale normale Acetabulum wurden entfernt. Micro-QCT mit 16 µm Auflösung, digitales Röntgen, DXA und Standard-Histologie wurden durchgeführt. Der histologische Mankin-Score wurde für die Beurteilung der Knorpel-Degeneration verwendet.

Resultate: Zwei osteonekrotische Femurköpfe in der Kontrollgruppe waren nach 12 Monaten eingebrochen, wohingegen keine aus der Alendronat-Gruppe eingebrochen waren. Komplexe Veränderungen in der 3D-Mikroarchitektur und Mineralisierung im trabekulären und kompakten Knochen im osteonekrotischen Femurkopf wurden gefunden. Inhibierung der Resorption des nekrotischen Knochens führte zu einem signifikanten Anstieg der BVF und vBMD in der trabekulären Region. Darüberhinaus wurde die Resorption des nekrotischen kompakten Knochens unterbunden. Arthrotischer subchondraler Knochen ist dicker, hat eine höhere Porosität und weist einen geringeren Mineralisierungsgrad auf. Alendronatbehandlung verhinderte die Resorption des arthrotischen subchondralen Knochens, erhöhte deren Mineralisierungsgrad und resultierte in einer signifikant geringeren Knorpeldegeneration.

Schlußfolgerung: Alendronat verhinderte die Resorption des nekrotischen Knochens, scheint aber nicht die Knochenneubildung zu behindern, wodurch der Einbruch des Femurkopfes verhindert werden konnte. Alendronat-Behandlung reduzierte den Grad der Hüftgelenksarthrose signifikant. Frühzeitige Alendronat-Therapie könnte beim Menschen den Einbruch des Femurkopfes verzögern oder vielleicht sogar verhindern.

Modulation of T3-induced MOPG-Promoter activity in NIH3T3 by Runx2

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Osteoprotegerin (OPG), a small glycoprotein expressed by osteoblasts (OB) as well as in cells of other tissues, inhibits the differentiation and activity of osteoclasts via interception of RANKL. Recently we have shown that thyroid hormone (T3) regulates OPG mRNA expression in the osteoblastic cell line MC3T3-E1. Usually the effect of T3 is mediated by a complex of T3 with thyroid hormone receptors (TR), which bind to a sequence of the promoter-DNA, a so-called thyroid hormone responsive element (TRE). In addition interactions of steroid hormone receptors with bone specific transcription factors are reported, i.e. vitamin D receptor (VDR) with the transcription factor Runx2 in ROS 17/2.8 cell line. Runx2 is an osteoblast-specific transcription factor, which contributes to the expression of osteoprotegerin and exists in several isoforms.

Methods: We isolated a 0.6 kb mouse OPG fragment by genome walking. Cloning and computer analysis of this fragment revealed the presence of one TRE- and two putative Runx2 binding elements suggesting a possible regulation of mOPG by T3 and Runx2. We evaluated whether Runx2 in 2 isoforms (Runx2 type I and Runx2 type II) could affect the basal and T3 regulated activity of OPG promoter in transient transfection assays in NIH3T3. Additionally we transfected TR for being independent of intracellular TR.

Results: We showed that T3 stimulated the activity of the 0.6 kb OPG-promoter fragment. Runx2 affected the OPG-promoter by attenuating the basal expression and by inhibiting the T3-stimulation, but there seemed to be no difference between the two types of Runx2. By mutation of the two putative Runx2 binding sites we were able to attenuate the T3-induction. In summary the T3-induction of mOPG promoter activity in NIH3T3 was affected by Runx2.

Autoantibodies to the translational regulators TIA-1 and TIAR occur frequently in sera of patients with SLE and correlate with presence of lupus nephritis and disease activity

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Rationale: TIA-1 and TIAR are RNA binding proteins which are involved in regulation of TNF expression by acting as translational silencers. Since TNF is pivotally involved in the pathogenesis of many autoimmune diseases we studied expression of TIA proteins in inflamed tissues and investigated sera of patients with rheumatic diseases for the presence of autoantibodies against them.

Methods: Using recombinant proteins sera of patients with SLE, Scleroderma, poly-/dermatomyositis, Sjögren's syndrom, RA, reactive arthritis and healthy controls were investigated by immunoblotting. Expression of TIA proteins in skin and kidney was studied by immunohistochemistry.

Results: AutoAb against TIA-1 and/or TIAR were detected in 61 % of SLE, 54 % of Scl and in 15–30 % of patients with other rheumatic diseases but not in sera of healthy controls. In SLE patients anti-TIAR auto-Ab were significantly associated with nephritis ($p = 0.0003$), while in Scl patients the presence of autoAb to TIA-1 and/or TIAR do not correlate with lung involvement. Moreover auto-Ab to TIA-1 and/or TIAR correlate with diseases activity (Anti-dsDNA and SIS) ($p = 0.01$). Epitope analysis revealed major epitopes to be located mainly within the first and second of the 3 RNA recognition motives. Immunohistochemical analyses showed TIA-1 and TIAR to be expressed in inflamed skin and kidney of lupus patients.

Conclusion: These data suggest that TIA proteins may play a regulatory role in inflamed tissues. The frequent presence of anti-TIAR auto-Ab in sera of patients with SLE and Scl may be a consequence of TIA overexpression which might lead to the formation of neoepitopes and activation of autoreactive T and B cells. Thus, TIA proteins might play a role in the pathogenesis of SLE and other systemic autoimmune disorders.

Inflammatory low back pain: High negative predictive value of contrast-enhanced color Doppler ultrasound in the detection of inflamed sacroiliac joints

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Introduction: To determine the value of microbubble contrast agents for color Doppler ultrasound (CDUS) in the detection of active sacroiliitis in comparison to magnetic resonance imaging (MRI).

Methods: Observational case-control study of one hundred-three consecutive patients (206 sacroiliac [SI] joints) with inflammatory low back pain according to the Calin criteria, and 30 controls (60 SI joints) without low back pain conducted at the University Hospital of Innsbruck. All patients and controls underwent unenhanced and contrast-enhanced CDUS, and MRI of the SI joints. Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) of unenhanced and contrast-enhanced CDUS were evaluated.

Results: Forty-three patients (41 %) with 70 of 206 sacroiliac (SI) joints (34 %) and none of the controls nor the 60 control SI joints demonstrated active sacroiliitis on MR images. Unenhanced CDUS showed a sensitivity of 17 %, a specificity of 96 %, a PPV of 65 % and a NPV of 72 %, and contrast-enhanced CDUS showed a sensitivity of 94 %, a specificity of 86 %, a PPV of 78 % and a NPV of 97 %. Detection of vascularity in the SI joint was increased by contrast administration ($p < 0.0001$). Clustered receiver operating curve (ROC) analysis demonstrated that enhanced CDUS (Az = 0.89) was significantly better than unenhanced CDUS (Az = 0.61) for the diagnosis of active sacroiliitis verified by MR imaging ($P < 0.0001$, two-sided test).

Conclusion: Microbubble contrast-enhanced CDUS is a sensitive technique with a high negative predictive value for detection of active sacroiliitis in comparison to MR imaging.

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Anti-CCP2-Assay: Bedeutung in der klinischen Routine-diagnostik und Erfahrung mit einem neuen automatisierten Immunoassay

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Einleitung: Der Nachweis von Antikörpern gegen zyklisch citrullinierte Peptide (anti-CCP) im Serum ist hochspezifisch für chronische Polyarthritis (cP; rheumatoide Arthritis, RA). Anti-CCP-Antikörper können mit kommerziell erhältlichen Enzymimmunoassays (ELISA) leicht bestimmt werden. Die zweite Generation der anti-CCP-Tests (anti-CCP2) zeigt neben einer hohen Spezifität (97–98 %) auch eine hohe, dem IgM-Rheumafaktor vergleichbare Sensitivität (75–80 %) für das Vorliegen einer cP. Die derzeit erhältlichen Immunoassays verwenden alle das gleiche Antigen einschließlich der haftenden Unterlage und unterscheiden sich nur in Details des Assayablaufs. In der vorliegenden Studie wurde eine neue automatisierte anti-CCP2-Methode auf ihre Brauchbarkeit in der klinisch-chemischen Routine getestet.

Methode: Sera von insgesamt 544 ambulant gesehenen Patienten mit Arthralgien wurden routinemäßig auf anti-CCP mit einem kommerziell erhältlichen anti-CCP2-ELISA (Inova Diagnostics Inc., USA) getestet und ein zweites Mal mit einem neuen automatisierten anti-CCP2-Assay (EliA-CCP, Pharmacia Diagnostica, Deutschland).

Ergebnisse: Beide Assays zeigten gute Übereinstimmung der Ergebnisse. 433 Proben waren in beiden Assays negativ, 68 Proben in beiden Assays positiv. Bezogen auf die vom Hersteller angegebenen Normwerte waren im Vergleich der beiden Assays (Inova/Pharmacia) 1 Probe negativ/grenzwertig, 13 Proben negativ/positiv, 9 Proben grenzwertig/negativ, 7 Proben grenzwertig/positiv, 3 Proben positiv/negativ. Sera mit diskrepanten Werten wurden mit beiden Assays ein zweites Mal analysiert, wobei sich in beiden Assays die Werte jeweils reproduzierten.

Schlußfolgerung: Der neue vollautomatisierte anti-CCP2-Assay EliA wurde in einer Probeversion getestet. Der Test war einfach durchzuführen, zeigte gut reproduzierbare Werte und die Ergebnisse waren mit einem gängigen kommerziell erhältlichen Assay vergleichbar mit einer Tendenz zur höheren Sensitivität.

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JNK1 is not essential for TNF-mediated joint disease

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Introduction: TNF signalling molecules are considered as promising therapeutic targets of anti-rheumatic therapy. Among them mitogen activated protein kinases are thought to be of central importance. Herein, we investigate the in vivo role tumor necrosis factor (TNF)-α signalling through c-jun N-terminal kinase- (JNK-) 1 in destructive arthritis.

Methods: Human TNF transgenic (hTNFtg) mice which develop inflammatory arthritis were intercrossed with JNK1-deficient (JNK1$^{-/-}$) mice. Animals (n = 35) of all 4 genotypes (wild-type, JNK1$^{-/-}$, hTNFtg, JNK1$^{-/-}$hTNFtg) were assessed for clinical and histological signs of arthritis.

Results: Clinical features of arthritis (swelling and grip strength) develop equally in hTNFtg and JNK1$^{-/-}$hTNFtg mice. Histological analyses reveal no differences in the quantity of synovial inflammation and bone erosions as well as in the cellular composition of the synovial infiltrate. Bone destruction and osteoclast formation are observed to a similar degree in hTNFtg and JNK1$^{-/-}$hTNFtg animals. Moreover, cartilage damage is comparable as indicated by proteoglycan loss in the articular cartilage of both hTNFtg and JNK1$^{-/-}$hTNFtg mice.

Conclusion: This suggests that JNK1 activation does not seem to be essential for TNF-mediated arthritis.

