Clinical characteristics and potential biomarkers for disease activity of patients with ANCA associated vasculitis: a monocenter study in China

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Abstract

Objective: The aim of this study was to describe the clinical and serological features of patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) in eastern China using data from a hospital-based study. And looking for indicators that can predict disease activity.

Methods: We retrospectively studied patients with newly diagnosed AAV evaluated from January 1, 2009, to December 31, 2018. In total, 219 patients diagnosed were classified according to the American College of Rheumatology classification criteria and/or revised Chapel Hill 2012 definitions, and their clinical and serological features were evaluated. The association of laboratory data with disease activity was assessed via regression models.

Results: Of 219 incident cases of AAV, 37/219 (16.9%) had granulomatosis with polyangiitis (GPA), 172/219 (78.5%) were microscopic polyangiitis (MPA), and 10/219 (4.6%) had eosinophilic granulomatosis with polyangiitis (EGPA). The mean age at diagnosis of patients with GPA were 51.5 years (male/female, 18/19), MPA were 61.7 years (male/female, 84/88), and EGPA were 49.8 years (male/female, 7/3), respectively. Patients with MPA were significantly older than GPA and EGPA at diagnosis (p<0.001). ANCAs tested positive in 207 (94.5%) of cases: 167 (80.7%) were MPO-ANCA and 40 (19.3%) were PR3-ANCA. Lung, skin, nervous system symptoms were the most common in EGPA. For GPA, ear–nose–throat (ENT) symptoms and lungs involvement were the most common. Renal and lung involvement occurs most frequently in MPA. In the multivariable logistic regression analysis, higher anti-MPO antibody (≥149.4 IU/ml), higher hypersensitive c-reactive protein (hs-CRP, ≥62.5 mg/L), lower hemoglobin (≤113.5g/L), and higher complement 4 (C4, >0.215 g/L) were proved to be independent risk factors for active disease. Further research showed that C4 had higher sensitivity (70.0%) and specificity (83.4%) than the other three indicators.

Conclusion: MPO-ANCA-positive MPA is the most common form of AAV in Chinese patients. Serum C4 concentrations at diagnosis might be a useful biomarker of disease activity in AAV.
1. Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) are a group of multisystem, autoimmune, inflammatory disease characterized by pauci-necrotizing vasculitis affecting small-to-medium blood vessels [1, 2]. Three different clinical forms have been differentiated: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA), formerly named Churg-Strauss syndrome. Two of the well-known autoantigen targets of ANCA are proteinase 3 (PR3) and myeloperoxidase (MPO) which are frequently associated with the disease. Usually, antibodies directed against PR3 antigen are associated with a C-ANCA pattern, whereas, MPO antigen are associated with a P-ANCA pattern [3]. The combination of C-ANCA-PR3 is strongly associated with GPA, whereas the combination of P-ANCA-MPO is predominant in MPA and EGPA. Occasionally, other autoantigens can also be targeted by ANCA, such as cathepsin G, lactoferrin, lysozyme, bacterial permeability increasing factor, hLAMP-2, and elastase [4].

AAV is a rare disease that can occur at any age. The clinical manifestations of the AAV are diverse and can be confined to one organ, or multiple organs and even life-threatening and prompt immunosuppressive treatment is crucial to reduce death by major-organ failure as well as long-term morbidity. Nevertheless, even if the diagnosis is correct and immunomodulatory therapy has been initiated, AAV tend to progress and the individual treatment response remains challenging to predict [5, 6].

Birmingham Vasculitis Activity Score (BVAS) had been used for assessing the activity and severity of AAV[5, 7], and it was reportedly associated with the poor prognosis of AAV. Besides BVAS, large-scale biomarker studies had been conducted to identify an ideal biomarker reflecting disease activity. Several biomarkers including c-reactive protein (CRP), complement 3a (C3a), C5a, and matrix metalloproteinase-3 (MMP-3) in the blood had been proposed for the evaluation of disease activity [8-11]. However, there has been no specific index for assessing the cross-sectional activity of AAV at diagnosis. Taking this into account, we investigated the variables at diagnosis which were correlated with active AAV and determined the initial predictors of cross-
sectional disease activity at diagnosis in immunosuppressive drug-naïve patients with AAV.

