Biological therapy improves myocarditis and disease activity in eosinophilic granulomatosis with polyangiitis patients

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Abstract

Background

Cardiac insufficiency is a major cause of mortality in eosinophilic granulomatosis with polyangiitis (EGPA). Despite the dosages-related cardiotoxicity, cyclophosphamide is usually prescribed to induce disease remission in the presence of myocarditis with heart involvement. There is an imperative need of novel medications to efficiently control disease activity and spare the use of cyclophosphamide.

Methods

A retrospective study was carried out in hospitalized EGPA patients from January 1, 2008 to December 31, 2019, focusing on the use of biologics including benralizumab (BEN, anti-IL-5 receptor), mepolizumab (MEP, anti-IL-5), omalizumab (OMA, anti-IgE) and rituximab (RTX, anti-CD20).

Results

Sixteen admitted patients, 8 females aged 10 to 70 years (40.4 ± 15.5), had higher disease activities (Birmingham Vasculitis Activity Score 16 to 39, 26.8 ± 6.9), poorer prognostic factors (five-factor score 1 [3, 4], 1.4 ± 0.5) and elevated eosinophil counts (2,314 to 26,781/µL, 11,108 ± 7,060). BEN, MEP, OMA and RTX were prescribed in one, two, one and 6 patients, respectively. Ten patients (63%) had myocarditis with impaired left ventricle ejection fraction and cardiac arrhythmia, and 7 received biological therapy without a combined use of cyclophosphamide. One patient obtained MEP with a 100 mg quadri-weekly × 13 regimen at induction for disease relapse. Six patients acquired RTX with a 375 mg/m² weekly × 4 regimen at induction for refractory activity or relapsing disease, or plus a yearly maintenance schedule in 5. All patients received serial cardiac magnetic resonance imaging, transthoracic echocardiography and 24-hour Holter monitor to evaluate the therapeutic responses in heart involvement. After biological therapy, there were improved cardiac dysfunction, lower eosinophil counts and clinical remission (4 complete, 3 partial) with a relapse-free follow-up (13 to 61 months, 39.1 ± 16.0) after induction.

Conclusions

In this single-center retrospective study, we observed improved cardiac dysfunction and disease activity after biological therapy in EGPA patients with myocarditis.

Background

Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare disease occurring exclusively in asthmatic patients [1], and classified as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) [2]. Among 3 AAV-related disorders, cardiac involvement is most commonly observed in EGPA patients with a frequency ranging from 16 to 92% [3, 4]. The therapy can be adapted for the presence of poor prognostic factors, i.e., five-factor score (FFS) [5]. In the existence of major organ damage with lethal manifestations like cardiac insufficiency, in addition to corticosteroids (CS), cyclophosphamide (CYC) is usually prescribed to induce disease remission [6]. Nevertheless, the majority of deaths in EGPA are caused by disease activity with heart involvement as a leading cause in spite of under combined CS and CYC therapy [4, 6, 7]. Furthermore, given the significant toxicity of CYC, it is generally acknowledged not to exceed 10 to 15 gram of exposure in EGPA patients due to the dosages-related cardiotoxicity [7]. Indeed, there is an imperative need of novel therapeutics to efficiently control disease activity and spare the use of CS and CYC [8].

Notably, eosinophils play a central role in the EGPA pathogenesis with eosinophilia of blood and tissue as the disease hallmark, and IL-5, a Th2 cytokine, is recognized as the key mediator in the development and maintenance of eosinophilia [1, 8, 9]. Through the autoantigen presentation and costimulatory signaling to Th2 cells, B cells can participate in the EGPA pathogenesis by inducing the release of IL-5, resulting in activation, maturation, survival and recruitment of eosinophils [1, 8, 10]. Current evidence supports the use of mepolizumab (MEP), an IL-5 monoclonal antibody (mAb), for induction treatment of refractory or relapsing EGPA with greater eosinophilia [1, 11]. Despite the ongoing clinical trials exploring the efficacy of benralizumab (BEN), an IL-5 receptor mAb, in EGPA, this biologics has acquired the orphan drug designation from the US regulator [1, 12]. Therapeutic responses to rituximab (RTX), a B cell-depleting mAb, have been observed in EGPA, and it has been recommended at induction for refractory activity or relapsing disease with a potential benefit in ANCA negativity [13]. Interestingly, MEP has been suggested to treat eosinophilic myocarditis in hypereosinophilic syndrome (HES) [14], and therapeutic effects of RTX on autoimmune myocarditis have been observed in microscopic polyangiitis and systemic lupus erythematosus [15, 16].

To identify myocarditis in EGPA, instead of investigating stable victims without admission, we analyzed hospitalized patients with disease activity and focused on the use of biologics in this retrospective study.

Methods

Patients enrollment

Under the approval of the Institutional Review Board, patients admitted to Departments of Internal Medicine and Pediatrics and fulfilling 1990 American College of Rheumatology (ACR) criteria for the EGPA classification [17], were analyzed from January 1, 2008 to December 31, 2019. Myocarditis was diagnosed according to the following criteria: (1) presenting symptoms consistent with heart failure, (2) raised concentrations of cardiac biomarkers, and (3) new or worsening changes including impaired left ventricle ejection fraction (LVEF, mild 46 to 55%, moderate 30 to 45%, severe below 30%) or plus wall motion...
abnormality on transthoracic echocardiography (ECC) or cardiac magnetic resonance imaging (cMRI) [15, 16, 18]. The additional endocardial involvement, i.e., endomyocarditis, was detected by cMRI. Cardiac rhythm was examined by 24-hour Holter monitor. Exclusion criterion was coronary artery disease with characterized findings in coronary angiography or cMRI, viral myocarditis with a confirmed history of infection, or preexisting heart diseases under medical therapy [15, 16].

