ORIGINAL ARTICLE

The clinical pattern differentiates ANCA-positive infective endocarditis patients from ANCA-associated vasculitis patients: a 23 years' retrospective cohort study in China and follow-ups

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Abstract

Objectives Patients with infective endocarditis (IE) may present rheumatic manifestations concurrent with various autoantibodies and thus mimic antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). This study aims to characterize the specific features in a long-term cohort of ANCA-positive IE patients and to perform comparative analysis with primary AAV patients.

Methods We performed a retrospective thorough review of 475 consecutive IE patients over 23 years, identifying 22 patients positive for proteinase 3 and/or myeloperoxidase and 36 treatment-naïve AAV patients. The clinical, laboratory, and follow-up data were collected to perform comparative analysis.

Results Our study illustrated that ANCA-positive IE patients were younger and had a shorter duration than AAV patients. Pulmonary lesions, ENT signs, peripheral neuropath, and proteinuria were more commonly seen in AAV patients, while heart valve involvement, spleen enlargement, and cerebral hemorrhage were more typical for IE patients (all $p < 0.05$). Besides, ANCA-positive IE patients presented a higher level of PR3-ANCA but lower C3 (both $p < 0.05$). Hyperleukocytosis and thrombocytopenia were more frequently found in AAV patients (both $p < 0.05$). No significant difference was noticed in the survival rate.

Conclusions Our study urges the early differential diagnosis of IE in ANCA-positive patients. It supports the claim that ANCA-positive IE patients and AAV patients do not share the same clinical spectrum. Echocardiography, serological profiles, and evaluation of multi-organ involvement might be required to improve diagnostic accuracy.

Key Points
●*Early differential diagnosis of ANCA-positive IE from AAV is challenging even for expert rheumatologists.*
●*Our study is so far one of the largest to include 22 ANCA-positive IE patients in one single center and spanning over 23 years. It is also the first study to include both ANCA-positive IE patients and AAV patients in one center.*
●*Our study aides to identify a clinical picture to differentiate ANCA-Positive IE Patients from AAV Patients.*

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Introduction

Infective endocarditis (IE) is a rare, life-threatening disease that has long-lasting effects even among patients who survive. In spite of the annual incidence estimated to be from 15 to 80 per million individuals varying in different population-based studies, it was increased in patients with prosthetic valves. Contemporary mortality rates for IE have exceeded approximately 25% even with the best available therapy [1, 2]. Despite the advances in diagnosis and

treatment, the prognosis for IE remains poor; in-hospital or 3-month mortality is 18–25% and has not decreased over time [3–7]. Consequently, early diagnosis remains indispensable to benefit patients with IE from antimicrobial therapy or cardiac surgery. Historically, IE was characterized by clinical manifestations such as cardiac murmur, embolic events, immunological vasculitis, and positive blood culture. However, a remarkable number of acute or subacute IE patients present few of these hallmark manifestations in modern era in China. Instead, these patients with IE often present early nonspecific symptoms such as fatigue, fever, arthralgia, myalgia, and serositis concurrent with various autoantibodies (ANCAs, rheumatoid factor (RF), anticardiolipin antibodies (aCLs), etc.), as we have previously reported [8]. The presence of the clinical variability complicates the identification and distinction of patients with IE from having a rheumatic disease, typically antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). In the meantime, the capacity to timely and stably exclude IE from AAV is also of great importance, to avoid unnecessary antibiotics and to concentrate considerations of immunosuppression therapy.

AAV is a heterogeneous group of clinical syndromes characterized by inflammatory infiltration of the walls of small and medium-sized blood vessels causing vascular and tissue necrosis, and the presence of ANCAs [9]. ANCAs against proteinase-3 (PR3) and/or myeloperoxidase (MPO) are considered major serological markers relevant to mainly small-vessel vasculitis, including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic GPA (EGPA), with up to 85–95% of patients presenting seropositive ANCAs according to different vasculitis types [10, 11]. Interestingly, previous reports have described that infection, particularly Hepatitis B virus and Streptococcus, may play an important role in triggering formation of ANCAs by the immune responses to infection and may even be regarded as the predominant pathogenetic mechanism [12]. Rheumatic manifestations, such as fever, myalgia, and arthralgia, are commonly seen in ANCA-related diseases. Moreover, it is worth mentioning that sporadic reports have described that PR3- or MPO-ANCAs are part of the pathophysiology in patients with IE [11, 13–16]. Thus, there is an urgent need to accurately understand the clinical presentations of patients with IE and patients with AAV.

On the other hand, as a systemic disease, IE results in characteristic pathological in multiple target organs, especially presenting rheumatic manifestations such as hematuria, multiple pulmonary nodules, cerebral hemorrhage, cerebral infarction, and glomerulonephritis [17]. To our knowledge, a few series have analyzed the prevalence and the frequency of ANCAs as well as the clinical and biochemical features and outcomes in patients with IE [18, 19], whereas few studies have compared the clinical manifestations and auxiliary examinations between ANCA-positive IE and AAV patients. Therefore, a comparative study looking for resemblances and dissimilarities between these two diseases may provide clues for differential diagnosis and treatment strategies.

This study aimed to characterize the clinical, laboratory, imaging features, and outcomes between 22 ANCA-positive IE patients and 36 AAV patients and to compare the similarities and differences of these two groups in a single center in China.

