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Serum soluble CD30 in early arthritis: a sign of inflammation but not a predictor of outcome

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ABSTRACT

Objective. *To evaluate serum soluble CD30 levels (sCD30) in an early arthritis series and assess their ability to predict the outcome in patients with rheumatoid arthritis (RA) and undifferentiated arthritis (UA) at one year follow-up.*

Methods. *Serum sCD30 levels were measured by ELISA from 92 adult patients with RA and UA at baseline and from 60 adult controls. The patients were followed up for one year in the Kuopio 2000 Arthritis Survey. Receiver operating characteristic (ROC) curves were constructed to determine cut off points of sCD30 in RA and UA that select the inflammatory disease from controls. Sensitivity, specificity and positive likelihood ratio, and their 95 % CIs were calculated for sCD30 levels in RA and UA.*

Results. *Median serum sCD30 levels were higher in RA 25.1 (IQ range 16.3-38.6) IU/ml ($p<0.001$) and in UA 23.4 (15.4-35.6) IU/ml ($p<0.001$) than in controls 15.1 (10.7-20.8) IU/ml. No differences were recorded between RA and UA ($p=0.840$). Serum sCD30 levels at baseline did not predict remission at one year follow-up.*

Conclusion. *Serum sCD30 levels were higher in RA and UA than in controls at baseline but they did not predict remission at one year follow-up in this series.*

Introduction

Synovial membrane of rheumatoid arthritis (RA) patients is highly infiltrated with inflammatory cells, especially T cells, which are also found in synovial fluid (1-2). Chronic inflammatory Th1-type responses of T cells are thought to be important in the pathogenesis of RA (3).

There has been great interest in the role of CD30 in the modulatory mechanisms of inflammation in RA (1-2, 4). CD30 molecule belongs to the tumour necrosis/nerve growth factor receptor superfamily and its surface expression on lymphocytes in the peripheral blood has been demonstrated to be low (1). Soluble CD30 (sCD30) molecule released upon T-cell activation by proteolytic cleavage is regarded as a measure of CD30 turnover and as a sign of the regulatory activity of these T cells (1-2, 4).

High levels of sCD30 have been found in peripheral blood and synovial fluid of patients with RA (1, 2). Furthermore, RA patients with active disease have also been shown to have significantly higher sCD30 levels than those with inactive disease (1). This is interesting, because CD30 positive T cells have been shown to secrete predominantly Th2 type cytokines (1). Moreover, it has been proposed in a small series of RA patients that high sCD30 levels in an early disease might predict a good response to disease modifying anti-rheumatic drugs (DMARDs) (4). In another study, CD30 positive T cells have been proposed to take part in the control of the inflammatory response in the joints of RA patients, as CD30 expression was found to be increased on a subset of activated synovial fluid T cells which produced anti-inflammatory Th2 type cytokines (1).

As new serological tests are sought for assessing the need of therapy and the predictor of prognosis for patients with early arthritides, sCD30 measurement seemed to be interesting and worthwhile for testing in further studies. The purpose of the present study was to evaluate serum sCD30 levels in an early arthritis series and their ability to correlate with the activity of the disease and to predict the outcome among Finnish patients with RA and undifferentiated arthritis (UA) at one year follow-up.

Patients and methods

Patients

Serum sCD30 levels were measured among 92 patients with RA or UA and 60 adult controls. The patients were followed up for one year in the Kuopio 2000 Arthritis Survey. The details of the survey have been described elsewhere (5, 6). The study was population based and patients with previously undiagnosed synovitis in at least one peripheral joint, or signs of inflammation in sacroiliac, glenohumeral or hip joints on the first visit in year 2000 were collected and followed up for a mean 13 months. Patients were

