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BRIEF REPORT

Subtypes in eosinophilic granulomatosis with polyangiitis classified according to rheumatoid factor

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Abstract

To investigate the relevance of RF in patients with EGPA, we reviewed consecutive patients who were newly diagnosed with EGPA from August 1998 to February 2019 in Keio University Hospital with RF titer at diagnosis available. We divided the patients according to the median level of RF titer of 75 IU/mL and compared clinical features between the two groups. Among 16 patients identified, 8 patients were in the RF high group and the other 8 patients were in the RF low group. All patients in the high RF group were negative for MPO-ANCA, whereas all in the low RF group was positive for MPO-ANCA with a mean titer of 103 IU/mL. The eosinophil count at diagnosis was significantly higher in the RF high group than the RF low group (20001/μL vs 5144/μL, $p < 0.01$). Gastrointestinal lesion was significantly more frequent in the RF high group, and parenchymal organ lesions, such as heart and renal organ involvement, were frequent in the RF low group. With principal component analysis, RF high and low groups were clearly divided by the combination of eosinophil count, MPO-ANCA titer, gastrointestinal lesions, musculoskeletal symptoms, and disease activity score. Those results suggest EGPA can be divided into two groups in association with RF.

Key Points
- *Our study showed that patients with EGPA can be separated into two groups according to RF titer.*
- *The two subtypes reflect different underlying pathogenesis in EGPA, and the optimal treatment for them may be different.*

Keywords Antineutrophil cytoplasmic auto-antibodies-associated vasculitis · Biomarker · Eosinophilic granulomatosis with polyangiitis · Rheumatoid factor

Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA), one of the antineutrophil cytoplasmic auto-antibodies (ANCA)-associated vasculitis, is a rare systemic vasculitis with allergic features such as asthma and eosinophilia, predominantly affecting small-sized vessels. The pathogenesis of EGPA is considered to be different from other two ANCA-associated vasculitis, microscopic polyangiitis, and granulomatosis with polyangiitis, because EGPA has unique characteristics of remarkable eosinophilia, elevated levels of Th2-cytokines [1], and lower positivity for myeloperoxidase (MPO)-ANCA [2]. The symptoms of EGPA are related to both its two sides of pathogenesis, small vessel inflammation, and eosinophilia similar to hyper-eosinophilic syndrome (HES).

Rheumatoid factor (RF) is an auto-antibody for self Fc portion of immunoglobulin (Ig) G and originally a biomarker for diagnosis of rheumatoid arthritis. However, RF positivity is also known to be as high as 35–45% in patients with EGPA [3], implying immunological role of RF in EGPA. This study aimed to identify clinical significance of RF in patients with EGPA.

Material and methods

Patients

Consecutive patients who had been newly diagnosed with EGPA from August 1998 to February 2019 in Keio

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Table 1 Characteristics of all patients ($n = 16$)

