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ORIGINAL ARTICLE

Serum levels of interleukin-32 and interleukin-6 in granulomatosis with polyangiitis and microscopic polyangiitis: association with clinical and biochemical findings

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ABSTRACT. *Background*: Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis is an autoimmune disorder of unknown etiology with dysregulated cytokines levels. *Objectives*: The main aim of this study was to assess the clinical correlation between antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis, granulomatosis with polyangiitis (GPA) serum levels of the microscopic polyangiitis (MPA), serum levels of the proinflammatory cytokines, interleukin (IL)-32 and interleukin-6. *Methods*: Study included 71 patients, 47 with GPA and 24 with MPA. Serum IL-32 and IL-6 concentrations were analyzed in all patients, and compared with levels observed in 10 controls. IL-32 and IL-6 were evaluated using DuoSet and Quantikine HS ELISA, respectively. IL-32 and IL-6 concentrations were correlated with disease-related clinical and laboratory findings. *Results*: IL-32 and IL-6 levels were significantly higher in GPA and MPA than in controls, especially IL-32 levels in GPA were elevated. IL-32 concentrations correlated positively with anti-proteinase 3 - ANCA (PR3-ANCA) levels in GPA ($P < 0.0001$), and with anti-myeloperoxidase ANCA (MPO-ANCA) in MPA ($P = 0.049$). IL-32 levels correlated positively with disease activity in GPA and MPA ($P < 0.0001$). GPA patients with pulmonary, cutaneous, and musculoskeletal involvement presented the highest IL-6 serum levels. Cutaneous manifestations correlated positively with IL-6 levels in MPA patients ($P = 0.05$). ANCA-positive patients with GPA expressed significantly high IL-6 levels ($P = 0.036$). No significant difference in IL-32 values was observed between ANCA-positive and ANCA-negative patients. *Conclusions*: Patients with GPA and MPA present higher serum IL-32 and IL-6 levels than controls. IL-32 levels correlate positively with disease activity.

Key words: granulomatosis with polyangiitis, interleukin-6, interleukin-32, microscopic polyangiitis, vasculitis, ANCA

INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is characterized by vasculitis of small vessels and the presence of ANCA in serum [1]. The main variants of AAV are granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) [1]. The serum presence of ANCA is thought to be a serologic marker of AVV; however, AAV cases with negative ANCA are also observed [1]. There are two types of ANCA: anti-proteinase 3 (PR3) – ANCA, and anti-myeloperoxidase (MPO) – ANCA [2]. PR3-ANCAs are mainly found in GPA, whereas MPO-ANCAs are mainly found in MPA [1, 2]. GPA and MPA often share overlapping clinical manifestations. It is still undefined whether GPA and MPA are two separate entities or two forms of the same disease [3].

The exact pathomechanisms contributing to the pathogenesis and relapses of AAV are still unknown. It was suggested that AAV presumably develops in genetically susceptible individuals as a result of autoimmune phenomena [4]. Beside the fact that ANCAs are considered to be pathogenic, the emerging evidence implies the additional role of T cells in AAV development [5]. The processes by which ANCA arise and the role of these autoantibodies in inducing GPA and MPA remain unclear. The main potential pathogenic mechanism of AAV development is ANCAs' binding to their receptors, PR3 and MPO which are located on neutrophils. These are first activated by cytokines priming, mainly by TNF-α, IL-1, or IL-18 [6, 7]. ANCA-PR3/ANCA-MPO complexes formed on neutrophils surfaces and induce their degranulation which in turn leads to endothelial cell injury and vasculitis [8]. In addition to that an

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increase in certain T-cell activation markers in AAV was observed [5, 8]. Imbalance between particular T-cell subsets was found in AAV patients [5, 8]. The exact mechanisms leading to such dysregulation have not been elucidated yet; however, nasal carriage of *Staphylococcus aureus* is considered to be a trigger point and risk factor for further disease relapses [8]. Ongoing infection can lead to neutrophil priming, increased expression of adhesion molecules on endothelial cells and switch between T cell subsets, mainly effector T cells (Teffs) and regulatory T cells (Tregs) [5]. Upregulated population of effector memory T cells (Tems), especially IL-17 producing T cells (Th17 cells), which are not properly balanced by regulatory Tregs, results in certain proinflammatory cytokines release. This subsequently promotes neutrophil priming [8]. Pro-inflammatory milieu induced by Tems and constant T cells disbalance leads to persistent, self-perpetuating state of autoimmune activation [8].

