
Imbalance between angiogenic and anti-angiogenic factors in sera from patients with large-vessel vasculitis

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ABSTRACT

Objective. To investigate serum levels of a panel of angiogenic inducers (VEGF, FGF-2, Angiopoietin 1, -2, soluble VCAM-1) and inhibitors (angiostatin, endostatin, pentraxin-3) in patients with giant cell arteritis (GCA) and Takayasu's arteritis (TAK), in order to gain further insights into the molecular mechanisms driving angiogenesis dysregulation in large-vessel vasculitis (LVV).

Methods. Sera were obtained from 33 TAK patients and 14 GCA patients and from two groups of age-matched normal controls (NC). Disease activity was assessed using 18F-FDG PET/CT and clinical indices including NIH/Kerr criteria and ITAS. Angiogenic and anti-angiogenic factor serum levels were evaluated using commercial ELISA kits. Pentraxin 3 (PTX3) serum levels were evaluated by non-commercial ELISA, as already described.

Results. Among the angiogenic factors, only VEGF serum levels were significantly higher in TAK patients compared to NC. No difference was found between angiogenic factor levels in GCA patients compared to those detected in NC. Anti-angiogenic factor (Angiostatin, Endostatin, PTX3) serum levels were significantly higher in both GCA and TAK patients compared to NC. Significant associations were observed between VEGF and PTX3 levels and disease activity evaluated using PET scan and clinical indices. Cluster analysis based on PET scan scores in TAK patients showed significant ordered differences in VEGF and angiostatin serum levels. Indeed, we noted a progressive increase of VEGF and angiostatin from NC to the cluster including patients with the highest and more diffuse scan positivity.

Conclusion. Our overall results demonstrate a circulating molecular profile

characterised by a prevailing expression of anti-angiogenic soluble factors.

Introduction

Large-vessel vasculitides (LVV) are immune-mediated vascular diseases that typically affect the aorta and its major branches. Giant cell arteritis (GCA) and Takayasu's arteritis (TAK) are the two major forms of LVV. GCA typically affects the extracranial branches of the carotid artery and is almost exclusively seen in people aged over 50 years, while TAK commonly affects subjects under the age of 40 (1, 2).

LVV are characterised by dysfunctional angiogenesis and by chronic inflammatory lesions within the vessel wall. Vascular remodelling in response to aberrant immune response leads to occlusion of the lumen and ischaemic damage of medium-sized arteries, whereas in the aorta, aneurysm formation, rupture or dissection are more frequently seen (3-5).

In vasculitis immune-mediated mechanisms, a dual role of angiogenesis has been hypothesised. Indeed, angiogenesis might be primarily involved in perpetuating inflammation and, subsequently, might be considered as a rescue mechanism in ischaemic damage (6).

The role of angiogenesis in LVV is also supported by histopathological analyses that have shown new capillary formation in the arterial media and intima, normally avascular (7, 8). In addition, a marked neoangiogenesis expanding the capillary network of the adventitia was recognised as a further pathogenic event. Adventitial vasa vasorum drive T cells and macrophages invasion of the vessel wall, and promote their differentiation. Recent studies have strengthened the role of interplay between tissue invasive T-cell and endothelial cells and stromal cells in the vessel wall, ultimately leading to formation of microvessels and

expansion of the intimal layer (9, 10). Angiogenesis is a complex and highly dynamic process that is finely tuned by circulating factors as well as locally produced mediators (11, 12).

Physiological formation of new blood vessels derives from the net balance between angiogenic inducers and inhibitors (positive and negative regulators of angiogenesis) (11, 12).

A wide array of factors is involved in this process. Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), angiopoietins, soluble adhesion molecules (such as soluble vascular cellular adhesion molecule sVCAM-1) are recognised as positive regulators of angiogenesis, whereas the major inhibitors include endostatin, angiostatin, thrombospondin-1, the first endogenous protein to be identified as an inhibitor of angiogenesis, and thrombospondin-2 (11-15).

