

## CONCISE REPORT

# Circulating tumour necrosis factor $\alpha$ and soluble tumour necrosis factor receptors in patients with different patterns of rheumatoid synovitis

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**Objective:** To examine the relation between the serum levels of tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), soluble tumour necrosis factor receptors (sTNF-R), and the histological pattern of rheumatoid synovitis.

**Methods:** An enzyme linked immunosorbent assay (ELISA) was used to measure TNF $\alpha$ , p55 sTNF-R, and p75 sTNF-R concentrations in the serum of 43 patients with rheumatoid arthritis (RA) and 34 patients with osteoarthritis (OA).

**Results:** Upon histological analysis two variants of rheumatoid synovitis emerged. Twenty six RA specimens presented only diffuse infiltrates of mononuclear cells. In the remaining 17 samples the formation of lymphocytic follicles with germinal centre-like structures was found. Serum concentrations of TNF $\alpha$ , p55 and p75 sTNF-R were raised in patients with RA compared with the OA control group ( $p < 0.001$  for all comparisons). Levels of TNF $\alpha$ , p55 and p75 sTNF-R were higher in the serum of patients with RA with follicular synovitis than in patients with diffuse synovitis ( $p < 0.001$ ,  $p < 0.01$ , and  $p < 0.05$ , respectively). Serum concentrations of TNF $\alpha$ , p55 and p75 sTNF-R correlated with markers of disease activity.

**Conclusion:** Different histological types of rheumatoid synovitis associated with distinct serum levels of TNF $\alpha$  and sTNF-R reflect varying clinical activity of the disease and support the concept of RA heterogeneity.

Tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) is supposed to have a pivotal role in the pathogenesis of rheumatoid arthritis (RA). The direct pathogenic effects of TNF $\alpha$  are amplified by its ability to induce other proinflammatory cytokines, such as interleukin 1, granulocyte macrophage colony stimulating factor, platelet derived growth factor, prostaglandin E<sub>2</sub>, and matrix metalloproteinases, involved in the degradation of synovial tissue. TNF $\alpha$  also stimulates the production of the soluble TNF receptors (sTNF-R), which may act as its natural inhibitors.<sup>1</sup> Rheumatoid synovium is infiltrated by lymphocytes, macrophages, and synoviocytes. Increased angiogenesis and the proliferation of the synovium lining layer may also be found. All these cells are thought to contribute to the synovial tissue destruction processes by several mechanisms, including TNF $\alpha$  production. Most rheumatoid synovia display diffuse infiltrate of mononuclear cells, without any further microanatomical organisation, and may be categorised as diffuse synovitis. In about one third of RA synovia formation of lymphoid follicles is found.<sup>2,3</sup> Such T-B cell conglomerates, which sometimes form germinal-like centres, seem to play an important part in the pathogenesis of RA. These RA synovia may be classified as follicular synovitis. Only individual patients with RA had necrobiotic granulomas with a fibrinoid necrotic centre lined by a collar of histiocytes.<sup>3</sup>

Patients with lymphoid follicles seem to have a greater degree of immunological activation, and greater potential for joint tissue destruction.<sup>3–5</sup> Moreover, different histological forms of rheumatoid synovitis have been found to be associated with a specific pattern of cytokine production in the synovium,<sup>3</sup> serum cytokine,<sup>4</sup> and matrix metalloproteinase levels.<sup>3</sup> Therefore, beside the genetic, biological, and clinical non-uniformity, the histological heterogeneity of RA has also been postulated.<sup>2,3,6</sup> This study was conducted to evaluate whether the serum concentrations of TNF $\alpha$  and sTNF-R reflect different histological appearances of RA synovitis.