The crucial role of cytokines and chemokines in AAV pathogenesis is strongly emphasized by various authors [6, 9]. However, the exact markers of these diseases are still unknown.

PR3 is a molecule that plays a critical role in GPA [10]. It was observed that PR3 released from activated neutrophils interacted with IL-32 [11]. PR3 molecule was described as a protein binding to IL-32 [11]. Additionally, it was able to activate protease-activated receptor 2 (PAR2) leading to increased IL-32 bioactivity [11]. PR3-induced activation of PAR2 on immune cells membranes led to proinflammatory effects, especially CD4 + T cells-related IFN-γ synthesis [12].

IL-32, a novel pluripotent cytokine, was found to be a mediator in various cellular mechanisms including cell differentiation, apoptosis, and immune responses [13]. The epithelial cells from healthy individuals express low levels of IL-32, whereas in various chronic diseases the concentration of this cytokine is significantly elevated [14]. This proinflammatory cytokine is considered to be a Th1-related molecule released by immune and nonimmune cells when initially stimulated by other proinflammatory factors such as IFN-γ, TNF-α, IL-1β, IL-12, and IL-18 [15]. It was described that IL-32 was involved in several autoimmune and inflammatory disorders by mediating NF-κB and p38 mitogen-activated protein kinase pathways. That subsequently led to production of other proinflammatory molecules, including IL-1β, IL-6, IL-8, TNF-α, chemokines, and macrophage inflammatory protein-2 (MIP-2) [11, 15-17].

The evidence of the exact role of IL-32 pathway in AAV is lacking. There were only a few studies analyzing this cytokine in GPA [10-12]. To the best of our knowledge, there has been only one study evaluating IL-32 in GPA patients, and there have been no reports on IL-32 expression in MPA [10]. Owing to the fact that IL-32 is presumably Th1-mediated cytokine and Th1 response in GPA is now strongly emphasized, we assessed the level of this cytokine and its clinical correlation in GPA. Since both GPA and MPA belong to AAV, we also compared IL-32 levels in these two entities.

IL-32 is able to stimulate the production of another proinflammatory cytokine, namely IL-6. For that reason we also investigated this molecule. The role of IL-6 in AAV has already been suggested; however, further investigation is required [18-24]. We were strongly encouraged to assess IL-6 levels and its clinical correlations in our patients, by Berti *et al.* research which revealed successful treatment of MPA patient with tocilizumab, a monoclonal antibody against IL-6 receptor [20].

The main aims of the study were: to analyze the levels of IL-32 and IL-6 in patients with GPA and MPA, and compare them with levels observed in healthy controls to determine if their levels differ in these groups; to determine specific IL-32 and IL-6 patterns in patients with active GPA and MPA; to find the potential correlation between IL-32 and IL-6 levels, and particular disease-related abnormalities in GPA and MPA, and to highlight the most significant one; to assess if measuring IL-32 and IL-6 levels in GPA and MPA could provide useful information about clinicopathological abnormalities in GPA and MPA.

Determining association between IL-32 and IL-6 levels, and various GPA/MPA-related abnormalities, and then measuring IL-32 and IL-6 levels in patients with GPA and MPA could help in monitoring and managing these diseases.

Here, we report the levels of IL-32 and their clinical correlation in GPA and, for the first time, in MPA. We also present the correlation between IL-32 and IL-6 and various disease-related abnormalities in GPA and MPA patients.

PATIENTS AND METHODS

Patients and controls

An institutional ethics committee approved the study. The study protocol complied with the Helsinki Declaration.

We recruited 71 patients with AAV who were admitted to our University Hospital between April 2016 and February 2018. All the patients were included after their informed consent. Significant patients' AAV-related records gathered before April 2016 were included in the study if appropriate. The American College of Rheumatology (ACR) classification criteria for vasculitis, and the Chapel Hill Consensus Conference (CHCC) classification were used to assign patients to GPA or MPA subgroup [25, 26]. In accordance with these criteria, 47 patients were classified as suffering from GPA and 24 individuals met the criteria for MPA *(table 1)*. The control group consisted of 10 healthy age-matched blood donors, 5 females and 5 males. Only healthy controls with no abnormalities in laboratory tests were included.

