# Interleukin-6 and soluble interleukin-6 receptor are elevated in large-vessel vasculitis: a cross-sectional and longitudinal study

L. Pulsatelli[1], L. Boiardi[2], E. Assirelli[1], G. Pazzola[2], F. Muratore[2], O. Addimanda[1,3], P. Dolzani[1], A. Versari[2], M. Casali[2], L. Magnani[2], E. Pignotti[1], N. Pipitone[2], S. Croci[2], R. Meliconi[1,3], C. Salvarani[2]

*[1]Rizzoli Orthopaedic Institute, Bologna; [2]Arcispedale Santa Maria Nuova-IRCCS, Reggio Emilia; [3]University of Bologna, Italy.*

*Lia Pulsatelli, BSc*
*Luigi Boiardi, MD*
*Elisa Assirelli, BSc*
*Giulia Pazzola, MD*
*Francesco Muratore, MD*
*Olga Addimanda, MD*
*Paolo Dolzani, BSc*
*Annibale Versari, MD*
*Massimiliano Casali, MD*
*Luca Magnani, MD*
*Elettra Pignotti, PhD*
*Nicolò Pipitone, MD*
*Stefania Croci, PhD*
*Riccardo Meliconi, MD*
*Carlo Salvarani, MD*

*Please address correspondence to:*
*Dr Lia Pulsatelli,*
*Laboratory of Immunorheumatology and Tissue Regeneration,*
*Rizzoli Orthopaedic Institute,*
*via di Barbiano 1/10,*
*40136 Bologna, Italy.*
*E-mail: lia.pulsatelli@ior.it*

*Received on November 17, 2016; accepted in revised form on February 13, 2017.*

*Clin Exp Rheumatol 2017; 35 (Suppl. 103): S102-S110.*



**Key words:** giant cell arteritis, Takayasu's arteritis, positron-emission tomography, interleukin-6, interleukin-6 receptor

*Funding: this work was supported by grants from the University of Bologna, Italy (RFO).*

*Competing interests: N. Pipitone has received honoraria <10,000 USD from GSK, UCB, Alfa-Wassermann, and uptodate.com; the other co-authors have declared no competing interests.*

## ABSTRACT

**Objective.** *To investigate serum levels of IL- 6 and soluble IL-6 receptor (sIL-6R) in patients with large-vessel vasculitis and their relationship with disease activity.*

**Methods.** *Sera were obtained from 33 Takayasu's arteritis (TAK) patients and 14 giant cell arteritis (GCA) patients, and from 60 age-matched normal controls (NCs). Disease activity was assessed using 18F-FDG PET/CT and clinical indices including NIH/Kerr criteria and ITAS. Among TAK patients with active disease at baseline, clinical records and serum samples from 11 TAK patients were available for the longitudinal study. IL-6 and sIL-6R serum levels were evaluated using commercial ELISA kits.*

**Results.** *IL-6 and sIL-6R serum levels were significantly higher in both GCA and TAK patients compared to NCs. IL-6 levels in TAK patients were significantly increased irrespective of disease phase, while a significant increase in sIL-6R concentrations was only found in TAK patients with active disease. Conversely, in GCA, IL-6 levels were significantly raised only in patients with active diseases, whereas sIL-6R levels appeared to be significantly higher irrespective of disease activity. Longitudinal analysis showed that levels of sIL-6R in TAK patients were significantly higher only at baseline, compared to NCs, whereas IL-6 levels were found to be significantly increased at each follow-up time point.*

**Conclusion.** *These overall results might suggest a role for sIL-6R as a potential biomarker for disease activity in TAK patients, whereas in GCA, modifications of IL-6 might better identify patients with active disease.*

## Introduction

Giant cell arteritis (GCA) and Takayasu's arteritis (TAK) are two forms of large-vessel vasculitis (LVV) that involve the aorta and its major branches. GCA typically affects the extracranial branches of the carotid artery and is almost exclusively seen in people aged over 50 years, while TA commonly affects subjects under the age of 40 (1, 2). Despite these differences, the similarities in clinical features (constitutional manifestations and symptoms related to arterial lesions) and the virtually identical histopathological changes of these two LVVs, have raised the question of whether GCA and TAK may be two faces of the same disease (3). Traditionally, LVV disease activity is monitored by defined criteria, including the National Institutes of Health (NIH)/Kerr criteria and the Indian Takayasu Activity Score (ITAS), which take into account clinical findings and (in the Kerr criteria only) laboratory parameters (erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)). Nevertheless, a considerable number of patients considered to be in clinical remission according to these criteria may be histologically active and show evidence of progressive vascular alterations on serial angiograms (4).

In addition, considering that about 5.4% of patients with GCA and 25% of patients with TAK do not show significant changes in ESR and CRP during flares (5, 6), the limitations of acute-phase response as a clinical activity biomarker in LVV are currently well recognised.

Imaging methods, such as computerised tomography (CT), colour Doppler ultrasonography and 18F-Fluorodeoxyglucose-positron emission tomography/CT (18F-FDG PET/CT) may aid in managing patients with LVV. However, while the role of imaging studies is relatively well established in diagnosing untreated patients, their function in assessing disease activity over

time is debated, which underlines the fact that there is a lack of international standards for assessing disease activity in LVVs (7-9).

The Outcome Measures in Rheumatology (OMERACT) Vasculitis Working Group has acknowledged the limitations of the Kerr index and ITAS, thus highlighting the need to develop a validated set of outcome measures for assessing disease activity in LVV and to ultimately define whether the similarities between GCA and TAK justify the application of the same set of outcome measures (7, 8).

Research into reliable circulating biomarkers has led to the identification of some inflammation-related molecules as potential tools for assessing disease activity in LVV (8, 10-12).

In this regard, IL-6 has emerged as a key player in the pathogenesis of numerous inflammatory disorders, including GCA and TAK (13, 14).

IL-6 is an important pleiotropic cytokine encompassing a broad spectrum of biological activities related to the regulation of inflammation, cell proliferation, immunomodulation, haematopoiesis and tumourigenesis (15, 16). Furthermore, several lines of evidence suggest that IL-6 may play a role in inflammation related angiogenesis (15, 17, 18)

IL-6 mediates its functions through two membrane proteins: the IL-6 receptor (IL-6R) and gp130, a signal transducer. While gp130 is practically ubiquitous, IL-6R expression appears to be limited to certain cell types. This lack of expression is compensated for by the presence of a soluble form of IL-6R (sIL-6R), which, after binding with IL-6, is able to associate to membrane gp130 and mediate intracellular signalling in IL-6R-negative cells (13, 19, 20).

