Serum proteins reflecting inflammation, injury and repair as biomarkers of disease activity in ANCA-associated vasculitis

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Abstract

Objective—To identify circulating proteins that distinguish between active anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and remission in a manner complementary to markers of systemic inflammation.

Methods—Twenty-eight serum proteins representing diverse aspects of the biology of AAV were measured before and 6 months after treatment in a large clinical trial of AAV. Subjects (n=186)

enrolled in the Rituximab in ANCA-Associated Vasculitis (RAVE) trial were studied. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were available for comparison. The primary outcome was the ability of markers to distinguish severe AAV (Birmingham Vasculitis Activity Score for Wegener's granulomatosis (BVAS/WG) 3 at screening) from remission (BVAS/WG=0 at month 6), using areas under receiver operating characteristic (ROC) curve (AUC).

Results—All subjects had severe active vasculitis (median BVAS/WG=8) at screening. In the 137 subjects in remission at month 6, 24 of the 28 markers showed significant declines. ROC analysis indicated that levels of CXCL13 (BCA-1), matrix metalloproteinase-3 (MMP-3) and tissue inhibitor of metalloproteinases-1 (TIMP-1) best discriminated active AAV from remission (AUC>0.8) and from healthy controls (AUC>0.9). Correlations among these markers and with ESR or CRP were low.

Conclusions—Many markers are elevated in severe active AAV and decline with treatment, but CXCL13, MMP-3 and TIMP-1 distinguish active AAV from remission better than the other markers studied, including ESR and CRP. These proteins are particularly promising candidates for future studies to address unmet needs in the assessment of patients with AAV.

INTRODUCTION

Granulomatosis with polyangiitis (Wegener's, GPA) and microscopic polyangiitis (MPA) are severe inflammatory diseases that share the features of necrotising vasculitis of small vessels in multiple organ systems and characteristic autoantibodies (anti-neutrophil cytoplasmic antibodies (ANCAs), with specificity for proteinase-3 (PR3) or myeloperoxidase (MPO)), and thus can be described together as ANCA-associated vasculitis (AAV). In addition, GPA features necrotising granulomatous inflammation. Thus there are many plausible avenues for discovery of new biomarkers in these diseases, based on activation of T and B cells, acute and chronic inflammation, damage to the microvasculature, and tissue damage and repair considered either generally or in an organ-specific manner. New biomarkers are needed for AAV because the course of disease after initial treatment is highly variable, and existing markers such as ANCA titres and non-specific markers of inflammation such as C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) have limited value.[1–8]

As the first step in a programme to identify markers that are useful in staging vasculitis activity, distinguishing vasculitis from other inflammatory diseases such as infections, and predicting response to treatment and risk of relapse, we assessed the ability of 28 proteins to distinguish severe AAV from remission and from healthy controls. We measured these proteins simultaneously in a large number of patients followed under standardised conditions in order to compare their ability to discriminate between disease states.

The proteins were chosen from a list of 108 proteins previously validated for assay on our custom microarray platform, in order to reflect a range of disease-related processes distinct from autoantibody specificity or systemic inflammation—broadly categorised as cytokines, chemokines, soluble receptors, markers of microvascular damage, and markers of tissue damage and repair. Some of these markers— such as ACE,[9] CCL5,[10] CCL17,[11] CXCL8 (interleukin-8, IL-8),[9][10][12][13] intercellular adhesion molecule-1 (ICAM-1),[9][14–17] interferon-γ (IFNγ),[9] interleukin-6 (IL-6),[12][15][18] interleukin-18 (IL-18),[19][20] IL-18 binding protein

(IL-18BP),[20] soluble interleukin-2 receptor (sIL-2R),[21–24] soluble IL-6 receptor (sIL-6R),[25] matrix metalloproteinase-3 (MMP-3),[2627] neutrophil gelatinase-associated lipocalin (NGAL, lipocalin-2),[28] osteopontin,[29] tissue inhibitor of metalloproteinases-1 (TIMP-1),[2627] soluble tumour necrosis factor receptors I or II (sTNF-RI/II),[22252830] and vascular cell adhesion molecule-1 (VCAM-1)[9141631]—have shown promise previously in smaller studies of AAV. The remaining proteins—clusterin, CXCL10 (IP-10), CXCL13 (BCA-1), basic fibroblast growth factor (bFGF), granulocyte colony-stimulating factor (G-CSF), granulocyte– monocyte colony-stimulating factor (GM-CSF), interleukin-15 (IL-15), kidney injury molecule-1 (KIM-1, TIM-1), nerve growth factor β (NGFβ), plasminogen activator inhibitor-1 (PAI-1) and platelet-derived growth factor, A and B subunits (PDGF-AB)—have not to our knowledge been studied previously in AAV.

METHODS

Study design

Subjects for this study were enrolled in the Rituximab in ANCA-Associated Vasculitis (RAVE) trial. Of the 197 subjects in RAVE, 11 were excluded on the basis of withdrawal from the study by month 4 (n=7), absence of serum collection at screening (n=2), or frequent absence of serum collection at other times throughout the trial (n=2). Of the remaining 186 subjects, all samples at screening were analysed, but data from the month 6 visit were analysed only from the 162 subjects who had completed 6 months without blinded cross-over to the other treatment and had serum collected at month 6.

The primary outcome of this study was the difference in marker level between active AAV (at screening) and remission (at month 6) in the same patients (n=137), as determined by analysis of the absolute changes in marker levels and the areas under receiver operating characteristic (ROC) curves (see below under Statistical analysis). Secondary outcomes included differences in marker levels in groups defined by treatment at the time of sample collection, comparison of active AAV with healthy controls, and comparison of remission with recurrent active disease (n=25) at month 6.

Summary of clinical trial and clinical outcome measures

RAVE was a randomised, double-blinded, multicentre clinical trial that compared standard remission-induction therapy using oral cyclophosphamide (CYC) and glucocorticoids with experimental treatment using the B-cell-depleting agent, rituximab (RTX), and glucocorticoids, in 197 patients with new or recurrent, severe AAV (GPA or MPA).[32] All patients tested positive for antibodies to either PR3 or MPO. Subjects randomised to receive CYC were switched to maintenance therapy with azathioprine (AZA) if they were clinically in remission between months 4 and 6. Subjects in the RTX arm were not placed on a maintenance agent, but the great majority still had no detectable B cells at month 6 and were therefore still considered to be 'on treatment' at this time point. Ongoing or recurrent severe disease during the first 6 months led to blinded cross-over to the other treatment arm for re- induction therapy or withdrawal from blinded treatment. Per protocol, glucocorticoid (prednisone) treatment was completely withdrawn before 6 months, although investigators had the option to restart prednisone at no more than 10 mg/day to control recurrent

symptoms of mild disease. The rates of achievement of remission were equivalent in the two treatment arms.[32]

Activity of vasculitis was assessed using the Birmingham Vasculitis Activity Score for Wegener's granulomatosis (BVAS/WG),[33] in which each severe disease manifestation is given 3 points and each non-severe (mild) manifestation is given 1 point.[33] Remission was defined as BVAS/WG=0. Every patient had a BVAS/WG of at least 3 at screening.

