

# Clinical and laboratory parameters which affect soluble interleukin-2 receptor levels in the serum and synovial fluids of patients with rheumatoid arthritis

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## Abstract

**Objective**—To investigate whether soluble interleukin-2 receptor (sIL-2R) could be a useful marker of disease activity in rheumatoid arthritis (RA); sIL-2R levels in serum and in synovial fluid were determined by enzyme-linked immunosorbent assay.

**Methods**—Sixty five serum and 27 synovial fluid samples were obtained from patients with RA. Twenty five serum and 28 synovial fluid samples from patients with osteoarthritis (OA) were used as controls. Furthermore, 10 synovial fluid samples from healthy volunteers were also examined. Variable laboratory and clinical data were compared with serum sIL-2R levels, in 26 patients with RA and serial samples from some patients were examined.

**Results**—Concentrations of sIL-2R in serum (median 81, range 40–350 pM) and synovial fluid (median 125, range 52–460 pM) from patients with RA were significantly higher than in serum (median 45, range 13–100 pM) and synovial fluid (median 37, range 15–140 pM) from patients with OA, and healthy control synovial fluid (median 2.5, range 0–10 pM). Serum sIL-2R levels correlated strongly with serum levels of C-reactive protein ( $p=0.0001$ ), and a significant correlation with erythrocyte sedimentation rate (ESR) ( $p=0.048$ ), IgG levels ( $p=0.028$ ), IgA levels ( $p=0.044$ ) and Lansbury Index ( $p=0.037$ ) was observed. However, serum sIL-2R levels showed no significant correlation with rheumatic factor, IgM or T cell subsets.

**Conclusion**—These findings indicate that sIL-2R levels in patients with RA reflect disease activity.

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Activated T cells,<sup>1</sup> B cells,<sup>2-4</sup> natural killer cells<sup>5</sup> and macrophages<sup>6,7</sup> express interleukin-2 receptors (IL-2R) on their surface. The IL-2R are composed of  $\alpha$ ,  $\beta$ ,  $\gamma$  heterotrimers. The 55 kd  $\alpha$  chain<sup>8,9</sup> binds IL-2 with low affinity ( $K_d=10^{-8}$  M), the 75 kd  $\beta$  chain binds IL-2 with intermediate affinity ( $K_d=10^{-9}$  M).<sup>10</sup> The  $\gamma$  chain is necessary for the formation of high

and intermediate affinity receptors, and is required for receptor-mediated internalisation of IL-2.<sup>10,11</sup> Following lymphocyte activation, the  $\alpha$  polypeptide chain, in addition to being expressed on the cell surface, is released in soluble form,<sup>12</sup> which is 8–10 kd less than their parent cell surface membrane receptor. Thus by detection of soluble IL-2 receptors (sIL-2R) in serum, lymphocyte activation can be assessed.

High concentrations of serum sIL-2R have been reported in patients with various autoimmune diseases<sup>13,14</sup> including rheumatoid arthritis (RA),<sup>15,16</sup> T cell leukaemia<sup>17</sup> and granulomatous disease.<sup>18</sup> Correlations between sIL-2R levels and disease activity have also been described in these diseases.<sup>13,16,19,20</sup> However, controversy exists on whether elevated levels of sIL-2R in the serum of RA patients reflects disease activity,<sup>15</sup> as several studies on sIL-2R regulation have demonstrated.<sup>21,22</sup>

In this study we measured the sIL-2R levels in serum and synovial fluid of patients with RA, and determined whether they correlated with clinical and laboratory parameters of disease activity.

## Materials and methods

### PATIENTS

Serum was obtained from 65 patients (14 men and 51 women) with RA, as defined by the criteria of the American Rheumatism Association,<sup>23</sup> and 23 patients (3 men and 20 women) with osteoarthritis (OA), clinically and radiologically diagnosed. Synovial fluid was obtained from 27 patients (5 men and 22 women) with RA and 28 patients with OA. Synovial fluid was also collected from 10 healthy volunteers (7 men and 3 women).

### CLINICAL EVALUATION

A number of clinical variables were examined in each patient. The erythrocyte sedimentation rate (ESR) was determined by the Westergren method. C-reactive protein (CRP) was quantified by a turbidometric method. Rheumatoid factor (RF) was measured by nephelometry and rheumatoid arthritis haemagglutinin (RAHA) was measured by microtitre methods. Serum immunoglobulin levels were determined by nephelometry.