Characteristics	All $N = 16$	RF high titer $N = 8$	RF low titer $N = 8$	p value
At diagnosis				
Demographic				
Age, years	61.8 (15.0)	63.5 (19.2)	55.6 (11.4)	1.00
Female, *N* (%)	12 (75)	6 (75)	6 (75)	0.72
Vasculitis pathologically proved, *N* (%)	3/6 (50)	1/4 (25)	2/2 (100)	0.40
Laboratory and clinical scores				
MPO-ANCA positivity, *N* (%)	8 (50)	0 (0)	8 (100)	< 0.01
MPO-ANCA titer, IU/mL	103.3 (81.8)	0 (0)	103.3 (81.8)	< 0.01
RF positivity, *N* (%)	12 (75)	8 (100)	4 (50)	0.04
RF titer, IU/mL	108.2 (115.2)	194.4 (104.6)	22.0 (23.1)	< 0.01
Anti-CCP antibody positivity, *N* (%)	0/9 (0)	0/6 (0)	0/3 (0)	1.00
White blood cell, /μL	21206 (10888)	27775 (10456)	13700 (5081)	0.01
Lymphocyte, /μL	1561 (516)	1414 (467)	1709 (551)	0.28
Eosinophil count, /μL	12572 (10763)	20001 (10680)	5144 (2838)	< 0.01
CRP, mg/dL	4.46 (4.52)	6.34 (5.4)	2.58 (2.57)	0.16
Albumin, g/dL	3.3 (0.6)	3.1 (0.6)	3.5 (0.6)	0.23
Creatinine, mg/dL	0.62 (0.16)	0.63 (0.19)	0.62 (0.14)	0.72
IgG, mg/dL	1660 (573)	1925 (649)	1395 (418)	0.13
IgM, mg/dL	107 (54)	98 (36)	117 (71)	0.56
IgA, mg/dL	216 (78)	233 (80)	196 (76)	0.52
IgE, IU/mL	1642 (2306)	2259 (3203)	1025 (523)	0.65
C3, mg/dL	125.1 (21.3)	121.1 (25.5)	129.0 (17.1)	0.48
C4, mg/dL	33.5 (10.1)	34.2 (11.8)	32.7 (8.7)	0.77
BVAS	20.0 (8.5)	21.0 (9.4)	19.0 (7.9)	0.96
Five Factor Score ≥ 1, *N* (%)	7 (44)	4 (50)	3 (38)	0.62
Clinical course				
Treatment				
Initial dose of PSL, mg	53.4 (11.1)	51.2 (10.6)	55.0 (12.0)	1.00
Steroid pulse, *N* (%)	3 (19)	2 (25)	1 (13)	0.50
IVCY, *N* (%)	7 (44)	3 (38)	4 (50)	0.50
Methotrexate, *N* (%)	1 (6)	0 (0)	1 (13)	0.50
Azathiopurine, *N* (%)	8 (50)	4 (50)	4 (50)	0.69
IVIg, *N* (%)	6 (38)	4 (50)	2 (25)	0.30
Prognosis				
Observation period, years	6.1 (4.6)	7.2 (4.9)	4.5 (4.1)	0.44
Flare rates, *N* (%)	3 (19)	1 (13)	2 (25)	0.61

ANCA, antineutrophil cytoplasmic antibody; *BVAS*, Birmingham Vasculitis Activity Score; *C3*, complement component 3; *C4*, complement component 4; *CCP*, cyclic citrullinated peptide; *CRP*, C-reactive protein; *IVCY*, intravenous cyclophosphamide; *IVIg*, intravenous immunoglobulin; *MPO*, myeloperoxidase; *PSL*, prednisolone; *RF*, rheumatoid factor

University Hospital were reviewed. Their diagnosis was all based on Watts's algorithm [4], with all patients except one fulfilling 1990 American College of Rheumatology (ACR) classification criteria [5]. This study was approved by the ethics committee (ETHICS COMMITTEE of Keio University School of Medicine, approval number: 20110136). The requirement for written informed consent from the patients was waived according to the regulations for retrospective cohort design study without any samples taken in Japan.

Data collection

Patients' clinical and serological information were collected from their medical records. The patients were divided into two groups