Hemmwirkungen der Leflunomid-Therapie auf den Knorpeldegradationsmarker COMP und die Aktivität von Matrixmetalloproteinase-9: eine sechsmonatige Multicenter-Pilot-Studie

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Einleitung: In der Pathogenese der Rheumatoiden Arthritis (RA) mit Bindegewebsschädigung und Entzündung führt eine enzymatische Schädigung der extrazellulären Knorpelmatrix zu Gelenksdestruktion und Funktionsverlust. Unter den destruierenden Enzymen spielen die Matrixmetalloproteinasen (MMPs) eine wesentliche Rolle. MMP-1 und MMP-9 können Komponenten der extrazellulären Matrix schädigen; MMP-9 spielt auch eine aktive Rolle bei der Diapedese von immunkompetenten Zellen und der Gewebsschädigung via insuffizientem Turnover der Bindegewebsmatrix. Das Cartilage Oligomeric Matrix Protein (COMP) ist ein Bestandteil der extrazellulären Knorpelmatrix. Erhöhte COMP-Serumspiegel korrelieren mit beschleunigter Gelenkserosion und geben Hinweise auf die zukünftige Progression wie auch auf die aktive Synovitis der RA. Ziel war es zu prüfen, ob eine sechsmonatige Therapie mit dem Basistherapeutikum Leflunomid bei Patienten mit RA einen Einfluß auf den Knorpelabbaumarker COMP, die Matrixmetalloproteinase-1 (MMP-1), und die Aktivität der Matrixmetalloproteinase-9 (MMP-9) besitzt.

Methoden: 36 Patienten (8 männlich, 28 weiblich) mit RA gemäß den ACR-Kriterien wurden in eine Multicenter-Pilotstudie aufgenommen. Während der sechsmonatigen Prüfperiode wurde eine tägliche Dosis von 20 mg Leflunomid (nach einer dreitägigen Anfangsdosis von 100 mg/die) – ein Isoxazolderivat und Hemmer der „De novo"-Pyrimidinsynthese – verabreicht. MMP-1-, MMP-9-Aktivität und COMP wurden ebenso wie das sehr sensitive Akute-Phase-Protein Serum-Amyloid A (SAA) mittels Enzymimmunoassays im Serum gemessen. Die Untersuchungen erfolgten vor, nach 3 und nach 6 Monaten Leflunomid-Therapie.

Resultate: Hohe Spiegel von aktivem MMP-9, COMP und SAA wurden in den Seren der Patienten mit RA vor Beginn der Leflunomid-Therapie im Vergleich zu normalen Kontrollseren gemessen. Eine signifikante Reduktion der MMP-9-Aktivitätsspiegel wurde bereits nach 3 Monaten Immunmodulation mit Leflunomid festgestellt, die auch nach 6 Monaten bestehen blieben ($p < 0{,}01$). Der Degradationsmarker COMP und der Entzündungsmarker SAA verringerten sich nach 6 Monaten signifikant ($p < 0{,}04$ bzw. $p < 0{,}01$). Parallel dazu fiel eine nichtsignifikante Tendenz zur Senkung von MMP-1 im Serum nach 6 Monaten auf.

Schlußfolgerung: Die Reduktion von MMP-9-Aktivität und des SAA während der sechsmonatigen RA-Therapie mit Leflunomid dürfte eine reduzierte enzymatische Schädigung des Knorpels und eine verminderte Entzündungsaktivität anzeigen; außerdem können die COMP-Resultate auf verminderte Gelenkserosionen nach einem halben Jahr Leflunomid-Therapie hinweisen.

Literatur:
Kullich WC, Aglas F, Niksic F, Czerwenka C, Mur E, Klein G. Inhibitory effects of leflunomide therapy on the cartilage degradation marker COMP and the activity of matrixmetalloproteinase-9: a 6-month multicentre pilot study. Ann Rheum Dis 2004; 63 (Suppl 1): 254.

Wird die Expression von knorpeldestruierenden Metalloproteinasen durch eine Dexibuprofen-Therapie beeinflusst?

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Einleitung: Die Arthrose ist durch vermehrten Abbau bzw. Degeneration von Knorpelgewebe charakterisiert, wobei Matrixmetalloproteinasen (MMPs) eine entscheidende Rolle spielen. Die Anwendung von schmerz- und entzündungshemmenden nichtsteroidalen Antirheumatika (NSAR) bei Arthrose ist insofern umstritten, da bekannt ist, daß einige NSAR den Knorpelabbau beschleunigen können, hingegen andere NSAR den Knorpelmetabolismus nicht beeinflussen dürften. Bei einer umfassenden Beurteilung der Arthrose ist neben klinischen Parametern neuerdings auch der Versuch interessant, den schädigenden enzymatischen Einfluß mittels Messung mehrerer MMPs zu erfassen.

Methoden: 40 Patienten mit schmerzhafter Gonarthrose (25 %) bzw. Coxarthrose (75 %) – beurteilt anhand des radiologischen Kellgren & Lawrence-Scores und einer Visuellen Analogskala-Schmerzbeurteilung von VAS > 3,5 – erhielten eine orale Verabreichung von 1.200 mg Dexibuprofen täglich (3 x 400 mg) über einen Zeitraum von 3 Wochen. Wirksamkeit und Verträglichkeit (5teilige Verbalskala) wurden durch Untersucher und Patienten beurteilt. Eine Beurteilung der Alltagsfunktionen wurde mit Hilfe des Western Ontario- and McMaster University Arthrose-Index (WOMAC) durchgeführt. Die Messung der Metalloproteinasen (MMP-1, MMP-3, MMP-8, MMP-9-Aktivität) wurde mittels 2seitigem Sandwich-ELISA durchgeführt.

Resultate: Die gegenüber einem normalen Kollektiv etwas erhöhten basalen mittleren Serumspiegel der untersuchten Metalloproteinasen veränderten sich unter der Pharmakotherapie mit dem NSAR Dexibuprofen nicht signifikant. Auch eine Analyse der Daten, gruppiert nach dem Kellgren & Lawrence-Score, zeigte ebenfalls keine signifikanten Änderungen in den jeweiligen

Subgruppen. MMP-3, MMP-8 und aktives MMP-9 korrelieren mit dem Schweregrad der Arthrose. Die Krankheits-Aktivitäts-Scores wie Schmerz und Alltagsfunktionen besserten sich signifikant während der Dexibuprofen-Therapie. Das Fehlen eines signifikanten Anstieges der MMPs deutet darauf hin, daß Dexibuprofen keine Verstärkung des Knorpelkatabolismus bewirkt.

Schlußfolgerung: Da bei keinem Patienten eine progressive MMP-Steigerung, gleichzeitig jedoch eine gute klinische Wirksamkeit bei hervorragender Verträglichkeit beobachtet wurde, kann die Therapie mit Dexibuprofen bei schmerzhaften Arthrosen der großen Gelenke als sicher und empfehlenswert angesehen werden.

Chemokine, welche die Anämie-entstehung bei rheumatoider Arthritis fördern, verringern sich unter einer Leflunomidtherapie

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Einleitung: Bei Patienten mit Rheumatoider Arthritis (RA) findet man häufig eine Anämie, die in gewissem Maß mit den Parametern der RA-Aktivität korreliert. Zusätzlich zu seinen proinflammatorischen Eigenschaften auf mononukleäre Phagozyten kann das Chemokin CCL3 (Makrophage Inflammatory Protein 1 alpha; MIP-1alpha) als potenter Hemmer der hämatopoetischen Zellproliferation die Pathogenese der Anämie beeinflussen. Das Chemokin CXCL10 (Interferon-gamma inducible Protein 10 kDa; IP-10) stimuliert immunkompetente T-Zellen; andererseits hemmt dieses pleiotrope Molekül sehr früh die hämatopoetischen Vorläuferzellen und die Angiogenese. Die Expression von Chemokinen wird im Rahmen entzündlicher Prozesse stark erhöht. Ziel war es, die Wirkung von Leflunomid, einem Basistherapeutikum der RA (DMARD), auf die eine Anämie chronischer Erkrankungen (ACD) auslösenden Chemokine bei Patienten mit RA zu untersuchen.

Methoden: 36 Patienten (8 männlich, 28 weiblich) mit RA (ACR-Kriterien) wurden in eine Multicenter-Pilot-Studie aufgenommen. ACD wurde definiert mit einer Hämoglobin-Konzentration < 11 mg/dl, MCV < 80 und einer Entzündungsaktivität mit erhöhten Akute-Phase-Reaktionen über dem Normbereich. Die peripheren Serumkonzentrationen von CCL3 (MIP-1alpha), CXCL10 (IP-10), Erythropoietin (EPO) und Serum-Amyloid A (SAA) wurden enzymimmunologisch vor und nach 3 sowie 6 Monaten Leflunomid-Therapie gemessen. Zusätzlich erfolgten die Routine-Labormessungen peripheres Blutbild und Serum-Eisen.

Resultate: Vor der Therapie hatten die RA-Patienten erhöhte CCL3-Spiegel, was auf eine Hemmung der hämatopoetischen Precursor-Zellen und eine verstärkte Entzündung durch Aktivierung von Monozyten/Makrophagen hinweist. Die Leflunomid-Therapie bei RA reduzierte signifikant die erhöhten CCL3-Spiegel nach 3 ($p < 0,005$) wie auch nach 6 Monaten ($p < 0,002$). Parallel zu der Reduktion von CCL3 wurde in 60 % der Fälle nach 6 Monaten Leflunomid-Therapie ein deutlicher Anstieg der Serumeisen-Spiegel in 55,6 % und ein Trend zur Normalisierung von EPO festgestellt. Es kann angenommen werden, daß die im Vergleich zu einer Eisenmangelanämie inadäquat erhöhten EPO-Spiegel bei RA die Hauptursache für die fehlende Signifikanz bei der EPO-Verminderung waren. CXCL10 verminderte sich signifikant nach 3 Monaten und bewirkt damit vermutlich eine verminderte Hemmung der Hämatopoese. Nach 6 Monaten war CXCL10 nicht signifikant vermindert, was auf einen möglichen antiangiogenetischen Effekt hinweist, der das synoviale Bindegewebswachstum mäßigen kann. Die Serumveränderungen von CCL3 korrelierten interessanterweise mit CXCL10 nach 3 und 6 Monaten Leflunomid. Die gleichzeitige Verminderung von CCL3 und dem Akute-Phase-Marker SAA nach 6 Monaten ($p < 0,01$) weist auf reduzierte Entzündungsvorgänge hin.

Schlußfolgerung: Eine Therapie mit Leflunomid über 6 Monate verbessert die ACD und die entzündliche Aktivität bei Patienten mit RA. Es wird angenommen, daß die unter der Therapie veränderte Chemokinexpression einen reduzierten inhibitorischen Einfluß auf die Proliferation der hämatopoetischen Precursorzellen wie auch eine reduzierte Aktivierung von mononukleären Phagozyten und T-Zellen bedeuten dürfte.

