Treatment of resistant rheumatoid arthritis by intra-articular infliximab injections: a pilot study

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Rheumatoid arthritis (RA) is a chronic inflammatory disease which is characterised mainly by synovial inflammation and joint destruction, as well as extra-articular manifestations.¹ Cytokines have a central role in the pathogenesis of this synovial inflammation. Tumour necrosis factor α (TNF α) is one of the dominant cytokines. Many studies have shown that TNF α is present in biologically significant amounts in RA synovial tissue and fluids, and the amount seems to parallel the extent of inflammation and bone erosion.^{2–3}

Persistent inflamed monarthritis in patients with RA is difficult to treat. Usually it is treated with local patches, intra-articular injections of steroids, or even with chemical, radioactive, or surgical synovectomy.⁴ The introduction of anti-TNF α treatments, especially the infusion of infliximab,⁵ prompted us to investigate the effectiveness and safety of intra-articular injection of infliximab in patients with RA and resistant monarthritis.

METHODS AND RESULTS

Five patients who fulfilled the American College of Rheumatology criteria for RA were studied.⁶ All were receiving treatment with disease modifying antirheumatic drugs (DMARDs). They presented an active inflammatory monarthritis, resistant to local treatment with corticosteroids for a period of at least three months. Written informed consent was obtained from the patient, who were given intra-articular infliximab, 100 mg, in two consecutive injections at a 24 hour interval after local anaesthesia. The primary end point was to examine the efficacy and safety of intra-articular infliximab administration in patients with RA who had a partial response to DMARDs and exhibited signs and symptoms of persistent inflammation of one large joint. The current treatment was maintained during the study. The secondary end point was the comparison of magnetic resonance imaging (MRI) findings before and six weeks after infliximab administration.⁷ Patients with a history or

presence of chronic infectious diseases, positive tuberculin skin test, or abnormal chest radiograph were excluded from the study.

Each patient had a complete physical and laboratory evaluation before and six weeks after treatment. The inflamed joint was examined and the following variables were evaluated: the degree of swelling and tenderness of the affected joint (mild 1+, moderate 2+, severe 3+), the pain score (visual analogue scale 0–10 cm), and the patient's and doctor's global assessment. In addition, a magnetic resonance (MR) examination of the inflamed joint was performed before and after treatment and the findings were read "blindly" and separately by two expert radiologists. The MR protocol consisted of sagittal short time inversion recovery scans and fat suppressed T₁ weighted sagittal, coronal, and axial scans before and after intra-venous contrast injection (Gd-DTPA). Intra-articular fluid collection and synovial thickening with enhancement were considered as findings of synovial inflammation.

Finally, acute phase reactants such as C reactive protein and erythrocyte sedimentation rate were evaluated in all patients.

Three female and two male patients with a mean (SD) age of 52.2 (8.5) years and mean (SD) disease duration of 11.3 (2.2) years were studied. Three had positive IgM rheumatoid factor. Four of the five patients responded well after the intra-articular injection of infliximab as evaluated by the reduction in the swelling and tenderness, by the decrease in the pain score, and by the improvement of laboratory variables (table 1). This clinical and laboratory improvement was associated with the improvement of MRI findings, which showed reduction of synovial fluid and of the enhancing inflammatory tissue (fig 1). One patient did not respond to intra-articular injection of infliximab and the MRI findings did not show any improvement. This patient had a synovitis flare in many joints and was treated successfully with intravenous

Table 1 Clinical and laboratory changes in patients with RA treated with intra-articular injections of infliximab