VEGF, FGF and PDGF are expressed in vasculitic lesions (7, 8, 16, 17), as well as the up-regulation of circulating levels of some proangiogenic factors was observed in patients with LVV (6, 18-20). On the other hand, pentraxin (PTX) 3, the prototypical long PTX that has been recognised as an antiangiogenic factor by binding to FGF-2 and inhibiting FGF-2-dependent endothelial cell proliferation and neovascularisation (21-23), is to be up-regulated at both vascular and circulating levels in LVV (19, 24, 25) and also in small-vessel vasculitis (26-28)

In order to gain further insights into the molecular mechanisms driving angiogenesis dysregulation in LVV, we investigated the serum levels of a panel of angiogenic inducers (VEGF, FGF-2, soluble vascular cellular adhesion molecule sVCAM-1, angiopoietin 1, -2) and inhibitors (angiostatin, endostatin, PTX3) in patients with GCA and TAK. Furthermore, we analysed the soluble factor levels according to disease activity assessed by means of 18F-Fluorodeoxyglucose-positron emission tomography/computerised tomography (18F-FDG PET/CT) and clinical indices including the National Institutes of Health (NIH)/Kerr criteria and the Indian Takayasu's Activity Score (ITAS).

Materials and 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, Italy were enrolled in the cross-sectional study. Patients were diagnosed according to the ACR classification criteria for TAK (29) and GCA (30), as appropriate.

As a tertiary reference centre for vasculitis, we admit patients with suspected, early, or established LVV for re-evaluation of treatment and/or disease activity. Among our patients, 36 (11 GCA) were on treatment: 19 received steroid only (prednisone: 16.07 ± 12.35 mg/day, mean \pm standard deviation), 13 received immunosuppressive agents (methotrexate, azathioprine, mycophenolate, cyclophosphamide) with or without steroid, and 4 received biologic agents (2 infliximab, 1 adalimumab, 1 tocilizumab).

Patients with LVV are offered a standardised screening at the first visit and then yearly in order to obtain a comprehensive evaluation of the disease activity and extension by means of duplex sonography, computerised tomography (CT), or magnetic resonance (MR), angiography (CTA, MRA), and 18F-FDG PET/CT. This protocol includes the determination of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) at the time of 18F-FDG PET/CT evaluation.

A semi-quantitative clinical evaluation was performed during their first visit at the unit (considered as the baseline in this study) using the ITAS (31) and the NIH/Kerr (32) 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 was considered active if one or more organ system scores positive (31). 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 (32). 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 or the appearance of new vascular lesions. MRA, CTA and/or colour Doppler ultrasound were used to assess luminal changes.

Clinical and 18F-FDG PET/CT data from an additional 20 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 Helsinki and the study was approved by the local ethics committee.

18F-FDG PET/CT protocol and imaging analysis

Positron emission tomography 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 (33, 34) 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 and 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 (AV, MC).

Evaluation of angiogenesis-related factor levels and acute-phase index assessment

Two groups of healthy subjects, matched for age to the GCA and TAK patients, respectively, were evaluated as normal controls (NC).

Venous blood was drawn from all the patients and all the NC 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 VEGF, sVCAM-1, FGF-2, endostatin, angiopoietin-1, angiopoietin-2, angiostatin were evaluated using commercial sandwich enzyme immunoassay following the manufacturer's instructions. We used ELISA kits from R&D Systems, Minneapolis, MN, USA for detection of VEGF, sVCAM-1, FGF, Angiopoietin-1 Angiopoietin-2 and endostatin; serum angiostatin levels were measured using ELISA kits from Cusabio College Park, MD, USA.

Serum levels of PTX3 were evaluated by means of a sandwich ELISA as previously described (35).

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 25th-75th 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.

Comparison of angiogenic/anti-angiogenic factor levels between the two independent groups was performed by non-parametric analysis using the Mann-Whitney test, since distribution of data did not fulfill the assumptions of normality and homoscedasticity.

The Spearman rank correlation was used to assess correlations between continuous variables. The Fisher Chi square test was performed to investigate the relationships between dichotomous variables.