All subjects were enrolled using a protocol and informed consent documents approved by institutional review boards at all participating sites.

Healthy controls

Sixty-eight subjects self-identified as being in good health were recruited separately at Boston University School of Medicine under a protocol approved by the institutional review board. Specifically, these persons denied having any autoimmune or inflammatory disease or cardiovascular disease, or taking immune-suppressive, antiplatelet or anticoagulant drugs, based on responses to a questionnaire.

Processing of serum samples

Serum was processed and stored at each study site, then shipped to a central repository, then shipped to the study laboratory. All samples remained frozen at −80°C until the day the assays were performed.

Biomarker assays

Antibody arrays—See online supplementary methods.

Additional laboratory data—Westergren ESR and CRP were assayed at the participating sites at the time of each study visit, and data were retrieved from the clinical report forms.

Statistical analysis

Distributions, correlations and adjustment for multiple testing—Distributions of marker values were evaluated for normality using Shapiro–Wilk and Kolmogorov–Smirnov tests, as well as visual inspection of histograms. Correlation between pairs of markers was measured using Spearman correlation coefficients. Findings in all analyses were considered significant at p 0.05 after adjustment for multiple comparisons (30 markers tested in each analysis) by calculating the false discovery rate as described by Benjamini and Hochberg.[34] All analyses were performed using SAS V.9.1 or InStat.

Distinguishing active AAV from remission or controls—Since very few of the markers showed normally distributed values among subjects with active vasculitis (online supplementary figure S1), data are reported as medians and inter-quartile range (IQR) and were analysed using non-parametric statistics (Wilcoxon signed rank test for univariate analyses and Wilcoxon rank sum test for bivariate analyses).

To further assess the ability of markers to distinguish active AAV from remission, ROC curves were constructed for each marker using logistic regression, with marker level as the predictor variable and one of two dichotomous outcomes: active AAV versus remission, or active AAV versus healthy controls. The area under each ROC curve (AUC or C-statistic) was calculated. The standard errors (SEs) of the AUCs for selected markers were calculated and used to determine whether one AUC was significantly greater than another.[35 36] An optimal cut-off point for each analysis was defined using the Youden Index[37]: the maximum of the sum of sensitivity (percentage of subjects with active AAV in whom marker level was elevated) and specificity (percentage of subjects in remission, or healthy controls, in whom marker level was not elevated). Positive likelihood ratios (LRs) (sensitivity/(1–specificity)) for active AAV versus remission or controls were calculated at these optimal cut-off points.

In analyses in which active AAV (at screening) was compared with AAV in remission, the dataset contained repeated measurements from the same subjects and thus had the potential to require adjustment. We reasoned that the balanced nature of the study (one active and one remission sample from each subject) obviated problems with repeated measures, but, to be certain, we performed a simulation in which each subject was randomly assigned to one of two groups, with one group used to provide the active AAV data and the other used to provide the remission data. This process was repeated 20 times, and the resulting ROC curves were averaged. Results were nearly identical (ie, within the error range expected from only 20 trials) with those obtained with a single analysis of the full dataset (data not shown).

RESULTS

Patient characteristics at screening and follow-up

The 186 subjects included 91 male and 95 female patients with median age 52 (IQR 44–66), all of whom had severe disease: median BVAS/WG 8, IQR 6–10, range 3–16. Of the 186 subjects evaluated at screening, 139 had been diagnosed with GPA and 46 with MPA; 124 were positive for anti-PR3 and 62 for anti-MPO; 90 had a new diagnosis of AAV, whereas 96 had established diagnoses and were experiencing relapses. At screening, 92 patients were receiving glucocorticoids, and 104 were receiving some immune-suppressive drug (glucocorticoids, other drugs, or both).

Samples from month 6 were also available for 162 patients who had not undergone re-induction therapy. Of these 162 subjects, 137 (85%) were in remission, and data from these 137 subjects were used for the primary analysis. The remaining 25 patients (15%) had active disease at month 6, and disease was usually mild (BVAS/WG 1 or 2 in 21/25 subjects). Of the 137 subjects in remission, 20 (15%) were receiving prednisone   10 mg/day, and the remaining subjects were off prednisone.

The 68 healthy controls included 28 men and 40 women, median age 41 (IQR 28–57).

Marker levels in active AAV, remission and healthy controls

Twenty-four of the 28 experimental markers, as well as ESR and CRP, were significantly different at times of active AAV compared with remission in the same subjects (table 1 and online supplementary figure S1). Levels of 20 proteins declined and four increased after

successful treatment. Twenty-four of the 28 markers were also significantly different (22 higher and two lower) in active AAV compared with healthy controls (table 1).

The ability of each marker to distinguish active AAV from remission or from healthy controls was quantified by measuring the AUC and calculating LR. Eleven proteins had AUC>0.7 for both comparisons, and three (CXCL13/BCA-1, MMP-3 and TIMP-1) stood out as the best-performing markers, with AUC>0.8 and positive LR 4.3–6.8 comparing active AAV with remission (p<0.05 compared with p=0.76 for ESR), and AUC>0.9 and positive LR 10.7–12.4 comparing active AAV with controls (table 2 and figure 1).

Four markers (CXCL13/BCA-1, G-CSF, IL-15 and TIMP-1) were significantly higher at month 6 in the 25 subjects with active, usually very mild, disease than in the 137 subjects in remission. Discrimination between mild disease and remission at month 6 was limited, with all AUC<0.7 (online supplementary table S1). However, power for detecting differences was low, and determining disease activity (versus consequences of damage) in such cases is challenging clinically.

Correlations among markers that distinguish active vasculitis from remission

Correlation coefficients between all pairs of markers are shown in figure 2. ESR and CRP correlated only weakly with all 28 experimental proteins. The three markers of greatest interest based on discrimination of active disease, remission and healthy controls also correlated weakly with each other: 0.20 for CXCL13/BCA-1 with MMP-3; 0.13 for CXCL13/BCA-1 with TIMP-1; and 0.17 for MMP-3 with TIMP-1.

In contrast, a group of 12 proteins correlated highly with each other: GM-CSF, IFNγ, IL-15, IL-18, IL-18BP, sIL-2Rα, IL-6, CXCL8/IL-8, CXCL10/IP-10, KIM-1, bFGF and G-CSF. Most of these markers had shown moderate ability to distinguish active AAV from remission or from healthy controls (tables 1 and 2). We confirmed the validity of the results for these 12 markers using individual ELISAs on 20 patients and 12 controls selected to include low, mid-range and extremely high values (data not shown).

