

BACKGROUND and aims: Although the participation of cytokines in the pathogenesis of rheumatoid arthritis (RA) seems to be unequivocal, their relationship with current serum markers of this disease is not clear. The present study analyses whether there is any correlation between the levels of tumour necrosis factor- α (TNF- α), interleukin-2 soluble receptor (sIL-2R) and the concentrations of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and β_2 -microglobulin in a group of 21 patients with RA, all rheumatoid factor positive.

Methods: The levels of TNF- α and sIL-2R were analysed in association with other parameters of inflammation (ESR, CRP and β_2 -microglobulin).

Results: In comparison with the control group, RA patients presented high median levels of both cytokines, TNF- α (6.4 pg/ml) and sIL-2R (56 pmol/L), as well as of ESR (34 mm/h), CRP (0.9 mg/dl) and β_2 -microglobulin (1.6 mg/dl) ($p < 0.01$). However, only ESR levels in the RA group significantly differ from the control group ($p < 0.01$). No correlation was found between the inflammatory parameters.

Conclusions: These results suggested that TNF- α and sIL-2R levels are up-regulated in RA patients but did not significantly differ from the control group. Due to the chronic course of this disease, other inflammatory markers must be identified in order to provide early therapeutic strategies to these patients.

Key words: Rheumatoid arthritis, Erythrocyte sedimentation rate, C-reactive protein, β_2 -Microglobulin, Cytokines

Tumour necrosis factor- α , interleukin-2 soluble receptor and different inflammatory parameters in patients with rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder of unknown aetiology¹ whose major distinctive feature is a chronic, symmetric, and erosive synovitis of peripheral joints. Its aetiology is unknown, and definitive diagnosis depends predominantly on characteristic clinical features, typical radiographic findings, the presence of rheumatoid factor (RF), and elevated erythrocyte sedimentation rate (ESR)/C-reactive protein (CRP).^{2,3} Failure to meet these criteria does not therefore exclude the diagnosis, especially during the early stages of the disease. Hence, diagnosis is often established months or even years after the first appearance of symptoms, at a stage where cartilage destruction has already propagated to an irreversible state.^{4,5}

Although the initiating event in RA has not yet been defined, a growing body of evidence indicates that cytokines may help the development and the perpetuation of the chronic inflammatory state. Among the cytokines involved in RA, tumour necro-

sis factor- α (TNF- α) is believed to be dominant.^{6,7} This cytokine is present in both the synovial cavity and the blood of RA patients.⁷ In relation to interleukin (IL)-2, this cytokine is generally considered to elicit pro-inflammatory effects that exacerbate T helper cell type 1-mediated disease states, such as autoimmune arthritis. It has also been demonstrated that IL-2 mRNA is markedly increased during the acute stage of collagen-induced arthritis, an animal model of rheumatoid arthritis.⁸ In this condition, IL-2 protein and the IL-2 soluble receptor (sIL-2R) are preferentially expressed at disease onset, in comparison with later stages of the disease.⁹ However, the studies that have addressed this theme have shown discordant results since they have reported evidence pro and against an association between the current proposed markers of this disease (CRP and ESR) and cytokines (TNF- α and sIL-2R). In the present study, we examined this issue again by evaluating whether there is any correlation between cytokine (TNF- α and sIL-2R) levels and inflammatory parameters such as ESR, CRP, and β_2 -microglobulin concentrations.

Table 1. Clinical and laboratorial features of the studied groups

Parameter	RA	Control
Sex (female:male)	18:3	7:0
Age (years)	36 (16–75)*	29 (20–75)
Haemoglobin (g/dl)	13.6 (10.1–15.1)	13.2 (11.7–14.5)
Haematocrit (%)	40.4 (29.7–46.3)	39.5 (36.7–45.1)
Platelet number ($\times 10^3 \text{ mm}^3$)	237 (148–795)	290 (205–344)
Granulocytes ($\times 10^3 \text{ mm}^3$)	4.7 (2.79–16.7)	4.2 (2.2–6.4)
White blood cell ($\times 10^3 \text{ mm}^3$)	6.3 (6.1–18.8)	6.9 (5.4–10.2)
IgG (mg/dl)	847 (549–1145)	160 (70.5–902.3)
IgM (mg/dl)	93.8 (36.7–749)	70.2 (56.9–86.1)
C3 (mg/dl)	108 (54–311)	88 (88–158)
C4 (mg/dl)	23 (10–75.4)	16.9 (10–83)

* Data presented as median (range) apart from the female:male ratio.