Methods

Patients

The patients were recruited retrospectively in this study. From 1997 to 2020, a total of 475 consecutive IE patients were diagnosed according to the modified Duke criteria [20] hospitalized in Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine. Among them, patients with long-term fever (fever ≥ 38 ℃ for more than 2 weeks) underwent the test for ANCAs. Therefore, 92 consecutive IE patients (confirmed by two qualified physicians: Jialin Teng and Chengde Yang) were tested for ANCAs. Twenty-two of them (23.91%) were positive for PR3-ANCA and/or MPO-ANCA. Clinical data were collected from these 22 patients based on inpatient medical records. Cardiac surgery and death were recorded during the period of hospitalization. We had ruled out the patients with primary rheumatic disease or immunosuppression. Moreover, we performed a thorough review of 94 consecutive AAV patients from 2015 to 2020. As a result, 36 (12 GPA, 22 MPA, and 2 EGPA) hospitalized primary AAV patients (confirmed by two qualified rheumatologists: Jialin Teng and Chengde Yang) were reviewed. Drug-induced AAV were excluded from this study. The diagnosis of GPA, MPA, and EGPA was evaluated according to clinical, laboratory, and pathological criteria [21]. In this study, either MPO-ANCA- or PR3-ANCA-positive patients were defined as ANCA-positive patients. The study was performed in accordance with the ethical standard of the Declaration of Helsinki and approved by the Committee of Ruijin Hospital (ID: 2016–62), Shanghai, China.

The medical records of patients with IE and AAV were all reviewed for general characteristics: age and sex, comorbidities, previous medical history, and clinical manifestations, especially constitutional symptoms and systemic manifestations: valve involvement, vascular embolic events, pulmonary features, renal failure, ENT involvement, neuropathic, and cutaneous abnormity as well as laboratory, microbiologic, and imaging data. The duration of the disease was deemed as the period from the first onset of a clinical symptom to a definite diagnosis. Outcomes consisting of cardiac surgery and in-hospital mortality were both

recorded during hospitalization, and the survival rates of two groups were performed.

Clinical, laboratory features, and imaging data

First, all patients were evaluated with the same protocol to perform comparative analysis. The protocol was as follows: clinical presentations: constitutional symptoms (fever ≥ 38 ℃, joint/muscle pain, edema, asthenia); systemic manifestations: heart: valve involvement; vascular: embolic events, pulmonary features, renal failure, neuropathic and cutaneous abnormity; transthoracic echocardiograph: vegetation or new valvular regurgitation and other abnormalities of a valve. In addition, each patient underwent chest computerized tomography (CT) scan and an abdominal ultrasound to identify multi-system involvement.

Second, laboratory data were recorded at the time of diagnosis. ANCA testing was performed using indirect immunofluorescence assay in ethanol-fixed peripheral blood neutrophils. The serum cytoplasmic (c-ANCA) and perinuclear ANCA (p-ANCA) were also determined by indirect immunofluorescence (Euroimmun, Germany). Furthermore, PR3- ANCAs and MPO-ANCAs in serum samples were detected with the use of a commercially available enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Germany). Elevated antibody titer above 20 IU/mL was considered positive. Besides, the following laboratory data were recorded: white blood cell counts in blood (WBC), hemoglobin (Hb), platelet (PLT), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum albumin (ALB), serum creatinine, hematuria, proteinuria, rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP), immunoglobulin G/A/M (IgG/A/M), complement 3 (C3), complement 4 (C4), antinuclear antibody (ANA), extractable nuclear antigen (ENA), anticardiolipin antibodies (aCLs), anti-β2-glycoprotein I antibodies (aβ2GPI), lupus anticoagulant (LAC), anti-double stranded DNA antibody (anti-dsDNA).

Complete blood counts were obtained using an automated hematology analyzer DXH800 (Beckman Coulter, USA). ESR was measured by an automated erythrocyte sedimentation analyzer SD-100 (Succeeder, China). CRP levels, RF, C3, and C4 were determined by nephelometry using an automated analyzer AU5800 (Beckman Coulter, USA). AST, ALT, and Alb were measured by colorimetric method using an automated analyzer AU5800 (Beckman Coulter, USA). ANA were measured by indirect immunofluorescent method (INNOVA, USA). Anti-dsDNA antibodies were measured using commercial ELISA kits by QUANTA LYSER 240. (INNOVA, USA). ENA were measured by immunoblotting using EUROBLine Master Plus (Euroimmun, Germany). Anti-CCP were measured using

chemiluminescent immunoassay method by BIO-FLASH automated chemiluminescence immunoassay analyzer (INNOVA, USA).ACL and anti-β2GPI antibodies were measured with ELISA kits (Euroimmun, Germany). The LAC levels for all patients were measured according to the criteria from the ISTH Subcommittee by Automated Coagulation Laboratory (ACL) 300R (Milan, Italy).

