Clinical Case Report

Medicine®

OPEN

Myeloperoxidase-antineutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis developed from ANCA negative renal limited vasculitis

A case report

Xiao-li Li, PhD[a], Peng-cheng Xu, PhD[a,*], Tong Chen, PhD[b], Tie-kun Yan, MSc[a], Jian-qing Jiang, MD[a], Jun-ya Jia, PhD[a], Li Wei, MSc[a], Wen-ya Shang, MSc[a], Shui-yi Hu, PhD[a]

Abstract

Rationale: The relationship between antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) and ANCA-negative vasculitis has not been elucidated.

Patient concerns: A 64-year-old female with edema and proteinuria was admitted. A kidney biopsy indicated focal proliferative nephritis with crescents in 25% of glomeruli. Serum ANCA was negative. Eighteen months later, systemic symptoms emerged and acute kidney injury occurred. Serum ANCA against myeloperoxidase (MPO) turned positive. Repeated kidney biopsy showed more severe lesion than last time. Immunoglobulin (Ig)G was purified from serum obtained before the first kidney biopsy. Weak ANCA which could not be detected in serum was found in IgG.

Diagnoses: MPO-ANCA-associated AAV developed from ANCA-negative renal-limited AAV.

Interventions: The patient was treated with glucocorticoid.

Outcomes: The serum creatinine decreased to 2.17 mg/dL a week later. MPO-ANCA turned negative when re-examined 3 weeks later. No relapse has been observed during follow-up for 6 months.

Lessons: This is the first reported case about the spontaneous transformation from ANCA-negative renal-limited AAV to ANCA-positive systemic vasculitis. There might be a slow process of epitope spreading in the pathogenesis of disease. Physicians should try their best to detect the ANCA in the diagnose and treatment of ANCA-negative AAV.

Abbreviations: AAV = antineutrophil cytoplasmatic antibodies-associated vasculitis, ANA = antinuclear antibody, ANCA = antineutrophil cytoplasmatic antibodies, C3 = complement 3, C4 = complement 4, CRP = C-reactive protein, IF = immunofluorescence, MPO = myeloperoxidase, PBS = phosphate-buffered saline, PR3 = proteinase 3.

Keywords: antineutrophil cytoplasmic antibody negative, case report, epitope spreading, vasculitis

1. Introduction

The relationship between antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) and ANCA-negative vasculitis has not been elucidated.[1–4] We reported a 64-year-old woman with proteinuria and normal kidney function. ANCA negative AAV could not be excluded because kidney biopsy indicated focal proliferative nephritis with crescents formation of 25% glomeruli. Treatment with angiotensin receptor blocker alone decreased proteinuria and kept kidney function stable. However, serum myeloperoxidase (MPO)-ANCA turned positive 18 months later. Acute kidney injury also occurred accompanied by extrarenal symptoms. We retrospectively purified immunoglobulin (Ig)G from the serum which was drawn and conserved before the first kidney biopsy and demonstrated the existence of weak ANCA. We believe this is the first reported case about the spontaneous transformation from ANCA-negative renal-limited AAV to ANCA-positive systemic vasculitis. The patient signed an informed consent form at admission to hospitalization. Ethical approval was not necessary because of the routine health care.

2. Case report

A 64-year-old woman arrived at our hospital with a 1-week history of edema. Pertinent physical examination findings were normal except a slight pitting edema of both lower extremities. Chest x-ray and abdominal ultrasonography showed no obvious abnormality. Blood routine and biochemical examinations were

Editor: Jinxian Xu.

Funding: This study is supported by 3 grants of National Natural Science Fund (no. 81200534, no. 81570630, and no. 81600554), a grant of Tianjin Research Program of Application Foundation and Advanced Technology (no. 15JCQNJC10700), and a grant of China Postdoctoral Science Foundation funded project (no. 2014M560191).

The authors have no conflicts of interest to disclose.

[a] Department of Nephrology, [b] Department of Hematology, Tianjin Medical University General Hospital, Tianjin, China.

[*] Correspondence: Peng-cheng Xu, Department of Nephrology, Tianjin Medical University General Hospital, Tianjin, China (e-mail: nkxpc@163.com).