Variables	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		p Value
	Before	After	Before	After	Before	After	Before	After	Before	After	
Degree of swelling in the joint	3	3	2	0	3	0	3	0	3	1	0.049
Degree of tenderness of the joint	2	2	3	1	3	1	3	1	2	0	0.046
Pain score	7.8	7.6	6.2	1.3	6.8	0.7	8.4	3.3	5.5	2.1	0.043
Patient's global assessment	7.2	6.9	5.6	1.7	7.6	2.3	8.2	3.6	4.6	3.8	0.080*
Doctor's global assessment	6.7	6.5	6.3	2.1	6.6	2.2	7.9	3.2	4.9	4.1	0.043
Erythrocyte sedimentation rate (mm/1st h)	60	58	41	24	75	13	28	20	46	30	0.043
C reactive protein (mg/l)	15	18	14	1	81	2	8	0.5	8	1	0.050
Remission follow up (months)	No	6		6		6			6		

For the statistical analysis Wilcoxon test for pairs was used; *non-statistical change.

Figure 1 Contrast enhanced T₁ weighted sagittal scan with fat suppression (A) demonstrating increased intra-articular fluid collection (arrowhead) and diffuse synovial thickening and enhancement (arrows); (B) showing disappearance of the intra-articular fluid and decrease of the enhancing inflammatory tissue (arrow) after intra-articular infliximab administration.

infusions of infliximab. No local or systemic adverse reactions were noted in our patients.

DISCUSSION

There is limited experience of intra-articular administration of infliximab in patients with RA. Dreher *et al* reported effectiveness with intra-articular infliximab in the local treatment of three patients with active RA, with an additional systemic effect.⁸ They noticed also a remarkable reduction of the daily cell count of the synovial fluid of one patient. Surprisingly, infliximab worked equally well in another patient with chondrocalcinosis, with immediate remission of clinical signs. Significant improvements were also seen by Lawless *et al* by injecting 1 mg of infliximab dorsally into the left wrist of a patient with monarticular erosive arthritis following silastic wrist prosthesis.⁹ On the other hand, Kellner *et al* published successful treatment of sarcoiditis in five patients with ankylosing spondylitis by intra-articular injection of 60 mg infliximab.¹⁰

Intra-articular administration of infliximab seems to be effective and safe in patients with RA with resistant monarthritis. Large, placebo controlled studies are required to validate our results.

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Coeliac disease associated with systemic sclerosis

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Systemic sclerosis (SSc) is a clinically heterogeneous disorder which affects the skin and internal organs such as the gastrointestinal tract, lungs, heart, and kidneys. Small bowel disease can present with a wide variety of symptoms, including intermittent bloating, abdominal cramps, chronic diarrhoea and, in a minority of patients, malabsorption.¹ Coeliac disease (CD) is a malabsorptive disorder resulting from inflammatory injury to the mucosa of the small intestine after the ingestion of wheat gluten. As CD has an immunological basis, an association between CD and other autoimmune disorders, such as type 1 diabetes mellitus and autoimmune thyroiditis, is not uncommon.^{2 3} We describe here a patient with SSc and insulin dependent diabetes mellitus in whom a diagnosis of CD was made at the age of 49.

CASE REPORT

A 49 year old white woman with insulin dependent diabetes mellitus diagnosed in 1980, with weak positive anti-islet cell antibodies, recently attended our clinic. In 1995, she complained of Raynaud's phenomenon, ischaemic digital ulcers, sclerodactyly, and telangiectasia. A diagnosis of SSc with limited scleroderma was made supported by the presence of antinuclear (1/160) and anticentromere antibodies. Lichen planus was diagnosed owing to hypochromic macular lesions on her thorax and arms. Associated sicca syndrome with anti SS-A/Ro antibodies was also diagnosed. Treatment with intravenous prostaglandins, calcium antagonists, and angiotensin converting enzyme inhibitors was started with good response.

Five years later, she presented with gastrointestinal symptoms, including dysphagia and oesophageal reflux, with barium studies that confirmed oesophageal hypomotility. She also presented with diarrhoea, with normal thyroid

function, and with no evidence of infection. Symptomatic management was started, with transient improvement.