Statistical analysis

Data was analyzed using R (R Foundation for Statistical Computing, version 3.6.2). Descriptive statistics are represented as the mean $\pm$ standard deviation for continuous variables and numbers, percentages for categorical variables. Statistical analysis was performed by t test, χ^2 test, Wilcoxon test, and Kruskal–Wallis test according to different variables as appropriate. We used logistic regression models with age and gender information to calculate the odds ratios between different clinical presentations and serum biomarkers. The fundamental principle of cluster analysis aimed to group individuals on the basis of clinical parameters and differentiate clusters from one another. Because only categorical variables were included in the algorithm, the commonly k-means clustering algorithm was used. The cluster analysis was performed by using the "cluster" package in R. Survival was assessed by long-rank testing using the "survival" package according to the Kaplan–Meier method in R. A two-tailed p value of less than 0.05 was considered statistically significant.

Patient and public involvement

Patients were not involved in the design, the recruitment to, and conduct of the study as the study was retrospective. The results were not shared with study participants.

Results

Among 92 consecutive IE patients who underwent ANCA examination, we focused on findings of serum samples for 22 (22/92, 23.91%) ANCA-positive IE patients (13 males, 49.95 ± 15.04). In addition, 36 (15 males, 60.22 ± 14.76) consecutively hospitalized patients with primary AAV were reviewed.

Demographic features of ANCA-positive IE patients

Among 22 ANCA-positive patients with IE, patients exhibited c-ANCA ($N = 21$) and p-ANCA ($N = 1$). In terms of ANCA ELISA tests, they were all 22 (100%)

PR3-ANCA-positive and 0 (0%) MPO-ANCA-positive. The clinical characteristics of patients with IE who presented with positive ANCA-antibodies were shown in Table 1. The 22 (100%) patients with positive PR3-ANCAs shared the same clinical pattern. Rheumatic manifestations were commonly seen in most cases in the initial signs or symptoms. Fever was found in 20 cases (90.91%), arthralgia was found in 12 cases (54.55%), edema in 5 cases (22.73%), pleural effusion in 3 cases (13.64%), and pericardial effusion in 4 individuals (18.18%) (Tables 1 and 2). Systemic symptoms were also noticed in ANCA-positive IE patients: heart murmur in 17 cases (77.27%); splenomegaly in 6 cases (27.27%); neuropathy in 7 cases (31.82%), including 4 cerebral hemorrhage (18.18%) and 3 cerebral infarctions (13.64%). Janeway lesions specific for IE were found in 2 individuals (9.09%) and purpura found in 2 cases (9.09%). Roth nodes were found in 1 case (4.55%) identifying eye involvement in IE (Tables 1 and 2).

Comparison of clinical characteristics between ANCA-positive IE patients and AAV patients

Clinical features of ANCA-positive IE patients and AAV patients were shown in Table 2. IE patients were significantly younger ($p < 0.01$), but there was no significant difference in gender between groups. As to clinical manifestations, IE patients tended to show a much shorter duration from the symptom onset to the disease diagnosis ($p < 0.01$). Interestingly, both ANCA-positive IE patients and AAV patients tended to have fever, arthralgia/myalgia, serositis, and edema. By contrast, ANCA-positive IE patients presented more a higher frequency of high fever ($p = 0.03$).

On the other hand, renal impairment was both frequently noticed in ANCA-positive IE patients (63.64%) and AAV patients (75.00%); hematuria was noticed in both groups while proteinuria was much frequently found in AAV patients ($p < 0.01$). Unfortunately, none of IE patients underwent the renal biopsy. Pulmonary involvement ($p < 0.01$), ENT signs ($p < 0.01$), and peripheral neuropathy ($p < 0.01$) were much more common in AAV patients. In contrast, ANCA-positive IE patients tended to have more heart murmur ($p < 0.01$), spleen enlargement ($p = 0.04$), and neurologic abnormality, typically cerebral hemorrhage ($p = 0.02$). As to the cutaneous and ocular involvement, although there were no significant differences, two IE patient presented typical Janeway lesions and only one IE patient presented Roth nodes, while purpura was noticed in both groups (Tables 1 and 2). The pattern of organ involvement was shown in Fig. 1. Regarding the numbers of involved organs, it demonstrated that more organs were affected in AAV than IE

patients, while more ANCA-positive IE patients had multi-organ involvement compared to AAV patients (Fig. 1).

Comparison of serological findings between ANCA-positive IE patients and AAV patients

ANCA-positive IE patients expressed anti-PR3 together with the presence of ANA, aCLs, anti-β2GPI, anti-dsDNA, and anti-CCP (Table 3). While all 22 (100%) ANCA-positive IE patients possessed positive anti-PR3 antibodies, AAV patients tended to have a higher tire of anti-PR3 ($p < 0.05$). There were no statistical differences in anemia, hypoalbuminemia, serum creatinine, elevated ESR, and CRP between two groups ($p > 0.05$). However, low levels of C3 were found in 21 (58.33%) patients in AAV patients compared with 5 (22.73%) patients in ANCA-positive IE patients ($p = 0.01$). Furthermore, hyperleukocytosis ($p = 0.03$) and thrombocytopenia ($p = 0.04$) were found more frequently in AAV patients. ANA-positive was found in 2 (9.09%) ANCA-positive IE patients, both were 1:160 ANA-positive (granular type). Eight (22.22%) ANA-positive were found in AAV patients: 3 with 1:160 ANA-positive (granular type), 2 with 1:160 ANA-positive (homogeneous pattern) and 3 with 1:320 ANA-positive (granular type). ENA-positive was found in 4 (11.11%) AAV patients: 2 with (5.56%) anti-Ro-52-positive and 2 with (5.56%) anti-SSA-positive. ENA-positive was not found in ANCA-positive IE patients. Positive blood culture was found in 14 (63.63%) ANCA-positive IE patients ($p < 0.01$). The detail of bacteria grown in blood culture is shown in Table 1. A heatmap of clinical and serological spectrum of both groups is presented in Fig. 2. The cluster analysis aimed to differentiate group individuals on the basis of clinical parameters and clusters from one another. Only categorical variables were evaluated in the algorithm. With regard to the variables in generating the solution, all 22 ANCA-positive IE patients and six AAV patients were classified in one cluster, while Group 2 contained the rest 30 AAV patients.

