MATTERS ARISING

Incidence of RA in people with persistently raised RF

A criticism of the study reported in the *Annals*[1] is that age was not taken into account in the evaluation of the probability of development of rheumatoid arthritis (RA) among symptom free subjects with persistently raised rheumatoid factor (RF). The prevalence of RF can be as high as 14.1% in apparently healthy people aged 67–95 (mean age 81).[2] RF is also 3.5 times more common in healthy elderly subjects (aged >65) than in their younger counterparts.[3] All these factors may alter the natural history of arthritis in elderly patients who have RF either in good health or in a non-arthritic presentation of RA.

The latter is exemplified by a patient admitted at the age of 76 with symptomatic, as well as echocardiographically validated rheumatoid pericarditis in the absence of arthritis. Rheumatoid arthritis latex fixation test (RA LFT) was positive with a titre of 1/160, antinuclear factor (ANF) titre was 1/250, and signs of active inflammatory disease included a platelet count of 750×10^9/l, and an erythrocyte sedimentation rate (ESR) of 98 mm/1st h (Westergren). Arthralgia of the hands and wrists developed for the first time two years later (when she was no longer taking steroids), with a subsequent RA LFT titre of 1/80 and an ANF titre of 1/320 about four months after the onset of arthralgia. Radiography showed narrowing of the joint spaces of the hands 12 months later, but there were as yet no erosions at this stage. Erosions were seen in March 1992, approximately two and a half years after the onset of arthralgia, when the RA LFT titre was 1/160, ANF titre 1/160, platelet count 421×10^9/l, ESR 18 mm/1st h. At her most recent attendance, on 2 February 2000, she was still very active, having continued to receive prednisolone (maximum dose 5 mg/d) continuously since 1989. Her only complaint was a little pain in the left thenar eminence and painful heels. RF was now 768 IU/ml, ANF titre 1/320, platelet count 340×10^9/l, ESR 42 mm/1st h. Antibodies against double stranded DNA had not been reported at any stage.

COMMENT
This case shows a remarkable dissociation between arthritic symptoms and levels of RF, perhaps signifying that when the immune status is altered in old age,[2] the relation between RF and the natural history of RA might be less clear than it is in younger people.

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Author's reply

It is certainly well documented that the incidence of raised rheumatoid factor (RF) increases with age. However, we are not aware of any study of different RF isotypes in this context, but our own unpublished observation indicates that it is mainly IgM RF that tends to increase in symptom free elderly people.

However, increased incidence of raised RF in elderly people is not relevant to the findings that we published recently in the *Annals*.[1] We simply observed increased prevalence and incidence of rheumatoid arthritis (RA) in elderly subjects who had one or more RF isotypes persistently raised, usually IgM and IgA, compared with those with a transient increase in RF or persistent increase in only one RF isotype. There was no significant age difference between these three groups of subjects studied.

Dr Jolobe's case history simply confirms what has already been often reported previously that an increase of RF often precedes clinical manifestation of RA.[2] It would have been interesting to know about the RF isotype pattern of his patient. We have noted that the pulmonary manifestation of RA is strongly associated with raised IgA RF.[3]

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LETTERS TO THE EDITOR

The HLA-B*2709 subtype in a patient with undifferentiated spondarthritis

In 1998, in this journal, we reported the cases of two B27 positive patients with undifferentiated spondyloarthropathy (uSpA) and showing dactylitis also affecting the synovial sheaths in the palm of the hand.[1] Neither patient had axial disease but showed peripheral manifestations of spondyloarthropathy (SpA), such as peripheral arthritis, peripheral enthesitis, and dactylitis.

Recently, one of our two patients (No 2) was subtyped and found to be B*2709 positive. As far as we know this subtype has never been found in patients with SpA.

DNA typing of HLA class I alleles was performed using a DNA sample prepared from peripheral blood lymphocytes by the salting out procedure.[2] The class 1 ABC SSP UNITRAY low resolution kit (Pel-Freez) was used. The primer sets amplify all alleles described by the International Nomenclature Committee of WHO in 1995[3] and in 1997.[4] Polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) was used. A control primer pair was present to verify the integrity of the PCR reaction. Molecular typing of B27 variants was carried out by a PCR-SSP technique with a DYNAL HLA-B27 kit (DYNAL AS, Oslo, Norway), which identifies all the phenotypically different HLA-B27 alleles, B*2701-11, recognised by the HLA Nomenclature Committee in 1973.[3] The typing results for our patients were: HLA-A*0101-02, *3201-02; HLA-B*0801, *2709; HLA-C*0102-03, *0701-07.