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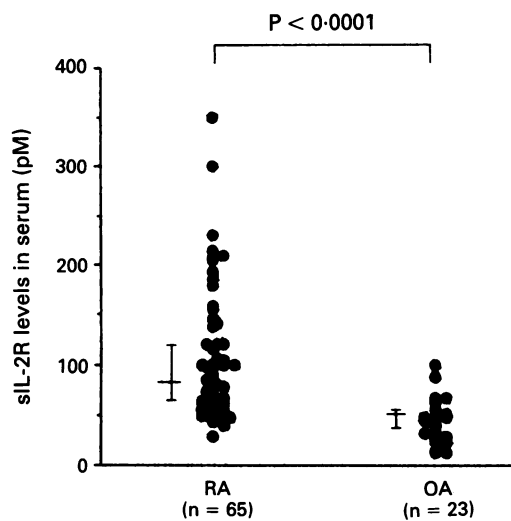


Figure 1 Serum soluble interleukin-2 receptor (sIL-2R) levels (pM) in patients with rheumatoid arthritis (RA) and osteoarthritis (OA). Bars represent the median  $\pm$  interquartile range.

Fresh heparinised blood was obtained from patients and separated peripheral blood mononuclear cells were stained for 2-colour flow cytometry. The number of cells which expressed the specific surface markers CD4 and CD8 were calculated. The modified Lansbury index<sup>24</sup> consisted of duration of morning stiffness, number of swelling joints, grip strength and ESR.

#### sIL-2R ASSAYS

sIL-2R levels in serum and fluid were measured by ELISA. Kits were purchased from Immunotech SA (Marseille, France). The kit contained a coating anti-sIL-2R monoclonal antibody and a second monoclonal antibody conjugated with alkaline phosphatase. Standards of sIL-2R ranging from 0–400 pM were provided.

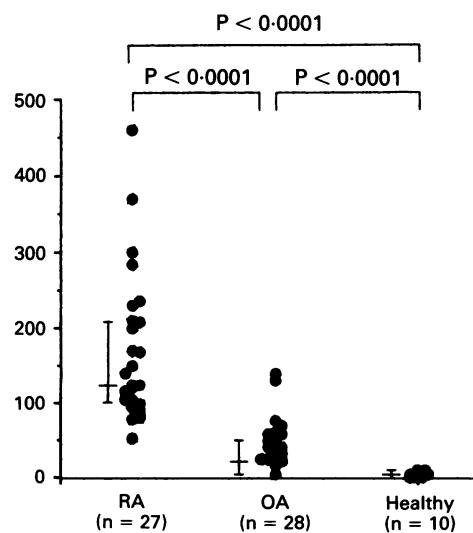


Figure 2 Soluble interleukin-2 receptor (sIL-2R) levels in synovial fluid from patients with rheumatoid arthritis (RA), osteoarthritis (OA) and healthy control subjects. Bars represent median  $\pm$  interquartile range.

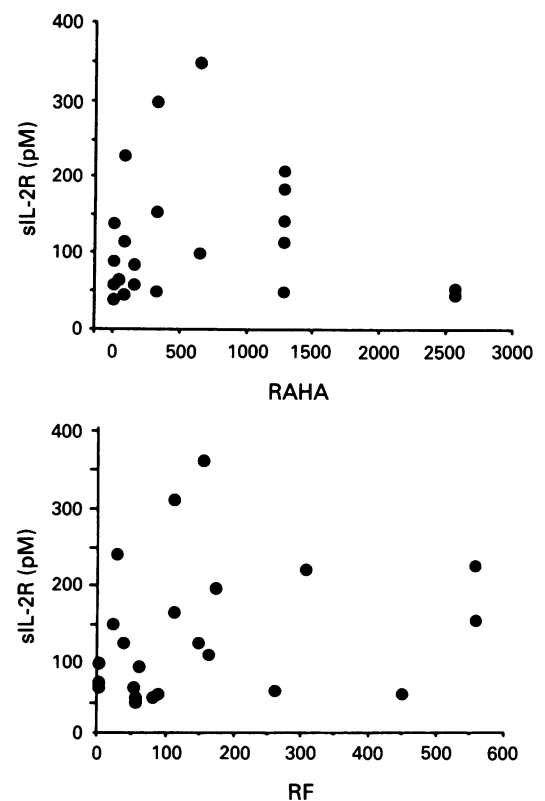


Figure 3 Correlation between serum levels of soluble interleukin-2 receptor (sIL-2R) and rheumatic factor (RF) or rheumatoid arthritis haemagglutinin (RAHA). No statistically significant correlation was found.