Medicine (2017) 96:51(e9128)

Received: 23 July 2017 / Received in final form: 15 November 2017 / Accepted: 16 November 2017

http://dx.doi.org/10.1097/MD.0000000000009128

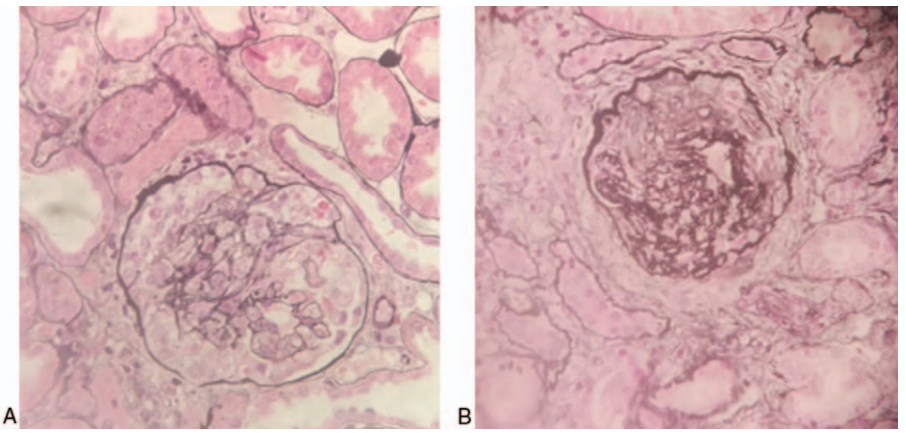

Figure 1. Manifestations of twice kidney biopsy. (A) Cellular crescent formation of a glomerulus of the first kidney biopsy. (B) Fibrous crescent formation of a glomerulus of the second kidney biopsy. There were tubular atrophy and interstitial fibrosis as well.

normal. Erythrocyte sedimentation rate was above normal (29 mm/h, normal range 0 to 20 mm/h). Urinalysis revealed hematuria 1+ and proteinuria 2+. Urine erythrocyte was 46/μL (normal range <10/μL). No urinary red blood casts was found. The 24-hour urine protein excretion revealed 2.5 g (normal range <0.15 g/24 h). Immunological examination showed that all the parameters including C-reactive protein (CRP), complement 3 (C3), and complement 4 (C4) were normal. Immunofluorescence (IF) showed that both antinuclear antibody (ANA) and ANCA were negative. The enzyme-linked immunosorbent assay (ELISA) showed antibody against myeloperoxidase (MPO), antibody against proteinase 3 (PR3), and antiglomerular basement membrane antibody were negative. Then kidney biopsy was performed (Fig. 1A). Among 12 glomeruli of the specimen, 3 had ischemic sclerosis, 1 had cellular crescents, and 2 had fibrocellular crescents. The others showed proliferation of the mesangium. IF showed neither immune complex nor complement deposition (IgA–, IgG–, IgM–, C3–, and C1q–). Focal proliferative nephritis with crescents formation was diagnosed and ANCA-negative AAV could not be excluded. The patient was then discharged with a treatment of losartan 100 mg/d because the patient did not agree with the immunosuppressive treatment at that time.