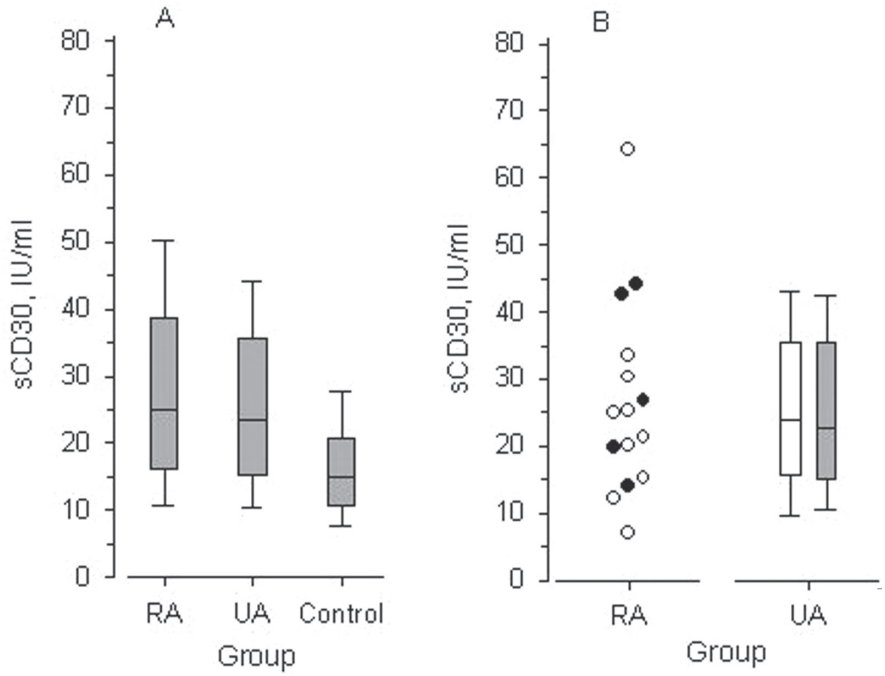

Fig. 1. A: Serum sCD30 levels at baseline in patients with rheumatoid arthritis (n=16), undifferentiated arthritis (n=76) and controls (n=60). **B:** Prediction of remission at one year follow-up among patient with rheumatoid arthritis and undifferentiated arthritis. Open circles show active disease (n=11) and black circles remission (n=5) in RA. Boxes show median levels of soluble sCD30 with interquartile ranges and Whiskers 10 and 90 % confidence intervals. White areas show active disease (n=33) and grey areas remission (n=43) in UA.

Table I. Demographic and clinical characteristics of 92 patients with early arthritis at baseline and at one year follow-up.

Characteristic	RA (n=16)	UA (n=76)	*p*-value
Sex (female) (%)	9 (56)	64 (84)	0.010
Mean age, years (SD)	58 (12)	49 (16)	0.036
Median delay from symptom onset to diagnosis (months, IQR)	6 (4, 7)	5 (2, 9)	0.238
Rheumatoid factor present (%)	11 (69)	11 (15)	<0.001
Anti-CCP antibodies (%)	11 (69)	9 (10)	<0.001
ESR at diagnosis (SD)	29 (24)	19 (21)	0.074
ESR at follow-up (SD)	8 (7)	7 (6)	0.348
Inflamed joint count at baseline (SD)	8 (3)	3 (3)	<0.001
Inflamed joint count at follow-up (SD)	2 (2)	1 (2)	0.081
Remission at follow-up (%)	5 (31)	43 (57)	0.066

RA: rheumatoid arthritis; UA: undifferentiated arthritis.

actively treated. At follow-up in RA, 81% of patients had a combination treatment with two or more DMARDs and 13% had a single therapy. One patient had no treatment. In UA, 49% of patients were on single DMARDs, mainly on hydroxychloroquine or sulphasalazine, 20% on a combination, one patient was on prednisolone alone, and 30% of patients had no treatment. Remission was defined by applying cross-sectionally five ACR remission criteria, excluding fatigue, on the follow-up visit (7).

Controls

Sixty subjects, stuff of the Department of Clinical Chemistry in the Kuopio University Hospital, were asked to give 10 ml blood to be controls in this study. The mean age of controls, 39 women and 21 men, was 34±12 years.

Methods

Serum sCD30 was analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) (Bender MedSystems GmbH, Vienna, Austria) according to the manufacturer's instructions. The detection limit was estimated to be 1.9 U/ml. Intra-assay precisions of serum sCD30 concentrations of 16.9 IU/ml (n=11), 27.5 IU/ml (n=9), and 44.7 IU/ml (n=5) were 20.2%, 5.9% and 6.8%, respectively. Inter-assay precision of serum sCD30 concentration of 34.9 IU/ml (n=13) was 13.5%.

Statistical analysis

The descriptive values were expressed as mean or median, standard deviation (SD) or interquartile range (IQR) and 95 per cent confidence intervals (95% CI). Comparisons of continuous data were carried out by using Student *t*-test or Mann Whitney test, when appropriate. Serum sCD30 levels were age and gender adjusted. Receiver operating characteristic (ROC) curves were constructed to determine cut off points of serum sCD30 in RA and UA that select the inflammatory disease from controls, with bias corrected accelerated bootstrap CIs (8). Sensitivity, specificity and positive likelihood ratio, and their 95% CIs were calculated for sCD30 levels in RA and UA.

Ethics

The study was approved by the Ethics Committee of the Kuopio University Hospital. All patients gave a written consent.

Results

Median serum sCD30 levels were higher in RA 25.1 (IQ range 16.3-38.6) IU/ml ($p<0.001$) and in UA 23.4 (15.4-35.6) IU/ml ($p<0.001$) than in controls 15.1 (10.7-20.8) IU/ml as is shown in Figure 1A. No differences were recorded in serum sCD30 levels between RA and UA (p=0.840). Demographic and clinical characteristics of the patients with RA and UA are shown in Table I. During follow-up the levels of erythrocyte sedimentation rate (ESR) and inflamed joint count decreased significantly in both RA and UA, for RA $p<0.004$ and <0.001 and for UA

Table II. Area under curve (AUC) of serum sCD30 levels with sensitivity, specificity and likelihood ratio among patients with rheumatoid arthritis (RA) and undifferentiated arthritis (UA).