Outcomes and survival curves of ANCA-positive IE patients and AAV patients

During a 4-month follow-up, among 22 ANCA-positive IE patients, 5 (22.73%) patients underwent cardiac surgery. Only one patient who had operation and another 3 (13.64%) ANCA-positive IE patients died of acute heart failure or septic shock. None of IE patients died of renal failure. Two deaths (5.56%) were registered in AAV patients: one patient died of pulmonary infection and the other died of septic shock. The survival curve of the two groups is shown in Fig. 3 ($p = 0.19$).

Table 1 Main characteristics of 22 ANCA-positive infective endocarditis patients

| Patients | Clinical characteristics of IE | | | | | | Outcome | |
	Disease duration (months)	Clinical presentations	Valve involvement	Vegetation	Other manifestations	Microorganism	Surgical treatment	In-hospital death
1	4	Fever, arthralgia	Mitral valve, Aortic valve	Yes	Pericardial effusion	Candida albicans	Yes	No
2	2	Arthralgia	Mitral valve	No	/	None identified	Yes	No
3	1	Fever,	Mitral valve	Yes	Pericardial effusion, Lacunar infarction	None identified	Yes	No
4	1	Fever	Mitral valve	Yes	Pleural effusion, Cerebral infarction	None identified	Yes	No
5	6	Fever, arthralgia	None	No	Janeway, Splenomegaly	Granulicatella adiacens	No	No
6	0.2	Fever	Mitral valve	Yes	Pleural effusion, Pericardial effusion, Osler, SAH, cerebral hemorrhage	Abiotrophia defectiva	No	No
7	0.2	Arthralgia	Tricuspid valve	No	Janeway, pulmonary embolism	Streptococcus sanguinis	No	No
8	1	Fever	Mitral valve, Aortic valve	Yes	Pleural effusion, Pericardial effusion, cerebral hemorrhage	None identified	Yes	Yes
9	0.6	Fever, arthralgia	None	No	/	α-hemolytic streptococcus	No	No
10	0.7	Fever	Aortic valve	Yes	Purpura	None identified	No	No
11	0.6	Fever, arthralgia	Mitral valve	Yes	Cerebral hemorrhage	None identified	No	No
12	0.7	Fever, edema	Mitral valve, Aortic valve	Yes	Splenomegaly	None identified	No	Yes
13	0.6	Fever, arthralgia	None	Yes	/	Streptococcus oralis	No	No
14	1	Fever, edema	Aortic valve	No	/	Enterococcus faecalis	No	No
15	0.4	Fever, edema, arthralgia	Mitral valve, Aortic valve	Yes	Splenomegaly	None identified	No	Yes
16	0.2	Fever	Mitral valve, Aortic valve	Yes	Splenomegaly	Streptococcus oralis	No	No
17	1.2	Fever	None	No	Purpura, SAH, cerebral hemorrhage	Streptococcus mitis, α-hemolytic streptococcus	No	No
18	1.3	Fever, arthralgia	None	No	/	Streptococcus mitis	No	Yes
19	0.5	Fever, edema, arthralgia	Mitral valve	Yes	Cerebral infarction, Splenomegaly	α-hemolytic streptococcus	No	No
20	2.1	fever, arthralgia	Aortic valve	Yes	/	α-hemolytic streptococcus	No	No
21	1.6	fever	Mitral valve	Yes	Splenomegaly	α-hemolytic streptococcus	No	No
22	0.5	Fever, edema, arthralgia,	Tricuspid valve	No	Pericardial effusion	Klebsiella pneumoniae	No	No