Data Collection

Demographical, clinical, laboratory, imaging and pathological data were analyzed, including age/sex, EGPA manifestations, disease activity (Birmingham Vasculitis Activity Score, BVAS) [19], prognosis assessment [5], myocarditis symptoms [15, 16], New York Heart Association Functional Classification (NYHAFC), ANCA titers (immunofluorescence, enzyme-linked immunosorbent assay), eosinophil counts, C-reactive protein (CRP) values, IgE levels, circulating B-cell numbers (CD19-positive lymphocytes), cardiac biomarker concentrations (cardiac troponin I, creatine kinase-MB, N-terminal pro-brain natriuretic peptide), cardiac image and rhythm findings, and pathological results. There was a comprehensive review in medication profiles including CS, immunosuppressive agents, biologics and cardiac medications with antiarrhythmic drugs for cardiac dysrhythmia and cardiac supportive agents (CSA) (angiotensin converting enzyme inhibitor, angiotensin-receptor blocker, β blocker, diuretic, inotrope) for heart failure. A complete remission was defined as the absence of disease activity corresponding to zero BVAS, and a partial remission was a reduction of no less than 50% in BVAS as compared with the baseline scores.

Statistical analysis

Data was expressed as the mean and standard deviation. Numerical data between two groups were compared by the Mann-Whitney test. BVAS, CRP levels and eosinophil counts before and after the biologic therapy were calculated by the Wilcoxon signed rank test. P value less than 0.05 was considered significant in this study.

Results

Characteristics of admitted patients

In Table 1, 16 admitted patients fulfilled 5 or 6 items (5.4 ± 0.5) of the ACR classification criteria [17], and had histopathological findings of tissue eosinophilia or plus vasculitis of small- to medium-sized vessels (Fig. 1). There were 8 females aged 10 to 70 years (40.4 ± 15.5), with positive ANCA against myeloperoxidase in 5 (31%), initial BVAS 16 to 39 (26.8 ± 6.9) and FFS 1 or 2 (1.4 ± 0.5). Laboratory parameters at the disease onset were eosinophil percentages 21 to 79% (46.6 ± 15.2) with total eosinophil counts 2,314 to 26,781/µL (11,108 ± 7,060), CRP values 19.1 to 183.3 mg/L (79.9 ± 51.6) and IgE levels 123 to 4,000 IU/mL (1,041 ± 1,002). Despite the challenge in differentiating idiopathic HES from EGPA due to eosinophilic tissue infiltration in both disorders, the presence of asthma is a characteristic diagnostic feature of EGPA [6, 7]. All enrolled patients had recurrent asthmatic attacks. Involvement of lung parenchymal, peripheral nerve system, skin, heart, sinus, joint, muscle, kidney, gastrointestinal tract and central nervous system was identified in 14, 14, 13, 10, 9, 7, 6, 4, 3 and one, respectively. Patients with cardiac manifestations had higher initial eosinophil counts than those without heart involvement (12,731 ± 6,346 versus 6,737 ± 4,439/µL, P = 0.056). In this series, two (cases no. 10 and 15) expired due to disease activity, and fourteen survived with complete remission in 5 and partial remission in 9. For the biologics use, 6 received RTX under 375 mg/m² weekly intravenous infusion (cases no. 2 to 7) due to refractory activity or relapsing disease. Two obtained MEP under 100 mg quadri-weekly subcutaneous injection (cases no. 1 and 13) due to disease relapse with a more than 90% inhibition (92.9 ± 1.3) of the baseline eosinophil counts. Case no. 16 acquired OMA under 150 mg bi-weekly subcutaneous injection due to relapsing disease at the age of 12 [20], and Ben with 30 mg quadri-weekly subcutaneous injection at the age of 16 with a 100% inhibition of the baseline eosinophil counts due to disease relapse [12]. For those receiving biologics, there were complete remission in 5 (cases no. 1, 2, 3, 4 and 16) and partial remission in 4 (cases no. 5, 6, 7 and 13). In particular, CS and immunosuppressants were not prescribed in cases no. 1 and 16 after biologic therapy.
Table 1

| No. | Age | Sex | Fever | Skin | Sinus | Joint | Muscle | Lung | Heart | GI | Renal | PNS | CNS | FFS | BVAS | Pathological findings | Eosin /µL | ANCA status |
|-----|-----|-----|-------|------|-------|-------|--------|------|-------|----|-------|-----|-----|-----|------|-----------------------|---------|-------------|
| 1   | 39F | Nil | Yes   | Yes  | Nil   | Nil   | Yes    | Yes  | Yes   | Nil| Nil   | Yes | Nil | 1   | 22  | Eosinophilia           | 10,140  | Posit       |
| 2   | 45M | Yes | Yes   | Yes  | Nil   | Nil   | Yes    | Yes  | Yes   | Nil| Yes   | Yes | Nil | 1   | 31  | Eosinophilia           | 6,090   | Nega        |
| 3   | 30M | Nil | Yes   | Yes  | Nil   | Yes   | Yes    | Yes  | Yes   | Yes| Nil   | Yes | Nil | 2   | 36  | Eosinophilia           | 11,567  | Nega        |
| 4   | 45F | Yes | Nil   | Yes  | Nil   | Yes   | Yes    | Yes  | Yes   | Nil| Yes   | Yes | Nil | 2   | 29  | Eosinophilia           | 16,947  | Nega        |
| 5   | 36M | Yes | Yes   | Yes  | Yes   | Yes   | Yes    | Yes  | Yes   | Nil| Nil   | Yes | Nil | 2   | 37  | Eosinophilia           | 17,424  | Posit       |
| 6   | 47F | Yes | Nil   | Yes  | Nil   | Nil   | Yes    | Yes  | Nil   | Nil| Nil   | Yes | Nil | 1   | 30  | Eosinophilia           | 26,781  | Nega        |
| 7   | 55F | Yes | Yes   | Yes  | Yes   | Yes   | Yes    | Yes  | Yes   | Nil| Nil   | Yes | Nil | 2   | 28  | Eosinophilia           | 5,806   | Nega        |
| 8   | 40M | Yes | Yes   | Yes  | Nil   | Nil   | Yes    | Yes  | Yes   | Nil| Nil   | Yes | Nil | 1   | 24  | Eosinophilia           | 23,392  | Nega        |
| 9   | 37F | Nil | Yes   | Yes  | Yes   | Yes   | Yes    | Yes  | Nil   | Nil| Nil   | Yes | Nil | 1   | 19  | Eosinophilia           | 11,139  | Posit       |
| 10  | 19F | Yes | Yes   | Nil  | Yes   | Yes   | Yes    | Yes  | Yes   | Yes| Yes   | Yes | Yes | 2   | 26  | Eosinophilia           | 8,019   | Nega        |
| 11  | 47M | Yes | Nil   | Nil  | Yes   | Yes   | Yes    | Yes  | Yes   | Nil| Yes   | Yes | Nil | 1   | 30  | Eosinophilia           | 9,750   | Posit       |
| 12  | 29F | Yes | Yes   | Nil  | Nil   | Nil   | Yes    | Yes  | Nil   | Nil| Nil   | Yes | Nil | 1   | 21  | Eosinophilia           | 12,816  | Nega        |
| 13  | 66M | Nil | Nil   | Yes  | Yes   | Yes   | Yes    | Yes  | Yes   | Nil| Nil   | Yes | Nil | 1   | 20  | Eosinophilia           | 2,541   | Nega        |
| 14  | 70M | Nil | Yes   | Yes  | Nil   | Nil   | Nil    | Nil  | Nil   | Nil| Nil   | Yes | Nil | 1   | 21  | Eosinophilia           | 3,744   | Nega        |
| 15  | 32M | Yes | Yes   | Nil  | Yes   | Yes   | Yes    | Yes  | Yes   | Nil| Nil   | Yes | Nil | 2   | 39  | Eosinophilia           | 2,314   | Posit       |
| 16  | 10F | Yes | Yes   | Nil  | Yes   | Yes   | Yes    | Yes  | Yes   | Nil| Nil   | Yes | Nil | 1   | 16  | Eosinophilia           | 9,259   | Nega        |