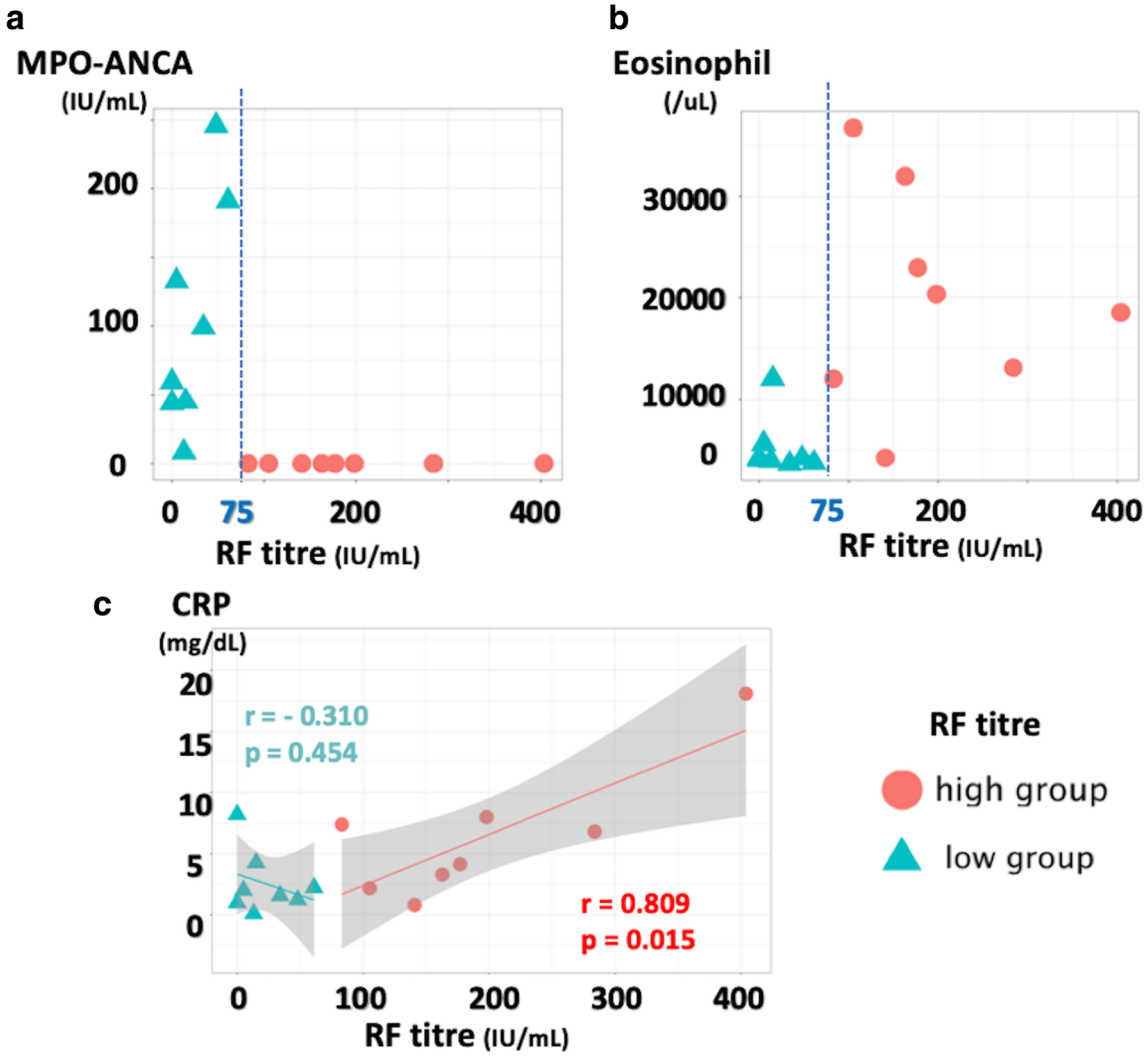

Fig. 1 A and B, Scatter plot of RF-titer and MPO-ANCA titer (**a**), eosinophil count (**b**) of all patients. **c**, F titer was significantly correlated with CRP level in only RF high titer group (Pearson correlation test)

ANCA, antineutrophil cytoplasmic antibody; CRP, C-reactive protein; RF, rheumatoid factor: MPO, myeloperoxidase

according to the median level of RF titer and compared regarding symptoms, involved organs, and serological features. The Birmingham vasculitis activity score (BVAS) was calculated according to the formula [6]. The RF and MPO-ANCA titers were measured by latex agglutination test (LZ test, Eiken Kagaku, Tokyo, Japan) and by chemiluminescent enzyme immunoassay (the STACIA MEBLux test, Medical & Biological Laboratories, Aichi, Japan), respectively.

