Effect of Protein A-Immunoadsorption Therapy on Serum Cytokines in AAV Patients
(Kesan Terapi A-Imunopenjerapan Protein ke atas Serum Sitokin pada Pesakit AAV)

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ABSTRACT
Protein A-immunoadsorption (IA) is an extracorporeal apheresis technique used in patients with autoimmune diseases which aims to remove pathogenic autoantibodies. Apart from the adsorption of immunoglobulins (IgG), IA may influence cellular and humoral immunity. The aim of this study was to observe the effect of protein A-IA on cytokine networks in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients. A total of 12 newly diagnosed AAV patients received IA therapy on days 1, 3, and 5. Serum levels of inflammatory cytokines were measured before and after treatment including members of the interleukin family, PDGF-BB, TNF-α and IFN-γ. IL-1β, IL-9, IL-17A, PDGF-BB, IFN-γ, and TNF-α were elevated in AAV patients compared to healthy individuals. There were no changes in the levels of any cytokines after the first IA session but after 3 sessions, IL-9, PDGF-BB, and TNF-α dramatically decreased. Moreover, reductions in IL-9 levels were positively correlated with the changes of myeloperoxidase-ANCA (MPO-ANCA). Our observations suggest that protein A-IA therapy does not directly adsorb cytokines, but removes autoantibodies (MPO-ANCA) which indirectly leads to changes in cytokine networks linked to cellular or humoral immunity.

Keywords: Cytokines; immunoadsorption; protein-A, ANCA; vasculitis

ABSTRAK
A-imunopenjerapan protein (IA) merupakan teknik ekstrakorporeum aferesis yang digunakan pada pesakit yang mempunyai penyakit autoimun bertujuan untuk menyingkirkan autoantibodi patogen. Selain daripada penjerapan immunoglobulin (IgG), IA boleh mempengaruhi imuniti sel dan humoral. Tujuan kajian ini adalah untuk melihat kesan protein A-IA ke atas rangkaian sitokin pada pesakit vasculitis (AAV) yang berkait dengan antibodi sitoplasmik antineutrofil (ANCA). Seramai 12 pesakit AAV yang baru didiagnos telah menerima terapi IA pada hari 1, 3 dan 5. Tahap serum daripada keradangan sitokin telah diukur sebelum dan selepas rawatan termasuk anggota daripada keluarga interleukin, PDGF-BB, TNF-α dan IFN-γ. IL-1β, IL-9, IL-17A, PDGF-BB, IFN-γ dan TNF-α menaik pada pesakit AAV berbanding dengan individu yang sihat. Tiada perubahan pada tahap dalam mana-mana sitokin selepas sesi pertama IA tetapi selepas sesi ketiga, IL-9, PDGF-BB dan TNF-α menurun dengan mendadak. Selain itu, penurunan dalam paras IL-9 berkorelasi secara positif dengan perubahan mieloperoxidase-ANCA (MPO-ANCA). Berdasarkan pemerhatian kami, terapi protein A-IA tidak menjerap sitokin secara langsung, tetapi mengeluarkan autoantibodi (MPO-ANCA) yang secara tidak langsung mengakibatkan perubahan kepada rangkaian sitokin yang berpaut kepada imuniti sel atau humoral.

Kata kunci: ANCA; imunopenjerapan; protein-A; sitokin; vaskulitis

INTRODUCTION
Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a grouping of multisystem autoimmune diseases that predominantly affects small vessels but may also involve medium-sized vessels (Jennette et al. 2013). The pathological characteristics of AAV involve necrotizing inflammation and fibrinoid necrosis in the vessel walls with its clinical manifestations largely dependent on the vascular beds involved. Kidneys
represent the most prominent organ involved although nearly all organ systems can be affected, including the upper and lower airways, skins, lungs, heart, and nervous system (Rowaiye et al. 2015). A variety of predisposing factors are now considered to initiate the development of AAV, such as microbial infection, genetic factors, environmental agents, and some drugs, but the specific mechanisms involved in its pathogenesis remain unclear.