The ability of sIL-6R to transduce the IL-6 signal in a variety of cells that harbor only gp130 but do not express transmembrane IL-6R has physiological and pathological implications. Indeed, circulating levels of sIL-6R are responsible for delivering the cytokine and widening the repertoire of IL-6-responsive cells to those that lack membrane-bound IL-6R. This process is known as the IL-6 trans-signalling pathway (13, 19, 20).

Mounting data suggest that blockade of the soluble IL-6 receptor (sIL-6R) might be beneficial for patients with refractory TAK and GCA (21-24).

Since reliable biomarkers for assessing clinical activity in LVV are still lacking, we aimed to investigate serum levels of IL-6 and IL-6R in patients with GCA and TAK, in order to evaluate their relationship with disease activity assessed by means of 18F-FDG PET/CT and clinical indices including the NIH/Kerr and ITAS criteria.

## Methods

*Patients and clinical assessment*

Between January 2003 and September 2010, 47 patients with LVV (33 with TAK and 14 with GCA) who had been referred to the Rheumatology Unit at the Arcispedale S. Maria Nuova in Reggio Emilia in Italy were enrolled in the cross-sectional study. Patients were diagnosed according to the ACR classification criteria for TAK (25) and GCA (26), as appropriate. A semi-quantitative clinical evaluation was performed during their first visit at the unit (considered as the baseline in this study) and during follow-up using the ITAS (27) and the NIH/Kerr (4) indices. The ITAS lists constitutional and organ manifestations with particular emphasis on cardiovascular features as well as inflammatory markers (ESR and CRP) and the physician's global opinion. A score is generated on the basis of present (=1) or absent (=0) clinical manifestations, and disease is considered active if one or more organ system scores positive (27). The NIH/Kerr index assesses four items: constitutional manifestations, raised ESR, manifestations of vascular ischaemia and angiographic features indicative of vasculitis. Disease was defined as active in the presence of at least two new or worsened items (4). For the purpose of this study, due to the heterogeneity of the angiographic modalities, worsening of angiographic findings was represented by the worsening of luminal changes in the affected vessel. Magnetic resonance angiography, computed tomography angiography and/or colour Doppler ultrasound were used to assess luminal changes.

Among the 33 TAK patients with active disease at baseline according to Kerr scores, clinical records and serum samples were available for the longitudinal study, over a mean follow-up period of 18 months (at baseline and at 9 months-T1 and 18 months-T2), from 11 patients, 2 of whom were untreated. Clinical and 18F-FDG PET/CT data from an additional 19 patients (5 with TAK and 15 with GCA) were also included in the cluster analysis (no serum samples were available from the patients in the latter group).

Written informed consent was obtained from all patients according to the Declaration of Helsinky and the study was approved by the local ethics committee.

*18F-FDG PET/CT protocol and imaging analysis*

PET scans were performed using a hybrid PET/CT scanner (Discovery, GE) with 3.30 min-emission scan/bed and CT- attenuation correction. All subjects fasted for ≥4 hours before the 18F-FDG injection (37 Mbq of 18F-FDG/13 kilograms of patient weight). Mean time from injection to acquisition was 60 minutes. Whole-body 18F-FDG PET/CT scanning was performed from mid-femora to external auditory meatus. Vascular uptake was visually graded using a four-point scale (28, 29) ranging from 0 to 3, where 0=no uptake, 1=low-grade uptake (lower than liver uptake), 2=intermediate-grade uptake (similar to liver uptake) and 3=high-grade uptake (higher than liver uptake).

Four aortic segments (ascending thoracic aorta, aortic arch, descending thoracic aorta and abdominal aorta) and the carotid, subclavian, axillary, iliac and femoral arteries were evaluated bilaterally. 18F-FDG vascular uptake scores ≥2 were considered ''positive'' for vasculitis and scores of 0 and 1 were considered ''negative''.

Assessment of the 18F-FDG PET/CT data was carried out by two nuclear medicine specialists who were blinded to clinical and pathological findings.

*Evaluation of IL-6 and sIL-6R circulating levels and acute-phase index assessment*

Two groups, each comprising 30 healthy normal controls (NCs), were matched

for age to the GCA and TAK patients, respectively.

Venous blood was drawn from all the patients and all the NCs between 8.00 a.m. and 10.00 a.m. and collected in tubes without anticoagulant. Serum was separated by centrifugation for 10 minutes 1000 x g. Samples were aliquoted and stored at -80°C until analysis. Serum levels of IL-6 and sIL-6R were evaluated using commercial sandwich enzyme immunoassay (ELISA) kits (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. ESR and CRP levels were measured at the same time points of sample collection for circulating cytokines.

*Statistical analysis*

Continuous data were expressed as median and 25$^{th}$-75$^{th}$ percentiles or as means and standard deviation depending on the distribution of data, unless otherwise indicated. The Kolmogorov Smirnov test was performed to test normality of continuous variables. the Levene test was performed to assess the homoscedasticity.

The ANOVA test was performed to assess the between groups differences of continuous, normally distributed and homoscedastic data, otherwise the Mann Whitney test was used. The ANOVA test followed by the Scheffè *post hoc* pairwise comparison was also used to assess the differences among groups of continuous, normally distributed and homoscedastic data, otherwise the Kruskal-Wallis test followed by the Dunn's *post hoc* correction for multiple comparisons was used. Ordered differences among classes were analysed using the Jonckheere Terpstra test.

The Friedman test for multiple comparisons of paired data was used to test hypotheses about medians of IL-6 and sIL-6R levels during follow up. IL-6 and sIL-6R concentrations at baseline, at T1 and at T2 were compared, respectively, to NCs by applying the Mann-Whitney test followed by Bonferroni's correction for multiple comparisons (a value of $p<0.017$ was considered significant after Bonferroni's correction).

The Spearman rank correlation was used to assess correlations between continuous or interval variables. The Fisher Chi square test was performed to investigate the relationships between dichotomous variables. The Pearson Chi square test evaluated by exact methods for small samples was performed to investigate the relationships between grouping variables.

Two-step Cluster analysis was performed to identify any subgroups of LVV patients with peculiar 18F-FDG PET/CT vascular uptake profiles. The classification was based on maximum likelihood and the number of clusters was chosen according to the BIC. A *post hoc* analysis among clusters was performed to identify which variables characterised each cluster. The *post hoc* analysis among the clusters was corrected for multiple comparison via the Bonferroni method and was based on the ANOVA test to check the equality of means for a continuous variable and the Pearson's chi square test to check the deviations from expected frequency for a categorical variable.

Statistical analysis was carried out using SPSS v. 19.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism for Windows v. 5.0 (CA, USA).