## PATIENTS AND METHODS

We studied 43 patients who fulfilled the American College of Rheumatology 1987 revised criteria for RA,<sup>7</sup> and 34 patients with osteoarthritis (OA) who comprised the control group. Synovial specimens were obtained during hip or knee joint orthopaedic surgery from all patients with RA and OA entered into the study. Blood samples were clotted for 30 minutes and then centrifuged for 10 minutes at 1000 *g*. Serum aliquots were frozen at  $-80^{\circ}\text{C}$ . Study procedures were approved by ethic committee. Table 1 shows the characteristics of the patient groups.

Clinical and laboratory evaluation included the number of tender joints (Ritchie's index),<sup>8</sup> number of swollen joints, erythrocyte sedimentation rate (ESR), disease activity score (DAS),<sup>9</sup> C reactive protein (CRP) concentration, and rheumatoid factor (RF) level. Steinbrocker's criteria were used for radiological assessment of the joint destruction.<sup>10</sup> Synovial samples underwent routine staining with haematoxylin and eosin. Histological evaluations, which included assessment of the mononuclear cell infiltrate density and their microanatomical organisation, were conducted as previously described.<sup>2–5</sup>

Serum TNF $\alpha$  was measured by a commercial enzyme linked immunosorbent assay (ELISA) kit (Bender MedSystems, Vienna, Austria). Concentrations of p55 sTNF-R and p75 sTNF-R were tested by ELISA kits from R&D Systems, Wiesbaden-Nordenstadt, Germany. Measurements were carried out according to the manufacturer's directions. The sensitivity of the assays was 5 pg/ml (TNF $\alpha$ ), 3 pg/ml (p55 sTNF-R), and 1 pg/ml (p75 sTNF-R).

Analysis of the data was performed using  $\chi^2$  test, unpaired Student's *t* test, Mann-Whitney U test, and Spearman's rank order test.

**Abbreviations:** CRP, C reactive protein; DAS, disease activity score; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; sTNF-R, soluble tumour necrosis factor receptor; TNF $\alpha$ , tumour necrosis factor  $\alpha$ .

**Table 1** Patient characteristics. Data are presented as means (SD) unless otherwise stated

	OA	Diffuse rheumatoid synovitis	Follicular rheumatoid synovitis	p <		
	(OA)	(A)	(B)	OA v A	OA v B	A v B
Women/men	25/9	21/5	14/3	NS	NS	NS
Age (years)	57.9 (13.0)	52.3 (12.1)	56.6 (13.8)	NS	NS	NS
Disease duration (years)	14.5 (11.1)	16.3 (8.1)	13.1 (6.2)	NS	NS	NS
ESR (mm/1st h)	16.7 (11.0)	46.2 (12.3)	59.4 (20.7)	0.001	0.001	0.05
CRP (mg/l)	6.3 (4.8)	32.4 (11.6)	42.3 (12.6)	0.001	0.001	0.05
Swollen joints	–	12.8 (3.3)	15.9 (4.1)	–	–	0.01
Ritchie index	–	12.5 (3.4)	14.4 (3.2)	–	–	NS
DAS	–	4.2 (0.5)	4.6 (0.6)	–	–	0.05
RF positive patients (No (%))	–	17 (65)	13 (76)	–	–	NS
DMARDs* (No (%))	–	17 (65)	15 (88)	–	–	NS
Sulfasalazine* (No (%))	–	7 (27)	3 (18)	–	–	NS
Methotrexate* (No (%))	–	10 (38)	13 (76)	–	–	0.05
Radiological stage III or IV† (No (%))	–	12 (46)	14 (82)	–	–	0.05

SD, standard deviation; OA, osteoarthritis; NS, not significant; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; DAS, disease activity score; RF, rheumatoid factor; DMARDs, disease modifying antirheumatic drugs.

\*Treatment in the last three months before surgery; †radiological stage of rheumatoid arthritis according to Steinbrocker.

