

Circadian rhythm of serum interleukin-6 in rheumatoid arthritis

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Abstract

Objectives—To test the hypothesis of a diurnal variation in circulating levels of interleukin-6 (IL-6) and/or tumour necrosis factor-alpha (TNF-alpha) in rheumatoid arthritis and other inflammatory connective tissue diseases.

Methods—Serum levels of IL-6 and TNF-alpha were measured at three hour intervals from 7:30 to 22:30 in 48 patients with different rheumatic diseases as well as ten healthy controls. In four of the patients with rheumatoid arthritis, serum IL-6 levels were measured before and after one week of treatment with prednisolone 15-20 mg daily.

Results—IL-6 and TNF-alpha could not be detected in serum from healthy controls. However, serum IL-6 levels were substantially increased in patients with rheumatoid arthritis. Furthermore, patients with rheumatoid arthritis showed a statistically significant circadian variation in levels of IL-6. Peak values appeared in the morning and low values in the afternoon and evening. In contrast, levels were low and stable in other connective tissue diseases. Levels of TNF-alpha were low in patients with rheumatoid arthritis and high in patients with other connective tissue diseases, but without circadian rhythm. After treatment with prednisolone, levels of serum IL-6 decreased significantly, but the circadian rhythm remained.

Conclusions—The circadian rhythm of circulating IL-6 might correspond to the circadian rhythm of symptoms in rheumatoid arthritis. The diurnal variation of IL-6, and possibly other cytokines, might explain the conflicting results previously reported on the inter-relationship between circulating IL-6 levels and disease activity in rheumatoid arthritis.

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The appearance of pro-inflammatory cytokines in joint tissue/synovial fluid and serum/plasma of patients with arthritic conditions, suggests that they play a role in the local and systemic inflammatory responses. However, in many clinical studies it has not been possible to relate known *in vitro* effects of pro-inflammatory cytokines to clinical and laboratory observations. IL-6 mediates the synthesis of acute phase proteins.¹⁻³ Substantially increased

concentrations of IL-6 have been reported in synovial fluid and serum/plasma from patients with rheumatoid arthritis.⁴⁻¹⁰ In some studies, a correlation was found between serum/synovial fluid IL-6 levels and parameters of disease activity in rheumatoid arthritis.^{3 7 11 12} This has been denied by others, who found poor or no association between the serum/plasma concentrations of this cytokine and acute phase proteins such as C-reactive protein.^{2 13 14} These conflicting results could be explained by diurnal variations in serum levels of certain cytokines. To test this hypothesis, we have measured serum IL-6 and TNF-alpha at intervals during the day in patients with rheumatic diseases. In this study we are able to report a prominent diurnal variation of IL-6 in patients with rheumatoid arthritis but not in patients with non-arthritic connective tissue diseases.

Patients and methods

PATIENTS

Thirteen patients, four male and nine female, who fulfilled the criteria of the American Rheumatism Association for classic rheumatoid arthritis¹⁵ and ten patients, one male and nine females, with inflammatory arthropathies other than rheumatoid arthritis (six with unspecified polyarthritis, two psoriatic arthropathies and two juvenile chronic arthritis) were also included in the study. Furthermore, 25 patients, six men and 19 women, fulfilling the international criteria for various connective tissue diseases (seven systemic lupus erythematosus, 13 systemic sclerosis, three primary Sjögren's syndrome and two mixed connective tissue disease) and 10 healthy controls (three men, 34-43 years and seven women, 35-52 years) were included in the study. Clinical data of the patients are shown in table 1. All patients were referred to the Section of Rheumatology, Uppsala University Hospital because of active disease and they were studied as inpatients after informed consent and according to the Declaration of Helsinki.

CLINICAL AND LABORATORY EVALUATION

Each patient was clinically evaluated according to Ritchie's index,¹⁶ by duration of morning stiffness and joint pain score at rest, using a 10 grade visual analogue scale.