The aim of this study was to describe the demographic data, clinical manifestations and serological characteristics in AAV patients, to evaluate the expression differences between active and inactive disease and to validate the most promising biomarkers for disease activity.

2. Patients and Methods

2.1 Patients

A total of 219 patients who were newly diagnosed as AAV in our department between January 1, 2009 and December 31, 2018 were enrolled in this study. The criteria for enrolment included patients who fulfilled the American College of Rheumatology 1990 criteria for the classification of GPA and EGPA; for MPA, a clinical diagnosis was made by an experienced rheumatologist in concordance with Chapel Hill 2012 consensus [2, 12, 13]. The exclusion criteria were age younger than 18 years, recurrent AAV, presence of other autoimmune diseases and a history of malignancies. The study was approved by the Institutional Review Board of Zhongshan Hospital and all patients included in the study provided informed consent to participate.

2.2 Data collection and disease assessment

The following data of each patient at baseline were obtained: demographics, organ involvement at disease onset, comorbidities, laboratory data (erythrocyte sedimentation rate (ESR), hypersensitive CRP (hs-CRP), white blood count (WBC), hemoglobin, platelets, serum creatinine, 24-hour urine protein excretion, serum albumin, serum globulin, Immunoglobulin (A, M, G, E), C3, C4, CH50 and ANCA ) and radiology findings. The disease activity of the enrolled patients was assessed in accordance with the BVAS 2003 [14], we stratified patients with AAV into three groups based on the tertile of BVAS and defined the lower limit of the highest tertile as the cutoff for the current severe AAV (BVAS at diagnosis ≥15). We defined active AAV as BVAS ≥15,
and finally, 149 patients with active AAV and 70 patients with inactive AAV were identified.

The disease severity of the enrolled patients was classified as localized, early systemic, generalized, and severe according to the European League Against Rheumatism (EULAR) recommendation for clinical studies in systemic vasculitis [15].

2.3. ANCA analysis

ANCA were determined by indirect immunofluorescence (IIF) tests and antigen-specific enzyme-linked immunosorbent assay (ELISA) for PR3-ANCA and MPO-ANCA in all patients at the time of diagnosis.

2.4 Statistical analysis

Statistical analysis was performed with SPSS statistics (version 20.0; SPSS Institute Inc., Cary, NC, USA). For descriptive statistics, continuous variables were presented as mean ± standard deviation (SD) and categorical variables were presented as number and percentage. Continuous variables were compared using Student t test or the Mann–Whitney U test depending on data distribution. Fisher or chi-square tests were used to compare categorical variables and one-way ANOVA was used to compare continuous ones in patients with GPA, MPA and EGPA subgroups. Pearson's correlation analysis was used to evaluate the associations between the different variables with BVAS. Logistic regression was performed to calculate the area under the curve (AUC) for each laboratory test in association with disease activity. The optimal cutoff of each laboratory test at diagnosis for estimating active AAV was calculated by using the area under the receiver operator characteristic curve (AUROC) and selecting the maximized sum of sensitivity and specificity. All tests were two-tailed, with P values < 0.05 being considered as significant.