Three months later, she was admitted with a necrotic ischaemic digital episode of the second finger of her right hand complicated with osteomyelitis, and this was accompanied by an exacerbation of her abdominal symptoms. Laboratory findings showed malabsorption markers and positive IgA and IgG antigliadin and anti-endomysial antibodies. Jejune biopsy showed severe intestinal atrophy with a moderate lymphoplasmacytic infiltrate in the lamina propria and an increase of the intraepithelial lymphocytes

Figure 1 Granzyme B immunohistochemical staining. A high number of intraepithelial T lymphocytes showing cytoplasmic cytotoxic granules were identified (EnVision system, peroxidase, $\times 600$).

Table 1 Characteristics of patients with CD and SSc

Characteristics	Case No (ref)					
	1 ³	2 ³	3 ⁵	4 ⁶	5 ⁶	6 [*]
Age/sex	21/M	54/F	27/F	35/F	40/M	49/F
Duration of SSc (years)	<1	9	1	3	13	8
Sicca symptoms	NR	NR	+	+	—	+
Malabsorption markers	NR	NR	+	+	—	+
Immunological profile	NR	NR	ANA+ (1/160) RF+ Thyroid abs	ANA+ (1/1280)	ANA+ (1/640) RF+ Centromere+	ANA+ (1/320) RF+ Centromere+
Antigliadin antibodies	NR	NR	+	+	NR	+
Anti-endomysial antibodies	NR	NR	NR	NR	NR	+
Intestinal biopsy with CD	+	+	+	+	+	+
Other autoimmune diseases	—	—	RA, HT ITP, PA	SS	— LP	DM

CD, coeliac disease; SSc, systemic sclerosis; RA, rheumatoid arthritis; HT, Hashimoto's thyroiditis; ITP, idiopathic thrombocytopenic purpura; PA, pernicious anaemia; DM, insulin dependent diabetes mellitus; LP, lichen planus; ANA, antinuclear antibodies; RF, rheumatoid factor; NR, not reported.

^{*}Present case.

(fig 1). A gluten-free diet was started, and her diarrhoea slowly improved.

DISCUSSION

A few publications have reported the coexistence of CD and SSc.³⁻⁷ Zammit-Maempel *et al* reported on a 63 year old man with CD confirmed by jejunal biopsy associated with sclerodactyly, but without other clinical or serological features related to SSc.⁴ We found six well documented cases of patients with CD and SSc, including the current one (table 1). The mean age was 37.7 years (range 21–54), with a mean duration of the SSc of 5.8 years (in one patient both diseases presented at the same time). Most of the patients were female (four women, two men) with limited scleroderma in five (83%) of the six cases. Three of the patients had sicca symptoms, in one of them with a salivary gland biopsy that confirmed a diagnosis of Sjögren's syndrome.

The presence of Sjögren's syndrome or sicca symptoms in three of the six patients with CD and SSc deserves special comment. Ilanen *et al* found that five (15%) of 34 patients with Sjögren's syndrome studied by an intestinal biopsy had concomitant CD.⁸ Additionally, they suggested that a close association between these two disorders can be partly explained by a similar genetic involvement, specially the HLA DR3-DQ2 haplotype.

Our patient and the previous cases collected emphasise the importance of studying patients with SSc with chronic diarrhoea and malabsorption not only by antigliadin and anti-endomysial antibody determination but also, in some cases, by an intestinal biopsy, in order to clarify the source of

the malabsorption, to obtain relief of symptoms, and to improve the prognosis of patients with SSc.

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Septic arthritis caused by *Moraxella catarrhalis* associated with infliximab treatment in a patient with undifferentiated spondarthritis

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Tumour necrosis factor α (TNF α) antagonists have been associated with reactivation of tuberculosis and development of other opportunistic infections.¹⁻⁴ We have recently observed a case of septic arthritis caused by *Moraxella* (*Branhamella*) *catarrhalis* in a patient treated with infliximab that we describe briefly here.

