Determination of anti-CCP antibodies in patients with suspected rheumatoid arthritis: does it help to predict the diagnosis before referral to a rheumatologist?

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Prognosis in rheumatoid arthritis (RA) depends critically on early diagnosis and timely treatment with immune modulating drugs. As a consequence, early referral and access of patients with suspected RA to rheumatologists is mandatory for the establishment of diagnosis and initiation of treatment.

Measurement of antibodies to cyclic citrullinated peptide (CCP) is a new and highly specific test for the diagnosis of RA. Detection of anti-CCP antibodies—in particular, if the second generation of anti-CCP2 tests is used—has been shown to be of prognostic significance and to be helpful in early diagnosis of RA.^{1–9}

The goal of this work was to investigate whether the measurement of anti-CCP antibodies alone or in combination with easily determinable parameters of a patient's complaints and routine laboratory tests might help to identify prospectively patients with a high probability for RA.

For this study 102 patients from a routine rheumatology clinic were examined. All were referred by general practitioners, orthopaedic surgeons, or other non-rheumatological subspecialties because of suspected RA. In all patients the value of the anti-CCP antibody (second generation anti-CCP2-test; Euroimmun, Lübeck, Germany), IgM rheumatoid factor (RF, as determined by nephelometry; Beckman Coulter, Krefeld, Germany), C reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were measured, and physical, laboratory, and radiological examinations were performed. RA was diagnosed according to the American College of Rheumatology (ACR) criteria revised in 1987.¹⁰ All

patients were questioned about the presence of morning stiffness of the joints and or muscles and about the presence of polyarticular pain, which was interpreted as positive if at least four tender joints were reported.

Sensitivity and specificity and—to obtain better information about the diagnostic value with a low pretest probability—the positive and negative predictive values (PPV and NPV) of the tests were calculated. For the latter, the following formulae were used:

$$PPV = a/(a+b); NPV = d/(c+d)$$

where a = test positive, disease positive; b = test positive, disease negative; c = test negative, disease positive; d = test negative, disease negative.

Moreover, the relative risk of fulfilling the ACR criteria for RA, whether or not the test criteria were present, was determined.

Twenty eight of the 102 patients fulfilled the diagnosis of RA according to the ACR criteria (pretest probability of 27%).¹⁰ The other patients were classified as having unclassified monarthritis, polyarthritis, or oligoarthritis (n = 21), arthralgias of unknown origin (n = 20), osteoarthritis of the fingers (n = 20), psoriatic arthritis (n = 4), fibromyalgia (n = 3), polymyalgia rheumatica (n = 2), cervicobrachialgia (n = 2), periostitis (n = 1), and reactive arthritis (n = 1).

If a patient was positive for anti-CCP, the PPV, or in other words the probability of fulfilling the ACR criteria for RA, increased to 55% (table 1).

This relatively low predictive value of the anti-CCP test was increased when it was combined with easily obtainable

Table 1 Values of the anti-CCP and the rheumatoid factor (RF) test alone or in combination with different laboratory parameters and patient's complaints for the diagnosis of RA

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	RR	95% CI
Anti-CCP	43	86	55	80	2.7	1.5 to 4.9
Anti-CCP plus ESR	21	95	60	76	2.5	1.3 to 4.7
Anti-CCP plus RF	36	91	59	79	2.8	1.6 to 4.9
Anti-CCP plus CRP	25	97	78	77	3.4	2.1 to 5.8
Anti-CCP plus polyarticular pain	43	95	78	81	4.0	2.4 to 6.8
Anti-CCP plus morning stiffness	29	99	89	78	4.1	2.7 to 6.5
RF	46	78	45	79	2.2	1.2 to 4.0
RF plus ESR	18	93	50	75	2.0	0.9 to 4.1
RF plus CRP	18	96	63	76	2.6	1.3 to 4.9
RF plus polyarticular pain	46	92	68	82	3.8	2.2 to 6.6
RF plus morning stiffness	30	97	80	78	3.7	2.2 to 6.0

The results of the positive predictive value (PPV) are highlighted. The patient group tested comprised 102 patients with suspected RA who were referred to a rheumatologist. Twenty eight of them fulfilled the ACR criteria for RA (pretest probability of 27%).