During follow-up for 18 months, proteinuria decreased gradually to a stable level of about 1 g/24 h. The edema relieved and serum creatinine kept normal. Serum ANCA was still negative when reexamined 6 and 12 months after discharge. However, 3 weeks before the second hospitalization, the patient felt fatigue and lost 1 kg of weight. The patient had a mild cough without hemoptysis. Chest CT scan showed increased lung marking without empty formation. She also had a mild pain of both knee joints. No rash, conjunctivitis, nasosinusitis, or hearing loss existed. No sign of peripheral neuropathy was found. The result of fecal occult blood test was negative. The serum creatinine increased to 2.82 mg/dL (normal 0.7–1.5 mg/dL). Losartan was stopped and the patient was admitted again. Laboratory test showed serum CRP increased to 5.4 mg/dL (normal range <0.8 mg/dL). Indirect IF showed perinuclear ANCA was positive. ELISA showed the level of ANCA against MPO was 64.3 RU/mL (normal range <20 RU/mL). The second renal biopsy was done (Fig. 1B). Among 19 glomeruli of the specimen, 4 had ischemic sclerosis, 3 had global sclerosis, 2 had cellular crescents, 3 had fibrocellular crescents, and 4 had fibrous crescents. There was moderate tubular atrophy, mild interstitial fibrosis, and mild mononuclear cell infiltration of the interstitium. IF showed neither immune complex nor complement deposition (IgA–, IgG–, IgM–, C3–, and C1q–). The patient was diagnosed as mixed category of MPO-ANCA-associated AAV according to the 2010 histopathological classification proposed by Berden et al.[5] The classification was mixed if <50% of the glomeruli were normal, crescentic or globally sclerotic. Then she was treated with intravenous methylprednisolone at a dose of 40 mg/day. The serum creatinine decreased to 2.17 mg/dL a week later. The patient was discharged with a maintain oral methylprednisolone treatment. MPO-ANCA turned negative when re-examined 3 weeks later. No relapse of AAV has been observed during follow-up for 6 months.

To investigate the relationship between twice onsets of the disease, we retrospectively purified IgG with affinity chromatography from the serum which was drawn and conserved before the kidney biopsies. Briefly, the serum was centrifuged for 20 minutes at 10,000 rpm. The supernatant 1 mL was loaded onto a protein-G agarose affinity column (GE Healthcare, USA) with a flow rate 1 mL/min. Bound IgG was eluted with 0.1 mol/L glycine, 0.5 mol/L NaCl (pH 2.7), and dialyzed against 0.01 M phosphate-buffered saline (PBS) (pH 7.4). The purified IgG was then concentrated to a volume of 1 mL with ultrafiltration. Standard IF assays were performed according to the manufacturer's instructions (Euroimmun, Lübeck, Germany). Serum of the patient with the first and second onset of disease, control serum, purified IgG of the patient with the first and second onset, and purified IgG of control, diluted 1/10 in PBS, were added to slides and incubated for 30 min at room temperature. The slides were washed twice with PBS and fluorescein isothiocyanate-conjugated second antibody was added and incubated for 30 minutes at room temperature. After washing with PBS twice, the slides were sealed with cover slips and observed at an exciting light of 490 nm. The IF test showed the existence of weak ANCA in purified IgG of the patient with the first onset of disease. Furthermore, we found this weak ANCA had a weak ability to bind MPO using ELISA. Briefly, purified human MPO (Merck) was coated to the wells of a polystyrene microtiter plate at 2.0 μg/mL in PBS. Purified IgG at a concentration equivalent to original plasma were diluted at 1:100 with PBS-Tween and incubated at 37°C for 1 hour. Binding was detected with alkaline phosphatase-conjugated goat antihuman IgG (Sigma) at a dilution of

