



Significance of autoantibodies in diagnostics of systemic vasculitis

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Abstract

Systemic vasculitis is a heterogeneous group of disorders characterized by inflammation and necrosis in the vessel wall. Patients usually present a quite broad spectrum of manifestations which vary in terms of vessels' size affected, organs involvement, and the extent of inflammatory process as well as an immunological diversity, including autoantibodies profile. Though, the diagnosis is based on clinical features, tissue biopsy, imaging investigations, and serologic tests. The main autoantibodies, important not only in the diagnosis but also in monitoring and prognosis of systemic vasculitides, are anti-neutrophil cytoplasmic antibodies (ANCA), anti-glomerular basement membrane antibodies (anti-GBM), anti-complement component C1q antibodies (anti-C1q), and cryoglobulins. Although other autoantibodies have been analyzed, their clinical utility still needs further investigation. The current work aimed to review the clinical associations of main autoantibodies in systemic vasculitis.

Keywords

Systemic vasculitis, anti-neutrophil cytoplasmic antibodies, anti-glomerular basement membrane antibodies, anti-complement component C1q antibodies, cryoglobulins

Introduction

Systemic vasculitides are characterized by the presence of various autoantibodies. They present different diagnostic specificity and sensitivity as well as clinical associations. The group of autoantibodies commonly used in clinical practice consists of anti-neutrophil cytoplasmic antibodies (ANCA), anti-glomerular basement membrane antibodies (anti-GBM), anti-complement component C1q antibodies (anti-C1q), and cryoglobulins. The revised 2017 international consensus recommendations summarize the new strategy for ANCA detection in small-vessel vasculitis putting attention to the primary use of monospecific immunoassays against proteinase 3 (PR3) and myeloperoxidase (MPO) without the categorical need for additional indirect immunofluorescence (IIF). Moreover, the presence of PR3-ANCA and MPO-ANCA have



led to the differentiation of distinct disease phenotype of ANCA-associated vasculitis (AAV): PR3-AAV, MPO-AAV, and ANCA-negative vasculitis [1].

The current review aimed to discuss the recent advances in the understanding of the role of ANCA as well as other autoantibodies in the diagnosis and management of the patients with systemic vasculitis.

ANCA

ANCA are considered a main biomarker of small-vessel vasculitis (AAV) such as granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic GPA (EGPA). The main target antigens for ANCA are PR3 and MPO located in primary granules of neutrophils and lysosomes of monocytes [2].

However, there is a plethora of antigens recognized by ANCA including human neutrophil elastase, lysosomal membrane protein-2, bactericidal/permeability protein, cathepsin G, lactoferrin, lysozyme, and many others. Of note, these additional antigens reactivities have no clinical value in the diagnosis of AAV. But they are detected in other disorders such as inflammatory bowel diseases, autoimmune liver diseases, infections, systemic connective tissue diseases, and drug-induced vasculitis [3].

Based on the international consensus statement on testing and reporting of ANCA published in 1999, the reference method for ANCA detection is IIF on ethanol fixed neutrophils which allows for differentiation of main types of ANCA-cytoplasmic (C-ANCA), perinuclear-ANCA (P-ANCA), and atypical-ANCA (A-ANCA). All the IIF-positive results should be followed by specific PR3 and MPO-ANCA immunoassays. To achieve the highest specificity, the combination of both techniques is recommended for the detection of ANCA in patients under investigation for AAV, since 10% of ANCA-positive GPA or MPA patients present only IIF-positive results [4].

The new revised 2017 international consensus recommendations are based on the results of the multicenter European Vasculitis Study Group (EUVAS) project which evaluated the diagnostic accuracy of novel technologies available for PR3 and MPO-ANCA detection [5, 6]. The comparison between IIF and monospecific tests detecting direct reactivities with PR3 and MPO brought a new view on ANCA testing and was a main assumption of these recommendations. Antigen-specific immunoassays should be the first-line tests. In case of negative or low positive test results and high clinical suspicion of AAV, the second antigen-specific test or IIF might be used to increase diagnostic specificity, which is of crucial importance, and this diagnostic approach should be addressed to patients with a clinical background strongly suggesting vasculitis [1]. ANCA have a low positive predictive value for AAV when they are tested in unselected patients [7].

In general, ANCA are detected in almost all patients with active GPA or MPA. In patients with limited disease, they are found with the frequency of about 60%, and in EGPA their prevalence usually does not exceed 40% [8].

PR3 is one of the target antigens for ANCAs and is present in primary granules of neutrophils, monocytes, and in the cytoplasm of endothelial cells. The IIF test shows the presence of C-ANCA (in isolated cases, P-ANCA, A-ANCA, or no reaction). As mentioned, PR3-ANCA are the main serological marker of GPA (up to 99% of patients), but they are also found in EGPA (10–20%) and MPA (10–25%), in which they are associated with higher mortality and worse prognosis in regarding kidney function. Of note, PR3-ANCA can also be induced by bacteria and parasites, as well as certain drugs, e.g., sulfasalazine, cocaine, or propylthiouracil which should be taken into account in differential diagnosis [1, 3].

