Original Article

Matrix Metalloproteinases and Their Tissue Inhibitors: an Evaluation of Novel Biomarkers in ANCA-Associated Vasculitis

(anti-neutrophil cytoplasmic antibodies / vasculitis / matrix metalloproteinases / tissue inhibitors of metalloproteinases / pregnancy-associated plasma protein A)

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Abstract. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) may play an important role in both inflammation with subsequent fibrosis and in repair and healing in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). We evaluated the circulating levels of MMPs, including pregnancy-associated plasma protein A (PAPP-A), and TIMPs in patients with AAV. PAPP-A, MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, TIMP-2 and selected parameters were measured in 100 AAV patients (36 patients with active disease and 64 patients in remission) and 34 healthy subjects. The levels of MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, TIMP-2, and PAPP-A in AAV were all found to be different to those of the controls. The MMP-7 and PAPP-A concentrations were increased in active disease in comparison to the controls (MMP-7: 13 ±.7 vs. 2 ± 0.6 ng/ml, PAPP-A: 14 ± 18 vs. 6.8 ± 2.6 ng/ml, both P < 0.005). The MMP-2 and TIMP-2 levels were increased in remission when compared to the controls (MMP-2: 242 ± 50 ng/ml vs. 212 ± 26 ng /ml, TIMP-2: 82 ± 14 ng/ml vs. 68 ± 93 ng/ml) and to the active AAV (MMP-2: 242 ± 50 vs. 219 ± 54 ng/ml, TIMP-2: 82 ± 14 ng/ml vs. 73 ± 15 ng/ml, all P < 0.005). MMP-3, MMP-7, TIMP-1, and PAPP-A correlated with serum creatinine. The serum levels of MMPs, TIMPs and PAPP-A are all altered in AAV. MMP-2, MMP-7 and TIMP-2 appear to be promising markers in distinguishing active AAV from remission. MMP-3, MMP-7, TIMP-1, and PAPP-A are associated with kidney function in AAV. Further studies are needed to delineate the exact roles of circulating MMPs, TIMPs and PAPP-A in patients with AAV.

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Abbreviations: AAV – anti-neutrophil cytoplasmic antibody-associated vasculitis, ANCA – anti-neutrophil cytoplasmic antibody, ANOVA – analysis of variance, BVAS – Birmingham Vasculitis Activity Score, CKD – chronic kidney disease, CRP – C-reactive protein, ECM – extracellular matrix, ELISA – enzyme-linked immunosorbent assay, FSGS – focal segmental glomerulosclerosis, GPA – granulomatosis with polyangiitis, HD – haemodialysis, IgA – immunoglobulin A, IgG – immunoglobulin G, IGFBP – insulin-like growth factor binding protein, MMP – matrix metalloproteinase, MPA – microscopic polyangiitis, MPO – myeloperoxidase, PAPP-A – pregnancy-associated plasma protein A, PR3 – proteinase 3, RLV – renal limited vasculitis, TIMP – tissue inhibitor of matrix metalloproteinase, TRACE – time-resolved amplified cryptate emission.

Introduction

Anti-neutrophil cytoplasmic antibodies (ANCAs) represent a group of autoantibodies, mainly of the IgG type, directed against those antigens expressed in the cytoplasm of neutrophil granulocytes and monocytes. The main cytoplasmic pattern (c-ANCA) against proteinase

3 (PR3) and the perinuclear pattern (p-ANCA) against myeloperoxidase (MPO) are the hallmarks of small-vessel vasculitic diseases including: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and renal limited vasculitis (RLV) (Falk and Jennette, 1997; Jennette and Falk, 1997). Vasculitic changes refer to inflammation and leukocyte infiltration of vessel walls leading to local inflammation, vessel wall damage, and vessel occlusion. GPA is distinguished by the presence of granulomatous inflammation (Jennette and Falk, 1997; Jennette et al., 2013). Many patients with ANCA-associated vasculitis (AAV) develop pauci-immune necrotizing crescentic glomerulonephritis, which is characterized by fibrinoid capillary necrosis and cellular crescents. These cellular, inflammatory crescents evolve and become acellular and fibrotic, leading to glomerulosclerosis. The progression of crescents from the cellular to the fibrotic stage requires interaction of infiltrating monocytes, proliferating parietal epithelial cells and extracellular matrix (ECM). Pauci-immune small vessel vasculitides are the most common cause of rapidly progressing glomerulonephritis in adults. Importantly, the extent of glomerular inflammation in renal biopsy predicts the renal outcome (Ruth et al., 2006).

Matrix metalloproteinases (MMPs) and pregnancy-associated plasma protein A (PAPP-A) are members of the metzincin superfamily of zinc-based proteinases. These metalloproteinases are capable of digesting not only ECM, but also some chemokines, cytokines, growth factors and other molecules, thereby modifying the tissue microenvironment (Lenz et al., 2000; Zakiyanov et al., 2019).