PPV, positive predictive value; NPV, negative predictive value; RR, relative risk; 95% CI, 95% confidence interval for relative risk; anti-CCP, anti-CCP values above normal (<2 U/l); RF, rheumatoid factor above normal (<10 U/l); CRP, C reactive protein value above normal (<5 mg/l); ESR, erythrocyte sedimentation rate >20 mm/1st h; morning stiffness, morning stiffness of at least 60 minutes; polyarticular pain, pain in at least four joints or muscular regions.

information about the patient's complaints: if a patient had a positive anti-CCP test and at the same time morning stiffness of at least 60 min the PPV was 89%. In fact, the only patient who was positive for both measures without fulfilling the ACR criteria for RA was thought by the examining rheumatologist to have early RA. Polyarticular pain is a symptom which was present in all patients with RA and, additionally, in the patients with arthralgias, undifferentiated polyarthritis, fibromyalgia and polymyalgia rheumatica. If anti-CCP positive patients had polyarticular pain, a PPV of 78% and a NPV of 81% was obtained. In contrast, the combination of anti-CCP and RF, as was suggested by other reports,²⁻⁹ did not result in a similar increase of the PPV or NPV (59% and 79%, respectively). The rather low sensitivity and specificity of the anti-CCP test (table 1) was in accordance with other reports with similar results obtained in unselected rheumatological patients who were not subjected to a prolonged follow up.¹¹

In conclusion, if information about morning stiffness of the joints or polyarticular pain is added, anti-CCP gives us a tool which more accurately predicts the presence of RA. This tool might be important to identify patients with early RA and facilitate a more precise selection of patients for rapid access to rheumatologists.

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Safety of 15-deoxyspergualin in the treatment of glomerulonephritis associated with active systemic lupus erythematosus

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Optimal treatment for patients with relapsing lupus nephritis remains unclear. The ability of 15-deoxyspergualin (gusperimus; 15-DSG) to suppress systemic lupus erythematosus (SLE)-like diseases has been demonstrated in animals and humans.¹⁻⁴ 15-DSG exerted no nephrotoxicity or hepatotoxicity but reversibly induced leucocytopenia.^{5,6}

In this study we aimed at evaluating the safety of 15-DSG in the treatment of glomerulonephritis associated with SLE.

CASE REPORTS

Table 1 shows the patient characteristics.

15-DSG was provided by Nippon Kayaku Co Ltd, Tokyo, Japan. Patients gave their informed consent, and 15-DSG 0.5 mg/kg normal body weight (height in cm minus 100)/day was self administered subcutaneously for 14 days, followed by a break of 7 days (= 1 cycle). The dose was adjusted

(dependent on efficacy or safety, or both) after cycles 4 and 6 to 0.35 mg/kg and 0.25 mg/kg, or 0.7 mg/kg and 1.0 mg/kg.

Patient 1

After a bolus, daily corticosteroids could be decreased to 5 mg after 9 weeks. The patient received six cycles of 15-DSG without major problems. Leucocyte counts were always $>4 \times 10^9$ cells/l, no infection was seen, no (serious) adverse events and occurred with the exception of parageusia. GN resolved and her SLE associated activity measures improved (table 1, fig 1). After the sixth cycle the patient was switched from 15-DSG to a combination of ciclosporin A (CSA) and azathioprine, and later azathioprine alone. Renal function was normal at the end of the trial, SELENA-SLEDAI (Safety of Oestrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index) decreased from 12 to 0, and the corticosteroid dosage was reduced to 5 mg/day.

Figure 1 Proteinuria during 15-DSG treatment in patients 1–3. 24 hour urine was collected at the day of entry into the study or the last days of the cycles as indicated. Owing to menstrual bleeding urine collection was not possible in patient 1 at the end of cycle 4 and in patient 2 at the end of cycles 4, 6, and 7.

Patient 2

Oral corticosteroids were increased to 40 mg/day and tapered to 5 mg/day within 9 weeks. Proteinuria decreased to 700–750 mg/day (fig 1; table 1). During the second cycle, she had herpes zoster exanthema (which completely disappeared after treatment with antiviral agents). The white blood cell count was always $>5 \times 10^9$ cells/l. Because dsDNA antibody titres remained high, the 15-DSG dosage was increased to 0.75 mg/kg/day for cycles 5 and 6, to 1.0 mg/kg/day for cycles 7–9. During cycle 5 she experienced bacterial bronchitis without fever. At the end of cycle 6 her anaemia worsened (possibly 15-DSG-related?).⁷ Within the ninth cycle, for the first time during treatment with 15-DSG, the urine sediment indicated a flare of GN. At this time, proteinuria was 890 mg/day (fig 1); creatinine was always normal. SELENA-SLEDAI decreased from 12 to 4 (end of cycle 8). The corticosteroid dosage was 5 mg/day at the end of the study.

Treatment was switched to 150 mg oral cyclophosphamide. During her last visit to our clinic the anaemia had improved (115 g/l), proteinuria was stable (1100 mg/day), and erythrocyturia was present without signs of active GN.