The antigen for MPO-ANCA is a protein located within the azurophilic granules of neutrophils. It is the main target antigen of P-ANCA, and exceptionally cytoplasmic staining of ethanol-fixed granulocytes is observed. Anti-MPO antibodies are considered the primary diagnostic marker of MPA (sensitivity 60–80%). These antibodies are also found in the course of other vasculitides in 18–60% of patients with EGPA and exceptionally in GPA. In addition, they are present in 30–40% of patients with anti-GBM disease and with varying frequency, e.g., in patients with systemic lupus erythematosus and systemic sclerosis, especially those with renal involvement. Factors that may induce MPO-ANCA include drugs such as hydralazine, propylthiouracil, penicillamine, thiamazole, allopurinol, or sulfasalazine [1, 3, 8].

The role of serological tests in monitoring the activity of the disease and supporting treatment decisions still remains a subject of experts' debate. Kemna et al. [9] showed that serial measurements of ANCA in patients lacking severe vasculitic manifestations are of limited significance because both ANCA rise, as well as constant titre, do not present significant utility in the prediction of relapses. Moreover, the risk of developing relapses in patients who became ANCA negative during follow-up was very low as long as no rise in ANCA level occurred. Additionally, in patients with renal involvement, ANCA rise is an important warning as the probability of relapse is > 11 higher in comparison with the period before the rise.

The Rituximab in ANCA-associated Vasculitis (RAVE) trial results confirmed that serial assessment of PR3-ANCA does not represent high clinical utility in the prediction of relapses in the general AAV patients' population. But, the rise of PR3-ANCA level was significantly associated with a higher risk of relapses in patients with renal involvement and alveolar hemorrhage, and treated with rituximab which should induce the careful monitoring of the patients with the special focus on kidney function [10].

However, one should note, that the European League Against Rheumatism (EULAR) recommendations strongly state that all treatment decisions in AAV patients' management need to be based on clinical evaluation, and not on changes in the ANCA levels alone [11].

Based on genetic investigations, PR3-ANCA, and MPO-ANCA-associated diseases might be classified as separate disorders. Similarly, the clinical pictures differ between PR3-AAV and MPO-AAV [12]. In patients with PR3-AAV granulomatous inflammation, extra-renal organ manifestations, and higher relapse rate are more frequent. On the other hand, in the course of MPO-AAV, the kidney-limited disease, severe renal scarring, and worse renal prognosis are the most dominant clinical features [13]. To sum up, the determination of ANCA specificity provides very useful information regarding disease phenotype, clinical course, outcomes, and treatment effectiveness [14].

Anti-GBM

Anti-GBM are associated with anti-GBM antibodies disease (formerly Goodpasture's syndrome). The type of vasculitis is classified as immune complex vasculitis affecting glomerular and/or pulmonary capillaries.

The major antigen for anti-GBM is the noncollagenous 1 domain of the $\alpha 3$ -chain of type IV collagen. The autoantibodies are detectable in almost all patients with anti-GBM antibodies disease. The presence of autoantibodies and rapidly progressive glomerulonephritis are the basis of the diagnosis.

Anti-GBM present high positive predictive value and according to experts' opinions should be tested especially in patients with rapid progression of renal failure with microscopic hematuria and/or pulmonary hemorrhage.

As mentioned, these autoantibodies may also coexist with MPO-ANCA. Anti-GBM disease with double positivity is characterized by a high risk of relapses and an early mortality rate. Patients usually present massive kidney and lung involvement in the initial phase of the disease and the therapy must be based on aggressive immunosuppressive treatment and plasmapheresis.

It is highlighted that double testing of both anti-GBM and ANCA should be made in patients with pulmonary-renal syndrome [15].

The recommended method to detect anti-GBM is enzyme-linked immunosorbent assay (ELISA) which is characterized by the highest sensitivity in comparison with the IIF test (e.g., false positive results in patients with diabetes) [3].

Anti-C1q

The target antigen for anti-C1q is a collagen-like region of the C1q complement component. These autoantibodies are markers for hypocomplementemic urticarial vasculitis syndrome (HUVS). Detection of anti-C1q in HUVS is considered a diagnostic component as they are present in 100% of patients [2]. With lower frequency, anti-C1q might be present in other vasculitis such as Behçet's disease with vascular involvement, infection-associated vasculitis, giant cell arteritis, or cryoglobulin-associated vasculitis [16].

These autoantibodies may also play a pathogenic role in systemic lupus erythematosus being associated with nephritis development [3].

Cryoglobulins

Cryoglobulins are immunoglobulins which precipitate *in vitro* at temperature < 37°C. Brouet classification of cryoglobulins distinguishes three types of cryoglobulinemia: type I refers to the presence of monoclonal immunoglobulins IgM, IgG, rarely IgA, and light chains without rheumatoid factor (RF) activity. This type usually associates with hematologic malignancies; type II is characterized by the presence of polyclonal immunoglobulins associated with the monoclonal IgM with the RF activity; type III represents a mixture of polyclonal IgM and IgG.