#### STATISTICAL ANALYSIS

All patient data were entered into a computer database and analysed using the Stat View 512+ program. The Mann-Whitney U test was performed for each set of variables. P values less than or equal to 0.05 were considered

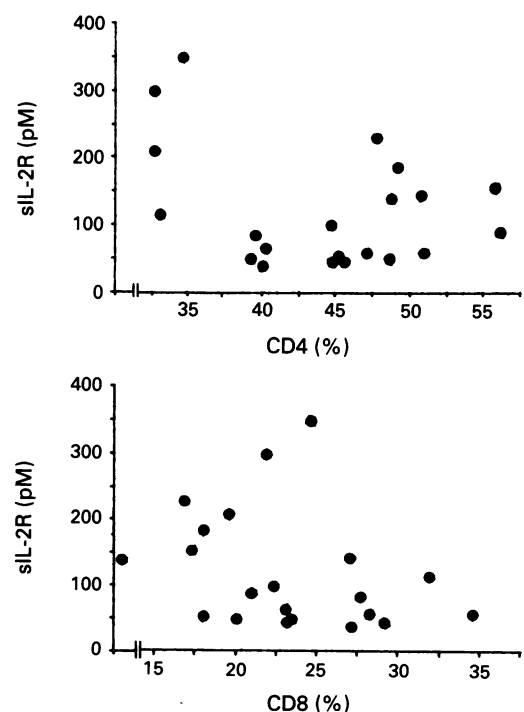


Figure 4 Correlations between serum levels of soluble interleukin-2 receptor (sIL-2R) and CD4 and CD8 T cell subsets in patients with rheumatoid arthritis. No statistically significant correlation was found.

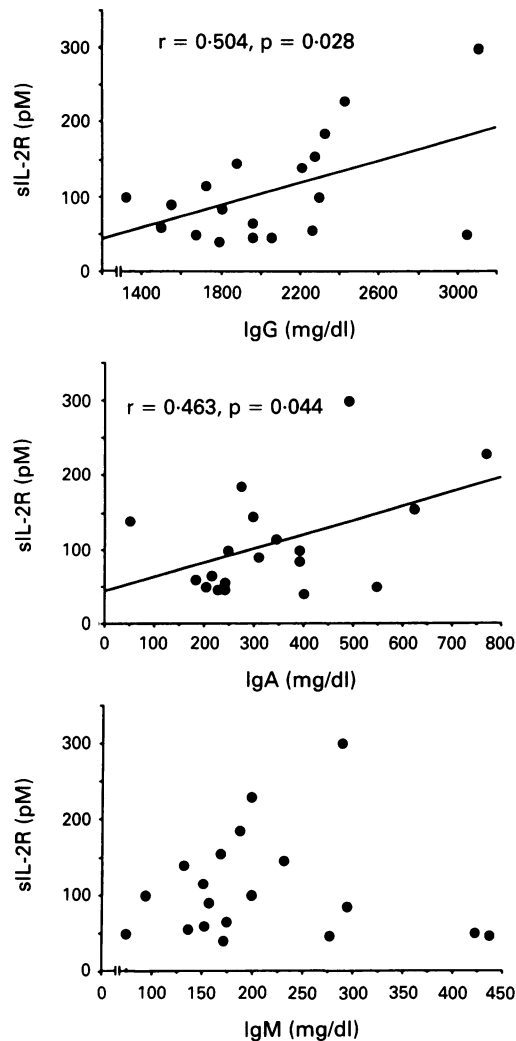


Figure 5 Correlation between serum levels of soluble interleukin-2 receptor (sIL-2R) and immunoglobulin in patients with rheumatoid arthritis. sIL-2R levels correlated positively with IgG ( $r = 0.504$ ,  $p = 0.028$ ) and IgA ( $r = 0.463$ ,  $p = 0.044$ ), but not with IgM.

significant. Regression analysis and correlations were also performed using the same programme.