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

	NC	GCA	NC	TAK
No. of subjects	21	14	25	33
Age, mean (SD)	64 (10)	61 (8)	40 (9.0)	42 (14)
Sex, no. female (%)	12 (57) ^a	8 (57)	17 (68) ^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-FDG PET/CT: 18F-Fluorodeoxyglucose-positron emission tomography/computerised tomography; ITAS: Indian Takayasu's activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; NA: not applicable.

^aSex ratio in NC groups was not significantly different compared to respective group of LVV patients (using Fisher's exact test) (NC vs. GCA: $p=1.000$; NC vs. TAK: $p=0.353$).

^bCorticosteroid with or without immunosuppressive drug (methotrexate/azathioprine/cyclophosphamide) /biological drugs.

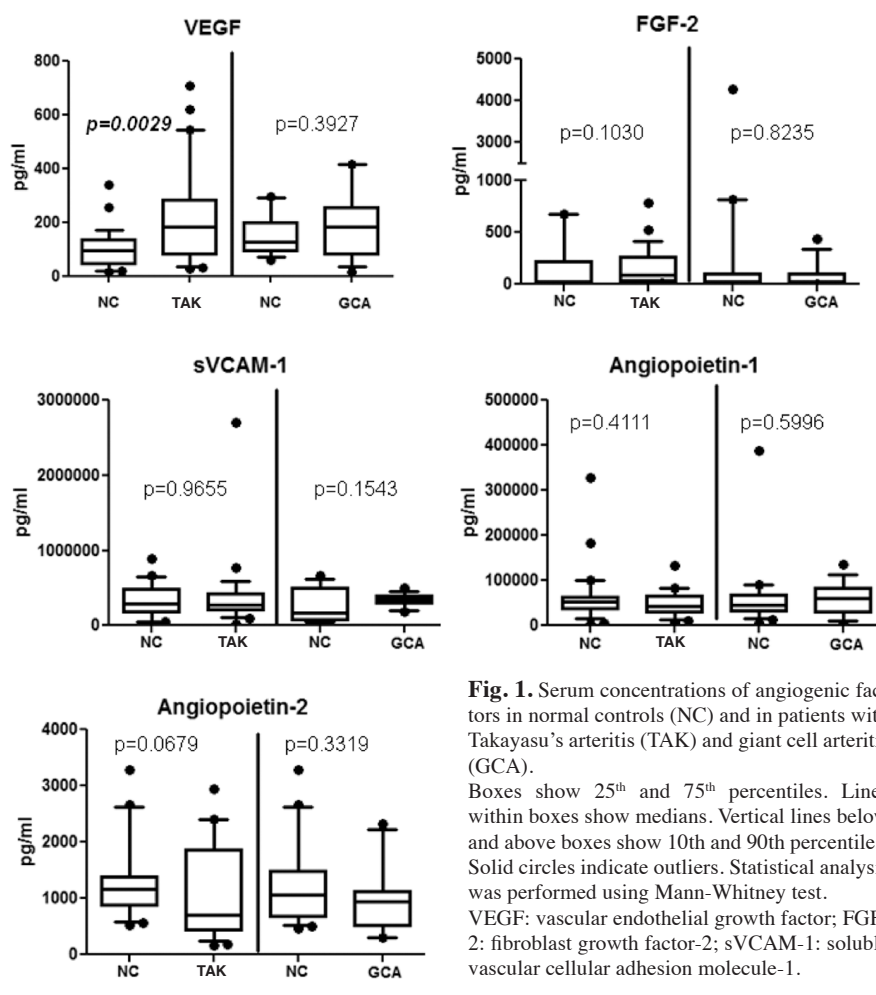


Fig. 1. Serum concentrations of angiogenic factors in normal controls (NC) and in patients with Takayasu's arteritis (TAK) and giant cell arteritis (GCA). Boxes show 25th and 75th percentiles. Lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. Solid circles indicate outliers. Statistical analysis was performed using Mann-Whitney test. VEGF: vascular endothelial growth factor; FGF-2: fibroblast growth factor-2; sVCAM-1: soluble vascular cellular adhesion molecule-1.

As already reported in a previous study (36), two-step Cluster analysis was performed to identify any subgroups of LVV patients with peculiar 18F-FDG PET/CT vascular uptake profiles.