**Table I.** Characteristics of healthy controls (NC), giant cell arteritis (GCA) patients and Takayasu's arteritis (TAK) patients at baseline.

| | NC | GCA | NC | TAK |
|---|---|---|---|---|
| No. of subjects | 30 | 14 | 30 | 33 |
| Age, mean (SD) | 60 (9) | 61 (8) | 33 (6.0) | 42 (14) |
| Sex, no. female (%) | 20 (67)[a] | 8 (57) | 23 (77)[a] | 27 (81) |
| FDG-PET/CT scan, no. positive (%) | NA | 6 (43) | NA | 20 (61) |
| Active disease according to Kerr score no. positive (%) | NA | 7 (50) | NA | 16 (48) |
| Active disease according to ITAS score, no. positive (%) | NA | 7 (50) | NA | 15 (45) |
| ESR, median (range), mm/hr | NA | 12.5 (2.0-120.0) | NA | 28.0 (2.0-116.0) |
| CRP, median (range), mg/dl | NA | 0.64 (0.08-9.59) | NA | 0.87 (0.08-13.39) |
| No. of treated patients (%)[b] | NA | 11 (79) | NA | 25 (74) |

18F-Fluorodeoxyglucose-positron emission tomography/computerised tomography (18F-FDG PET/CT); Indian Takayasu Activity Score (ITAS ); ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; NA: not applicable.

[a]Sex ratio in NC groups was not significantly different compared to respective group of LVV patients (by Fisher's exact test);

[b]Corticosteroid with or without immunosuppresive drug (Methotrexate/azathioprine/cyclophosphamide).

## Results

*IL-6, sIL-6R levels in patients and controls at baseline*

The baseline characteristics of NCs, GCA and TAK patients are reported in Table I.

IL-6 and sIL-6R serum levels were significantly higher in both GCA and TAK patients compared to NCs (IL-6: TAK *vs*. NC $p<0.0001$, GCA *vs*. NC $p<0.005$; sIL-6R: TA *vs*. NC: $p<0.01$, GCA *vs*. NC: $p<0.0001$) (Fig. 1). No significant difference was found between the GCA and TAK patients.

Baseline IL-6 correlated with both ESR and CRP (rho=0.56, $p<0.001$, rho=0.51; $p<0.005$) in TAK patients, but not in GCA patients. No correlation was found between baseline sIL6R levels and either ESR or CRP in both LVVs.

*IL-6, sIL-6R levels and disease activity at baseline*

When stratifying LVV patients according to 18F-FDG PET/CT assessment, similar levels of IL-6 and sIL-6R were observed in patients with an active scan compared to those with an inactive scan in both LVVs (Fig. 2-3).

Similarly, when disease activity was evaluated according to ITAS and NIH/Kerr indices, no difference was found between the soluble factor levels in patients with active disease compared to those with inactive disease.

Compared to NCs, IL-6 levels in TAK patients were significantly increased irrespective of the disease phase (active or inactive), whereas a significant increase in sIL6R concentrations was only found in TAK patients with active disease, as assessed by both 18F-FDG

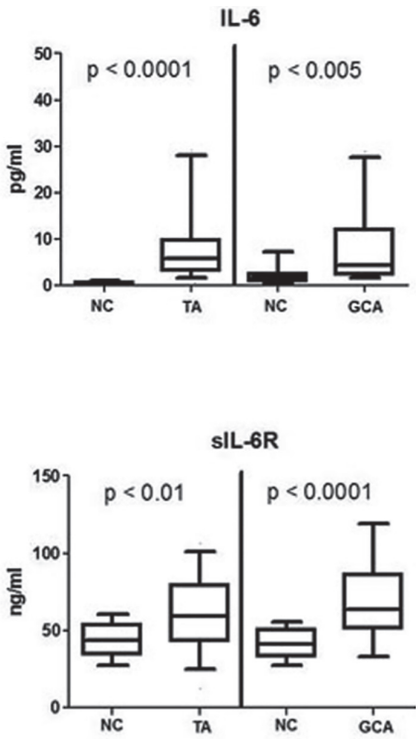


**Fig. 1.** Serum concentrations of IL-6 and sIL-6R in NCs and in patients with TAK and GCA. Boxes show 25th and 75th percentiles. Lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles.

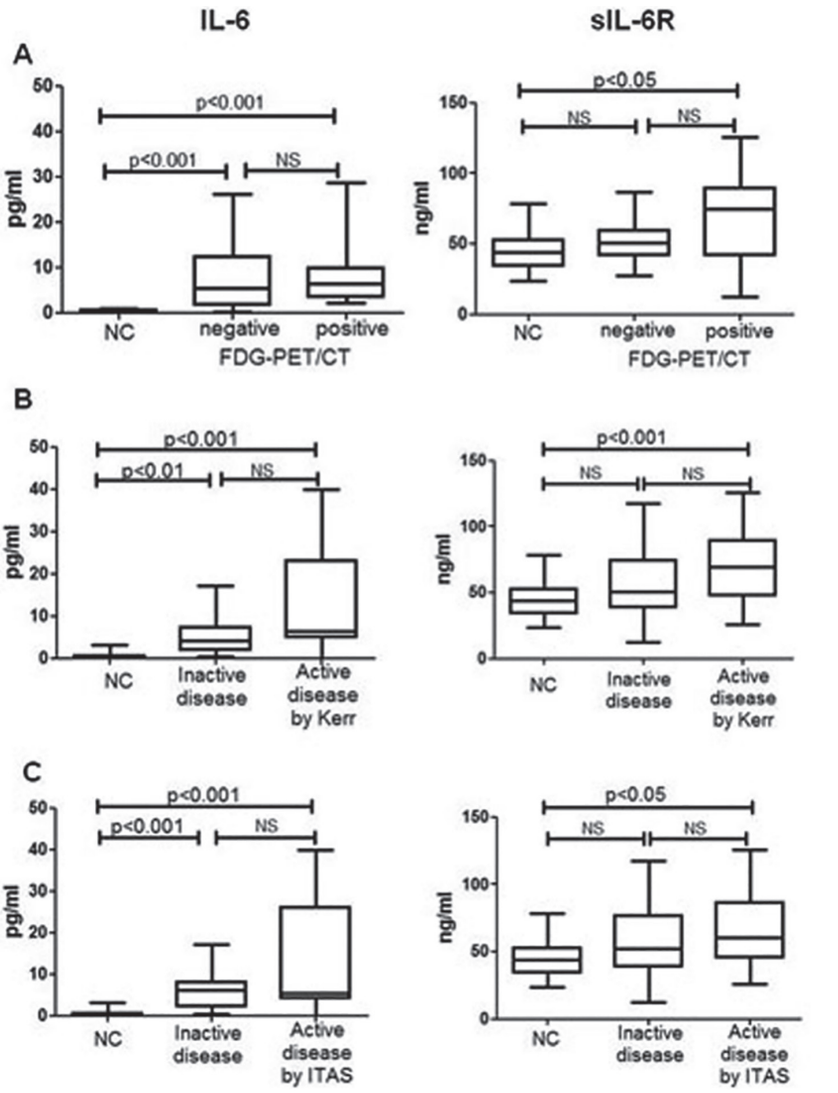


**Fig. 2.** IL-6 and sIL-6R circulating levels in NCs and in TAK patients with active and inactive disease. (**A**) Disease activity assessed by 18F-FDG PET/CT. (**B**) and (**C**) disease activity assessed by clinical indices (NIH/Kerr and ITAS criteria, respectively).
Boxes show 25th and 75th percentiles. Horizontal lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. NS: Not significant.