Effects of treatment on marker levels

Table 3 shows marker levels in groups at screening defined by newly diagnosed AAV versus relapse or by use of prednisone at the time of screening. Several markers were significantly higher in subjects with newly diagnosed AAV than in subjects with relapsing disease; all of these markers were also nominally higher in subjects not taking prednisone than in those already taking prednisone, although the differences often did not reach statistical significance.

The effects of different treatments in the first 6 months were examined using subjects who were in remission at month 6 and had remained in their original treatment groups. As shown in table 4, after adjustment for multiple comparisons, only one marker (IL-15) was significantly different in patients who received CYC/AZA versus RTX, and only one marker (CXCL13/BCA) was significantly different comparing patients on and off prednisone at month 6, although the sample size for the latter analysis was small.

Overall, these results suggest that recent treatment did not have a dramatic effect on marker levels at screening, and that the changes seen in marker levels from screening to month 6 were not driven by effects of particular treatments.

DISCUSSION

This study of markers of active AAV identifies several novel markers (CXCL13/BCA-1, NGFβ, bFGF, GM-CSF, G-CSF, IL-15, IL-18BP, sIL-6R, KIM-1 and NGAL), confirms several (IFNγ, sIL-2R, IL-6, CXCL8/IL-8, MMP-3, TIMP-1), and suggests that some previously studied markers may not be valuable (ICAM-1, VCAM-1). Multiple markers appeared to distinguish between active AAV and remission better than did ESR or CRP, but, more importantly, provided complementary information to ESR and CRP based on low correlation coefficients.[38]

Markers that distinguish between clinically obvious disease states are the most promising ones for further studying the more important and challenging goals of biomarker development. These goals have not been enumerated for vasculitis; we propose that they include staging of current internal organ involvement, distinguishing active vasculitis from infections or other conditions, distinguishing mildly active vasculitis from remission, predicting permanent and/or progressive damage to organ systems, predicting response to treatment or risk of relapse, and assessing efficacy of treatment early in its course. These goals are thematically similar to what have been proposed by expert panels in other rheumatic diseases.[39][40]

The strengths of this study include the large study population, standardised prospective collection of detailed clinical information, inclusion of enough healthy controls to provide good estimates of normal ranges, and testing and comparison of many novel markers drawn from diverse aspects of autoimmune inflammatory disease processes. As above, the main limitation of the study is that it represents only a first step in identifying clinically useful biomarkers. Clinical use will require studies with longer follow-up, many samples associated with mildly active disease, and comparison with other disease states, as well as assessment of marker variability within and between individuals,[40][41] technical validation of assay methodologies,[42] and evaluation of pre-analytical factors such as posture, time of day, effects of food or exercise, and stability during processing and storage. The three markers of greatest interest in distinguishing active disease from remission in this study clearly have limited capacity to distinguish active AAV from other inflammatory conditions and infections.

MMP-3 has previously been identified by our research group using the same cohort,[43] and the current study provides a useful technical validation of that finding, since different assay methodologies were used. Multiple cell types and tissues could contribute to circulating MMP-3 levels, and elevated levels have been detected in inflammatory diseases presumed to be pathophysiologically distinct, such as GPA,[26][27] Takayasu's arteritis[44] and multiple forms of inflammatory arthritis.[45]

TIMP-1 (in plasma rather than serum) has been previously reported to be elevated in active GPA compared with remission or healthy controls.[26] This marker is not specific for AAV, since it circulates at high levels in sepsis.[46] The tissue source in AAV of this widespread antiproteinase is not clear but is probably not circulating leucocytes, since transcription of TIMP-1 was found to be much higher in peripheral blood mononuclear cells from patients in remission than in patients with active GPA.[26]

Elevation of CXCL13/BCA-1 in AAV is a particularly interesting finding in considering pathophysiology, although there are clearly limits to what can be inferred about mechanisms of disease from circulating levels of a locally produced, locally acting protein. This chemokine is important in the development of secondary and ectopic lymphoid tissues and is critical in B cell homing via the receptor, CXCR5.[47] The importance of B cells in AAV was shown in the RAVE Trial.[32] Circulating CXCL13/BCA-1 has not been measured in AAV, but levels have been reported to be elevated in patients with cryoglobulinaema,[48] systemic lupus erythematosus,[49–51] multiple sclerosis,[52 53] HIV infection[54] and sepsis.[49] Although elevation of CXCL13 in all of these diseases, including AAV, implies a connection with abnormalities in B cell function, CXCR5 is also expressed on subpopulations of CD4 Tcells and dendritic cells[47]; similarly, although follicular dendritic cells are the main producers of CXCL13 in lymph nodes,[47] it is not clear which cells are the source of elevated circulating levels in disease states.

