Annals of the Rheumatic Diseases 1995; **54:** 674–677

Clinical significance of interleukin-6 measurement in early rheumatoid arthritis: relation with laboratory and clinical variables and radiological progression in a three year prospective study

Miek A van Leeuwen, Johanna Westra, Pieter C Limburg, Piet L C M van Riel, Martin H van Rijswijk

Abstract

***Objective*—To evaluate the clinical significance of interleukin-6 (IL-6) measurements in relation to laboratory and clinical measures of disease activity and radiological progression in early rheumatoid arthritis (RA).**

***Methods*—A prospective study was performed in 51 patients with early RA during the first three years of the disease, with monthly clinical and laboratory assessments and biannual radiographs of the hands and feet. IL-6 was measured by enzyme linked immunosorbent assay (ELISA). Cross sectional (n = 51) and longitudinal (n = 20) correlations between plasma IL-6 concentrations and values of C reactive protein (CRP), serum amyloid A protein (SAA), erythrocyte sedimentation rate (ESR), haemoglobin (Hb), platelets, and joint scores were calculated, and correlations made between time integrated values of IL-6, CRP and ESR, and radiological progression over three years (n = 20).**

***Results*—Significant correlations were found between IL-6 and the acute phase response and platelets, but variable results were obtained for the correlation between IL-6 and Hb. In contrast to a significant correlation between time integrated values of CRP or ESR and radiological progression, time integrated values of IL-6 did not correlate with radiological progression over three years follow up.**

***Conclusion*—The course of disease activity and the radiological progression of joint damage are better reflected by CRP, SAA, and ESR values than by plasma IL-6 concentrations, particularly in stages of low disease activity.**

(*Ann Rheum Dis* 1995; **54:** 674–677)

Rheumatoid arthritis (RA) is a systemic disease characterised by chronic inflammation of synovial joints. Chronic synovitis often leads to destruction of joint cartilage and bone, and is systemically accompanied by a marked acute phase response. Cytokines play a central role in both the local events and the systemic acute phase response. Interleukin-1 (IL-1) and tumour necrosis factor α (TNFα) are considered to be the major cytokines mediating inflammatory joint destruction and have been shown to be potent inducers of IL-6 synthesis.[1 2] Though it is often present in high concentrations in the synovial fluid, IL-6 has little influence on cartilage and bone destruction, but appears to act rather at a systemic level, where its major effect is the induction of the hepatic synthesis of acute phase proteins.[3] In addition, IL-6 has been shown to play a regulatory role in platelet production in vivo,[4] and evidence is increasing that IL-6 is one of the cytokines involved in the aetiology of anaemia of chronic disease.[5] In RA, measurement of the acute phase response by erythrocyte sedimentation rate (ESR) or C reactive protein (CRP) concentration is generally used to monitor disease activity, and the course of changes in CRP concentrations over time have been shown to be related to the progression of radiological joint damage in RA.[6] As the inflamed synovium is thought to be the main source of plasma IL-6 in RA, theoretically, IL-6 concentrations could yield a more direct reflection of joint inflammation and destruction compared with acute phase reactants.

The aim of the present study was to evaluate the clinical significance of plasma IL-6 measurements in relation to clinical and laboratory measures of disease activity and to radiological progression during the first years of RA. In addition, we analysed the relation between IL-6 and haemoglobin and platelet counts in early RA.

Patients and methods

PATIENTS

We analysed the data of 51 patients, selected from a cohort of 149 patients with RA, who had completed a three year follow up study: 31 had active disease (clinical indication for therapeutic joint aspiration) at the time of investigation and 20 were selected randomly from those patients (132/149) who developed radiological joint damage within the first three years of the disease. All patients met the 1958 ARA criteria for classic or definite RA and had joint symptoms for less than one year at entry to the study.[6] Monthly blood samples were available in addition to monthly clinical data, comprising the number of tender and swollen

Department of Rheumatology, University Hospital Groningen, The Netherlands
M A van Leeuwen
J Westra
P C Limburg
M H van Rijswijk

Department of Rheumatology, University Hospital Nijmegen, The Netherlands
P L C M van Riel

Correspondence to:
Miek A van Leeuwen, Department of Rheumatology, University Hospital, PO Box 30.001, 9700 RB Groningen, The Netherlands.