M, male; *F,* female; *SAH*, subarachnoid hemorrhage

Table 2 Clinical features of ANCA-positive IE patients versus AVV patients

| Variables	Patients		*p* value
	ANCA-positive IE patients (*n*=22)	AAV patients (*n*=36)	
Age at diagnosis	49.95 ± 15.04	60.22 ± 14.76	< 0.01**
Age > 50 at diagnosis	11 (50.00)	30 (83.33)	< 0.01**
Gender, men/total	13 (59.09)	15 (41.67)	0.19
Duration, months	1.30 ± 1.38	6.88 ± 8.64	< 0.01**
Clinical features			
Fever	20 (90.91)	23 (63.89)	0.03*
Joint/muscle pain	12 (54.55)	24 (66.67)	0.41
Edema	5 (22.73)	7 (19.44)	0.75
Serositis	7 (31.82)	9 (25.00)	0.76
Pleural effusion	3 (13.64)	5 (13.89)	> 0.99
Pericardial effusion	4 (18.18)	7 (18.42)	> 0.99
Organ involvement			
Renal	14 (63.64)	27 (75.00)	0.39
Hematuria (> 3/HP)	14 (63.64)	23 (63.89)	> 0.99
Proteinuria	5 (22.73)	22 (61.11)	< 0.01**
Both hemauria and proteinuria	5 (22.73)	18 (50.00)	0.05*
Heart valve	17 (77.27)	5 (13.89)	< 0.01**
ENT	0	19 (52.77)	< 0.01**
Pulmonary	3 (13.64)	21 (58.33)	< 0.01**
Neuropathic	7 (31.82)	7 (19.44)	0.35
Cerebral hemorrhage	4 (18.18)	0	0.02*
Cerebral infarction	3 (13.64)	7 (19.44)	0.73
Peripheral neuropathy	0	14 (38.89)	< 0.01**
Splenomegaly	6 (27.27)	2 (5.56)	0.04*
Hepatomegaly	0	1 (2.78)	> 0.99
Cutaneous	4 (18.18)	8 (22.22)	> 0.99
Eye	1 (4.55)	8 (22.22)	0.13
Outcomes			
Surgical treatment	5 (22.73)	0	< 0.01**
In-hospital death	4 (18.18)	2 (5.56)	0.19

All continuous variables and numbers are represented as the mean ± standard deviation. All categorical variables are presented as n (percentage). *p value of less than 0.05 was considered statistically significant; **p value less than 0.01. *HP*, high power objective; *ENT*, ear, nose, and throat

Discussion

In the past decades, early diagnosis of IE remains challenging due to various nonspecific symptoms such as fatigue, fever, and arthralgia at the early stage. In spite of the advances in diagnosis and treatment, most studies have shown a growing trend towards higher incidence with high in-hospital mortality [3–7]. However, few case series reported positive ANCA in 18 to 33% of patients with IE [19, 21]. The findings of our study further assessed the prevalence of ANCAs in IE patients, which was 22 out of 92 patients (23.91%).

Early in 2010, Branka Bonaci-Nikolic et al. [22] discussed the tight connection of prolonged infections with ANCAs,

most frequently chronic HBV infection and Streptococcal and Staphylococcal infections, of which the latter two turned out to be estimated as the predominant pathogenic bacteria of IE [23]. Although the mechanism of seropositive ANCAs in IE patients is still unclear, Staphylococcus, which owns homologous peptides to PR3, may implicate as a trigger factor for an autoantibody response [22]. To note, an attempt to validate the diagnosis and explicit standard clinical and serologic criteria for differentiating ANCA-positive IE patients from AAV patients is of critical importance. Nevertheless, it remains challenging for many rheumatologists for ages, especially at the initial disease stage, since no predictive factors have reached a consensus. Thus, positive ANCA-tests must be carefully interpreted.
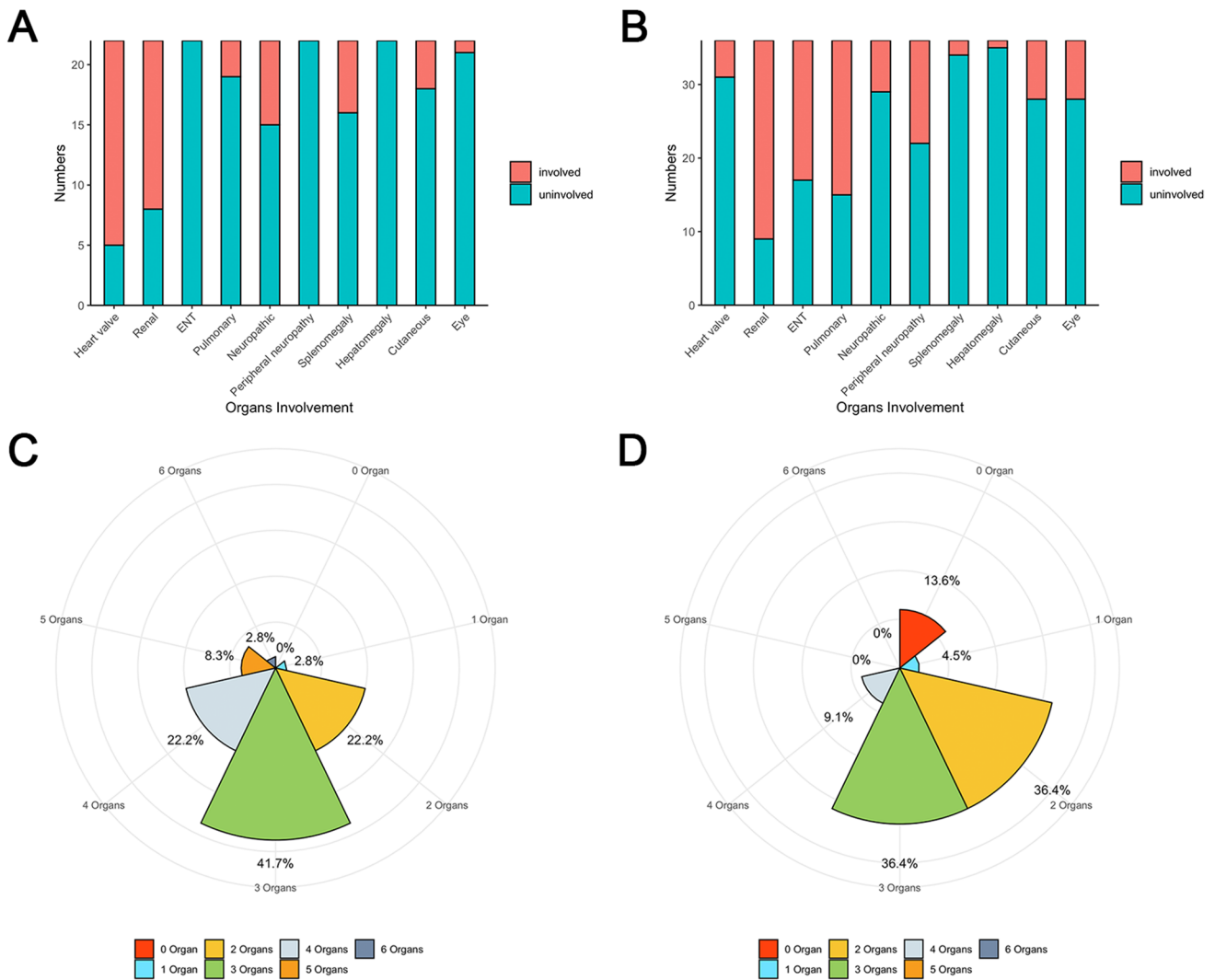
Fig. 1 Multi-organ involvement in ANCA-positive IE patients and AAV patients. **A** and **B** Distribution of multi-organ involvement in ANCA-positive IE patients and AAV patients; **A** organ involvement in ANCA-positive IE patients; **B** organ involvement in AAV patients.