Finally, the poor correlation between the best-performing markers and ESR and CRP indicates that the use of multiple markers will improve on the performance of any individual marker, which will be important in approaching the outcomes of greatest need for biomarker development in AAV: distinguishing active vasculitis from infections or other inflammatory diseases, distinguishing mild disease from remission, staging of current organ-system involvement, and prediction of response to treatment, future relapse, and other long-term outcomes. Large longitudinal studies such as the RAVE trial will provide the essential framework for development and validation of models in pursuit of these clinically relevant goals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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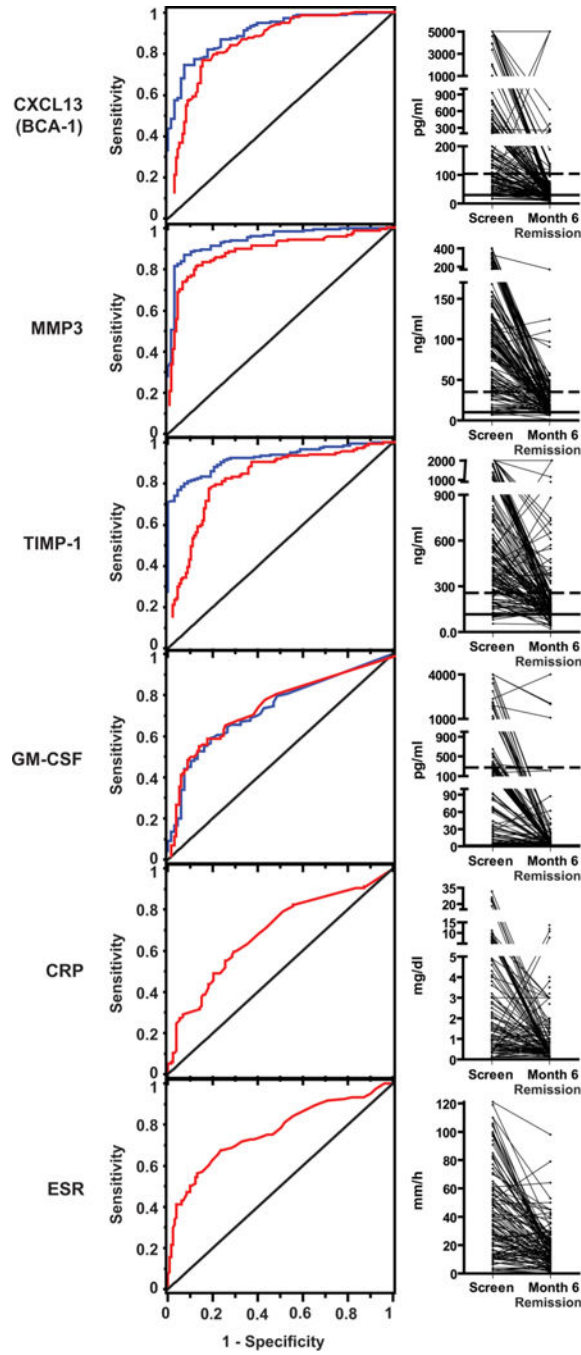
Figure 1.
Levels of selected markers in severe active ANCA-associated vasculitis (AAV), AAV in remission, and in healthy controls. Left panels: receiver operating characteristic curves showing the ability of selected markers to distinguish severe active AAV (at screening) from remission at month 6 (blue curves), and severe active AAV from healthy controls (red curves). The diagonal line indicates what would be expected with no discrimination between groups. Erythrocyte sedimentation rate and C-reactive protein were not measured in healthy controls. Right panels: levels in individual patients. All subjects had severe active AAV at
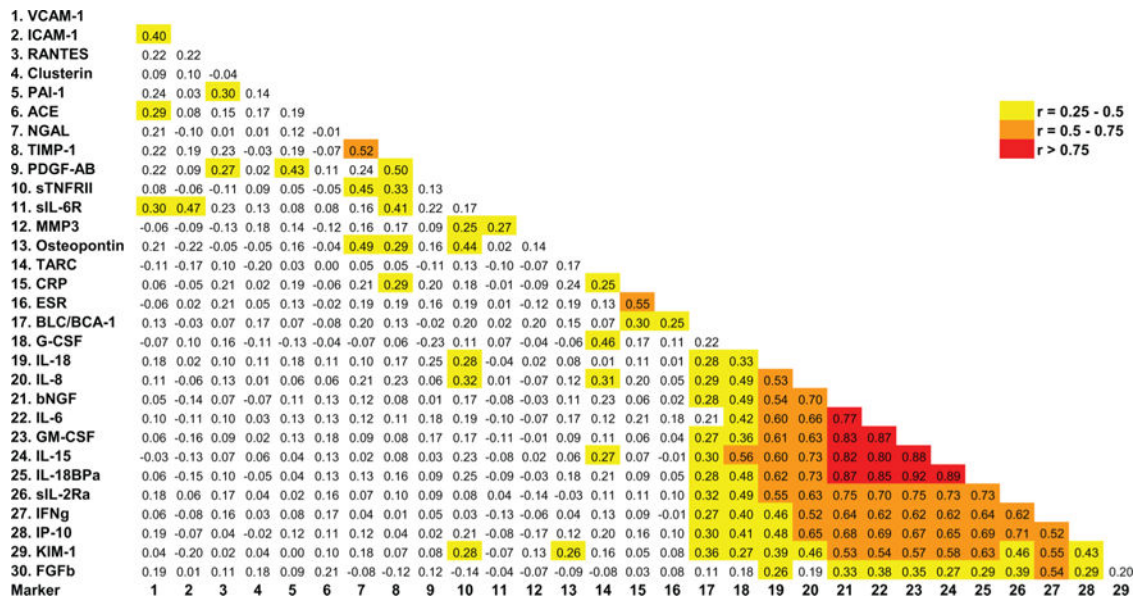
screening and were in remission at month 6. The medians and 95th centiles among healthy controls are shown with horizontal solid and dotted lines, respectively. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GM-CSF, granulocyte–monocyte colony-stimulating factor; MMP3, matrix metalloproteinase-3; TIMP-1, tissue inhibitor of metalloproteinases-1.