To confirm these results HLA-B locus sequence based typing was performed. A unique DNA amplification, encompassing exon 1 to intron 3, and four fluorescent sequencing reactions, covering exon 2 and 3, were used.[5] Two intronic amplification primers generated a 1 kb length product useful for direct sequencing. For complete subtyping of the allelic variants PCR-SSP was used. Cycle sequencing reactions allowed the incorporation of fluorescently labelled dideoxy terminators for detection on a DNA automated sequencer (ABI PRISM 377, Perkin Elmer). Data processing and allele assignment were performed automatically with specific analysis software that compares the sequencing results against a sequence library and provides individual allele assignment for each sequence. The HLA-B class 1 high resolution typing of our sample was HLA-B*0801/2709 in agreement with the low resolution typing performed by PCR-SSP.

SpA has a strong association with the HLA-B27 molecule. Studies in humans and transgenic rodents suggest a direct involvement of HLA-B27 in the pathogenesis of the disease. Thirteen subtypes of HLA-B27 (B* 01–13) have been described, differing from each other by one or more amino acid changes, mainly in the peptide-binding site.[6 7] Of these B*2701, 02, 03, 04, 05, 07, 08, and 10 are associated with ankylosing spondylitis (AS). B*2711–13 are rare, which has precluded assessing their putative association with AS. B*2706 is not associated with AS in South East Asia. However some B*2706 positive patients with AS have been reported in China.[8] It has been suggested that the B*2706 might protect against SpA. Recently, a study on families in which both B*2704 and B*2706 occurred has suggested that B*2706, although not associated with SpA, does not protect against SpA.[9]

B*2709 has been found in Sardinia and in continental Italy, where the frequency of HLA-B27 in the general population is around 2%. B*2709 accounts for 25% of HLA-B27 subtypes in Sardinia and 3% in continental Italy.[10] D'Amato and coworkers have tested 35 Sardinian patients with AS and 40 Sardinian B27 positive healthy subjects by genomic typing.[10] None of the patients with AS were found to be B*2709 positive, in contrast with 25% among the healthy controls. The authors suggested that B*2709 is not

associated with AS. B*2709 differs from B*2705 by a single substitution (His *v* Asp) at position 116, which is located in the F pocket of the peptide-binding site. In the opinion of D'Amato and his colleagues the substitution at position 116 might exclude the acceptance of arthrogenic peptide from the B*2709.

Our patient was born in the south of Italy, she is B27 positive, and has uSpA with an erosive and disabling peripheral arthritis. Our case, also, suggests that the B*2709 might be associated with SpA and that the negative association found in Sardinian patients with AS[10] should be confirmed in other studies. These should include the full spectrum of SpA and not be limited to AS.

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Y chromosome microchimerism in rheumatic autoimmune disease

It is well known that some features of chronic graft-versus-host disease (GVHD) resemble those of other rheumatic autoimmune diseases, such as systemic sclerosis (SSc), Sjögren's syndrome (SS), and primary biliary cirrhosis (PBC). Furthermore, the development of systemic lupus erythematosus (SLE)-like diseases has been seen in murine models of GVHD.[1] The pathogenesis of rheumatic autoimmune diseases is still unknown. One possibility that has been suggested is that these diseases are associated with pregnancy because of their strong female predilection and, especially in SSc, a peak incidence after parturition. In 1996 Bianchi *et al* reported that fetal cells could survive in the maternal circulation for up to 27 years after parturition, a phenomenon termed fetal microchimerism.[2] These observations led the hypothesis that persistent fetal cells in the maternal circulation could mediate a graft-versus-host reaction, resulting in autoimmune disease.