Cellular immunity and humoral immunity are thought to play an essential role in the pathogenesis of AAV (Xiao et al. 2016). Currently AAV is primarily associated with ANCA directed against either myeloperoxidase (MPO-ANCA) or against leukocyte proteinase 3 (PR3-ANCA) with PR3-ANCA or MPO-ANCA autoantibodies detected in more than 90% of patients. Notably, B cell-derived ANCAs can induce significant amounts of pro-inflammatory cytokines through stimulating the proliferation or polarization of T cells, and even activating the complement system. Due to the pivotal role of immunomodulation in this disease, treatment strategies typically involve glucocorticoids and immunosuppressors, such as prednisone, cyclophosphamide, mycophenolate mofetil, and azathioprine. Nevertheless, while these agents can alleviate the condition to some extent, such treatment is also frequently accompanied by serious drug toxicity and high recurrence rates (Lilliebladh et al. 2018).

Glucocorticoids in combination with other immunosuppressants are central component of the management of AAV in induction and maintenance therapy. Except for traditional drug therapies, the effect of the extracorporeal apheresis can rapidly remove pathological circulating factors in attempt to reduce their deleterious effects. Various apheresis techniques such as plasma exchange (PE) and immunoadsorption (IA) have been explored for the treatment of AAV, especially in severe patients (Casian & Jayne 2011). In contrast to PE, IA treatment involving columns coupled with staphylococcal protein A is highly selective since only antibodies and immune complexes are removed (Gaskin & Pusey 2001). Importantly, this approach preserves other important plasma constituents such as albumin and coagulation factors. Moreover, no replacement fluid is needed with IA since the patient’s own plasma is reinfused after treatment, thereby allowing for larger plasma volumes to be treated compared with PE. IA is therefore more effective in clearing IgG, with a low rate of complications such as hemorrhage, anaphylaxis, and transfusion-related lung injury.

Although the cost of protein-A IA columns is high, they can be reused approximately 10 times with the same patient. This aspect reduces the global cost of IA when the technique is used to treat a patient over several sessions and could be even lower than for classical PE techniques (Maillard et al. 2015). However, economic benefits aside, it is vitally important to ensure the clinical efficacy of this approach in the treatment of AAV. Although some research has been previously conducted, relatively little information is currently available on the potential usefulness of IA in AAV, and not all studies have yielded uniform results. As early as 1991, a study of 10 patients on dialysis (5 with microscopic polyangiitis (MPA), 2 with granulomatosis with polyangiitis (GPA), and 3 with systemic lupus erythematosus (SLE)) showed that IA therapy could reduce autoantibodies to undetectable levels and impressively allowed 9 patients to cease dialysis (Palmer et al. 1991). Nevertheless, the results from a multi-center randomized trial from Sweden treating AAV patients showed the efficacy of IA compared to PE was similar in terms of renal function improvements and overall survival (Stegmayr et al. 1999). Moreover, while several studies have demonstrated that IA therapy was effective in ANCA clearance and produced favorable clinical outcomes, the underlying mechanism whereby IA benefits AAV patients is still undefined.

Some researchers consider that the possible mechanisms of IA involve effects beyond the removal of pathogenic autoantibodies. These advantages may include improvement of the immune system, including reticuloendothelial function, and effects on the distribution of T cells subsets that can alter their immunoregulatory abilities (Baggi et al. 2008; Goto et al. 2003). This view is projected from findings made in other autoimmune diseases where IA has shown immunoregulatory abilities (Baggi et al. 2008; Goto et al. 2001). However, for AAV patients, there is only scare data available and moreover, whether IA affects the expression of inflammation-related cytokines in AAV is also largely unknown. Therefore, the aim of this study was to investigate the possibility that protein A-IA therapy influences cellular immunity and cytokine networks by examining IA effects on circulating cytokine levels.

**MATERIALS AND METHODS**

**PATIENTS**

A total of 12 patients diagnosed with AAV belonging to the MPA clinical phenotype and positive for MPO-ANCA were included in the study. All patients fulfilled Chapel
The Hill Consensus Conference (CHCC) 2012 definition of vasculitis and received protein-A IA therapy before immunosuppressive drugs and hemodialysis were applied. Patients with malignant disorders and infection were excluded from the study. Patients data, including gender, age, and Birmingham vasculitis activity score (BVAS) were recorded. The following laboratory data were measured before IA therapy: hemoglobin, immunoglobulin G (IgG), serum creatinine, eGFR, white blood cell (WBC) and lymphocyte counts, total protein quantification of urine for 24 h and hematuria. Six healthy, age and sex-matched volunteers were included as normal controls. No immunosuppressive treatment (such as corticoids, cyclophosphamide or other immunosuppressants) were given during the treatment course. All patients received 3 consecutive protein A-IA treatments every other day (days 1, 3, and 5). Peripheral venous blood was withdrawn immediately before the first IA (Pre-IA) treatment and at the end of every IA session (Post-IA), and deposited in EDTA tubes. The eluent from protein A column was also collected. All samples were centrifuged at 2000 × g for 15 min at 4 °C and stored at -80 °C for later cytokine determination. The study was approved by the Human Research Ethics Committees in the Second Xiangya Hospital of Central South University (2018YFC1314000) with written informed consent obtained from all patients.

