Serum Interleukin-6 Receptor in Polymyalgia Rheumatica: A Potential Marker of Relapse/Recurrence Risk

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Objective. To investigate the modulation of systemic levels of soluble interleukin-6 receptor (sIL-6R) and soluble gp130 (sgp130) in untreated and treated polymyalgia rheumatica (PMR) patients during a follow-up period of at least 24 months in order to evaluate the relationship of these molecules with clinical outcome and their feasibility to provide a prognostic tool in clinical practice.

Methods. We analyzed sIL-6R and sgp130 serum levels in 93 PMR patients, and 46 age-matched normal controls, at disease onset and at 1, 3, 6, 12, and 24 months of followup during corticosteroid therapy by enzyme-linked immunosorbent assay.

Results. No difference in sIL-6R and sgp130 levels was observed between PMR patients and normal controls at disease onset or during followup. A significant correlation was found between the number of relapses and sIL-6R concentrations at baseline and after 1, 3, and 12 months of therapy. No correlation was found between sgp130 levels and the number of relapses. Cox multivariate analysis indicated that the best model for predicting relapses was identified by sIL-6R levels and the hemoglobin value at baseline. We found that high sIL-6R levels combined with low hemoglobin values resulted in a 10.1-fold increased risk of relapse.

Conclusion. Our data support the identification of a potential prognostic marker of PMR outcome that might have important implications in clinical practice. Because targeting sIL-6R with blocking antibodies has proven useful in other rheumatic disorders, our results could suggest the opportunity to evaluate sIL-6R–blocking treatment in patients with PMR and elevated levels of sIL-6R at disease onset.

INTRODUCTION

Polymyalgia rheumatica (PMR) is characterized by aching in the shoulder, pelvic girdle, and neck associated with morning stiffness. The symptoms of PMR appear to be related to synovitis of the proximal joints and extraarticular synovial structure (1). The pathogenesis of PMR still remains obscure despite more than 2 decades of intensive research and the wealth of information now available about giant cell arteritis (GCA), which often occurs with PMR (1,2). Because of the insufficient knowledge about the pathogenesis of PMR, the only available and effective treatment is corticosteroids, which have a dramatic effect on the symptoms and laboratory signs of inflammation. Despite the rapid action of steroids, some immunoinflammatory features persist in treated patients (3,4). Some evidence suggests that there are 2 subsets of patients with PMR: those with a mild, self-limiting disease requiring 1–2 years of treatment, and others with a more chronic relapsing disease course that may require steroid treatment for several years or indefinitely (1). Furthermore, relapses are not uncommon in patients with isolated PMR, occurring in approximately 30–50% of patients (5,6).

In the last 10 years, studies have aimed at identifying reliable predictors of clinical outcome, thus enabling the detection of patients with PMR at higher risk of experienc-
Patients and Methods

Patients. Ninety-three consecutive untreated patients with PMR (24 men, 69 women, mean age 74 years, range 53–86 years) were prospectively assessed. All patients were diagnosed according to the following criteria: 1) persistent pain (for at least 1 month) involving 2 of the following areas: neck, shoulder, and pelvic girdle; 2) morning stiffness lasting >1 hour; 3) rapid response to prednisone (≥20 mg/day); 4) age >50 years; and 5) absence of other diseases capable of causing musculoskeletal symptoms (11). All patients were clinically assessed by the same rheumatologist at presentation, once monthly for the first 6 months, then every 3 months during the followup period (12–96 months). All patients were treated with prednisone of followup during corticosteroid therapy and in 46 age-matched normal controls. Serum levels of sIL-6R and sgp130 in untreated and treated patients with PMR monitored by clinical and laboratory parameters during a followup period of at least 24 months in order to evaluate the relationship of these molecules with clinical outcome and their feasibility to provide a prognostic tool in clinical practice.