ANCA: anti-neutrophil cytoplasmic antibody, AZ: azathioprine, BVAS: Birmingham Vasculitis Activity Score, Ben: benralizumab, CNS: central nervous system, C cyclophosphamide, Eosin: eosinophil, F: female, FFS: Five-factor score, GI: gastrointestinal, M: male, MEP: mepolizumab, MPO: myeloperoxidase, No.: number nervous system, RTX: rituximab

**Characteristics Of Myocarditis Presentation**

Ten patients (cases no. 1 to 10), 6 females aged 20 to 56 years (39.9 ± 10.1) with negative ANCA in 7 (70%), met the diagnostic criteria of myocarditis in this study, a presentation at disease onset in 6 and at relapse in 4. Their BVAS were 13 to 37 (25.9 ± 6.5) at the diagnosis of myocarditis, and FFS were 1 or 2 (1.5 ± 0.5). There was lower LVEF (31 to 55%, 42.1 ± 9.2%) with 5 moderate impairment (cases no. 3, 4, 5, 7 and 10) and 5 mild impairment (cases 1, 2, 5, 8 and 9). Cases no. 4, 5, 8 and 10 had concurrent pericardial effusion, consistent with the diagnosis of myopericarditis [18], and cases no. 1, 4, 5 and 7 had coexistent endocarditis, an ominous manifestation in EGPA associated with overt heart failure [21]. Patients with additional pericardial or endocardial involvement,
indicative of diffuse heart involvement, had lower LVEF than those without such a presentation (for pericarditis, $38.8 \pm 7.6\%$ versus $44.3 \pm 10.1\%$; for endocarditis, $38.5 \pm 11.2\%$ versus $44.5 \pm 7.6\%$). For myocarditis-related cardiac arrhythmia, 9 had sinus tachycardia with additional ventricular extrasystoles in 3 (no. 3, 4 and 10) and atrial extrasystoles in 2 (no. 1 and 10). In particular, one had sinus bradycardia complicated with sinus pause (no. 2). All received antiarrhythmic drugs and CSA for their underlying cardiac dysfunction.

**Myocarditis Patients Receiving Biologics**

Table 2 shows clinical, laboratory and medication profiles, and MPA- or RTX-related therapeutic indication and regimen in 7 patients receiving biologic therapy.

| No. | *Age /Sex | ANCA status | Biologic Tx indication | Biologic regimen (course) | @B-cell change | @BVAS change | @Eosinophil counts change (Inhibition %) | @CRP change | Side effects | #Follow-up time | Biologics therapeutic response | CS and immunosup before/after |
|-----|-----------|-------------|------------------------|--------------------------|----------------|-------------|----------------------------------------|-------------|--------------|----------------|-----------------------------|-----------------------------|
| 1   | 39F       | Positive    | Induction for relapsing | 100 mg weekly × 4 RTX, (1) | 203 to 311/µl | 13 to 0 | 1,080 to 67/µL (93.8%) | 16.3 to 1.9 mg/L | ISR | 13 m | Complete remission | AZ, CS, CYC | Nil |
| 2   | 47M       | Negative    | Induction for relapsing, maintenance | 375 mg/m² weekly × 4 RTX, (4) | 35 to 0/µl | 23 to 0 | 1,170 to 149/µL (87.3%) | 24.0 to 1.8 mg/L | Low IgM | 61 m | Complete remission | AZ, CS, CYC | AZ, *low-dose C |
| 3   | 31M       | Negative    | Induction for relapsing, maintenance | 375 mg/m² weekly × 4 RTX, (3) | 103 to 0/µl | 29 to 0 | 826 to 95/µL (88.5%) | 8.0 to 2.2 mg/L | Low IgG/M | 50 m | Complete remission | AZ, CS, CYC | +low-dose C |
| 4   | 46F       | Negative    | Induction for refractory, maintenance | 375 mg/m² weekly × 4 RTX, (3) | 71 to 0/µl | 20 to 0 | 882 to 159/µL (82.0%) | 23.0 to 1.1 mg/L | Nil | 47 m | Complete remission | CS, CYC / AZ, *low-dose C | |
| 5   | 36M       | Positive    | Induction for refractory, maintenance | 375 mg/m² weekly × 4 RTX, (3) | ND to 0/µl | 15 to 4 | 1,206 to 322/µL (73.3%) | 17.9 to 3.2 mg/L | Nil | 40 m | Partial remission | CS, CYC / AZ, *low-dose C | |
| 6   | 48F       | Negative    | Induction for refractory, maintenance | 375 mg/m² weekly × 4 RTX, (2) | 159 to 0/µl | 21 to 3 | 755 to 172/µL (77.2%) | 51.6 to 1.5 mg/L | Low IgM | 38 m | Partial remission | CS, CYC / +low-dose C | |
| 7   | 56F       | Negative    | Induction for relapsing | 375 mg/m² weekly × 4 RTX, (1) | 316 to 0/µl | 20 to 6 | 1,006 to 437/µL (56.6%) | 35.4 to 6.4 mg/L | IR at 1st infusion | 25 m | Partial remission | AZ, CS, CYC | AZ |