Beeinflussung von Chemokinen und Entzündung bei rheumatoider Arthritis unter der Therapie mit Leflunomid

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Einleitung: Chemokine sind potente Zellaktivatoren und Schlüsselmoleküle in der Regulation der Zellwanderung zum spezifischen Ort der Entzündungsvorgänge bei Rheumatoider Arthritis (RA). Das Chemokinsystem ist damit auch ein potentielles Ziel für die Therapie bei RA. Die Wirksamkeit einer Therapie mit einem erfolgreichen Basistherapeutikum wie Leflunomid kann möglicherweise mit Veränderungen der Chemokin-Expression einhergehen.

Ziel: Ziel war es, die Wirkung einer Leflunomid-Therapie auf die Expression von CC- und CXC-Chemokinen und auf die Akute-Phase-Reaktion von Patienten mit RA zu prüfen.

Methoden: In einer Multicenter-Studie wurde die Wirkung von Leflunomid auf drei CC- und ein CXC-Chemokin über einen Zeitraum von 6 Monaten bei 36 RA-Patienten, die die ACR-Kriterien erfüllten, untersucht. Die Konzentrationen des Chemokins CCL3 (Macrophage Inflammatory Protein 1α; MIP-1α), CCL4 (Regulated on Activation, Normal T-cell Expressed and Secreted; RANTES), CCL17 (Thymus and Activation Regulated Chemokine) und CXCL10 (Interferon-gamma inducible Protein 10 kDa; IP-10) wurden enzymimmunologisch im Serum vor, nach 3 und 6 Monaten Leflunomid-Therapie gemessen; auch die Spiegel des Akute-Phase-Markers Serum Amyloid A (SAA), eines sensitiven Markers für Entzündungsprozesse, wurde mittels EIA geprüft.

Ergebnisse und Diskussion: Nach 6 Monaten Leflunomid-Therapie wurden signifikant reduzierte Konzentrationen von CCL3 und CCL17 festgestellt; CCL3 erreichte schon nach 3 Monaten signifikant verringerte

Spiegel und korrelierte mit der Entzündungsaktivität, gleichzeitig verringerten sich auch die Serumkonzentrationen des Akute-Phase-Parameters SAA. Die CCL5-Konzentrationen zeigten nach 3 Monaten Therapie nur eine mäßige Tendenz zur Verminderung, nach 6 Monaten fiel ein nicht ganz erklärbarer Anstieg auf. Die CXCL10-Spiegel waren nach 3 Monaten signifikant reduziert, nach 6 Monaten jedoch erhöht. Es ist anzunehmen, daß diese Beobachtung mit jener des CCL5-Verhaltens in Verbindung steht, da CXCL10, das bekanntlich antiinflammatorische und antiangiogenetische Effekte bei der RA hat, die von CCL5 stimulierte Zellmigration hemmt.

Schlußfolgerung: Die Reduktion von CCL17 – einem starken Aktivator der Plättchenaggregation, welche eine weitere Chemokin-Ausschüttung aus den α-Granula bewirkt –, wie auch die deutliche Reduktion von CCL3 – einem Makrophagen-aktivierenden Chemokin –, weist auf eine immunomodulatorische Potenz von Leflunomid bei RA hin, in deren Rahmen eine Verminderung der Entzündungsprozesse – erkennbar an den verminderten SAA-Spiegeln – bewirkt wird. Eine längerfristige Therapie mit dem DMARD Leflunomid dürfte das Chemokinmuster von RA-Patienten verändern.

Literatur:
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Intravenous application of omega-3-fatty acids in patients with active rheumatoid arthritis: the ORA-1 trial

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Background: Unsatured fatty acids are supposed to exert a beneficial therapeutic effect in inflammatory diseases. This study to our knowledge constitutes the first investigation dealing with an intravenous application of omega-3-fatty acids in Rheumatoid Arthritis (RA) patients.

Objectives: To assess the therapeutic efficacy and tolerability of the intravenous application of omega-3-fatty acids in active RA patients.

Methods: 34 RA patients (27 yrs to 85 yrs; 29 female, 5 male; 26 RF+) were enrolled into this open pilot study. Patients had to have active RA, defined as a DAS28 higher than 4, at the screening visit (V0), despite ongoing DMARD-Therapy, and corticosteroids up to 10 mg prednisolone/day as well as NSAIDS. From V0 no change of the background therapy was allowed during the observation period. Patients received 2 ml/kg (= 0.1–0.2 g fish oil/kg) fish oil emulsion (Omegaven®) on 7 consecutive days in addition to the background therapy. The DAS28 was assessed before (V1), at day 8 (V2) and four weeks thereafter (V3). Primary efficacy measure was a decrease of the DAS28 greater than 0.6 at V2. Moreover the DAS28 at V3, the mHAQ, the ACR criteria of response and the SF-36 were applied as secondary efficacy parameters. Tolerability was assessed by laboratory measures and the reporting of side-effects.

Results: 33 patients completed the trial. The mean DAS28 at V0 amounted to a mean of 5.42, at V1 to 5.45 ($p = 0.32$). 19 (16 female) of the 34 pats (58 %) achieved a reduction of the DAS28 > 0.6 at V2, 9 of them > 1.2 (27 %). At V3 15 pts showed a DAS28 reduction > 0.6 (44 %), 12 of them > 1.2 (36 %). The mean DAS28 at V2 amounted to 4.51 ($p = 0.00029$ to V1) and at V3 to 4.71 ($p = 0.011$ to V1). No statistical difference was found between V2 and V3. In the 19 primary responders a mean reduction of the DAS28 of 1.52 and 1.06 at V2 and V3 respectively could be observed, indicating highly significantly different DAS28 values at these time points compared to V1. ACR 20 % responses at V2 and V3 were seen in 10 and 5 pts respectively, ACR 50 % in 5 respectively 3 pts at V2 and V3. Overall tolerability, including local reactions at the infusion site, was excellent. One patient dropped out for a severe flare of the disease, another severe adverse event was given by an acute lumbar disc herniation. Three upper respiratory tract infections and one phlebitis had to be observed additionally.

Conclusion: Intravenously applied omega-3-fatty acids were seen to be, at least in part, efficacious in a reasonable number of RA patients as additional therapeutic procedure. Moreover, their application was considerably well tolerated. A controlled trial of longer duration is warranted to reply to the question whether the intravenous application of omega-3-fatty acids could really constitute a therapeutic option in RA patients.

Enzyme Immunoassay for N-terminal C-type Natriuretic Peptide (NT-proCNP)

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CNP belongs to well characterised group of natriuretic peptides like atrial natriuretic (ANP) and brain natriuretic peptide (BNP), which play a pivotal role as natural vasorelaxants as well as bio-markers in cardiac diseases. All NPs are synthesised as protein precursors which are cleaved into the biologically active, C-terminal hormones and N-terminal fragments, which can be found in human serum, and as CNP in contrast to ANP and BNP, which predominantly derive from the heart, is also synthesised in the brain, vascular endothelium and bone and does not only act as vasorelaxant, but also as a neurotransmitter and stimulator of long bone growth. Therefore the physiological role of CNP is not only studied in cardiac disease [1], but also in bone developmental biology [2] and generally in bone research [3]. We developed an Enzyme Immunoassay prototype to provide a research tool for those studies. We chose epitopes (amino acids 1-19 and 30-50) of the N-teminal fragment (NT-proCNP) to raise antibodies in sheep, because NT-proCNP is more stable and present in higher concentrations than the active peptide hormone. First data on assay performance and on the serum levels of patients with renal disorders compared to a healthy control group are presented. We believe that plasma measurements of NT-proCNP will be a valuable tool for clinical and basic research in the fields of cardiac and bone diseases.

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THE POLYMYALGIA RHEUMATICA DISEASE ACTIVITY SCORE (PMR-AS) IN DAILY PRACTICE

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Background: In December 2003 EULAR response criteria for polymyalgia rheumatica (PMR), comprising morning stiffness, the ability to elevate the upper limbs, CRP/ESR, the visual analogue scale of pain (VAS pain) and the VAS of physician's global assessment, have been published. Subsequently a disease activity score, the PMR-AS, consisting of these parameters, resulting in a number expressing disease activity was developed and validated.

Objectives: To compare the PMR-AS with the current gold standard of monitoring, namely ESR and patient's global assessment, in PMR patients with the PMR-AS. Additionally, to assess whether, and if how, the PMR-AS is related to patient satisfaction.

Methods: 78 PMR patients (mean 65.97yrs; 38 to 84 yrs; 50 female, 28 male) before and on corticoids were enrolled into this cross-sectional evaluation. At the occasion of a regular control visit, in 9 pts at their first presentation, the PMR-AS was determined. Simultaneously patient satisfaction (PATSAT) from 1 (excellent) to five (completely unsatisfied), according to the school mark system, the ESR and the VAS of patient's global assessment (VAS pat global) were recorded. Patients were classified with respect to the disease activity categorizing according to the PMR-AS. Statistical analyses comprised tests for normal distribution, regression and correlation analyses (Pearson's r) as well as the Friedman test.

Results: The median PMR-AS amounted to 3.75 (0.1 to 29.2), indicating overall low disease activity. 58 pts were in low, 12 pts in medium and 8 pts (all at their first presentation) were in high disease activity. Median satisfaction was 2; the median ESR totalled to 15 mm/1st hour (2–80) and the median VAS pat global was 15 (0–95). The PMR-AS was significantly correlated to PATSAT ($r = 0.826$); to VAS pat global ($r = 0.711$) and to the ESR ($r = 0.64$) (all $p < 0.0001$). Regression analysis revealed that the PMR-AS, PATSAT, VAS pat global as well as patient's age were not related. Disease activity categorizing was highly significantly correlated to PATSAT, VAS pat global and the ESR (all $p < 0.0001$).

Conclusion: PATSAT, ESR and the VAS pat global were seen to be highly significantly correlated to the PMR-AS and the corresponding disease activity categories. Moreover, the PMR-AS, PATSAT, VAS pat global, and the ESR were seen to be not age dependent. The PMR-AS constitutes an easily applicable tool to monitor PMR-patients. Another advantage constitutes the possibility to compare patients independently of preceding examinations.

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DIMINISHED 5'EXONUCLEASE ACTIVITY IN B CELLS FROM SLE PATIENTS

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Purpose: Recently, we have shown that B cells from SLE patients utilize in the complementarity determining region (CDR) 3 significantly more often D segments in a hydrophobic reading frame than normal B cells. To analyze whether the dominant hydrophobic reading frame usage in B-cells from SLE patients is the result of an altered activity of the molecular mechanisms which are responsible for the generation of the CDR 3, we compaired this major antigen binding site from SLE antibodies with that from a healthy individual and a preterm infant.

Methods: CDR 3 from individually sorted B-cells from two SLE patients (n = 39 and n = 39, respectively) were analyzed for their composition using the V BASE Sequence Directory and GeneWorks software (Intelligenics, Inc.) and compared with that from a preterm infant (n=142, reported in Blood 2001; 97: 1511) and from individually sorted normal peripheral blood (PB) B-cells (n = 71). To eliminate the effect of antigen-specific influences only unmutated VDJ-rearrangements (= 98 % homology to the respective germline sequence) were analyzed. The Wilcoxon Rank test was used for statistical analysis (StatView for Windows; SAS Institute Inc.; Version 5.0.1). Threshold for significance was $p < 0.05$.