Table 1. Demographic, clinical, and serologic findings of 93 patients with polymyalgia rheumatica*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Male/female</td>
<td>24 (26)/69 (74)</td>
</tr>
<tr>
<td>Age at disease onset, median (range) years</td>
<td>74 (53–86)</td>
</tr>
<tr>
<td>Duration of therapy, median (range) months</td>
<td>18 (6–96)</td>
</tr>
<tr>
<td>Duration of followup, median (range) months</td>
<td>36 (12–96)</td>
</tr>
<tr>
<td>Starting prednisone dosage, median (range) mg/day</td>
<td>17.5 (10–25)</td>
</tr>
<tr>
<td>Patients with ≥1 relapse</td>
<td>47 (50.6)</td>
</tr>
<tr>
<td>Patients with ≥2 relapses</td>
<td>24 (25.9)</td>
</tr>
<tr>
<td>Systemic symptoms or signs (fever, anorexia, weight loss)†</td>
<td>44 (47.3)</td>
</tr>
<tr>
<td>Morning stiffness, median (range) hours†</td>
<td>3 (1–6)</td>
</tr>
<tr>
<td>Hip involvement†</td>
<td>69 (74)</td>
</tr>
<tr>
<td>Neck involvement†</td>
<td>72 (77)</td>
</tr>
<tr>
<td>Distal symptoms†</td>
<td>31 (33)</td>
</tr>
<tr>
<td>Tenosynovitis†</td>
<td>3 (3.2)</td>
</tr>
<tr>
<td>Pitting edema†</td>
<td>14 (15.1)</td>
</tr>
<tr>
<td>Carpal tunnel syndrome†</td>
<td>12 (12.9)</td>
</tr>
<tr>
<td>Peripheral synovitis†</td>
<td>14 (15.1)</td>
</tr>
<tr>
<td>ESR, median (range) mm/hour†</td>
<td>68 (14–128)</td>
</tr>
<tr>
<td>CRP level, median (range) mg/dl†</td>
<td>4.8 (0.7–18.2)</td>
</tr>
<tr>
<td>Fibrinogen, median (range) mg/dl†</td>
<td>560 (290–788)</td>
</tr>
<tr>
<td>Hemoglobin, median (range) gm/dl†</td>
<td>11.6 (8.2–15)</td>
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* Values are the number (percentage) unless otherwise indicated. ESR = erythrocyte sedimentation rate; CRP = C-reactive protein. † At disease onset.

Evaluation of sIL-6R and sgp130 circulating levels. Venous blood from all patients and controls was collected in tubes without anticoagulant, and serum was separated by centrifugation for 10 minutes at 1,000 g. Samples were divided into aliquots and stored at −80°C until analysis. We analyzed sIL-6R and sgp130 serum levels in PMR patients at disease onset and at 1, 3, 6, 12, and 24 months of followup during corticosteroid therapy and in 46 age-matched normal controls. Serum levels of sIL-6R and sgp130 were evaluated using highly sensitive, commercial sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

Statistical analysis. All continuous data are expressed as the median and 25th to 75th percentiles under the hypothesis of non-normality verified by the Kolmogorov-Smirnov test.

In univariate analysis, the Mann-Whitney test (2 independent groups) or the Kruskal-Wallis test (≥3 independent groups) was used to test hypotheses about medians of different groups. Ordered differences among classes were collected during clinical evaluation at the start of therapy and during the followup period.

Written informed consent was obtained from all patients, and the study was approved by the ethics committee of the hospitals involved.

IL-6 mediates functions through 2 membrane proteins: the IL-6 receptor (IL-6R) and gp130, the signal transducing element. Whereas gp130 is practically ubiquitous, IL-6R expression appears to be limited to certain cell types. This lack of expression is compensated by the presence of a soluble form of IL-6R, which, after binding with IL-6, is able to attach to membrane gp130 and mediate intracellular signaling in IL-6R-negative cells (10). Because the biologic activity of IL-6 strictly depends on the interrelationship among these molecules, we investigated the modulation of systemic levels of soluble IL-6R (sIL-6R) and soluble gp130 (sgp130) in untreated and treated patients with PMR and GCA. Furthermore, studies have shown that persistently elevated levels of IL-6 are an indicator of disease activity in patients with PMR monitored by clinical and laboratory parameters during a followup period of at least of 24 months in order to determine if patients with PMR who are at a higher risk of relapsing (5).
analyzed by the Jonckheere-Terpstra test. Wilcoxon’s test for paired data was used to test hypotheses about medians of sgp130 and sIL-6R levels during followup. Spearman’s correlation analysis was used to assess relationships between variables. The Cox regression survival analysis (with Wald’s statistics) was performed to test if clinical and laboratory parameters were related to relapses.