Marker	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1. VCAM-1																													
2. ICAM-1	0.40																												
3. RANTES	0.22	0.22																											
4. Clusterin	0.09	0.10	-0.04																										
5. PAI-1	0.24	0.03	0.30	0.14																									
6. ACE	0.29	0.08	0.15	0.17	0.19																								
7. NGAL	0.21	-0.10	0.01	0.12	-0.01																								
8. TIMP-1	0.22	0.19	0.23	-0.03	0.19	-0.07	0.52																						
9. PDGF-AB	0.22	0.09	0.27	0.02	0.43	0.11	0.24	0.50																					
10. sTNFRII	0.08	-0.06	-0.11	0.09	0.05	-0.05	0.45	0.33	0.13																				
11. sIL-6R	0.30	0.47	0.23	0.13	0.08	0.08	0.16	0.41	0.22	0.17																			
12. MMP3	-0.06	-0.09	-0.13	0.18	0.14	-0.12	0.16	0.17	0.09	0.25	0.27																		
13. Osteopontin	0.21	-0.22	-0.05	-0.05	0.16	-0.04	0.49	0.29	0.16	0.44	0.02	0.14																	
14. TARC	-0.11	-0.17	0.10	-0.20	0.03	0.00	0.05	0.05	-0.11	0.13	-0.10	-0.07	0.17																
15. CRP	0.06	-0.05	0.21	0.02	0.19	-0.06	0.21	0.29	0.20	0.18	-0.01	-0.09	0.24	0.25															
16. ESR	-0.06	0.02	0.21	0.05	0.13	-0.02	0.19	0.19	0.16	0.19	0.01	-0.12	0.19	0.13	0.55														
17. BLC/BCA-1	0.13	-0.03	0.07	0.17	0.07	-0.08	0.20	0.13	-0.02	0.20	0.02	0.20	0.15	0.07	0.30	0.25													
18. G-CSF	-0.07	0.10	0.16	-0.11	-0.13	-0.04	-0.07	0.06	-0.23	0.11	0.07	-0.04	-0.06	0.46	0.17	0.11	0.22												
19. IL-18	0.18	0.02	0.10	0.11	0.18	0.11	0.10	0.17	0.25	0.28	-0.04	0.02	0.08	0.01	0.11	0.01	0.28	0.33											
20. IL-8	0.11	-0.06	0.13	0.01	0.06	0.06	0.21	0.23	0.06	0.32	0.01	-0.07	0.12	0.31	0.20	0.05	0.29	0.49	0.53										
21. bNGF	0.05	-0.14	0.07	-0.07	0.11	0.13	0.12	0.08	0.01	0.17	-0.08	-0.03	0.11	0.23	0.06	0.02	0.28	0.49	0.54	0.70									
22. IL-6	0.10	-0.11	0.10	0.03	0.13	0.13	0.12	0.11	0.18	0.19	-0.10	-0.07	0.17	0.12	0.21	0.18	0.21	0.42	0.60	0.66	0.77								
23. GM-CSF	0.06	-0.16	0.09	0.02	0.13	0.18	0.09	0.08	0.17	0.17	-0.11	-0.01	0.09	0.11	0.06	0.04	0.27	0.36	0.61	0.63	0.83	0.87							
24. IL-15	-0.03	-0.13	0.07	0.06	0.04	0.13	0.02	0.08	0.03	0.23	-0.08	0.02	0.00	0.27	0.07	-0.01	0.30	0.56	0.60	0.73	0.82	0.80	0.88						
25. IL-18BPa	0.06	-0.15	0.10	-0.05	0.04	0.13	0.13	0.16	0.09	0.25	-0.09	-0.03	0.18	0.21	0.09	0.05	0.28	0.48	0.62	0.73	0.87	0.85	0.92	0.89					
26. sIL-2Ra	0.08	0.06	0.17	0.04	0.02	0.16	0.07	0.10	0.09	0.08	0.04	-0.14	-0.03	0.11	0.11	0.10	0.32	0.49	0.55	0.63	0.75	0.70	0.75	0.73	0.73				
27. IFNg	0.06	-0.08	0.16	0.03	0.08	0.17	0.04	0.01	0.05	0.03	-0.13	-0.06	0.04	0.13	0.09	-0.01	0.27	0.40	0.46	0.52	0.64	0.62	0.62	0.62	0.64	0.62			
28. IP-10	0.19	-0.07	0.04	-0.02	0.12	0.11	0.12	0.04	0.02	0.21	-0.08	-0.17	0.12	0.20	0.16	0.10	0.30	0.41	0.48	0.65	0.68	0.69	0.67	0.65	0.69	0.71	0.52		
29. KIM-1	0.04	-0.20	0.02	0.04	0.00	0.10	0.18	0.07	0.08	0.28	-0.07	0.13	0.26	0.16	0.05	0.08	0.36	0.27	0.39	0.46	0.53	0.54	0.57	0.58	0.63	0.46	0.55	0.43	
30. FGFb	0.19	0.01	0.11	0.18	0.09	0.21	-0.08	-0.12	0.12	-0.14	-0.04	-0.07	-0.09	-0.08	0.03	0.08	0.11	0.18	0.26	0.19	0.33	0.38	0.35	0.27	0.29	0.39	0.54	0.29	0.20

r = 0.25 - 0.5
r = 0.5 - 0.75
r > 0.75

Figure 2.

Correlations between marker levels. Spearman correlation coefficients for all pairs of experimental markers as well as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are shown. Background colour indicates strength of association (red, >0.75; orange, 0.5–0.75; yellow, 0.25–0.5; white, <0.25). BCA-1, CXCL13; FGFb, basic fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–monocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IL-8, CXCL8; IL-18BP, interleukin 18 binding protein; IP-10, CXCL10; KIM-1, kidney injury molecule-1; MMP-3, matrix metalloproteinase-3; NGAL, neutrophil gelatinase-associated lipocalin; NGFβ, nerve growth factor β; PAI-1, plasminogen activator inhibitor-1; PDGF-AB, platelet-derived growth factor, A and B subunits; RANTES, CCL5; sIL-2R, soluble IL 2 receptor; sIL-6R, soluble IL 6 receptor; sTNF-RII, soluble TNF receptor II; TARC, CCL17; TIMP-1, tissue inhibitor of metalloproteinases-1; VCAM-1, vascular cell adhesion molecule-1.

Table 1

Marker levels in severe active ANCA-associated vasculitis (screening) and remission among the patients who were in remission at month 6

Marker	Screening (n=137)	Month 6 remission (n=137)	Difference (n=137)	p Value*	Healthy controls (n=68)	p Value**
Cytokines						
G-CSF (pg/ml)	20.4 (8.01;45.9)	10.5 (5.63;23.7)	5.78 (−0.74;26.0)	<0.0001*	7.58 (4.89;12.7)	<0.0001**
GM-CSF (pg/ml)	27.6 (2.28;269)	1.17 (<0.98;4.99)	16.8 (0.00;241)	<0.0001*	1.39 (<0.98;7.27)	<0.0001**
IFNγ (pg/ml)	<0.49 (<0.49;2.01)	<0.49 (<0.49;<0.49)	0.00 (0.00;0.95)	<0.0001*	<0.49 (<0.49;<0.49)	0.011**
IL-6 (pg/ml)	2.14 (<0.69;19.8)	<0.49 (<0.49;0.77)	1.36 (0.00;18.3)	<0.0001*	<0.49 (<0.49;<0.49)	<0.0001**
IL-15 (pg/ml)	21.6 (7.65;109)	5.69 (2.60;13.5)	14.6 (1.65;92.4)	<0.0001*	2.87 (2.18;4.31)	<0.0001**
IL-18 (pg/ml)	57.4 (37.2;101)	51.9 (31.0;85.8)	8.49 (−23.4;37.7)	0.023*	36.2 (20.1;60.8)	<0.0001**
Osteopontin (ng/ml)	65.0 (38.8;101)	54.4 (37.8;80.9)	5.48 (−15.9;37.8)	0.013*	36.2 (29.5;42.4)	<0.0001**
Chemokines						
BCA-1 (pg/ml)	170 (74.2;489)	32.0 (18.2;55.6)	116 (27.6;416)	<0.0001*	29.6 (19.8;44.8)	<0.0001**
IL-8 (pg/ml)	19.5 (7.33;51.2)	7.09 (3.59;15.3)	7.60 (−2.26;40.4)	<0.0001*	3.00 (1.30;5.19)	<0.0001**
IP-10 (pg/ml)	11.1 (6.01;23.7)	13.2 (7.68;25.0)	−0.87 (−7.95;7.95)	0.73	3.28 (2.19;5.30)	<0.0001**
RANTES (ng/ml)	60.3 (33.4;107)	52.3 (30.8;90.0)	0.00 (−16.6;31.3)	0.43	58.3 (27.7;91.4)	0.11
TARC (pg/ml)	520 (234;1535)	655 (347;>2500)	−62.7 (−439;92.4)	0.0046*	177 (115;367)	<0.0001**
Soluble receptors						
IL-18BP (pg/ml)	116 (21.6;768)	14.6 (<6.11;55.1)	87.7 (1.46;537)	<0.0001*	13.9 (6.11;46.5)	<0.0001**
sIL-2R (pg/ml)	<2.44 (<2.44;153)	<2.44 (<2.44;<2.44)	0.00 (0.00;120)	<0.0001*	<2.44 (<2.44;<2.44)	0.0005**
sIL-6R (ng/ml)	27.4 (21.1;43.1)	21.9 (15.4;33.0)	4.27 (−3.12;12.5)	0.0002*	15.8 (11.7;20.0)	<0.0001**
sTNF-RII (pg/ml)	2671 (1306;4855)	2417 (1350;5808)	122 (−2068;1606)	0.86	499 (295;687)	<0.0001**
Tissue damage and repair						
ACE (ng/ml)	105 (73.8;144)	178 (130;252)	−70.9 (−137; −24.6)	<0.0001*	96.7 (81.4;115)	0.076
bFGF (pg/ml)	3.05 (<0.98;34.4)	<0.98 (<0.98;9.77)	0.00 (0.00;28.5)	<0.0001*	2.39 (<0.98;11.0)	0.42
KIM-1 (pg/ml)	242 (73.4;744)	45.6 (17.2;127)	134 (27.1;691)	<0.0001*	19.8 (8.08;128)	<0.0001**
MMP-3 (ng/ml)	96.6 (46.6;148)	15.6 (11.8;29.1)	79.1 (27.4;126)	<0.0001*	10.2 (7.02;15.5)	<0.0001**
NGFβ (pg/ml)	9.11 (3.15;37.0)	2.48 (1.25;4.32)	5.11 (0.67;31.4)	<0.0001*	2.11 (0.77;4.53)	<0.0001**