## RESULTS

### Histological findings

Mononuclear cell infiltrates, comprising mainly lymphocyte and macrophage-like cells, were found in RA synovia specimens. Twenty six samples demonstrated diffuse lymphocyte infiltration, with no specific microanatomical organisation, and were categorised as diffuse rheumatoid synovitis. Formation of the lymphocyte follicular conglomerates, sometimes with germinal centre-like structures, was localised in 17 specimens. These RA synovia were classified as follicular rheumatoid synovitis. Synovial lining layer proliferation, rare giant-like cells, and new vessel formations were also seen in rheumatoid samples. The presence of the necrobiotic granulomas was not noticed. OA synovial specimens disclosed only mild mononuclear cell infiltrates. Figure 1 presents representative examples of OA and the two different forms of rheumatoid synovitis.

### Demographic and clinical results

Differences in sex profile, age, or disease duration between patients with both histological forms of RA and with OA were not seen. ESR and CRP concentration were higher in patients with RA than in the OA group (in all cases  $p < 0.001$ ), especially in patients with follicular rheumatoid synovitis (table 1). Patients with RA with the follicular type of synovitis also had more swollen joints and a higher DAS than those with diffuse synovitis ( $p < 0.01$ ,  $p < 0.05$ , respectively). About 65% and 76% of patients with RA, with diffuse and follicular synovitis respectively, were seropositive (table 1). All patients had been taking non-steroidal anti-inflammatory drugs (data not shown). Disease modifying antirheumatic drugs were more often used among patients with the follicular histological form of the RA, but the difference was only significant for methotrexate ( $p < 0.05$ ) (table 1). Advanced joint destruction (III or IV stage according to Steinbrocker's radiological classification) was more often found in patients with lymphocytic follicular conglomerates than in those without ( $p < 0.05$ ) (table 1).

### Serum concentrations of tumour necrosis factor $\alpha$ (TNF $\alpha$ ) and soluble tumour necrosis factor receptors (sTNF-R)

Serum of all patients with RA and of those with diffuse or follicular synovitis contained higher levels of TNF $\alpha$  than did OA serum ( $p < 0.001$  for all comparisons) (fig 2A). TNF $\alpha$  concentration was especially increased in patients with follicular type of synovitis, as compared with diffuse synovitis ( $p < 0.001$ ). Figure 2B shows that the concentration of p55 sTNF-R was

also raised in all patients with RA with both histological types of synovitis relative to patients with OA ( $p < 0.001$  for all comparisons). p55 sTNF-R dominated in the RA group with follicular synovitis as compared with other patients with RA ( $p < 0.01$ ). Serum p75 sTNF-R concentrations were also increased in all patients with RA with diffuse or follicular synovitis as compared with patients with OA ( $p < 0.001$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively) (fig 2C). p75 sTNF-R was found in highest concentrations among patients with the follicular histological type of RA as compared with diffuse synovitis ( $p < 0.05$ ).