## Results

Figure 1 shows that serum sIL-2R levels were considerably elevated in patients with RA

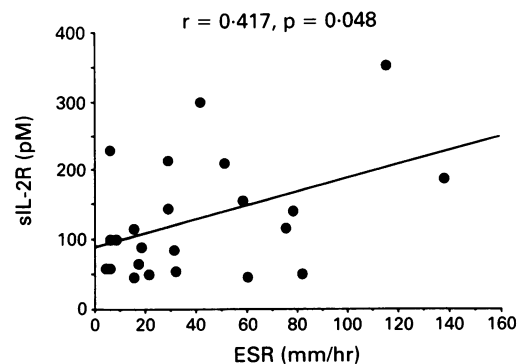


Figure 6 Correlation between serum levels of soluble interleukin-2 receptor (sIL-2R) and erythrocyte sedimentation rate (ESR) in patients with rheumatoid arthritis. A statistically significant correlation was found ( $r = 0.417$ ,  $p = 0.048$ ).

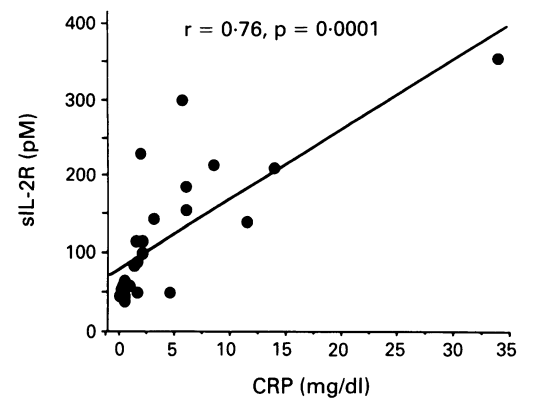


Figure 7 Correlation between serum levels of soluble interleukin-2 receptor (sIL-2R) and C-reactive protein (CRP) in patients with rheumatoid arthritis. A statistically significant correlation was found ( $r = 0.76$ ,  $p = 0.0001$ ).

(median 81, range 40–350 pM) compared with the levels in OA patients (median 45, range 13–100 pM) ( $p < 0.0001$ ).

Figure 2 shows that sIL-2R levels in the synovial fluid of patients with RA (median 125, range 52–460 pM) were significantly higher than in OA patients (median 37, range 15–140 pM) and in healthy controls (median 2.5, range 0–10 pM) ( $p < 0.0001$ ).

We also examined relationships between serum sIL-2R levels and clinical and laboratory parameters. There was no correlation between serum sIL-2R and either RF or RAHA (fig 3). The relative percentages of CD4 and CD8 cells in the T cell subsets showed no significant correlations with sIL-2R levels (fig 4). IgG ( $r = 0.504$ ,  $p = 0.027$ ) and IgA ( $r = 0.463$ ,  $p = 0.043$ ) levels correlated significantly with serum sIL-2R levels, however, IgM did not (fig 5). Although ESR showed only a weak correlation with sIL-2R levels ( $r = 0.417$ ,  $p = 0.048$ ), CRP correlated strongly ( $r = 0.753$ ,  $p = 0.0001$ ) (fig 6 and 7). The Lansbury index also exhibited a positive correlation with serum sIL-2R levels ( $r = 0.47$ ,  $p = 0.037$ ) (fig 8).

Serum sIL-2R levels and CRP levels were determined from three patients with RA at different times during the course of their treatment. sIL-2R values decreased along with a concomitant decrease in CRP levels (data not shown).

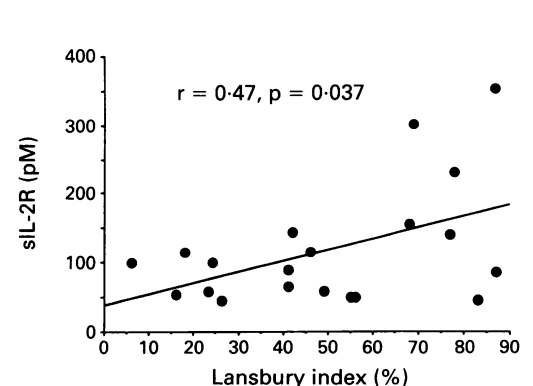


Figure 8 Correlation between serum levels of soluble interleukin-2 receptor (sIL-2R) and modified Lansbury index in patients with rheumatoid arthritis. A statistically significant correlation was found ( $r = 0.47$ ,  $p = 0.037$ ).