Marker	Screening (n=137)	Month 6 remission (n=137)	Difference (n=137)	p Value[*]	Healthy controls (n=68)	p Value[**]
PDGF-AB (pg/ml)	4298 (1573;6585)	3260 (879;5374)	798 (−617;2980)	0.0003 [*]	8877 (5812;11510)	<0.0001 [**]
TIMP-1 (ng/ml)	477 (302;862)	166 (125;233)	269 (67.1;638)	<0.0001 [*]	117 (65.4;163)	<0.0001 [**]
Inflammation and vascular injury						
Clusterin (μg/ml)	77.1 (65.3;89.3)	73.0 (59.4;85.9)	5.52 (−8.12;18.2)	0.0079 [*]	71.9 (61.2;83.0)	0.0085 [**]
CRP (mg/dl)	1.2 (0.5;4.0)	0.5 (0.3;1.2)	0.7 (0.0;3.3)	<0.0001 [*]	ND	ND
ESR (mm/h)	37 (16;60)	14 (7;22)	19 (2;39)	<0.0001 [*]	ND	ND
ICAM-1 (ng/ml)	463 (307;933)	537 (345;882)	−61.3 (−198;103)	0.004 [*]	281 (226;337)	<0.0001 [**]
NGAL (ng/ml)	271 (176;399)	172 (129;237)	80.3 (6.96 ;214)	<0.0001 [*]	117 (92.4;150)	<0.0001 [**]
PAI-1 (pg/ml)	1491 (<977;5650)	1202 (<977;4719)	0.00 (−1449;2325)	0.39	3307 (1083;7236)	0.0008 [**]
VCAM-1 (ng/ml)	133 (95.4;174)	148 (108;224)	−7.75 (−84.4;26.9)	0.0035 [*]	139 (84.3;188)	0.9

Values are median (IQR).

[*]
p<0.05 by Wilcoxon signed rank test, after adjustment for multiple comparisons.

[**]
p<0.05 by Wilcoxon rank sum test comparing screening to healthy controls, after adjustment for multiple comparisons.

BCA-1, CXCL13; bFGF, basic fibroblast growth factor; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–monocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IL-8, CXCL8; IL-18BP, interleukin 18 binding protein; IP-10, CXCL10; KIM-1, kidney injury molecule-1; MMP-3, matrix metalloproteinase-3; NGAL, neutrophil gelatinase-associated lipocalin; NGFβ, nerve growth factor β; PAI-1, plasminogen activator inhibitor-1; PDGF-AB, platelet-derived growth factor, A and B subunits; RANTES, CCL5; sIL-2R, soluble IL 2 receptor; sIL-6R, soluble IL 6 receptor; sTNF-RII, soluble TNF receptor II; TARC, CCL17; TIMP-1, tissue inhibitor of metalloproteinases-1; VCAM-1, vascular cell adhesion molecule-1.

Table 2

Distinction of active ANCA-associated vasculitis (AAV) from remission or from healthy controls: receiver operating characteristic (ROC) analyses and likelihood ratios

Marker	Active AAV versus remission					Active AAV versus controls				
	AUC*	OCP†	Sensitivity‡	Specificity‡	LR+§	AUC*	OCP†	Sensitivity‡	Specificity‡	LR+§
BCA-1	0.86	70	77 (69–83)	85 (78–90)	5.13	0.91	70	75 (68–81)	93 (84–98)	10.7
ESR	0.76	23	68 (59–76)	76 (68–83)	2.83	ND				
GM-CSF	0.72	10	60 (51–68)	82 (74–88)	3.33	0.72	11	59 (52–66)	81 (70–89)	3.11
IL-6	0.75	0.68	75 (67–82)	71 (62–78)	2.59	0.77	0.51	77 (70–83)	78 (66–87)	3.50
IL-15	0.74	17	58 (50–67)	82 (75–88)	3.22	0.86	4.5	87 (81–92)	78 (66–87)	3.95
IL-18BP	0.74	93	54 (45–63)	85 (78–91)	3.60	0.73	22.5	73 (66–79)	69 (57–80)	2.35
KIM-1	0.76	90	72 (64–80)	69 (59–75)	2.32	0.74	43	86 (80–91)	63 (51–75)	2.32
MMP-3	0.89	38	82 (74–88)	88 (81–93)	6.83	0.94	29.5	87 (81–92)	93 (84–98)	12.4
NGAL	0.73	193	74 (66–81)	64 (55–72)	2.06	0.89	190	72 (65–78)	91 (82–97)	8.00
NGFβ	0.74	4.5	67 (58–74)	77 (69–84)	2.91	0.74	5.7	61 (53–68)	82 (71–91)	3.39
TIMP-1	0.83	270	78 (70–85)	82 (74–88)	4.33	0.93	237	80 (73–85)	93 (84–98)	11.4

*
AUC, area under the ROC curve. An AUC of 1 indicates perfect discrimination between groups; an AUC of 0.5 indicates no discrimination.

†
OCP, optimal cut-off point, which is the maximum sum of sensitivity and specificity; for units, see table 1.