*Age at biologic therapy, @Calculation before and after therapy, ‡Follow-up time after initiating induction therapy, *5 mg/day prednisolone; AZ: azathioprine, B Birmingham Vasculitis Activity Score, CRP: C-reactive protein, CS: corticosteroid, CYC: cyclophosphamide, Dx: diagnosis, F: female, IR: infusion reactions, ISR injection site reaction, M: male, MEP: mepolizumab, ND: not determined, No.: number, m: month, RTX: rituximab, yr: year

An ANCA-positive 39-year-old female (case no. 1) received MPA without a concurrent CYC use at induction due to relapsing disease. The regimen was 100 mg quadri-weekly × 13 subcutaneous injection. No disease relapse after a 13-month follow-up period after the initiation of induction treatment. An observed side effect was injection site reaction.

Six patients (cases no. 2 to 7), 3 females aged 31 to 56 years (44.0 ± 9.0), 5 with negative and one with positive ANCA (case no. 4), received RTX without a combined use of CYC at induction due to refractory activity in 3 and relapsing disease in 3. The regimen was 375 mg/m² weekly × 4 intravenous infusion at induction in 6, or pulse at maintenance in 5 with a yearly administering schedule. Five accepted multiple therapeutic courses, 2 to 4 (3.0 ± 0.7). There was no disease relapse after initiating RTX induction with a follow-up period of 25 to 61 months (43.5 ± 12.2). All had completely depleted B-cell numbers (0/µl) after induction, and received daily trimethoprim/ sulfamethoxazole prophylaxis against Pneumocystis infection. Despite the presence of RTX-related lower immunoglobulin concentrations in 3 and infusion reactions in one, there were no documented infection episodes.
Besides lower CRP levels (25.2 ± 14.4 to 2.6 ± 1.8 mg/L, \( P = 0.016 \)) after biologic therapy, there was a decrease in BVAS (20.1 ± 5.2 to 1.9 ± 2.5, \( P = 0.016 \)) with a complete remission in 4 patients and a partial remission in 3. Peripheral eosinophil counts were reduced from 989 ± 174 to 200 ± 132/µL (\( P = 0.016 \)) with a 79.8 ± 12.4% inhibition of the baseline values. Before biological therapy, all received the use of CS and CYC at induction, and azathioprine was prescribed at maintenance before the relapsing disease in cases no. 1, 2, 3 and 7. The accumulated CYC dosages were beyond 15 gram in cases no. 2 and 3, and 10 gram in cases no. 4, 5 and 6. During biological therapeutic period, all received cardiac medications. After biological therapy, one received azathioprine alone, 2 obtained low-dose CS (5 mg/day prednisolone) alone, and 3 acquired both drugs. For the CSA prescription, case no. 2 received an angiotensin-receptor blocker and cases no. 3, 4, 6 and 7 obtained an angiotensin converting enzyme inhibitor.

**Efficacy In Cardiac Manifestations**

Table 3 demonstrates the myocarditis-related clinical and imaging findings before and after biological therapy. At the onset of myocarditis, all had clinical symptoms with NYHAFC II in 3 and III in 4, cardiac dysrhythmia, elevated cardiac biomarker concentrations, and lower LVEF with mild impairment in 3 and moderate impairment in 4 (31 to 55%, 40.9 ± 10.5%). LV dilation or global hypokinesia were found in 6 patients (cases no. 2 to 7). Myocardial edema was not identified in case no. 5 due to an initial cMRI performed after 2 RTX therapeutic courses. Mid-wall myocardium delayed gadolinium enhancement (DGE) were detected in all. Furthermore, case no. 4 and 5 had pericardial effusion (myopericarditis) and cases no. 1, 4, 5 and 7 had endocardium DGE (endomyocarditis).
| No. | Involved cardiac area | Symptoms, NYHAFC before/after | Rhythm before/after | Biomarkers before/after | Image findings of cMRI and ECG before (*during) biologic therapy | Image findings of cMRI and ECG after biologic therapy |
|-----|-----------------------|------------------------------|--------------------|-------------------------|---------------------------------------------------------------|-------------------------------------------------|
| 1   | Endocardium, myocardium | Dyspnea, palpitation / Nil | ST with PAC, PAT / NSR | Elevated / Normalized | Mildly impaired LVEF, Myocardial edema, Curvilinear mid-wall DGE at basal LV, LV mid-cavity, Diffuse endocardial DGE at global LV | Normalized LVEF, Reduced myocardial edema, Reduced mid-wall DGE, Reduced endocardial DGE |
| 2   | Myocardium | Dyspnea / Nil | SB with SP / NSR | Elevated / Normalized | Dilated LV, Mildly impaired LVEF, Myocardial edema, Multifocal mid-wall DGE at basal LV, LV mid-cavity IVS | Normalized LV size, Normalized LVEF, Resolved myocardial edema, Reduced mid-wall DGE |
| 3   | Myocardium | Dyspnea, orthopnea, palpitation / Nil | ST with PVC / NSR | Elevated / Normalized | Dilated LV with global hypokinesia, Moderately impaired LVEF, Myocardial edema, Patchy mid-wall DGE at LV mid-cavity IVS and inferolateral wall, apical LV anterolateral wall | Normalized LV size and motion, Normalized LVEF, Resolved myocardial edema, Reduced mid-wall DGE |
| 4   | Endocardium, myocardium, pericardium | Dyspnea, orthopnea, chest pain / Nil | ST with PVC / NSR | Elevated / Normalized | LV global hypokinesia, Moderately impaired LVEF, Pericardial effusion, Myocardial edema, Curvilinear mid-wall DGE at global LV, Diffuse endocardial DGE at global LV | Normalized LV motion, Normalized LVEF, Resolved pericardial effusion, Resolved myocardial edema, Reduced mid-wall DGE, Reduced endocardial DGE |
| 5   | Endocardium, myocardium, pericardium | Dyspnea, orthopnea, chest pain / Nil | ST / NSR | Elevated / Normalized | LV global hypokinesia, Moderately impaired LVEF, Pericardial effusion, *Resolved myocardial edema, *Spotty mid-wall DGE at basal LVinferolateral wall, *Endocardial DGE at basal LV, LV mid-cavity | Normalized LV motion, Normalized LVEF, Resolved pericardial effusion, Resolved myocardial edema, Reduced mid-wall DGE, Reduced endocardial DGE |