3. Results

3.1 Demographic data of all patients at baseline
Demographic data, ANCA status and disease severity of the 219 patients who were newly diagnosed of AVV (37 GPA, 172MPA and 10 EGPA; 109 men and 110 women) are summarized in Table 1. There was no significant gender difference for all AAV diseases. The mean age at diagnosis was 59.5±15.4 years. Patients with MPA were more than 10 years older on average than those with GPA or EGPA (61.7±13.9 years vs 51.5±17.5 years vs 49.8±22.7 years, respectively, p<0.001). MPO-ANCA was detectable in 94.2% of patients with MPA, in none of those with GPA, and in 50.0% of those with EGPA. In contrast, PR3-ANCA was detectable in 86.5% of those with GPA, in 10.0% of the patients with EGPA and in 4.1% of those with MPA. Overall, ANCA testing was negative at diagnosis in 5.5% of the entire AAV patients, in 13.5% of GPA patients, in 2.3% of MPA patients and in 30% of the EGPA patients. The BVAS were 17.3, and all these patients had similar BVAS scores at diagnosis. We observed that compared with GPA and EGPA, there was significantly more generalized and severe (organ-threatening) disease states, especially more renal involvement in MPA.

3.2 Clinical characteristics of all patients at diagnosis

The detailed information on organ involvement described and compared among patients with GPA, MPA and EGPA is presented in Table 2. Most patients were pyrexial on first admission. For all AVV patients at diagnosis, the most common organ involvement was lung (74.9%) and kidney involvement (62.6%). In GPA, the most common clinical features at diagnosis were pulmonary (78.4%), followed by ear–nose–throat (ENT) symptoms and renal manifestations (67.6% and 54.1%, respectively). Lung and kidney involvement were most common in patients with MPA (74.4% and 65.7%, respectively). Lung involvement was presented at diagnosis in 70% of patients with EGPA. Cutaneous involvement was present in 50% of patients with EGPA, and renal manifestation and nervous system involvement were each present in 40% of EGPA.

3.3 Laboratory data of all patients at baseline
Laboratory data are shown in Table 3. The mean hemoglobin, WBC and platelets counts were 102.7 g/L, 10.4×10^9/L and 292×10^9/L, respectively. The average value of proteinuria and Scr in AAV patients were 0.89 g/24h and 160.87 µmol/L. Proteinuria and Scr in patients with MPA were dramatically higher than those in GPA and EGPA (0.99±1.03 vs 0.54±0.55 vs 0.49±0.48; 181.44±210.57 vs 85.57±86.81 vs 85.7±49.18, respectively), corresponding to the kidney involvement mentioned above. Concerning the laboratory results of acute reactants, the mean ESR and hs-CRP were 70.7 mm/h and 62.4 mg/L, which showed no difference among the entire AAV patients. In terms of immunoglobulin, the IgE of EGPA was higher than that in GPA and MPA, but there was no statistical difference (322.9±628.3 vs 210.5±507.3 vs 130.0±151.9, p=0.548). Also, IgG4 levels were significantly higher in subjects with EGPA (3.3±1.8 g/L) than in the other two groups (GPA 1.5±1.4 g/L, MPA 1.6±1.4 g/L, p=0.039). With respect to IgM, IgA and IgG levels, no clinically meaningful differences were detected between the groups.

3.4 Comparison of laboratory variables at diagnosis between AAV patients in the active and inactive groups

The baseline features of patients in the active and inactive groups are compared in Table 4. Age and gender were comparable between these two groups (both p>0.05). Anti-MPO antibody titers were found significantly higher in active group (130.2±82.0 vs 101.2±79.0 IU/ml, p=0.014) and PR3 antibody titers were similar between both groups (p=0.2). The active group also tended to have higher levels of platelets (293.6±103.2 vs 238.2±80.2×10^9/L, p=0.00), hs-CRP (81.0±57.0 vs 45.4±36.8 mg/L, p=0.00), C4 (0.32±0.09 vs 0.19±0.06g/L) than inactive group. Hemoglobin levels were significantly lower in active group than in inactive group (98.8±21.2 vs 111.1±23.0 g/L, p=0.00). These patients also had lower levels of serum albumin (31.1±5.5 vs 33.5±5.7 g/L, p=0.003), serum globulin (31.0±7.5 vs 34.0±8.5 g/L, p=0.009), IgG (14.7±5.6 vs 18.1±7.7 g/L, p=0.001). However, the mean ESR level showed no difference between these two groups (p=0.088).
3.5 Correlation of baseline features and BVAS