Gelatinases MMP-2 and MMP-9 are capable of degrading the basement membrane and collagens. MMP-2 degrades fibronectin and laminin and has significantly less affinity towards types IV and V collagen than MMP-9 (Olson et al., 1998). MMP-2 and MMP-9 form proenzyme complexes with their endogenous tissue inhibitors TIMP-2 and TIMP-1 (Gomez et al., 1997), which have been shown to be expressed in human glomeruli (Carome et al., 1993). Stromelysin-1, also known as MMP-3, degrades fibrillar collagens. Matrilysin, also known as MMP-7, displays broad proteolytic activity against a variety of ECM substrates including collagens, elastin, laminin, fibronectin, and casein (Bäck et al., 2010; Oelusarz et al., 2013). PAPP-A cleaves insulin-like growth factor-binding proteins (IGFBPs), such as IGFBP-4 (Lawrence et al., 1999), thus increasing IGF bioavailability (Boldt and Conover, 2007; Kalousová et al., 2012).

Enhanced glomerular and interstitial expression of MMPs and TIMPs, namely MMP-2, MMP-3, MMP-9, and TIMP-1, in active ANCA-associated glomerulonephritis has been specifically localized in crescentic glomeruli and tubulointerstitial infiltrates. In comparison, normal or sclerotic glomeruli showed no MMP and TIMP expression (Sanders et al., 2004). The urinary MMP-2, MMP-9 and TIMP-1 have been proposed as potential indicators of kidney damage in AAV patients (Sanders et al., 2007). In addition, marked changes in the circulating levels of MMPs, TIMPs and PAPP-A have been observed in patients with biopsy-verified primary and secondary glomerular diseases (Faber-Elmann et al., 2002; Robak et al., 2006; Bauvois et al., 2007; Zakiyanov et al., 2013). Monitoring the circulating levels of MMP-3 and TIMP-1 has the potential to improve upon clinically available tests in assessing current disease activity and prognosis in AAV (Monach, 2014). Recently, in large vessel vasculitis, T cells depending on monocytes producing MMP-9 have been identified as being capable of invading the vessel wall and causing vasculitis (Watanabe et al., 2018).

To evaluate the roles of MMPs, TIMPs and PAPP-A in AAV and glomerulonephritis, we examined the circulating levels of MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, TIMP-2, and PAPP-A in AAV patients with active disease and those in maintained remission. We also assessed the possible relationships between the serum levels of the studied markers and inflammation or kidney function in AAV patients.

Material and Methods

Patients

One hundred AAV patients were identified at the Department of Nephrology, First Faculty of Medicine, Charles University and General Faculty Hospital in Prague, Prague, Czech Republic. All patients fulfilled the revised Chapel Hill Consensus criteria for a diagnosis of either GPA (formerly Wegener's granulomatosis) or MPA (Jennette et al., 2013). The disease activity was assessed clinically, biochemically, and histologically. The activity of vasculitis was evaluated using the Birmingham Vasculitis Activity Score (BVAS) (Stone et al., 2001). The disease activity and organ involvement were assessed clinically, as well as by using routinely performed tests including blood count, urine analysis, proteinuria, measurement of the serum levels of C reactive protein (CRP), serum creatinine, and albumin. Additionally, to evaluate organ involvement, either renal biopsy or computer tomography of the lungs was performed in the majority of the AAV patients. All patients had at some time tested positive for antibodies against either proteinase 3 or myeloperoxidase. Twenty-seven patients with active disease were newly diagnosed, and nine were patients undergoing a full disease relapse. Blood samples from the patients with active disease were obtained either before initiation of induction therapy or shortly afterwards. Sixty-four patients in remission had no symptoms related to active disease and had a BVAS of 0. The majority of the patients (91 %) were treated using pulses of cyclophosphamide and corticosteroids.

The control group included 34 blood donor subjects. The recorded data was used for the purpose of this study. The clinical features of the AAV patients, divided into two groups, and the control subjects were summarized in Table 1.