*cMRI done before the initiation of biologic therapy in all patients except no. 4 before initiating the 3rd RTX infusion course. cMRI: cardiac magnetic resonance imaging, DGE: delayed gadolinium enhancement, ECG: ECG: echocardiograph, IVS: interventricular septum, LVEF: left ventricle ejection fraction, No.: number, NSR: normal sinus rhythm, NYHAFC: New York Heart Association Functional Classification, PAC: premature atrial contraction, PAT: paroxysmal atrial tachycardia, PVC: premature ventricular contraction, SB: sinus bradycardia, SP: sinus pause, ST: sinus tachycardia
| No. | Involved cardiac area | Symptoms, NYHAFC before/after | Rhythm before/after | Biomarkers before/after | Image findings of cMRI and ECG before (*during) biologic therapy | Image findings of cMRI and ECG after biologic therapy |
|-----|-----------------------|-------------------------------|---------------------|-------------------------|---------------------------------------------------------------|-----------------------------------------------|
| 6   | Myocardium            | Dyspnea, Nil / II / I         | ST                  | Elevated / Normalized   | Dilated LV / Mildly impaired LVEF / Myocardial edema Curvilinear mid-wall DGE at basal LV anteroseptal wall, spotty mid-wall DGE at LV mid-cavity antero-lateral wall | Normalized LV size / Resolved myocardial edema / Reduced mid-wall DGE |
| 7   | Endocardium, myocardium | Dyspnea, orthopnea, palpitation, dyspnea III / II | ST                  | Elevated / Normalized | LV global hypokinesia / Moderately impaired LVEF / Myocardial edema Curvilinear mid-wall DGE at basal LV, LV mid-cavity Endocardial DGE at LV mid-cavity | Normalized LV motion / Normalized LVEF / Resolved myocardial edema / Reduced mid-wall DGE / Unresolved endocardial DGE |

* cMRI done before the initiation of biologic therapy in all patients except no. 4 before initiating the 3rd RTX infusion course. cMRI: cardiac magnetic resonance imaging, DGE: delayed gadolinium enhancement, ECG: ECG: echocardiograph, IVS: interventricular septum, LVEF: left ventricle ejection fraction, No.: number, NSR: normal sinus rhythm, NYHAFC: New York Heart Association Functional Classification, PAC: premature atrial contraction, PAT: paroxysmal atrial tachycardia, PVC: premature ventricular contraction, SB: sinus bradycardia, SP: sinus pause, ST: sinus tachycardia

In addition to improved NYHAFC with normalized LVEF, biomarker concentrations and cardiac rhythm, case no. 1 had reduced myocardial edema and myocardium DGE after MEP induction. Furthermore, there was evidently lessened endocarditis as shown in her follow-up cMRI (Fig. 2).

Cases no. 2 to 7 had improved NYHAFC, normalized biomarker concentrations, cardiac rhythm, LVEF and LV size/motion, resolved myocardial edema, and reduced myocardium DGE after RTX therapy. Case no. 4 had worsening endocardium DGE in spite of RTX induction; however, reduced endocardial involvement was found after maintenance therapy with another two therapeutic courses. Case no. 7 had unresolved endocardium DGE after RTX induction treatment. Serial cMRI in cases no. 3 and 4 were shown in Figs. 3 and 4, respectively.

Biologics was not prescribed in another 3 patients with cardiac presentations at disease onset, myocarditis in case no. 9 and myopericarditis in cases 8 and 10. They received combined CS and CYC treatment at induction. Improved myocardial dysfunction and a partial BVAS response were observed in cases no. 8 and 9. Nevertheless, case no. 10 with moderately impaired LVEF had persistent cardiac dysfunction without disease remission, and further succumbed to the EGPA activity.

**Discussion**

Cardiac involvement with heart failure in EGPA is a major cause of early death and a poor long-term prognostic factor [4, 22]. Myocarditis usually presents as non-ischemic cardiomyopathy with heart failure and arrhythmia [18]. cMRI can serve as a non-invasive tool for evaluating the myocardium and the endocardium, assessing the extent of heart involvement and helping the evaluation of therapeutic responses. Combined T2-weighted and post-gadolinium T1-weighted cMRI images provide the best diagnostic sensitivity and specificity [18, 23]. T2-weighted images are allowed to detect myocardial edema, whereas T1-weighted DGE can identify myocardial and endocardial fibrosis in addition to acute inflammation. In this study, the diagnosis of myocarditis and endomyocarditis were based on both T2- and T1-weighted images, and the therapeutic responses were evaluated by serial follow-up of cMRI. Six patients had myocardial edema before biological therapy, and 7 had mid-wall myocardium DGE and 4 had additional endocardium DGE before or during biological therapy. Notably, there were reduced myocardial edema and lessened endocardium DGE after MEP therapy, and resolved myocardial edema and reduced myocardium DGE after RTX treatment.

A randomized control trial of MEP therapy in EGPA with a 300 mg quadri-weekly × 13 regimen for 52 weeks, has demonstrated the efficacy at induction for relapsing and refractory disease as well as for CS-dependent victims [24]. As comparing MEP with placebo, there was a larger proportion of patients in
remission, with lower relapse rates and benefiting from CS dose tapering. Nevertheless, owing to no specific dose evaluation in that study, it remains to be
determined whether 300 mg is superior to 100 mg dosage for EGPA therapy [8]. Subsequent trials with a regimen of 100 mg quadri-weekly have been carried
out in EGPA with relapsing disease [25, 26]. In particular, a clinic cohort with disease relapse under the long-term CS use received low-dose MEP therapy for 16
weeks, resulting in clinical improvement with weaning off CS in all enrolled patients [25]. In this study, a similar 100 mg dosage was prescribed in a EGPA
victim for 52 weeks at induction for relapsing disease (case no. 1), leading to a complete remission with sparing the use of CS. Currently, despite the
established efficacy of MEP, optimal regimens in treating EGPA remain to be a research focus.

