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:

\[
PPV = \frac{a}{a+b}; \quad NPV = \frac{d}{c+d}
\]

where \(a = \text{test positive, disease positive}; \ b = \text{test positive, disease negative}; \ c = \text{test negative, disease positive}; \ d = \text{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

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

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, did not result in a similar increase of the PPV or NPV (59% and 79%, respectively). The rather low sensitivity reports, did not result in a similar increase of the PPV or NPV (59% and 79%, respectively). The rather low sensitivity

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.

CASE REPORTS

Table 1 shows the patient characteristics.

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 >4×10^9 cells/l, no infection was seen, no (serious) adverse events and occurred with the exception of paraguesia. 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.

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

H-M Lorenz, M Grunke, J Wendler, P A Heinzel, J R Kalden

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. 14-17 15-DSG exerted no nephrotoxicity or hepatotoxicity but reversibly induced leucocytopenia. 18

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

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.

REFERENCES


Accepted 14 March 2005
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 x 10^7 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). 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. 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.

ACKNOWLEDGEMENTS
We thank our patients for their collaboration and help in performing this trial.

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P A Heinzel, Euro Nippon Kayaku, Frankfurt, Germany
PAH is an employee of Euro Nippon Kayaku, a branch of the mother company Nippon Kayaku, Tokyo, Japan, which provided the drug for this trial free of charge.

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

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Accepted 15 March 2005

REFERENCES

Autoimmune hepatitis associated with infliximab in a patient with psoriatic arthritis

V Germano, A Picchianti Diamanti, G Baccano, E Natale, A Onetti Muda, E Priör, G Valesini

We read with interest the debate about liver toxicity of infliximab in psoriatic arthritis (PsA). 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), antismooth 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 perportal 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, even if, as Provenzano pointed out, 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), 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. 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, 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.

Moreover, patients with PsA seem more prone to liver toxicity during infliximab treatment than those with RA. 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.
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. 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 documented 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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Accepted 14 March 2005

REFERENCES
Alleviation of polyarticular syndrome in multicentric reticulohistiocytosis with intravenous zoledronate

C P Mavragani, K Batziou, K Aroni, D Pikazis, M N Manoussakis


A 67 year old previously healthy woman 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 nontender 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, antinuclear antibodies, and rheumatoid factor were within normal limits. A hand x ray examination showed erosive deformations 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 malignancies1-3 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 activity.

On the day of surgery, the patient was given intravenous combination therapy of carboplatin and cyclophosphamide. The patient was subsequently removed by laparotomy and diagnosed as well circumscribed mass at the left parametrial space, which magnetic resonance imaging showed the presence of a small pelvic computed tomographic scan was normal; however, 

...in our patient it resulted only in resolution of the cutaneous nodules. Recently, the beneficial role of tumour necrosis factor blockers has been suggested; however, these are contraindicated in patients with concomitant neoplasia. Intravenous alendronate has been also advocated in the management of MRH. In our patient, the administration of the parenteral bisphosphonate zoledronate, so far used for the treatment of osteoporosis and of hypercalcaemia of malignancy, 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, to inhibit the metalloprotease activity and matrix metalloproteinase-9 expression of infiltrating macrophages, and to induce apoptosis of macrophage-like cells. 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.

ACKNOWLEDGEMENTS
We are indebted to Professor HM Moutsopoulos for inspiration and fruitful suggestions.

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Acetaminophen may act through β endorphin

H Sprott, H Shen, S Gay, A Aeschlimann

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. 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. 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.

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. 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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Accepted 7 February 2005

REFERENCES

Figure 1  Acetaminophen reduces plasma β-endorphin levels in patients with symptomatic osteoarthritis (box plot graphs). BEND, β-endorphin; T0, basic values (no significant difference between the groups, p = 0.956); T1, plasma levels after 1 month’s oral treatment; T3, plasma levels after 3 months’ oral treatment.
Refractory adult onset Still’s disease and hypersensitivity to non-steroidal anti-inflammatory drugs and cyclo-oxygenase-2 inhibitors: are biological agents the solution?

E H J G Aarntzen, P L C M van Riel, P Barrera

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 (TNFα) blocking agents1–2 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) enzyme4–6 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. TNFα 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 acetylsalicyclic acid for 10 years. In 1993, she developed an allergic reaction with angio-oedema to naproxen (fig 1), and later also to acetylsalicyclic acid 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 TNFα blocking agents are only partially effective in the treatment of refractory AOSD. Partial or limited efficacy of these agents has also been also observed in patients with systemic onset juvenile idiopathic arthritis.7–8 Our case and several other reports suggest that it is not TNFα
but IL1 which has a pivotal role in the pathogenesis of AOSD and systemic onset juvenile idiopathic arthritis. 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.