To further analyze the differences of the above laboratory data between patients with different levels of disease activity score (BVAS), Pearson's correlation analysis was conducted as shown in Table 4. PR3 antibody titers, platelets and hs-CRP levels were positively correlated with BVAS ($r=0.189, 0.149, 0.265$, respectively; $p<0.05$ for all the correlations). However, hemoglobin levels, serum albumin, serum globulin, IgA, IgM and IgG levels were negatively correlated with BVAS ($r=-0.263, -0.223, -0.187, -0.215, -0.216, -0.245$, respectively; all $p<0.05$).

3.6 Potential risk factors associated with disease activity in AAV patients

To determine the independence of associations between baseline parameters and disease activity, all parameters in the univariate analysis ($p<0.1$) were included in a multivariate regression analysis. The logistic regression analysis identified the following factors as independent risk factors of disease activity: MPO antibody titer (OR=3.214 [95% CI=1.003–10.038], $p=0.045$), hemoglobin (OR=0.106 [95% CI=0.024–0.479], $p=0.004$), hs-CRP (OR=3.908 [95% CI=1.22–12.521], $p=0.022$) and C4 (OR=4.858 [95% CI=1.243–18.690], $p=0.021$) (Table 5). Cut-off values to evaluate sensitivity, specificity and the area under the curve (AUC) were further calculated (Table 6). The optimal cut-off level of serum C4 for predicting active AAV was determined to be 0.215 g/L based on the area under the ROC curve (AUC=0.710, 95% CI 0.638-0.783; sensitivity 70%, specificity 83.4%). A hs-CRP cut-off value of 62.5mg/L yielded a sensitivity of 62.3% and specificity of 77.1%, while the AUC was 0.696. Hemoglobin (cut-off value 113.5 g/L) had a sensitivity of 50% and specificity of 81.2% (AUC 0.659), anti-MPO antibody titer had a sensitivity and specificity of 57.7% and 65.7%, respectively (AUC 0.605). The above results indicated that C4 had a higher sensitivity and specificity than the other three indicators. Furthermore, serial and parallel tests were conducted using C4 and other independent biomarkers for AAV activity, as shown in Table 6. The results indicated that when C4 was combined with hs-CRP for serial test, the specificity improved to 94.3%. When combined with C4 and hemoglobin for parallel test, the sensitivity improved to 96.7%.
4. Discussion

To our knowledge, this is the first study regarding the characteristics of AAV in eastern China. The age, gender, and patterns of organ involvement in our patients were similar to those described in Europe and other countries [6, 16-19]. At the time of diagnosis, the mean age was significantly higher in patients with MPA consistent with that reported by other studies [6, 16].

Our findings concur with previous reports that ENT involvement was predominant in GPA but rare in EGPA and MPA, renal involvement was almost constant in MPA but exceptional in EGPA; neurological involvement was notably more prevalent in EGPA than in GPA and MPA [5, 6, 17, 18, 20]. However, these clinical characteristics cannot distinguish different types of AAV, just like laboratory tests, they are also not specific.

Our study showed that MPO-ANCA were much more common than PR3-ANCA in Chinese patients with AAV in accordance with other Asian populations that MPA was approximately two to three times more common than GPA in China and Japan [21-23]. However, GPA is more common than MPA in European and American patients [23, 24]. With respect to ANCA, we confirmed that C-ANCA-PR3 antibodies were mainly associated with GPA, and P-ANCA-MPO antibodies with MPA and EGPA, but caution should be exerted in view of its negativity in a milder form of the disease, or positivity in other disorders. MPO-ANCA was reported to contribute to severe renal involvement and a percentage of Interstitial lung disease (ILD) in patients with AAV. Studies showed that renal lesions and inflammation in renal biopsy from MPA patients were more severe than GPA [25]. Our study found that serum creatinine and proteinuria levels in patients with MPA were higher than those with GPA and EGPA, further confirming these studies.

