

# Serum IL-1 $\beta$ levels are associated with the presence of erosions in recent onset rheumatoid arthritis

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## Abstract

### Objective

To study interleukin (IL)-1 $\beta$  levels in recent onset RA patients treated either with combination DMARD therapy (sulfasalazine, methotrexate, hydroxychloroquine) or a single DMARD therapy.

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### Methods

Serum IL-1 $\beta$  levels were measured before the treatment and 6 months after the institution of either single or combination DMARD therapy using a high sensitivity ELISA method. Radiographic evaluation of the hands and feet was performed at 0 and 24 months.

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### Results

Significant correlations ( $r = 0.28$ , 95% CI 0.10-0.45) were found between IL-1 $\beta$  levels measured at 0 and 6 months. The IL-1 $\beta$  levels at 0 months correlated significantly ( $r = 0.23$ , 95% CI 0.03-0.4,  $p = 0.021$ ) with the baseline number of eroded joints at 0 months but not with radiographic joint damage at 24 months. The baseline level of IL-1 $\beta$  was a better indicator for the presence of eroded joints than the baseline level of serum CRP. No significant changes in IL-1 $\beta$  levels were observed during the first 6 months of anti-rheumatic treatment in either group. No statistically significant difference between IL-1 $\beta$  levels in the patients with or without the shared epitope could be observed.

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### Conclusions

The serum IL-1 $\beta$  level is significantly associated with the presence of erosions at the onset of RA but its predictive value is attenuated or lost when single or combination DMARD medication is instituted. Measuring IL-1 $\beta$  at the time of diagnosis in a single patient cannot be used to estimate the erosive nature of the disease or the prognosis.

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### Key words

Rheumatoid arthritis, treatment, cytokine.

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## Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that affects about 0.5-1% of adults worldwide. It is associated with disability, morbidity and also increased mortality (1). The treatment of RA, particularly in its early stages, is complicated by the lack of reliable predictors of a severe disease course and poor prognosis (2). In spite of extensive research, the pathogenesis of RA remains unclear. Several proinflammatory cytokines are overexpressed in the inflamed joints of RA patients. Of these cytokines, tumour necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$  have been implicated as key cytokines in the inflammatory reaction and in the formation of structural damage (3).

The IL-1 cytokine family consists of four primary members, of which IL-1 $\alpha$ , IL-1 $\beta$  and IL-18 are proinflammatory, whereas IL-1 receptor antagonist (IL-1ra) is anti-inflammatory. IL-1 $\alpha$  is mostly intracellular but IL-1 $\beta$  can frequently be found in the circulation (4). IL-1 $\beta$  can induce the expression of metalloproteinases as well as proinflammatory cytokines (5). Polymorphism in the IL-1 $\beta$  gene has also been shown to be associated with more severe disease (6). Injection of IL-1 $\beta$  into normal rabbit joints induces severe arthritis (7) and a chronic inflammatory arthropathy develops in IL-1ra-deficient mice (8). Furthermore, administration of neutralizing antibodies to IL-1 $\beta$  prevents bone and cartilage destruction in the model of collagen-induced arthritis (9, 10). These findings emphasize the role of IL-1 $\beta$  in the pathogenesis of arthritis and structural joint damage in particular.

Elevated IL-1 $\beta$  levels have been found in the sera of RA patients and a correlation between IL-1 $\beta$  levels and disease activity has also been observed (11, 12). A decrease in IL-1 $\beta$  levels during sulfasalazine therapy has been reported (13). However, no studies examining the relationship between serum IL-1 $\beta$  levels and the erosiveness of RA have been published. Likewise, there are no published studies that have compared the effect of different types of anti-rheumatic drug therapy on the serum level of IL-1 $\beta$ , and their correlations to

the disease outcome and the development of erosions.

Therefore, in this study we examined the serum levels of IL-1 $\beta$  in patients with recent onset RA who had not previously received disease modifying anti-rheumatic drugs (DMARDs) and (i) assessed the association of serum IL-1 $\beta$  with erosions, and (ii) compared the effect of single drug and combination therapy with three DMARDs on serum IL-1 $\beta$  levels.