Types II and III are considered mixed cryoglobulinemia and are related to viral infections (mainly hepatitis C virus) and connective tissue diseases [17].

The main symptoms of cryoglobulinemia were first described by Meltzer et al. [18] and are known as a triad of rash, arthralgia, and weakness. More recent observations differentiate between two clinical syndromes caused by circulating cryoglobulins: hyperviscosity syndrome and cryoglobulinemic vasculitis.

Hyperviscosity syndrome is the main clinical presentation of type I of cryoglobulinemia (although most patients are asymptomatic) and includes manifestations such as digital ischemia, Raynaud's phenomenon, renal failure, and neurological symptoms e.g., vision disorders, deafness, confusion, and coma [19].

Cryoglobulinemic vasculitis develops in patients with type II and III cryoglobulinemia and is illustrated by Raynaud's phenomenon, livedo reticularis, palpable purpura, arthralgia, myalgia, glomerulonephritis, and peripheral neuropathy. Sometimes the course might be very severe including rapidly progressive glomerulonephritis, central nervous system vasculitis, and/or pulmonary vasculitis [20].

Cryoglobulins are detected using a simple laboratory protocol that is based on *in vitro* observation of cold precipitation in serum [20].

Other autoantibodies

Anti-endothelial cell antibodies (AECA) are detected in various types of systemic vasculitis including AAV, Kawasaki's disease, Behçet's disease, IgA vasculitis, polyarteritis nodosa, Takayasu arteritis, or giant cell arteritis. They may play a pathogenic role in vasculitis as well as serve as biomarkers of disease activity. However, because of the lack of specificity for any disease, high heterogeneity, and methodological aspects (specific and sensitive, well-standardized immunoassays are not available for the routine use), these autoantibodies are not included in the AAV and other vasculitides diagnostic protocol [21].

Similarly, the clinical value of other autoantibodies like antiphospholipid, antinuclear, anti-ferritin, anti-laminin, or anti-alpha-enolase is still unclear and further studies are needed to determine their clinical and diagnostic potential [3].

Conclusions

Autoantibodies are very useful diagnostic markers for small vessel vasculitis including AAV and immune complex vasculitis. They also may serve as biomarkers of disease activity and indirectly support therapeutic decisions. However, there is a wide spectrum of serological markers with unclear clinical potential.

No specific autoantibodies have been shown for medium and large vessel vasculitis.

Take home messages

The most important information about the role of autoantibodies in small vessel vasculitides diagnosis and management are listed below:

(1) Autoantibodies associated with clinical manifestations and prognosis in vasculitis are ANCA, anti-GBM, anti-C1q, and cryoglobulins.

(2) The AAVs are: GPA, MPA, and EGPA.

(3) According to new ANCA testing recommendations monospecific immunoassays for PR3- and MPO-ANCA are the first-line tests in AAV.

(4) IIF is not the recommended screening method for AAV and might be used as a second-line test in case of negative or low positive results of PR3 and MPO immunoassays.

(5) ANCA can be present in other than vasculitis clinical disorders.

(6) ANCA may be useful in assessment of activity of the disease in patients with renal involvement and treated with rituximab, however, the therapeutic decisions should be based on clinical evaluation and not on serological markers only.

(7) Serological variants (PR3-AAV and MPO-AAV) provide very useful information regarding disease phenotype, clinical course, outcomes, and treatment effectiveness.

(8) The clinical value of other autoantibodies like AECA, antiphospholipid, antinuclear, and others is still unclear and not included in the vasculitis diagnostic protocol.

Abbreviations

AAV: anti-neutrophil cytoplasmic antibodies-associated vasculitis

ANCA: anti-neutrophil cytoplasmic antibodies

anti-C1q: anti-complement component C1q antibodies

anti-GBM: anti-glomerular basement membrane antibodies

EGPA: eosinophilic granulomatosis with polyangiitis

GPA: granulomatosis with polyangiitis

IIF: indirect immunofluorescence

MPA: microscopic polyangiitis

MPO: myeloperoxidase

MPO-AAV: myeloperoxidase-anti-neutrophil cytoplasmic antibodies-associated vasculitis

MPO-ANCA: myeloperoxidase anti-neutrophil cytoplasmic antibodies

P-ANCA: perinuclear-anti-neutrophil cytoplasmic antibodies

PR3: proteinase 3

PR3-AAV: proteinase 3-anti-neutrophil cytoplasmic antibodies-associated vasculitis

PR3-ANCA: proteinase 3-anti-neutrophil cytoplasmic antibodies

Declarations

Author contributions

KF: Conceptualization, Formal analysis, Writing—original draft, Writing—review & editing. MB: Validation, Writing—review & editing, Supervision. Both of the authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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