Statistical analysis

Continuous values are shown as mean ± standard deviation (SD). Differences between the groups were analyzed using the Mann-Whitney *U* test for continuous variables, and chi-square or Fisher's exact test for categorical data. For easy visualization of the correlation among clinical variables and how those involve in discrimination of patients, we used hierarchical cluster analysis and primary component analysis (PCA). These analyses were performed using normalized clinical variables with mean and variance to arrange all variables in same level. In hierarchical cluster analysis, the ward D2 was used as a clustering method [7]. We used the formula for the Euclidean distance to measure the distance between each variable. In PCA, we selected two components with highest contribution in maximizing the variance of patients explained by clinical information. The RStudio statistical package (v. 1.1.463) was used for analysis.

Results

Clinical and serological characteristics

Among 22 patients with EGPA identified, 6 patients were excluded due to lack of information about RF titer at diagnosis,

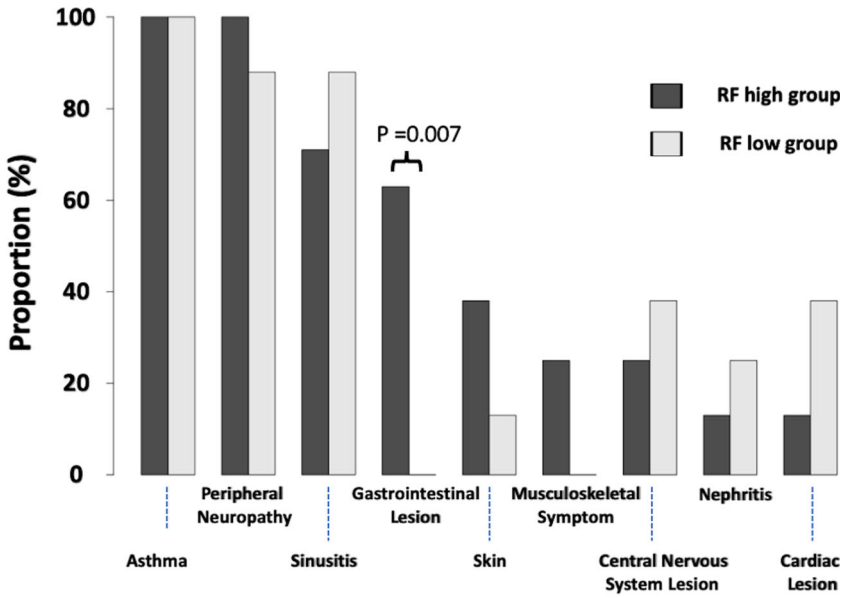

Fig. 2 Distribution of organ lesion. RF, rheumatoid factor

resulting in 16 patients enrolled. Their clinical characteristics are shown in Table 1. The mean age was 61.8 years, and 75% were female. Among them, 8 patients were in the RF high group (RF ≥ 75 IU/mL) and 8 patients were in the RF low group (RF < 75 IU/mL). A scatter plot of the titers of RF and MPO-ANCA (Fig. 1A) and the count of eosinophil (Fig. 1B) confirmed that those patients could be clearly separated with the median RF level of 75 IU/L. Those two groups had distinct characteristics that the RF high group showed negative MPO-ANCA and extreme eosinophilia while the RF low group showed positive MPO-ANCA and mild eosinophilia (mean eosinophil count, 20001/μL vs 5144/μL, $p = 0.001$, Table 1). RF titers were significantly correlated with CRP levels in the RF high group ($r = 0.809$, $p = 0.015$, Fig. 1C) but not in the RF low group.

The distribution of organ lesions was also different between the two groups. Musculoskeletal, gastrointestinal, and skin lesions were more frequent in the RF high group (Fig. 2). Particularly, gastrointestinal lesions, ischemic colitis, and eosinophilic enteritis were observed in 60% of the RF high group while none in the RF low group. On the other hand, parenchymal organ involvement, such as the heart and kidneys, tended to be frequent in the RF low group although it was not significant.