C and **D** Numbers of involved organs in ANCA-positive IE patients and AAV patients. **C** Numbers of involved organs in ANCA-positive IE patients; **D** numbers of involved organs in AAV patients

To our knowledge, our study is so far one of the largest to include 22 ANCA-positive IE patients in one single center and spanning over 23 years. It is also the first study to recruit both ANCA-positive IE patients and AAV patients. Among our 22 ANCA-positive IE patients, all of them showed combined patterns of positivity, referring to c-ANCA/PR3-ANCA. The prevalence of ANCA in IE patients has been previously noted [14, 24, 25] and only eight positive MPO-ANCA IE patients were reported [18]. In contrast, among our 36 AAV patients, 12 (33.33%) subjects showed combined patterns of positivity referring to c-ANCA/PR3-ANCA, and 29 (80.56%) subjects showed combined patterns of positive p-ANCA/MPO-ANCA, and 5 (13.89%) patients were positive in both c-ANCA/PR3-ANCA and p-ANCA/MPO-ANCA (Table 3). Elevated PR3-ANCA levels might arouse suspicion of the diagnosis of an

infectious disease. In addition, the finding that various autoantibodies were positive in ANCA-positive IE patients such as RFs in 10 patients (45.45%), aCLs in 2 (9.09%, anti-dsDNA in 4（18.18%, and anti-CCP in 4（18.18%） patients signified the prevalence of autoantibodies probably due to a nonspecific hyperimmune response [26].

In our study, IE patients with ANCA, compared with AAV patients, presented a younger age while a shorter disease course at onset. Considering multiple organ involvement, typical symptoms of AAV including ENT signs, pulmonary lesions, and peripheral neuropathy were less observed in ANCA-positive IE patients. In addition, Osler's nodes and Janeway lesions typical for IE might help identify IE patients from AAV patients. The results also demonstrated that manifestations such as lower platelet count, lower C3, and

**Table 3** Laboratory features of ANCA-positive IE patients versus AVV patients

Variables	Patients		p value
	ANCA-positive IE patients (n=22)	AAV patients (n=36)	
WBC, ($\times 10^9$/L)	9.23 ± 3.87	10.85 ± 5.32	0.03*
Anemia	18 (81.81)	22 (71.43)	0.53
Platelet count, ($\times 10^9$/L)	228.00 ± 109.6	323.80 ± 136.50	0.04*
Hypoalbuminemia, (< 35 g/L)	16 (72.73)	26 (72.22)	> 0.99
ALT, (U/L)	31.11 ± 28.83	24.33 ± 20.60	0.52
AST, (U/L)	35.11 ± 25.92	23.94 ± 13.67	0.24
ESR, (mm)	64.73 ± 35.98	59.31 ± 39.66	0.59
CRP, (mg/L)	51.13 ± 56.43	62.83 ± 58.81	0.46
Serum Cr, (μmol/L)	88.00 ± 20.21	101.00 ± 12.55	0.59
Serum Cr > 150 μmol/L	1 (4.55)	5 (13.89)	0.39
Positive blood culture	14 (63.64)	2 (5.56)	< 0.01**
Autoantibodies			
Anti-PR3	22 (100.00)	12 (33.33)	< 0.01**
Anti-PR3 titer	53.38 ± 39.76	103.20 ± 43.79	0.05*
Anti-MPO	0	29 (80.55)	< 0.01**
Anti-PR3 and MPO	0	5 (13.89)	< 0.01**
Positive RF	10 (45.45)	22 (61.11)	0.29
ANA-positive	2 (9.09)	8 (22.22)	0.29
ENA-positive	0	4 (11.11)	0.29
LAC-positive	2 (9.09)	5 (13.89)	0.70
ACLs-positive	2 (9.09)	4 (11.11)	> 0.99
Anti-β2GPI-positive	1 (4.55)	1 (2.78)	> 0.99
Anti-dsDNA-positive	4 (18.18)	1 (2.78)	0.06
Anti-CCP-positive	4 (18.18)	2 (5.56)	0.19
Low C3, (< 9 0 g/L)	5 (22.73)	21 (58.33)	0.01*
Low C4, (< 10 g/L)	1 (4.55)	4 (11.11)	0.64