Patient 3

After a bolus, corticosteroids could be tapered to 5 mg/day (end of cycle 3). Maximum proteinuria was 10 300 mg/day at entry into 15-DSG treatment (fig 1; table 1). By the end of cycle 3, proteinuria had decreased to <1000 mg/day (fig 1). Within cycles 3 and 4 she had a prolonged urogenital infection, possibly related to leucocytopenia (3.9×10^9 cells/l at the end of cycle 3, 1.7×10^9 cells/l at the end of cycle 4). She now experienced a flare of her GN (increased proteinuria up to 7100 mg/day at the end of cycle 5; fig 1). We increased the corticosteroids to 60 mg/day, continued 15-DSG as previous success was excellent, but decreased the dosage to 0.35 mg/kg/day because of (possibly 15-DSG-related?) leucocytopenia and the infectious episodes. In addition, we prescribed low dose CSA (2.5 mg/kg/day) as 15-DSG had been successfully used together with CSA.⁸ Subsequently, corticosteroids could be tapered to 5 mg/day within 7 weeks. Proteinuria decreased to 780 mg/day (cycle 9; fig 1); creatinine was always normal. No infections were reported during the 15-DSG + CSA treatment. She is now receiving CSA alone. SELENA-SLEDAI decreased from 14 to 6; the corticosteroid dosage was reduced to 5 mg/day at the end of the study.

CONCLUSION

As far as we know, this is the first report on safety of 15-DSG in the treatment of active SLE-GN. Two of the three patients had non-severe infectious episodes, but otherwise 15-DSG was well tolerated. We are currently conducting a phase I/II trial with 15-DSG in patients with SLE and active GN which will also focus on efficacy measurement.

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Table 1 Details of the patients' history, especially previous immunosuppressive treatment, signs of SLE-GN activity or general SLE activity at entry, and indicators for response during/after 15-DSG treatment							
Patient No	Age (years)	First diagnosed	Organ involvement in history and diagnostic criteria	Previous treatment	Signs of SLE-GN activity/general SLE activity	Indicators for response	Additional comments
1	49	1975	Pericarditis, mesangial GN (biopsy 4 years before 15-DSG), pulmonary involvement, arthralgias, serology	CYC IV or CYC by mouth 1986–90, CSA 1990–96, plasma exchange intermittently, chloroquine plus leflunomide 1997–98, CSA, in part with LJP394, 1999–2002	Increasing proteinuria (max 8 g/day), active urine sediment, increase of serum creatinine (max 120 µmol/l), arterial hypertension, anaemia, dsDNA Ab titres rising, C3/C4 decreased/arthralgias	Loss of proteinuria and haematuria, detection of auto-Ab, arthralgias, C3, C4 normal, serum creatinine normal	15-DSG 0.5 mg/kg/day for 6 cycles, followed by AZA plus CSA, later AZA alone
2	23	1993	Mesangioproliferative GN, later diffuse proliferative mesangial GN (biopsy 3 months before 15-DSG), arthralgias, serology	CYC IV 1993–94, MTX 1994–95, AZA 1995–2002, CYC IV 2002	Increasing proteinuria despite CYC (max 6 g/day), active urine sediment, arterial hypertension, anaemia, dsDNA Ab titres rising, C3/C4 decreased/arthralgias, fatigue	Loss of oedema, fatigue, arthralgias, active urine sediment, arterial hypertension improvement in: proteinuria (1.1 g/day) anaemia, required steroid dosage unchanged: dsDNA Ab titres, C3, C4	15-DSG 0.5 mg/kg/day cycles 1–4, 0.75 mg/kg/day cycles 5–6, 1 mg/kg/day cycles 7–9; herpes zoster cycle 2, bacterial bronchitis cycles 5+6, GN flare at the end of cycle 9 during 15-DSG treatment
3	29	1998	Arthritis, leucocytopenia, malar rash, serology, mesangial GN (biopsy 2 months before 15-DSG)	Antimalarial drugs 1998–99, MTX 1999–2000, AZA 2000, CYC IV 2000–01, AZA 2001–02, MMF 2002–03	Increasing proteinuria despite MMF (max 10.3 g/day), active urine sediment, arterial hypertension, anaemia, dsDNA Ab titres rising, C3/C4 decreased, oedema/rash, leucocytopenia, mucosal ulcers, arthralgias, night sweat	Loss of oedema, anaemia, arthralgias, haematuria, mucosal ulcers, night sweat improvement in: proteinuria (0.78 g/day), C3, C4, dsDNA Ab, required steroid dosage, arterial hypertension unchanged: rash, leucocytopenia (15-DSG + SLE induced), Sm-, RNP-Ab	15-DSG 0.5 mg/kg/day cycles 1–4, flare cycles 4+5 after infection (leucocytopenia!), steroids increased, 15-DSG 0.35 mg/kg/day +CSA cycles 5–9

CYC, cyclophosphamide; CSA, ciclosporin A; MTX, methotrexate; AZA, azathioprine; MMF, mycophenolate mofetil.