Owing to non-inferiority to CYC, RTX with a 375 mg/m² weekly × 4 regimen can be prescribed in severe AAV at induction as the first-line therapy [2]. A 24-month
RTX maintenance therapy regimen with 1 gram every 6 months has shown a lower relapse rate in refractory or relapsing AAV [27]. Another RTX maintaining therapy
with a regimen of 500 mg fortnightly at 6, 12 and 18 months, has also demonstrated a sustained remission with overall survival superior to the azathioprine-based
protocol in newly diagnosed or relapsing AAV [28]. Despite the exclusion of EGPA from AAV in above studies, therapeutic effects of RTX at induction in refractory or relapsing EGPA have been shown in 2 larger trials with 1 gram fortnightly and 375 mg/m² weekly × 4 regimens [20, 29]. Furthermore, a regimen
with 1 gram fortnightly every 6 months has been observed to be effective as maintenance treatment [29]. A recent investigation reveals a beneficial outcome in the RTX therapy with multiple therapeutic courses (mean 4.6 courses) for relapsing disease and remission maintenance in EGPA patients [30]. In this study, therapeutic benefits were observed in 5 patients receiving multiple therapeutic courses (mean 3.0 courses) for refractory activity or relapsing disease with a 375 mg/m² weekly × 4 regimen at induction and plus a yearly maintenance schedule.

Up to 95% of AAV patients other than EGPA have the presence of ANCA [6, 7]. Nevertheless, this autoantibody is only identified in around 40% of EGPA
patients, implicating the existence of different disease subsets with distinct pathogenic mechanisms according to the ANCA status [31]. ANCA-negative EGPA
patients are less likely to have typical features of other AAV disorders, but more susceptible to cardiac manifestations [2, 8]. EGPA patients have elevated
circulating Th17 frequencies and IgG4 levels correlating with their disease activity [32, 33]. RTX therapy has been observed to improve disease activity through
reducing synovial Th17 numbers in rheumatoid arthritis, and induce therapeutic responses by lowering serum IgG4 levels in IgG4-related disease [34, 35].
Moreover, for AAV not including EGPA, disease remission and relapse-free survival after RTX therapy have been observed to be irrelevant to the presence of
ANCA [36].

EGPA patients with heart involvement have been shown to have higher eosinophil numbers in peripheral blood than those without cardiac manifestations [37],
as also demonstrated in our patients. In addition, there were significantly lower circulating eosinophil counts in this study after biological therapy. In addition to
the presence of vasculitis lesions, persistent eosinophilia can cause myocardium damage, typically in the form of eosinophilic myocarditis with EGPA as an
underlying cause [14]. Furthermore, the endocardium and the underlying myocardium are involved in the eosinophilic endomyocarditis, the most characteristic
cardiabnormality in HES [38]. By using the cMRI survey, 13 (27%) in a 49-patient EGPA cohort were documented to have this specific form of heart
involvement [21], a similar occurrence (4 of 16 patients, 25%) in the present series. In eosinophilic myocarditis-related cardiac manifestations, heart injury is
due to a direct eosinophil-mediated cytotoxicity, eosinophil-degranulation products released from eosinophils and the recruitment of inflammatory leukocytes
by eosinophil-derived cytokines/chemokines [6, 11]. Interestingly, stabilization of cardiac dysfunction in eosinophilic myopericarditis with severely impaired
LVEF has been observed in an asthmatic patient receiving MEP therapy, 100 mg quadri-weekly for 28 weeks [39]. In this study, there were significantly reduced
peripheral eosinophil counts to a more than 90% inhibition after anti-IL5 therapy (cases no. 1 and 13) [40], and normalized myocardial contractility with significantly reduced endocarditis in EGPA endomyocarditis under the same therapeutic dosages for 52 weeks (case no. 1). Moreover, we demonstrated improved cardiac dysfunction after RTX therapy in 5 ANCA-negative EGPA patients with myocardial involvement. The therapeutic efficacy of RTX in EGPA myocarditis with ANCA negativity appears to include an action mechanism by suppressing IL-5-mediated eosinophilia through the depletion of B cells [1, 10].

Conclusions
In this monocentric retrospective study, we observed improved cardiac dysfunction and disease activity after biologic therapy in EGPA patients with
myocarditis.

Abbreviations

ACR: American College of Rheumatology; ANCA: Anti-neutrophil cytoplasmic antibody; AAV: Anti-neutrophil cytoplasmic antibody-associated vasculitis; BEN:
Benralizumab; cMRI: Cardiac magnetic resonance imaging; BVAS: Birmingham Vasculitis Activity Score; CRP: C-reactive protein; CS: Corticosteroid; CSA: Cardiac supportive agents; CYC: Cyclophosphamide; DGE: Delayed gadolinium enhancement; ECG: echocardiograph; EGPA: Eosinophilic granulomatosis with polyangiitis; HES: Hypereosinophilic syndrome; LVEF: Left ventricle ejection fraction; MEP: Mepolizumab; NYHAFC: New York Heart Association Functional Classification; OMA: Omalizumab; RTX: Rituximab

Declarations

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References

1. Harish A, Schwartz SA. Targeted anti-IL-5 therapies and future therapeutics for hypereosinophilic syndrome and rare eosinophilic conditions. Clin Rev Allergy Immunol 2020; published online ahead of print, 2020 Jan 9.

2. Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. Nat Rev Rheumatol. 2019;15:91–101.

3. Knockaert DC. Cardiac involvement in systemic inflammatory diseases. Eur Heart J. 2007;28:1797–804.

4. Misra DP, Shenoy SN. Cardiac involvement in primary systemic vasculitis and potential drug therapies to reduce cardiovascular risk. Rheumatol Int. 2017;37:151–67.