Hierarchical cluster analysis and principal component analysis based on clinical characteristics

Hierarchical cluster analysis based on clinical characteristics revealed that the cluster with gastrointestinal lesion, the eosinophil count, and the levels of CRP and RF titer were enriched in the RF high titer group (Fig. 3A).

To confirm whether this grouping is appropriate, we separated all patients by these clinical and laboratory parameters by using two-dimensional PCA. First, we performed pre-PCA to select variables which maximize the variance of patients effectively. Then, variables above average, red-dotted lines in Fig. 3B, in either dimension 1 or 2, were used for final-PCA except RF titer. Figure 3C shows the final-PCA, suggesting the two subgroups divided by the median RF titer was very much alike with the separation by clinical and laboratory variables without RF titer. The largest red round and blue triangle indicated the central location of each RF high and low group, respectively. The patients in the RF high group localize mainly in quadrant with positive value of both dimensions, which was associated with high BVAS, eosinophilia, and the presence of gastrointestinal lesions and musculoskeletal symptoms, whereas the patients in the RF low group exists in quadrant with negative value of both dimensions, which associated with high MPO-ANCA titer.

Discussion

Our study showed that patients with EGPA can be separated into two groups according to RF with distinct clinical and laboratory characteristics. Those two separable groups suggest that EGPA has two clinical subtypes that reflect different underlying pathogenic mechanism in EGPA. One is classically defined vasculitis with mild eosinophilia, with high MPO-ANCA and parenchymal organ involvement, and the other is rather resembling to HES, showing remarkable hypereosinophilia with lack of ANCA involving mucocutaneous and musculoskeletal lesions (Fig. 4).

The relationship of MPO-ANCA positivity and symptoms related to vasculitis in EGPA has been well known. Organ involvements in positive ANCA are mainly associated with histopathological vasculitis patterns such as nephritis, whereas patients negative for ANCA are more inclined to non-vasculitic