All continuous variables and numbers are represented as the mean ± standard deviation. All categorical variables are presented as n (percent). *p value of less than 0.05 was considered statistically significant. **p value less than 0.01. *WBC*, white blood cell; *ALT*, alanine transaminase; *AST*, aspartate transaminase; *ESR*, erythrocyte sedimentation rate; *CRP*, C-reactive protein; *Cr*, creatinine; *Anti-PR3*, anti-proteinase-3; *Anti-MPO*, anti-myeloperoxidase; *RF*, rheumatoid factor; *ANA*, anti-nuclear antibody; *ENA*, extractable nuclear antigen; *LAC*, lupus anticoagulant; *ACL*, anticardiolipin antibodies; *Anti-β2GPI*, anti-β2-glycoprotein I antibodies; *Anti-dsDNA*, anti-double stranded DNA antibody; *Anti-CCP*, anti-cyclic citrullinated peptide; *C3*, complement 3; *C4*, complement 4

positive blood culture might be considered useful clinical parameters in ANCA-positive IE. Moreover, Chirinos JA et al. [15] have noticed that splenomegaly or hepatosplenomegaly was more often seen in ANCA-positive IE patients. By contrast, we found splenomegaly in 6 patients out of 22 patients (27.27%). Comparing ANCA-positive IE patients with AAV patients, they possess similarities as nonspecific symptoms (fatigue, fever, arthralgia, myalgia, and serositis), muti-organ involvement, and positive autoantibodies. By contrast, these features raised high suspicion of IE: young age at diagnosis, short disease duration, heart valve involvement, cerebral hemorrhage, splenomegaly, Janeway lesions, Osler's nodes positive blood culture, anti-PR3 positive, and highanti-PR3 titer. ANCA-positive IE tends to have high anti-PR3 titer, and both IE and AAV patients only carry low ANA titer. However, renal, ENT, and pulmonary involvement and peripheral neuropathy were much more commonly seen in AAV patients. Our study is the first study to summarize the similarities and differences between ANCA-positive IE and AAV. The criteria