Table 1. Clinical features of vasculitis patients and controls

	Controls	Vasculitis		
		All	Remission	Active
N	34	100	64	36
Gender (F : M)	14 : 20	48 : 52	35 : 29	13 : 23
Age, years	31 (26–44)	59 (53–68)	61 (54–68)	59 (39–66)
Diagnosis Granulomatosis with polyangiitis (GPA) Microscopic polyangiitis (MPA)		 44 56	 32 32	 12 24
ANCA Anti-PR3 Anti-MPO		 45 55	 32 32	 13 23
BVAS		0 (0–13.5)	0	15 (12–19)
Lung involvement (ever)		66	41	25
Kidney involvement (ever)		94	60	34
CRP (mg/l)	5.4 (4.2–7)	4.2 (1.5–14)	3.1 (1.4–7.4)	14 (2.5–44)
Serum creatinine (umol/l)	91 (80–96)	166 (99–277)	141 (95–203)	226 (111–345)
Albumin (g/l)	48 (41–53)	40 (17–52)	43 (30–52)	36 (17–44)
Haemoglobin (g/l)	145 (127–168)	118 (76–180)	122 (76–170)	105 (80–140)
Proteinuria (g/day)		0.47 (0–3.4)	0.22 (0–2.25)	0.97 (0–3.4)

Data presented as N, median (interquartile range). ANCA – anti-neutrophil cytoplasmic antibodies; Anti-PR3 – anti-proteinase 3; Anti-MPO – anti-myeloperoxidase; BVAS – Birmingham Vasculitis Activity Score; CRP – C-reactive protein.

Written informed consent and laboratory samples were obtained from all subjects according to ethical guidelines. The study complies with the Declaration of Helsinki and was approved by the local Institutional Ethical Committee.

Blood samples

Serum was separated from the blood samples obtained from AAV patients with active disease, those in remission, and the healthy controls. Fasting blood samples from each patient were collected via a puncture of the cubital vein simultaneously with blood collection for routine examination. All blood samples were centrifuged for 10 min at 1,450 *g* (4 °C). The sera were stored at –80 °C until analysis.

Biochemical analysis

MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, and TIMP-2 levels were determined immunochemically with enzyme-linked immunosorbent assay (ELISA) using standard kits according to the manufacturer's protocols (Quantikine; R&D Systems, Minneapolis, MN, www.rndsystems.com). These assays employ the quantitative sandwich enzyme immunoassay technique. The results are expressed in ng/ml (nanograms per millilitre). PAPP-A was assessed immunochemically with the time-resolved amplified cryptate emission (TRACE) technology based on non-radiating energy transfer in a Kryptor analyser (Brahms Gmbh, Thermo Fisher Scientific, Hennigsdorf, Germany, www.brahms.de). This method employs PAPP-A assessment using two different monoclonal antibodies: 1) conjugated with europium cryptate and 2) conjugated with fluorescent agent XL 665. PAPP-A present in serum samples is sandwiched between these two conjugates. The detected fluorescent signal emitted at the moment of the antigen-antibody complex formation is proportional to the antigen concentration. The results of PAPP-A measurements are expressed in mIU/l (miliinternational units per litre). Routine biochemical parameters were assessed using standard laboratory methods. The blood count was measured with an automated haematological analyser.

Statistical analysis

Statistical analyses were performed using Statistics Toolbox™ MATLAB® software (The MathWorks™, Inc., Natick, MA, www.mathworks.com). Data is presented as the mean ± SD, or medians with an interquartile range for continuous variables. Comparisons between groups were conducted with unpaired sample *t*-tests and ANOVA for normally distributed continuous variables, and the Mann-Whitney U test and Kruskal-Wallis ANOVA for non-normal distributions. Variables with non-normal distributions were log-transformed where appropriate. The associations between the analysed parameters were assessed using Spearman rank correlation coefficient. All results were considered statistically significant at $P < 0.05$.

Results

The levels of MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, TIMP-2, and PAPP-A in AAV were all different from the controls. The levels of MMP-3, MMP-7,

MMP-9, TIMP-1, and PAPP-A were higher in patients with AAV in comparison to the controls. The MMP-2 (Fig. 1) and TIMP-2 levels (Fig. 2) were higher in patients in remission in comparison both to the controls ($P < 0.001$) and to patients with active disease, $P < 0.03$. When compared to the controls, the levels of MMP-3

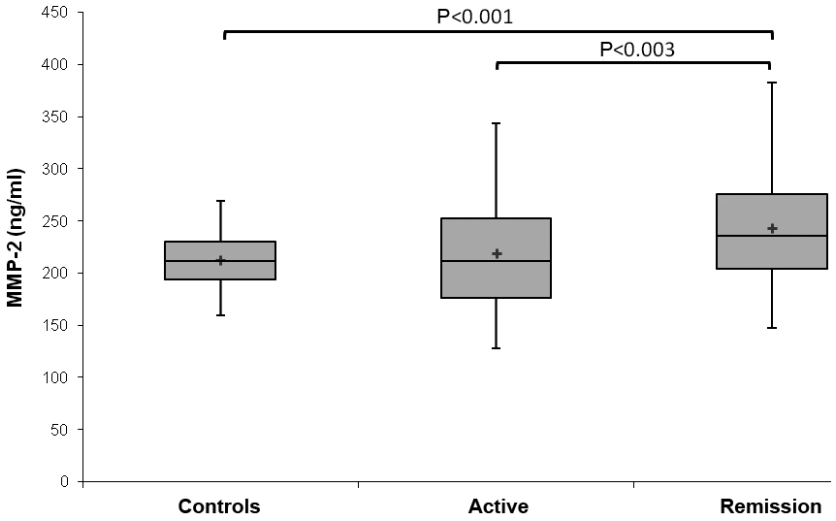

Fig. 1. Levels of MMP-2 in AAV patients with active disease, patients in remission, and controls
MMP-2 remission (242 ± 50 ng/ml) versus controls (212 ± 26 ng/ml ($P < 0.001$), active (219 ± 54 ng/ml) versus remission ($P < 0.003$)