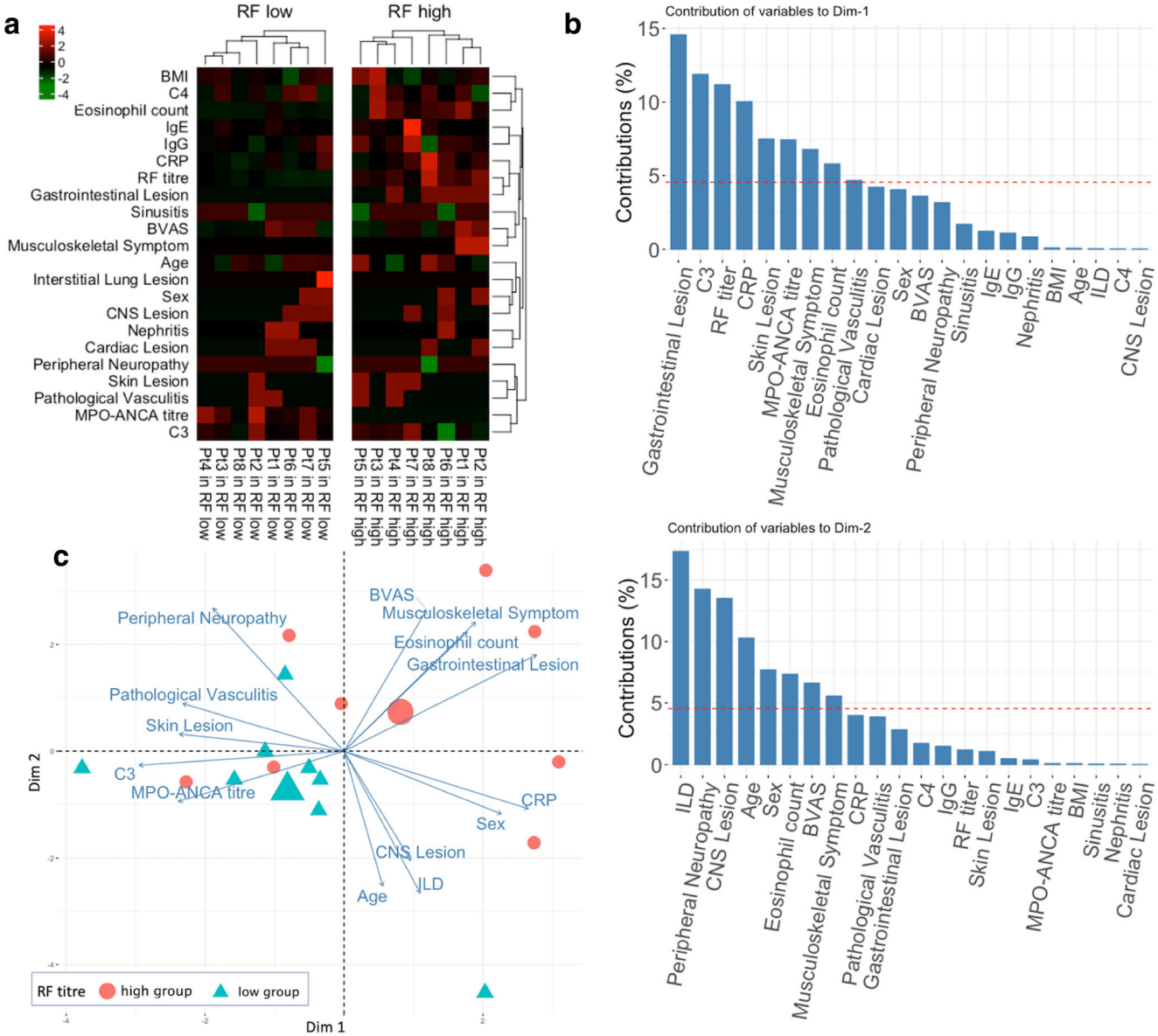

Fig. 3 **a**, Hierarchical clustering analysis by clinical information. Columns and rows show patients and clinical variables respectively. **b**, Contribution rate of variables in dimension 1 (above) and 2 (below) of pre-PCA. Red-dotted lines shows average of contribution rate, respectively. C, Final-PCA by selected variables after pre-PCA. ANCA, antineutrophil cytoplasmic antibody; BMI, body mass index; BVAS, Birmingham Vasculitis Activity Score; CNS, central nervous lesion; CRP, C-reactive protein; ILD, interstitial lung disease; PCA, principal component analysis; Pt, patient; RF, rheumatoid factor; MPO, myeloperoxidase

organ involvement such as general symptom [8, 9]. Our findings were consistent with those previous study results that MPO-ANCA positivity is associated with mesenchymal organ involvement.

The interesting point of our study is that those two subgroups can be separated by RF titer. The combination of high titer RF with remarkable eosinophilia without MPO-ANCA in the RF high group might reflect the over-expression of IL-5, the key cytokine for development, proliferation and growth of eosinophil [1]. A study reported that IL-5 was essential promoting factor of splenic B-1a cell that produces IgM-RF in experimental mixed cryoglobulinemia model mice [10]. Moreover, eosinophil count in patients with asthma and chronic eosinophilic pneumonia, which accompany upregulation of IL-5 and respond well to anti-IL-5 therapy [11–13], was also positively associated with RF titer [14, 15]. Furthermore, in the sub-analysis of phase 3 trial for anti-IL-5 therapy in EGPA, patients with higher eosinophil count at baseline showed better response to the agent [16]. The optimal treatment for the subgroup of EGPA (the RF high group with remarkable hypereosinophilia without ANCA) in this study may be anti-IL-5 drug rather than high-dose glucocorticoids.