Results: In SLE B cells the 5'exonuclease activity was significantly lower compaired to B cells from a healthy adult and a preterm infant. Thus, the mean ± SE 5'exonuclease activity in SLE was 2.6 ± 0.5 nucleic acids (NA) versus 4.1 ± 0.6 NA ($p = 0.03$) and 4.0 ± 0.5 NA ($p = 0.026$), respectively. In contrast, no significant differences in the 3'exonuclease activity were observed. Furthermore, there was a significant difference between the 5' and 3'endonuclease activity within each group analyzed. Thus, in SLE B cells the mean ± SE 5' and 3' endonuclease activity was 7.4 ± 1.0 NA and 3.6 ± 1.0 NA ($p = 0.007$), respectively; in normal adult and preterm B cells 6.5 ± 0.7 NA versus 4.2 ± 0.7 NA ($p = 0.01$) and 6.5 ± 0.6 NA versus 4.2 ± 0.6 NA (0.000), respectively

Conclusion: Our data suggest that in SLE recombinational bias in the bone marrow is induced by an altered 5'exonuclease activity. This might be one of the reasons for the predominant usage of hydrophobic reading frames of D segments.

ANA DEVELOPMENT FOLLOWING TREATMENT WITH THREE DIFFERENT TUMOUR NECROSIS FACTOR ALPHA-BLOCKING AGENTS

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Background: The administration of tumour necrosis factor alpha (TNFα) blocking agents may lead to the development of abnormal titres of antinuclear antibodies (ANA) and rarely of lupus-like syndromes.

Objective: To investigate whether TNFα-antibodies or TNFα-receptor constructs differently induce antinuclear antibody production in patients with rheumatoid arthritis (RA) in clinical practice.

Methods: Clinical and serological data from 48 consecutive patients with active

Table 2: Mueller T et al. ANA development

	ANA negative before treatment	ANA development at follow-up
Infliximab	17	8 (47 %)
Adalimumab	12	3 (25 %)
Etanercept	8	2 (25 %)

RA (32 female), aged from 24 to 77 (median 54) years, who were treated with infliximab (23 patients), etanercept (8) or adalimumab (17) for at least six months were analyzed retrospectively. Before and after a median observation period of 27 weeks ANA were measured by indirect immunofluorescence and antibodies to double-stranded DNA (ds-DNA) using a commercial ELISA. Based on healthy control populations, ANA-titres >1:80 and ds-DNA antibodies > 55 IU/ml were considered abnormal. Results were compared to data from an extension of this cohort to 91 patients by including the diagnoses of ankylosing spondylitis and psoriatic arthropathies and a mean observation period of 76 weeks.

Results: Before treatment ANA were negative in 37 patients and all patients were negative for ds-DNA antibodies. During the observation period 13 patients developed de novo ANA and in 3 patients antibodies to ds-DNA became detectable. In the infliximab-group 8 of 17 patients developed ANA **(Table 2)** and one patient antibodies to ds-DNA. In three of the 12 ANA-negative patients treated with adalimumab abnormal ANA were detected and two of them showed elevated ds-DNA antibodies. In the etanercept group two of 8 patients developed ANA, and none of the patients became positive for ds-DNA antibodies. Data from the extension cohort yielded analogous numbers. In the whole observation period no lupus like syndrome was observed.

Conclusion: In a clinical practice setting, an increase in the titres of autoantibodies was observed with all three TNF-blockers currently on the market. The analysis of large databases of patients treated for long periods is necessary to investigate the clinical significance of this phenomenon.

The Importance of Micro-architecture and Mineralization parameters for pathogenesis of Idiopathic Osteoporosis in Male Patients with Fragility Fractures

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Aim: Transiliac bone biopsies from male patients (n = 24, mean age: 47 ± 12) with idiopathic osteoporosis and fragility fractures (vertebral and non vertebral) were investigated for microarchitecture and bone mineralization density distribution (BMDD) to elucidate their contribution to fracture risk.

Patients and Methods: Microarchitecture was described by trabecular bone volume (BV/TV), trabecular thickness (Tb.Th) and trabecular connectivity (N.Bf./B.Ar.). Mean calcium content (CaMean), the variation of calcium content (CaWidth) and the percentage of low mineralized matrix (CaLow) measured by quantitative backscattered electron imaging (qBEI) were used to characterize BMDD. The data were compared to published normative data. The biochemical parameters and the results of osteodensitometry (BMD measured by DXA) were compared to those of a healthy male control group (n = 35, mean age: 49 ± 9).

Results: The clinical parameters and the biochemical markers of bone turnover of the patients did not differ from the control group. The mean value of T-score (neck: –2.1 ± 0.8 SD, spine: –2.6 ± 0.9 SD, n = 24) of the patient group was significantly ($p < 0.001$) different from that of the control group (neck: –0.7 ± 1.1 SD, spine: –0.2 ± 0.9 SD, n = 35) but mostly in osteopenic levels. The analysis of the biopsies revealed that patients with fragility fractures were characterized at the tissue level by reduced Tb.Th. (–12.9 %) and lower trabecular linking / connectivity. At the material level CaMean (–5.8 %, $P < 0.0001$) was decreased, CaWidth (+15.9 %, $P < 0.0001$) increased and there was found a dramatically higher portion of CaLow (+73.2 %, $P < 0.0001$). No correlation was found between BMD of lumbar spine or hip and number of clinical fractures.

Conclusion: We conclude from these data, that hypomineralization at the material level but not BMD is linked to fracture risk in idiopathic male osteoporosis.

Ultraschall bei Patienten mit klinischem Verdacht auf Gicht: Sollten die klinisch stummen Grosszehengrundgelenke ergänzend untersucht werden, wenn die Beschwerden in einer anderen Körperregion lokalisiert sind?

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Studienziel: Die Körperperipherie mit ihrer niedrigeren Temperatur neigt zu Uratkristallniederschlägen, wobei bevorzugt der Bereich der Großzehengrundgelenke betroffen ist. Der Ultraschall ist als sensitive Methode zum bildgebenden Nachweis von Uratkristalldepots bekannt. Unserer Beobachtung nach kann der Uratkristallnachweis im akut entzündeten Gichtgelenk mißlingen, so daß das Studienziel war, zu untersuchen, ob die zusätzliche Untersuchung der klinisch stummen Großzehengrundgelenke die Ultraschalldiagnoserate der Gicht erhöht.

Patienten und Methode: In einer prospektiven Studie wurden 26 erwachsene Patienten (3 Frauen und 23 Männer) mit klinischem Verdacht auf Gicht, deren aktuelle Beschwerden nicht in den Großzehengrundgelenken lokalisiert waren, sowohl in der Region der Beschwerden, als auch an beiden Großzehengrundgelenken mittels Ultraschall untersucht. Die Ultraschalluntersuchungen wurden mit hochqualitativen Breitband-Matrix-Schallköpfen (8–14 MHz) durchgeführt. Ultraschallzeichen für diese Studie, welche verdächtig auf Uratkristallablagerungen gewertet wurden, waren reflexreiche Stippchen und echodichte wolkige Areale in Gelenksflüssigkeit, in Synovia und um Gelenke. Endgültige Diagnosen

wurden entweder mittels mikroskopischem Uratkristallnachweis aus Aspiraten und/oder charakteristischen klinischen und laborchemischen Parametern erstellt.

Ergebnisse: Bei 13 Patienten wurde eine endgültige Diagnose einer Gicht gestellt, 9 Patienten wiesen eine andere Form von Arthritis auf und 4 litten an einer Arthrose. Ultraschallzeichen von Uratkristallablagerungen fanden sich in 9 von 13 Gichtpatienten (69 %) in der Region der klinischen Beschwerden und in 12 dieser Patienten (93 %) an den klinisch stummen Großzehengrundgelenken. Die additive Ultraschalluntersuchung der Großzehengrundgelenke in Ergänzung zur Ultraschalluntersuchung der klinisch auffälligen Körperregion erhöhte die Gichtdetektionsrate von 69 % (9 von 13 Patienten) auf 100 % (13 von 13 Patienten).

Schlußfolgerung: Die Ultraschalluntersuchung beider klinisch stummer Großzehengrundgelenke in Ergänzung zur Ultraschalluntersuchung der klinisch betroffen Regionen erhöhte die Ultraschalldetektionsrate der diagnostisch richtungsweisenden Uratkristallablagerungen beträchtlich und sollte daher immer erfolgen.

AN UPSTREAM REGION OF MOUSE OSTEOPROTEGERIN BINDS ESTROGEN RECEPTOR AND CONTRIBUTES TO ESTROGEN RESPONSIVENESS

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The mechanism of estrogen regulation of adult skeletal metabolism involves the RANK/RANKL/OPG system. Disturbances in this pathway result in patho-physiological processes, such as osteoporosis or osteolytic metastasis. It was shown that estrogens regulate OPG expression but it was not shown up to now whether estradiol (E2) directly modulates OPG gene expression via binding of the estrogen receptor alpha (ER-alpha) to the OPG promoter.

We used genomic walking to isolate the 5'flanking region of the OPG gene and by computer analyses we identified four putative estrogen response elements (EREs). The transcriptional activity of the isolated OPG promoter was investigated by transfection experiments. The binding site for the ERalpha to the OPG promoter was identified with ERalpha/Promoter-DNA coimmunoprecipitation and electromobility shift assay (EMSA).

We isolated 2031 bp of the 5'flanking region of the mouse OPG gene. Computer analysis revealed four putative EREs, additionally to typical promoter elements. In transient transfections of luciferase-promoter constructs into ST2 and ROS17/2.8 cells, E2 treatment resulted in an upregulation, whereby in U2OS and MCF-7 cells E2 treatment resulted in a downregulation of the reporter activity. Coimmunoprecipitation of ER-alpha with the promoter-DNA revealed a binding of the ER-alpha to the OPG promoter within the proximal 385 bp, and with EMSA the range from –158 to –133 was verified to be a binding site for the ERalpha. Specific mutation of this ERE abolished all E2 mediated effects on the transcription as well as the binding of the ERalpha to the OPG promoter. The identified ERE is homologue to a functional ERE in the rabbit uteroglobin promoter.

In summary, we identified an ERE in the OPG promoter, which contributes to the regulation of OPG through estrogens in osteoblasts.

THE VALIDATION OF M-SACRAH (A MODIFIED AND SIMPLIFIED SCORE FOR ASSESSMENT AND QUANTIFICATION OF CHRONIC RHEUMATOID AFFECTIONS OF THE HAND) IN RHEUMATOID ARTHRITIS PATIENTS

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Background: We developed a patient administered score for the assessment of disease activity and therapeutic efficacy in hand osteoarthritis (HOA) as well as rheumatoid arthritis (RA) patients (SACRAH). The original score proved its reliability and showed construct validity by a high Cronbach's alpha, which was also suggesting the redundancy of some questions. For this reason and in order to shorten the score targeting an easier and quicker application in daily clinical practice, we modified the questionnaire (23 questions) and eliminated 11 questions, leading to 12 remaining (8 function domain, 2 stiffness domain, 2 pain domain). This modified score (M-SACRAH) was validated in a cohort of 60 HOA patients, who completed both questionnaires. The M-SACRAH showed a high correlation with the original score and proved to be as reliable but less time consuming and less complicated than the SACRAH.