Disease activity was assessed using Birmingham Vasculitis Activity Score (BVAS) version 3 (scoring range: 0-63) [27]. BVAS v.3 score of 0 represented disease remission, whereas BVAS $\geq$1 indicated active disease. A total of 71 patients were examined during the active phase of the disease to minimize the bias. Blood samples were collected from patients at presentation or within 14 days of presentation, and before drugs administration. Appropriate treatment for AAV was introduced immediately after the biological material was taken. Only the patients who

Table 1
Patients' characteristics.

	GPA	MPA
Number of patients, n (%)	47 (66.2)	24 (33.8)
Gender (female/male), n (%)	26/21 (55.3/44.7)	13/11 (54.2/45.8)
Age, median (IQR)	55 (40-61)	64.5 (47.5-74.5)
Disease duration in years, median (IQR)	3 (1-5)	2 (1-5)
ANCA positivity, n (%)[a]	40 (85.1)	22 (91.7)
PR3-ANCA positivity, n (%)[a]	38 (80.9)	0 (0)
MPO-ANCA positivity, n (%)[a]	3 (6.4)	22 (91.7)
ANCA negativity, n (%)	7 (14.9)	2 (8.3)
BVAS, median (IQR)	9 (5-12.5)	8 (4-10.5)
Disease severity stage		
Localised, *n* (%)	10 (21.3)	2 (8.3)
Early systemic, *n* (%)	17 (36.2)	3 (12.5)
Generalised, *n* (%)	16 (34.0)	14 (58.3)
Severe, *n* (%)	4 (8.5)	5 (20.8)
Organ involvement, n (%)		
Pulmonary	33 (70.2)	16 (66.7)
Kidney	27 (57.4)	22 (91.7)
ENT	41 (87.2)	9 (37.5)
Nervous	10 (21.3)	0 (0)
Cutaneous	9 (19.1)	8 (33.3)
Musculoskeletal	11 (23.4)	7 (29.2)
Ocular	8 (17.0)	4 (16.7)
Cardiovascular	10 (21.3)	8 (33.3)
Gastrointestinal	3 (6.4)	4 (16.7)
S. aureus presence, n (%)	11 (23.4)	4 (16.7)

[a] Anytime in patients' history.

were not receiving treatment for vasculitis (*e.g.*, glucocorticosteroids, immunosuppressants, biologic drugs), at the time of clinical assessment and blood samples collection were included in the study to minimize drug-related bias.

Disease severity stage of studied patients was measured using European Vasculitis Study Group (EUVAS) recommendations [28].

Laboratory tests including complete blood count (CBC), urinalysis, serum ANCA and creatinine levels, erythrocyte sedimentation rate (ESR), C-reactive protein (CPR), and procalcitonin (PCT) were done during hospitalization. Renal function was assessed using serum creatinine levels and urinalysis. Nasal swabs were taken to detect *S. aureus* nasal carriage. Swabs in standard transport tubes were immediately transported to microbiological laboratory for assessment. ANCAs profile and their serum levels were determined by indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA). In patients with ANCA-positivity detected by IIF, ELISA was used to assess the serum levels of PR3-ANCA and MPO-ANCA. The norms for all the studied laboratory parameters are presented in *table 2*. The separate blood sample from every patient was collected to assess serum IL-32 and IL-6 levels.

Cytokine assessment

IL-6 serum concentration was determined by Quantikine HS ELISA (RnD Systems). The detection range of the assay was 0.039-10 pg/ml. IL-32 serum concentration was evaluated using DuoSet ELISA (RnD Systems). The detection range of the assay was 50-5000 pg/ml. The samples exceeding the detection limit were 10-fold diluted and then reassayed.

The correlation between IL-32/IL-6 levels, and various clinical and biochemical variables was examined. The IL-32 and IL-6 levels in the studied patients were compared with the levels detected in the healthy controls (HC).

Statistics

Statistical analysis was performed using SPSS v. 23 software. Not normally distributed data were presented as median, IQR. Correlation analysis was performed using the nonparametric Spearman's test. The Mann-Whitney U test and chi square test were used for comparison of unpaired data. In case of multiple comparisons, the nonparametric Kruskal-Wallis test was used. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

GPA patients versus *MPA patients* versus *healthy controls (HC)*

The comparison of clinical features of patients with GPA and MPA is presented in *table 1*. Laboratory findings in both the groups are described in *table 2*.