PET/CT and clinical indices (Fig. 2).
In contrast, IL-6 levels in GCA were significantly raised only in patients with active diseases, whereas sIL-6R levels appeared to be significantly higher irrespective of disease activity compared to NCs (Fig. 3).
Furthermore, a significant positive correlation was found between sIL-6R levels and 18F-FDG PET/CT total score in TAK patients (rho=0.382, $p<0.05$), but not in GCA patients.

*IL6/sIL6R level longitudinal analysis*
The longitudinal analysis was performed on 11 TAK patients with clinically active disease at baseline according to Kerr scores. During the follow-up period, the percentage of patients with active disease progressively decreased (100% at baseline, 18% at T1, 18% at T2) (Fig. 4).
No difference was found between IL6 and sIL6R levels at baseline compared to those estimated at each time point of follow up (Fig. 4).
In addition, IL-6 and IL-6R levels at each follow-up time point were compared to NCs. Levels of sIL-6R were significantly higher only at baseline ($p<0.017$) (a value of $p<0.017$ was considered significant after Bonferroni's correction) compared to NCs, whereas IL-6 levels were found to be significantly increased at each follow-up time point compared to NCs ($p<0.0001$) (Fig. 4).

*Cluster analysis of 18F-FDG PET/CT vascular uptake*
We analysed 18F-FDG PET/CT vascular uptake in four aortic segments (ascending thoracic aorta, aortic arch, descending thoracic aorta and abdominal aorta) and the carotid, subclavian, axillary, iliac and femoral arteries were analysed, bilaterally, by cluster analysis to identify any subgroups of LVV patients with peculiar 18F-FDG PET/CT vascular uptake profiles.
For this analysis, data were obtained by 18F-FDG PET/CT assessment performed in 67 patients with LVV (38 TAK and 29 GCA).
On this basis, 3 clusters were identified (Fig. 5A) and patients' characteristics including age, sex, ESR, CRP, circulating levels of IL-6 and sIL-6R were compared in the 3 clusters.
Cluster 1 (11 TAK, 4 GCA) was characterised by mean vascular uptake scores between 0 and 1, with higher uptake being noted in three aortic seg-

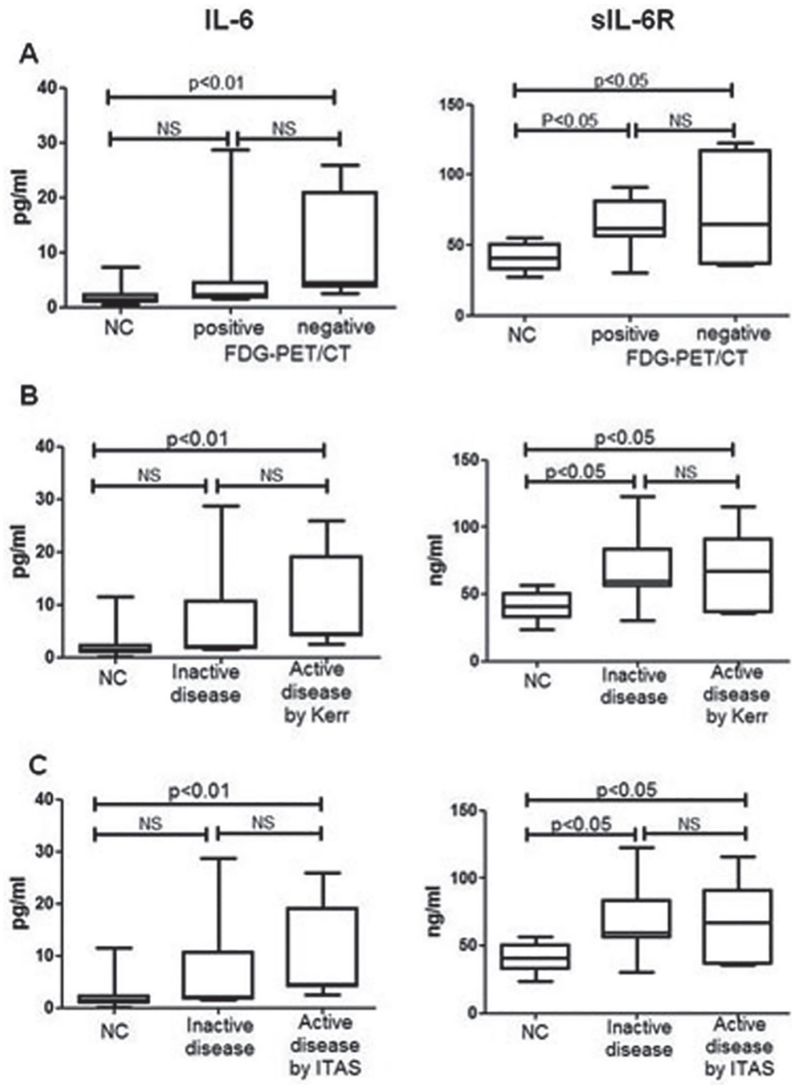


**Fig. 3.** IL-6 and sIL-6R levels in NCs and in GCA patients with active and inactive disease.
(**A**) Disease activity assessed by 18F-FDG PET/CT. (**B**) and (**C**) Disease activity assessed by clinical indices (NIH/Kerr and ITAS criteria respectively).
Boxes show 25th and 75th percentiles. Horizontal lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. NS: Not significant.

ments (ascending thoracic aorta, aortic arch, descending thoracic aorta).
Cluster 2 (8 TAK, 5 GCA) includes patients with mean vascular uptake scores between 0 and 1.7 with maximum uptake seen in femoral arteries.
Cluster 3 (19 TAK, 20 GCA) comprises patients with a mean vascular uptake score between 0.8 and 1.7 with the maximum uptake occurring in three aortic segments (ascending thoracic aorta, aortic arch, descending thoracic aorta).
The number of GCA and TAK patients in each cluster was not significantly different.
We observed significant, ordered differences in ESR and CRP values among clusters, with the highest values being seen in Cluster 3 (Jonckheere Terpstra test: ESR, $p<0.05$; PCR, $p<0.005$) (Fig. 5B).
Considering levels of IL-6 and sIL-6R (available for 47 patients: 33 with TAK and 14 with GCA) and age, no significant, ordered differences were observed across the clusters.
We also stratified IL-6 and sIL-6R levels of LVV patients included in each cluster according to diagnosis and then we compared results to NCs.
Given the small sample size of the GCA patient group in Cluster 1 and Cluster 2, this analysis was limited to TAK patients.
When considering these patients, each cluster was characterised by higher IL-6 levels compared to the NC group (NCs *vs*. Cluster 1: $p<0.01$; NCs *vs*. Cluster 2 and *vs*. Cluster 3: $p<0.001$); on the other hand, sIL-6R levels appeared to be significantly elevated only in Cluster 3 ($p<0.01$) (see supplementary Fig. S1).
In the three clusters, the percentage of patients with positive 18F-FDG PET/CT findings was 27%, 25% and 89%, respectively.