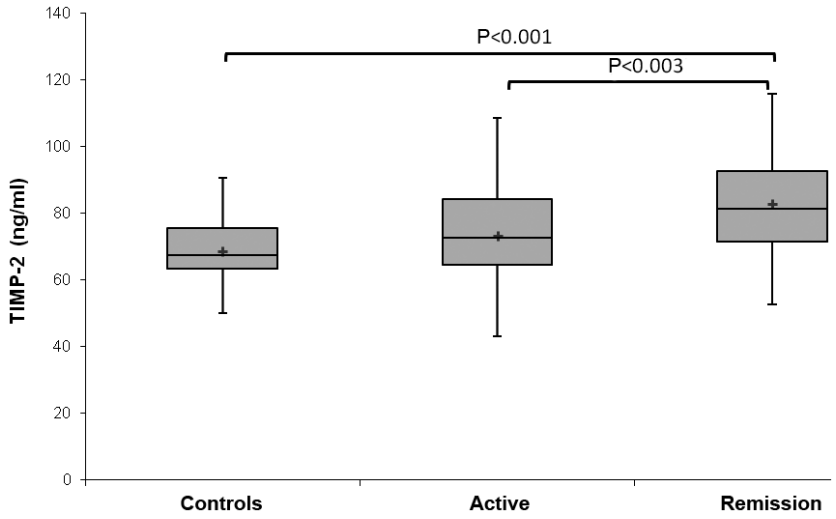

Fig. 2. Levels of TIMP-2 in AAV patients with active disease, patients in remission, and controls
TIMP-2 remission (82 ± 14 ng/ml) versus controls (68 ± 9 ng/ml) ($P < 0.001$), active (73 ± 15 ng/ml) versus remission ($P < 0.003$)

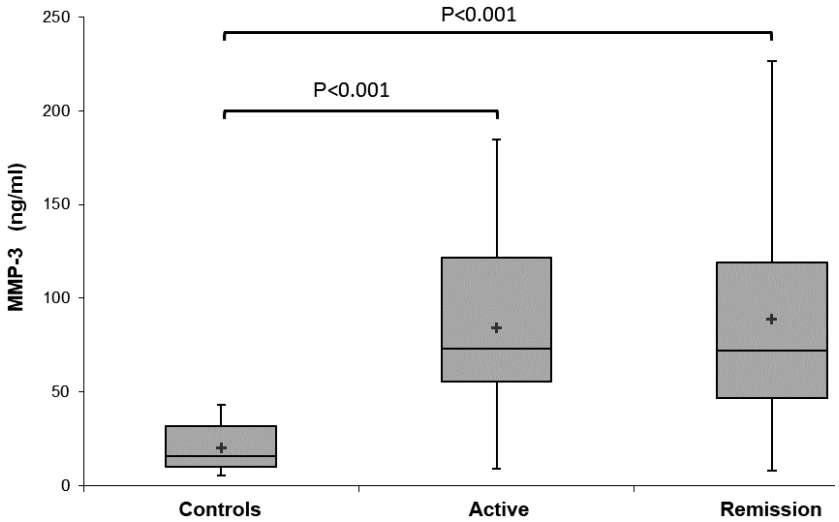

Fig. 3. Levels of MMP-3 in AAV patients with active disease, patients in remission, and controls
MMP-3 remission (84 ± 48 ng/ml) versus controls (20 ± 12) ($P < 0.001$), active (86 ± 53 ng/ml) versus controls ($P < 0.001$)

(Fig. 3), MMP-7 (Fig. 4), MMP-9 (Fig. 5), TIMP-1 (Fig. 6), and PAPP-A (Fig. 7) were higher both in patients with active disease and in patients in remission, all P < 0.001. The MMP-7 (Fig. 4) and PAPP-A (Fig. 7) concentrations were increased in patients with active disease compared to the controls, both P < 0.001.