‡
Sensitivity and specificity, at OCP, determined by values greater than OCP in active AAV and less than OCP in remission or controls unless otherwise noted. 95% confidence intervals (asymptotic method) are shown. ND, not done.

§
LR+, positive likelihood ratio at the OCP, which equals sensitivity/(1−specificity).

BCA-1, CXCL13; ESR, erythrocyte sedimentation rate; GM-CSF, granulocyte–monocyte colony-stimulating factor; IL, interleukin; IL-18BP, interleukin 18 binding protein; KIM-1, kidney injury molecule-1; MMP-3, matrix metalloproteinase-3; NGAL, neutrophil gelatinase-associated lipocalin; NGFβ, nerve growth factor β; TIMP-1, tissue inhibitor of metalloproteinases-1.

Table 3

Marker levels in patients with newly diagnosed or relapsing ANCA-associated vasculitis (AAV), and in patients treated or not treated with glucocorticoids at the time of screening

Marker	Newly diagnosed or relapsing AAV			Glucocorticoid treatment at screening		
	Relapsing (n=96)	New (n=90)	p Value	Yes (n=92)	No (n=94)	p Value
Cytokines						
G-CSF	23.4 (11.5;56.3)	17.2 (6.60;41.2)	0.032	21.5 (10.2;47.6)	22.7 (8.79;49.2)	0.81
GM-CSF	39.0 (2.78;243)	18.1 (1.72;269)	0.4	26.7 (1.92;236)	23.3 (2.66;269)	0.85
IFNγ	<0.49 (<0.49;2.06)	<0.49 (<0.49;2.11)	0.87	<0.49 (<0.49;0.79)	<0.49 (<0.49;3.56)	0.4
IL-6	2.52 (0.53;19.9)	1.93 (0.69;20.0)	0.92	2.04 (<0.49;20.4)	2.12 (0.63;19.8)	0.87
IL-15	30.2 (10.4;110)	17.9 (7.23;99.3)	0.31	26.8 (9.11;102)	20.6 (8.28;116)	0.4
IL-18	58.0 (34.5;113)	52.1 (30.7;101)	0.31	56.5 (38.6;145)	56.6 (30.7;99.4)	0.22
Osteopontin	49.0 (34.0;75.5)	76.5 (45.7;116)	0.0004 *	50.7 (32.2;81.4)	68.9 (46.3;107)	0.0021
Chemokines						
BCA-1	125 (53.4;320)	232 (84.5;771)	0.011 *	153 (62.9;474)	199 (74.5;603)	0.65
IL-8	19.1 (7.11;44.8)	17.9 (5.71;53.3)	0.94	17.4 (5.82;44.8)	19.9 (7.18;51.2)	0.48
IP-10	12.4 (6.55;23.2)	9.92 (5.21;23.4)	0.54	13.3 (6.63;21.8)	9.85 (5.09;23.7)	0.35
RANTES	59.2 (34.0;113)	64.2 (36.0;93.4)	0.66	56.5 (35.4;89.5)	74.7 (36.5;117)	0.19
TARC	869 (238;1901)	444 (227;999)	0.042	504 (187;1582)	595 (287;1841)	0.23
Soluble receptors						
IL-18BP	160 (22.7;828)	80.3 (20.2;969)	0.73	119 (14.5;834)	134 (22.6;825)	0.74
sIL-2Rα	<2.44 (<2.44;206)	<2.44 (<2.44;250)	0.98	<2.44 (<2.44;206)	<2.44 (<2.44;250)	0.91
sIL-6R	25.6 (19.5;33.0)	29.0 (22.7;44.8)	0.021	26.4 (20.2;37.0)	26.5 (21.5;43.3)	0.71
sTNF-RII	1912 (1167;3885)	3110 (1646;5392)	0.012 *	2039 (1123;4532)	2886 (1481;4661)	0.054
Tissue damage and repair						
ACE	113 (76.2;166)	106 (70.1;145)	0.38	113 (73.2;156)	105 (72.0;150)	0.96
bFGF	3.86 (<0.98;50.6)	2.86 (<0.98;37.4)	0.54	3.20 (<0.98;55.1)	2.86 (<0.98;37.4)	0.38
KIM-1	190 (49.5;709)	274 (89.6;1267)	0.08	157 (48.5;698)	298 (113;1550)	0.011
MMP3	83.3 (48.0;156)	101 (47.1;146)	0.77	94.6 (54.8;160)	88.7 (37.0;136)	0.077
NGFβ	9.57 (4.08;43.9)	6.68 (2.05;35.6)	0.17	9.19 (3.69;40.6)	8.85 (2.61;37.9)	0.76
PDGF-AB	2769 (968;5002)	4682 (2322;7392)	0.0072 *	3541 (1261;5940)	4136 (1440;7338)	0.2

Marker	Newly diagnosed or relapsing AAV			Glucocorticoid treatment at screening		
	Relapsing (n=96)	New (n=90)	p Value	Yes (n=92)	No (n=94)	p Value
TIMP-1	389 (236;673)	525 (311;1149)	0.01 *	473 (290;730)	430 (253;1055)	0.78
Inflammation and vascular injury						
Clusterin	76.7 (65.3;87.0)	80.9 (66.4;96.1)	0.24	79.0 (66.5;94.8)	75.6 (65.7;88.8)	0.17
CRP	0.8 (0.39;2.45)	1.96 (0.6;6.3)	0.0026 *	0.9 (0.3;2.3)	1.9 (0.6;4.9)	0.003
ESR	29 (13;52)	49 (26;80)	0.0002 *	29 (13;53)	48 (25;70)	0.005
ICAM-1	413 (292;775)	555 (346;1005)	0.02	450 (305;974)	484 (317;819)	0.96
NGAL	223 (153;342)	291 (222;431)	0.0057 *	224 (161;340)	283 (204;428)	0.045
PAI-1	1089 (<977;4761)	1002 (<977;6481)	0.77	1887 (<977;5817)	<977 (<977;4509)	0.28
VCAM-1	120 (87.8;168)	144 (106;177)	0.026	127 (95.3;179)	138 (95.5;77.9)	0.52

Values are median (IQR). For units, see table 1.

*
p<0.05 comparing newly diagnosed with relapsing AAV, still significant after adjustment for multiple comparisons. No marker met this standard comparing subjects treated or not treated with glucocorticoids, although several unadjusted p values were<0.05.