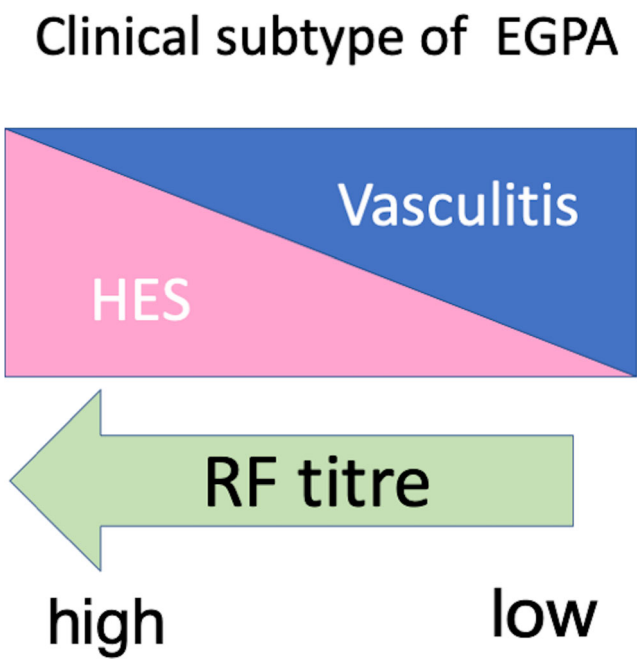

Fig. 4 Conception of clinical subclasses in EGPA. EGPA, Eosinophilic granulomatosis with polyangiitis; HES, hyper eosinophilic syndrome; RF, rheumatoid factor

Sinico RA et al. demonstrated that eosinophilic infiltration to perivascular lesions was significantly more frequent in ANCA negative patients with EGPA, whereas necrotizing vasculitic pattern was more common in ANCA positive patients [8]. In addition, patients with negative ANCA in the study Sokolowska BM et al. reported had higher blood eosinophil counts and more severe asthma compared with patients with positive ANCA [17]. Our results are consistent with those results in that patients with EGPA could be divided according to RF titers into two groups; one with positive MPO-ANCA with classical vasculitis and the other with negative MPO-ANCA with hyper eosinocytosis presenting with HES-like symptoms. We expect that patients in the RF high group would have tissue infiltration of eosinophils rather than extensive vessel wall inflammation. Indeed, 2 of 2 (100%) patients who underwent a biopsy in the RF low group showed apparent vasculitic pathology, whereas only 1 of 4 (25%) patients in the RF high group proved to have vasculitic findings. However, the small sample size with the smaller number of patients with pathological findings available hampered a further analysis. Future studies with a larger number of patients are needed to clarify this point.

Recently, Moon JS et al. also investigated the association RF positivity and organ involvement in ANCA-associated vasculitis including EGPA [3]. It is interesting that there are some differences and similarities between their study and our current one. First, the positivity of MPO-ANCA is 15.8% in the RF (−) group in Moon's study while 100% in the RF low group in our study. While they divided patients by RF negativity/positivity, we used RF titer (not simple positivity) because very low RF titer could have been non-specific reaction. It could make difference in MPO-ANCA positivity and relevant organ involvements. Second, genetic background might lead to the difference. ANCA status is reported to be with associated with genetic factors [18]. Third, there might be some skew in both studies because the number of patients was small in both studies. In contrast, the relationship of RF positivity to cutaneous lesions is common. The relationship of RF high titer with hypereosinophilia, one of the interesting points of our study, was not mentioned in Moon's study. Considering a critical role of eosinophil in the pathogenesis of EGPA, our study implies new concept, leading to next research agenda such as whether RF titer also could be a new predictive marker for efficacy of IL-5 inhibitor, and whether RF has immunological function in allergic status. Our study only implies the possibility of two subtypes of EGPA which can be classified by co-existing RF in EGPA. The sample size was too small to draw a conclusion, which could generate bias and possible deviation by chance. But we believe it warrants cooperated cohort studies with a large sample size and also basic researches.

Compliance with ethical standards

Disclosures YK, JI and, YO have nothing to be declared. TT has consultant fees from GlaxoSmithKline.

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