At the start of the study, four patients were under treatment. One of the patients with rheumatoid arthritis received sulphasalazine

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Table 1 Clinical and laboratory data from 48 patients participating in the study. The laboratory values are presented as means, with range in brackets

Patients	Number of patients (male/female)	Mean age in years	Disease duration in years	Hb (g/l)	ESR (mm/h)	CRP (g/l)	Haptoglobin (g/l)	WBC (10^9)	Platelets (10^9)
RA	13 (4/9)	59 (25-80)	10 (1-28)	112 (93-126)	58 (30-105)	48 (0-88)	3.6 (2.5-5.2)	8.8 (7-12)	404 (280-357)
JCA	2 (0/2)	24 (21-27)	11 (7-15)	117 (114-119)	29 (26-31)	40 (27-52)	2.5 (2.2-2.8)	7.1 (6-9)	455 (211-412)
PsoAr	2 (0/2)	76 (70-83)	0.3 (0.2-0.5)	126 (122-130)	40 (32-48)	18 (0-36)	2.9 (-)	5.3 (-)	300 (233-368)
PolyAr	6 (1/5)	54 (21-78)	1.0 (0.2-1.3)	114 (100-129)	48 (20-80)	35 (0-132)	3.7 (1.3-5.3)	6.3 (4-9)	327 (148-497)
SSc	13 (6/7)	62 (50-74)	7.1 (2-25)	125 (97-146)	29 (5-110)	4.2 (0-23)	2.3 (0.6-4.2)	5.0 (3-7)	274 (159-475)
SLE	7 (-/7)	44 (19-76)	11 (2-20)	119 (101-140)	43 (7-110)	15 (0-86)	2.8 (0.8-6.0)	5.3 (3-7)	272 (172-370)
pSS	3 (-/3)	59 (54-64)	9 (6-15)	135 (122-147)	23 (13-35)	< 10	2.7 (1.6-4.0)	7.5 (6-9)	272 (176-342)
MCTD	2 (-/2)	30 (28-31)	10 (10-10)	109 (106-112)	51 (18-84)	< 10	2.0 (1.6-2.5)	2.3 (1.7-3)	215 (187-243)

Abbreviations: Hb = haemoglobin; WBC = white blood cells; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; RA = rheumatoid arthritis; JCA = juvenile chronic arthritis; PsoAr = psoriatic arthropathy; PolyAr = polyarthritis unspecified; SSc = systemic sclerosis; SLE = systemic lupus erythematosus; pSS = primary Sjögren's syndrome; MCTD = mixed connective tissue disease.

and one patient methotrexate. One patient with systemic lupus erythematosus was treated with chloroquine phosphate and one patient with mixed connective tissue disease received azathioprine. Of the remaining 44 patients, none received second line drugs. Most of the patients with rheumatoid arthritis and other arthritic diseases received NSAIDs in the morning and in the evening. None of the patients was treated with systemic or local corticosteroids.

The first blood sample was drawn at 7:30 after overnight fasting and while the patient was still in bed. Thereafter blood samples were collected at 10:30, 13:30, 16:30, 19:30 and 22:30. The serum samples were stored for one hour at room temperature, centrifuged at room temperature and aliquoted before being stored at -70°C for later analysis of IL-6 and TNF-alpha. Furthermore, the first morning sample at 7:30 was analysed for the following: haemoglobin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum haptoglobin, white blood cell and platelet count.

The effects of a potent anti-inflammatory treatment on the serum levels of IL-6 and TNF-alpha in patients with rheumatoid arthritis were also studied. Therefore a second set of blood samples was drawn from four patients' after one week of treatment with the glucocorticoid prednisolone (15-20 mg daily).

CYTOKINE ANALYSIS

The levels of free human IL-6 and TNF-alpha in serum were measured using commercial ELISA-type kits (MEDGENIX Diagnostics, Brussels, Belgium). The two tests were based on solid phase enzyme amplified sensitivity immunoassay technique (EASIA). They were performed in microtitre plates using several monoclonal antibodies directed against distinct epitopes on the two molecules. Purified recombinant human IL-6 and TNF-alpha were used as standards. The lowest standard point for the IL-6 assay was 20 pg/ml while the minimum detectable concentration was estimated to be 3 pg/ml. The corresponding figures for the TNF-alpha assay were 15 pg/ml and 3 pg/ml, respectively. The IL-6 EASIA was shown not to cross-react with IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, GM-CSF, IFN-alpha and gamma, TNF-alpha and beta, while the TNF-alpha assay was shown not to cross-react with TNF-beta, IL-1, IL-2, IFN-alpha, beta and gamma as specified by the manufacturer.