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 haptons, resulting in anaphylaxis upon next exposure. Our case shows that these reactions are not mediated by TNFz and not altered by TNFz neutralisation.

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Accepted 24 June 2005

Published Online First 13 July 2005

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

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. 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. Floating sections were immunostained using a panel of primary antibodies, including the pan-neuronal marker anti-neurofilament protein (PNFP), 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, Meissner corpuscles (MCs), and myelinated papillary endings per square millimetre on confocal images using image analysis software (Scion Image). On 250 μm below the basement membrane.

The mean (SD) density of blood vessels measured in mm2 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 pili muscles compared with controls. These findings, evident in apparently unaffected areas (figs 1C and D 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 mm2 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 (r2 = 0.51, p<0.05 at the thigh and r2 = 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 mechanoceptors and predegenerative
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, 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

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**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 sclerodermitic 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 μm. E, epidermis; ENFs, epidermal nerve fibres; BM, basement membrane; V, blood vessels; SNP, subepidermal neural plexus; APM, arrector pilorum muscle.
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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Competing interest statement: No author has any competing interest regarding this report.

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Accepted 30 March 2005

### REFERENCES


### Table 1

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Disease duration (years)</th>
<th>Subset</th>
<th>ENF thigh*</th>
<th>Dermal vessels density thigh†</th>
<th>ENF leg*</th>
<th>Dermal vessels density leg†</th>
<th>ENF fingertip*</th>
<th>Meissner corpuscles‡</th>
<th>Epithaloid intrapapillary endings‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>1</td>
<td>lSSc</td>
<td>15.8</td>
<td>8.1</td>
<td>10.3</td>
<td>8.7</td>
<td>7.4</td>
<td>20.5</td>
<td>42.7</td>
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<tr>
<td>2</td>
<td>43</td>
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<td>dSSc</td>
<td>7.3</td>
<td>7.8</td>
<td>9.0</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>1</td>
<td>lSSc</td>
<td>6.0</td>
<td>2.2</td>
<td>3.5</td>
<td>11.3</td>
<td>1.9</td>
<td>8.6</td>
<td>18.3</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
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<td>lSSc</td>
<td>16.6</td>
<td>9.6</td>
<td>9.3</td>
<td>9.2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>34</td>
<td>6</td>
<td>dSSc</td>
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<td>4.7</td>
<td>(0.0)</td>
<td>1.5</td>
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<tr>
<td>6</td>
<td>65</td>
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<td>dSSc</td>
<td>2.0</td>
<td>11.8</td>
<td>(0.0)</td>
<td>3.4</td>
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<tr>
<td>7</td>
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<tr>
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<td>60</td>
<td>8</td>
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<td>7.1</td>
<td>(0.0)</td>
<td>9.6</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>70</td>
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<td>23.8</td>
<td>16.2</td>
<td>3.5</td>
<td>15.1</td>
<td>63.9</td>
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<tr>
<td>11</td>
<td>70</td>
<td>5</td>
<td>lSSc</td>
<td>12.3</td>
<td>9.7</td>
<td>11.5</td>
<td>13.6</td>
<td>2.2</td>
<td>15.0</td>
<td>37.2</td>
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</table>

Mean (SD):

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td>ENF thigh*</td>
<td>56.0 (12.6)</td>
</tr>
<tr>
<td>Dermal vessels density thigh†</td>
<td>4.3 (3.7)</td>
</tr>
<tr>
<td>ENF leg*</td>
<td>9.1 (8.1)</td>
</tr>
<tr>
<td>Dermal vessels density leg†</td>
<td>6.4 (2.9)</td>
</tr>
<tr>
<td>ENF fingertip*</td>
<td>7.4 (7.1)</td>
</tr>
<tr>
<td>Meissner corpuscles‡</td>
<td>8.7 (4.7)</td>
</tr>
<tr>
<td>Epithaloid intrapapillary endings‡</td>
<td>3.8 (2.5)</td>
</tr>
</tbody>
</table>

Significance:

<table>
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<tr>
<th>p</th>
<th>p</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>0.005</td>
<td>0.05</td>
<td>0.34</td>
</tr>
</tbody>
</table>

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 μm²/100 μm² 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.