PROTEIN A-IA PROTOCOL
Vascular access for the procedure involved jugular or femoral vein catheter placement before performing IA using protein A-IA columns (KONPIA, Guangzhou Koneen Bioscience Co. Ltd, China) with heparin sodium anticoagulation. A single column was used for each patient in the trial, alternating between adsorption and elution procedures. Plasma flow ranged between 30 and 40 mL/min, and the total volume of plasma used for adsorption was 4000 - 6000 mL per session.

CYTOKINE AND ANCA DETERMINATIONS
Inflammation-related cytokines including IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-17A, PDGF-BB, IFN-γ, and TNF-α were detected by using the Bio-Plex Multiplex Immunoassay System according to the manufacturer’s instructions (human cytokine standard 27-Plex kit, Bio-Rad, USA). MPO-ANCA were measured by ELISA (Quanta Lite MPO IgG Elisa, INOVA Diagnostics, USA).

RESULTS
The clinical characteristics of the 12 AAV patients enrolled in the study are summarized in Table 1. All patients received protein A-IA therapy in the active disease phase following diagnosis. The average Birmingham vasculitis activity score (BVAS) was 17.2 ± 3.3 and severe kidney damage was recorded in all patients (eGFR baseline was 17.17 ± 10.07 mL/min). After 3 IA sessions, serum IgG decreased by 75.3% (from 15.95 to 3.93 g/L), indicating the effective clearance of IgG by protein A-IA. Accompanying this change, we found WBC and lymphocyte counts were elevated, along with declining levels of MPO-ANCAs in the AAV patients. However, there was no significant improvements in renal function in the short term (Table 2).

In addition to the clinical indicators mentioned, we also measured the effects of IA on a panel of cytokines which included IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IL-17A, PDGF-BB, and TNF-α. Baseline comparisons with normal controls showed dramatically elevated serum levels of a number of cytokines in AAV patients including PDGF-BB (1728.25 vs 73.28 pg/mL, P < 0.001), TNF-α (70.13 vs 27.34 pg/mL, P < 0.001), IL-17A (8.14 vs 5.35 pg/mL, P < 0.01), IL-1β (0.64 vs 0.46 pg/mL, P < 0.01), IFN-γ (8.14.25 vs 3.43 pg/mL, P < 0.01) and IL-9 (41.2 vs 13.72 pg/mL, P < 0.001) (Figure 1). Other cytokines measured were not significantly different between the two groups or undetectable (data not shown).

Thereafter, we determined whether the pathologically elevated serum levels of cytokines were eliminated by protein A-IA therapy. Our analysis compared cytokine serum concentrations in pre-IA (1st), post-IA (1st) and post-IA (3rd). Notably, between the pre-IA (1st) and post-IA (1st) samples, there were no differences in any of the cytokines measured (P < 0.05) (Table 3). We further assessed cytokines in the eluent but none were detected, suggesting the column did not directly adsorb any of the cytokines being measured. Interestingly, three cytokines (PDGF-BB, TNF-α, IL-9) were progressively decreased after 3 IA sessions, the
average being 43.6, 23.9, and 17.8%, respectively, of starting levels (Figure 2). Besides cytokines, we found that MPO-ANCA levels were cleared following every session of protein A-Ia treatment. The average reduction rates from pre-Ia (1st) to post-Ia (3rd) were 30.3% of the initial levels (Table 3, Figure 2).

Finally, we examined the relationship between changes in the levels of the three Ia-responsive cytokines (PDGF-BB, TNF-α, IL-9) and MPO-ANCA (Table 4). This analysis showed that only IL-9 was positively correlated with reductions in MPO-ANCA concentrations after 3 Ia sessions (r=0.584, P=0.046), while changes in PDGF-BB and TNF-α levels were not correlated with MPO-ANCA.