Diagnosis	AUC (95% CI) †	Cut point	Sensitivity (95% CI)	Specificity (95% CI)	LR (95% CI)
RA	0.75 (0.59 to 0.89)	≥25	0.56 (0.30 to 0.80)	0.88 (0.77 to 0.95)	4.82 (2.12 to 10.69)
UA	0.73 (0.65 to 0.82)	≥23	0.57 (0.45 to 0.68)	0.82 (0.70 to 0.90)	3.09 (1.81 to 5.52)

†95% confidence interval obtained by bias corrected bootstrapping (5000 replications).

$p<0.001$ and <0.001, respectively. Serum sCD30 levels selected inflammatory arthritides from controls, the detailed characters are shown in Table II. Serum sCD30 levels at baseline did not predict remission at one year follow-up, $p=0.75$ in RA and $p=0.88$ in UA as is shown in Figure 1B.

Synovial fluid samples were available for three patients with RA and nine patients with UA. In this small group the median serum sCD30 levels were in RA and in UA 15.2 IU/ml (IQ range 6.9-40.3) and 19.6 IU/ml (IQ range 10.7-38.6), respectively. The median synovial fluid sCD30 level in RA was 70.8 IU/ml (IQ range 41.7 -93.1) and in UA 16.6 IU/ml (IQ range 13.0-25.3). All three patients with RA and 3/9 patients with UA had active disease at follow-up.

In controls in the groups under 30 years of age (n=29) and 30 years or over (n=31) the median serum sCD30 level were 20.8 (IQ range 13.9-25.9) IU/ml and 12.5 (IQ range 8.1-16.3) IU/ml, respectively, $p<0.001$. All RA patients were over 30 years of age at diagnosis. In UA, 8/76 patients were under 30 years of age but there were no significant differences in the serum sCD30 levels between the age groups, $p=0.290$.

Discussion

In this study serum sCD30 levels were higher in RA and UA patients than in controls at baseline, which accords with the results of earlier studies (1, 2, 4, 9). We could confirm that elevated sCD30 levels were found to be an inflammation associated phenomenon, but we did not find highly elevated serum sCD30 values in our patients and neither could we find an association of baseline sCD30 levels with treatment response.

The values of sCD30 have repeatedly been higher in synovial fluid than in serum (1, 2). Synovial CD30 positive T cells have been shown to play a role in control of inflammatory response (1, 2), and serum sCD30 levels have been suggested to reflect such cell activity. In our series, three patients with RA showed higher sCD30 levels in synovial fluid than in serum, whereas among nine patients with UA the serum and synovial fluid sCD30 levels were more equal and in three patients the levels were even lower in synovial fluid than in serum. Although the sCD30 levels were high in synovial fluid at baseline in RA patients and the patients were treated with DMARDs, they had an active disease at follow-up reflecting the chronic course of the disease. The patients with UA had a milder disease.

Recently, high serum sCD30 levels were proposed to represent activated regulatory CD30 positive T cells which are attempting to down-modulate inflammation in the inflamed joint in RA and, therefore, to be favourable to the prognosis of the disease (1). Furthermore, responders to DMARDs had higher sCD30 basal levels than non-responders in a series of 14 RA patients reported by Gerli *et al*. (4). This is at variance with our results as the increased values of sCD30 in our RA patients at baseline had no association with response to DMARDs or remission at one year follow-up.

The number of patients in the study of Gerli *et al*. (4) was too small to draw definite conclusions, but, however, this discrepancy might be explained due to the difference in technique. Some other studies using the same sCD30 ELISA method as by Gerli *et al*. have shown similar high values of sCD30 in RA patients (1, 2). The values were also higher compared with those of our RA patients. Strikingly, using another kind of radioimmunoassay (RIA) method RA patients were also found to have increased sCD30 values compared with healthy controls (9), but the levels of RA patients and healthy controls in that study were even lower compared with ours. Regardless of the techniques used increased serum sCD30 values have definitely been associated with RA.

Supposing that high sCD30 values predict good response to DMARDs and, furthermore, that our results reflect true modulatory response, the increased but not high values of our patients would predict poor response to DMARDs. Of our patients, only 25% in RA and 58% in UA were in remission, although the levels of the ESR and the joint count decreased significantly. Among RA patients those who have high sCD30 values may be capable of producing Th2 type cytokines down regulating unspecifically chronic proinflammatory Th1 response and specifically benefit from DMARDs.

Ageing is accompanied by decreased immune system responses and cell mediated immunity (10).

The levels of sCD30 have also been found to be age dependant (11-13). The serum levels of sCD30 have been highest in young children and the levels have decreased with ageing correlating with the maturation of immune system towards Th1 pattern (11). In the control group of our study the sCD30 levels were higher in younger persons but in UA group we could not record such a difference which, however, may be due to a small number of cases of young UA patients.

In this study of early arthritides, the levels of serum sCD30 were increased but were not very high in RA and UA patients at baseline compared with controls, nor did they differ between RA and UA. We could not confirm the prognostic value of increased sCD30 values in assessing the outcome of inflammatory arthritides at one-year follow-up.

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