5. Guillevin L, Pagnoux C, Seror R, Mahr A, Mouthon L, Toumelin PL, French Vasculitis Study Group (FVSG). The Five-factor score revisited: assessment of prognostic of systemic necrotizing vasculitides based on the French Vasculitis Study Group (FVSG) cohort. Medicine. 2011;90:19–27.

6. Wu EY, Hernandez ML, Jennette JC, Falk RJ. Eosinophilic granulomatosis with polyangiitis: clinical pathology conference and review. J Allergy Clin Immunol Pract 2018, 6:1496–1504.

7. Goffredi A, Mariti F, Oliva E, Buzio C. Eosinophilic granulomatosis with polyangiitis: an overview. Front Immunol. 2014;5:549.

8. Trivoli G, Terrier B, Vaglio A. Eosinophilic granulomatosis with polyangiitis: understanding the disease and its management. Rheumatology. 2020;59(Supplement_3):ii84–94.

9. Leru PM. Eosinophilic disorders: evaluation of current classification and diagnostic criteria, proposal of a practical diagnostic algorithm. Clin Transl Allergy. 2019;9:36.

10. Pepper RJ, Fabre MA, Pavesio C, Gaskin G, Jones RB, Jayne D, Pusey CD, Salama AD. Rituximab is effective in the treatment of refractory Churg-Strauss syndrome and is associated with diminished T-cell interleukin-5 production. Rheumatology. 2008;47:1104–5.

11. Ennis D, Lee JK, Pagnoux C. Mepolizumab for the treatment of eosinophilic granulomatosis with polyangiitis. Expert Opin Biol Ther. 2019;19:617–30.

12. Nagase H, Ueki S, Fujieda S. The roles of IL-5 and anti-IL-5 treatment in eosinophilic diseases: Asthma, eosinophilic granulomatosis with polyangiitis, and eosinophilic chronic rhinosinusitis. Allergol Int. 2020;69:178–86.

13. Raffray L, Guillevin L. Treatment of eosinophilic granulomatosis with polyangiitis: a review. Drugs. 2018;78:809–21.

14. Cheung CC, Constantine M, Ahmadi A, Shiou C, Chen LYC. Eosinophilic myocarditis. Am J Med Sci. 2017;354:486–92.

15. Wang CR, Tsai Ys, Li WT. Lupus myocarditis receiving the rituximab therapy- a monocentric retrospective study. Clin Rheumatol. 2018;37:1701–7.

16. Wang CR, Tsai Ys, Tsai HW. Acute myocarditis in anti-neutrophil cytoplasmic antibody-positive microscopic polyangiitis patients receiving the rituximab therapy. J Rheumatol. 2019;46:1645–6.

17. Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY, Lightfoot RW, McShane DJ, John A. Mills JA, Stevens MB, Wallace SL, Zvaifler NJ. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum. 1990;33:1094–100.

18. Cooper LT Jr. Myocarditis. N Engl J Med 2009, 360:1526–1538.

19. Mukhtyar C, Lee R, Brown D, Carruthers D, Dasgupta B, Dubey S, Flossmann O, Hall C, Hollywood J, Jayne D, Jones R, Lanyon P, Muir A, Scott D, Young L, Luqmani RA. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). Ann Rheum Dis. 2009;68:1827–32.
20. Jachiet M, Samson M, Cottin V, Kahn JE, Le Guenno G, Bonniaud P, Devilliers H, Bouillet L, Gondouin A, Makhlfouf F, Meaux-Ruault N, Gil H, Bienvenu B, Coste A, Groh M, Giraud V, Dominique S, Godeau B, Puéchal X, Khounatra C, Ruivard M, Le Jeune C, Mouthon L, Guillemin L, Terrier B. French Vasculitis Study Group: **Anti-IgG Monoclonal Antibody (Omalizumab) in Refractory and Relapsing Eosinophilic Granulomatosis With Polyangiitis (Churg-Strauss): Data on Seventeen Patients.** *Arthritis Rheumatol.* 2016;68:2274–82.

21. Neumann T, Manger B, Schmid M, Krogel C, Hansch A, Kaiser WA, Reinhardt D, Wolf G, Hein G, Mall G, Schett G, Zwerina J. Cardiac involvement in Churg-Strauss syndrome: impact of endomyocarditis. *Med (Baltim).* 2009;88:236–43.

22. Moosig F, Bremer JP, Hellmich B, Holle JU, Hul-HuJk K, Ljadue M, Matthis C, Metzler C, Nölle B, Richardt G, Gross WL. A vasculitis center based management strategy leads to improved outcome in eosinophilic granulomatosis and polyangiitis (Churg-Strauss, EGPA): monocentric experiences in 150 patients. *Ann Rheum Dis.* 2013;72:1011–7.

23. Friedrich MG, Sechtem U, Schulz-Menger J, Holmvang G, Alakija P, Cooper LT, White JA, Abdel-Aty H, Gutberlet M, Prasad S, Aletras A, Laissy JP, Paterson I, Filipcuk NG, Kumar A, Pauschinger M, Liu P. International Consensus Group on Cardiovascular Magnetic Resonance in Myocarditis: *Cardiovascular magnetic resonance in myocarditis: A JACC White Paper.* *J Am Coll Cardiol.* 2009;53:1475–87.

24. Wechsler ME, Akuthota P, Jayne D, Khoury P, Klion A, Langford CA, Merkel PA, Moosig F, Specks U, Cid MC, Luqmari N, Brown J, Mallett S, Philipson R, Yancey SW, Steinfeld J, Weller PF, Gleich GJ, EGPA Mepolizumab Study Team. Mepolizumab or placebo for eosinophilic granulomatosis with polyangiitis. *N Engl J Med.* 2017;376:1921–32.

25. Favero P, Bonatti G, Bini F, Vaghi A, Pesci A. Mepolizumab as the first targeted treatment for eosinophilic myocarditis with polyangiitis: a review of current evidence and potential place in therapy. *Ther Clin Risk Manag.* 2018;14:2385–96.

