

Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity

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Abstract

In rheumatoid synovium interleukin 6 (IL-6) is the most abundantly expressed cytokine. Increased serum levels have been previously reported in patients with rheumatoid arthritis (RA). In this study serum IL-6 levels were measured in a well defined cohort using a bioassay (B9 cells) and levels were correlated with conventional clinical and laboratory indices of disease activity.

Levels were significantly higher in serum from patients with RA (median 55 IU/ml; interquartile range 28-139) compared with serum from disease (median 7 IU/ml; 1-23) and normal controls (median 10 IU/ml; 7-12). No difference was observed between men and women. Levels did not correlate with disease duration. Significant associations were observed between IL-6 and C reactive protein and between the Ritchie articular index and duration of morning stiffness. No other correlations were observed. The value of these findings in the monitoring of RA and as an indicator of response to second line treatment needs to be established.

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Interleukin 6 (IL-6) is the most abundantly expressed cytokine in rheumatoid synovium.¹ The *in vitro* activities of this pleiotropic cytokine have been catalogued and several activities previously ascribed to interleukin 1 (IL-1) are now believed to be mediated also by IL-6. In particular IL-6 mediates acute phase protein synthesis and terminal B cell differentiation.²

Previous studies have shown that IL-6 levels are higher within synovial fluid than in serum.^{3,4} In most patients synovial fluid macrophages do not spontaneously produce IL-6, suggesting that IL-6 is derived from cells within the synovium.⁵ Using cytokine probes Firestein *et al* have shown that synoviocyte IL-6 is derived from non-T lymphocytes, type B synovial lining cells and fibroblasts.¹ Synoviocyte derived IL-6 in patients with rheumatoid arthritis (RA) is enhanced by IL-1 and tumour necrosis factor⁵ and can stimulate hepatocyte synthesis of acute phase proteins *in vitro*.

In this study we measured serum IL-6 levels in a well defined cohort of patients with RA

and estimated the *in vivo* importance of this cytokine in mediating some of the clinical and laboratory changes observed in active RA.

Patients and methods

PATIENTS

The patients studied consisted of 93 patients with RA seen at one clinic and referred for second line drug treatment. Patients selected for this study did not have coexisting diseases and were not receiving drugs other than non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics. To avoid any diurnal variations in disease activity all patients were seen in the morning.

CONTROLS

Normal controls matched for age and sex were selected from a general population survey determining risk factors for coronary artery disease. Disease controls included patients with osteoarthritis receiving analgesics and NSAIDs.

CLINICAL ASSESSMENTS

All clinical assessments were made by an independent nurse familiar with clinical trial methodology and included the Ritchie articular index, duration of morning stiffness in minutes, and pain score rated on a 10 cm visual analogue scale.

LABORATORY ASSESSMENTS

These included erythrocyte sedimentation rate (Westergren), full blood count, platelets (Coulter S) and serum C reactive protein (CRP) measured by laser nephelometry. Serum samples for subsequent determinations of IL-6 were stored at -20°C.

INTERLEUKIN 6 ASSAY

An IL-6 dependent cell line was used (B9). The cells were maintained as previously described with one modification: hybridoma growth factor (Cambio, Cambridge, United Kingdom) was used instead of stimulated human mononuclear cell supernatant.⁶ The assay was carried out in 96 well flat bottomed microtitre plates. A total of 5000 B9 cells/well were cultured in RPMI 1640 containing antibiotics, 5% heat inactivated fetal calf

serum, 5×10^{-5} M mercaptoethanol, and serial twofold dilutions of heat inactivated test serum. Proliferation was assessed using a spectrophotometric method after a 72 hour incubation in a 5% CO₂/humidified atmosphere. Each dilution was tested in triplicate. Test sample IL-6 values (IU/ml) were calculated from a standard curve obtained using a reference preparation known to contain