STATISTICAL ANALYSIS

Values are given as means, SEM (range). Non parametric tests, Wilcoxon signed rank tests and the Spearman rank correlation coefficient, were used to analyse the data between groups. One factorial ANOVA was used to analyse the significance in repeated measurements. A p value of 0.05 was considered significant.

Results

CLINICAL DATA

Clinical data are presented in table 1. All patients with rheumatoid arthritis had active disease measured by Ritchie's index [15.4, 2.2 (range: 7-28)] and with morning stiffness [1.5 hours, 0.5 hours (5-300 min)]. Pain at rest, as judged by visual analogue scale, was estimated by the patients with RA to be 2.8, 0.7 (0-6) and pain at movement to be 4.6, 0.9 cm (0-8), respectively.

INTERLEUKIN-6

Figure 1 presents serum levels of IL-6 measured from 7:30 to 22:30 in 13 patients with RA, ten patients with arthropathies other than rheumatoid arthritis and 25 patients with inflammatory connective tissue diseases. The mean serum concentrations of IL-6 in patients with RA decreased significantly during the day ($p < 0.001$) from 95.9 (27) pg/ml at 7:30 to 27 (8.2) pg/ml at 22:30. A non-significant reduction of IL-6 was also observed in the smaller patient groups with other arthritic

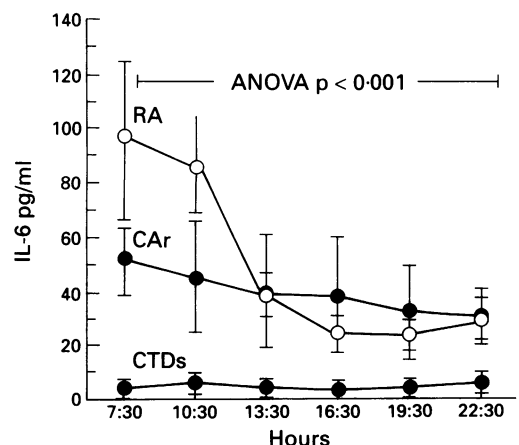


Figure 1 Diurnal variation in serum interleukin-6 (IL-6) in 13 patients with rheumatoid arthritis (RA), in ten patients with chronic non-RA arthritides (CAr) and in 25 patients with various connective tissue diseases (CTDs).

Table 2 The circadian variation of serum IL-6 in various inflammatory connective tissue diseases. The data are presented as mean (SEM)

	IL-6 (pg/ml)					
	Time: 07:30	10:30	13:30	16:30	19:30	22:30
Patients (n)						
RA (13)	96 (21)	85 (15)	37 (8)	24 (8)	23 (7)	27 (8)
Non-RA arthritides (10)	50 (13)	43 (19)	38 (17)	37 (20)	31 (14)	29 (13)
SSc (13)	<5	6 (3)	7 (3)	5 (3)	6 (4)	7 (3)
SLE (7)	<5	<5	<5	<5	<5	<5
pSS (2)	<5	12 (12)	<5	<5	<5	<5
MCTD (2)	15 (0)	11 (0)	5 (5)	7 (7)	8 (8)	16 (4)
Healthy controls (10)	<5	<5	<5	<5	<5	<5

Abbreviations: RA = rheumatoid arthritis; SSc = systemic sclerosis; SLE = systemic lupus erythematosus; pSS = primary Sjögren's syndrome; MCTD = mixed connective tissue disease.

conditions (fig 1 and table 2). Patients with non arthritic diseases had low IL-6 levels during the day without diurnal variation. In healthy controls the IL-6 levels were not detectable. Four patients with RA had a second evaluation after one week of treatment with the glucocorticoid prednisolone (15–20 mg daily). Their serum IL-6 levels decreased significantly at 7:30, 10:30 and 13:30 ($p < 0.01$ and 0.05). However, they still showed a similar pattern of diurnal rhythm, although this was not significant by ANOVA analysis (fig 2).