26. Moyano VA, Cuestas El, Albiero JA, Ferreyra Dillon R, Pereyra BL, Perrone VE, Guendulain S, Lugones JI, Casas JP. Eosinophilic granulomatosis with polyangiitis treated with mepolizumab at a dose of 100 mg/month. *Arch Bronconeumol* 2020; 56:253–254.

27. Alberici F, Smith RM, Jones RB, Jones RB, Roberts DM, Willcocks LC, Chaudhry A, Smith KG, Jayne DR. Long-term follow-up of patients who received repeat-dose rituximab as maintenance treatment for ANCA-associated vasculitis. Rheumatology. 2015;54:1153–60.

28. Terrier B, Pagnoux C, Perrodeau É, Karras A, Khounatra C, Aumaitre O, Cohen P, Decaux O, Desmurs-Clavel H, Maurier F, Gobert P, Quémeneur T, Blanchard-Delaunay C, Bonnotte B, Carron PL, Daugas E, Ducet M, Godfrin P, Hamidou M, Lidove O, Limal N, Puéchal X, Mouthon L, Ravaud P, Guillemin L, French Vasculitis Study Group. Long-term efficacy of remission-maintenance regimens for ANCA-associated vasculitides. *Ann Rheum Dis.* 2018;77:1150–6.

29. Thiel J, Troilo A, Salzer U, Schleyer T, Halmischlag K, Rizzi M, Frede N, Venhoff A, Voll RE, Venhoff N. Rituximab as induction therapy in eosinophilic granulomatosis with polyangiitis refractory to conventional immunosuppressive treatment: a 36-month follow-up analysis. *J Allergy Clin Immunol Pract.* 2017;5:1556–63.

30. Theis D, Langford CA, Hoffman GS, Villa-Forte A. Long-term use of rituximab for eosinophilic granulomatosis with polyangiitis. *Arthritis Rheumatol.* 2015;67(suppl 10):s891.

31. Radice A, Bianchi L, Sinico RA. Anti-neutrophil cytoplasmic autoantibodies: methodological aspects and clinical significance in systemic vasculitis. *Autoimmun Rev.* 2013;12:487–95.

32. Saito H, Tsurikisawa N, Tsuburai T, Oshikata C, Akiyama K. Cytokine production profile of CD4 + T cells from patients with active Churg-Strauss syndrome tends toward Th17. *Int Arch Allergy Immunol.* 2009;149(Suppl 1):61–5.

33. Vaglio A, Strehl JD, Manger B, Mariti F, Alberici F, Beyer C, Rech J, Sinico RA, Bonatti F, Battistelli L, Distler JH, Schett G, Zwerina J. IgG4 immune response toward Th17. *Int Arch Allergy Immunol.* 2009;149(Suppl 1):61–5.

34. Khosroshahi A, Bloch DB, Deshpande V, Stone JH. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. *Arthritis Rheum.* 2010;62:1755–62.

35. van de Veerdonk FL, Lauwers Y, Marinissen RJ, Timmermans K, Di Padova F, Koenders MI, Gutierrez-Roelens I, Durez P, Netea MG, van der Meer JW, van den Berg WB, Joosten LA. The anti-CD20 antibody rituximab reduces the Th17 cell response. *Arthritis Rheum.* 2011;63:1507–16.

36. Jones RB, Ferraro AJ, Chaudhry AN, Brogan P, Salama AD, Smith KG, Savage CO, Jayne DR. A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum.* 2009;60:2156–68.

37. Miloslavsky E, Unzony S. The heart in vasculitis. *Rheum Dis Clin North Am.* 2014;40:11–26.

38. Ogbo RU, Rosing DR. *Horne MK 3rd. Cardiovascular manifestations of hypereosinophilic syndromes.* *Immunol Allergy Clin North Am.* 2007;27:457–75.

39. Song T, Jones DM, Homsy Y. *Therapeutic effect of anti-IL-5 on eosinophilic myocarditis with large pericardial effusion.* *BMJ Case Rep* 2017, bcr-2016-218992.

40. Pouliquen IJ, Kornmann O, Barton SV, Price JA, Ortega HG. Characterization of the relationship between dose and blood eosinophil response following subcutaneous administration of mepolizumab. *Int J Clin Pharmacol Ther.* 2015;53:1015–27.
Histopathological findings of tissue eosinophilia and vasculitis changes in skin biopsy specimens from case no. 2. (A) Interstitial infiltration of abundant eosinophils in the dermis. Hematoxylin and eosin stain 100×. (B) Necrotizing vasculitis in a small vessel with marked infiltration of eosinophils in the vessel wall. Hematoxylin and eosin stain 200×.

Serial cMRI images in case no. 1 before and after MEP therapy. (A-B) Pre-MEP treatment short-axis post-gadolinium delayed enhancement images showed acute edema (white asterisk) at the mid-wall of LV septal wall and endocarditis at the LV septal and inferior walls (white arrows). (C-D) After induction treatment short-axis post-gadolinium delayed enhancement images disclosed some septal mid-wall fibrosis (white asterisk) and residual endocarditis (white arrows) with improvement at the LV septal and inferior walls. Post-inflammatory myocardial insult with wall thinning was noted at the LV septal wall (D, white open bracket).
Figure 3

Serial cMRI images in case no. 3 before and after RTX therapy. (A-B) Pre-RTX treatment cMRI images revealed patchy edema in short-axis T2-weighted images (A, white arrows) and post-gadolinium delayed enhancement images (B, white arrowheads). (C-D) After induction and two courses of maintenance therapy, resolved myocardial edema (C) and some mid-wall fibrosis at the sepal and inferior walls of LV mid-cavity (D, white arrowheads).
Serial cMRI images in case no. 4 before and after RTX therapy. (A-B) Pre-RTX treatment cMRI images revealed mid-wall edema in short-axis T2-weighted images (A, white arrows) and post-gadolinium delayed enhancement images (B, white arrowheads). Regional endocarditis in the anterior wall at LV mid-cavity (B, asterisk). (C-E) After induction therapy, follow-up images showed resolved myocardial edema (C), some mid-wall fibrosis (D, white arrowheads) and interval worsening of endocarditis (D and E, asterisks) seen from short-axis and 4-chambered sections. (F) The endocardial change revealed improvement after two courses of maintenance therapy (F, asterisks).