Methods

Subjects

The patients with RA were enrolled from the out-patient clinical service of the University Hospital-UFSC by reviewing their medical records. Only patients with positive results for RF who fulfilled the American College of Rheumatology diagnostic criteria for RA^{10,11} were selected for inclusion in the study. Exclusion criteria were the presence of other acute or chronic inflammatory diseases. Apparently healthy persons were asked to participate as controls if they had negative RF values and an absence of acute and chronic diseases. All patients and subjects gave informed consent, and the Medical Ethics Committee of the Universidade Federal of Santa Catarina approved the study protocol.

Procedures

All laboratory specimens for the study were collected at the time the patients came to the clinical laboratory for other reasons. Blood samples were drawn from the antecubital vein in the fasting state. Some evaluations such as ESR, RF and haematological parameters were made as part of a standard battery of assessments requested by their doctors. Samples were collected in different tubes according to the type of analysis. ESR and haematological parameters (red and white blood cell indices: number, haematocrit, haemoglobin, platelet number) were obtained from the samples using different anticoagulant solutions: sodium citrate and ethylenediaminetetracetic acid, respectively. Silicone-coated tubes were used for other analyses [cytokines, CRP, RF, immunoglobulins G and M (IgG and IgM), complement components (C3 and C4), β_2 -microglobulin and anti-nuclear antibody (ANA) assays]. After being centrifuged, coded serum aliquots were stored at -20°C and analysed for cytokines, CRP, RF, IgG, IgM, C3, C4, β_2 -microglobulin and ANA in a single batch at the end of the study. The

samples from the control group were submitted to the same procedures.

Measurements

Serum cytokine assays

On the day of the measurements of the studied parameters, the samples were thawed to room temperature. Both TNF- α and sIL-2R were measured by enzyme-linked immunosorbent assay (Boehringer Mannheim Biochemical, Indianapolis, Indiana, USA) methodologies. The ranges of the values detected by these assays were: TNF- α , 5–1000 pg/ml; and sIL-2R, 50–100 pmol/l. The principle of these tests is based on the two-step sandwich techniques. Briefly, sample aliquots and standards (20 μl) were transferred to the wells of a microtitre plate (TNF- α , 5–1000 pg/ml; sIL-2R, 5–100 pmol/l) and incubated at room temperature (4 h). Then the samples were washed three times with washing solution (composition: NaCl, 137 mM; KCl, 2 mM; and phosphate, 10 mM; pH 7.6), followed by the addition of 200 μl of substrate solution into the wells. The microtitre plate was then protected from light and maintained at room temperature (20–30 min). The reaction was stopped by adding 50 μl of sulphuric acid (1 mol/l). One minute later, the concentration of cytokines in each well was determined (Organon Teknika, Durham, North Carolina, USA). When indicated by the manufacturer, the procedures were carried out on a shaker (250 r.p.m.).

CRP, RF, IgG and IgM, complement components (C3, C4) and ANA assays

CRP (cut-off, 0.8 mg/dl), RF (cut-off, < 20 IU/ml), IgG (reference range, 694–1618 mg/dl), IgM (reference range, 60–263 mg/dl), C3 (reference range, 88–210 mg/dl) and C4 (reference range, 16–47 mg/dl) were measured by nephelometry (Beckman Coulter Array, Brea, California, USA), β_2 -microglobulin (1.01–1.73 mg/dl) was determined by the chemiluminescence method (Immulate 2000; DPC Medlab, Dade, Boehringer, Deerfield, Illinois, USA) and leuko-