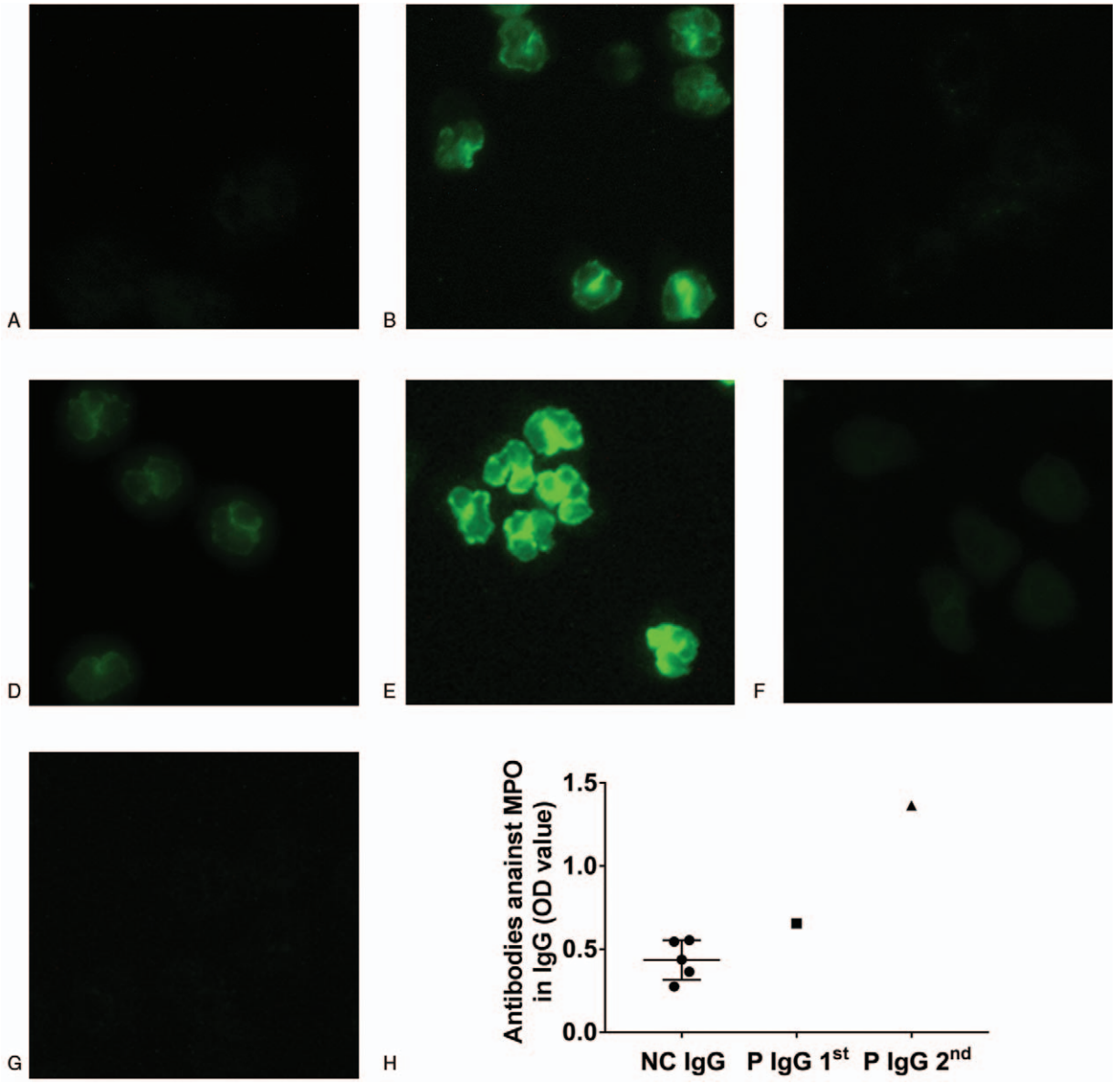

Figure 2. Detection of ANCA. (A) Detection of ANCA with serum of the patient with the first onset of disease. (B) Detection of ANCA with serum of the patient with the second onset of disease. (C) Detection of ANCA with serum of a normal control. (D) Detection of ANCA with the purified IgG of the patient with the first onset of disease. (E) Detection of ANCA with the purified IgG of the patient with the second onset of disease. (F) Detection of ANCA with the purified IgG of a normal control. (G) Negative control using only the second antibody. (H) Levels of the MPO-ANCA in purified IgG of the patient with the first and second onset of disease (ELISA result). IgG obtained from 5 normal people were made as controls. ANCA = antineutrophil cytoplasmic autoantibody, ELISA = enzyme-linked immunosorbent assay, IF = immunofluorescence, Ig = immunoglobulin, MPO = myeloperoxidase, NC = normal control, OD = optical density, P = patient (IF result, ×400).

1:20,000. The results were recorded at 405 nm. The results are shown in Figure 2.

3. Discussion

The diagnosis of ANCA-negative AAV is not as easy as ANCA-positive AAV, especially when there is no extrarenal symptoms. Crescent formation without fibrinoid necrosis can also be seen in other glomerulonephritis besides AAV. For this patient, we were not very convinced of the diagnosis of ANCA-negative AAV after the first kidney biopsy since the patient did not have kidney dysfunction, serum ANCA positivity, or extrarenal symptoms. The long-time stability of disease with no need of any immunosuppressive treatment and the negative results of multiple tests of serum ANCA almost made us exclude the diagnosis of AAV. It was the discovery of the weak ANCA in purified IgG that helped us to confirm the diagnosis of ANCA-negative AAV finally. The 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides defined ANCA-negative AAV as a subtype of AAV and emphasized that patients with ANCA-negative AAV might have ANCA that could not be detected with current methods or might have ANCA of as-yet-undiscovered specificity.[6] As far as we know, this might be the first reported case about the spontaneous transformation from ANCA-negative renal-limited AAV to ANCA-positive systemic vasculitis after a long interval.