CASE REPORT

The patient, a 45 year old man, was referred to us in September 1999 for evaluation of an eight year history of recurrent episodes of arthritis of the knees and elbows and of recurrent episodes of inflammatory swelling with pitting oedema over the dorsum of the hand. One year before the onset of arthritis he had undergone replacement surgery for an insufficient aortic valve. Physical examination disclosed swelling, effusion, and tenderness in the right knee, together with tenosynovitis of the left tibialis posterior and flexor digitorum longus. Spine movement was not limited and chest expansion was not restricted. Routine laboratory evaluation was normal and HLA typing was positive for B27. Radiographs of the knees showed only soft tissue swelling, and pelvis and spine radiographs were normal. There were no symptoms of gut disease, and ileocolonoscopy was not performed. Our diagnosis was undifferentiated spondarthritis (uSpA).

In the following months the patient developed severe recurrent episodes of peripheral arthritis, peripheral enthesitis, and tenosynovitis. At the beginning he was treated with steroid injections, non-steroidal anti-inflammatory drugs, and short courses of oral steroids. In the following months he was given sulfasalazine, cyclosporin A, and methotrexate, with no improvement.

In May 2002, because of the severity of the clinical situation, we decided to treat the patient with infliximab, after obtaining his informed consent. He received the drug at a dose of 5 mg/kg by intravenous infusion at 0, 2, and 6 weeks. A fourth infusion was given two months after the third one. The improvement was only partial. The patient continued to have less severe peripheral manifestations of spondarthritis.

In October 2002, one month after the fourth infusion, he presented with a severe arthritis of the right knee. He denied any airway complaints. Arthrocentesis yielded 50 ml of purulent fluid. Laboratory evaluation showed only an erythrocyte sedimentation rate (ESR) of 95 mm/1st h and a C reactive protein (CRP) level of 172 mg/l (normal <5). Earlier values were 2 mm/1st h and 0.7 mg/l, respectively. Culture of the purulent synovial fluid grew *Moraxella catarrhalis*, and the blood culture was negative. The infection

was cured with a two week course of antibiotic treatment with ciprofloxacin at a dosage of 1 g/day and teicoplanin at a dose of 200 mg/day. Joint drainage was performed on two occasions. The ESR was 2 mm/1st h and CRP was 20.5 mg/l after the end of treatment.

DISCUSSION

Both the two TNF α antagonists, the mouse-human monoclonal IgG1 monoclonal antibody infliximab and the 7 kDa IgG1 recombinant fusion protein etanercept, have been shown to be effective in ankylosing spondylitis and psoriatic arthritis.^{5,6} Severe uSpA, unresponsive to sulfasalazine, is another possible indication for TNF α neutralising treatment.⁷ Our patient with severe uSpA unresponsive to sulfasalazine and methotrexate partially improved with infliximab. Unfortunately, the drug was stopped owing to the infection caused by *Moraxella catarrhalis*.

Opportunistic infections, including tuberculosis,¹ aspergillosis,² listeriosis,³ and histoplasmosis,⁴ are potential complications of treatment with TNF α blocking agents. *Moraxella catarrhalis*, a component of the normal bacterial flora of the upper airways and possibly the female genital tract, has recently emerged as a cause of different illnesses, including sinusitis, otitis media, conjunctivitis, laryngitis, bronchitis and pneumonia, and systemic infections in immunocompromised patients.⁸ There are also reports of septic arthritis due to *Moraxella catarrhalis*.^{9,10} Our patient developed *Moraxella catarrhalis* arthritis after the third infusion of infliximab. He was immunocompromised also because he had received immunosuppressive disease modifying treatment with methotrexate in the eight months before beginning the anti-TNF α blocking therapy.

In conclusion, we suggest that *Moraxella catarrhalis* should be included in the list of opportunistic organisms inducing infection associated with anti-TNF α blocking therapy.