## Discussion

Currently, disease activity assessment in vasculitis remains a challenge in clinical practice. Several soluble factors have been investigated in TAK and GCA patients with the aim of identifying the key molecules responsible for driving the pathogenic mechanism features and which could also be useful for monitoring disease activity and response to treatment (11, 30-33).
The role of the IL-6/IL-6R system in LVV is supported by a body of evidence: serum IL-6 has been shown to be raised in LVV patients (10-12, 34); IL-6 is expressed by vascular lesions (12, 14, 34, 35), the IL-6/IL-17 axis is one of the major pathways involved in pathogenic mechanisms (14), and anti-IL6R antibody (tocilizumab) treatment is effective in ameliorating clinical manifestations (36-38).
In this study, we performed a paired evaluation of circulating levels of IL-6/sIL-6R in patients with GCA and TAK, in order to evaluate their relationship with disease activity.
In line with previously reported findings (8, 10, 31, 32, 39, 40), we can also confirm the elevated levels of IL-6 in LVV compared to NCs. In addition, we also found increased sIL-6R levels compared to healthy subjects in both LVVs.
In a previous study (41) on sIL-6R serum levels in polymyalgia rheumatica (PMR), a disease closely related to GCA, we found no difference in sIL-6R levels between PMR and NCs, either at disease onset or during follow-up. Since, in this study, elevated levels of sIL-6R were observed in GCA patients, these findings might suggest a

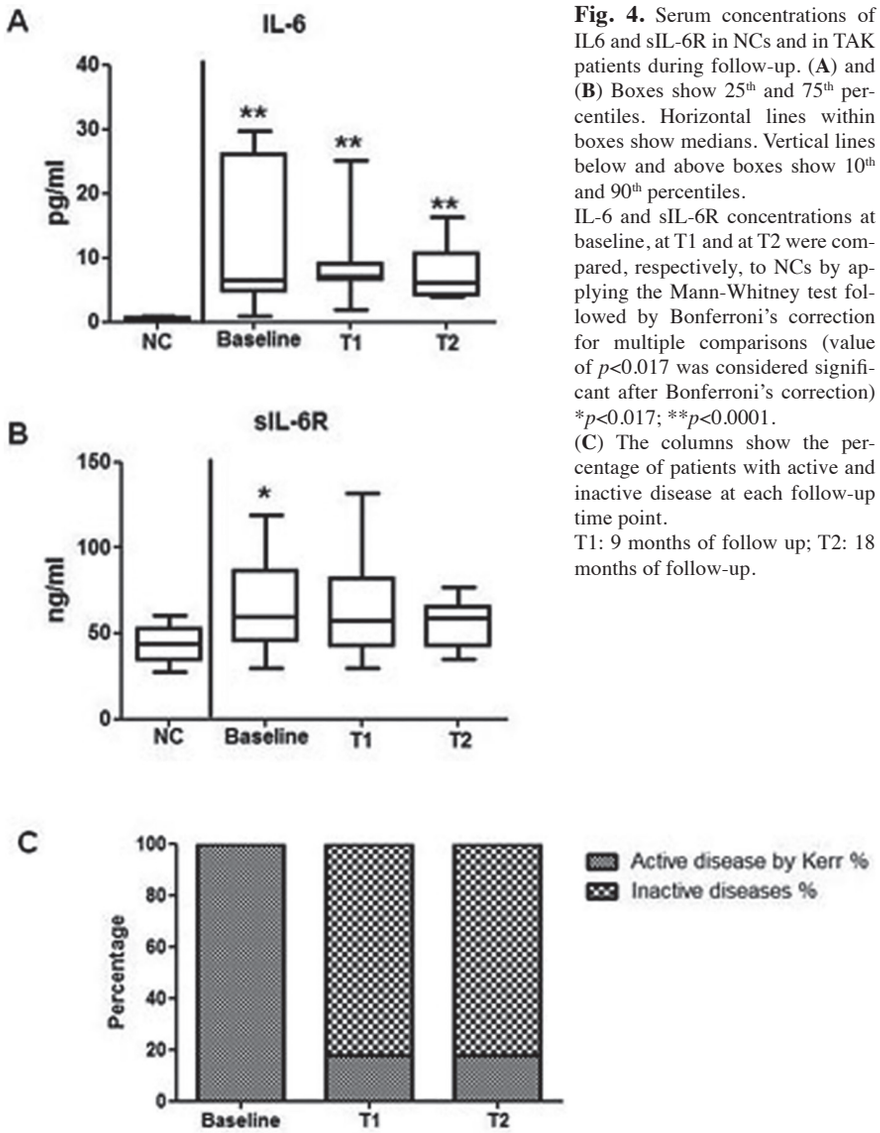


**Fig. 4.** Serum concentrations of IL6 and sIL-6R in NCs and in TAK patients during follow-up. (**A**) and (**B**) Boxes show 25th and 75th percentiles. Horizontal lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles.
IL-6 and sIL-6R concentrations at baseline, at T1 and at T2 were compared, respectively, to NCs by applying the Mann-Whitney test followed by Bonferroni's correction for multiple comparisons (value of $p<0.017$ was considered significant after Bonferroni's correction) *$p<0.017$; **$p<0.0001$.
(**C**) The columns show the percentage of patients with active and inactive disease at each follow-up time point.
T1: 9 months of follow up; T2: 18 months of follow-up.