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Treatment of SLE-GN with 15-DSG was approved by the local ethics committee.

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Autoimmune hepatitis associated with infliximab in a patient with psoriatic arthritis

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We read with interest the debate about liver toxicity of infliximab in psoriatic arthritis (PsA).^{1,2} We describe the case of a 53 year old woman with a 4 year history of refractory PsA who developed transaminasitis during infliximab treatment.

CASE REPORT

Despite combination treatment (ciclosporin 300 mg/day, fluocortolone 10 mg/day, and methotrexate (MTX) 15 mg/week intramuscularly), disease activity was still high, and intravenous infliximab at 3 mg/kg was administered initially at weeks 0, 2, 6, 14 and then every 6 weeks. Ciclosporin was withdrawn.

Within 3 weeks she improved, fluocortolone was gradually stopped while methotrexate (MTX) 20 mg/week intramuscularly, was continued. After the sixth infusion, she developed a mild transaminasitis and MTX, initially tapered, was stopped. After the eighth infusion transaminases continued to rise and in the absence of any other plausible cause, infliximab was withdrawn.

She was admitted to our department with persistently high values of aspartate aminotransferase and alanine aminotransferase and a flare of PsA.

The erythrocyte sedimentation rate was 30 mm/1st h, C reactive protein 170 mg/l, aspartate aminotransferase 143 IU (normal 5–40), alanine aminotransferase 234 IU (normal 5–55), anti-parietal cell antibodies and liver and kidney microsomal antigen were absent, and serology for hepatitis viruses, cytomegalovirus and Epstein-Barr virus, was negative. The new appearance of anti-dsDNA (IgG) 1/20 (indirect immunofluorescence on *Crithidia luciliae*), anti-smooth muscle antibodies (ASMA) 1/640 was observed, while the titre of antinuclear antibodies (ANA), previously present at a serum dilution of 1/80, increased to 1/160. Liver ultrasonography showed steatosis. A liver biopsy revealed

intense and diffuse portal lymphoplasmacytic, granulocytic inflammatory infiltration and severe interface hepatitis. Mild periportal fibrosis was also evident (figs 1A and B). Fluocortolone 20 mg daily was started and the joints improved. Within a few months, transaminases declined and finally normalised; ANA remained positive, while anti-dsDNA and ASMA disappeared.

DISCUSSION

Feletar *et al* found a high incidence of liver toxicity in patients with PsA treated with infliximab,¹ even if, as Provenzano pointed out,² in some cases this association was debatable because of the concomitant use of MTX and the lack of exclusion of viral infections. In one of the largest studies on the use of infliximab in rheumatoid arthritis (RA),³ no liver disease was recorded, but recently two possible cases of liver disease associated with infliximab use have been observed in Crohn's disease and PsA.^{4,5} In our patient the chronological relationship between transaminasitis and treatment (fig 2), linked to the peculiar histology, is suggestive of autoimmune hepatitis induced by infliximab. The high titre of ASMA, notoriously associated with autoimmune hepatitis,⁶ supports this hypothesis.

Our patient was concomitantly treated with MTX for almost 30 weeks. MTX can produce steatosis, fibrosis and, ultimately, cirrhosis; its hepatotoxicity in psoriasis is well known. A 2.5–5.0-fold increase in liver damage for psoriasis compared with RA has been reported.⁷

Moreover, patients with PsA seem more prone to liver toxicity during infliximab treatment than those with RA.¹ A dissimilar toxicity profile for disease modifying antirheumatic drugs in various diseases has been linked to differences in pathophysiology, genetic background, drinking behaviour, and age.

Figure 1 (A) A liver biopsy revealed intense and diffuse portal lymphoplasmacytic, granulocytic inflammatory infiltration and severe interface hepatitis; Diffuse macro-microvesicular steatosis (over 60% hepatocytes), intranuclear glycogen accumulation, and hyaline degeneration of hepatocytes (Mallory bodies) were also noted. Mild periportal fibrosis was evident with formation of incomplete septa and perisinusoidal and perivenular fibrosis. (B) Lobular evidence of confluent necrosis and inflammatory infiltrates.

Figure 2 Chronological relationship between transaminasitis and infliximab in the patient. AST, aspartate aminotransferase; ALT, alanine aminotransferase; T, time in weeks; MTX, methotrexate; CyA, ciclosporin A; ANA, antinuclear antibodies; ASMA, anti-smooth muscle antibodies; α-DNA, anti-DNA antibodies.