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Lack of association between angiotensin converting enzyme gene polymorphism and Korean Behçet's disease

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The histological hallmark of Behçet's disease (BD) is a vasculitis, and endothelial dysfunction has a role in the development of the vascular lesions in BD.^{1,2} Angiotensin converting enzyme (ACE) plays a part in the renin-angiotensin and kallikrein-kininogen systems by producing angiotensin II from angiotensin I and by inactivating bradykinin. The ACE gene is located on the long arm of chromosome 17, and insertion and deletion (I/D) polymorphism of this gene is strongly related to the levels of circulating ACE: the serum levels of ACE in the DD genotype, homozygote for the deletion allele, are about twice as high as those in the II genotype, homozygote for the insertion allele.³ In addition, the DD genotype is associated with endothelial dysfunction, as the stimulated endothelial or donated nitric oxide response is blunted.⁴ Moreover, angiotensin II may participate in the vascular pathogenesis of several diseases through vascular smooth muscle cell contraction and proliferation, monocyte adhesion, and platelet aggregation. However, to our knowledge, there have been no studies on the relationship between the ACE gene and BD. Thus, we studied the potential association between ACE I/D gene polymorphism and Korean BD.

PATIENTS, METHODS, AND RESULTS

The study group included 70 patients with BD (27 men and 43 women; mean (SD) age 38.1 (7.8)) fulfilling the international study group criteria⁵ and 106 healthy controls (37 men and 69 women; mean (SD) age 37 (11.5)). All the subjects were ethnically homogenous Koreans, unrelated to each other. The cumulative history of severe manifestations was investigated during the disease course, as described in our previous study.⁶ Informed consent was obtained from all the subjects.

ACE I/D polymorphism was determined by polymerase chain reaction genotyping.⁷ The statistical significance was evaluated using χ^2 test, *t* test, or one way analysis of variance test. Values of *p* < 0.05 were considered significant, and these were corrected by multiplying the values by the number of alleles in certain cases.

Table 1 shows that the distribution of genotypes and alleles of the ACE gene did not differ significantly between patients with BD and controls (*p* > 0.05), and it was in Hardy-Weinberg equilibrium. In a comparison of clinical variables including sex, clinical manifestations, severe manifestations, and positivity of HLA-B51, no

Table 1 The distribution of angiotensin converting enzyme genotypes and alleles in the patients with BD and controls

	No	Genotype			Allele
		DD	ID	II	D v I
<i>For study groups</i>					
Patients with BD	70	17 (24%)	28 (40%)	25 (36%)	0.44:0.56
Controls	106	20 (19%)	44 (42%)	42 (40%)	0.40:0.60
<i>For patients with BD</i>					
Male (female)	27 (43)	9 (8)	12 (16)	6 (19)	0.56:0.44 (0.37:0.73)
Genial ulcerations	61 (9)	13 (4)	26 (2)	22 (3)	0.43:0.57 (0.56:0.44)
Skin lesions	65 (5)	15 (2)	26 (2)	24 (1)	0.43:0.57 (0.60:0.40)
Ocular lesions	20 (50)	5 (12)	6 (22)	9 (16)	0.40:0.60 (0.46:0.54)
Positive pathergy test	24 (46)	4 (13)	10 (18)	10 (15)	0.37:0.63 (0.48:0.52)
Intestinal lesions	16 (54)	2 (15)	9 (19)	5 (20)	0.41:0.59 (0.45:0.55)
Peripheral arthritis	16 (54)	6 (11)	6 (22)	4 (21)	0.56:0.44 (0.41:0.59)
Vascular lesions	11 (59)	2 (15)	5 (23)	4 (21)	0.41:0.59 (0.45:0.55)
CNS lesions	7 (63)	2 (15)	2 (26)	3 (22)	0.43:0.57 (0.44:0.56)
Severe manifestations	27 (43)	5 (12)	12 (16)	10 (15)	0.41:0.59 (0.46:0.54)
Positivity of HLA-B51	37 (33)	9 (8)	13 (15)	15 (10)	0.42:0.58 (0.47:0.53)

The values in parentheses in the data for patients with BD are for the patients lacking each criterion: there were no significant differences in the comparison of genotype and allele frequencies between any two groups, including patients with BD v controls, male v female patients with BD, and patients with BD with criterion v without criterion (all $p > 0.05$).

significant associations were found in the frequencies of genotypes and alleles between patients with BD with and without each criterion (all $p > 0.05$). Although the D allele frequency was significantly higher in male patients than in female patients, the significance was lost when multiplying by the number of alleles ($p = 0.033$, $p_{\text{corr}} = 0.066$).