Table 2
Laboratory findings in GPA and MPA patients.

	GPA (n = 47)	MPA (n = 24)
CRP (mg/l)[†,a]	4 (4-24.15)	4 (4-8.09)
ESR (mm/h)[†,b]	25 (8-42)	10 (8-37)
PCT (ng/ml)[†,c]	0.05 (0.025-0.135)	0.05 (0.05-0.235)
Serum creatinine (mg/dl)[†,d]	1 (1-1.83)	2.62 (1.44-3.8)
Serum urea (>43 mg/dl)[e], *n (%)*	20 (42.6)	20 (83.3)
PR3-ANCA level (RU/ml)[†,f]	16.6 (3-115.56) min-max [1->1000]	0 (0-0)
MPO-ANCA level (RU/ml)[†,g]	0 (0-0) min-max [0-71.51]	124.5 (0-161.72) min-max [0-201]
IL-6 (pg/ml)[†]	2.91 (1.37-5.86)	4.81 (2.03-9.42)
IL-32 (pg/ml)[†]	387.0 (83.5-5442.5)	202.0 (41.5-1186.0)
Urinalysis – abnormalities, n (%)		
Proteinuria	19 (40.43)	15 (62.5)
Microscopic hematuria	22 (46.81)	15 (62.5)
Gross hematuria	10 (21.28)	13 (54.17)
Leukocyturia	16 (34.04)	8 (33.3)
CBC – abnormalities, n (%)		
Leukocytosis	18 (38.3)	15 (62.5)
Neutrophilia	27 (57.45)	15 (62.5)
Lymphopenia	24 (51.06)	13 (62.5)
Anaemia	21 (44.68)	8 (33.3)
Thrombocytosis	7 (14.89)	0 (0)

n: number of patients (%); min-max: minimum and maximum level; CRP: C-reactive protein; ESR; erythrocyte sedimentation rate; CBC: complete blood count; PCT: Procalcitonin.
[†] Median, IQR.
[a] CRP norm (0-5 mg/l).
[b] ESR norm (0-10 mm/h).
[c] PCT norm (0-0.1 ng/ml).
[d] Serum creatinine norm (0.7-1.3 mg/dl).
[e] Serum urea norm (17-43 mg/dl).
[f] PR3-ANCA norm (0-19.999 RU/ml).
[g] MPO-ANCA norm (0-12 RU/ml).

IL-32

IL-32 levels in studied groups are presented in *table 3*. We observed statistically significant higher levels of serum IL-32 in patients with GPA than in HC ($P = 0.01$). There was also a nonsignificant borderline trend toward higher IL-32 levels in patients with MPA in comparison with HC ($P = 0.075$). The levels of IL-32 were significantly higher in individuals with GPA than in those with MPA ($P = 0.042$).

IL-32 in GPA (table 4)

The correlation between IL-32 levels and analyzed variables is presented in *table 4*.
We observed strong, statistically significant positive correlation between IL-32 levels and BVAS ($P < 0.0001$). There was a trend toward higher IL-32 levels in patients with severe stage of the disease in comparison with less serious stages ($P = 0.052$). A positive significant correlation was found for the IL-32 and PR3-ANCA levels ($P < 0.0001$). Although IL-32 levels were higher in younger patients and those with shorter disease duration, these differences did not reach statistical significance ($P = 0.078$ and $P = 0.152$, respectively).

There was no significant correlation between IL-32 levels and organ involvement, sex and analyzed biochemical parameters (CRP, ESR, PCT, CBC, MPO-ANCA levels, creatinine levels and urinalysis).

IL-32 in MPA (table 5)

There was a significant positive correlation between IL-32 levels and BVAS in MPA ($P < 0.0001$). We also found significant positive associations between IL-32 levels and both serum creatinine and MPO-ANCA levels ($P = 0.014$ and $P = 0.049$, respectively). In addition to that we observed not statistically significant trend toward higher IL-32 levels in patients with nasal *S. aureus* carriage ($P = 0.053$). We also found positive association between IL-32 levels and proteinuria in urinalysis ($P = 0.025$).
No significant correlation between IL-32 level and organ involvement, age, sex, disease stage, ANCA presence, disease duration, CPR, PCT, ESR, and CBC was found.