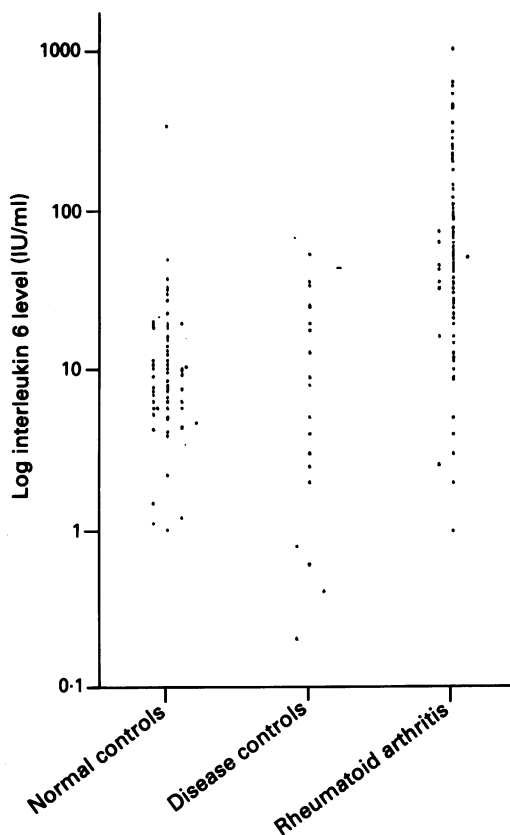
400 IU/ml of IL-6 (Cambio). Relative to an international IL-6 standard (National Institute for Biological Standards, United Kingdom, IL-6 standard Ref. No 88/514) our reference preparation had 91% of the IL-6 activity quoted by the manufacturer.

To confirm that the assay was measuring IL-6, neutralisation studies of random test serum samples were undertaken using a goat polyclonal antibody to IL-6 (British Biotechnology, Oxford, United Kingdom). The antibody (10 mg/ml) was serially diluted in RPMI 1640 in microtitre plates. To each well 50 µl of heat inactivated serum was added to a final volume of 100 µl. Plates were incubated for one hour at 37°C. The IL-6 activity was then measured in a 50 µl aliquot of neutralised serum as described.

Table 1 Clinical and laboratory indices of disease activity

| Disease activity parameter | Value (median (interquartile range)) |
|--------------------------------------|---|
| Haemoglobin (g/l) | 121(109-129) |
| White cell count ($\times 10^9/l$) | 7.9(6.6-9.2) |
| Platelets ($\times 10^9/l$) | 392(336-489) |
| ESR* (mm/hour) | 48(30-70) |
| CRP* (mg/l) | 34(16-67) |
| Ritchie articular index | 12(9-19) |
| Morning stiffness (minutes) | 90(30-180) |
| Pain (cm) | 5(2-7) |

*ESR=erythrocyte sedimentation rate; CRP=C reactive protein.



Interleukin 6 levels were measured in 70 normal controls, 20 patients with osteoarthritis as disease controls, and 93 patients with RA. There was no difference in levels between normal and disease controls. Levels were higher in patients with RA than in normal and disease controls.

STATISTICAL METHODS

The median and interquartile ranges are reported. Spearman rank correlation coefficients were calculated for all variables measured. The Mann-Whitney U test was used to estimate the differences between groups.

Results

The median age of the 93 patients studied was 56 years (interquartile range 47-67) with a median disease duration of eight years (range 3-15). Of the patient group 71 were women. All patients had active synovitis and table 1 gives parameters of disease activity.

INTERLEUKIN 6 LEVELS

In our laboratory the lower limit of IL-6 activity detected by B9 cells was 0.15 IU/ml (2 pg/ml). The intra-assay and interassay precision values of the assay were 14 and 18% respectively. The IL-6 activity could be completely neutralised in samples from patients with RA using an IL-6 antibody.

Serum IL-6 values were significantly higher in patients with RA (median 55 IU/ml; 28-139) than in disease (20 subjects, median 7 IU/ml; 1-23) ($p < 0.0001$) and normal controls (70 subjects, median 10 IU/ml; 7-12) ($p < 0.0001$) (figure). There was no difference in IL-6 values between men (median 71 IU/ml; 33-219) and women (median 52 IU/ml; 28-111) with RA ($p = 0.3$).