The mean (SD) ages at onset of patients with BD in each genotype were 33.3 (8.3) (DD), 33.3 (8.0) (ID), and 32.5 (8.2) years (II), and no differences were seen between groups ($p > 0.05$). In a comparison of patients with BD with and without each genotype (DD, ID, II), frequencies of clinical variables (sex, clinical manifestations, severe manifestations, positivity of HLA-B51, mean age at onset) did not differ significantly (all $p > 0.05$) (data not shown).

DISCUSSION

Genetic susceptibility to BD is affected by multiple genes, such as major histocompatibility complex (MHC) and non-MHC genes. Endothelial nitric oxide synthase (eNOS) gene polymorphisms are noted to be associated with the pathogenesis of various vascular diseases, including coronary artery disease (CAD), myocardial infarction (MI), hypertension, and renal diseases. Recently, we reported that Glu298Asp polymorphism of the eNOS gene was another susceptibility gene for Korean BD and other rheumatic diseases with vasculitis.⁸

ACE gene polymorphism is also reported to be a risk factor for CAD, MI, hypertension, and renal diseases. Furthermore, the DD ACE genotype is associated with endothelial dysfunction, which is believed to have an important role in the development of the vascular lesions in BD.²⁻⁴ On the other hand, associations between the ACE gene and non-Behçet's rheumatic diseases with vascular involvement have been inconsistently reported.⁹⁻¹⁰ We therefore considered that the ACE gene might be another candidate gene for BD. However, we could not detect any significant correlation between BD and ACE gene polymorphism. Because of the well known regional differences in the disease expression of BD, further studies in other ethnic populations will be required.

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Spontaneous pregnancy in a woman with lupus and thyroiditis despite imminent premature ovarian failure

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We report a case of spontaneous pregnancy in a woman with lupus and clinical and hormonal changes suggesting imminent premature ovarian failure (POF).

CASE REPORT

A French woman born in 1962 had had Raynaud's phenomenon, photosensitivity, and subacute cutaneous lupus since the age of 15 years, which was treated with hydroxychloroquine. She underwent abortions for unwanted pregnancies in 1986, 1987, and 1988. Various progestin contraceptives were prescribed but rapidly stopped. In 1990 she presented with giant cell thyroiditis with normal thyroxine, thyroid stimulating hormone 6.1 mU/l (normal 0.1–3.2), and negative antithyroperoxidase and antithyroglobulin antibodies. L-Thyroxine 100 µg daily was started. In August 1992 endometriosis was discovered.

In February 1997 she complained of polyarthritides and sustained climacteric symptoms. She had regular menses with a short luteal phase. Her mother became menopausal at the age of 50. Follicle stimulating hormone (FSH) was increased (95 U/l) with normal oestradiol and karyotype. She was advised to become pregnant rapidly because of imminent POF. Antinuclear antibodies, anti-dsDNA, anti-extractable nuclear antigen and anticardiolipin were negative, total complement (CH₅₀) 34 U (normal 35–55), C3 0.66 g/l (normal 0.7–1.3), C4 0.1 g/l (normal 0.1–0.3). Antithyroglobulin became positive at 1000 U/ml (normal <100). Articular symptoms remitted with additional treatment with diclofenac.