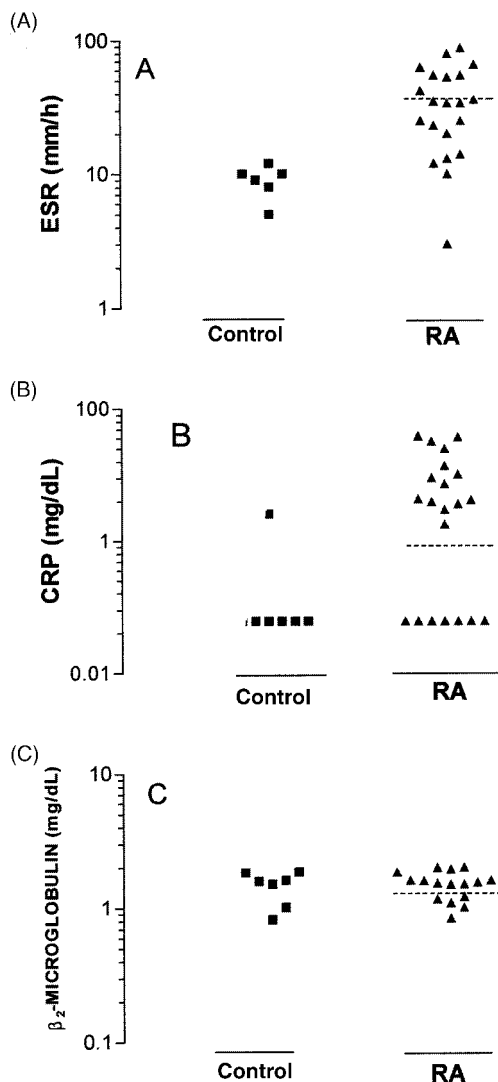


FIG. 1. Individual values of ESR (A), CRP (B) and β_2 -microglobulin (C) in both control and RA groups. The dotted line indicates the median.

cyte indexes were determined on an automatic counting machine (Beckman-Coulter). The ESR (cut-off values: female, < 20 mm/h; male, < 15 mm/h) was measured by the Westergreen method (automatic Ves-mastic-20, Miami, Florida, USA). ANA tests were evaluated by indirect immunofluorescence assays using human epithelial cell (HEP-2) slides.¹⁰

Statistical analysis

When indicated, the Mann-Whitney test, single linear regression test or correlation (Pearson) analysis were used. For all analyses, $p < 0.05$ was used to assess overall differences. The results are expressed as the median and its range.

Results

The present study included a series of 21 patients; all fulfilled the American College of Rheumatology Diag-

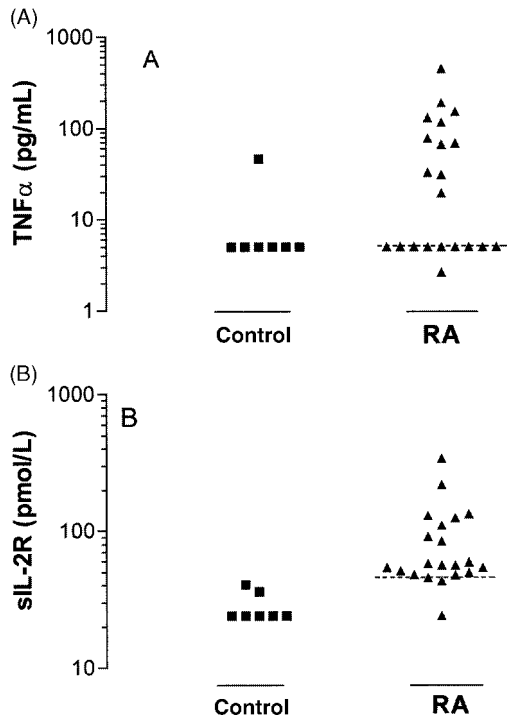


FIG. 2. Individual values of TNF- α (A) and sIL-2R (B) in both control and RA groups. The dotted line indicates the median.

nostic Criteria for RA,^{10,11} and seven apparently healthy individuals were used as controls. The gender ratio was 18 females to three males for RA patients and seven females to zero males for the control group. All patients were white. The median age of the groups was 36 years (range 16-75 years) for the RA group and 29 years (range 20-75 years) for the control group.

Table 1 presents some of the features of the studied groups. As shown, no evidence of anaemia or changes in either platelet or white cell numbers, levels of IgG and IgM or complement factors (C3 and C4) were detected in RA patients (Table 1) ($p > 0.05$). In comparison with the control group, RA patients presented increased levels of ESR, CRP, and β_2 -microglobulin ($p < 0.01$) (Fig. 1A-C). The median levels of ESR in RA and groups were 34 mm/h (range 3.0-88 mm/h), whereas those for CRP and β_2 -microglobulin concentrations were 0.9 mg/dl (range 0.08-58 mg/dl) and 1.6 mg/dl (range 0.84-2.0 mg/dl), respectively. In this studied group of RA patients, only ESR levels significantly differ from control group ($p < 0.01$). Both TNF- α (median 6.4 pg/ml, range 2.8-450 pg/ml) and sIL-2R (median 56 pmol/l, range 12.5-341 pmol/l) levels were also enhanced in the RA patients (Fig. 2A,B) but they did not differ in comparison with control data.