IL-6

3.5.1. IL-6 levels in studied groups are presented in *table 3*.

Table 3
IL-32 and IL-6 levels in studied groups.

	GPA, *n* = 47 Median (IQR) * [min-max]	**MPA, *n* = 24**	**HC, *n* = 10**	**GPA:MPA, *P*-value**	**GPA:HC, *P*-value**	**MPA:HC, *P*-value**
IL-32 (pg/ml)	387.0 (83.5-5442.5)* [0-52460.0]	202.0 (41.5-1186.0)* [0-6439.0]	78 (0-199)* [0-398.0]	***P* = 0.042**	***P* = 0.01**	*P* = 0.075
IL-6 (pg/ml)	2.91 (1.37-5.86)* [0.51-40.0]	4.81 (2.03-9.42)* [0.82-20.0]	1.15 (0.59-1.64)* [0.47-1.81]	*P* = 0.146	***P* = 0.007**	***P* = 0.001**

HC: healthy controls. Significant *P*-values (<0.05) are in bold.

Table 4
Correlation between IL-32 and IL-6 levels, and various disease-related abnormalities in GPA.

	IL-32		**IL-6**	
	r	***P*-value**	***r***	***P*-value**
Age	-0.259	0.087	0.097	0.519
Sex	-	0.256	-	0.235
Disease duration	-0.212	0.152	-0.217	0.143
Disease stage	-	0.052	-	0.129
BVAS	0.841	**<0.0001**	0.081	0.588
Pulmonary involvement	-	0.123	-	**0.002**
Kidney involvement	-	0.832	-	0.897
ENT involvement	-	0.713	-	0.588
Nervous involvement	-	0.262	-	0.222
Ocular involvement	-	0.256	-	0.246
Cardiovascular involvement	-	0.876	-	0.370
Cutaneous involvement	-	0.645	-	**0.005**
Musculoskeletal involvement	-	0.405	-	**0.024**
Gastrointestinal involvement	-	0.541	-	0.433
ANCA presence anytime	-	0.61	-	**0.036**
MPO-ANCA presence anytime	-	0.256	-	0.632
PR3-ANCA presence anytime	-	0.684	-	**0.043**
MPO-ANCA level[a]	-0.169	0.256	0.063	0.676
PR3-ANCA level[a]	0.605	**<0.0001**	0.129	0.388
ESR[a]	0.094	0.531	0.447	**0.002**
PCT[a]	-0.083	0.580	0.354	**0.015**
CRP[a]	0.091	0.542	0.829	**<0.0001**
Creatinine level[a]	-0.111	0.459	-	0.591
Urea level above normal[a]	-	0.682	-	0.675
Nasal S. aureus presence[a]	-	0.652	-	0.958
Urinalysis[a]	-	0.427	-	0.643
Leukocyturia		0.241		0.302
Proteinuria		0.931		0.530
Hematuria		0.507		0.543
Gross hematuria		0.566		0.640
Complete blood count[a]	-	0.842	-	0.318
Leukocytosis		0.809		0.906
Neutrophilia		0.897		**0.006**
Lymphopenia		0.417		**0.02**
Anemia		0.881		0.095
Thrombocytosis		0.490		0.473

NSC: no significant correlation; *r*: correlation coefficient. Significant *P*-values (<0.05) are in bold.
[a] At the time of assessment.

Table 5
Correlation between IL-32 and IL-6 levels, and various disease-related abnormalities in MPA.