The Cox regression survival analysis (with backward Wald’s method) was also performed as a multivariate analysis to test if the set of variables, significant at the univariate analysis, produced a good model for predicting relapse, and to identify the relative effect on increasing this risk. The model was adjusted for age at diagnosis, sex, and initial prednisone dose. After dividing the parameters in the model based on a cutoff value, a Kaplan-Meier survival analysis with the Breslow’s test was performed. For all tests, \( P \) values less than 0.05 were considered significant. Statistical analysis was performed using the Statistical Package for the Social Sciences software, version 14.1 (SPSS, Chicago, IL).

RESULTS

The demographic, clinical, and laboratory characteristics of the patients are shown in Table 1. No difference in sIL-6R and sgp130 levels was observed between PMR patients and normal controls at disease onset or during followup (Figure 1).

A significant correlation was found between the number of relapses and sIL-6R concentrations at baseline (rho = 0.334, \( P = 0.01 \)) and after 1 month (rho = 0.246, \( P = 0.023 \)), 3 months (rho = 0.253, \( P = 0.021 \)), and 12 months of therapy (rho = 0.233, \( P = 0.035 \)). In addition, after dividing the parameters in the model based on outcome (no relapses, 1 relapse, or at least 2 relapses), we observed significant ordered differences in sIL-6R levels among classes at baseline, with the highest sIL-6R concentration corresponding to the group of PMR patients who experienced at least 2 relapses (Jonckheere-Terpstra test: \( P < 0.01 \)) (Figure 2). No correlation was found between sgp130 levels (at baseline and during followup) and the number of relapses. Clinical and serologic parameters that, by univariate Cox analysis, were individually significantly associated with relapses are reported in Table 2.

These variables were included in the Cox multivariate analysis adjusted for age, sex, and initial prednisone dose, and the model including sIL-6R levels and hemoglobin value at baseline was the best model for predicting relapses (likelihood ratio test of the model: \( P < 0.0005 \); sIL6R: relative risk 1.02, 95% confidence interval [95% CI] 1.01–1.03, \( P < 0.0005 \); hemoglobin: relative risk 0.614, 95% CI 0.45–0.85, \( P = 0.003 \)). To obtain results with an easier application to clinical practice, we established a cutoff value by evaluating the distribution of these variables in PMR patients who experienced relapse and considering the 95% CI upper limit corresponding to 11.5 gm/dl for hemoglobin and the 95% CI lower limit corresponding to 56 ng/ml for sIL-6R. Cox regression survival analysis, performed by dividing patients based on sIL-6R levels (<56 ng/ml or \( \geq 56 \) ng/ml) and hemoglobin values (<11.5 gm/dl or \( \geq 11.5 \) gm/dl) at diagnosis onset, showed that high levels (\( \geq 56 \) ng/ml) of sIL-6R at baseline were significantly associated with a 4.6-fold higher risk (95% CI 2.45–8.65, \( P < 0.0005 \)) of having at least 1 relapse compared with circulating sIL-6R levels <56 ng/ml. PMR patients with a low hemoglobin value at disease onset (<11.5 gm/dl) had a 2.2-fold higher risk of having at least 1 relapse (95% CI 1.2–4.0, \( P = 0.01 \)) compared with PMR patients with a higher hemoglobin value.

The results obtained by Cox regression survival analysis
testing the reliability of the relapse-predicting models including both sIL-6R levels and hemoglobin values at baseline are shown in Table 3. We found that high sIL-6R levels combined with low hemoglobin values increased the risk of relapse by 10.1 times compared with sIL-6R levels <56 ng/ml and hemoglobin values ≥11.5 gm/dl at baseline.