## Patients and methods

### Patients

This study is based on the material collected in association with a previous study by Möttönen *et al.* (14). Of the 195 patients in the original study, 132 were included in the present study. All the patients fulfilled the revised criteria of the American Rheumatism Association for rheumatoid arthritis (15). The age of the patients was between 18 and 65 years and the duration of symptoms less than 2 years. Patients who had used DMARDs in the past, or had received glucocorticoid therapy within the previous 2 weeks were excluded. Three patients in the combination treatment group and 4 patients in the single therapy group had used corticosteroid at some time before the onset of the study. The protocol was approved by the national health authorities and the ethical committees in all participating hospitals. All the patients gave their informed written consent.

### Treatment

The patients enrolled in the study were randomized to treatment with either combination DMARD therapy plus prednisone (starting dose 5mg/day) or a single DMARD with or without prednisolone (14). In the single therapy group, glucocorticosteroid was used if disease activity according to the treating physician was high. In this group 27% started glucocorticosteroid at the onset of the study and an additional 36% started glucocorticosteroid within a median of 6 weeks from the initiation of the trial (starting dose 5mg of prednisolone per day). If the clinical improvement at 3 months was less 50%, the prednisolone dose was increased to 7.5 mg daily. The

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maximum prednisolone dose allowed during the study was 10 mg per day. Both treatment strategies were aimed at achieving remission. In the single DMARD therapy sulphasalazine (2 g daily) was used as the initial DMARD in all patients. If the patient experienced an adverse event or if the clinical response was less than 25% (defined as less than 25% improvement in two of the three criteria, *i.e.* the number of swollen joints, the number of tender joints, and ESR or CRP) at 6 months, sulphasalazine was replaced with methotrexate (7.5-15 mg per week). 21% of patients were switched from sulphasalazine to methotrexate because of side effects. The use of oral prednisolone up to 10 mg per day was allowed if clinically indicated because of active disease (14).

The combination therapy initially consisted of sulphasalazine 500 mg twice daily, methotrexate 7.5 mg weekly, hydroxychloroquine 300 mg daily, and prednisolone 5 mg daily and this therapy was continued for 3 months if no side effects occurred. If the clinical improvement after 3 months of therapy was less than 50% in at least two of the three criteria (swollen joints, tender joints, and ESR or CRP), the doses of methotrexate and prednisolone were increased to 10 mg weekly and 7.5 mg daily respectively. Thereafter the dose adjustments were allowed to mimic clinical practice.

Clinical assessments of the patients were carried out at baseline and at months 1, 3, 4, 5, 6, 9, 12, 18, and 24. The assessment included: counts of the tender (68 joints examined) and swollen (66 joints examined) joints; the patient's overall assessment on a visual analogue scale (from 0 = no symptoms to 100 = most severe disease); the patient's assessment of pain on a visual analogue scale (from 0 = no pain to 100 = most severe pain); the duration of morning stiffness; the physician's global assessment of disease activity on a visual analogue scale; the patient's self assessment of disability using the Stanford Health Assessment Questionnaire (16); and measurement of the ESR and CRP. The use of intra-articular glucocorticoid injections (methylprednisolone

or triamcinolone hexacetonide) was allowed in both treatment groups. The ACR response rates at 6, 12 and 24 months (17) and remission using the preliminary ARC criteria (18) were evaluated; however, fatigue and the duration of the absence of symptoms were excluded.

#### *Determination of IL-1 $\beta$ levels and the shared epitope*

Serum IL-1 $\beta$  levels were measured in sera obtained before the institution of therapy and 6 months afterwards using a high sensitivity solid-phase enzyme-linked immunosorbent assay (ELISA). The assay was performed according to the manufacturer's recommendations (R&D systems, UK). The detection limit of the assay was less than 0.1 pg/ml. Values below the detection limit were assigned a value of 0 and included in the analysis. To determine the presence of shared epitopes, DNA was extracted from anti-coagulated blood samples using a salting out method. Thereafter, HLA-DRB1 alleles were determined by sequence-specific polymerase chain reaction (PCR) amplification (19). As the induction of remission during the first 6 months has been shown to be associated with a good 5-year outcome (20) and as the treatment after 6 months was not fixed, the study focused on IL-1 $\beta$  levels during the first 6 months of treatment.