Some reports indicated that patients with negative ANCA had a greater degree of proteinuria and a higher prevalence of

nephrotic syndrome than patients with positive ANCA.[7,8] According to previous studies, ANCA-negative AAV has less extrarenal manifestations than ANCA-positive AAV. For the current patient, proteinuria was the sole clinical symptom at first. After the serum MPO-ANCA turned positive, the acute kidney injury occurred and extrarenal symptoms also emerged. This indicated the MPO-ANCA might accelerate the disease. By now, the pathogenesis of AAV has not been elucidated. This is even true for the ANCA-negative AAV. The activation of alternative complement pathway has been considered to be very important in the development of both ANCA-positive and ANCA-negative AAV,[9] but the activation of alternative complement pathway should be secondary to the activation of netrophils. The neutrophils-activating ability of ANCA has been demonstrated by many in vitro and in vivo experiments. However, the existence of ANCA seems not to be necessary in the pathogenesis of AAV and the initial factor of AAV has not been confirmed yet. Actually, although ANCA can activate neutrophils, it is not the sole reason for the activation of neutrophils in AAV because the activation of neutrophils has also been demonstrated in ANCA-negative AAV.[10,11] The ANCA-positive AAV can relapse in the ANCA-negative status.[12] One reasonable speculation about the pathogenesis of AAV is that neutrophils are activated initially without the need of ANCA. Then the activated neutrophils release ANCA antigens to the immune system and induce the generation of ANCA which can activate more neutrophils and accelerate the disease.

According to previous studies, about 10% to 40% of patients with pauci-immune crescentic glomerulonephritis were ANCA negative.[13–16] Although lacking autoantibodies, targeting B cells for the treatment of ANCA-negative AAV has been demonstrated effective.[17] We believe that more and more ANCA-negative AAV will be reclassified as ANCA-positive AAV along with the development of examination technique.[18] Although the positivity of ANCA detection in ANCA-negative AAV can be elicited with combined use of different methods, the spontaneous seroconversion of ANCA in ANCA-negative AAV has not been reported by previous studies. For the current patient, since the kidney function was normal and the diagnosis of AAV was not definite, no immunosuppressive treatment was given after the first kidney biopsy. This might be the critical reason for the seropositivity of ANCA after 18 months. To clarify the diagnosis, we purified the IgG from the serum obtained before the first kidney biopsy and found that there was weak ANCA in the IgG which could not be detected in serum. The research of Roth et al[19] might help to explain this phenomenon. In that study, the researchers found that many patients with ANCA-negative AAV had antibodies recognizing a special linear epitope of MPO (aa 447–459), but the antibodies could only be detected with purified IgG since there were fragments of ceruloplasmin in sera which could interfere the binding between MPO and the antibodies. It is noteworthy that natural anti-MPO antibodies exist in normal people and these natural antibodies only can be detected with purified IgG.[20,21] So we speculate there should be a slow process of epitope spreading in the pathogenesis of disease for the current patient. Interestingly, although ANCA seemed to be pathogenic for this patient, neither immune complex nor complement deposition was found in 2 kidney biposies. It should be explained by the fact that the antigen of ANCA exists in neutrophils not in endothelial cells of glomeruli.

In conclusion, this case offers a new clue for the understanding of the relationship between ANCA-negative and ANCA-positive AAV. For some patients, ANCA-negative status might be a transient stage in the development of AAV. Physicians should try their best to detect the ANCA in the diagnoses and treatment of ANCA-negative AAV.

References

[1] Chen M, Kallenberg CG, Zhao MH. ANCA-negative pauci-immune crescentic glomerulonephritis. Nat Rev Nephrol 2009;5:313–8.