The Kaplan-Meier survival analysis with Breslow’s test (Figure 3) performed in 4 groups of PMR patients on the basis of sIL-6R and hemoglobin levels showed significant differences in cumulative rates of relapse-free survival according to both sIL-6R levels and hemoglobin values, highlighting the lowest rate in patients with both high sIL-6R levels and low hemoglobin values. Furthermore, sIL-6R appeared to have a stronger influence on the lowering of cumulative rates of relapse-free survival (Figure 3).

We evaluated the levels of sIL-6R in the 2 groups of PMR patients divided according to sIL-6R levels at disease onset (low and high sIL-6R levels) (Figure 4A). In the group with low sIL-6R levels, sIL-6R did not show significant modification during followup; conversely, in the group with high sIL-6R levels, statistically significant decreasing levels were observed by months 1, 6, and 12 (Figure 4A).

We also evaluated the modifications of hemoglobin values in the 2 groups of patients divided according to high and low hemoglobin levels at disease onset (Figure 4B). In both groups, hemoglobin values increased significantly during followup, but in the group with low hemoglobin values, hemoglobin levels at each followup time point were significantly lower than the corresponding values in the group with high hemoglobin values ($P < 0.0005$) (Figure 4B).

## DISCUSSION

Several proinflammatory cytokines have been investigated in patients with PMR and GCA to identify key molecules in driving pathogenic mechanism features that could also be useful in recognizing subsets of patients with chronic, relapsing disease. Identifying a serologic marker with prognostic value would be an essential achievement for guiding the optimal use of corticosteroids that are still the treatment of choice for patients with PMR and GCA.

Local and systemic production of tumor necrosis factor α (TNFα), IL-1, CC chemokine ligand 2 (CCL2/monocyte chemotactic protein 1), and CC chemokine ligand 5 (CCL5/ RANTES) has been found in patients with PMR and GCA.

![Figure 2](image-url)
In patients with GCA, TNFα and CCL2 have been associated with persistence of disease activity (9,17). This evidence supports the idea that TNFα is implicated in the pathogenesis of these diseases, but the recent results of trials with infliximab, which failed to show any benefit induced by the treatment (18–20), have led to the conclusion that TNF does not play a major role in sustaining disease activity, at least in the majority of patients.

PMR is probably a heterogeneous disorder in which some patients experience a regular disease course of a few months to 2 years before stopping steroid treatment, whereas a subset of patients exhibit a prolonged and relapsing course necessitating very long or persistent steroid therapy. The mechanisms of immunoinflammation responsible for this subset of patients are still unknown, but some persistent immune mechanisms have been observed. Indeed, high levels of circulating IL-6 are generally lowered by therapy, but they may remain elevated in some patients, even during long-term treatment (3,5,7). In addition, elevated spontaneous production of vascular endothelial growth factor by PMR peripheral blood mononuclear cells has been shown not to be modulated by steroid treatment (4).

Therefore, in at least some patients, an underlying, more vigorous immune activation may be treatment resistant. The identification of patients with this type of PMR from the very onset of the disease could help in devising and implementing treatment regimens that are more suitable for long-term immune suppression while avoiding the inevitable side effect of prolonged corticosteroid therapy.

In addition to the general agreement concerning elevated circulating IL-6 levels in untreated patients with PMR and GCA (3,5,7,8,14,15) that appear closely correlated with clinical manifestation and disease activity (3,5,8), there is evidence of local expression of IL-6 in the temporal arteries of patients with GCA (9,12). Furthermore, genetic studies have identified a polymorphism in the IL-6 promoter gene that discerns a subgroup of PMR patients with higher risk of relapse/recurrence (21). Although the polymorphism of IL-6 at position −174 was not associated with an increased risk of relapse or recurrence in another population-based study (22), relapse of isolated PMR was associated with HLA–DRB1*0401 (23). Moreover, in the same group of patients, although intercellular adhesion molecule 1 (ICAM-1) polymorphism alone was not associated with disease severity, the presence of both HLA–DRB1*0401 and the ICAM-1 codon 241 GG homozygosity was significantly associated with increased risk of PMR relapse (24).