A *post hoc* analysis among clusters was performed to identify which variables characterised each cluster (36). In the present study, comparison of angiogenesis-related factor levels among

clusters was performed by applying the Kruskal-Wallis test followed by the Dunn's post-hoc correction.

Ordered differences among classes were analysed using the Jonckheere Terpstra test. Statistical analysis was carried out using SPSS version 19.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism for Windows v. 5.0 (CA, USA).

Results

Angiogenesis-related factor levels in patients and controls

The baseline characteristics of NC, GCA and TAK patients are reported in Table I. Among the angiogenic factors, only VEGF serum levels were significantly higher in TAK patients compared to NC ($p=0.0029$) (Fig. 1).

No difference was found for angiogenic factor serum levels between GCA patients and NC.

Anti-angiogenic factor (Angiostatin, Endostatin, PTX3) serum levels were significantly higher in both GCA and TAK patients compared to NC (Fig. 2). No significant difference was found between GCA and TAK patients.

In addition, we did not find a significant difference between the treated and untreated patients in our series (Data in Supplementary file: Table S1).

No correlation was found between VEGF and anti-angiogenic factor (Angiostatin, Endostatin, PTX3) serum levels. Furthermore, no correlation was observed between angiogenic/anti-angiogenic factors and either ESR or CRP in both LVVs (data not shown).

Among the angiogenic factors, as only VEGF serum levels were significantly modified in LVV patients compared to NC, we also evaluated the VEGF/Angiostatin, VEGF/endostatin and VEGF/PTX3 ratios in order to better investigate the reciprocal modification between VEGF and the anti-angiogenic factors. Only the VEGF/endostatin ratio was significantly lower in GCA patients compared to NC ($p=0.0325$) (Suppl. Fig. S1).

Angiogenesis-related factor levels and disease activity at baseline

When stratifying LVV patients according to 18F-FDG PET/CT assessment, no significant increase in all the factor levels was observed in patients with an

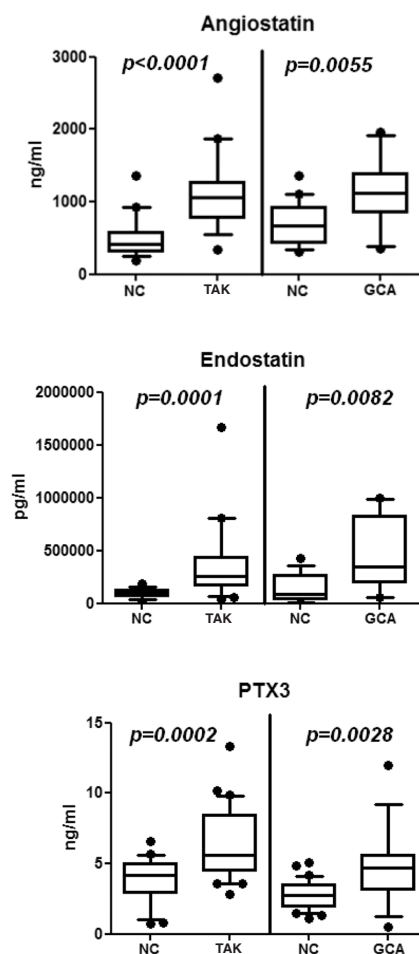


Fig. 2. Serum concentrations of anti-angiogenic factors in normal controls (NC) and in patients with Takayasu's arteritis (TAK) and giant cell arteritis (GCA).

Boxes show 25th and 75th percentiles. Lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. Solid circles indicate outliers. Statistical analysis was performed using Mann-Whitney test. PTX3: pentraxin 3.

active scan, compared to those with an inactive scan, except for PTX3 in TAK patients (Suppl. Fig. S2). Similar results were obtained when considering VEGF/Angiostatin, VEGF/endostatin and VEGF/PTX3 ratios (data not shown).

Furthermore, no significant correlation was found between soluble factor levels and 18F-FDG PET/CT total score in either TAK or GCA patients.