role of sIL-6R as a potential biomarker for the identification of GCA patients with polymyalgic features. Prompt recognition of GCA has a relevant clinical impact leading to prompt glucocorticoid treatment, which prevents the cranial ischaemic complications of GCA.
However, when LVV patients were stratified according to disease activity as assessed by PET/CT and ITAS and NIH/Kerr scores, respectively, no difference was seen between IL-6 and IL-6R levels in patients with active disease compared to inactive patients.
Currently, the role of IL-6 as a biomarker of disease activity in LVV is still debated. Indeed, even if several lines of evidence have identified IL-6 as a potentially useful molecule for assessing disease activity in LVV (10, 30, 32, 33, 39), other findings, in agreement with our results, showed similar IL-6 serum levels in LVV patients with active and inactive disease (11, 12, 31). When comparing IL-6 and sIL-6R serum levels found in active and inactive LVV patients to NC circulating levels, specific findings were shown to characterise TAK and GCA.
Compared to NCs, IL-6 levels in TAK patients were significantly increased, irrespective of disease phase (active or inactive disease), whereas a significant increase in sIL6R concentrations was only found in TAK patients with active disease as assessed by both 18F-FDG PET/CT and clinical indices.
In contrast, in GCA, IL-6 levels were significantly raised only in patients with active diseases, whereas sIL-6R levels appeared to be significantly higher irrespective of disease activity compared to NCs.
In addition, a significant positive correlation was found between sIL-6R levels and 18F-FDG PET/CT total score in TAK patients only.
These overall results might suggest a role for sIL-6R as a potential biomarker for disease activity in TAK patients, whereas in GCA, modifications of IL-6 might better identify patients with active disease.
The findings from the longitudinal study and the cluster analysis in TAK patients further support the hypothesis concerning the potential role of sIL-6R in assessing disease activity in TAK patients. Indeed, the longitudinal study showed that, compared to NCs, sIL-6R levels appeared to be significantly increased only at baseline, when all the patients were in active disease according to the Kerr score, whereas IL-6 serum concentration remained significantly higher at each time point during follow up.
Cluster analysis identified three clusters, each typified by a peculiar 18F-FDG PET/CT vascular uptake profile and by a progressive increase in the average visual score. Considering TAK patients, each cluster was characterised by higher IL-6 levels compared to the NC group. On the other hand, sIL-6R levels appeared to be significantly elevated only in the TAK patients from the cluster with the highest vascular uptake score in most of the arterial compartments and by the highest percentage of patients with positive 18F-FDG PET/CT findings.
The interesting results obtained from the cluster analysis should be addressed in a future, large-scale multicentre study. In particular, a future longitudinal study may confirm whether different 18F-FDG PET/CT vascular uptake profiles are correlated to peculiar clinical outcomes, such as the risk of developing relapses or recurrences.
Vascular endothelial cells lack membrane-bound IL-6R (13, 42, 43) and the response of these cells to IL-6 is only achievable via the IL-6 trans-signalling pathway (13, 42, 43). Thus, sIL-6R is the limiting factor for conferring IL-6 sensitivity to vascular endothelial cells (44).

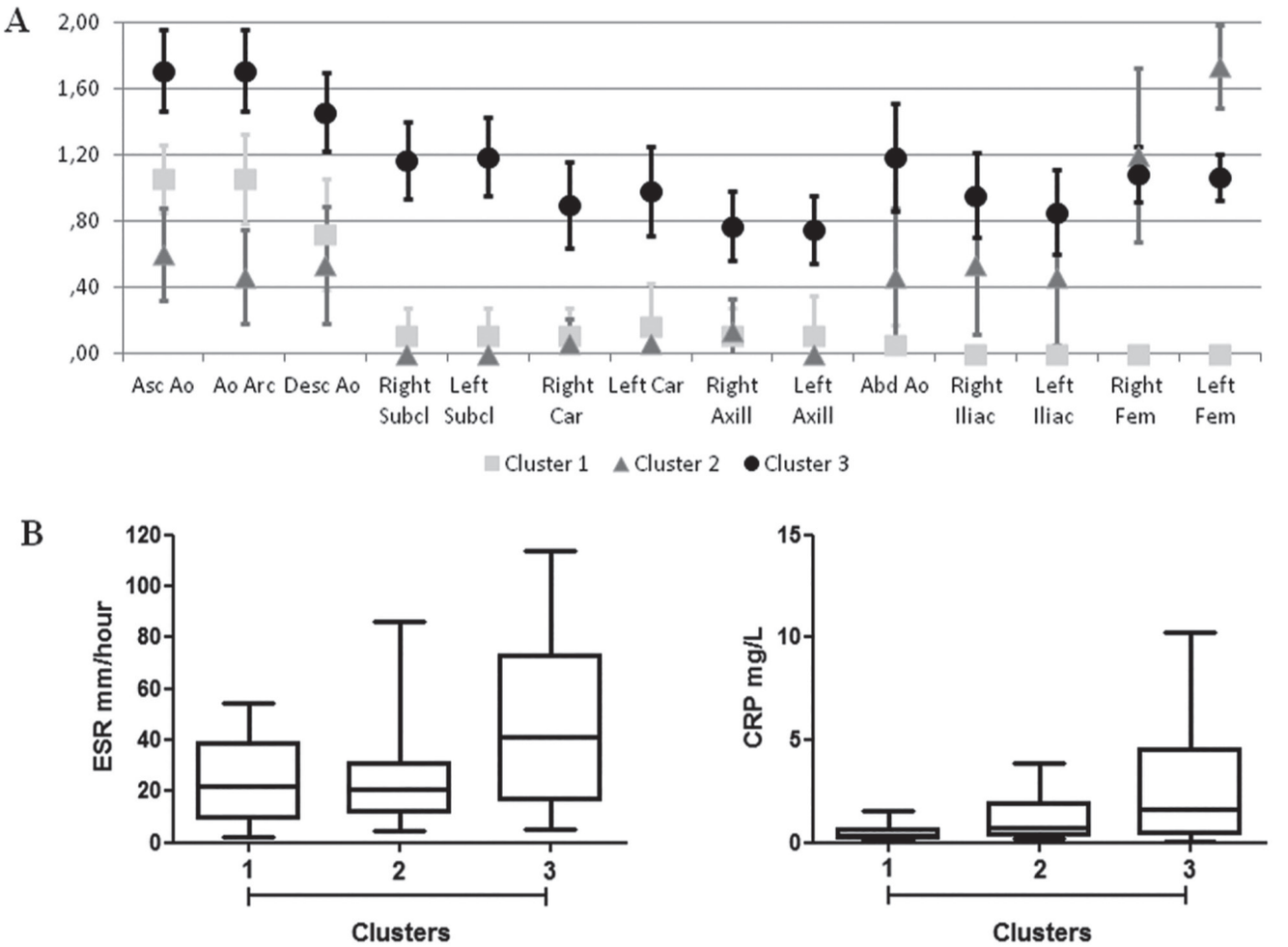


**Fig. 5.** 18F-FDG PET/CT vascular uptake profile (**A**) and acute-phase index levels (**B**) characterising the three clusters.
(**A**) Squares/triangles/circles show means, vertical lines show 95% Confidence interval of the mean.
(**B**) Boxes show 25th and 75th percentiles. Horizontal lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. Ordered differences in ESR and CRP values among clusters were analysed using the Jonckheere Terpstra test (ESR, $p<0.05$; PCR, $p<0.005$).