Accepted for publication 11 April 1995

joints (52 joint score) and Ritchie articular index (RAI). Posteroanterior radiographs of the hands and feet were made every six months and joint damage was assessed according to Sharp *et al*[7] with some modifications. The maximum total score was 448.

Patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying antirheumatic drugs (DMARDs), low dose corticosteroids, or combinations of these, as clinically indicated.

ASSAYS

CRP and serum amyloid A protein (SAA) were measured by enzyme linked immunosorbent assay (ELISA)[8 9] in EDTA plasma, haemoglobin and platelet counts were measured using standard procedures, and ESR was measured according to Westergren.

Measurements of IL-6 were performed in EDTA plasma by ELISA,[10] using a combination of the anti-IL-6 monoclonal antibody (MAb) IL-6-16 and biotinylated polyclonal sheep antibody Sh-a-IL-6 (both kindly provided by Dr L Aarden, Amsterdam). Measurements were performed in serial dilutions (starting at 1:3) in the presence of an excess of normal sheep serum. As standard, we used recombinant human IL-6 (rhIL-6) from Genzyme (Boston, MA), with a range of detection of 3–100 pg/ml. Control experiments revealed that rhIL-6, spiked to normal serum at different concentrations, was recovered in this ELISA to an extent of 95–115%. Both intra-assay and interassay variability were <10%.

STATISTICS

Correlation coefficients were calculated with the Spearman rank test. Mean correlations were assessed after Z transformation.

Results

PATIENT CHARACTERISTICS AND TREATMENT

The 51 patients studied included 38 women and 13 men; at entry to the study they were aged 16–72 years (median 52), and 50 were IgM rheumatoid factor positive (>10 IU/ml). No significant differences with regard to demographic and initial clinical and laboratory data were apparent between the 20 patients selected for the longitudinal studies and the remaining patients from the cohort who developed radiological damage (data not shown). The initial DMARD was hydroxychloroquine (n = 35), intramuscular gold (n = 10), or D-penicillamine (n = 2). Four patients were treated with NSAIDs only.

Table 1 Plasma concentrations (concn) of IL-6 and acute phase reactants, haemoglobin and platelets, and their interrelationships (Spearman correlations)

	Plasma concn	*Correlation with IL-6*	*Correlation with CRP*
IL-6 (ng/l)	51 (5–662)	—	0·564***
CRP (mg/l)	34 (0·8–340)	0·564***	—
SAA (mg/l)	36 (2–1000)	0·605***	0·891***
ESR (mm/1st h)	50 (5–135)	0·744***	0·654***
Hb (g/l)	124 (81–152)	–0·311*	–0·417**
Thrombocytes ($\times 10^9$/l)	307 (145–624)	0·565***	0·577***

Plasma concns are median (range) of n = 51. IL-6 = Interleukin-6; CRP = C reactive protein; SAA = serum amyloid A; ESR = erythrocyte sedimentation rate; Hb = haemoglobin.
*p < 0·05; **p < 0·01; ***p < 0·001.

RELATION BETWEEN PLASMA IL-6 AND THE OTHER SYSTEMIC VARIABLES

For the *cross sectional study* (n = 51), the results of the samples taken at the time of joint aspiration (n = 31) and the samples at entry in the follow up study (n = 20) were used. Table 1 shows the values of the respective variables and the correlations between plasma IL-6 or CRP and these variables.

In the *longitudinal study* we evaluated the interrelationship between the different parameters during the first three years of the disease in 20 patients. The within individual correlations between plasma IL-6 concentrations and values of the concurrent laboratory and clinical variables (36 observations per patient) were calculated. Table 2 shows the means of the within individual correlation coefficients and 95% confidence intervals (95% CI), assessed after Z transformation of the individual correlation coefficients. The 95% CI was significant ($p \leq 0{\cdot}05$) for all mean correlations.