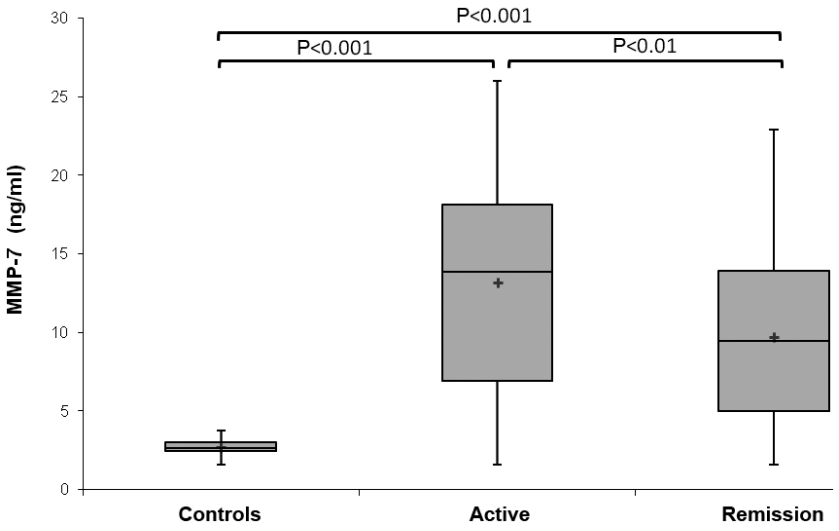

Fig. 4. Levels of MMP-7 in AAV patients with active disease, patients in remission, and controls
MMP-7 remission (9 ± 5 ng/ml) versus controls (2 ± 0.6 ng/ml) (P < 0.001), active (13 ± 7 ng/ml) versus controls (P < 0.001), remission versus active (P <0.01)

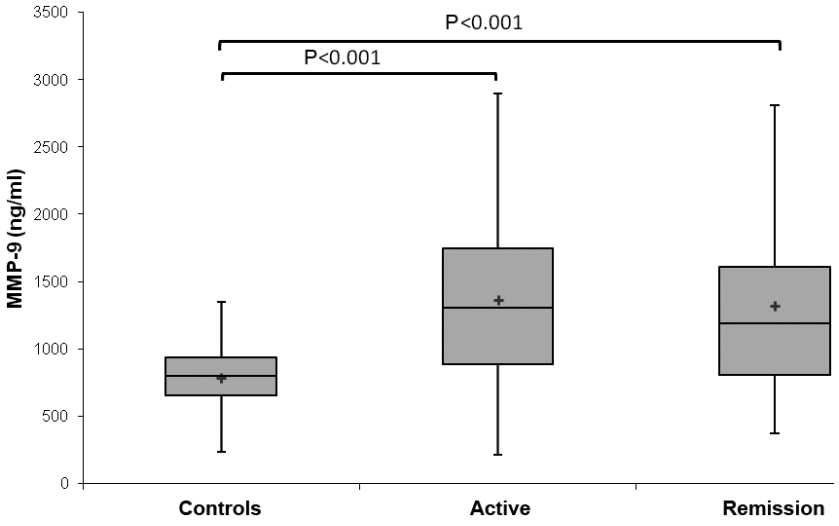

Fig. 5. Levels of MMP-9 in AAV patients with active disease, patients in remission, and controls.
MMP-9 remission (1290 ± 670 ng/ml) versus controls (784 ± 300 ng/ml) (P < 0.001), active (1360 ± 700 ng/ml) versus controls (P < 0.001)

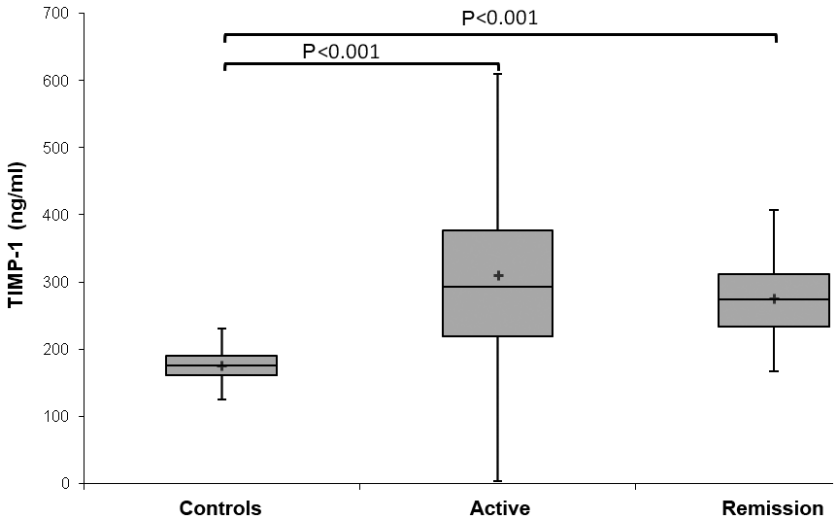

Fig. 6. Levels of TIMP-1 in AAV patients with active disease, patients in remission, and controls
TIMP-1 remission (276 ± 55 ng/ml) versus controls (176 ± 25 ng/ml) (P < 0.001), active (310 ± 129 ng/ml) versus controls (P < 0.001)