Furthermore, the role of IL-6/sIL-6R systems in inflammatory diseases is supported by the evidence that different IL-6 activities may be mediated by the IL-6 classic-signalling (via the membrane bound receptor) and trans-signalling pathways (via the soluble IL-6R), respectively. Indeed, IL-6 via the membrane-bound receptor induces protective, regenerative and anti-inflammatory effects on its target cells, while IL-6 response via the sIL-6R is pro-inflammatory (45).

In addition, the role of sIL-6R is not limited to mediating IL-6 signalling, but it may directly control the cellular release of inflammatory mediators. In this regard, evidence that sIL-6R, by itself, induces chemokine release and adhesion molecule expression by endothelial cells has already been reported (44).

Genetic polymorphisms have been found to influence disease susceptibility and the clinical spectrum of GCA (46). In this regard, a single base-change variation at the promoter region (transition G to C position -174 in the 5-region) has been observed in the human IL-6 gene (47). Interestingly, in biopsy-proven GCA patients with PMR manifestations, IL-6 allele C of this polymorphism was increased in frequency compared with GCA patients without PMR (47). This increased frequency of allele C in the whole group of biopsy-proven GCA with PMR manifestations was mainly caused by the subgroup of HLA-DRB1*04–negative patients (47). This suggests that IL-6 promoter polymorphism at position -174 may modulate the phenotypic expression of PMR in biopsy-proven GCA.

Two main limitations of this study should be addressed.

Firstly, a relatively small number of GCA groups were investigated, which specifically affected the statistical power of the analysis of patient subgroups obtained following stratification according to the specific variables. In addition, given the small simple size of GCA patients, this group was only partially investigated. Indeed, the longitudinal study and the analysis performed on IL6/sIL-6R levels in the three groups identified by cluster analysis were limited to TAK patients.

Secondly, the great majority of LVV patients evaluated in the study were in treatment with corticosteroids either with or without immunosuppressive drugs. This issue should be considered when interpreting the results, since

drug regimen might influence IL-6/sIL-6R circulating levels.

The main point of interest raised by this study lies in the novel and original results concerning the role of sIL-6R circulating levels in LVV, in particular in TAK patients. Indeed, to our knowledge, no similar data have been previously reported.

## Conclusions

Considering the central role of sIL-6R as a limiting factor for biological responsiveness to IL-6, our results suggest that in LVV both components of the IL-6/sIL-6R system should be evaluated, since paired modification may better reflect IL-6 involvement in inflammatory mechanisms.

Furthermore, in this study, peculiar findings have been shown to characterise TAK and GCA; indeed in TAK, sIL-6R appears to be a potential biomarker for monitoring disease activity, whereas in GCA patients, active disease might be better identified by IL-6 modifications.

This evidence could strengthen the hypothesis that GCA and TAK are two forms of LVV with distinctive biological features, thus supporting the school of thought which considers GCA and TAK as two distinct disorders rather than as two faces of a single LVV form. Further studies on larger patient populations should focus on evaluating the role of sIL-6R and IL-6 as potential biomarkers for monitoring disease activity in TAK and GCA patients, respectively.

## Acnowledgement

The authors thank Alexandra Teff for English language assistance.