When disease activity was evaluated according to NIH/Kerr and ITAS indices, only VEGF serum levels appeared to be significantly up-regulated in GCA patients with active disease, whereas in terms of all the other soluble factors, no difference was found in patients with active disease compared

to those with inactive disease (Suppl. Fig. S3, S4). No significant changes of VEGF/Angiostatin, VEGF/endostatin and VEGF/PTX3 ratios were found in patients with active disease compared to those with inactive disease (data not shown).

Cluster analysis of 18F-FDG PET/CT vascular uptake

As already reported in a previous study (36), 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, 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) and, on this basis, 3 clusters were identified and were characterised as previously detailed (36) (Fig. 3).

We stratified the angiogenic and anti-angiogenic factor levels of LVV patients included in each cluster (available for 47 patients: 33 with TAK and 14 with GCA) according to diagnosis and then we compared the angiogenesis-related factors levels in each cluster to NC.

Given the small sample size of the GCA patient group in Cluster 1 and Cluster 2 (36), this analysis was limited to TAK patients.

When considering these patients, VEGF, angiostatin and PTX3 levels appeared to be significantly elevated only in Cluster 3 compared to the NC group (Figs. 4-5), whereas endostatin levels were found to be higher in Clusters 1 and 3 (Fig. 5).

Furthermore, significant ordered differences in VEGF and angiostatin serum levels among clusters were also observed, with the highest concentration corresponding to Cluster 3 (Jonckheere Terpstra test: $p=0.001$ and $p<0.0005$ respectively) (Figs. 4-5).

As already reported, in the three clusters, the percentage of patients with positive 18F-FDG PET/CT findings was 27%, 25% and 89%, respectively (36).