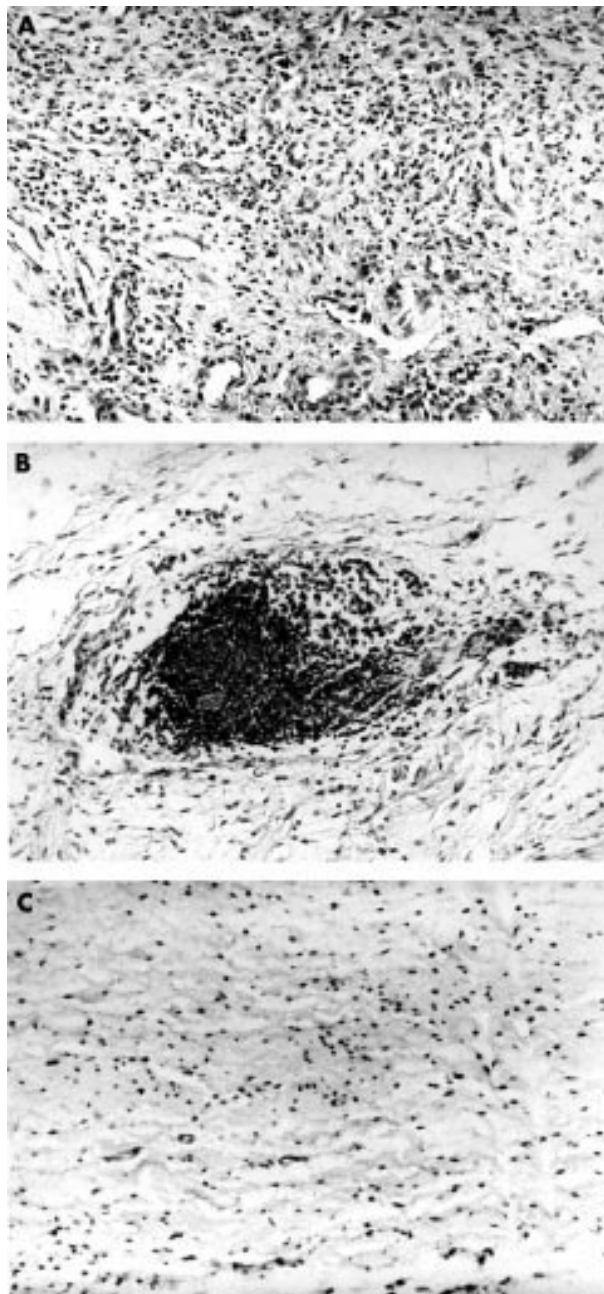
### Relationship between serum levels of TNF $\alpha$ or sTNF-R and clinical findings

Serum levels of TNF $\alpha$  correlated with ESR ( $r_s = 0.438$ ;  $p < 0.01$ ), CRP ( $r_s = 0.340$ ;  $p < 0.05$ ), number of swollen joints ( $r_s = 0.408$ ;  $p < 0.01$ ), and DAS ( $r_s = 0.402$ ;  $p < 0.01$ ). Circulating concentrations of p55 sTNF-R correlated with ESR ( $r_s = 0.413$ ;  $p < 0.01$ ), CRP ( $r_s = 0.385$ ;  $p < 0.05$ ), number of swollen joints ( $r_s = 0.423$ ;  $p < 0.01$ ), Ritchie index ( $r_s = 0.419$ ;  $p < 0.01$ ), and DAS ( $r_s = 0.441$ ;  $p < 0.01$ ). Serum levels of p75 sTNF-R correlated with ESR ( $r_s = 0.529$ ;  $p < 0.001$ ), CRP ( $r_s = 0.329$ ;  $p < 0.05$ ), number of swollen joints ( $r_s = 0.348$ ;  $p < 0.05$ ), Ritchie index ( $r_s = 0.363$ ;  $p < 0.05$ ), and DAS ( $r_s = 0.396$ ;  $p < 0.01$ ). No associations between patient age, disease duration, or RF and serum TNF $\alpha$  or sTNF-R levels were found.