In our study, lung involvement occurred in most of the AAV patients. For lung lesions, patients with MPA seemed to be more prone to have ILD in line with some studied that ILD developed more frequently in patients with MPO-ANCA-positive AAV, mainly in those with a diagnosis of MPA [26]. A high ratio of MPO-ANCA
positivity to PR3-ANCA positivity and a high prevalence of ILD also had been reported in Asian countries [4, 27].

Humoral immunity had been shown to play an important role in the pathogenesis of AAV for the efficacy of B-cell depletion with rituximab in refractory AAV cases [28]. Previous reports showed that IgG4 are increased in GPA and EGPA and correlated with disease activity [29, 30]. Similarly, in this study, we found elevated serum IgG4 levels in patients with AAV, and the significantly increased IgG4 production is much more pronounced in EGPA than that in MPA and GPA. This was confirmed by our study that the difference between EGPA and GPA was highly significant.

Despite our increasing understanding of the pathogenesis of AAV, there is still no reliable biomarker to assess disease activity in AAV. At present, ESR, CRP, and changes in ANCA titers are still the most commonly used markers of disease activity in the clinic practice [11, 31, 32]. In our study, multivariate analysis was used to find the potential independent predictors related to disease activity. Similar to reports in other cohorts of patients with AAV, levels of hs-CRP were significantly up-regulated in active AAV and associated with disease activity [31, 32]. However, inflammatory processes besides vasculitis could raise its respective values, leading to an impractical disease marker. Serial ANCA titre, although full of controversy, was the most frequently used tool to assess disease activity. These findings were corroborated in our study, further supporting a role of elevated MPO-ANCA titre in active disease and independently correlated with disease activity. A low hemoglobin was reported to be imparted an increased risk of death, possibly as markers of the severity of the systemic inflammation. Moreover, low hemoglobin had been considered to be a negative prognostic factor of AAV [5, 33]. Interestingly, our observations revealed that hemoglobin levels were correlated with disease activity and also as an independent predictor for activity, in accordance with other studies who described lower hemoglobin levels were associated with severe AAV [33].

As an important part of the immune system, the complement system plays a central role in innate and adaptive immunity, and abnormalities in complement had been associated with many autoimmune diseases, including systemic lupus erythematosus
(SLE), atypical hemolytic-uremic syndrome (aHUS), sepsis and cryoglobulinemia[34-36]. Recently, studies from experimental animal models, in vitro experiments, and clinical observations had demonstrated that activation of alternative complement pathway played a critical role in the pathogenesis of AAV [36, 37]. The fact was supported by studies that higher plasma levels of alternative complement pathway components such as Bb, C3a, and C5a were observed in patients with AAV than in healthy control[37]. Moreover, other studies had shown that low baseline C3 levels predicted severe AAV and implied bad outcomes and poor response to immunosuppressive treatment [9, 38]. Inconsistent with their research, we found no correlation between C3 and disease activity. The inconsistency of the research results may be attributed to the study population, detection methods and the cut-off values.

In our present study, we found that plasma levels of C4 were significantly higher in patients with AAV in active stage compared with that in inactive stage. So far, there had been few studies regarding the C4 and disease activity. A previous study demonstrated the plasma level of C4d, a product derived from activation of C4 in patients with AAV was significantly higher than that in normal controls, showing that the classical complement pathway was activated in AAV[10]. However, the authors could not conclude whether C4 represented an independent factor predicting the cross-sectional severity and activity of AAV. To the best of our knowledge, we have first demonstrated that C4 could predict disease activity at diagnosis in patients with AAV. Our study showed that baseline serum C4 was significantly correlated with BVAS for AAV patients, and furthermore, C4 was also the independent predictor of disease activity in multivariable regression analysis. However, evidence from the anti-MPO-induced AAV mouse models demonstrated C4 knockout (KO) mice developed necrotizing crescentic glomerulonephritis (NCGN) comparable to wild type mice [37]. All of the above suggested that the classic pathway was activated in patients with AAV and correlated with disease activity, but they were probably not pathogenic. The complement system, especially classic pathway had been reported to play an important role in removing waste material from necrotic or apoptotic cells, as well as the clearance of circulating immune complexes. The possible reasons for the increased C4 levels
along with disease activity in our study may be as follows. On one hand, as the degree of AAV inflammation increases, more necrotic and apoptotic cells were produced and more C4 was needed to clear the waste material; On the other hand, C4, like several other factors of complement system, belong to the acute phase proteins which increased rapidly in inflammatory conditions, such as vasculitis.