	IL-32		**IL-6**	
	r	***P*-value**	***r***	***P*-value**
Age	0.186	0.383	0.35	0.094
Sex	-	0.256	-	0.908
Disease duration	-0.101	0.638	0.216	0.311
Disease stage	-	0.063	-	0.385
BVAS	0.872	**<0.0001**	0.212	0.319
Pulmonary involvement	-	0.854	-	0.603
Kidney involvement	-	0.753	-	0.210
ENT involvement	-	0.385	-	0.551
Nervous involvement	n/a		n/a	
Ocular involvement	-	0.276	-	0.561
Cardiovascular involvement	-	0.110	-	0.806
Cutaneous involvement	-	0.389	-	**0.05**
Musculoskeletal involvement	-	0.687	-	0.525
Gastrointestinal involvement	-	0.815	-	0.333
ANCA presence anytime	-	0.142	-	0.347
MPO-ANCA presence anytime	-	0.142	-	0.347
PR3-ANCA presence anytime	n/a		n/a	
MPO-ANCA level[a]	0.406	**0.049**	0.257	0.226
PR3-ANCA level[a]	n/a	n/a		
ESR[a]	-0.281	0.183	0.329	0.116
PCT[a]	0.376	0.070	0.455	**0.025**
CRP[a]	0.017	0.526	0.499	**0.013**
Creatinine level[a]	0.494	**0.014**	0.485	**0.016**
Urea level above normal[a]	-	0.213	-	0.201
Nasal S. aureus presence[a]	-	0.053	-	0.506
Urinalysis[a]	-	0.185	-	0.386
Leukocyturia		0.806		**0.022**
Proteinuria		**0.025**		0.493
Hematuria		0.22		0.095
Gross hematuria		0.399		**0.02**
Complete blood count[a]	-	0.721	-	0.449
Leucocytosis		0.906		0.598
Neutrophilia		0.499		0.603
Lymphopenia		**0.0432**		0.817
Anemia		0.539		0.118
Thrombocytosis		0.294		0.531

NSC: no significant correlation; n/a: not applicable; r: correlation coefficient. Significant *P*-values (<0.05) are in bold.
[a] At the time of assessment

Although the levels of IL-6 were slightly higher in patients with MPA than in GPA, the difference did not reach statistical significance ($P = 0.146$). In contrast, IL-6 levels in patients with GPA and MPA were significantly higher than in HC ($P = 0.007$ and $P = 0.001$, respectively).

IL-6 in GPA (table 4)
We noted that patients with higher CRP levels presented significantly higher IL-6 amounts ($P = 0.0001$). Higher IL-6 levels also correlated with higher ESR and PCT levels ($P = 0.002$ and $P = 0.015$, respectively). Additionally, IL-6 levels significantly correlated with various organs involvements. Higher IL-6 levels were expressed by patients with pulmonary, cutaneous, and musculoskeletal disease-related manifestations ($P = 0.002$, $P = 0.005$ and $P = 0.024$, respectively).
Beside the fact that individuals with PR3-ANCA presence displayed higher IL-6 levels than seronegative patients ($P = 0.043$), no significant correlation between PR3-ANCA levels and IL-6 levels was detected ($P = 0.388$). Moreover, patients with neutrophilia and lymphopenia expressed higher IL-6 levels ($P = 0.006$ and $P = 0.02$, respectively).
Although we noted higher IL-6 levels in patients with shorter disease duration, this difference did not reach statistical significance ($P = 0.143$).

Serum amounts of IL-6 did not correlate with creatinine and MPO-ANCA levels, urinalysis, *S. aureus* carriage, age, sex, disease stage, and BVAS.

IL-6 in MPA (table 5)
Elevated IL-6 amounts positively correlated with PCT, CRP, creatinine levels, leukocyturia, and gross hematuria ($P = 0.025$, $P = 0.013$, $P = 0.016$, $P = 0.022$ and $P = 0.02$, respectively). Besides cutaneous involvement, no other organ involvement correlated with IL-6 levels. Patients with disease-related cutaneous manifestations expressed significantly higher IL-6 levels than subjects with other organs involvement ($P = 0.05$). We noted higher amounts of IL-6 in individuals with higher MPO-ANCA levels. Nevertheless, this observation did not reach statistical significance ($P = 0.226$).
We did not find any significant correlations between IL-6 levels and *S. aureus* carriage, ANCA presence, age, sex, disease stage, duration, and BVAS.

Correlation between IL-32 and IL-6
There was no significant correlation between IL-32 and IL-6 levels in the studied group. The levels of both cytokines in patients and healthy controls are presented in *table 3*.