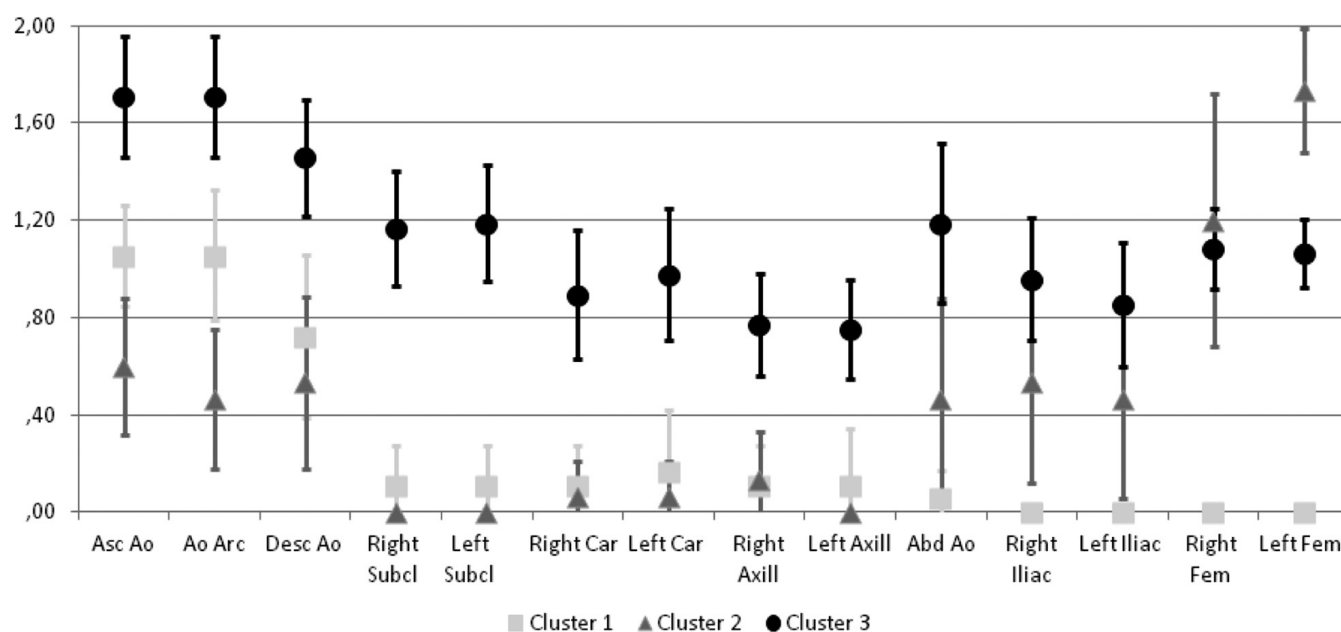


Fig. 3. 18F-FDG PET/CT vascular uptake profile characterising the three clusters (Pulsatelli *et al.*, *Clin Exp Rheumatol* 2017; 35 (Suppl. 103): S102-10, with permission of *Clinical and Experimental Rheumatology*). Squares/triangles/circles show means, vertical lines show 95% Confidence Interval of the mean.

Discussion

In this study, we performed a paired evaluation of circulating levels of a set of positive (VEGF, FGF-2, Angiopoietin-1, -2, sVCAM-1) and negative (endostatin, angiostatin, PTX3) regulators of angiogenesis in patients with GCA and TAK, in order to gain further insight into the angiogenesis-driving mechanisms in LVV.

Our overall results demonstrated a circulating molecular profile characterised by a prevailing expression of anti-angiogenic soluble factors in LVV.

Indeed, among the angiogenic factors, only VEGF serum levels were significantly higher in TA patients compared to NC. On the other hand, anti-angiogenic factor (angiostatin, endostatin, PTX3) serum levels were significantly higher in both GCA and TAK patients compared to NC. The cluster analysis limited to TAK patients and the VEGF/endostatin ratio (which was significantly lower in GCA patients compared to NC) further confirms the imbalance towards an anti-angiogenic pattern.

The up-regulation of the anti-angiogenic mediators could, on the one hand, counterbalance the inflammation-induced angiogenesis, but, on the other, it might contribute to ischaemic damage.

Up to now, the modulation of anti-angiogenic factors has barely been investigated.

Recently, plasma levels of the N-terminal fragment of Chromogranin-A (CgA), which have a recognised inhibitory effect on angiogenesis, have been found markedly increased in TAK patients, confirming an up-regulation of the anti-angiogenic inducers in this LVV (37).

Furthermore, a genome-wide association study has identified several variants within *PLG* gene associated with the risk of developing GCA. Interestingly, this gene encodes a secreted blood zymogen that can be converted to plasmin and angiostatin and, considering the opposing role of these factors in the angiogenic process, *PLG* risk alleles could contribute to the imbalance of the angiogenic mechanism in GCA (38).

It is noteworthy that in other vasculitides angiogenic factors are elevated. Indeed, in Kawasaki disease (KD), a systemic vasculitis that predominantly affects infants and children, significantly higher VEGF levels and lower endostatin levels have been observed (39).

In small vessel ANCA-associated vasculitis, increasing levels of VEGF, sVCAM-1, Angiopoietin-2 and PTX3 have been reported (27, 40-42).

Thus, considering the above-mentioned results, our findings may support the hypothesis of a distinctive proangiogenic/anti-angiogenic profile in LVV.

In contrast with our results, high circulating levels of VEGF have been previously reported in GCA patients (19, 43, 44).

In these papers, VEGF up-regulation in GCA may be due to the inclusion of various subgroups of GCA patients (*e.g.* biopsy-proven GCA *versus* clinically diagnosed GCA patients, and GCA patients with very recent optic nerve ischaemia *versus* patients without optic ischaemia), which typically showed higher levels of VEGF compared to the other GCA patients.

It should also be brought to mind that the variability of VEGF circulating levels is also determined by a functional genetic variant that affects gene expression and circulating protein levels (45) and by platelet release in serum samples (43, 46, 47).