In the clinical settings, BVAS might be affected by subjective complaints, such as discomfort, myalgia or arthralgia and it needs more tests including chest imaging in a patient suspected of pleural effusion or pleurisy. However, since C4, hs-CRP and hemoglobin which are routinely measured at every visit, it is more convenient to evaluate the severity of AAV. Our present study suggested that C4 with high sensitivity and specificity for predicting disease severity may be a new biomarker of disease activity that could help clinicians quickly identify patients with disease activity.

Several limitations of this study should be considered. The main limitations of this study were the cross-sectional, retrospective, observational design and the laboratory data were collected by reviewing medical records. Secondly, the number of patients evolved was relatively limited. Nevertheless, and taking into account our limitations, future studies with a larger number of participants and prospective cohorts compared with control groups to ascertain their clinical utility for AAV.

5. Conclusions
In conclusion, our study has for the first time indicated that baseline C4 values can be an independent predictor for assessing disease activity in AAV.
Abbreviations
ANCA: antineutrophil cytoplasmic antibody; AAV: antineutrophil cytoplasmic antibody-associated vasculitis; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3; ENT, ear-nose-throat; CNS, central nervous system; hLAMP-2, human lysosomal-associated membrane protein 2; BVAS, Birmingham Vasculitis Activity Score; MMP-3, matrix metalloproteinase-3; WBC, white blood cell; Scr, serum creatinine; ESR, erythrocyte sedimentation rate; hs-CRP, hypersensitive c-reactive protein; Ig, Immunoglobulin; C3, complement 3; EULAR, European League Against Rheumatism; IIF, indirect immunofluorescence; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation; AUC: area under ROC curve; ILD, Interstitial lung disease; SLE, systemic lupus erythematosus; aHUS, atypical hemolytic-uremic syndrome; NCGN, necrotizing crescentic glomerulonephritis; OR, odds ratio; CI, confidence interval; PPV: positive predictive value; NPV: negative predictive value; 95% CI: 95% confidence interval;

Competing interests
The authors have no conflicts of interest to disclose.

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Ethics approval and consent to participate
The study was approved by the Institutional Review Board of Zhongshan Hospital and all patients included in the study provided informed consent to participate.

Consent for publication
Not applicable.
Authors’ contributions
All authors meet the authorship requirements. Yun Liu and Lindi Jiang were involved in the study concept and design; Yun Liu contributed to the acquisition, analysis, and interpretation of data and manuscript writing. Lili Ma, Zongfei Ji and Huiyong Chen contributed to the acquisition data and gave suggestions. Rongyi Chen, Ying Sun, Xiufang Kong helped to provide statistical method support. Yuan Ji and Yingyong Hou provide pathological data. All authors had read and approved the final manuscript for submission.

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Availability of Data and Materials
The datasets analysed during the current study are not publicly available due to the protection of patient privacy for all data comes from clinical patients but are available from the corresponding author on reasonable request.