DISCUSSION

The most important findings in our study were the increased levels of IL-32 in GPA patients, the positive correlation between IL-32 levels and disease activity (BVAS) in patients with GPA, and the positive correlation between IL-32 levels and BVAS in MPA patients.
IL-6 levels were significantly higher in GPA and MPA patients than in HC. Increased IL-6 levels correlated positively with gross hematuria, leukocyturia, and higher serum creatinine levels in MPA. The positive correlation between IL-6 and both, CRP and PCT levels was observed in GPA and MPA, and also between IL-6 and ESR levels in GPA. GPA patients with positive PR3-ANCA expressed higher IL-6 levels than seronegative individuals.
Some of our findings were consistent with the previous study conducted by Bae *et al*. and may suggest that measuring IL-32 could be a useful tool in GPA monitoring [10]. The authors reveled that among three cytokines, namely IL-32, IL-6, and TNF-α, IL-32 expressed the strongest association with GPA [10]. They found that increased IL-32 levels in GPA resulted from the upregulation of transcription and translation of IL-32 gene [10]. The authors speculated that IL-32 promoter region or the actual IL-32 exon region in genome of patients with GPA could differ from that observed in healthy individuals [10]. Additionally, it was suggested that increased levels of IL-32 could be a consequence of the significant interaction between PR3 and IL-32 [11]. The authors speculated that PR3 influenced IL-32 in two separate ways, enzymatic and nonenzymatic [11]. The nonenzymatic way was presumably less important in GPA biology than the enzymatic one [11, 12]. The enzymatic activity of PR3 was based on cleaving IL-32. It led to creation of more active form of IL-32 and consequently its enhanced biological activity [11].
Because of the fact that PR3 released from activated neutrophils in GPA patients stimulates further production of PR3-ANCA, the level of PR3-ANCA autoantibodies may consequently interact with IL-32 levels [10]. Positive correlation between IL-32 antibodies and PR3 antibodies was detected by Bae *et al*. [10]. Consistently with Bae *et al*. results, our patients with increased serum PR3-ANCA levels also expressed increased serum IL-32 amounts. Bae at al. revealed strong and significant positive association between BVAS and IL-32 levels in GPA, which was similar to our results [10]. The association between BVAS and IL-32 was higher than observed for IL-32, and PR3, IL-6 and TNF-α in this study [10].
Additionally, our patients with more severe disease stages displayed higher IL-32 levels. Interestingly, increased IL-32 levels detected in synovial tissues in patients with rheumatoid arthritis correlated positively with disease severity [14]. In light of the fact that one of the most important biologic activities of IL-32 in GPA is inducing the production of other proinflammatory cytokines, we speculated that increased IL-32 levels in patients with more severe disease could be a consequence of more intensive systemic inflammation in these individuals [12].
To the best of our knowledge, there has been no clinical report measuring IL-32 levels and its clinical correlation in MPA patients so far. We found that IL-32 levels in MPA were higher than in HC; however, the difference did not reach statistical significance. We observed positive correlation between IL-32 levels and both MPO-ANCA presence and IL-32 serum creatinine levels. Disease activity expressed by BVAS in MPA patients positively correlated with IL-32 levels, similarly to patients with GPA.
According to our results, increased serum level of IL-32 is associated with elevated levels of PR3-ANCA in patients with GPA, and MPO-ANCA levels in patients with MPA. Since we did not find significant difference in serum IL-32 amounts between ANCA-positive and ANCA-negative patients, we speculate that measuring IL-32 levels may be a useful tool in shedding the light on potential AAV development, especially in patients with negative ANCA. Our results also suggested that high IL-32 levels might correlate with higher disease activity. According to this, assessing IL-32 levels may potentially help in monitoring disease activity. It might reflect disease activity in a more specific way than CRP, PCT, or ESR. Our results also revealed that GPA patients with short disease duration had higher IL-32 and IL-6 levels than those with longer history of the disease. Yet, this observation was not statistically significant. Because of the fact that in patients with short history of GPA symptoms establishing the proper diagnosis is often difficult, finding a new marker of the disease is of great importance. It prompted us to suspect that increased levels of IL-32 and IL-6, which were observed at initial stage of GPA in our cohort, could be a phenomenon that may possibly shed the light on potential GPA development in patients in whom the diagnosis has not already been established.