In January 1998, FSH was 68 U/l and oestradiol 10 pg/ml, but cycles were still occasionally ovulatory. In the subsequent months, climacteric symptoms remitted. In October 1998 she became spontaneously pregnant. Pregnancy was uneventful with hydroxychloroquine and L-thyroxine. Caesarean section was indicated at 38 weeks because of abnormal cardiotocography, and she delivered a 2910 g healthy girl.

DISCUSSION

Our patient had an intermediate status between cutaneous and systemic lupus erythematosus. She developed imminent POF at the age of 35, without any other cause of ovarian failure. In particular, she did not take thalidomide.¹ Menopause is defined by the cessation of menstruation for ≥12 months with low oestradiol and high gonadotrophins. Menopause is considered premature when it occurs at <40 years. POF is suggested in cases of amenorrhoea of ≥4 months and confirmed by persistent FSH levels >40 U/l at least twice with a one month interval. Despite regular menses, our patient had clinical symptoms and hormonal changes suggesting imminent POF.

Premature menopause and POF affect 1–2% of women in the general population.² Although known causes of POF include X chromosome deletions, radiation, chemotherapy, and genetic defects of the gonadotrophin hormones or receptors, one third to one half of cases remain idiopathic.

A significant proportion of patients with apparently idiopathic POF have evidence for an autoimmune aetiology because of positive autoantibodies.³ However, confirmation of an autoimmune cause requires an ovarian biopsy. Antiovarian and other self tissue antibodies are present in up to one third of women with POF, but the tests are not well standardised and poorly correlated with ovarian histology. Autoimmune POF may occur as an isolated event, be part of the polyglandular autoimmune syndrome, or associated with other autoimmune diseases. Antibodies directed against the corpus luteum were found to be present in 22% of women with systemic lupus erythematosus (SLE) aged <40 years,⁴ but they did not correlate with age, race, SLE activity, other autoantibodies, and treatment.⁴

Our patient had endometriosis, which is associated with an increased prevalence of various autoantibodies: antinuclear, anti-ribonucleoproteins, anti-smooth muscle, anticardiolipin, and lupus anticoagulant.⁵ Endometriosis is characterised by abnormal aromatase activity in endometrial tissue, which leads to local production of oestrogen, inducing prostaglandin E₂ (PGE₂) formation. PGE₂ stimulates aromatase expression and establishes a positive feedback cycle. Endometriosis is associated with a twofold increased risk of SLE.⁶ Oestradiol might cause autoimmune changes by mechanisms which have not been clearly elucidated. Association of endometriosis with POF⁷ suggests that the primary mechanism is hormonal rather than immune.

POF is characterised by occasional recurrent ovarian activity, which occurs more often than in the natural menopause. Patients with POF still have a 5–10% chance of conceiving after diagnosis.⁸ Glucocorticoids were tried without documented efficiency in autoimmune POF.⁹ Women with hypergonadotrophic hypogonadism may become able to ovulate when treated with oestrogen alone or in combination with gonadotrophins, and may even conceive after treatment with gonadotrophin releasing hormone analogue and gonadotrophins.¹⁰ In our patient, it is difficult to ascertain whether diclofenac and hydroxychloroquine favoured the spontaneous pregnancy because POF does not imply definitive sterility. About 80% of pregnancies after POF resulted in the birth of a healthy child. However, no treatment has been found which efficiently restores fertility in prospective controlled studies.

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Doppler ultrasound identifies increased resistive indices in SSc

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Cutaneous lesions in patients with systemic sclerosis (SSc) are characterised by fibrosis as well as by changes in the microvasculature. Various methods, including nailbed capillaroscopy, laser Doppler flow monitoring, thermography, and plethysmography, have been used to evaluate distal digital vascularisation and to assess the microvascular damage.^{1–4} In studies using Doppler flowmetry and iontophoresis, patients with SSc showed reduced vasodilatory reserve of the skin microvasculature in response to ischaemia.^{5–6} A new colour Doppler ultrasound (DU) technique of the nail bed appears to be able to detect and quantify early vascular damage in patients with connective tissue disease.⁷ However, none of these methods has been generally introduced and accepted in clinical routine.