However, our patient had received less than a third (900 mg in the past 4 years) of the cumulative dose known to be a risk for hepatic toxicity.⁸ In this case, infliximab might have led to the acute damage—that is, severe portal inflammation and initial neoductulogenesis, whereas MTX might have been responsible for the chronic hepatic injury—that is, mild fibrosis and steatosis. The introduction of corticosteroids probably hastened liver recovery with subsequent normalisation of transaminases.

The appearance of autoantibodies, occasionally associated with mild and transitory autoimmune diseases, during anti-tumour necrosis factor α treatment has been documented¹⁹ and reflects the complex relationship between tumour necrosis factor α blockage and autoimmunity. This report confirms the need to monitor liver enzymes carefully and perform liver biopsy, if necessary, not only in patients with PsA using combination treatment with MTX and infliximab

but also for those using infliximab alone, especially in the presence of pre-existent serological signs of autoimmunity such as ANA. Such signs might be a risk factor for further development of clinical autoimmunity during infliximab treatment.

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Alleviation of polyarticular syndrome in multicentric reticulohistiocytosis with intravenous zoledronate

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A 67 year old previously healthy women presented with a 12 month history of generalised symmetric arthralgias and bilateral hand contractures. Her past medical history was unremarkable, except for heavy smoking.

On physical examination, she had tight incapacitating flexion contractures of both hands, and small cutaneous non-tender well circumscribed nodules (3–6 mm diameter) on the dorsum of the fingers and over the proximal and distal interphalangeal joints (fig 1). A symmetric polyarthritis affecting the shoulders, elbows, proximal and distal interphalangeal joints, and the knees was prominent. Blood counts, biochemical profile and inflammatory markers, anti-nuclear antibodies, and rheumatoid factor were within normal limits. A hand x ray examination showed erosive deforming arthropathy of the styloid processes. Biopsy samples of a skin nodule and of synovial membrane disclosed infiltrates of multinucleated giant cells and histiocytes, indicative of multicentric reticulohistiocytosis (MRH). The infiltrating histiocytes were macrophages, as illustrated by positive staining for CD68 marker and negative staining for S-100 (Langerhans' dendritic cell marker) and HHF-35 actin (fibroblast marker).

The well described association of MRH with malignancies^{1,2} had prompted the screening for underlying malignancies, which disclosed a 10-fold increase of CA-125. A pelvic computed tomographic scan was normal; however, magnetic resonance imaging showed the presence of a small well circumscribed mass at the left parametrial space, which was subsequently removed by laparotomy and diagnosed as poorly differentiated ovarian adenocarcinoma without metastatic spread. The patient was given intravenous combination treatment of carbaplatine and cyclophosphamide.

Three months later, the cutaneous nodules resolved, but the polyarthritis persisted and the patient gradually developed a generalised painful stiffness of the trunk and extremities, which required the use of narcotic analgesics

and confined her to bed. Intravenous methylprednisolone pulses were also administered, without response. Based on the recently reported effectiveness of intravenous alendronate for MRH,³ zoledronate (4 mg) was given intravenously, because alendronate was unavailable locally. Two weeks later, the stiffness and arthralgias were dramatically reduced. The patient is now completely free of pain and ambulatory.

DISCUSSION

MRH is a rare disorder of unknown cause, characterised by destructive symmetric arthritis associated with cutaneous papulonodular lesions. In about one third of patients, musculoskeletal symptoms may precede or follow an underlying malignancy (such as breast and ovarian cancer, melanoma or mesothelioma).² MRH should be differentiated from fibroblastic rheumatism, which is also rare.⁴ Although strict differentiating histological criteria are lacking, multinucleated foreign body-type giant cells appear to denote MRH, whereas the predominance of myofibroblasts and excessive collagen production characterises fibroblastic rheumatism.⁵ The inclusion of fibroblastic rheumatism in the broader spectrum of non-Langerhans' cell histiocytosis has been recently proposed.⁶

To date, the decision for systemic therapeutic intervention in MRH remains largely empirical. Treatment with steroids and various cytotoxic agents is of questionable efficacy,² and in our patient it resulted only in resolution of the cutaneous nodules. Recently, the beneficial role of tumour necrosis factor blockers has been suggested⁷; however, these are contraindicated in patients with concomitant neoplasia. Intravenous alendronate has been also advocated in the management of MRH.⁴ In our patient, the administration of the parenteral bisphosphonate zoledronate, so far used for the treatment of osteoporosis⁸ and of hypercalcaemia of malignancy,⁹ dramatically alleviated the incapacitating joint symptoms.