Together with IL-6, other inflammatory parameters such as ESR and CRP have been evaluated as candidate prognostic markers (5,7,8). A previous study reported an increased risk of relapse in a subset of PMR patients with persistently elevated levels of CRP and IL-6 (5), whereas

![Figure 3. Kaplan-Meier curve. Cumulative rate of relapse-free survival (RFS) for the different subsets of patients with polymyalgia rheumatica divided according to both soluble interleukin-6 receptor (sIL-6R) levels and hemoglobin (Hb) values at diagnosis.](image-url)
Weyand et al (7) identified the value of ESR at disease onset together with the lack of response of circulating IL-6 to corticosteroid therapy as potential predictive tools in identifying subsets of PMR patients with different treatment requirements.

We present evidence that elevated sIL-6R levels at disease onset together with the lack of response of circulating IL-6 to corticosteroid therapy as potential predictive tools in identifying subsets of PMR patients with different treatment requirements.

Figure 4. Serum concentration of A, soluble interleukin-6 receptor (sIL-6R) and B, hemoglobin (Hb) values at baseline and during corticosteroid treatment in the 2 subsets of patients with polymyalgia rheumatica divided according to sIL-6R levels and Hb values at diagnosis. Boxes are the 25th and 75th percentiles. Line within the boxes is the median. Vertical lines below and above the boxes are the 10th and 90th percentiles. Horizontal gray lines define the 25th and 75th percentiles of normal range. Circles indicate outliers. Followup values are compared with values at disease onset: * \( P < 0.01; ** \( P < 0.0005.\)
ease onset (associated with low hemoglobin values) are associated with a significantly elevated risk of relapse/recurrence. Several studies have demonstrated that serum levels of sIL-6R may offer prognostic information. The role of sIL-6R as a prognostic factor has been recognized in multiple myeloma, kidney transplant, and primary hyperparathyroidism (25–27).

The biologic significance of elevated levels of sIL-6R in patients with PMR who experienced relapses and the relationship with clinical manifestations can only be hypothesized. The main sources of circulating sIL-6R are hepatocytes and leukocytes (10) and 2 independent cellular processes have been identified to control sIL-6R production: messenger RNA splicing (DS) and proteolytic cleavage shedding (PC) (10). Serum levels of the DS isoform substantially decrease with age, whereas total circulating levels remain unaltered (10). Studies of rheumatoid arthritis (RA) demonstrated that DS sIL-6R does not account for the increase in sIL-6R found in the serum of these patients, suggesting that the PC isoform is the relevant component of circulating sIL-6R (10). Because PMR characteristically occurs in older patients, and because some clinical, histologic, and serologic manifestations in PMR resemble those seen in elderly patients who develop RA, we could speculate that, in the same manner, PC sIL-6R is the prevailing isoform in the serum of patients with PMR, but experimental support is needed.

Interestingly, CRP is an activator of IL-6R shedding by human neutrophils (28). In addition, sIL-6R could also be released by platelets following activation (29). These could also be important contributions in sustaining mechanisms involved in vascular activation (30). Indeed, there is evidence that IL-6R/IL-6 complexes induce chemokine release and adhesion molecule expression by endothelial cells (ECs), and consequently leukocyte recruitment (30,31). In fact, indirect evidence of EC activation in PMR has been reported (32–35). Modur et al demonstrated that EC activation does not require the addition of exogenous IL-6, indicating that the primary agonist for EC response is sIL-6R and not the cytokine itself (30), even if the issue of whether or not ECs express membrane-associated IL-6R is at least controversial (36,37). This evidence led us to hypothesize that in patients with PMR, high systemic levels of IL-6R might represent an efficient stimulus for persistent systemic immune activation even when IL-6 levels are lowered in response to therapy.

In conclusion, our data support the identification of sIL-6R as a potential prognostic marker of PMR outcome that might have important implications in clinical practice. In addition, sIL-6R may be involved in the pathogenesis of the systemic immune activation that occurs in patients with PMR, even if we have to take into consideration the ability of sIL-6R to neutralize the effect of IL-6 on cells carrying membrane-bound IL-6R. Should this pathogenic hypothesis be confirmed, it would allow the opportunity to evaluate sIL-6R–blocking treatment in patients with PMR and elevated levels of sIL-6R at disease onset, because targeting sIL-6R with blocking antibodies has proven useful in other rheumatic disorders (38,39).

**REFERENCES**