#### *Evaluation of structural damage*

Radiographic evaluation of the hands and feet was done at entry and at 24 months using standard films with anterior-posterior projections. Radiographic findings for 32 joints were scored by one radiologist who was blinded to the clinical data, using the Larsen method (21).

#### *Statistical analysis*

The results were expressed as means or medians with the standard deviation (SD) or interquartile range (IQR) and 95% confidence intervals (95% CI). Statistical comparison was made using the Mann-Whitney U or Wilcoxon's tests with the Monte Carlo *p*-value and the Hodges-Lehmann estimation of the shift of medians. As non-parametric tests were used, single high values did not have any significant effect on the overall statistical significance. Correlation coefficients were calculated by the Spearman method.

## **Results**

### *Demographics of the patients at baseline*

The demographic parameters of the patients in the single and combination therapy groups are shown in Table I. It can be seen that the demographic parameters, as well as the disease activity and the number of erosions present in hand and foot radiographs, were very similar in the two treatment groups.

**Table I.** Base-line demographic, clinical and radiographic characteristics of patients.

Characteristic	Treatment Group	
	Combination (n = 49)	Single (n = 57)
<i>Demographic</i>		
Sex (female/male)	30/19	40/17
Age (years), mean (SD)	49 (9)	48 (11)
Duration of disease (months), median (IQR)	6 (4, 12)	8 (4, 12)
Rheumatoid factor present (%)	32 (65)	40 (70)
Shared epitope (%)	27 (55)	27 (47)
<i>Measures of disease activity</i>		
Erythrocyte sedimentation rate (mm/hr), mean (SD)	34 (19, 54)	34 (22, 56)
C-reactive protein (mg/ml), median (IQR)	19 (5, 40)	21 (5, 49)
Number of swollen joints, median (IQR)	13 (8, 16)	13 (10, 16)
Number of tender joints, median (IQR)	17 (13, 24)	17 (14, 28)
<i>Radiographic</i>		
Number of x-ray pictures available	49	54
Erosions in hand or foot radiographs, n(%)	20 (41)	29 (54)
Larsen score, median (IQR)	0 (0, 4)	2 (0, 6)

### Serum IL-1 $\beta$ levels in RA patients prior to the antirheumatic treatment

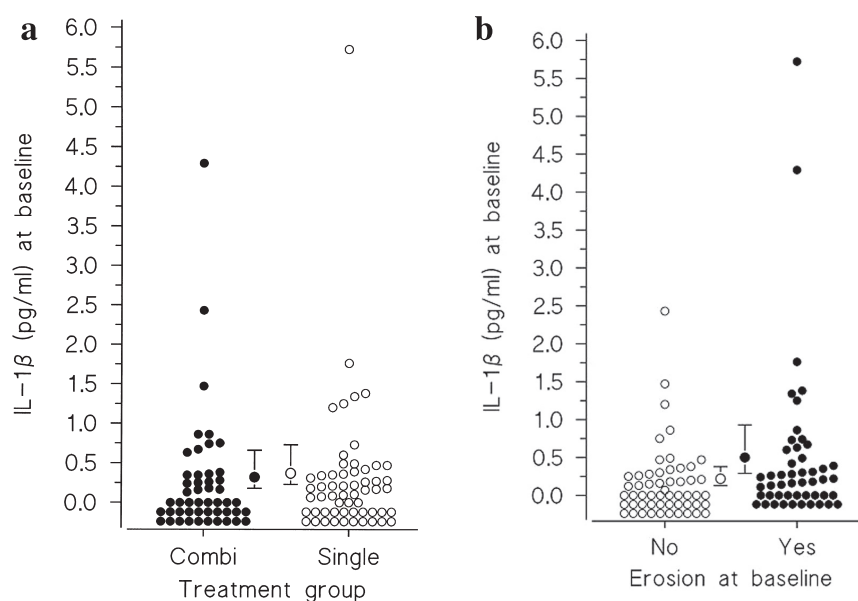
Prior to the anti-rheumatic treatment, detectable levels of IL-1 $\beta$  (>0.1 ng/ml) were found in the serum of 70 of the 132 patients studied. The median baseline serum level of IL-1 $\beta$  in untreated RA patients was 0.35 (IQR 0.23–0.55) pg/ml. No difference was observed in the baseline serum IL-1 $\beta$  levels between the single therapy and combination therapy groups (Fig. 1A). As shared epitopes have been shown to be associated with more severe disease, we studied whether patients with a shared epitope had higher IL-1 $\beta$  levels, but no statistically significant difference in IL-1 $\beta$  levels between patients with and without the shared epitope were observed (data not shown). Baseline IL-1 $\beta$  levels did not predict the chance of a patient achieving remission or an ACR 20% response at any time.