## DISCUSSION

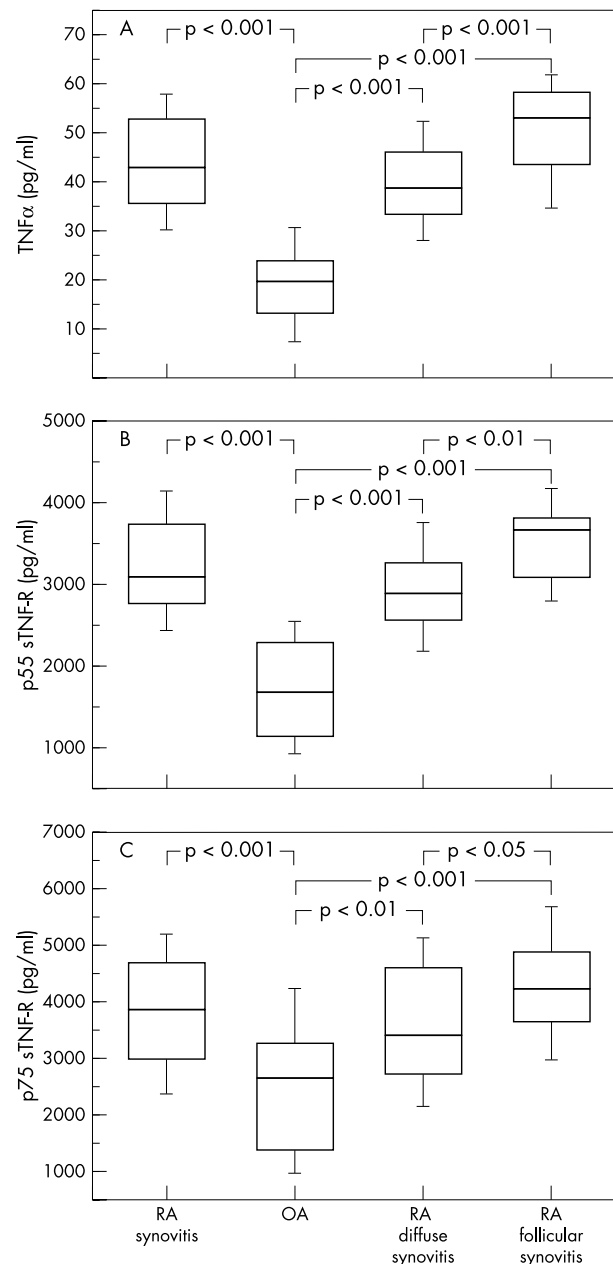
RA is a multigene disorder with genetic polymorphisms influencing a wide spectrum of its clinical presentations. Disease progression, pattern of joint disease, and extra-articular manifestations are highly variable.<sup>6</sup> Several data suggested also that RA is histopathologically heterogeneous.<sup>2,3</sup> Histological analyses in this and in previous studies showed that most rheumatoid synovia are characterised by differences in the density of the diffuse infiltrate of mononuclear cells, and lack any further specific microanatomical organisation. Such synovia may be classified as diffuse synovitis.<sup>3</sup> In about one third of RA synovia, categorised as follicular synovitis, the formation of lymphoid conglomerates was demonstrated.<sup>2,3</sup> The presence of lymphoid follicles was associated with a greater degree of immunological activation, and greater potential for joint tissue destruction.<sup>2-5</sup> All these findings support the concept of the clinical and histological heterogeneity of RA.

Mononuclear cells infiltrating the RA synovium are thought to contribute to the joint tissue destruction processes by several mechanisms, including the proinflammatory cytokines. A



**Figure 1** Histological findings in RA and OA synovia. Typical specimens are presented for the analysed groups of patients. (A) RA synovium sample displaying diffuse lymphocyte infiltrates with no additional specific microanatomical organisation. (B) RA specimen with the presence of lymphocytic follicular aggregates. (C) OA synovium with mild mononuclear cell infiltration. Original magnification  $\times 200$ .

pivotal role in the disease process is thought to be played by TNF $\alpha$ .<sup>1</sup> Previously, we demonstrated a high concentration of serum TNF $\alpha$  in patients with RA with the follicular histological form of rheumatoid synovitis.<sup>4</sup> In this present study also, which included a larger group of patients, we showed that circulating TNF $\alpha$  dominates in patients with RA with follicular synovitis. Furthermore, in contrast with the previous study, serum TNF $\alpha$  concentration was correlated not only with ESR but also with CRP level, the number of swollen joints, and the DAS. Several studies have shown higher TNF $\alpha$  concentrations in the serum of patients with RA than in those with OA or in healthy controls.<sup>11, 12</sup> Others observed also positive correlation of serum TNF $\alpha$  with the Lansbury index, ESR, and CRP.<sup>11</sup>



**Figure 2** Serum concentrations of TNF $\alpha$  (A), p55 sTNF-R (B), and p75 sTNF-R (C) in the studied patient groups. Measurement was based on an ELISA technique. Box plots represent median (line), 25th and 75th centiles (box), and whiskers indicate the 10th and 90th centiles.