Nelson *et al* have previously carried out a quantitative assay for male DNA in women with SSc and normal women who had delivered at least one son.[3] They indicated that the mean number of male cell DNA equivalents among controls was 0.38 cells/16 ml whole blood and 11.1 among patients with SSc. In addition, Artlett *et al* have shown Y chromosome-specific sequences in the DNA extracted from peripheral blood in 32 of 69 women with SSc (46%) as compared with 1 of 25 normal women (4%).[4] They also reported that those allo-cells were T lymphocytes and infiltrated lesional skin. These findings support the hypothesis that fetal microchimerism may contribute to the pathogenesis of SSc. However, this is still controversial because Murata *et al* have recently reported that there is no significant difference in the presence of fetal DNA in peripheral blood between Japanese patients with SSc and healthy women with non-quantitative assay.[5] Here we report further studies of fetal microchimerism in SSc, SLE, and SS.

We assayed for a specific Y chromosome sequence in the DNA extracted from peripheral blood by a nested polymerase chain reaction (PCR) in 20 patients with SSc, 21 patients with SLE, 18 patients with SS, and 41 healthy volunteers. All patients and healthy volunteers were Asian-Japanese women who had delivered at least one son. The nested PCR was done using the primers Y1–1, Y1–2, Y1–3, and Y1–4, which are specific for a part of the Y chromosome sequence, DYZ1, as described previously.[4 6] The identity of the detected PCR product was confirmed by nucleotide sequencing. The results from healthy volunteers and test groups were compared by Fisher's extract probability test.

Table 2 Comparison of clinical findings of DYZ1 positive and negative systemic sclerosis groups

	DYZ1		
	Positive (n=10)	*Negative (n=10)*	*Total (n=20)*
Barnett's type,			
I	3	4	7
II	3	5	8
III	4	1	5
Autoantibodies			
Antinuclear factor	10	8	18
Topoisomerase I	4	1	5
Centromere (PBC*)	3 (3)	8 (0)	11 (3)
RNP	4	0	4
SS-A(Ro)	2	3	5
SS-B(La)	0	0	0
RA	3	1	4
ssDNA	2	1	3
Mitochondria	2	0	2
Smooth muscle	1	0	1

*PBC = primary biliary cirrhosis.

Y chromosome-specific DNA was detected in 10 of the 20 patients with SSc (50%), eight of 41 healthy volunteers (20%, p=0.017), and six of 18 patients with SS (33%). No Y chromosome-specific DNA was detected in any of the patients with SLE (table 1). The DYZ1 was most commonly found in Barnett's type III (four of five). The DYZ1 positive patients with SSc also had a variety of antibodies including anti-RNP, antimitochondrial, and anti-smooth muscle antibodies that may reflect polyclonal activation of immune cells. Anticentromere antibodies were detected more commonly in the DYZ1 negative group (eight of 10). All three patients with SSc who had PBC were DYZ1 positive and had anticentromere antibodies (table 2).

Our data confirm that male DNA is found more commonly in women with SSc than in normal women. Interestingly, DYZ1 was not detected in patients with SLE and there was no significant difference between patients with SS and healthy volunteers. These data suggest that fetal microchimerism may be a phenomenon which is strongly associated with the pathogenicity of SSc and not with the related autoimmune diseases, SLE and SS.

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Table 1 Patients' characteristics

	SSc	*SLE*	*SS‡*	*Healthy controls*
Patients (n)	20	21	18	41
Age (years, mean (range))	56.1 (44–74)	50.2 (34–82)	54.8 (27–74)	53.2 (39–59)
Duration of illness (years, mean (range))	10.2 (1–26)	11.9 (1–24)	8.7 (1–19)	
DYZ1 positive (No (%))	10* (50)	0† (0)	6 (33)	8 (20)

*p=0.017, systemic sclerosis (SSc) *v* healthy volunteers.
†p=0.028, healthy volunteers and systemic lupus erythematosus (SLE).
‡SS = Sjögren's syndrome.

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Marker of erosive progression in RA

Urokinase plasminogen activator (uPA) catalyses the formation of the proteolytic enzyme plasmin, which plays a part in tissue degradation and remodelling,[1] and seems to have an important role in the erosive growth of pannus in rheumatoid arthritis (RA).[2] The action of uPA is localised and intensified by a cell bound receptor (uPAR),[3] expressed by some malignant cells and some inflammatory cell types, including activated synoviocytes in the marginal zone between pannus and cartilage in RA synovial tissue.[4]

The uPAR may become cleaved at the cell surface bound anchor, forming a free soluble receptor (suPAR) which is detectable in steady, low concentrations in healthy controls, but with raised concentrations in patients with disseminated malignant disease.[5]

Recently, in a cross sectional study, we found increased concentrations of suPAR in plasma of patients with RA compared with controls and patients with other types of inflammatory rheumatic disorders.[6] This finding raises the question, whether suPAR might be an easily accessible plasma marker of erosive activity in the synovial joint space in RA.