There was no correlation between IL-6 and age in patients with RA ($r = -0.1$; $p = 0.2$) or normal controls (data not shown) or in disease

Table 2 Spearman rank correlations between laboratory and clinical variables measured. Numbers given are r(p) values. Serum IL-6 values showed some association with serum C reactive protein levels, Ritchie articular index, and pain

| | IL-6 | Hgb | WBC | PLT | ESR | CRP | RAI | AMS | Pain |
|------|------|------------|-----------|-------------|-------------|-------------|------------|------------|------------|
| IL-6 | | -0.06(0.6) | 0.05(0.6) | 0.1(0.3) | 0.2(0.1) | 0.2(0.05) | 0.3(0.01) | 0.2(0.03) | 0.0(0.1) |
| Hgb | | | 0.3(0.01) | -0.4(0.001) | -0.6(0.001) | -0.5(0.001) | -0.05(0.6) | -0.01(0.9) | -0.04(0.7) |
| WBC | | | | 0.4(0.001) | -0.00(0.9) | 0.2(0.09) | -0.1(0.2) | 0.1(0.2) | -0.00(0.9) |
| PLT | | | | | 0.5(0.001) | 0.6(0.001) | 0.03(0.7) | 0.2(0.06) | 0.1(0.3) |
| ESR | | | | | | 0.7(0.001) | -0.01(0.9) | 0.04(0.6) | 0.01(0.9) |
| CRP | | | | | | | -0.06(0.6) | 0.2(0.1) | -0.08(0.4) |
| RAI | | | | | | | | 0.4(0.003) | 0.1(0.3) |
| AMS | | | | | | | | | 0.1(0.3) |

IL-6=interleukin 6; Hgb=haemoglobin; WBC=white blood cell count; PLT=platelet count; ESR=erythrocyte sedimentation rate; CRP=C reactive protein; RAI=Ritchie articular index; AMS=duration of morning stiffness.

duration ($r=-0.08$; $p=0.4$). Table 2 gives correlations between IL-6 and disease activity parameters.

Discussion

The observations in this study extend results of previous studies showing increased levels of IL-6 in serum and synovial fluid from patients with RA.^{3 4 7 8} We confirm that serum IL-6 values are increased in RA, are independent of age, duration of RA, and patient gender. Of the laboratory indices measured, only IL-6 showed an association with the Ritchie articular index. Serum IL-6 values account for only 9% of the variation in the Ritchie articular index, however. A better association might have been obtained if an articular index taking into account joint surface area⁹ or a more sensitive indicator of joint disease such as radionuclide joint scanning had been used.¹⁰ No associations were observed with duration of morning stiffness and pain score. We and others have shown that IL-6 levels can be modulated by sodium aurothiomalate.^{11 12}

Two additional observations of interest were noted. Firstly, IL-6 levels were weakly associated with CRP concentrations and, secondly, there was no correlation with platelet count. In vitro studies show that IL-6 is the principal cytokine driving hepatocyte acute phase protein synthesis.² We found that in vivo IL-6 accounts for only 4% of CRP values, a finding similar to that observed by Holte *et al.*⁷ A similar poor association was apparent between IL-6 and fibrinogen, another acute phase reactant, in our control population. Houssiau *et al* have, however, reported a better association between IL-6 and CRP.³ The reasons for the difference in findings may be due to differences in the assays used, a smaller number of patients studied or a selection bias.

Our findings suggest that in vivo acute phase protein synthesis may be due to several cytokines acting sequentially or together to modulate acute phase protein synthesis. In support of this Gatner *et al* have reported that in vitro IL-1 and IL-6 have a synergistic effect on hepatocyte CRP expression¹³ and transforming growth factor β inhibits the expression of some acute phase reactants induced by IL-6.¹⁴

The poor correlation between IL-6 and platelet count was also surprising as this cytokine shows some homology with thrombopoietin, has the capacity to induce megakaryocyte maturation in vitro synergistically with interleukin-3, and increases the platelet count in experimental animals

(reviewed by Hirano¹²). A similar poor association between IL-6 and secondary thrombocytosis has been reported elsewhere.¹⁵ Therefore in patients with RA thrombopoietic factors other than IL-6 appear to be more important.

In conclusion, the results of this study show that of the laboratory parameters measured only IL-6 showed some association with the Ritchie articular index. The value of this observation in monitoring patients with RA and selecting those suitable for second line drug treatment needs to be determined further in a routine clinical setting and in trials of disease modifying drugs. Some of our observations were contrary to those that would be predicted from the in vitro effects of this cytokine, confirming the complexity of the cytokine network in vivo.

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