Most patients with a significant correlation ($p < 0{\cdot}05$) between IL-6 and CRP also showed a significant correlation between IL-6 and the other laboratory and the clinical variables. In the few patients without a significant correlation between IL-6 and CRP, drug treatment did not differ from that received by the other patients. There was a significant within individual correlation between CRP and SAA values in all patients ($p < 0{\cdot}001$), and no differences appeared to exist between CRP and SAA in relation to IL-6.

RELATION BETWEEN IL-6 AND RADIOLOGICAL PROGRESSION

In our patients the radiological score at entry to the study ranged from 0 to 37 (median 3), and the radiological progression score during the three year follow up was from 0 to 203 (median 50). As radiological progression is essentially a cumulative phenomenon, the results of the monthly values of the different process variables were transformed into time integrated values (area under the curve) according to the trapezoidal rule. Table 3

Table 2 Within individual correlations between plasma IL-6 and laboratory and clinical variables during three years follow up (20 patients, 36 observations per patient)

	Mean	*95% CI*
CRP	0·595	0·544 to 0·641
SAA	0·606	0·566 to 0·651
ESR	0·575	0·553 to 0·623
Hb	–0·206	–0·133 to –0·277
Thrombocytes	0·496	0·437 to 0·551
No of swollen joints	0·471	0·410 to 0·528
No of tender joints	0·423	0·359 to 0·483
RAI	0·484	0·423 to 0·541

Values are mean and 95% CI of within individual correlations after Z transformation.
IL-6 = Interleukin-6; CRP = C reactive protein; SAA = serum amyloid A; ESR = erythrocyte sedimentation rate; Hb = haemoglobin; RAI = Ritchie articular index.

Table 3 Correlations between radiological progression and time integrated values of IL-6, CRP and ESR over 3 years follow up (n = 20)

	Correlation	p
IL-6	0·226	<0·40
CRP	0·477	<0·05
ESR	0·604	<0·01

IL-6 = Interleukin-6; CRP = C reactive protein; ESR = erythrocyte sedimentation rate.

shows the between individual correlations between radiological progression after three years and the time integrated values of IL-6, and the time integrated values of CRP and ESR during 36 months. A weak, non-significant correlation was found between time integrated IL-6 values and radiological progression, compared with a significant correlation between time integrated CRP and ESR values and radiological progression.

Discussion

In previous studies, we and others have demonstrated a highly significant correlation between the acute phase response, joint swelling, and radiological progression during the first years of RA.[6] In the present study we have analysed the clinical value of IL-6 measurements in the monitoring of disease activity and radiological progression in early RA, based on the supposed role of IL-6 as mediator between the inflammatory process in the joints and the systemic acute phase response.

In the cross sectional study in 51 patients we found highly significant correlations between IL-6 and both the acute phase proteins and platelets, and a significant but weaker correlation between IL-6 and Hb. In the longitudinal study in 20 patients during three years follow up, similar results were found with highly significant within individual correlations between IL-6 and the different variables in the majority of the patients, but in fewer patients for Hb. However, the correlation between time integrated IL-6 values and radiological progression during three years follow up was not significant.

The relations between plasma IL-6 and acute phase proteins, and haematological variables in RA reported in the literature have been contradictory.[11-13] Differences in sensitivity of the assays and in patient selection may, in part, explain these discrepancies. In addition, most of the studies were cross sectional. In our longitudinal study, the within individual correlations between plasma IL-6 and the other process variables varied between patients. The significant correlation between IL-6 and platelet counts in most patients is in agreement with the potent thrombopoietic activity of IL-6 in vitro,[4] and with clinical reports of reactive thrombocytosis in inflammatory diseases.[14 15] We found both patients with (n = 9) and patients without (n = 11) a significant correlation between IL-6 and Hb. Although a decrease in Hb values after administration of rhIL-6 has been described,[4] anaemia of chronic disease appears to be of multifactorial origin, IL-6 being one of these factors,[5] and the relative role of IL-6 compared with other factors may differ between patients.