Consistently with other authors, our patients with active MPA and GPA expressed higher serum IL-6 levels than HC [19, 20, 23]. We found that MPA patients displayed slightly higher IL-6 amounts than GPA patients, similarly to Ohlsson *et al*. results [19]. There was a positive association between IL-6 and PR3-ANCA presence in GPA individuals, and no significant association between IL-6 and MPO-ANCA presence in MPA patients. In contrast, Ohlsson *et al*. observed higher IL-6 levels in MPO-ANCA-positive patients than in PR3-ANCA-positive ones [19]. Consistently with our results, no significant association between ANCA titers and IL-6 amounts was observed by Ohlsson *et al*. [19]. Interestingly, it was found that biopsy samples obtained from inflamed tissues of AAV patients revealed IL-6 expression [20]. It strongly suggested the potential role of this cytokine in inflammatory process in AAV [20]. Elevated levels of IL-6 in GPA were also reported by Bae *et al*. [10]. In contrast to our and Ohlsson *et al*. results, Berti *et al*. found that MPA patients expressed lower IL-6 levels than GPA patients [24].

We also revealed that GPA and MPA individuals with cutaneous involvement had significantly elevated IL-6 levels. A similar observation was made for GPA patients with pulmonary and musculoskeletal involvement. According to this, we speculated that IL-6 could be a potential marker of organ involvement in AAV, especially the cutaneous one.

In contrast to Ohlsson *et al*. results presenting positive association between IL-6 amounts and disease activity in AAV patients, we did not observe such correlation in our GPA cohort [19]. According to our study, high IL-6 levels correlated positively with ESR, CRP, PCT in GPA patients, whereas in MPA patients high IL-6 levels were observed in those with higher PCT, CRP, and creatinine levels. The significant association between IL-6 amounts and CRP in AAV patients was also found in other studies [19, 23]. The potential role of IL-6 in decreased renal function and chronic renal diseases was also observed by other authors [19, 29].

This study has several important strengths. First, this is the second available clinical study evaluating IL-32 in GPA. However, it was conducted on a significantly more representative cohort than the pioneer one. It is also the first report examining IL-32 in MPA. Additionally, this research assessed the correlation between both, IL-32 and IL-6, and various disease-related clinical and laboratory abnormalities in GPA and MPA. Reports on this matter are lacking.

This study also has some limitations. First, it was conducted on a quite small cohort with a relatively scanty subgroup of seronegative individuals. It included only patients with active disease, thus the results cannot be generalized to all patients with GPA and MPA, especially to those in remission. Moreover, IL-32 and IL-6 serum levels were assessed in every patient only once. For that reason we did not know exactly if the levels remained unchanged in time intervals. Additionally, we only measured the serum levels of circulating cytokines without measuring their peripheral blood mononuclear cell (PBMC) production. Therefore, we could not prove that these cytokines have a role in pathophysiology of the disease. Finally, the number of controls was limited. Because of the fact that IL-32 and IL-6 levels in every person from our control group reached the reference value for the healthy population set by the manufacturer, the number of controls included in the study was limited to such small group.

Based on available data, IL-32 may act as a potential marker of GPA that could have significant therapeutic consequences. The exact reason why IL-32 and IL-6 levels are elevated in AAV remains unclear. The genetic investigation of the exact IL-32 and IL-6 genome regions in patients with GPA and MPA is warranted to establish whether it is different in these two entities, and whether it differs from genome observed in healthy individuals. Moreover, studies on stimulated cells derived from GPA and MPA patients to assess cellular IL-32 and IL-6 secretion as well as studies on cytokines expression in biopsy specimens of affected tissues are justified. Studies comparing IL-32 and IL-6 levels in ANCA positive and ANCA negative are warranted to establish if these cytokines could help in diagnosis and monitoring of AAV, especially in seronegative patients. Further, long-term clinical studies analyzing IL-32 and IL-6, conducted on larger cohorts of patients, could lead to better disease understanding.

CONCLUSIONS

Our findings indicate that patients with active systemic ANCA vasculitis express different serum IL-32 and IL-6 levels than healthy controls. The important questions for the future are whether measurements of these cytokines could be a novel tool in monitoring GPA and MPA, and whether they could provide useful clinical information.

Key points

- Patients with GPA and MPA express higher serum IL-32 and IL-6 levels than healthy controls.
- IL-32 concentrations correlate positively with levels of anti-proteinase 3-ANCA (PR3-ANCA) in GPA, and with anti-myeloperoxidase ANCA (MPO-ANCA) in MPA.
- IL-32 levels correlate positively with disease activity (expressed by BVAS) in GPA and MPA.
- Measuring expression of IL-32 and IL-6 in GPA and MPA might help in monitoring these diseases, and could be a promising factor in managing these diseases

Disclosure. The authors declare they have no conflicts of interests.

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