Objectives: To validate the modified score in a cohort of RA patients.

Methods: 55 consecutive RA patients (median age 60 (22–77), 46 female / 9 male) completed both, the original SACRAH-questionnaire (23 VAS) + patient's global assessment (PGA) + physician's global assessment (PhGA) as well as the M-SACRAH (12 VAS). For all patients ESR (1st hour) and CRP (mg/dl) values were determined and the DAS28 was calculated.

Results: Median (range) SACRAH was 24.7 (0–84.3) vs. M-SACRAH 27.0 (0–86.7), p = n.s. The correlation coefficient for the total score amounted to $r = 0.99$, $p < 0.0001$ (Spearman rank correlation). A highly significant correlation was also seen between SACRAH and M-SACRAH domain scores (function: $r = 0.97$; stiffness: $r = 0.95$; pain: $r = 0.95$; $p < 0.0001$). The median ESR (1st hour) amounted to 33.5 (5–79), the median CRP to 1.05 (0–10). The median DAS28 reached 4.19 (1.61–7.18). The DAS28 was found to be significantly correlated to both the SACRAH ($r = 0.61$) and the M-SACRAH ($r = 0.6$). Median PGA amounted to 36 (0–90), and was found to be significantly correlated to the M-SACRAH ($r = 0.79$). Median PhGA was 25 (0–75), Spearman's rho for the correlation with the M-SACRAH reached $r = 0.56$. Cronbach's alpha for the M-SACRAH amounted to 0.95, indicating high reliability of the score.

Conclusion: The M-SACRAH proved to be as reliable but less time consuming and less complicated than the originally developed score in the assessment of patient reported disease activity in RA patients. A reasonable good correlation to the DAS28, thus mirroring actual disease activity, was to be observed. Internal consistency could be proven by Cronbach's alpha.

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Effects of the intravenous application of omega-3-fatty acids on the metabolism of active rheumatoid arthritis patients

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Background: Numerous beneficial health effects of unsaturated fatty acids are well known. Assuming a beneficial therapeutic effect in inflammatory diseases, we – as a novelty – intravenously administered omega-3-fatty acids to rheumatoid arthritis (RA) patients.

Objectives: To evaluate the therapeutic efficacy of intravenously administered omega-3-fatty acids on the clinical appearance of active RA patients. Here the effects on the patients' glucose-, fat- and coagulation-metabolism are reported.

Methods: 34 RA patients (27 yrs to 85 yrs, 29 female, 5 male) according to the ACR criteria gave their written informed consent to be enrolled into this open pilot study. All patients had active RA, defined as a DAS28 higher than 4.0 at the screening visit, 26 were seropositive and 8 seronegative. These individuals received one fish oil emulsion (Omegaven®) at a dosage of 2 ml/kg body weight (= 0.1–0.2 g fish oil/kg) i.v. per day for a seven day-period. The individual medication was left unaltered during the whole study. Clinical parameters and questionnaires to assess the functional handicap concerning activities of daily life and the psychological impairment were determined; these are reported elsewhere. Chemical parameters like ESR, CRP, PCC, PTT, fasting lipids (cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) and fasting glucose were measured immediately before treatment initiation (U1), the day after the last infusion (U2) and after a period of four weeks (U3). All patients were told not to change their usual diet until the end point of the study (U3) and agreed on that. Possible changes along the five weeks-observation period were statistically tested employing an analysis of variance for repeated measures (ANOVA).

Results: Omegaven®-infusions were generally well tolerated. The mean body mass index (BMI) did not change significantly during this time (U1: 27.6 ± 3.6 vs. U3: 27.5 ± 3.6), thus giving proof of the unaltered regimen. Among the measured blood parameters, no statistically significant differences between U1 and U3 were to be observed. Only LDL-cholesterol significantly changed and showed an initial increase which was followed by a final decrease at U3 (U1: 124 mg/dl ± 27 vs. U2: 133 mg/dl ± 31 vs. U3: 117 mg/dl ± 43).

Conclusion: Intravenous infusion of omega-3-fatty acids appeared to be well tolerated and did not lead to any change in the BMI or in the measured metabolic parameters of the observed RA patients. The unchanged levels of HDL-cholesterol and the decrease of LDL-cholesterol are in line with the results of protocols testing the oral supplementation of omega-3-fatty acids in overweighed patients.

Beschreibung des Receptor activator of nuclear factor κB ligand (RANKL) bei Sus scrofa

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Einleitung: Zytokine nehmen eine zentrale Rolle in der Regulation der Differenzierung der Zellen des mononukleär-phagozytären Systems, zu dem auch die Osteoklasten gehören, ein. Von besonderer Bedeutung ist in diesem Zusammenhang ein osteoklastogenetisches Zytokin der TNF-Superfamilie, der Receptor activator of nuclear factor κB ligand (RANKL, TNFSF11). RANKL wird unter anderem von Knochenmarksstromazellen, Osteoblasten und Lymphozyten produziert und natürlicherweise von Osteoprotegerin (OPG) antagonisiert (Lacey et al., 1998; Yasuda et al., 1998). Eine Vielzahl humaner knochen- und gelenkspathologischer Prozesse, wie die Osteoporose oder die rheumatoide Arthritis, werden über das RANK/RANKL/OPG-System mediiert (Sattler et al., 2004; Yoneda et al., 2004).

Material und Methode: Porcine Knochenmarkszellen wurden durch Spülen des Femurschaftes von Saugferkeln gewonnen. Nach Auftrennung der Zellsuspension über Ficoll-Paque wurden die Interphasezellen über eine Woche in α-MEM ±10^{-8} M 1α,25(OH)2D3 kultiviert. Osteoklasten wurden histochemisch mittels TRAP-Färbung dargestellt. Die Zellkulturüberstände wurden mittels kommerziellem ELISA auf solubles RANKL untersucht. Porcine RANKL-Sequenzen wurden unter Verwendung muriner RANKL-spezifischer Primer (Lee et al., 1999) amplifiziert und die erhaltenen Sequenzen mittels PHYLIP®-software mit humanen und murinen Sequenzen verglichen.

Ergebnisse und Diskussion: In den 1α,25(OH)2D3-sublimierten Kulturen, die eine signifikant höhere Anzahl an Osteoklasten hervorbrachten ($p < 0,01$), wurde die Bildung von solublem RANKL deutlich hochreguliert. Dies entspricht dem humanen und dem murinen System. Der Homologiegrad zwischen den untersuchten humanen und porcinen RANKL-Sequenzen beträgt 79 %. Die festgestellten Parallelen zwischen Mensch und Schwein stimmen optimistisch, daß porcine Knochenkultursysteme auf breiterer Basis als Modell für humane osteologische Fragestellungen dienen werden können.

The overexpression of osteoprotegerin (OPG) is not sufficient to inhibit cathepsin K, the main enzyme in matrix degradation, in patients with longstanding rheumatoid arthritis

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Background: Osteoprotegerin is a soluble decoy receptor, produced by osteoblastic cells and among others by cells of the synovium in rheumatoid arthritis (RA).The receptor activator of NF-kappa B ligand (RANKL) is responsible for osteoclast activation and maturation and inhibits

osteoclast apoptosis. OPG is known to inhibit the function of RANKL, thus preventing osteoclast maturation and influencing the levels of cathepsin K. Cathepsin K is essential for normal bone resorption which depends on the production of cathepsin K by osteoclasts and its secretion into the extracellular department. This leads to a degradation of organic matrix between the osteoclasts and bone surface by cleavage of collagen type I and II. The overexpression of cathepsin K, induced by proinflammatory cytokines like interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-alpha), due to the increase of cathepsin K expressing cells proves this protease as an important tool for bone resorption.

Objective: The aim of this study was to investigate the relationship between OPG and cathepsin K levels in the serum of patients with longstanding RA.

Methods: We measured serum levels of OPG, RANKL and cathepsin K in 100 patients with active, longstanding RA. The results were analysed by Spearman Correlation Statistics and Wilcoxon two sample test.

Results: We detected elevated serum levels of cathepsin K (median Q1–Q3 54.8 pmol/l) compared to a healthy control group (median Q1-Q3 12.7 pmol/l, $p = 0.0003$). Cathepsin K did not show a correlation with OPG ($p = 0.64$) and RANKL ($p = 0.81$), although OPG was also overexpressed in this RA group. Cathepsin K ($p = 0.59$) and RANKL ($p = 0.60$) are not age dependent, in contrast to OPG ($p < 0.0001$). The radiological destruction calculated with the Larsen score correlates significantly with cathepsin K ($p = 0.004$) and as we could demonstrate in earlier studies also with OPG ($p = 0.007$), but not with RANKL ($p = 0.26$). To give better insights in this metabolism we calculated the Cathepsin K/OPG ratio, which was significantly correlated with the Larsen score ($p = 0.05$); the cathepsin K/RANKL ratio correlates with the cathepsin K/OPG ratio ($p < 0.0001$) and weakly with the Larsen score ($p = 0.05$).

Discussion: OPG and RANKL together regulate the bone metabolism among other cytokines, Cathepsin K has a potent aggrecan-degrading activity, whereby the aggrecan cleavage products increase the collagenolytic effects of this protease against collage type I and II; a dysbalance of this system may be partly responsible for the skeletal complications of RA. The increased levels of OPG, which we found in our cohort seem to be an ineffective protective mechanism in RA, because it cannot prevent bone destruction reflected by the elevated serum levels of cathepsin K. So the inhibition of cathepsin K by selective inhibitors like aldehyde- or ketoamide-substrate analogues may provide an effective tool to prevent irreversible bone damages in RA.

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Serum levels of cartilage oligomeric matrix protein (COMP) are elevated in rheumatoid arthritis, but not in inflammatory rheumatic diseases as psoriatic arthritis, reactive arthritis, Raynauds syndrome, scleroderma, systemic lupus erythematosus, vasculitis and Sjögren's syndrome

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Background: During recent years a lot of interest has been focused on serum biomarkers in the clinic to evaluate ongoing disease process in the cartilage and bone. Cartilage oligomeric matrix protein in serum (sCOMP) is a biomarker for the cartilage turnover and is elevated in patients with rheumatoid arthritis (RA), osteoarthritis and articular trauma. Concentration of sCOMP is a valuable parameter for the assessment of therapy response in RA. Elevated serum levels of sCOMP showed a significant correlation with the progression of the Larsen score within five years in a patient group with established RA and it is described as prognostic factor in early RA. Inflammatory synovium has been considered as a potential tissue source of sCOMP since the molecule has been detected in the synovium in both, RA and osteoarthritis.

Objective: The aim of our investigation was to study if increased sCOMP is a specific marker for joint destruction comparing sCOMP between RA patients and patients with other inflammatory rheumatic diseases with non-destructive arthritis.