However, some investigators found no associations between the serum TNF $\alpha$  concentration and clinical markers of disease activity such as ESR or CRP.<sup>12</sup> Our findings confirm our previous suggestion that follicular synovitis reflects greater severity of RA than diffuse synovitis, and that the serum TNF $\alpha$  levels may be useful as a marker of disease activity.<sup>4</sup>

TNF $\alpha$  binds to two cell surface receptors (TNF-R) of molecular weight 55 kDa and 75 kDa, respectively. sTNF-R are thought to be generated not only locally by synovial cells but also by circulating peripheral blood cells. The production of sTNF-R is up regulated by TNF $\alpha$ . It was suggested that the serum sTNF-R level might reflect not only TNF $\alpha$  expression but also intravascular cell activation associated with the more severe form of RA.<sup>13</sup> TNF-R shedding reduces surface receptor expression, limiting cell sensitivity to the biological mediators.



sTNF-R may also simply act as natural inhibitors of this proinflammatory cytokine, reducing disease activity.<sup>13</sup> However, increased production of these receptors within the synovial joint seems to be insufficient to neutralise the pathological effects of TNF $\alpha$ .

Increased p55 sTNF-R concentrations in RA serum compared with patients with OA and healthy subjects have been already demonstrated.<sup>13, 14</sup> The concentration of p55 sTNF-R was found to be especially raised in the serum of patients with clinically active RA. Furthermore, significant correlations were noticed between serum levels of p55 sTNF-R and ESR or CRP in patients with RA.<sup>14</sup> However, others failed to correlate p55 sTNF-R with markers of disease activity such as ESR and CRP.<sup>15</sup> Our study showed that the serum p55 sTNF-R concentrations were higher in all patients with RA in comparison with patients with OA. p55 sTNF-R dominated in patients with follicular synovitis. Furthermore, we noted that serum p55 sTNF-R correlated with ESR, CRP, the number of swollen joints, Ritchie index, and DAS. All these observations suggest that RA is more severe in patients with follicular synovitis.

Here we also report the raised serum p75 sTNF-R concentration in all patients with RA and in both histological types of the disease. Other also found increased p75 sTNF-R levels in RA serum compared with patients with OA and healthy subjects.<sup>13, 14</sup> Furthermore, the concentration of p75 sTNF-R was shown to be particularly raised in the serum of patients with clinically active RA.<sup>14</sup> We also found the highest serum concentration of p75 sTNF-R in patients with RA with follicular synovitis, who are considered to have a more severe form of disease, in comparison with patients with diffuse synovitis. In our study we demonstrated the association of serum levels of p75 sTNF-R with such disease activity variables as ESR, CRP, number of swollen joints, Ritchie index, and DAS. Others, also, have correlated p75 sTNF-R with ESR and CRP levels in patients with RA.<sup>14</sup> However, some investigators failed to find such a correlation.<sup>15</sup>

In our study we demonstrated significantly raised concentrations of TNF $\alpha$  and its soluble receptors in RA serum in comparison with patients with OA. These molecules dominated in patients with the follicular form of synovitis and their levels correlated with laboratory and clinical markers of disease activity. Our findings suggest greater activity of the disease in patients with RA with follicular synovitis than in those with diffuse synovitis. Therefore, serum concentrations of TNF $\alpha$  and its soluble receptor concentrations are associated with RA activity and related to histological manifestation of the disease. These data support the theory of RA heterogeneity and suggest the possibility of various responses to the disease treatment. Therefore, the heterogeneity of RA should be considered in the design of treatment.

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