### Association of baseline serum IL-1 $\beta$ levels with the presence of erosions

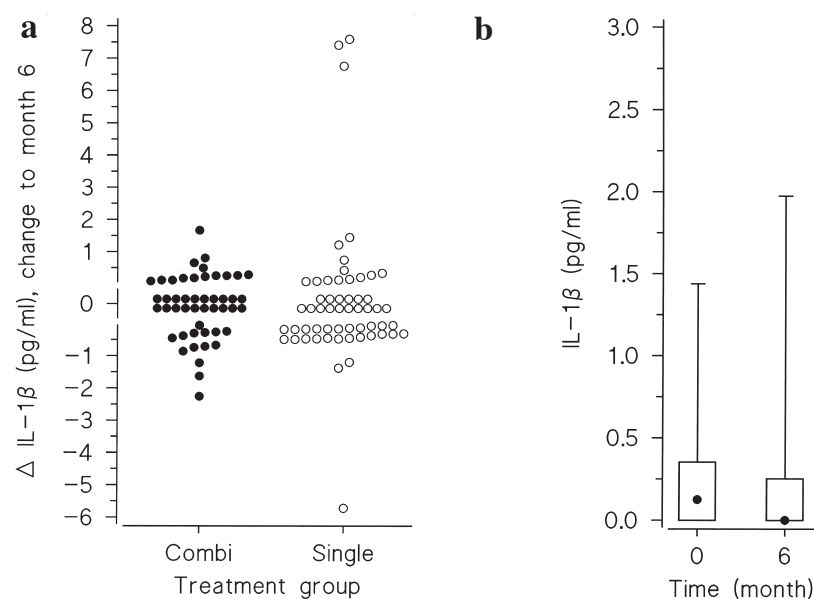
A significant association was observed between baseline IL-1 $\beta$  levels and the number of eroded joints ( $r = 0.24$ , 95% CI 0.06 to 0.41,  $p = 0.021$ ) (Fig. 1B). Multivariate analysis of the association between serum IL-1 $\beta$  levels and the number of eroded joints was significant even after adjustment for the duration of symptoms, sex, age, the number of swollen joints, rheumatoid factor and ESR. It is noteworthy that the level of serum IL-1 $\beta$  was a better indicator of erosiveness than the CRP level. As a matter of fact, no statistically significant correlation was observed between CRP values and the Larsen scores before the onset of therapy. However, the baseline IL-1 $\beta$  level did not seem to predict the later erosiveness of the disease after a period of anti-rheumatic therapy, either single or combination, as no correlation was found between IL-1 $\beta$  levels at 0 and 6 months and the presence of erosions at 24 months.

### Serum IL-1 $\beta$ levels and anti-rheumatic treatment

In the patients studied, a significant correlation (Spearman) was observed between the serum IL-1 $\beta$  levels meas-



**Fig. 1.** (a) Baseline levels of serum IL-1 $\beta$  in the single therapy and combination therapy groups at 0 months before the institution of anti-rheumatic therapy (A). (b) Baseline levels of IL-1 $\beta$  in patients with and without erosions at baseline ( $p = 0.021$  between groups).