References
1. Pagnoux C. Updates in ANCA-associated vasculitis. *Eur J Rheumatol* 2016, 3:122-133.
2. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013, 65:1-11.
3. Csernok E, Moosig F. Current and emerging techniques for ANCA detection in vasculitis. *Nat Rev Rheumatol* 2014, 10:494-501.
4. Chen M, Yu F, Zhang Y, Zhao MH. Antineutrophil cytoplasmic autoantibody-associated vasculitis in older patients. *Medicine (Baltimore)* 2008, 87:203-209.
5. Heijl C, Mohammad AJ, Westman K, Hoglund P. Long-term patient survival in a Swedish population-based cohort of patients with ANCA-associated vasculitis. *RMD Open* 2017, 3: e000435.
6. Mohammad AJ, Jacobsson LT, Westman KW, Sturfelt G, Segelmark M. Incidence and survival rates in Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and polyarteritis nodosa. *Rheumatology (Oxford)* 2009, 48:1560-1565.

7. Luqmani RA, Exley AR, Kitas GD, Bacon PA. Disease assessment and management of the vasculitides. *Baillieres Clin Rheumatol* 1997, 11:423-446.

8. Monach PA, Warner RL, Tomasson G, Specks U, Stone JH, Ding L, et al. Serum proteins reflecting inflammation, injury and repair as biomarkers of disease activity in ANCA-associated vasculitis. *Annals of the Rheumatic Diseases* 2013, 72:1342-1350.

9. Choi H, Kim Y, Jung SM, Song JJ, Park Y-B, Lee S-W. Low serum complement 3 level is associated with severe ANCA-associated vasculitis at diagnosis. *Clinical and Experimental Nephrology* 2018, 23:223-230.

10. Gou SJ, Yuan J, Chen M, Yu F, Zhao MH. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. *Kidney Int* 2013, 83:129-137.

11. Kronbichler A, Kerschbaum J, Grundlinger G, Leierer J, Mayer G, Rudnicki M. Evaluation and validation of biomarkers in granulomatosis with polyangiitis and microscopic polyangiitis. *Nephrol Dial Transplant* 2016, 31:930-936.

12. Leavitt RY, Fauci AS, Bloch DA, Michel BA, Hunder GG, Arend WP, et al. The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum* 1990, 33:1101-1107.

13. Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). *Arthritis Rheum* 1990, 33:1094-1100.

14. Mukhtyar C, Lee R, Brown D, Carruthers D, Dasgupta B, Dubey S, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis* 2009, 68:1827-1832.
15. Hellmich B, Flossmann O, Gross WL, Bacon P, Willem Cohen-Tervaert J, Guillemin L, et al. EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on antineutrophil cytoplasm antibody-associated vasculitis. *Annals of the Rheumatic Diseases* 2007, 66:605-617.

16. Sada KE YM, Harigai M, Fujii T, Dobashi H, Takasaki Y, Ito S, Yamada H, Wada T, Hirahashi J, Arimura Y, Makino H; Research Committee on Intractable Vasculitides, the Ministry of Health, Labour and Welfare of Japan. Classification and characteristics of Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis in a nationwide, prospective, inception cohort study. *Arthritis Res Ther* 2014, 16:R101.

17. Sinico RA, Radice A. Antineutrophil cytoplasmic antibodies (ANCA) testing: detection methods and clinical application. *Clin Exp Rheumatol* 2014, 32: S112-S117.

18. Pavone L, Grasselli C, Chierici E, Maggiore U, Garini G, Ronda N, et al. Outcome and prognostic factors during the course of primary small-vessel vasculitides. *J Rheumatol* 2006, 33:1299-1306.

19. Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med* 1983, 98:76-85.

20. Mukhtyar C, Flossmann O, Hellmich B, Bacon P, Cid M, Cohen-Tervaert JW, et al. Outcomes from studies of antineutrophil cytoplasm antibody associated vasculitis: a systematic review by the European League Against Rheumatism systemic vasculitis task force. *Annals of the Rheumatic Diseases* 2007, 67:1004-1010.

21. Liu LJ, Chen M, Yu F, Zhao MH, Wang HY. Evaluation of a new algorithm in classification of systemic vasculitis. *Rheumatology (Oxford)* 2008, 47:708-712.