Patients and methods: Serum levels of COMP and CRP were measured in 150 patients: 77 patients with seropositive erosive RA, 15 patients with psoriatic arthritis (PsA), 10 patients with reactive arthritis (ReA), 12 patients with primary Raynaud's syndrome (RS), 11 patients with scleroderma (SCL), 9 patients with systemic lupus erythematosus (SLE), 7 patients with leucocytoclastic vasculitis (VA), 5 patients with primary Sjögren's syndrome (SS) and 4 patients with CRST-syndrome & primary biliary cirrhosis (CRST & PBC). COMP was measured by an ELISA according to the recommendation of the manufacturer (AnaMar Medical; cut off point: 10 U/L). Statistical evaluation was calculated by paired t-test.

Results: Elevated sCOMP levels were detected only in patients with RA and PsA. We found a significant difference between sCOMP in RA and PsA ($p = 0.01$), ReA ($p < 0.0001$), RS ($p = 0.0005$), SCL ($p = 0.003$), SLE ($p = 0.0002$), VA ($p = 0.0004$), SS ($p = 0.0007$), but not in CRST & PBC ($p = 0.06$). In RA sCOMP did not differ significantly between Steinbrocker stages II–IV. Serum levels of COMP were not significantly associated with CRP ($p = 0.78$).

Discussion: COMP is described as indicator for the current extent of cartilage destruction in RA. In our patient group elevated serum levels of COMP could only be detected in patients with RA and in few cases of PsA, but not in patients with ReA, RS, SCL, SLE, VA, SS. In none of the diseases sCOMP was associated with inflammation markers as CRP in agreement with other studies. In a previous study we found elevated COMP levels even in patients with low clinical prognostic factors (ESR, CRP, rheumatoid factor, DAS), but a correlation with delta Larsen over five years in this group. Our results further confirm the conclusion that COMP serum levels are highly specific markers for the cartilage degradation process in RA and not related to a non-specific inflammatory process in an arthritic joint.

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CATHEPSIN K: A NOVEL PARAMETER FOR THE MEASUREMENT OF BONE DESTRUCTION IN THE SERUM OF PATIENTS WITH ESTABLISHED RHEUMATOID ARTHRITIS

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Background: The Osteoprotegerin (OPG) / receptor activator of NF-kappa B ligand (RANKL) system is an important part of the regulation of bone metabolism. OPG, a decoy receptor for RANKL, is known to inhibit the function of RANKL, thus preventing osteoclast maturation and influencing the levels of cathepsin K. Cathepsin K is a cystein protease, which main function consists in the degradation of protein components of bone matrix. It is produced by osteoclasts and cleaves proteins like collagen type I and II and osteonectin and therefore it plays a role in bone resorption in diseases like rheumatoid arthritis.

Objective: The aim of this study was to investigate serum cathepsin K levels as parameter for the bone destruction measured by the Larsen score in patients with established rheumatoid arthritis.

Patients and methods: Serum levels of cathepsin K were measured in the sera of 100 patients suffering from RA according to the criteria of ACR. All patients received disease modifying antirheumatic drugs, whereby methotrexate was the most commonly used. Each blood examination consisted of the determination of cathepsin K, rheumatoid factor, C-reaktive protein, erythrocyte sedimentation rate and blood count. An enzyme immunoassay for cathepsin K developed by Biomedica Austria was used. The disease activity was documented by disease activity index (DAS), Ritchie index and the radiological destruction calculated by Larsen score. For the statistical evaluation a Spearman rank correlation was used.

Results: The cathepsin K serum levels of patients with RA (median Q1–Q3 54.8 pmol/l) compared to the healthy control group (median Q1–Q3 12.7 pmol/l) were significantly elevated ($p = 0.0003$). We found a statistically significant correlation between Cathepsin K and the Larsen score (mean 54.8) ($p = 0.004$). The highest levels of Cathepsin K were observed in patients with the highest Larsen score. Cathepsin K levels showed an increase with the progression of radiological destruction ($p = 0.035$). It was not associated with CRP and ESR. Serum levels of cathepsin K correlate with the DAS of the whole cohort ($p = 0.04$).We found no correlation with sex and age. The most frequently used DMARD was Methotrexate (n = 42), followed by Leflunomide (n = 10) and Sulfasalzine (n = 10), 22 patients had no DMARD at the time of examination. The lowest cathepsin K levels were evident in the leflunomide group, but no significant difference between these groups could be demonstrated.

Discussion: Cathepsin K is a part in the regulation of bone metabolism and is elevated in patients with rheumatoid arthritis compared to a healthy control group. The up-regulation of cathepsin K and the correlation with the Larsen score as parameter for radiological changes mirrors the destruction of bone structures in inflammatory diseases like rheumatoid arthritis. Cathepsin K seems to be a valuable parameter for the assessment of bone metabolism in patients with established RA and its measurement will probably contribute to develop targeted therapies to prevent further bone destruction.

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THERAPEUTIC EFFECTS OF GEMCITABINE ON CUTANEOUS MANIFESTATIONS IN AN ADAMANTIADES-BEHÇET'S DISEASE-LIKE MOUSE MODEL

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Introduction: Immunosuppressive agents are widely used for treatment of Adamantiades-Behçet's disease (ABD). The aim of this project was to study effects and side-effects of the new immunosuppressive agent gemcitabine (2',2'-difluorodeoxycytidine, dFdC), a pyrimidine synthesis inhibitor, on skin lesions of a herpes simplex virus-induced ABD-like mouse model.

Methods: For the dose-escalation study, ICR mice were treated intraperitoneally with dFdC over 5 days. For the efficacy study, ICR mice were inoculated with herpes simplex virus, classified as having ABD according to a revised Japanese classification, and then 18 ABD-mice were randomly assigned to placebo, 0.06 or 0.12 µg of dFdC /day over 5 days. Serum levels of interleukin- (IL-) 4, IL-6, IL-10, tumor necrosis factor-α and interferon-γ were determined using ELISA assays.

Results: After application of 3 µg dFdC over 5 days, alanine aminotransferase increased ($p = 0.032$), but all other kidney and liver parameters were unchanged. In ABD-mice, 5 days of dFdC treatment with 0.06 or 0.12 µg of dFdC /day resulted in a dose-dependent improvement of cutaneous manifestations by more than 60 % ($p = 0.017$). There was no significant change of cytokine levels and none of the cytokine levels correlated with response to treatment.

Conclusion: DFdC shows promising effects to improve cutaneous lesions in the herpes simplex virus-induced ABD-like mouse model. In this animal model, effects of dFdC on the cytokine profile remained inconclusive.

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Immunoadsorption (IAS) in lupus-nephritis: Reduction of proteinuria and rapid improvement of disease activity

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Purpose. To analyze the effects of IAS, a novel method of extracorporeal immunoglobulin removal used as a rescue therapy in severe SLE, on proteinuria and overall disease activity in SLE patients with renal involvement.

Patients and Methods. 16 SLE patients (2 m/14 f, mean age 29.9 ± 10.7 yrs., mean disease duration 77 ± 97 months) with severe SLE and renal involvement were treated with IAS when intravenous cyclophosphamide (IVCP) was contraindicated or failed to halt disease progression. We analyzed the effects of short-term IAS (within the first 3 months) and long-term IAS after 6 and 12 months. A reduction of proteinuria was determined as the primary outcome variable and a reduction in SLE activity (as indicated by SIS) and pre-treatment dsDNA levels were defined as secondary outcome variables. A decrease of at least 50 % was regarded a full response (r50), a decrease of 20 % was considered a partial response (r20). Patients not improving by at least 20 % were regarded non-responders (nr).

Results. 50 % of patients had an r50 in proteinuria within the first 3 months (13 % r20, 37 % were nr) leading to a decrease in mean (± SD) proteinuria from 6.5 ± 4.7 g/24 h to 2.5 ± 2.6 ($p < 0.05$). In addition, 81 % each had a r50 in the secondary outcomes (both SIS and anti-dsDNA-ab reduction), resulting in decrease in mean SIS (from16 ± 6 to 6 ± 3, $p < 0.05$) and anti-dsDNA-ab (from 460 ± 785 IU/lo to 107 ± 196, $p < 0.002$). IAS was continued in 11 patients. By 6 months, 64 % had reached the r50 in proteinuria (27 % a r20, 9 % were nr). We also observed further reductions in SIS (r50 = 91 %) and dsDNA-ab (r50 = 54 %). After one yr. of IAS treatment, most patients had significantly improved: 82 % had reached a response in the primary outcome criterium proteinuria (we observed a r50 in 64 % and a r20 in 18 %); with regard to the secondary outcome parameters, a reduction in SIS was achieved in all patients (r50 = 73 %, r20 in 27 %) and 91 % had a reduction in anti-dsDNA-ab levels (r50 in 73 %, r20 in 18 %). In the group of patients under long-term IAS, proteinuria had decreased from 6.7 ± 4.8 to 2.7 ± 2.5 ($p < 0.01$) within one yr., while SIS fell from 15 ± 6 to 5 ± 2 ($p < 0.001$) and and anti-dsDNA-ab from 391 ± 679 to 53 ± 53 ($p < 0.01$). Infectious complications and side effects were rarely observed under IAS.

Conclusion. IAS was associated with reduced proteinuria and improved overall disease activity in our cohort of negatively selceted patients,. These findings suggest that IAS, and extended IAS, in particular, may be of therapeutic benefit in SLE.

Autoreactive T-cells to histon H1 and core histones in SLE patients

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Background: B-cell hyperactivity leading to the formation of anti-nuclear and anti-ds-DNA autoantibodies (ab) is a hallmark of SLE. Histon H1 is located near the DNA linker region and may thus be better accessible to (auto-) immune responses than the core histones (H2–H4). H1 constitutes the major antigen (ag) in LE cell formation and anti-H1-ab are found in up to 60 % of SLE patients.

Objective: We observed T cell reactivity to purified H1 ag as well as to H2A, H2B, H3, H4 antigens in SLE patients and healthy controls in order to further elucidate the role of T cells and their influence on antibody production in human SLE.

Methods: PBMC of 42 SLE patients and 22 healthy controls were exposed to histone ags and a stimulation index (SI) ≥ 2 was considered specific. We then proceeded to draw H1-specific T cell clones (TCC) by cultivation and limiting-dilution cloning of T cell lines. T cell phenotyping was done by FACS; the T cell subset was determined by detecting IL4 and IFNγ in the supernatants by ELISA.

Results: After stimulation with H1 ag, SLE patients showed an elevated SI (2.3 ± 1.5, mean ± SD) when compared to HC (1.5 ± 0.4, $p = 0.01$) and a positive response in 19 of 42 patients (45 %) compared to 3 of 22 (14 %) responders among HC ($p = 0.02$). The proliferative response to H2A was slightly elevated in SLE (SI of 2.9 ± 2.4 vs. 1.7 ± 0.8 in HC, $p < 0.05$) and did not differ after stimulation with H2B, H3 and H4. The generated H1 specific TCC from SLE patients (n = 5) and healthy controls (n = 3) did not reveal a significant difference in SI. They showed a CD4+/CD8-/CD28+ a/b phenotype and produced IFNγ, suggesting that they were of Th1 phenotype.