Table 2 shows that a significant correlation was only found between TNF- α and sIL-2R ($p < 0.01$). However, this statistical significance may be artificial since no statistical significance was found for the

Table 2. +Correlation *r* values among the studied variables (*n* = 27)

Variables	Linear Regression <i>r</i>	Pearson correlation <i>r</i>
ESR versus CRP (log)	0.53	0.53
ESR versus sIL-2R	0.40	0.40
ESR versus RF	0.10	0.10
ESR versus TNF- α	0.11	0.11
ESR versus β_2 -microglobulin	0.05	-0.13
RF versus β_2 -microglobulin	0.41	0.41
RF versus CRP (log)	0.19	0.19
RF versus sIL-2R	0.16	0.16
RF versus TNF- α	0.18	0.17
CRP (log) versus sIL-2R	0.06	0.06
CRP (log) versus β_2 -microglobulin	0.004	-0.064
CRP (log) versus TNF- α	-0.17	-0.17
β_2 -Microglobulin versus TNF- α	0.25	0.25
β_2 -Microglobulin versus sIL-2R	0.03	-0.17
TNF- α versus sIL-2R	0.70**#	0.70**#

* $p < 0.01$, log-logarithm values. # No statistical significance was found, however, when the highest level of both cytokines from one patient of the RA group was removed.

data of a patient that presented the highest levels of both cytokines from the RA group.

Discussion

The results presented show that the median levels of TNF- α , sIL-2R and CRP, although slightly increased in RA patients, did not differ from a group of apparently healthy subjects. In the RA group, these findings were associated with high levels of ESR, whereas β_2 -microglobulin concentrations also did not differ. Overall, these changes are representative of a spectrum of effects that are triggered in response to the autoimmune injury that characterizes RA.

Several clinical and experimental lines of evidence point to TNF- α as an important inflammatory mediator in RA.^{6,7} It is well known that this cytokine is found at high levels in the synovial fluids of RA patients, and that it contributes to both bone and cartilage destruction.¹² On the other hand, experimental models of arthritis in mice have demonstrated that TNF- α injection accelerates arthritis, whereas anti-TNF- α injection prevents it.⁶ In our work, we observed that the median blood levels of this cytokine did not significantly differ in comparison with those measured in the control group. Considering that the studied RA patients were also RF-positive, an indicator of disease severity, the relevance of these findings remains to be established.^{13,14}

Few studies in the literature have evaluated the levels of sIL-2R in RA patients. Suenaga *et al.*¹⁵ have demonstrated that an increased concentration of sIL-2R in the serum of patients with joint pain is a predictor for the future development of RA. Spadaro *et al.*¹⁶ observed that treatment of RA patients with methotrexate for 6 months was able to decrease the levels of both sIL-2R and IL-6. On the other hand, the levels of sIL-2R did not correlate with any future bone

or joint changes within 1 year of observation.¹⁷ In the present study, the serum concentrations of sIL-2R did not significantly differ in comparison with those of controls, whereas ESR levels but not CRP and β_2 -microglobulin were significantly increased. Altogether, these inflammatory indices seem to independently reflect a final pathway of multifactorial events.

Another point that deserves consideration is the fact that, despite adequate control of symptoms in this disease, the available therapeutic measures do not prevent further progression of this disease that results in high levels of disability.^{18,19} Advances in this field are urged and the provision of adequate therapy is now a common goal for several chronic diseases. In this regard, ultra-sensitive assays of polymerase chain reaction have been used both as markers and prognostic predictors of cardiovascular disease.^{20,21} However, treatment evaluation was not addressed in our work and the small number of RA patients did not permit further conclusions in relation to the polymerase chain reaction levels that were measured by ultra-sensitive assay.

In conclusion, the present results show that, in this group of RA patients, all RF negative, the elevated levels of ESR did not correlate with the studied cytokine levels. According to these findings, other inflammatory markers must be searched in order to provide early therapeutic strategies to these group of patients.

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