## Discussion

In this study, we confirmed that sIL-2R levels in serum and synovial fluid of patients with RA were significantly elevated compared with patients with OA. Furthermore, in patients with RA, synovial fluid sIL-2R levels (median 125 pM) were higher than serum sIL-2R levels (median 81 pM) ( $p = 0.0002$ ). In OA patients, however, synovial fluid and serum sIL-2R levels were approximately equivalent. Thus measurement of synovial fluid sIL-2R levels appears to be useful diagnostically for differentiation between arthropathy of RA and OA. These results are compatible with the report by Keystone<sup>15</sup> suggesting that sIL-2R may be a very sensitive indicator of in situ immune activation. Low levels of sIL-2R in OA synovial fluid indicated significant macrophage infiltration, which may have been the source of sIL-2R.

There have been several reports that serum levels of sIL-2R correlate with disease activity in RA patients.<sup>16-20</sup> Campen<sup>16</sup> described a strong correlation between serum sIL-2R and joint tenderness. Keystone, however,<sup>15</sup> showed that serum sIL-2R levels did not correlate with disease activity. We examined several laboratory parameters that correlated with serum sIL-2R levels. Our data indicated that CRP showed strongest correlations with serum sIL-2R levels, and following treatment, we observed distinct variations in serum sIL-2R levels associated with concurrent changes in CRP levels (data not shown). In contrast to CRP, ESR showed a weak correlation with serum sIL-2R levels. The ESR is generally considered to be the best available serological marker for monitoring disease activity in patients with RA. However, some exceptions have been demonstrated in RA patients whose disease was in remission.<sup>25-26</sup> When considered in connection with the Lansbury index, it is clear that serum sIL-2R levels in RA patients reflect disease activity.

In this study, immunoglobulins (Ig), IgG and IgA, but not IgM, correlated with sIL-2R levels in serum. This is because activated T cells stimulate B cells to secrete Ig,<sup>27-28</sup> but we have no explanation for the lack of correlation between IgM and sIL-2R levels in serum.

Neither serum IgM-RF levels nor RAHA correlated to sIL-2R levels. There is a possibility that circulating RF may non-specifically bind to the coating IgG monoclonal antibody used in the ELISA. To explore this possibility, several serum samples with high-titre RF were treated by RF absorption, and tested for the presence of sIL-2R and IgM-RF. The level of sIL-2R was found to be comparable with that in paired untreated samples, even when RF had been removed (data not shown). RF and RAHA may not always reflect disease activity, compatible with the above results.

The release of sIL-2R appears to be chiefly a characteristic marker of T lymphocyte activation. In this study, the T cell subsets and sIL-2R levels in serum of RA patients showed no significant correlations. However, we did not examine activation of peripheral blood T

cells, for example, expressing IL-2R or HLA-DR, so we cannot discuss here the direct relationship between sIL-2R and T cell subsets. Wagner *et al*<sup>29</sup> demonstrated that T cells are likely to be the source of elevated serum sIL-2R after administration of human recombinant IL-2. Wood *et al*<sup>30</sup> described the source of the sIL-2R in serum as probably derived from the synovium itself rather than from peripheral blood mononuclear cells. It has been reported that the peripheral blood of patients with RA contains a reduced number of CD8 cells, and that the synovial fluid of these patients contains a significantly different ratio of T cell subsets.<sup>31</sup> Symons *et al*<sup>32</sup> showed that synovial fluid levels of sCD4 correlated positively with sIL-2R levels but no correlation with sCD8 levels was observed. Thus T cell subsets in peripheral blood do not reflect the T cell subsets at the site of inflammation, so examination of T cell subsets in the synovial fluid would provide more accurate information on the relationship with sIL-2R levels.

In conclusion, measurement of sIL-2R levels in serum and synovial fluid of patients with RA was useful in assessing disease activity.

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