The precise mechanism of bisphosphonate action on MRH is unclear. However, after intravenous injection, bisphosphonates have been previously shown to deposit in the reticuloendothelial system,¹⁰ to inhibit the metalloprotease activity and matrix metalloproteinase-9 expression of infiltrating macrophages,¹¹ and to induce apoptosis of macrophage-like cells.¹² Therefore, one may speculate that their favourable effect in MRH is due to the inhibition of tissue infiltration by histiocytes, possibly through induction of apoptosis.

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Figure 1 Contraction flexures and cutaneous nodules in the dorsum of the fingers (arrows).

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Acetaminophen may act through β endorphin

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Acetaminophen, also known as paracetamol, is a non-steroidal anti-inflammatory drug (NSAID) with potent antipyretic and analgesic actions but with very weak anti-inflammatory activity. The mechanism of action of acetaminophen is still not clearly understood. It has no known endogenous high affinity binding sites. In addition, acetaminophen does not appear to share with NSAIDs the ability to inhibit peripheral cyclo-oxygenase (COX) activity.¹ Although various biochemical studies point to inhibition of central COX-2 activity, the existence of a COX activity that is selectively susceptible to acetaminophen (COX-3?) is an alternative hypothesis.² However, this may hold true only for the dog. Database analysis of human COX-1 showed a frame shift induced by intron 1, possibly showing COX-3 to be a virtual protein in humans.³

Our studies in osteoarthritis provide evidence of a clear effect of acetaminophen on β endorphin levels in plasma (fig 1) compared with rofecoxib 25 mg/day.⁴ Plasma β endorphin levels decreased in 10 patients with osteoarthritis after 1 month of treatment with up to 4 g/day acetaminophen orally ($p=0.017$) as well as after 3 months of treatment ($p=0.028$). Whereas, there were no changes in the rofecoxib group after 1 month ($p=0.73$) and 3 months ($p=1.00$), respectively.

Acetaminophen may play a part in the delivery of peripheral β endorphin to their receptors and thereby relieve pain.

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Figure 1 Acetaminophen reduces plasma β endorphin levels in patients with symptomatic osteoarthritic (box plot graphs). BEND, β endorphin; T0, basic values (no significant difference between the groups, $p=0.965$); T1, plasma levels after 1 month's oral treatment; T3, plasma levels after 3 months' oral treatment.

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Refractory adult onset Still's disease and hypersensitivity to non-steroidal anti-inflammatory drugs and cyclo-oxygenase-2 inhibitors: are biological agents the solution?

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A adult onset Still's disease (AOSD) is an autoimmune disorder characterised by periodic high fever, arthritis, and typical evanescent rashes. Non-steroidal anti-inflammatory drugs (NSAIDs) are the preferred treatment. In severe cases several disease modifying antirheumatic drugs, thalidomide, and intravenous immunoglobulin have been used. More recently, successful treatment with tumour necrosis factor α (TNF α) blocking agents^{1–2} and interleukin (IL) 1 neutralisation³ has also been reported.

Hypersensitivity to NSAIDs, often characterised by urticaria, angio-oedema, and asthma, has been well documented, and several studies indicate that anaphylactic reactions are related to the inhibition of cyclo-oxygenase-1 (COX-1) enzyme^{4–6} and that selective COX-2 inhibitors can be safe in these patients. Here we report a case of AOSD complicated by coexisting hypersensitivity to acetaminophen (paracetamol), aspirin, NSAIDs, and also to selective COX-2 inhibitors. TNF α neutralisation controlled the fever, but not the AOSD related rashes and polyarthritis or the anaphylactic reactions to NSAIDs and COX-2 inhibitors. Treatment with IL1 receptor antagonist led to full remission of the AOSD.

CASE REPORT

A patient, with known AOSD for 22 years, was admitted to our centre with a 3 week history of spiking high fever, chills, skin rash, cough, and a sore throat. Physical examination disclosed a typical AOSD related rash, polyarthritis, and enlarged inguinal lymph nodes without hepatosplenomegaly. Laboratory examination showed an increased acute phase reaction and a normochromic normocytic anaemia; white

blood cell count, platelet count, and liver function tests were normal. Serological tests for viral infections, toxoplasma, *Bartonella*, blood and urine culture rheumatoid factor, and antinuclear antibodies were all negative.