**Fig. 2.** (a) The change in serum IL-1 $\beta$  levels during 6 months of anti-rheumatic treatment in the single and combination therapy groups. (b) IL-1 $\beta$  levels at the onset of the study and after 6 months of anti-rheumatic treatment, shown as box plots.

ured at baseline and at 6 months ( $r = 0.28$ , 95% CI 0.10–0.45), suggesting that patients with high IL-1 $\beta$  levels before therapy continue to have higher levels after 6 months of therapy. However, there were no significant differences in IL-1 $\beta$  levels before and after 6 months of anti-rheumatic treatment for either treatment group (Fig. 2). Furthermore, the changes in IL-1 $\beta$  levels were similar in those patients who reached

remission (0.24 pg/ml, 95% CI -0.89 to 1.08)] and in those patients who did not reach remission (-0.12 pg/ml, 95% CI -0.23 to 0.01) and there was no significant difference between these changes ( $p = 0.16$ , Mann Whitney).

### Discussion

Several studies using animal models or human synovial tissue have pointed to a role of IL-1 $\beta$  in the process of the



formation of erosions (8). However, to the best of our knowledge, the present study is the first to show a significant association between serum IL-1 $\beta$  levels and the grade of radiographic erosions in recent onset RA. This finding supports the concept that IL-1 $\beta$  does indeed play a significant role in erosion. It is noteworthy that in the same analysis no association was observed between serum CRP and the presence of erosions, suggesting that the role of IL-1 $\beta$  in the formation of erosions does not merely reflect the degree of inflammation. It should however be noted that in other studies an association between CRP and erosions has been reported (22).

Such an association was evident in our study before the institution of anti-rheumatic treatment, but was lost after the onset of single or combination therapy. This is not surprising as many DMARDS, such as sulfasalazine (13), methotrexate (23) and glucocorticosteroids (24, 25) can reduce the production of cytokines. As there was no significant change in IL-1 $\beta$  levels during therapy, the loss of association between IL-1 $\beta$  levels and erosions at 6 months could be due to increased erosiveness. In the present study, in addition to DMARDs all the patients in the combination group and most (67%) in the single therapy group also used glucocorticoids (up to 10 mg per day prednisolone) during the first 6 months of the study, a drug that generally has an effect on cytokine levels. Baseline IL-1 $\beta$  levels were not associated with the presence of the shared epitope. However, IL-1 $\beta$  could be associated with IL-1 $\beta$  gene polymorphism, which has been linked to more severe disease (6). The baseline IL-1 $\beta$  levels did not predict the chance of reaching remission or the ACR 20% response at any time. As there was also a great variation in IL-1 $\beta$  values and many negative values, it seems evident that IL-1 $\beta$  levels cannot be used in a single patient as a means to predict the erosive nature or the prognosis of the disease.

The IL-1 $\beta$  levels did not change significantly during the first 6 months of treatment with either single or combi-

nation therapy. There was a strong correlation between IL-1 $\beta$  levels at baseline and after 6 months of treatment, which further suggests that the IL-1 $\beta$  levels in particular patients remain similar irrespective of the anti-rheumatic therapy. In many studies anti-rheumatic treatment has been shown to reduce the production of cytokines by mononuclear cells in the blood (26, 27). However, the effect of treatment on serum cytokine levels is not so clear. In some studies a correlation between IL-1 $\beta$  levels and disease activity (11, 12) has been observed and others a decrease in IL-1 $\beta$  and other cytokines has been observed during therapy with sulfasalazine (13) or glucocorticosteroids (24). However, in several studies no effect of treatment or no detectable IL-1 $\beta$  levels were observed in RA patients (27-29). In all of the reports the levels of IL-1 $\beta$  tended to be very low. Whether increasing the sensitivity of the assay to detect values below the detection limit could influence the results is not clear. It is, however, unlikely that even with a more sensitive assay the IL-1 $\beta$  level could be used to predict the erosive nature of the disease.

To conclude, serum IL-1 $\beta$  levels were significantly associated with the number of eroded joints at the onset of RA but the predictive value was attenuated or lost when DMARD medication, either single or combination, was instituted. The variation in IL-1 $\beta$  levels in individual patients is so large that measuring IL-1 $\beta$  at the time of diagnosis in a single patient cannot be used to estimate the erosive nature or the prognosis of the disease.

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