### Table 1. Baseline clinical features of AAV patients

| No | Age(y) | Gender | eGFR(mL/min) | BVAS(scores) | Proteinuria | Hematuria |
|----|--------|--------|--------------|--------------|-------------|-----------|
| 1  | 56     | M      | 18           | 18           | ++          | +++       |
| 2  | 65     | F      | 7            | 16           | +           | +++       |
| 3  | 67     | M      | 10           | 23           | -           | +         |
| 4  | 47     | M      | 14           | 17           | +++         | +++       |
| 5  | 39     | F      | 27           | 17           | +++         | +++       |
| 6  | 55     | M      | 12           | 23           | +           | +++       |
| 7  | 75     | M      | 20           | 13           | +           | +++       |
| 8  | 55     | M      | 10           | 16           | ++          | +++       |
| 9  | 55     | M      | 13           | 14           | +++         | +++       |
| 10 | 54     | F      | 6            | 19           | ++          | +++       |
| 11 | 70     | M      | 31           | 13           | +           | +++       |
| 12 | 55     | M      | 38           | 17           | +           | +++       |

*eGFR: Estimated glomerular filtration rate; M: Male; F: Female; BVAS: Birmingham vasculitis activity score*

### Table 2. Differences of laboratory biomarkers after 3 Ia sessions (n=12)

| Clinical index | Pre-Ia               | Post-Ia(3rd)         | P value |
|----------------|----------------------|----------------------|---------|
| WBC (10⁹/L)    | 7.97 (4.65, 9.25)    | 9.47 (7.60, 13.29)   | 0.028   |
| L (10⁹/L)      | 0.79 (0.63, 1.07)    | 1.13 (0.88, 1.47)    | 0.013   |
| Hb (g/L)       | 81.5 (66.0, 90.8)    | 82.5 (66.5, 87.8)    | 0.326   |
| IgG (g/L)      | 15.95 (14.78, 18.00) | 3.93 (3.14, 5.20)    | 0.002   |
| SCr (μmol/L)   | 479.1 (265.4, 640.4) | 342.70 (260.8, 669.2)| 0.200   |
| 24hUTP (mg/d)  | 1587.9 (1287.6, 3204.5) | 1699.5 (910.5, 3054.1) | 0.182   |
| MPO-ANCA (IU/ml)| 89.78 (66.80, 116.97)| 62.57 (30.08, 86.18) | 0.002   |

*WBC: white blood cell; L: lymphocytes; Hb: hemoglobin; IgG: immunoglobulin G; SCr: serum creatinine; 24hUTP: total protein quantification of urine for 24 hours. MPO-ANCA: MPO Anti-neutrophil cytoplasmic antibody; PR3-ANCA: PR3 Anti-neutrophil cytoplasmic antibody. Wilcoxon rank test was used. Difference between groups were used by Wilcoxon rank test*

### Table 3. Changes of cytokines and MPO-ANCA after IA (n=12)
### TABLE 4. Correlation analysis between cytokines with MPO-ANCA (n=12)

The levels of IL-1β, IL-9, IL-17A, PDGF-BB, TNF-α and IFN-γ were expressed as pg/mL. MPO-ANCA was expressed as IU/mL. Difference between groups were used by Wilcoxon rank test. ** means $p < 0.01$ vs Pre-IA, *** means $p < 0.001$ vs Pre-IA.
FIGURE 2. A-D: Changes of cytokines after IA in AAV patients (A: PDGF-BB; B: TNF-α; C: IL-9; D: MPO-ANCA.). E: Percentage of changes in cytokines after the 3rd IA compared to pre-IA

** means p < 0.01 vs Pre-IA, ## means p < 0.01 vs Pre-IA
Variables | Coefficient (with Δ MPO-ANCA) | P value
--- | --- | ---
Δ PDGR-BB | r=0.452 | 0.169
Δ TNF-α | r=0.526 | 0.079
Δ IL-9 | r=0.584 | 0.046

Δ means the variety of concentrations between pre-IA and post-IA (3RD). Correlations between variables were determined by Spearman rank correlation coefficient.