## References

1. WEYAND CM, GORONZY JJ: Medium- and large-vessel vasculitis. *N Engl J Med* 2003; 349: 160-9.
2. BORG FA, DASGUPTA B: Treatment and outcomes of large vessel arteritis. *Best Pract Res Clin Rheumatol* 2009; 23: 325-37.
3. MAKSIMOWICZ-MCKINNON K, CLARK TM, HOFFMAN GS: Takayasu arteritis and giant cell arteritis: a spectrum within the same disease? *Medicine* (Baltimore) 2009; 88: 221-6.
4. KERR GS, HALLAHAN CW, GIORDANO J *et al*.: Takayasu arteritis. *Ann Intern Med* 1994; 120: 919-29.
5. MAKSIMOWICZ-MCKINNON K, CLARK TM, HOFFMAN GS: Limitations of therapy and a guarded prognosis in an American cohort of Takayasu arteritis patients. *Arthritis Rheum* 2007; 56: 1000-9.
6. SALVARANI C, HUNDER GG: Giant cell arteritis with low erythrocyte sedimentation rate: frequency of occurence in a population-based study. *Arthritis Rheum* 2001; 45: 140-5.
7. DIRESKENELI H, AYDIN SZ, KERMANI TA *et al*.: Development of outcome measures for large-vessel vasculitis for use in clinical trials: opportunities, challenges, and research agenda. *J Rheumatol* 2011; 38: 1471-9.
8. ALIBAZ-ONER F, AYDIN SZ, DIRESKENELI H: Advances in the diagnosis, assessment and outcome of Takayasu's arteritis. *Clin Rheumatol* 2013; 32: 541-6.
9. ELEFANTE E, TRIPOLI A, FERRO F, BALDINI C: One year in review: systemic vasculitis. *Clin Exp Rheumatol* 2016; 34 (Suppl. 97): S1-6.
10. VAN DER GEEST KS, ABDULAHAD WH, RUTGERS A *et al*.: Serum markers associated with disease activity in giant cell arteritis and polymyalgia rheumatica. *Rheumatology* (Oxford) 2015; 54: 1397-402.
11. ALIBAZ-ONER F, YENTUR SP, SARUHAN-DIRESKENELI G, DIRESKENELI H: Serum cytokine profiles in Takayasu's arteritis: search for biomarkers. *Clin Exp Rheumatol* 2015; 33: (Suppl. 89): S32-5.
12. SAADOUN D, GARRIDO M, COMARMOND C *et al*.: Th1 and Th17 cytokines drive inflammation in Takayasu arteritis. *Arthritis Rheumatol* 2015; 67: 1353-60.
13. CALABRESE LH, ROSE-JOHN S: IL-6 biology: implications for clinical targeting in rheumatic disease. *Nat Rev Rheumatol* 2014; 10: 720-7.
14. WEYAND CM, GORONZY JJ: Immune mechanisms in medium and large-vessel vasculitis. *Nat Rev Rheumatol* 2013; 9: 731-40.
15. MIHARA M, HASHIZUME M, YOSHIDA H, SUZUKI M, SHIINA M: IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci* (Lond) 2012; 122: 143-59.
16. MIDDLETON K, JONES J, LWIN Z, COWARD JI: Interleukin-6: an angiogenic target in solid tumours. *Crit Rev Oncol Hematol* 2014; 89: 129-39.
17. HERNANDEZ-RODRIGUEZ J, SEGARRA M, VILARDELL C *et al*.: Elevated production of interleukin-6 is associated with a lower incidence of disease-related ischemic events in patients with giant-cell arteritis: angiogenic activity of interleukin-6 as a potential protective mechanism. *Circulation* 2003; 107: 2428-34.
18. O'NEILL L, ROONEY P, MOLLOY D *et al*.: Regulation of Inflammation and Angiogenesis in Giant Cell Arteritis by Acute-Phase Serum Amyloid A. *Arthritis Rheumatol* 2015; 67: 2447-56.
19. JONES SA, HORIUCHI S, TOPLEY N, YAMAMOTO N, FULLER GM: The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *Faseb J* 2001; 15: 43-58.
20. ROSE-JOHN S: IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci* 2012; 8: 1237-47.
21. SALVARANI C, MAGNANI L, CATANOSO M *et al*.: Tocilizumab: a novel therapy for patients with large-vessel vasculitis. *Rheumatology* (Oxford) 2012; 51: 151-6.
22. PAZZOLA G, PADOVANO I, BOIARDI L *et al*.: Tocilizumab in glucocorticoid-naive large-vessel vasculitis. *Clin Exp Rheumatol* 2013; 31 (Suppl. 75): S59-61.
23. SALVARANI C, MAGNANI L, CATANOSO MG *et al*.: Rescue treatment with tocilizumab for Takayasu arteritis resistant to TNF-alpha blockers. *Clin Exp Rheumatol* 2012; 30 (Suppl. 70): S90-3.
24. OLIVEIRA F, BUTENDIECK RR, GINSBURG WW, PARIKH K, ABRIL A: Tocilizumab, an effective treatment for relapsing giant cell arteritis. *Clin Exp Rheumatol* 2014; 32 (Suppl. 82): S76-8.
25. AREND WP, MICHEL BA, BLOCH DA *et al*.: The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis Rheum* 1990; 33: 1129-34.
26. HUNDER GG, BLOCH DA, MICHEL BA *et al*.: The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum* 1990; 33: 1122-8.
27. MISRA R, DANDA D, JAYASEELAN L, SIVAKUMAR R, LAWRENCE A, BACON P: ITAS and DEI.TAK - scores for clinical disease activity and damage extent in Takayasu aortoarteritis (TA). *Rheumatology* (Oxford) 2008; 47: 101.
28. WALTER MA, MELZER RA, SCHINDLER C, MULLER-BRAND J, TYNDALL A, NITZSCHE EU: The value of [18F]FDG-PET in the diagnosis of large-vessel vasculitis and the assessment of activity and extent of disease. *Eur J Nucl Med Mol Imaging* 2005; 32: 674-81.
29. MELLER J, STRUTZ F, SIEFKER U *et al*.: Early diagnosis and follow-up of aortitis with [(18)F]FDG PET and MRI. *Eur J Nucl Med Mol Imaging* 2003; 30: 730-6.
30. NORIS M, DAINA E, GAMBA S, BONAZZOLA S, REMUZZI G: Interleukin-6 and RANTES in Takayasu arteritis: a guide for therapeutic decisions? *Circulation* 1999; 100: 55-60.
31. SUN Y, MA L, YAN F *et al*.: MMP-9 and IL-6 are potential biomarkers for disease activity in Takayasu's arteritis. *Int J Cardiol* 2012; 156: 236-8.
32. PARK MC, LEE SW, PARK YB, LEE SK: Serum cytokine profiles and their correlations with disease activity in Takayasu's arteritis. *Rheumatology* (Oxford) 2006; 45: 545-8.
33. WEYAND CM, FULBRIGHT JW, HUNDER GG, EVANS JM, GORONZY JJ: Treatment of giant cell arteritis: interleukin-6 as a biologic marker of disease activity. *Arthritis Rheum* 2000; 43: 1041-8.
34. KONG X, SUN Y, MA L *et al*.: The critical role of IL-6 in the pathogenesis of Takayasu arteritis. *Clin Exp Rheumatol* 2016; 34 (Suppl. 97): S21-S7.
35. HERNANDEZ-RODRIGUEZ J, SEGARRA M, VILARDELL C *et al*.: Tissue production of pro-inflammatory cytokines (IL-1beta, TNFalpha and IL-6) correlates with the intensity of the systemic inflammatory response and

with corticosteroid requirements in giant-cell arteritis. *Rheumatology* (Oxford) 2004; 43: 294-301.
36. FERFAR Y, MIRAULT T, DESBOIS AC *et al*.: Biotherapies in large vessel vasculitis. *Autoimmun Rev* 2016; 15: 544-51.
37. LORICERA J, BLANCO R, CASTANEDA S *et al*.: Tocilizumab in refractory aortitis: study on 16 patients and literature review. *Clin Exp Rheumatol* 2014; 32 (Suppl. 82): S79-89.
38. LORICERA J, BLANCO R, HERNANDEZ JL *et al*.: Tocilizumab in patients with Takayasu arteritis: a retrospective study and literature review. *Clin Exp Rheumatol* 2016; 34 (Suppl. 97): S44-53.
39. ROCHE NE, FULBRIGHT JW, WAGNER AD, HUNDER GG, GORONZY JJ, WEYAND CM: Correlation of interleukin-6 production and disease activity in polymyalgia rheumatica and giant cell arteritis. *Arthritis Rheum* 1993; 36: 1286-94.
40. DASGUPTA B, PANAYI GS: Interleukin-6 in serum of patients with polymyalgia rheumatica and giant cell arteritis. *Br J Rheumatol* 1990; 29: 456-8.
41. PULSATELLI L, BOIARDI L, PIGNOTTI E *et al*.: Serum interleukin-6 receptor in polymyalgia rheumatica: a potential marker of relapse/recurrence risk. *Arthritis Rheum* 2008; 59: 1147-54.
42. ROMANO M, SIRONI M, TONIATTI C *et al*.: Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* 1997; 6: 315-25.
43. HOU T, TIEU BC, RAY S *et al*.: Roles of IL-6-gp130 signaling in vascular inflammation. *Curr Cardiol Rev* 2008; 4: 179-92.
44. MODUR V, LI Y, ZIMMERMAN GA, PRESCOTT SM, MCINTYRE TM: Retrograde inflammatory signaling from neutrophils to endothelial cells by soluble interleukin-6 receptor alpha. *J Clin Invest* 1997; 100: 2752-6.
45. SCHAPER F, ROSE-JOHN S: Interleukin-6: Biology, signaling and strategies of blockade. *Cytokine Growth Factor Rev* 2015; 26: 475-87.
46. GONZALEZ-GAY MA, AMOLI MM, GARCIA-PORRUA C, OLLIER WER: Genetic markers of disease susceptibility and severity in giant cell arteritis and polymyalgia rheumatica. *Semin Arthritis Rheum* 2003; 33: 38-48.
47. GONZALEZ-GAY MA, HAJEER AH, DABABNEH A *et al*.: IL-6 promoter polymorphism at position-174 modulates the phenotypic expression of polymyalgia rheumatica in biopsy-proven giant cell arteritis. *Clin Exp Rheumatol* 2002; 20: 179-84.