[2] Miloslavsky EM, Lu N, Unizony S, et al. Myeloperoxidase-antineutrophil cytoplasmic antibody (ANCA)-positive and ANCA-negative patients with granulomatosis with polyangiitis (Wegener's): distinct patient subsets. Arthritis Rheumatol 2016;68:2945–52.

[3] Shah S, Havill J, Rahman MH, et al. A historical study of American patients with anti-neutrophil cytoplasmic antibody negative pauci-immune glomerulonephritis. Clin Rheumatol 2016;35:953–60.

[4] Yoo J, Kim HJ, Ahn SS, et al. Clinical and prognostic features of Korean patients with MPO-ANCA, PR3-ANCA and ANCA-negative vasculitis. Clin Exp Rheumatol 2017;35(suppl 103):111–8.

[5] Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. J Am Soc Nephrol 2010;21:1628–36.

[6] Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013;65:1–1.

[7] Sampathkumar K, Ramakrishnan M, Sah AK, et al. ANCA negative pauci-immune glomerulonephritis with systemic involvement. Indian J Nephrol 2010;20:43–7.

[8] Chen M, Yu F, Wang SX, et al. Antineutrophil cytoplasmic autoantibody-negative Pauci-immune crescentic glomerulonephritis. J Am Soc Nephrol 2007;18:599–605.

[9] Sethi S, Zand L, De Vriese AS, et al. Complement activation in Pauci-immune necrotizing and crescentic glomerulonephritis: results of a proteomic analysis. Nephrol Dial Transplant 2017;32(suppl_1):i139–45.

[10] Wang F, Chen M, Zhao MH. Neutrophil degranulation in antineutrophil cytoplasmic antibody-negative pauci-immune crescentic glomerulonephritis. J Nephrol 2009;22:491–6.

[11] Xing GQ, Chen M, Liu G, et al. Renal neutrophils infiltration in antineutrophil cytoplasmic antibodies-negative pauci-immune crescentic glomerulonephritis. Am J Med Sci 2010;340:474–80.

[12] Komagata Y, Nomura K, Yoshihara K, et al. AP-VAS 2012 case report: MPO-ANCA-negative relapse of MPO-ANCA-associated vasculitis. CEN Case Rep 2013;2:180–3.

[13] Hedger N, Stevens J, Drey N, et al. Incidence and outcome of pauci-immune rapidly progressive glomerulonephritis in Wessex, UK: a 10-year retrospective study. Nephrol Dial Transplant 2000;15:1593–9.

[14] Eisenberger U, Fakhouri F, Vanhille P, et al. ANCA-negative Pauci-immune renal vasculitis: histology and outcome. Nephrol Dial Transplant 2005;20:1392–9.

[15] Hung PH, Chiu YL, Lin WC, et al. Poor renal outcome of antineutrophil cytoplasmic antibody negative Pauci-immune glomerulonephritis in Taiwanese. J Formos Med Assoc 2006;105:804–12.

[16] Sokolowska BM, Szczeklik WK, Wludarczyk AA, et al. ANCA-positive and ANCA-negative phenotypes of eosinophilic granulomatosis with polyangiitis (EGPA): outcome and long-term follow-up of 50 patients from a single Polish center. Clin Exp Rheumatol 2014;32:S41–7.

[17] Shah S, Hruskova Z, Segelmark M, et al. Treatment of severe renal disease in ANCA positive and negative small vessel vasculitis with rituximab. Am J Nephrol 2015;41:296–301.

[18] Tateyama K, Kodama S, Kishibe K, et al. A novel strategy with combined assays for detection of anti-neutrophil cytoplasmic antibody (ANCA) in clinically ANCA-negative granulomatosis with polyangiitis patients. Auris Nasus Larynx 2017;44:735–41.

[19] Roth AJ, Ooi JD, Hess JJ, et al. Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. J Clin Invest 2013;123:1773–83.

[20] Cui Z, Zhao MH, Segelmark M, et al. Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. Kidney Int 2010;78:590–7.

[21] Xu PC, Cui Z, Chen M, et al. Comparison of characteristics of natural autoantibodies against myeloperoxidase and anti-myeloperoxidase autoantibodies from patients with microscopic polyangiitis. Rheumatology (Oxford) 2011;50:1236–43.