In TAK patients, we found a significant increase in VEGF circulating levels compared to NC. These results may be considered in line with those obtained by Dogan *et al.* (20), that showed higher, albeit without statistical significance, VEGF levels in TA patients compared to NC. In the same study, in contrast with our results, the authors reported a significant increase in VEGF levels in patients with active disease compared to those with inactive disease. These conflicting results should be interpreted

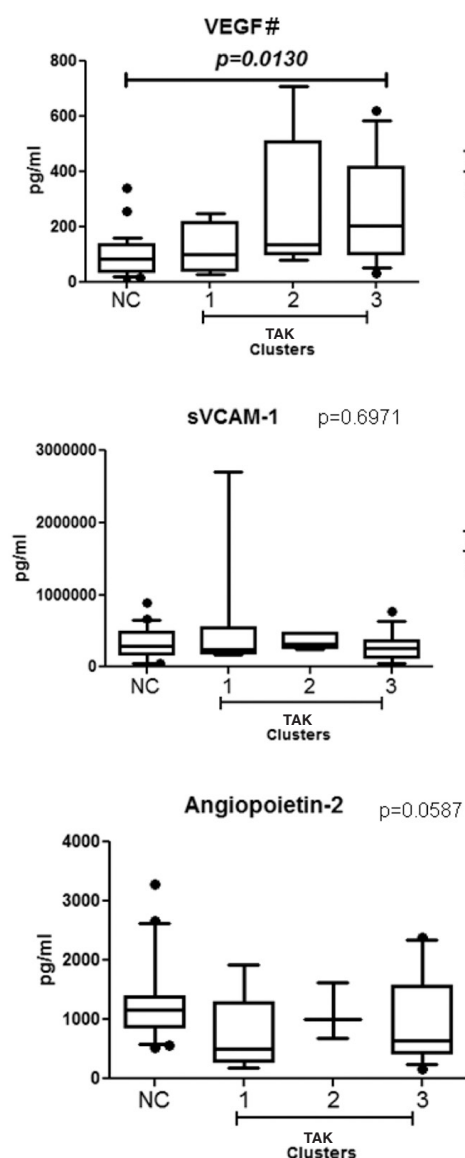


Fig. 4. Angiogenic factor levels in three TAK groups identified by cluster analysis. Boxes show 25th and 75th percentiles. Horizontal lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. Solid circles indicate outliers. Comparison of angiogenic factor levels among clusters was performed using the Kruskal-Wallis test followed by the Dunn's *post-hoc* correction. #Significant ordered differences among clusters (Jonckheere Terpstra test): $p=0.001$. TAK: Takayasu's arteritis; VEGF: vascular endothelial growth factor; FGF-2: fibroblast growth factor-2; sVCAM-1: soluble vascular cellular adhesion molecule-1.

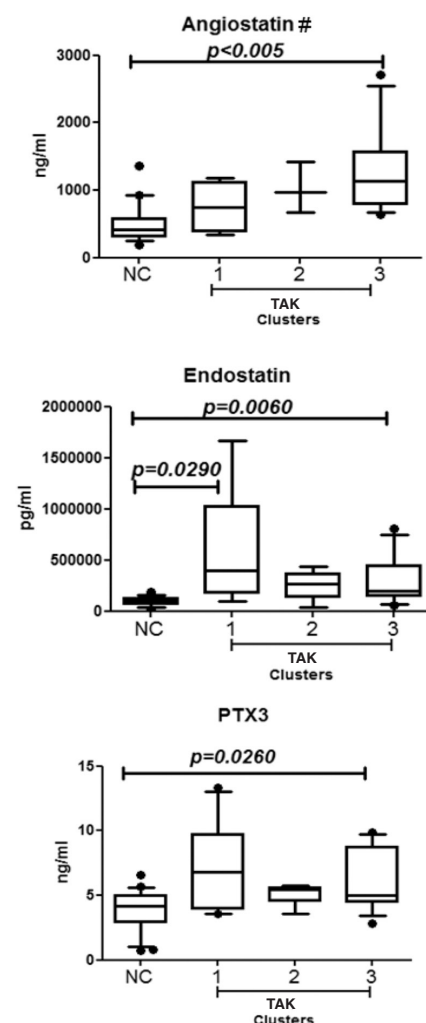


Fig. 5. Anti-angiogenic factor levels in three TAK groups identified by cluster analysis. Boxes show 25th and 75th percentiles. Horizontal lines within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. Solid circles indicate outliers. Comparison of anti-angiogenic factor levels among clusters was performed using the Kruskal-Wallis test followed by the Dunn's *post-hoc* correction. #Significant ordered differences among clusters (Jonckheere Terpstra test): $p<0.0005$. TAK: Takayasu's Arteritis; PTX3: pentraxin 3.