DISCUSSION

According to current clinicopathological classifications, the AAV group of rare syndromes includes MPA, GPA, and eosinophilic GPA (EGPA) (Jennette et al. 2013). However, these disease classifications are based on histology and clinical manifestations rendered at a specific times but can be subject to change as the disease develops. Moreover, biopsies are not routinely obtained in most cases. In general, PR3-ANCA is associated with GPA (75%), while MPO-ANCA is found in cases of MPA (60%) and EGPA (30%) (Bosuuy et al. 2017; Gioffredi et al. 2014). However, the specificity of ANCA partially overlaps with the different clinical syndromes. Accumulating evidence suggests that ANCA specificity better discriminates between genetic backgrounds (Lyons et al. 2012; Rahmattulla et al. 2016), epidemiology (Watts et al. 2015), therapeutic response (Unizony et al. 2016), relapse risk (Hogan et al. 2005), long-term prognosis (Tanna et al. 2015; Wallace et al. 2019) and comorbidities (Kronbichler et al. 2019) than a classification based on the clinical phenotype alone (Cornec et al. 2016). In China in particular, MPA accounts for more than 80% of AAV patients and therefore, MPO-ANCA constitutes the major antibodies detected clinically (Li et al. 2016). Based on this situation, we focused our study on MPO-ANCA-positive patients.

The pathogenesis of AAV is still unclear at present, although many researchers have naturally focused on investigating immunological changes along with effects on related cytokines. Hyperactivation of B cells and T cells induced by PR3-ANCA and MPO-ANCA (Martinez Valenzuela et al. 2019; Szczeklik et al. 2017), together with the disruption of cytokine networks orchestrate the immunological response (Ishizaki et al. 2017; Monach et al. 2013). In the present study, we observed some inflammation-related cytokines including IL-1β, IL-9, IL-17A, PDGF-BB, TNF-α, and IFN-γ were significantly increased in patients, demonstrating that T-cell immunity and cytokine networks were likely involved in the occurrence and development of AAV. Previous work has highlighted the involvement of IL-1β, IL-17A, TNF-α, and IFN-γ in AAV but the identification of IL-9 and PDGF-BB here represents new insights into the pro-inflammatory environment associated with AAV.

In addition to being a diagnostic marker, a pathogenic role for ANCA and the association of ANCA with disease activity is well established. However, vasculitis can occur in AAV without ANCA, and the utility of measuring ANCA antibody titers to predict disease activity or prognosis is controversial (Aljuhani et al. 2021; Kemna et al. 2015; Watanabe et al. 2018). A recent meta-analysis concluded that rising or persistent levels of ANCs during remission is only modestly predictive of future disease relapse in AAV patients (Tomasson et al. 2012). IA in the treatment of autoimmune diseases is recognized to be able to remove pathogenic autoantibodies and to quickly alleviate symptoms. It can be seen from our study that both MPO-ANCA and serum IgG levels were obviously reduced after IA. The benefits of eliminating autoantibodies in AAV are implicit but whether the removal of total immunoglobulins can also affect the immune system or otherwise help to eliminate elevated inflammatory cytokine levels is still unknown. In support, Braun and Risler (1999) reported that protein A-IA therapy may cause immunomodulation of the humoral and cellular immune systems in autoimmune diseases beyond its immunoglobulin-reducing effects. Additionally, Bulut et al. (2013, 2010) observed that protein A-IA therapy in patients with idiopathic cardiomyopathy (IDCM) produced changes in circulating T cells lasting 3-6 months levels involving increases in regulatory T cells (CD4+, CD25+, CD127low) with decreases in activated T cells (CD4+/CD69+ and CD8+/CD69+ cells) and CD28+ T cells (co-stimulatory cells). However, in this regard published data concerning AAV patients is presently limited.

In our study, the level of cytokines before and
after a single IA treatment had no discernible changes. This result implied that protein A-IA does not eliminate cytokines directly by adsorption and this was supported by our analyses of column eluents. This result was not unexpected since it is generally known that protein A has a high affinity for IgG binding through interactions with immunoglobulin Fe and Fab domains which are absent in cytokines (Inganäs 1981). We also confirmed in our study that even a single IA procedure could reduce the levels of MPO-ANCA, which represent the IgG subclass of antibodies. Notably, after 3 IA sessions, the MPO-ANCA concentrations was 30.3% lower than that of the original levels. Although the protein A-IA procedure had no direct effect on cytokines, we did observe that a selected subset of cytokines, namely IL-9, PDGF-BB, and TNF-α, were dramatically decreased after the 3rd session of IA. The exact mechanism of this phenomenon remains unclear, but we propose two broad avenues that should be considered as possible explanations.