IL-6 appeared to correlate very well to the acute phase proteins and clinical variables in most patients. A weak within individual correlation between IL-6 and these variables was found in only a few patients. This occurred predominantly in patients with low disease activity and low levels of IL-6 and acute phase proteins during a substantial period of three years follow up. It would appear that changes in the lower range of disease activity are more accurately reflected by CRP and SAA than by IL-6. It is conceivable that, at the hepatic level, the concentrations of IL-6 required for the synthesis of acute phase proteins are too small to be detectable in the circulation, or that the local hepatic concentrations of IL-6 bound to the receptor are greater than those of free IL-6 in the circulation. In patients with high levels of IL-6 and acute phase proteins, this appeared to be of less influence, as was supported by the results of the cross sectional study which comprised samples mainly obtained at stages of high disease activity. These arguments may also be valid for the discrepancy between the time integrated IL-6 values and the time integrated acute phase protein values in their relation to radiological progression, with weak and highly significant correlations, respectively.

We conclude that, based on the higher correlations between CRP and ESR and both clinical measurements and radiological progression, compared with the correlations with IL-6, there is as yet no case for measurement of IL-6 levels instead of acute phase proteins as indicators of disease activity and radiological progression in clinical practice.

This study was supported by a grant from 'Het Nationaal Reumafonds', The Netherlands.

1 Arend W P, Dayer J M. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum* 1990; **33:** 305–15.
2 Guerne P A, Zuraw B L, Vaughan J H, Carson D A, Lotz M. Synovium as a source of interleukin 6 in vitro. *J Clin Invest* 1989; **83:** 585–92.
3 Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci USA* 1987; **84:** 7251–5.
4 Van Gameren M M, Willemse P H B, Mulder N H, *et al.* Effects of recombinant human interleukin-6 in cancer patients; a phase I-II study. *Blood* 1994; **84:** 1434–41.
5 Means R T, Krantz S B. Progress in understanding the pathogenesis of the anemia of chronic disease. *Blood* 1992; **80:** 1639–47.
6 Van Leeuwen M A, van der Heijde D M F M, van Rijswijk M H, *et al.* Interrelationship of outcome measures and process variables in early rheumatoid arthritis. A comparison of radiological damage, physical disability, joint counts and acute phase reactants. *J Rheumatol* 1994; **21:** 425–9.
7 Sharp J T, Young D Y, Bluhm G B, *et al.* How many joints in the hands and wrists should be included in a score of radiologic abnormalities used to assess rheumatoid arthritis? *Arthritis Rheum* 1985; **28:** 1326–35.
8 Van Leeuwen M A, van Rijswijk M H, Marrink J, Westra J, de Jong H J. CRP measurements in rheumatic disorders. In: Peeters H, ed. *Protides of the biological fluids*, vol 34. Oxford: Pergamon Press, 1986; 315–8.
9 Hazenberg B P C, Limburg P C, Bijzet J, van Rijswijk M H. Monoclonal antibody based ELISA for human SAA. In: Natvig J B, Forre O, Husby G, *et al.*, eds. *Amyloid and amyloidosis 1990.* Dordrecht, Boston, London: Kluwer Academic Publishers, 1991, 898–901.

10 Van Leeuwen M A, Westra J, Limburg P C, van Riel P L C M, van Rijswijk M H. Interleukin-6 in relation to other proinflammatory cytokines, chemotactic activity and neutrophil activation in rheumatoid synovial fluid. *Ann Rheum Dis* 1995; **54:** 33–8.
11 Holt I, Cooper R G, Denton J, Meager A, Hopkins S J. Cytokine inter-relationships and their association with disease activity in arthritis. *Br J Rheumatol* 1992; **31:** 725–33.
12 Dasgupta B, Corkill M, Kirkham B, Gibson T, Panayi G. Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. *J Rheumatol* 1992; **19:** 22–5.
13 Madhok R, Crilly A, Watson J, Capell H A. Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity. *Ann Rheum Dis* 1993; **52:** 232–4.
14 Hollen C W, Henthorn J, Koziol J A, Burstein S A. Elevated serum interleukin-6 levels in patients with reactive thrombocytosis. *Br J Haematol* 1991; **79:** 286–90.
15 de Benedetti F, Massa M, Robbioni P, Ravelli A, Burgio G R, Martini A. Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. *Arthritis Rheum* 1991; **34:** 1158–63.