In the past the AOSD had followed a polycyclic course, which had been successfully treated with several NSAIDs alone or in combination with acetaminophen for 10 years. In 1993, she developed an allergic reaction with angio-oedema to naproxen (fig 1), and later also to acetaminophen and sodium salicylate. Methotrexate was used for the next 10 years, but frequently corticosteroids were needed to treat the AOSD exacerbations. To avoid chronic use of corticosteroids, etanercept was started in May 2003 and the corticosteroids were tapered. This led to exacerbations of a mild polyarthritis and worsening of the rash but no fever. Because selective COX-2 inhibitors may be safe in patients with intolerance to NSAIDs,^{4–5} rofecoxib was successfully added to etanercept without intolerance. However, a second challenge with rofecoxib resulted in severe angio-oedema and urticarial rash and the same occurred after challenges with celecoxib and etoricoxib. IL1 receptor antagonist was started in December 2004, leading to a full remission of all AOSD related symptoms despite the withdrawal of long term steroid treatment.

DISCUSSION

Our case illustrates that TNF α blocking agents are only partially effective in the treatment of refractory AOSD. Partial or limited efficacy of these agents has also been observed in patients with systemic onset juvenile idiopathic arthritis.^{7–8} Our case and several other reports suggest that it is not TNF α

Figure 1 Angio-oedema after NSAID ingestion. Reproduced with the patient's permission.

but IL1 which has a pivotal role in the pathogenesis of AOSD³ and systemic onset juvenile idiopathic arthritis.⁹ In these diseases and in other rare disorders with a single amino acid mutation in the NALP-3 gene which results in increased IL1 secretion, IL1 blockade seems to be the preferred treatment.¹⁰

Furthermore, our case suggests that hypersensitivity to NSAIDs is not exclusively mediated by COX-1 blockade, but can also be provoked by selective COX-2 inhibitors that can function as haptens, resulting in anaphylaxis upon next exposure.⁵ Our case shows that these reactions are not mediated by TNF α and not altered by TNF α neutralisation.

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Distal degeneration of sensory and autonomic cutaneous nerve fibres in systemic sclerosis

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We studied innervation and dermal vasculature in affected and apparently normal skin of sclerodermic patients to evaluate the involvement of different nerve fibre groups and to determine a possible correlation with vascular damage in this disease. Immunohistochemical analysis and confocal microscopic examination of skin biopsy samples were used.

METHODS AND RESULTS

We obtained 3 mm punch skin biopsy samples from the distal thigh and distal leg in 11 consecutive 34–70 year old female patients with systemic sclerosis (SSc), identified by the American College of Rheumatology classification criteria.¹ We excluded patients who had been exposed to potentially neurotoxic exogenous or endogenous conditions. The skin appeared sclerotic in 4/11 patients in the leg and in 3/11 in the thigh (table 1). In four patients a further skin sample from fingertip was taken to evaluate myelinated fibres. None of the patients complained of sensory disturbances, and neurological and neurophysiological evaluations were normal except in two patients, in whom a conduction velocity study showed the presence of an entrapment syndrome. Patient morphological findings were compared with data from a group of 16 healthy volunteers (nine male, seven female, age range 34–65 years).

Skin biopsy specimens were processed according to previously published procedures.² Floating sections were immunostained using a panel of primary antibodies, including the pan-neuronal marker anti-protein gene product (PGP) 9.5, anti-myelin basic protein for myelinated fibres, anti-vasoactive intestinal peptide (VIP) to mark autonomic

nerve fibres, and anti-collagen IV to visualise basement membrane and blood vessels.

We quantified, as previously described, epidermal nerve fibres (ENFs) per linear millimetre,³ Meissner corpuscles (MCs), and myelinated papillary endings per square millimetre⁴ on confocal images using image analysis software (Neurolucida, Microbrightfield Inc, Colchester VT, USA; ScionImage, Scion Corporation, Frederick, MD, USA). On the same images used to quantify ENF density, we measured blood vessel density in $\mu\text{m}^2/100 \mu\text{m}^2$ of dermal tissue within 250 μm below the basement membrane.

We found a significant loss of ENFs in sclerodermic patients in all the examined sites (table 1) without a distal-proximal gradient, a poor subepidermal neural plexus, and a reduced innervation of sweat glands, blood vessels, and arrector pilorum muscles compared with controls. These findings, evident in apparently unaffected areas (figs 1E and F compared with 1A and B), were more severe in clinically involved skin (figs 1C and D compared with 1A and B) and affected both sensory and autonomic unmyelinated nerve fibres as demonstrated by PGP and VIP immunostainings.