In a pilot study we followed up outpatients with RA to evaluate the relation between suPAR and disease activity. Plasma suPAR was measured and other clinical and paraclinical variables of disease activity determined in these patients on two or more occasions during a 12 month period. The present study included all patients (n=16) for whom comparable radiographs of the wrists and hands were obtainable, and also, when relevant, other symptomatic joints, taken before and after the period of suPAR measurements. The *x* ray films of participating patients were read independently by a radiologist unaware of the patient's clinical status and suPAR values. An enzyme linked immunoabsorbent assay (ELISA) was used to measure suPAR in plasma, as previously described.[5 6]

Table 1 Period average values of corresponding paraclinical and clinical variables of 16 patients with rheumatoid arthritis followed up prospectively and subsequently divided into two groups with or without progressive erosive changes on radiographs. Values are medians with range

	Erosive progression (n=5)	*No erosive progression (n=11)*
suPAR† (µg/l)	1.51 (0.93–2.73)*	1.03 (0.56–2.09)*
CRP† (mg/l)	11.4 (6.1–30.1)	11.0 (4.2–29.5)
ESR† (mm/1st h)	24 (15–24)	16 (7–58)
Tender joints (of 28)	6 (3–20)	4 (0–17)
Swollen joints (of 28)	4 (1–8)	2 (0–10)

*p<0.05, non-parametric Mann-Whitney test.
†suPAR = soluble urokinase plasminogen activator in plasma; CRP = C reactive protein; ESR = erythrocyte sedimentation rate.

The study group comprised 11 women and five men with a median age of 53.5 years (range 25–80) and a median disease duration of 57 months (range 5–360). Fifteen patients were rheumatoid factor positive and 10 had bony erosions on prestudy radiographs. Antirheumatic treatment included methotrexate (11 patients), hydroxychloroquine (two), sulfasalazine (one), and low dose steroids (eight). Clinical evaluation and measurement of suPAR, erythrocyte sedimentation rate (ESR), and C reactive protein (CRP) were done a median number of three times, and the time interval between radiographs was a median of 22 months.

Table 1 shows the results of the study. We found significantly higher suPAR concentrations ($p<0.05$) in plasma from those patients with RA whose *x* ray findings showed disease progression than in the patients who had no radiographic signs of progression, but the differences in ESR, CRP, and clinical variables were not significantly different.

This study was a pilot study in a clinical setting and conclusions must be drawn cautiously. The main problems, apart from the small number of patients, are, firstly, that in some of the patients prestudy radiographs were one to two years old. However, this would tend to diminish the differences found between the erosive progressive and non-erosive progressive groups as patients in remission, or with low activity in the study period, could be classified as progressive due to previous activity. Secondly, another possible bias, tending to increase the difference in suPAR between the two groups in this study, is that patients with high clinical activity would probably have had more extensive *x* ray examinations, increasing the chance of finding new erosions. We did not, however, find a difference in the number of radiographically investigated joints between our two groups of patients.

In conclusion, we find that this study indicates that plasma suPAR may be an easily accessible plasma marker of erosive progression in RA, and further studies on the subject are warranted.

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CORRECTION

Epidemiology of whiplash (Barnsley L. Ann Rheum Dis 2000;59:394; Reply: Ferrari R, Russell AS. Ann Rheum Dis 2000;59:395–6.)

The Editor of the *Annals* regrets that we inadvertently published a reply to Dr Barnsley from Drs Ferrari and Russell that contained some misinformation, and offers his apologies to Dr Barnsley.

Possibly, Drs Ferrari and Russell were confusing Dr Barnsley with someone else. Firstly, Dr Barnsley is a man and not a woman, as they stated. Secondly, Dr Barnsley did not attend the World Whiplash Congress in Vancouver and has not read the transcripts of it and thus could not be, as Drs Ferrari and Russell commented, "well aware of an impressive study presented there".

(Note: Corrections printed in the journal also appear on the *Annals* web page (www.annrheumdis.com) and are linked to the original publication.)