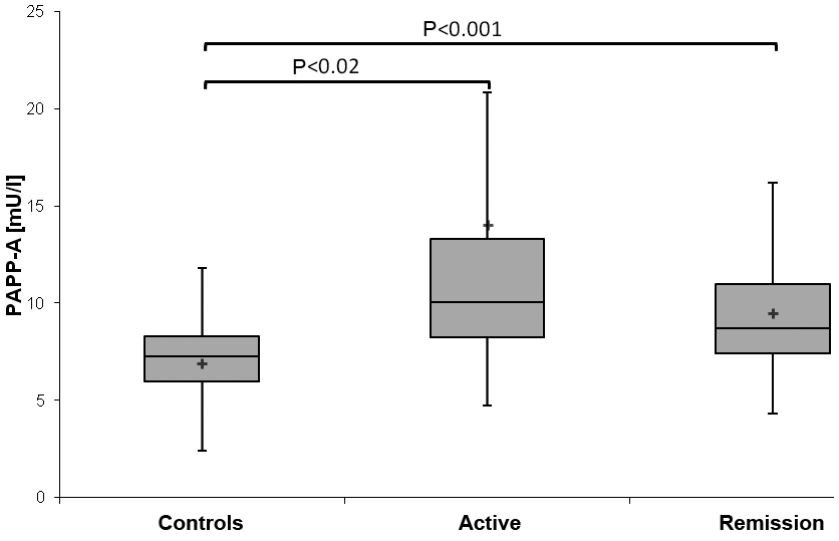

Fig. 7. Levels of PAPP-A in AAV patients with active disease, patients in remission, and controls
PAPP-A remission (9.4 ± 2.7 mIU/l) versus controls (6.8 ± 2.6 mIU/l) ($P < 0.001$), active (14 ± 18 mIU/l) versus controls ($P < 0.001$)

The levels of MMP-2, MMP-3 and TIMP-2 were higher in patients with combined immunosuppression than in patients with no immunosuppression or minimal doses of corticosteroids; all $P < 0.03$. The levels of MMP-7 and TIMP-1 were higher in patients with kidney failure in chronic kidney disease (CKD) stage 5 when compared to the patients with better kidney function in CKD 1–4; all $P < 0.03$.

Although the control subjects were younger, the ages of patients with active disease and patients in remission were similar. Only PAPP-A, MMP-2 and TIMP-2 correlated with age. No statistically significant differences in the studied markers were found between the male and female AAV patients.

As shown in Table 2, which focuses on AAV patients, the studied markers correlated not only with inflammatory parameters such as CRP and BVAS, but also with markers of kidney dysfunction such as proteinuria and serum creatinine. No correlations between the studied markers and ANCA titres were found.

Table 2. Correlation analysis of parameters

Parameter	MMP-2	MMP-3	MMP-7	MMP-9	TIMP-1	TIMP-2	PAPP-A
Age	0.29***					0.29***	0.38*
S-Creatinine		0.39*	0.68*		0.28**		0.23***
CRP				0.35*	0.43**		
Albumin			–0.32*	–0.22***	–0.27*	0.27***	
Haemoglobin		–0.23*	–0.55*		–0.39*		
Proteinuria	0.27*	0.26**	0.62*		0.23***		
MMP-2		0.28*	0.26**			0.67*	0.37*
MMP-3	0.28*					0.22***	
MMP-7	0.26**			0.26**	0.37*	0.22**	0.26**
MMP-9			–0.22***				
TIMP-1			0.37*				0.24***
TIMP-2	0.67*	0.22**	0.22**				0.25**
PAPP-A	0.37*		0.26**		0.24***	0.25***	
BVAS			0.21***			–0.28*	0.21***
Number of leukocytes				0.44*			
Number of thrombocytes	–0.27**				0.21***		

Correlation between marker levels. Spearman correlation coefficients for all markers as well as age, serum creatinine, serum albumin, C-reactive protein (CRP), Birmingham Vasculitis Activity Score (BVAS), proteinuria, haemoglobin, the number of leukocytes and the number of thrombocytes are shown. * $P < 0.001$; ** $P < 0.01$; *** $P < 0.04$

Discussion

In this study we explored the simultaneous assessment of MMPs, TIMPs and PAPP-A in relation to the disease activity and kidney dysfunction in patients with AAV. The serum levels of MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, TIMP-2, and PAPP-A were all altered in AAV patients. The differences in the detected levels of MMP-2, MMP-7 and TIMP-2 appear to be promising markers in distinguishing active disease from remission. Another important finding is the divergent association of the studied MMPs, TIMPs and PAPP-A in AAV patients to inflammatory parameters and to markers of renal dysfunction.