METHODS

This study aimed at assessing the digital blood flow of patients with SSc by DU. We compared the resistive indices (RIs) of 14 healthy subjects and 19 patients with SSc. Patients with SSc were classified as affected by limited SSc or diffuse SSc according to the criteria proposed by LeRoy *et al.*⁸ The measurements were performed with an Ultramark 9 HDI duplex Doppler ultrasound (HDI; Advanced Technology Laboratories) after at least 15 minutes of thermal acclimatisation in our ultrasound laboratory. A 10 MHz probe was used for visualising digital vessels (Doppler filter 100 Hz, minimal flow velocity 10 cm/s). The outcome variable was the RI of the distal palmar arteries (arteriae digitales palmares propriae) of the thumb and the forefinger of the right and left hand (dig I and II). The arteries were identified by colour DU. The Doppler samples were obtained at the distal part of the digital artery, and the RI was determined by analysis of the spectral waveforms (fig 1). The RI was calculated according to the standard formula:

$$RI = (\text{peak}_{SV} - \text{end}_{DV}) / \text{peak}_{SV}$$

where SV = systolic velocity; DV = diastolic velocity.

The RI of each of the digital arteries was determined in duplicate, and the mean of the resulting eight measurements was used for statistical analysis. Measurements were incomplete in seven patients with SSc, because it was impossible to identify all four digital arteries. In these seven patients we used the available measurements for statistical analysis. Statistical analysis was performed using the Mann-Whitney rank test.

Figure 1 Picture of a colour Doppler ultrasound (A) and the associated spectral waveform (B) of the distal artery of the left thumb (patient with diffuse disease).

RESULTS

Table 1 shows the clinical characteristics of patients and controls. The mean of all measurements of all fingers showed a significantly higher RI for patients with SSc (limited and diffuse disease) (RI = 0.66) than for healthy controls (RI = 0.59; $p = 0.01$). However, there was a considerable overlap between the two groups. Individual digital analysis

Table 1 Patients characteristics

	SSc	Controls
Male	8	8
Female	11	6
Age (mean) years:		
Limited disease	50.3* (p=0.001)	30.9
Diffuse disease	44.2 (p=0.11)	
Raynaud's phenomenon	17	None
Unknown	1	None
Smoking	1	3
Unknown	1	None
Limited disease	10	—
Diffuse disease	9	—
Immunosuppressive drugs	3	None
Vasodilators	5	None

Figure 2 Mean resistive indices and 95% confidence interval of all measurements of patients with limited disease (n=10), diffuse disease (n=9), and controls (n=14).

showed that the mean of the RI of the left thumb ($p = 0.01$) and the right thumb ($p = 0.035$) were significantly higher in patients with SSc than in normal controls. In contrast, there was no significant difference between the right and left forefinger, and the RI of the individual fingers of the patients did not show a consistent correlation.

We analysed patients with diffuse and limited disease separately, and found no significant difference between healthy controls and patients with limited disease (fig 2). However, patients with diffuse disease showed a significantly

higher RI of the left thumb ($p = 0.005$), the right thumb ($p = 0.033$), the left forefinger ($p = 0.031$), the right forefinger ($p = 0.046$), and for the mean of all measurements ($p = 0.013$) in comparison with the healthy controls. In addition, no significant difference between the patients with limited and diffuse disease was found when calculating the mean of all measurements, but in individual digital analysis patients with diffuse disease showed a significantly higher RI of the right forefinger ($p = 0.04$).

In summary DU is an economic and simple non-invasive investigation technique, which may help to provide more information on the status of the digital microvasculature in patients with SSc. The increased RI values may reflect structural changes in digital arterial walls associated with a low vessel compliance, but, owing to the overlap of the RI between both groups, the diagnostic value of the RI measurements in the present group of patients was limited.

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