to differentiate ANCA-positive IE patients from AAV patients were summarized in supplementary Table 1. Previously, a poorer prognosis in IE patients with positive ANCA than those with negative ANCA was reported [27]. Here, it showed no significant difference of survival rate between ANCA-positive IE patients and AAV patients. Instead, a higher frequency cardiac surgery was found in ANCA-positive IE patients.
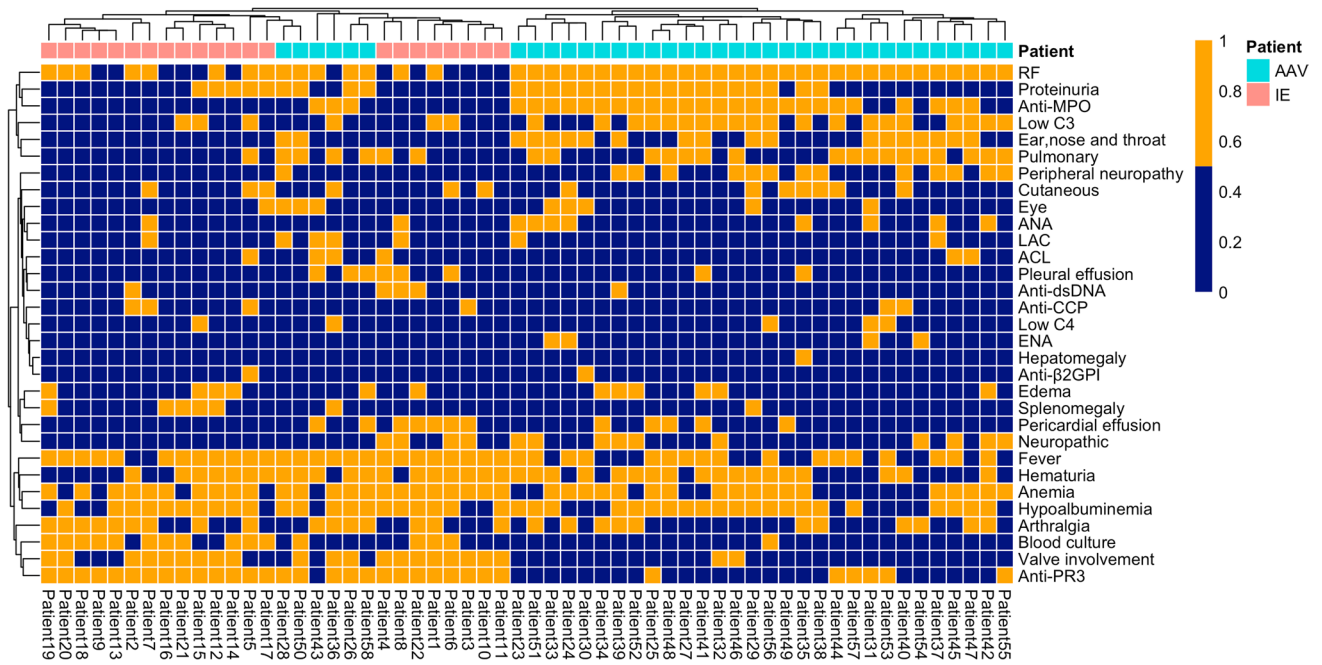
Fig. 2 Cluster of clinical of and laboratory manifestations of ANCA-positive IE patients and AAV patients
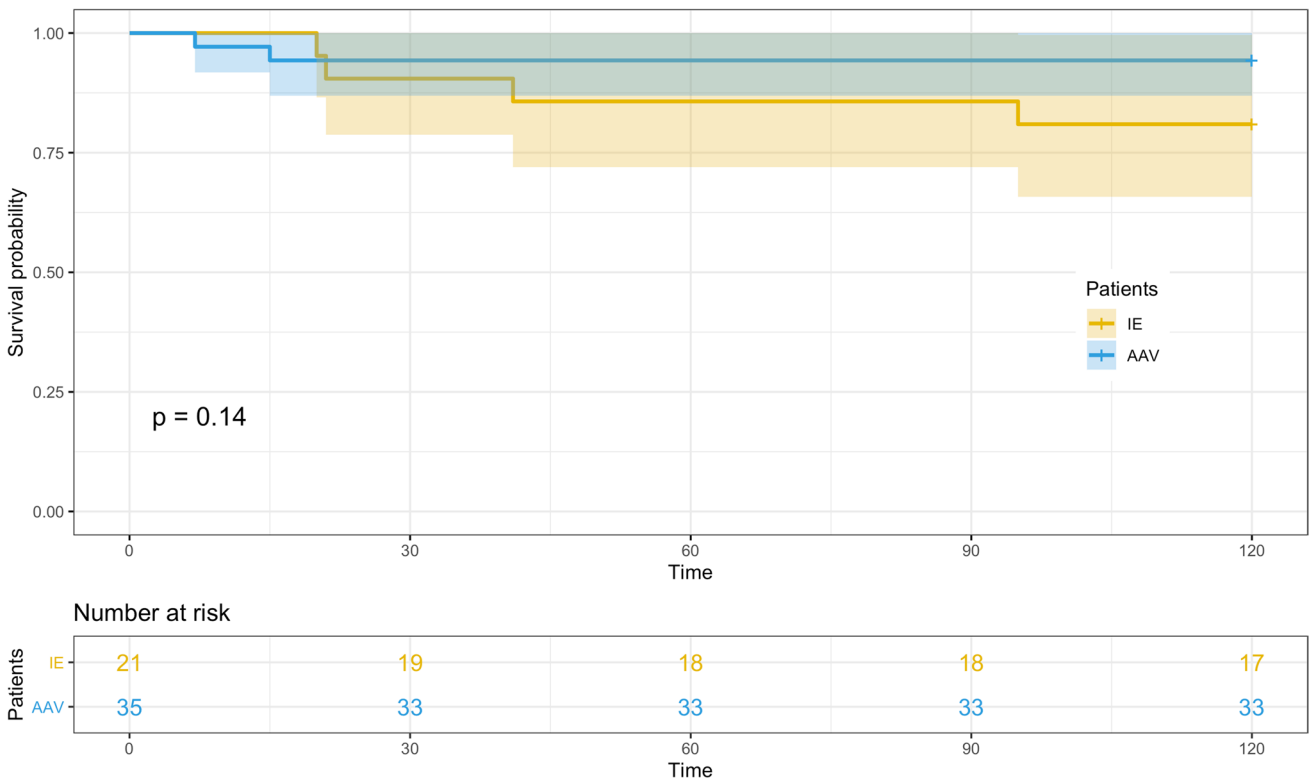
Fig. 3 Kaplan–Meier survival curves

The presence of ANCA in IE patients has been previously reported [3–7]. However, many questions about the presentations of abnormalities in ANCA-positive IE patients remain unsolved. J. J Yang et al. [28] have described the expression of ANCA antigens on the surface of apoptotic neutrophils instead of primed viable neutrophils, suggesting that the bacterial infection could play a role in producing apoptotic blebs as a trigger of autoimmune reaction. The mechanism behind this phenomenon requests further studies.

In conclusion, our study urges the early interpreting of differential diagnosis of IE in ANCA-positive patients and supports the claim that ANCA-positive IE patients and AAV patients do not share the same clinical spectrum. It is of critical importance to identify and thoroughly rule out of IE in ANCA-positive patients. However, the pathogenic mechanisms remain unknown and require further exploration.

Data availability No additional data available.

Compliance with ethical standards

Ethics approval and consent to participate The study was performed in accordance with the ethical standard of the Declaration of Helsinki and approved by the Committee of Ruijin Hospital (ID: 2016–62), Shanghai, China.

Disclosures None.

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