The circulating MMP-2 levels differed significantly only between AAV patients in remission and controls in this study. In our previous study, the MMP-2 levels were comparable to those found in healthy subjects (Zakiyanov et al., 2013), probably due to a small cohort of the examined population. The increased levels of MMP-2 in patients in remission could illustrate enhanced gelanolytic activity in patients in the convalescent phase of their disease. MMP-2 has been reported to be implicated in mesangioproliferative changes and/or tubulointerstitial changes (Turck et al., 1996; Cheng and Lovett, 2003). MMP-2 and TIMP-1 correlated with tubulointerstitial damage and creatinine clearance at biopsy, but not with kidney MMP expression and glomerular inflammation (Sanders et al., 2007). Another study showed that while AAV patients in remission showed increased gene expression of several MMPs and TIMPs, patients with active disease showed only selective up-regulation of MMP-2 and MMP-8 and down-regulation of TIMP-1 and TIMP-3 (Bjerkeli et al., 2004). Furthermore, there was an increase in total MMP activity observed particularly in the supernatants of the peripheral mononuclear cells of patients with active disease, proposing a matrix cleaving effect (Bjerkeli et al., 2004). MMP-2 correlated with PAPP-A, MMP-3, MMP-7, and proteinuria in this study. These correlations suggest that MMP-2 activity may represent a connection between the possible causative risk factors for kidney and vessel damage in both AAV patients and CKD patients.

In contrast, the MMP-9 levels were elevated in patients with active disease and those in remission. Additionally, MMP-9 positively correlated with some of the determined variables of inflammation such as CRP and the number of leukocytes. However, there was a negative correlation with albumin, suggesting the role of MMP-9 in inducing inflammatory cytokine production and their activation in AAV patients (Keeling and Herrera, 2008). The migration of immune cells to the sites of vasculitis from the bloodstream requires proteolysis of the basement membrane, causing further damage by ECM degradation. *In vitro*, T cell and dendritic cell migration is, in part, MMP-9 dependent (Van den Steen et al., 2000). The positivity of immunohistochemical staining of MMP-2, MMP-3, TIMP-1, and specifically MMP-9 in renal biopsies was located mainly in the glomeruli with cellular crescents and fibrinoid necrosis and in the tubulointerstitial tissue, but not in the unaffected or sclerotic glomeruli (Sanders et al., 2004), which supports the role of MMPs and specifically of MMP-9 in patients with active AAV. Recently, among the 135 tested biomarker candidates in targeted proteomic analysis, MMP-9 was able to discriminate the patients with highly active AAV from those in remission (Ishizaki et al., 2017).

In this study, the MMP-3 circulating levels were increased in both groups of patients: in patients with active disease and in patients in remission. In AAV patients, MMP-3 correlated with serum creatinine levels and proteinuria. The levels of MMP-3 in AAV should be evaluated with caution. Although MMP-3 reportedly induces leukocyte migration to the sites of inflammation (Monach et al., 2013), it also stimulates angiogenesis (Monach et al., 2011). The activity of MMP-3 in AAV is therefore possibly more linked to the markers of kidney damage, suggesting a greater role of MMP-3 in matrix degradation. Subsequent studies identified that MMP-3 was increased in severe active AAV (Monach et al., 2011), and both MMP-3 and TIMP-1 were suggested as promising markers to distinguish active AAV from the disease in remission (Monach et al., 2013, Monach, 2014).

We are not aware of any previous study dedicated to investigating the MMP-7's role in AAV and glomerulonephritis. Indeed, monocytes and macrophages have been noted to produce MMP-7 in response to severe inflammation (Busiek et al., 1995), and MMP-7 is known to play a role in the inflammatory recruitment of neutrophils via a chemotactic agent (Swee et al., 2008). MMP-7 displays broad proteolytic activity against a variety of ECM substrates, including collagens, proteoglycans, elastin, laminin, fibronectin, and casein. MMP-7 is upregulated in acute Kawasaki disease vasculopathy and was suggested as a promising circulating disease marker in this medium vessel vasculitis (Reindel et al., 2013). In this study, MMP-7 correlated significantly with markers of inflammation and markers of kidney injury. The most promising finding for further research, according to our results, is that MMP-7 distinguishes better between clinically active disease and disease in a state of remission.