considering the acknowledged limitation of currently utilised criteria to monitor disease activity in LVV (48). In line with previously reported findings, we also confirm the elevated levels of PTX3 in LVV compared to NC. Currently, the role of PTX3 as a biomarker of disease activity in LVV is still debated. Indeed, even if several lines of evidence have identified PTX3 as a potentially useful molecule for assessing disease activity in LVV (49, 50), other findings, in agreement with our results, did not show a significant association of PTX3 serum levels and disease activity in LVV (24, 25). This discrepancy may be due to the different definitions of active disease adopted in the studies. In two previous studies on VEGF (51) and on PTX3 (52) serum levels in poly-

myalgia rheumatica (PMR), a disease closely related to GCA, we found significantly higher VEGF levels compared to NC, whereas no difference in PTX3 levels was found between PMR and NC, either at disease onset or during follow-up. Since, in this study, opposing results have been shown in GCA (no elevation of VEGF together with an up-regulation of PTX3 levels), these findings might suggest a role for combined evaluation of VEGF and PTX3 as a potential biomarker for the identification of an occult GCA in 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. A direct comparison

study on GCA and PMR patients should verify this hypothesis. Two main limitations of this study should be addressed. Firstly, the relatively small sample size of the GCA group which specifically affected the statistical power of the analysis of patient subgroups obtained following stratification according to the specific variables. For this reason, the GCA group was only partially investigated and the analysis performed on pro-angiogenic/anti-angiogenic factors levels

in the three groups identified by cluster analysis were limited to TAK patients. Secondly, since our patients were enrolled in a tertiary Rheumatology Centre with special interest in vasculitis, the great majority of LVV patients evaluated in the study were already on corticosteroid treatment, either with or without immunosuppressive drugs/biologic agents (53).

The effect of treatment on angiogenic/anti-angiogenic factors has been previously described. Several *in vitro* and *in vivo* studies on cancer and placenta angiogenesis reported a down-regulation of VEGF expression induced by glucocorticoids (54, 55).

In a recent study, VEGF and angiopoietin-2 serum levels have been showed to be lowered in glucocorticoid-treated GCA patients compared to baseline levels (56).

In Behçet's disease, conflicting results were reported concerning serum angiopoietin modification by therapies. Indeed, cyclophosphamide and steroids have been shown to increase angiopoietin-1 (57), whereas other observations have revealed that several medications, such as steroids, cyclosporine, colchicine, did not have any effects on serum angiopoietin-1 and angiopoietin-2 (58). Among the anti-angiogenic factors, increased endostatin levels were observed in RA patients treated with a combination of disease-modifying anti-rheumatic drugs (59). Similarly, PTX-3 circulating levels were up-regulated by glucocorticoids in mouse and human treated with dexamethasone (60). Opposing results were reported by our previous study performed on PMR patients. Indeed, in this study a significant decrease of PTX3 serum levels compared to baseline was observed during corticosteroid treatment (52). Similarly, tocilizumab (TCZ) has been shown to lower circulating levels of PTX3 in GCA patients (61).

We did not find any difference with regard to angiogenic/anti-angiogenic factor serum levels between treated and untreated LVV patients. However, this finding should be evaluated taking into account that the small sample size of the untreated group should affect the statistical power of the analysis. There-

fore, our main results (*i.e.* normal levels of angiogenic factors and increased anti-angiogenic factor levels) may be a consequence of the treatment effects on factor serum levels.

Considering that specific medications may modify serum levels of angiogenesis-related factors differently and the conflicting findings previously reported, the influence of drug regimen on circulating levels of angiogenic/anti-angiogenic molecules should be investigated more specifically in future studies.

Finally, the main point of interest raised by this study lies in the novel and original results concerning the imbalance of the circulating molecular pattern towards an anti-angiogenic profile. This pattern of angiogenic/anti-angiogenic response should be evaluated in treatment-naïve patients in the early phase of the disease.

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Competing interests

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