First, after the removal of circulatory MPO-ANCA and IgG by IA, changes in the immune status of patients, especially the proportion and function of Th1/Th2 cells, results in reductions in the level of pro-inflammatory cytokines. This scenario was inferred in Bernd Hehmke’s study published in 2000, which showed that some patients with refractory rheumatoid arthritis showed severely depressed T cell activity which could be recovered to normal or near-normal function after 3 IA sessions. He proposed that alterations of cellular immunity were accompanied by the elimination of immunoglobulins (Hehmke et al. 2000). Indeed, we also discovered that reductions in IL-9 levels were positively correlated with decrease in MPO-ANCA, suggesting that protein A-IA may play an immunomodulatory role indirectly by eliminating autoantibodies.

A second explanation involves the hypothesis that IA has a direct effect on the function and distribution of T lymphocytes, where changes in cytokines levels lag changes in cellular immune status. Notably we also found that there were increased peripheral blood lymphocyte numbers found after 3 IA sessions. Schneidewind-Müller et al. (2002) discovered that protein-A IA could alter the lymphocytic subpopulation cluster distribution and the changes of some inflammatory proteins as early as day 7 post-treatment, reaching a peak between days 21 and 36. This timeframe is consistent with our observation that IL-9, PDGF-BB, and TNF-α were dramatically reduced after the 3rd IA treatment administered on day 5. Additionally, we also found that changes of IL-9 levels were positively correlated with reductions in MPO-ANCA, suggesting that IL-9 might be an indicator of disease activity.

As with any intervention, the benefits need to be balanced against the risks. Certainly, treatment strategies to control disease in AAV have significantly reduced mortality rates, but complications involving infection-associated risks have been reported after long-term follow-up. Frequent causes of death within the first year of diagnosis are infection (48%) and active vasculitis (19%), and those after 1 year were cardiovascular (CV) disease (26%), malignancy (22%), and infection (20%) (Flossmann et al. 2011). Patients with higher disease burden, higher cumulative exposure to glucocorticoids, and those with kidney involvement have the highest risk of infection (Kronbichler et al. 2015). After 12 months from diagnosis, CV events become the leading cause of death of AAV patients (Kronbichler et al. 2020a; Lai et al. 2014), primarily involving the inflammation of blood vessels. Glucocorticoid-induced obesity, insulin resistance, dyslipidemia and hypertension are all risk factors for CV events which influences vascular function, atherogenesis and vascular remodeling. The occurrence of malignancy is not uncommon due to the pro-tumorigenic effects of immunosuppressive therapy, such as bladder cancer induced by chemotherapy such cyclophosphamide (Le Guenno et al. 2011; Mahr et al. 2013). In addition to solid cancers, malignant hemopathies of lymphoid or myeloid origin are rare, but do also occur. It was speculated that inflammatory stimulation or immune imbalance in AAV pathologies may be involved (Philipponnet et al. 2017). Considering these issues, IA therapy has advantages as an adjunct therapy in AAV where patients require lower exposure to immunosuppressive drugs to restore immune balance.

Current treatment strategies are highly efficient at inducing remission, with response rates of up to 90% in patients with AAV. Maintenance immunosuppression aims to prevent relapses but there is some uncertainty as to how long maintenance therapy should be continued to ensure remission. Moreover, the differences between true pathophysiological remission and the absence of clear evidence of disease activity is difficult to determine. The association between changes in ANCA-titers and relapse are still debated but a growing number of studies suggest that changes of circulating cytokines are linked to disease pathogenesis and activity (Kronbichler et al. 2020b). Our research findings support this view although there are some limitations that should be taken into consideration.

As a rare disease, large sample sizes are difficult
to achieve and consequently our study may have been underpowered due to the small cohort size. Therefore, the significance of IA treatment effects in AAV should be elucidated within a larger patient cohort. Moreover, the observation times we reported were short, and side effects and long-term outcomes are still lacking. Nevertheless, we will continue to carry out long-term follow-up of these patients and collect further data about complications, such as the incidence of infections and CV events. Lastly, improvements in the quality of life of our patients will always be a fundamental objective for our further research.

CONCLUSIONS
Our preliminary study found that protein A-IA therapy could efficiently adsorb and reduce ANCA levels in AAV patients following a short-term course of 3 treatments and this was accompanied by significant reductions in the levels of circulating inflammatory cytokines. We draw conclusions from the results that imbalances in cytokine networks exist in AAV patients, which may be linked to its pathogenesis. Further research should pay close attention to the interrelationships between circulating cytokines and pathophysiologic or protective mechanisms in AAV, and elucidate the immunomodulatory role of protein A-IA therapy in the treatment of AAV and other autoimmune diseases.

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