TUMOUR NECROSIS FACTOR-ALPHA

The serum levels of TNF-alpha did not show any significant differences during the day in any of the patient groups. In contrast to the IL-6 levels, the serum levels of TNF-alpha were lower in patients with rheumatoid arthritis compared with the other patient groups (fig 3 and table 3).

In patients with RA, significant correlations were found between the calculated total amount of serum IL-6 during the day (area under the curve) and the platelet count ($r = 0.6$; $p < 0.01$), and between the maximum IL-6 value in the morning and the platelet count ($r = 0.7$; $p < 0.01$). No correlation was found between morning serum levels of IL-6 or calculated IL-6 area under the curve and clinical indices of disease activity such as morning stiffness, joint pain score or Ritchie's index in patients with rheumatoid arthritis, nor was there any correlation with laboratory parameters of disease activity such as ESR, CRP or haptoglobin. However, when all arthritic patients were included in the calculations, the maximum IL-6 levels in the morning correlated to Ritchie's index ($r = 0.5$; $p < 0.05$) and CRP ($r = 0.4$; $p < 0.05$).

Table 3 The circadian variation of serum tumour necrosis factor alpha (TNF-alpha) in various inflammatory connective tissue diseases. The data are presented as means (SEM)

	TNF-alpha (pg/ml)					
	Time: 07:30	10:30	13:30	16:30	19:30	22:30
Patients (n)						
RA (13)	<5	<5	<5	<5	<5	<5
Non-RA arthritides (10)	7 (4)	8 (5)	7 (4)	6 (4)	6 (4)	6 (4)
SSc (13)	18 (4)	18 (5)	18 (5)	17 (4)	19 (5)	18 (4)
SLE (7)	29 (12)	30 (12)	28 (12)	24 (11)	29 (12)	17 (6)
pSS (2)	<5	<5	<5	<5	<5	<5
MCTD (2)	88 (50)	81 (37)	68 (67)	92 (48)	85 (45)	88 (47)
Healthy controls (10)	<5	<5	<5	<5	<5	<5

RA = rheumatoid arthritis; JCA = juvenile chronic arthritis; PsoAr = psoriasis associated arthritis; PolyAr = Polyarthritis UNS; SSc = systemic sclerosis (scleroderma); SLE = systemic lupus erythematosus; pSS = primary Sjögren's syndrome; MCTD = mixed connective tissue disease.

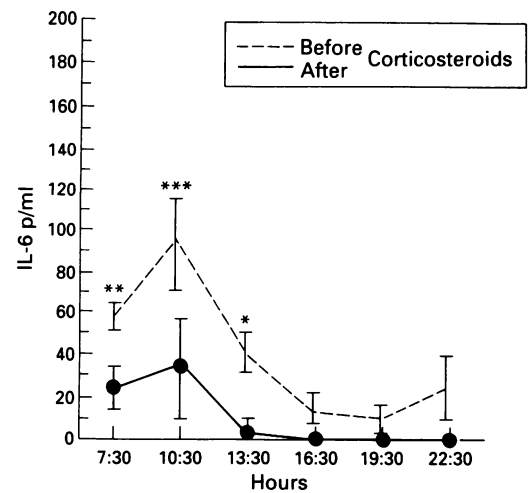


Figure 2 Diurnal variation in serum interleukin-6 (IL-6) in four patients with rheumatoid arthritis before and after one week of treatment with the glucocorticoid prednisolone (15–20 mg/d).

Discussion

Our findings demonstrate that the circulating levels of IL-6 are increased in the morning in patients with rheumatoid arthritis and decline significantly from early in the afternoon to late in the evening. On the contrary, the circulating levels of TNF-alpha were not increased and remained stable during the day. In other inflammatory connective tissue diseases like systemic lupus erythematosus, systemic sclerosis, primary Sjögren's syndrome and mixed connective tissue disease, the IL-6 levels were low while the TNF-alpha levels were increased but without diurnal variation.