22. Kamali S, Artim-Esen B, Erer B, Ozdener L, Gul A, Ocal L, et al. Re-evaluation of 129 patients with systemic necrotizing vasculitides by using classification
algorithm according to consensus methodology. *Clinical Rheumatology* 2011, 31:325-328.

23. Fujimoto S, Watts RA, Kobayashi S, Suzuki K, Jayne DRW, Scott DGI, et al. Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the UK. *Rheumatology* 2011, 50:1916-1920.

24. Fujimoto S, Uezono S, Hisanaga S, Fukudome K, Kobayashi S, Suzuki K, et al. Incidence of ANCA-associated primary renal vasculitis in the Miyazaki Prefecture: the first population-based, retrospective, epidemiologic survey in Japan. *Clin J Am Soc Nephrol* 2006, 1:1016-1022.

25. Hauer HA BI, van Houwelingen HC, Ferrario F, Noël LH, Waldherr R, Jayne DR, Rasmussen N, Bruijn JA, Hagen EC. Renal histology in ANCA-associated vasculitis: differences between diagnostic and serologic subgroups. *Kidney Int* 2002, 61:80-89.

26. Hervier B, Pagnoux C, Agard C, Haroche J, Amoura Z, Guillevin L, et al. Pulmonary fibrosis associated with ANCA-positive vasculitides. Retrospective study of 12 cases and review of the literature. *Ann Rheum Dis* 2009, 68:404-407.

27. Huang H, Wang YX, Jiang CG, Liu J, Li J, Xu K, et al. A retrospective study of microscopic polyangiitis patients presenting with pulmonary fibrosis in China. *BMC Pulm Med* 2014, 14: 8.

28. Cartin-Ceba R, Keogh KA, Specks U, Sethi S, Fervenza FC. Rituximab for the treatment of Churg-Strauss syndrome with renal involvement. *Nephrol Dial Transplant* 2011, 26:2865-2871.

29. Vaglio A, Strehl JD, Manger B, Maritati F, Alberici F, Beyer C, et al. IgG4 immune response in Churg-Strauss syndrome. *Ann Rheum Dis* 2012, 71: 390-393.

30. Brouwer E, Tervaert JW, Horst G, Huitema MG, van der Giessen M, Limburg PC, et al. Predominance of IgG1 and IgG4 subclasses of anti-neutrophil
cytoplasmic autoantibodies (ANCA) in patients with Wegener's granulomatosis and clinically related disorders. *Clin Exp Immunol* 1991, 83:379-386.

31. Sanders JS, Huitma MG, Kallenberg CG, Stegeman CA. Prediction of relapses in PR3-ANCA-associated vasculitis by assessing responses of ANCA titres to treatment. *Rheumatology (Oxford)* 2006, 45:724-729.

32. Tedesco M, Gallieni M, Pellegata F, Cozzolino M, Alberici F. Update on ANCA-associated vasculitis: from biomarkers to therapy. *J Nephrol* 2019, 32:871-882.

33. Kim MK, Choi H, Kim JY, Song JJ, Park YB, Lee SW. Multivariable index for assessing the activity and predicting all-cause mortality in antineutrophil cytoplasmic antibody-associated vasculitis. *J Clin Lab Anal* 2019, e23022.

34. Markiewski MM, Lambris JD. The role of complement in inflammatory diseases from behind the scenes into the spotlight. *Am J Pathol* 2007, 171:715-727.

35. Wang FM, Yu F, Tan Y, Song D, Zhao MH. Serum complement factor H is associated with clinical and pathological activities of patients with lupus nephritis. *Rheumatology (Oxford)* 2012, 51:2269-2277.

36. Dragon-Durey MA, Loirat C, Cloarec S, Macher MA, Blouin J, Nivet H, et al. Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2005, 16:555-563.

37. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* 2007, 170:52-64.

38. Crnogorac M, Horvatic I, Kacinari P, Ljubanovic DG, Galesic K. Serum C3 complement levels in ANCA associated vasculitis at diagnosis is a predictor of patient and renal outcome. *J Nephrol* 2018, 31:257-262.