The mean (SD) density of blood vessels measured in $\mu\text{m}^2/100 \mu\text{m}^2$ of dermal tissue, was 6.4 (2.9) and 8.7 (4.7), respectively, in the thigh and leg of patients with SSc. These values significantly correlated with the density of epidermal nerve fibres in both sites ($r^2 = 0.51$; $p < 0.05$ at the thigh and $r^2 = 0.58$; $p < 0.05$ at the leg). In glabrous skin we found a significant reduction of MC density compared with controls, with a number of intrapapillary myelinated fibres still within the normal range. Moreover, evident structural abnormalities of the surviving mechanoreceptors and predegenerative

Figure 1 Confocal micrographs of cutaneous innervation in thigh skin samples. (A) Normal skin, (C) affected, and (E) apparently unaffected skin from a patient with SSc: samples are triple stained to visualise nerve fibres (rPGP in yellow-green), basement membrane and vessels (mColIV in red), and endothelium and epidermis (ULEX Europaeus in blue). Sweat gland images in a healthy subject (B) and in sclerodermic patients from affected (D) and apparently normal (F) skin, are double stained to visualise nerve fibres (rPGP in yellow-green) and vessels (mColIV in red). A derangement of dermal architecture, of subepidermal neural plexus, and a marked reduction of ENF density are evident in affected and, although to a lesser extent, in apparently normal skin of patients with SSc (C and E compared with A). A loss of nerve fibres innervating dermal annexes such as arrector pilorum muscles (E compared with A) and sweat glands (D and F compared with B) in skin from sclerodermic patients is also evident. Bar = 50 μ m. E, epidermis; ENFs, epidermal nerve fibres; BM, basement membrane; V, blood vessels; SNP, subepidermal neural plexus; APM, arrector pilorum muscle.

aspects of myelinated fibres, such as swellings or vacuolisation, were present.

DISCUSSION

Our data indicate that the cutaneous nerves in SSc are impaired. This mainly involves the unmyelinated sensory and autonomic nerve fibres, but does not completely spare the large fibres. The observation that the loss of ENFs was more

significant in subjects with an evident reduction of vascular bed suggests that ischaemia may have a role in determining the neuropathic process. However, we cannot rule out the possibility that early biohumoral changes, demonstrated in apparently unaffected skin,^{5,6} may induce both neural and vascular damage. We speculate that the abnormalities of terminal innervation seen in the skin may be present in multiple organs in SSc. This neuropathic process, affecting

Table 1 Clinical and morphological data in sclerodermic patients compared with mean values in the control group

Patient	Age	Disease duration (years)	Subset	ENF thigh*	Dermal vessels density thigh†	ENF leg*	Dermal vessels density leg†	ENF fingertip*	Meissner corpuscles‡	Myelinated intrapapillary endings‡
1	62	1	lSSc	15.8	8.1	10.3	8.7	7.4	20.5	42.7
2	43	13	dSSc	7.3	7.8	9.0	7.1	–	–	–
3	63	1	lSSc	6.0	2.2	3.5	11.3	1.9	8.6	18.3
4	62	2	lSSc	16.6	9.6	9.3	9.2	–	–	–
5	34	6	dSSc	(0.0)	4.7	(0.0)	1.5	–	–	–
6	65	5	dSSc	2.0	11.8	(0.0)	3.4	–	–	–
7	46	2	dSSc	(0.0)	3.0	(3.1)	2.8	–	–	–
8	41	1	lSSc	18.7	8.0	10.5	12.0	–	–	–
9	60	8	dSSc	(0.0)	7.1	(0.0)	9.6	–	–	–
10	70	3	lSSc	20.5	8.3	23.8	16.2	3.5	15.1	63.9
11	70	5	lSSc	13.3	9.7	11.5	13.6	2.2	15.0	37.2
<i>Mean (SD)</i>										
Patients	56.0 (12.6)	4.3 (3.7)		9.1 (8.1)	6.4 (2.9)	7.4 (7.1)	8.7 (4.7)	3.8 (2.5)	14.8 (4.9)	40.5 (18.8)
Controls	52.6 (10.0)	–		27.2 (7.7)	–	19.1 (8.8)	–	7.5 (3.6)	29.7 (11.2)	51.9 (20.7)
Significance				p<0.0001§ p<0.005¶		p<0.001§ p<0.05*		p<0.05§	p<0.005§	p=0.34§

ENF density values from affected skin are shown in parentheses.

lSSc, limited cutaneous systemic sclerosis; dSSc, diffuse cutaneous systemic sclerosis.

*Expressed as the number of epidermal nerve fibres/mm; †expressed in $\mu\text{m}^2/100 \mu\text{m}^2$ of dermal tissue; ‡ expressed as the number of structures/mm²;

§comparison of density values in the control group and in all skin samples from patients with SSc; ¶comparison of density values in the control group and in samples of apparently unaffected skin in patients with SSc.

primarily unmyelinated nerve fibres, may contribute to the production of abnormalities that are common in SSc, like visceral dysmotility and cardiac arrhythmias.

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