Conclusion: Our data reveal that 45 % of SLE-patients have a significant T cell reactivity (SI ≥ 2) to histon H1 indicating that the antibody response might be T cell driven. In addition, all TCC were CD4+, which further supports the importance of these T cells in SLE and the formation of LE cells, in particular.

Detrimental effect of 3-Tesla high-field magnetic resonance to chondrocyte activity is temporary

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Objective: Today magnetic resonance imaging (MRI) is a highly sensitive method in the diagnosis of cartilage damage; however, the effects of high magnetic flux densities on cartilage metabolism are currently unknown. In this study we investigated the effect of a 3 Tesla MRI device on the biosynthetic activity of articular chondrocytes.

Materials: Metacarpophalangeal joints were obtained from 15 three month old calves and divided into 3 groups. Group 1 was left untreated and served as control. Group 2 was exposed to a static magnetic field (3 Tesla), while group 3 was subjected to a pulsed magnetic field (constant 3 Tesla, additional 0.0135 Tesla pulsed field, pulse rate 0.5 s) for the duration of a standard knee-joint examination. Directly after exposure cartilage was removed from the joints and biosynthesis of extracellular matrix macromolecules was measured by [^{35}S]Sulfate incorporation and values were normalized to hydroxyproline content. In a

second step cartilage explants were exposed to the magnetic field as described above and proteoglycan synthesis was measured on days 0, 3 and 6. Furthermore, to investigate a possible differentiation towards osteogenesis, alkaline phosphatase (ALP) activity was determined. Chondrocyte apoptosis was assessed by Annexin V staining and TUNEL-FITC labelling using FACS analysis 24 hours after exposure.

Results: The exposure to the magnetic fields, either static or pulsed, resulted in a significant decrease in cartilage macromolecule synthesis (control (mean ± SEM cpm/µg hydroxyproline): 6137.1 ± 446.5 vs static field: 3865.7 ± 415.6, $p < 0.002$; vs pulsed field: 3177.8 ± 256.5, $p < 0.00002$). In time response studies cartilage metabolic activity recovered and reached proteoglycan synthesis levels comparable to control cultures after 3 days post exposure. The release of proteoglycans into the supernatant remained unchanged when compared to controls. Annexin V staining as well as TUNEL labelling revealed no significant increase in the number of apoptotic cells in the static and pulsed magnetic field group. There was no difference in ALP activity between the 3 groups.

Conclusion: Although high-field magnetic resonance provides a better signal-to-noise ratio with the possibility to gain higher spatial resolution, our data demonstrate reduced matrix synthesis by articular chondrocytes under the influence of high magnetic flux densities. The 3 Tesla MRI may mimic mechanical stress and induce an inadequate movement of fluid and electrolytes thereby temporarily compromising chondrocyte metabolism.

A CORTICOSTEROID SIDE EFFECT QUESTIONNAIRE (SCSEQ) DISCRIMINATES BETWEEN CORTICOSTEROID USING AND NOT-USING PATIENTS WITH RHEUMATOID ARTHRITIS (RA) AND POLYMYALGIA RHEUMATICA (PMR)

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Background: Corticosteroids (CS) are widely used in the treatment of RA and PMR patients. Side effects related to CS are commonly reported, but because of their heterogeneity difficult to quantify and to compare, e.g. in clinical studies. As a consensus of the authors a questionnaire comprising 8 questions (7 for male patients), covering the most frequent corticosteroid side effects, was developed targeting a possible quantification of corticosteroid tolerability.

Objectives: To assess the discrimination capacity of this questionnaire, its internal consistency and validity.

Methods: The questions were formulated in a general way emphasizing the recently applied medication, but without using the term CS. However, they were targeting the most commonly reported CS side-effects (mood change, depression, infections, weight gain, hypertension, skin reactions, muscle weakness, menstruation cycle changes). Possible answers were yes (1) or no (0). The SCSEQ was completed by 44 regular out-patients with (mean 6.42 mg/day; 2.5 to 25 mg) and without CS therapy suffering from RA and PMR, resulting in a possible score between 0 and 8, for male patients 7 respectively, for each individual. Patients were age and gender matched. Internal consistency testing was carried out by the split-half method; additionally, factor analysis was performed. Discrimination capacity was calculated by comparing the scores of the individual patients and the numbers of positive answers to each question in CS-users and non-users. Moreover a possible CS dose relationship was assessed by regression analysis.

Results: The half-split procedure resulted in a Spearman-Brown coefficient of 0.763 for CS-users and 0.682 for non-users indicating reasonable internal consistency. Factor analysis revealed that each single question contributes significantly to the score, but to different extents. The mean number of positive answers to each single questions was 10.38 (3–22) in CS users and 3.88 (1–10) in non-users ($p = 0.009$). The median score in CS-users amounted to 2 (0–6), and to 0 (0–3) in non-users ($p < 0.0001$). Regression analysis revealed that the score was CS dose dependent ($p = 0.02$). Mood change (question 1; $n = 22$), weight gain (question 4; $n = 15$) and muscle weakness (question 7; $n = 12$) were most frequently answered positively by CS users. Analysis of the negative answers in the same way gave congruent results.

Conclusion: The pilot analysis of the SCSEQ presented here indicates internal consistency. Moreover, this tool is able to discriminate between CS-users and non-users. Ongoing investigations are intended to prove its validity to score corticosteroid side effects.

SPONTANE REMISSION EINES MARGINALZONEN-LYMPHOMS NACH ABSETZEN DER INFLIXIMAB-METHOTREXAT-KOMBINATIONSTHERAPIE

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Fallbericht: Eine 64-jährige Patientin mit 1997 diagnostizierter, seropositiver chronischer Polyarthritis (cP) wurde 2002 bei ausgeprägten Gelenksschwellungen trotz bestehender Therapie mit Methotrexat (MTX) und Sulfasalazin (SAL) an unser Zentrum überwiesen. Bei hochgradiger klinischer Aktivität (DAS284v von 5,56) leiteten wir eine Therapie mit Infliximab in Kombination mit MTX ein. In Woche 12 zeigte sich ein gutes Therapieansprechen (DAS284v von 2,87). In der klinischen Kontrolle vor Verabreichung der 16. Infliximab-Infusion berichtete die Patientin über eine kontinuierlich „verstopfte Nase". Die Patientin wurde an einen HNO-Facharzt weiter überwiesen. In der durchgeführten Epipharyngoskopie wurde ein kleiner Tumor im Epipharynx nachgewiesen. In der Computertomographie (CT) und Magnetresonanztomographie (MRT) wurde die Diagnose „hyperplastisches lymphatisches Gewebe" gestellt. Aufgrund des erhöhten Malignomrisikos bei Patienten mit hochaktiver cP [1–4] und DMARD-Therapie [5–7] wurde eine Biopsie des Tumors durchgeführt. Histologisch konnte die Diagnose eines Marginalzonen-Lymphoms gestellt werden. Nach Diagnosestellung wurde die Infliximab-MTX-Therapie abgesetzt. Aufgrund der niedrigen Malignität, sowie der Möglichkeit von Spontanremissionen wurde keine onkologische Therapie eingeleitet. Engmaschige klinische Kontrollen, sowie eine erneute MRT und Biopsie der betroffenen Region nach drei Monaten erfolgten. Nach drei Monaten fanden sich weder im MRT noch in den Rebiopsien Hinweise für das Marginalzonen-Lymphom. Weitere Kontrollen wurden nach sechs und neuen Monaten durchgeführt und erbrachten ebenso unauffällige Befunde.

Diskussion: Patienten mit hochaktiver cP haben ein erhöhtes Risiko für Malignome [1–4]. Ein Teil dieser Tumore ist möglicherweise als therapieinduziert anzusehen [5–7]. Unsere Patientin wies beide Risikofaktoren, hohe Krankheitsaktivität und aggressive antirheumatische Therapie, für die Entwicklung einer lymphoproliferativen Erkrankung auf. Aufgrund der raschen und vollständigen Remission des Marginalzonen-Lymphoms nach Absetzen der Infliximab-MTX-Therapie ist von einer therapieinduzierten Erkrankung auszugehen. Für die klinische Routine in Fällen wie dem hier beschriebenen empfehlen wir rasches Absetzen der möglicherweise auslösenden Medikamente, sowie engmaschige klinische Kontrollen. Da Remissionen möglich sind, sollten initial keine onkologischen Therapiemaßnahmen eingeleitet werden.

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Einfluss von Bone morphogenetic protein-5 auf Osteoklasten und Osteoblasten

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Einleitung: Neuerdings stellen Bone Morphogenetic Proteins (BMPs) einen vielversprechenden Ansatz knochenrekonstruktiver Therapien dar. Knochenumbau resultiert aus einem Gleichgewicht von Formation (Osteoblastenfunktion) und Resorption (Osteoklastenfunktion) welches der Steuerung durch systemische Hormone und lokale Faktoren unterliegt. Bone Morphogenetic Proteins (BMPs) sind lokale Faktoren mit der Fähigkeit, die Differenzierung von mesenchymalen Stammzellen zu Osteoblasten zu induzieren. Für BMP 2, 4 und 7 zeigten In vitro-Studien, daß sie sowohl die Differenzierung von Osteoklasten als auch die Aktivität von Osteoblasten dosisabhängig fördern. Bis dato existieren keine In-vitro Untersuchungen über die Auswirkungen von BMP-5 auf Knochenzellen, obwohl im Tiermodell die bedeutende Rolle von BMP-5 für die Ossifikation von Knochendefekten gezeigt werden konnte. Das Ziel dieser Studie war es, die Effekte von BMP-5 auf Osteoklasten und Osteoblasten in vitro zu untersuchen.

Methodik: Murine Osteoklasten wurden mittels TRAP-Assays und Resorption-pit-Assays untersucht: Murine Osteoblasten wurden hinsichtlich Alkalischer Phosphatase-Aktivität (ALP) und Proliferation überprüft. Die Bildung von Osteoklasten wurde durch Osteoprotegerin (OPG) gehemmt.

Ergebnisse: Unsere Ergebnisse zeigten einen biphasischen Verlauf der Anzahl der TRAP+/MNC durch BMP-5, die Konzentration mit dem stärksten stimulierenden Effekt betrug 1ng/ml. Der pit formation assay bestätigte die Resorptionsfunktion dieser Osteoklasten. Der ALP-Assay zeigte einen dosisabhängigen Aktivitätsanstieg der ALP durch BMP-5 mit dem stärksten Effekt bei 300 ng/ml. Auch die Zunahme der Proliferation der Osteoblasten war dosisabhängig mit dem stärksten Effekt bei 300 ng/ml. Die Steigerung der Anzahl der TRAP+/MNCs wurde durch den Zusatz von Osteoprotegerin dosisabhängig gehemmt.