The synovial fluid in rheumatoid arthritis contains substantially increased amounts of IL-6 and more modestly increased amounts of TNF-alpha.^{2 5 17} Using cytokine probes, it has been claimed that IL-6 in rheumatic synovial fluid originates from type B synovial lining cells and fibroblasts.^{1 18 19} It is uncertain to what extent the locally produced IL-6 contributes to the circulating levels of IL-6 in rheumatoid arthritis. However, it is reasonable to assume that a considerable amount of IL-6 is transported from the joint tissue to the general

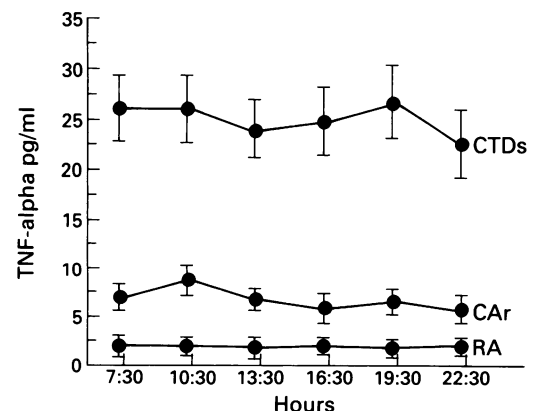


Figure 3 Diurnal variation of serum tumour necrosis factor alpha (TNF-alpha) in 13 patients with rheumatoid arthritis (RA), in ten patients with chronic non-RA arthritides (CAr) and in 25 patients with various connective tissue diseases (CTDs).

circulation by lymphatic transport and passive capillary leakage. During the morning, with increased muscular and joint activity, the drainage from the joint tissue should be enhanced²⁰ and could possibly induce a transient increase of the circulating concentrations of substances which have accumulated to a high degree in the inflamed joints.²¹

The circulating levels of IL-6 might also be influenced by the circadian secretion of cortisol. Glucocorticosteroids are endogenous inhibitors of IL-6 synthesis²² and IL-6 stimulates secretion of corticotropin by the pituitary gland.⁹ Although giving glucocorticoids suppressed production of IL-6, there was still a diurnal variation so that glucocorticoids alone cannot be the whole explanation. It has recently been proposed that impairment of the endogenous control of inflammation by the HPA axis could contribute to the development of chronic inflammation.²³ The high morning levels of circulating IL-6 in rheumatoid arthritis might agree with this hypothesis. These patients would thus need a stronger centrally acting inflammatory stimulus to release adequate amounts of endogenous cortisol. It has also been reported that certain neuropeptides, with high amplitude circadian rhythm, have prominent effects of IL-6 production.²⁴ Our arthritic patients were on NSAIDs during the study period. Available data on the effects of NSAIDs on cytokine synthesis makes it less likely that NSAIDs have had an effect on our observations.

The circadian rhythm of serum IL-6 might explain the confusing results previously obtained trying to correlate serum and synovial fluid IL-6 levels to signs of disease activity in rheumatoid arthritis.^{2 3 7 11-14} In an attempt to consider the circadian peak appearance of IL-6 in rheumatoid arthritis, we calculated the IL-6 area under the curve. We found that this estimation of IL-6 correlated to the increased platelet found in rheumatoid arthritis. IL-6 increases the platelet count in experimental animals.^{25 26} Maximum IL-6 levels in the morning were also related to Ritchie's index and CRP, respectively in arthritic patients.

The circadian rhythm of IL-6, and possibly other cytokines, in rheumatoid arthritis should be considered by those who regard cytokine measurements a possible tool for monitoring disease activity and selection of patients for second line therapy. Assuming that the morning IL-6 peak contributes to some clinical features of arthritic diseases, it would be worthwhile to try to minimise the IL-6 influence by adequate timing of the intake of drugs inhibiting IL-6.

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