BCA-1, CXCL13; bFGF, basic fibroblast growth factor; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–monocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IL-8, CXCL8; IL-18BP, interleukin 18 binding protein; IP-10, CXCL10; KIM-1, kidney injury molecule-1; MMP-3, matrix metalloproteinase-3; NGAL, neutrophil gelatinase-associated lipocalin; NGFβ, nerve growth factor β; PAI-1, plasminogen activator inhibitor-1; PDGF-AB, platelet-derived growth factor, A and B subunits; RANTES, CCL5; sIL-2R, soluble IL 2 receptor; sIL-6R, soluble IL 6 receptor; sTNF-RII, soluble TNF receptor II; TARC, CCL17; TIMP-1, tissue inhibitor of metalloproteinases-1; VCAM-1, vascular cell adhesion molecule-1.

Table 4

Marker levels in patients in remission at month 6, stratified by treatment

Marker	Treatment group			Glucocorticoids at month 6		
	CYC/AZA (n=60)	RTX (n=72)	p Value	Yes (n=20)	No (n=117)	p Value
Cytokines						
G-CSF	9.56 (4.67;19.6)	11.9 (6.81;29.9)	0.19	21.9 (4.03;36.6)	10.2 (6.14;20.7)	0.24
GM-CSF	1.38 (<0.98;4.84)	1.08 (<0.98;8.33)	0.72	1.18 (<0.98;7.66)	1.17 (<0.98;4.68)	0.88
IFNγ	<0.49 (<0.49;<0.49)	<0.49 (<0.49;<0.49)	0.15	<0.49 (<0.49;<0.49)	<0.49 (<0.49;<0.49)	0.84
IL-6	<0.49 (<0.49;0.73)	<0.49 (<0.49;0.97)	0.35	0.59 (<0.49;1.18)	<0.49 (<0.49;0.73)	0.2
IL-15	9.93 (3.80;16.4)	4.11 (1.77;11.1)	0.0014*	4.93 (2.11;13.8)	5.69 (2.60;13.5)	0.69
IL-18	49.7 (27.2;83.3)	45.7 (31.1;88.0)	0.49	45.2 (28.0;128)	52.8 (33.1;84.8)	0.99
Osteopontin	60.4 (37.7;98.4)	50.5 (37.2;72.9)	0.072	54.4 (38.6;76.4)	54.5 (37.6;81.9)	0.82
Chemokines						
BCA-1	38.4 (23.5;60.0)	23.9 (16.8;49.2)	0.0053	64.3 (35.1;191)	26.9 (17.1;48.2)	<0.0001*
IL-8	6.40 (3.78;13.0)	8.33 (3.49;22.1)	0.41	6.65 (1.76;24.5)	7.23 (3.74;14.8)	0.72
IP-10	12.6 (7.23;25.4)	12.9 (7.68;23.5)	0.98	16.6 (6.89;30.0)	13.1 (7.68;24.2)	0.71
RANTES	67.0 (35.5;100)	45.3 (29.2;84.2)	0.08	62.6 (42.8;200)	51.1 (30.2;89.5)	0.16
TARC	555 (247;2500)	700 (393;2500)	0.36	653 (491;2161)	655 (322;2500)	0.58
Soluble receptors						
IL-18BP	13.0 (6.11;42.4)	18.4 (<6.11;65.2)	0.5	12.0 (<6.11;49.3)	16.4 (<6.11;55.7)	0.51
sIL-2Rα	<2.44 (<2.44;<2.44)	<2.44 (<2.44;<2.44)	0.19	<2.44 (<2.44;<2.44)	<2.44 (<2.44;<2.44)	0.49
sIL-6R	21.2 (16.0;33.9)	22.5 (15.2;32.8)	0.89	23.0 (15.2;35.7)	21.7 (15.5;32.4)	0.81
sTNF-RII	2039 (1137;4009)	2902 (1470;6569)	0.087	1528 (978;3285)	2524 (1445;5985)	0.079
Tissue damage and repair						
ACE	190 (127;253)	170 (132;260)	0.83	165 (142;253)	180 (129;251)	0.91
bFGF	<0.98 (<0.98;11.6)	<0.98 (<0.98;4.53)	0.88	3.15 (<0.98;21.0)	<0.98 (<0.98;3.75)	0.16
KIM-1	40.6 (17.3;111)	53.9 (15.9;134)	0.67	28.2 (12.8;119)	49.3 (18.5;127)	0.3
MMP-3	14.9 (11.0;26.0)	16.6 (12.9;30.5)	0.39	29.3 (16.1;43.0)	14.9 (11.6;24.0)	0.0039
NGFβ	2.30 (0.94;4.21)	2.71 (1.35;4.29)	0.24	2.83 (0.75;7.17)	2.35 (1.26;4.03)	0.58
PDGF-AB	2437 (701;4814)	3807 (1463;5864)	0.018	3049 (748;3967)	3295 (886;5610)	0.34
TIMP-1	169 (125;233)	162 (129;263)	0.9	212 (144;355)	160 (125;224)	0.078

Marker	Treatment group			Glucocorticoids at month 6		
	CYC/AZA (n=60)	RTX (n=72)	p Value	Yes (n=20)	No (n=117)	p Value
Inflammation and vascular injury						
Clusterin	78.3 (64.6;91.3)	68.2 (57.5;83.8)	0.024	69.4 (57.2;82.6)	73.0 (60.0;86.8)	0.26
CRP	0.4 (0.3;1.2)	0.5 (0.3;1.0)	0.78	0.5 (0.3;0.8)	0.5 (0.3;1.3)	0.77
ESR	17 (9;26)	11 (7;21)	0.034	19 (7;28)	12 (7;22)	0.15
ICAM-1	461 (318;905)	607 (356;895)	0.25	786 (458;1088)	490 (329;791)	0.14
NGAL	169 (99.9;215)	179 (137;287)	0.028	157 (119;222)	174 (130;243)	0.61
PAI-1	1200 (<977;3733)	1645 (<977;5217)	0.49	2545 (<977;5358)	1202 (<977;3969)	0.38
VCAM-1	155 (116;230)	138 (102;216)	0.36	208 (126;455)	145 (107;209)	0.027

Values are median (IQR). For units, see table 1.

*
p<0.05 comparing groups, still significant after adjustment for multiple comparisons.

BCA-1, CXCL13; bFGF, basic fibroblast growth factor; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–monocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IL-8, CXCL8; IL-18BP, interleukin 18 binding protein; IP-10, CXCL10; KIM-1, kidney injury molecule-1; MMP-3, matrix metalloproteinase-3; NGAL, neutrophil gelatinase-associated lipocalin; NGFβ, nerve growth factor β; PAI-1, plasminogen activator inhibitor-1; PDGF-AB, platelet-derived growth factor, A and B subunits; RANTES, CCL5; sIL-2R, soluble IL 2 receptor; sIL-6R, soluble IL 6 receptor; sTNF-RII, soluble TNF receptor II; TARC, CCL17; TIMP-1, tissue inhibitor of metalloproteinases-1; VCAM-1, vascular cell adhesion molecule-1.