Diskussion: Die biphasische Steigerung der Anzahl der TRAP+/MNCs von BMP-5 in vitro steht im klaren Gegensatz zu bisher bekannten Effekten von BMP-2, -4 und -7 auf die Generation von Osteoklasten. Andererseits kommt es bei Osteoblasten durch BMP-5 zu einer Steigerung sowohl der ALP-Aktivität als auch der Proliferation. Die Regulation der Osteoklastengeneration durch BMP-5 unterliegt der Steuerung durch Osteoblasten. Trotz der unterschiedlichen Dosis-Wirkungskurve der TRAP+/MNCs durch BMP-5, ist wie bei anderen BMPs der RANKL-RANK Mechanismus an der Osteoklastendifferenzierung beteiligt. Da die Effekte auf Osteoklasten und Osteoblasten in unterschiedlichen Konzentrationen auftreten, könnte BMP-5 einen positiven Nettoeffekt auf die Knochenneubildung haben.

A modified disease activity score with fibrinogen as a tool for the assesment of disease activity in patients with rheumatoid arthritis

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Purpose: To determine the correlation between fibrinogen and disease activity in RA patients, as an alternative tool to assess disease activity in daily routine.

Rationale: Regarding the costs for the lifelong therapy of patients with rheumatoid arthritis (RA), the monitoring of disease activity gains increasing importance in everyday therapeutic management. Most disease activity-scores are based on clinical joint assesssment and erythrocyte sedimentation rate (ESR) as a laboratory parameter for inflammatory activity. The multitude of factors influencing ESR (anemia, immunoglobulin level, renal failure, sex), the lack of standardisation of ESR-analysis and its decreasing usage in daily routine throughout medicine raise the question of an alternative. The first thought might be CRP, but because of different test kits with various reference values, it is hard to compare the results. Another alternative might be fibri-

nogen, which is regarded superior to ESR, since it seems to be independent of the above mentioned factors. In addition its analysis is standardized and can be done by almost every laboratory. However there is little data of its value as tool for determining inflammatory activity in RA to be used in disease activity scores.

Methods: The disease activity in 79 patients (57 female, 22 male; mean age (SD) 57 (13) yrs) with established RA according to ACR criteria, was assessed using the disease activity score 28 [DAS28 = (0.56v(tender joints 28) + 0.28v(swollen joints 28) + 0.70ln(ESR)) * 1.08 + 0.16] and a modified DAS 28 [mDAS28 = (0.56v(tender joints 28) + 0.28v(swollen joints 28) + 0.70ln(fibrinogen/10)) * 1.08 + 0.16]. As a sign of articular inflammation the sum of tender and swollen joints (STSW) were used. All subjects gave their written, informed consent before participating in the study. Blood was drawn from all patients at the time they were clinically evaluated and plasma fibrinogen (using nephelometry), CRP (using immunoassay) and ESR were determined. Correlation between disease activity scores (DAS28, mDAS28), ESR, STSW, CRP and fibrinogen was tested using the Z-test. P-values < 0.05 were regarded as significant. (StatView for Windows; Version 5.0.1, SAS)

Results: The two scores (DAS28, mDAS28) strongly correlated with each other ($r = 0.95$, $p < 0.0001$). The correlation between STSW and fibrinogen and ESR were adequate (fibrinogen $r = 0.331$, $p < 0.028$; ESR $r = 0.399$, $p < 0.0002$). Futhermore, the serum CRP-levels significantly correlated with both disease activity scores (mDAS28 $r = 0.514$, $p < 0.0001$; DAS28 $r = 0.557$, $p < 0.0001$) as well as with STSW ($r = 0.541$, $p < 0.0001$).

Conclusion: Our results suggest, that fibrinogen is a valuable tool for assessing disease activity in patients with RA and in our opinion fibrinogen could replace ESR.

Significant increase of IgG- and IgA-rheumatoid factor in patients with rheumatoid arthritis during prolonged treatment with etanercept

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Background: Rheumatoid arthritis (RA) is a chronic, progressive disease characterized by continous activation of the immune system. Although, the initiating event of RA is still unknown, recent research has demonstrated the pivotal role of T-cells and certain cytokines. Targeting this molecule with soluble receptors, i.e. etanercept, or antibodies, like infliximab, a new class of highly effective antirheumatic drugs has been developed. In contrast to T cells the role of B cells in RA is still contraversial. Nevertheless, B cells appear to be of importance in the pathogenesis of RA. The products of B cells such as the rheumatoid factor (RF) or anticyclic citrullinated peptide (aCCP) antibodies are well established indicators of this disease. Several reports have also indicated that a high titer of RF is associated with increased disease activity, progressive joint damage, and extra-articular manifestations especially in patients with elevated RF-IgG and RF-IgA isotypes.

Aim: To study the longterm effect of anti-TNF therapy on the production of antibodies we measured the serum levels of Ig and certain autoantibodies in RA patients treated with etanercept.

Methods: 13 consecutive sero-positive RA patients treated with etanercept were included in the study after informed consent and approval by the local ethics committee were obtained. Patients fullfilled the ACR-criteria for the diagnosis of RA. Patients were followed for 9 months, and clinical efficacy of treatment was evaluated using the disease activity score (DAS). Serum samples were collected at baseline and after 9 month. For detection of RF isotypes (RF IgA, IgM, IgG), aCCP commercially available ELISA-kits were used. Serum immunoglobulin levels were determined by nephelometry. The Wilcoxon Signed Rank Test was used for statistical analysis. Threshold for significance was $p < 0.05$.

Results: All RA patients showed a rapid, significant and sustained response to etanercept therapy. There was a significant decrease in the DAS after the first month that was constant for the whole observation period. At baseline the mean DAS ± SE was 5.6 ± 0.3 and after 9 month 3.7 ± 0.2 ($p = 0.002$). Suprisingly, a significant increase in RF-IgG and RF-IgA serum levels after 9 months of anti-TNF-therapy was observed. Thus, RF-IgG raised from 20.6 ± 8.1 to 33.8 ± 11.5 IU/ml ($p = 0.04$) and RF-IgA from 19.5 ± 4.8 to 30.5 ± 5.9 IU/ml ($p = 0.01$). No significant change in the mean-serum level of IgM, IgG, IgA, aCCP and RF-IgM before and after 9 months of treatment with etanercept was found.

Conclusion: These data suggest that etanercept seems to have a pivotal effect on RF-producing B cells either directly or indirectly. Thus, the down-stream isotypes of RF, i.e. IgG and IgA, were significantly elevated after 9 months of treatment. Furthermore this increase was not found for the other RA-specific autoantibodies aCCP and RF-IgM. Interestingly, RF-IgG and -IgA has been linked to the severity of erosive arthritis and extra-articular manifestation of RA. Further studies are warranted to evaluate whether the combination of drugs that specifically suppress B cells and T cells, might lead to a prolonged and sufficient response in patients with RA.

Vascular endothelial growth factor expression in patients suffering from Raynaud's phenomenon

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Purpose: To determine the vascular endothelial growth factor (VEGF) levels in patients with Raynaud's phenomenon.

Rationale: Recently, it has been shown that patients with thrombangitis obliterans (TAO) have an elevated serum level of

VEGF compaired to healthy individuals. This disease is characterized by the triade of claudication of the affected extremity, Raynaud's phenomenon and migratory superficial vein thrombophlebitis. Raynaud's phenomenon that is the episodic digital blanching, cyanosis, and rubor of the fingers or toes following cold exposure and subsequent rewarming, is not limited to TAO but is also a common early symptom of other diseases, including Raynaud's disease, scleroderma, CREST and mixed connective tissue disease. To test whether the observed elevation of VEGF is a common feature of Raynaud's phenomenon or is limited to pathophysiological alterations in TAO we analyzed the VEGF-levels, as well as several other growth factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF) and platelet derived growth factor (PDGF) in patients with primary and secondary Raynaud's phenomenon.

Methods: A total of 68 patients (49 female, 19 male; mean age (SD) 54 (11) yrs) with diagnosed Raynaud's phenomenon (21 patients with Raynaud's disease, 13 patients with CREST, 22 patients with TAO, 6 patients with MTCD and 6 patients with scleroderma) were included in the study. All subjects gave their written, informed consent before participating in the study. Blood was collected by venepuncture from all patients and after clotting, it was centrifuged for collection of serum. All serum samples were stored at –20 °C and VEGF-levels were analyzed by commercial available Luminex™ kit (Human VEGF, EGF, FGF and PDGF BeadmatesTM, Upstate, NY). The Wilcoxon Rank test was used for statistical analysis (StatView for Windows; SAS Institute Inc., Version 5.0.1). Threshold for significance was $p < 0.05$.

Results: There was a significant increase in the VEGF-levels in patients with TAO compaired to Raynaud's disease and scleroderma. Thus, in TAO the mean ± standard error was 189 pg/ml ± 52, in Raynaud's disease 92 pg/ml ± 20 ($p=0.04$), and in scleroderma 50 pg/ml ± 18 ($p = 0.03$). In contrast there was no difference in the VEGF-levels in patients with TAO compaired to patients with MCTD (185 pg/ml ± 72) and CREST (185 pg/ml ± 98). The analysis of all other growth factor serum levels revealed no difference between all examined diseases.

Conclusion: Our results suggest, that in contrast to TAO, MCTD and CREST patients with scleroderma and Raynaud's disease have a limited capacity to produce VEGF and therefore an impaired ability to develop new collaterals.

OSTEOCALCIN-MRNA IS DETECTABLE IN HYPERTROPHIC CHONDROBLASTS OF THE EPIPHYSEAL GROWTH PLATE IN MICE

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Osteocalcin (OCN) is the most abundant non-collagenous protein of the bone matrix and is expressed in mature osteoblasts. Although mice depleted in both genes have higher bone mass, the exact function of this small protein is still unknown. It was reported that its expression is restricted to osteoblasts, but recently it was also shown by PCR that hypertrophic chondrocytes of the epiphyseal growth plate express it.

In-situ hybridization was performed on demineralized paraformaldehyde fixed metatarsal bones of newborn mice. DIG-labeled anti-sense transcripts of the mouse OCN-cDNA were used as probe and the sense as negative control. Hybridization was performed at 60 °C with 50 % formamide (FA) in 6 x SSC overnight. After washing with 50 % FA at 60 °C and short washes with TBS, 1:100 anti-DIG antibodies coupled with horse radish peroxidase in blocking solution was bound to the hybridized RNA. Developing was performed with DAB-substrate for 1 h at 37 °C. Slides were counterstained with hemalaun.

In untreated normal mice aged 3 and 7 days, OCN mRNA was strongly expressed in the hypertrophic zone of metatarsal bone. This mRNA was also strongly expressed in osteoblasts at the zone of secondary ossification at the epiphyseal growth plate, as expected. To a lesser extent, it was also present in the zone of proliferating cartilage, mainly in chondroblasts arranged in vertical columns. Signal intensity was high in the transitional zone between columnar and hypertrophic chondroblasts and decreased in apoptotic chondroblasts. Our results based on in-situ hybridization support the previous findings by PCR that hypertrophic chondroblasts express OCN.

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