The circulating levels of TIMP-1 were elevated in both patients with active disease and in patients in remission, and in AAV patients, they were related to markers of kidney dysfunction. TIMP-1 can activate normal human granulocytes and protect them from programmed cell death during inflammation (Chromek et al., 2004). High TIMP-1 levels were also associated with fatal outcomes in sepsis (Lauhio et al., 2011). TIMP-1 has been previously reported to be elevated in active AAV patients (Monach et al., 2011). The renal tissue expression of TIMP-1 along with MMP-2, MMP-3, MMP-9 was detected in glomeruli with cellular crescents and fibrinoid necrosis; tubulointerstitial expression of TIMP-1 and MMP-9 correlated with markers of neutrophil and

monocyte infiltration and renal function at the time of biopsy in ANCA-associated glomerulonephritis (Sanders et al., 2004). Enhanced urinary MMP-2, MMP-9 and TIMP-1 levels were also found in AAV patients (Sanders et al., 2007). The plasma levels of MMP-8 and TIMP-1 were associated with C-reactive protein (CRP) levels (Bjerkeli et al., 2004). TIMP-1 in targeted proteomic analysis was confirmed to be the best biomarker to differentiate between mildly active AAV and remission (Ishizaki et al., 2017).

The circulating levels of TIMP-2 were significantly elevated only in AAV patients in remission when compared with the healthy controls. In contrast to TIMP-1, TIMP-2 is a constitutive protein. TIMP-2 levels in septic patients were found to be significantly lower when compared to the controls (Mühl et al., 2011). TIMP-2 was found to be correlated with age, albumin, and inversely with the BVAS score in this study.

The increased circulating levels of PAPP-A in AAV patients with active disease were even more pronounced than in patients in remission in this study. However, in our previous cohort of 32 AAV patients, the serum concentrations of MMP-2, MMP-9 and PAPP-A were no different from the levels found in the healthy control subjects (Zakiyanov et al., 2013). The most plausible explanation for the elevated PAPP-A levels in the AAV patients with active disease in this study lies in the declining kidney function, which is supported by the correlation of PAPP-A to serum creatinine, since it is known from previous studies that PAPP-A levels correlate with the level of renal function (Kalousová et al., 2012). In addition, the PAPP-A expression represents an enhanced local inflammatory state causing myocyte vessel injury in vasculitis due to its metalloproteolytic activity, but it also indicates a systemic inflammatory burden, possibly contributing to the damage of vessels due to accelerated atherosclerosis in AAV (de Leeuw et al., 2005).

A limitation of our study is that the control subjects used for comparison to AAV patients were not age-matched, but the age of AAV patients in remission and patients with active disease was comparable. In this study, only the levels of MMP-2, TIMP-2 and PAPP-A correlated with age. The circulating levels of the enzymes regulating extracellular matrix turnover are variable and change during life both in aging and in acquired disease states. However, there is a significant discordance between the studies examining the effects of age on MMPs and TIMPs in healthy adults. One study found no changes in MMP-2 and MMP-9 and decreased TIMP-1 and TIMP-2 with increasing age (Tayebjee et al., 2004). In contrast, another study found an elevation in TIMP-1 with increasing age (Sundstrom et al, 2004), while another study observed that MMP-2, MMP-7, TIMP-1, TIMP-2 increased and MMP-9 decreased as a function of age (Bonnema et al., 2007). Similar to our study, the measurements of MMPs and TIMPs were done using a validated ELISA method, but we measured the serum samples, while the majority of studies used the ELISA method in the measurement of plasma concentrations. In addition, the kidney function decreases with age as well as in patients with kidney impairment, which might influence the circulating concentrations of the studied markers. Moreover, the applied immunosuppressant therapy, specifically glucocorticoids (Van Lint and Libert, 2006; Conklin et al., 2018), might have repressed the activity of the studied biomarkers and explain why the levels of MMP-2 were different when comparing the AAV patients in remission to the controls. These confounding factors might lessen the differences found in the studied markers between the diseased groups and the controls. Despite these intrinsic limitations, our work allowed simultaneous evaluation of a number of MMPs, TIMPs, and PAPP-A in both active and remitted AAV patients.

In conclusion, the serum levels of MMPs, TIMPs and PAPP-A are altered in patients with AAV and glomerulonephritis. The differences in the detected levels of MMP-2, MMP-7 and TIMP-2 in AAV appear to be promising markers in distinguishing active disease from remission. MMP-3, MMP-7, TIMP-1, and PAPP-A are associated with kidney function in AAV. Further studies are needed to delineate the exact roles of circulating MMPs, TIMPs and PAPP-A in patients with AAV.

Conflict of interest

The authors declare that they have no competing interests.

Authors' contribution

Oskar Zakiyanov conceived the study, was responsible for the study design and coordination, and drafted the manuscript. Zdeňka Chocová, Zuzana Hladinová, Vladimíra Bednářová, Jan Vachek, and Petra Wurmová were inestimable in sample data collection and clinical data collection. Vitězslav Kříha performed the statistical analysis. Marta Kalousová and Karin Malíčková were responsible for laboratory processing and coordinated the biochemical analysis of the samples. Marta Kalousová, Tomáš Zima, Zdenka Hrušková, and Vladimír Tesař provided expert opinions, took part in data interpretation and manuscript preparation. All authors read and approved the final manuscript.

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