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Review

Anti-neutrophil cytoplasm antibodies (ANCA): Recent methodological advances—Lead to new consensus recommendations for ANCA detection

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ABSTRACT

The current practice for detection of anti-neutrophil cytoplasm antibodies (ANCA) directed against proteinase 3 (PR3) and myeloperoxidase (MPO) has been screening by indirect immunofluorescence (IIF) followed by an antigen specific tests for PR3- and MPO-ANCA. However, ANCA diagnostics have undergone many technical developments that have affected the 1999 international consensus recommendations, and lead to a revision of the existing ANCA detection strategy. Recent European multicentre studies have compared the diagnostic performance of various ANCA detection methods and demonstrated that PR3- and MPO-ANCA immunoassays yielded the highest diagnostic accuracy. New guidelines for ANCA testing have been developed based on these data. According to the revised 2017 international consensus recommendations, testing for ANCA in small vessel vasculitis can be done by PR3- and MPO-ANCA immunoassays, without the categorical need for IIF. Thus, IIF can be discarded completely, or can be used as confirmation assays instead a screening test.

Clearly, though, the new testing strategy for ANCA in vasculitis must identify the ANCA target antigen, as PR3- and MPO-ANCA serotype correlate well with disease expression. Furthermore, recent studies have shown that AAV can be classified based on ANCA serotype, since PR3- and MPO-ANCA- diseases are strongly associated with distinguishable genetic alleles, different clinical and histological features. ANCA presence and the antigen specificity also may have important value as a prognostic factor and may serve as a guide for immunosuppressive therapy.

In the current review, we summarize the novelties in ANCA testing, present the 2017 revised international consensus on ANCA testing in vasculitis, evaluate the diagnostic significance of ANCA, and discuss the role of ANCA serotypes in the diagnostic work-up of patients with AAV.

1. Introduction

Since the 1980s, the detection of anti-neutrophil cytoplasm antibodies (ANCA) is a well-established diagnostic test for a distinct form of small vessel vasculitis (ANCA-associated vasculitis = AAV), including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA). Patients with GPA are predominantly proteinase 3 (PR3)-ANCA-positive, whereas those with MPA and EGPA are predominantly myeloperoxidase (MPO)-ANCA-positive, although ANCA specificity overlaps only partially with these clinical syndromes (Csernok et al., 2006; Kallenberg, 2015).

However, the spectrum of disorders with positive ANCA results has since broadened to include a range of other inflammatory and infectious diseases, calling into question the diagnostic implications of ANCA positivity. Furthermore, the diagnostic utility of ANCA detection depends on the type of immunoassays performed and the clinical setting (Kallenberg, 2015).

According to the 1999 international recommendations for ANCA detection, screening for ANCA is generally done by IIF, and a positive IIF test should be followed by an antigen-specific test (Savige et al., 1999, 2003). Since the publication of this international consensus on ANCA testing, many new developments in detection of PR3- and MPO-ANCA have come to light. In general, currently available antigen-specific immunoassays are more sensitive and specific than IIF testing for diagnosing AAV, and this has raised the questions with respect to the positioning of ANCA IIF. In an effort to address these problems, a multicenter study by the European Vasculitis Study Group (EUVAS) has investigated the diagnostic value of PR3- and MPO-ANCA tests and IIF. Data of that study confirmed that PR3- and MPO-ANCA immunoassays

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are equal or even exceed the diagnostic performance of IIF (Csernok et al., 2016, Damoiseaux et al., 2017, Bossuyt et al., 2017a). These results enabled us to revise the existing international recommendations and to develop of a new testing strategy for ANCA in vasculitis (Bossuyt et al., 2017a, 2017b).

Whereas PR3-and MPO-ANCA are highly specific for GPA and MPA, these autoantibodies have very little diagnostic value in non vasculitic conditions. ANCA detection in a population with a low pretest probability of small vessel vasculitis is expected to yield a large number of ANCA positive results (McAdoo et al., 2012; Cohen Tervaert and Damoiseaux, 2012).

The prognostic value of the use of serial ANCA monitoring in the prediction of disease relapse is still controversial (reviewed in Fussner and Specks, 2015). Currently, several studies are ongoing that investigate the value of serial ANCA measuring for guiding treatment in GPA. Data from these trials will show the utility of ANCA levels in monitoring treated and relapsing disease.

The ANCA-associated vasculitides are a heterogeneous group of rare syndromes characterized by necrotizing inflammation of small/medium-sized blood vessels and the presence of ANCA. Several clinical and pathological classification systems exist that aim to define homogeneous groups among patients with AAV, however, considerable debate surrounds this classification. Existing classification systems have relied on combinations of different clinical, radiographic and histological findings, but have not included ANCA specificity. Accumulating evidence suggests that ANCA specificity could be better than clinical diagnosis for defining groups of patients, as PR3-ANCA and MPO-ANCA are associated with different genetic backgrounds, epidemiology, and pathogenesis (reviewed in Cornec et al., 2016).

In this review, we will discuss recent developments in ANCA detection, their diagnostic significance, their role in disease classification, and their value in follow-up of patients with PR3-ANCA associated vasculitis.

2. New developments in the methodology of ANCA testing

The 1999 international consensus for ANCA detection recommended screening by IIF on ethanol-fixed neutrophils, and if positive, follow up with PR3- and MPO-ANCA immunoassays (Savige et al., 1999). Although this consensus is still widely applied, in the last decade the position of IIF is being questioned. Since adequate ANCA detection requires distinct methods (the combination of IIF with antigen-specific assays) that are most often performed step-wise, the diagnostic work-up of a new patient can be time consuming and delayed. Furthermore, a positive immunofluorescence ANCA result is less frequently associated with a diagnosis of small vessel vasculitis than a positive PR3-/MPO-ANCA ELISA (Holle et al., 2010a, 2010b). The lack of expertise in ANCA pattern interpretation in even experienced laboratories is an ongoing issue and IIF is labour intensive and the automation is not yet worldwide available. Consequently, in many centres ANCAIIFT has already been discarded (Novikov et al., 2016).

Furthermore, several new developments in the ANCA detection techniques, in particular PR3- and MPO-ANCA detection, such as addressable-laser-bead immuno-assays (ALBIA), chemiluminescent immuno-assays (CLIA), dot-/line-immuno-assays, and fluorescent-enzyme immuno-assays (FEIA) have been introduced (for review Cohen Tervaert and Damoiseaux, 2012; Radice et al., 2013; Csernok and Moosig, 2014). Besides, there have been advances in assay setup (antigen presentation) with development of second (capture-based) and third (anchor-based) generation assays. Most often these novel assays have been clinically evaluated as an isolated entity, and this obviously hampers comparability due to distinct sample selection, study design and data analysis.

In a multicenter EUVAS evaluation, the performance of manual and automated IIF was compared to the performance of various antigen-specific immunoassays for ANCA detection. Four international vasculitis centers [Klinikum Bad Bramstedt, Germany; Staten Serum Institute, Denmark; Maastricht University Medical Centre, The Netherlands, and University Hospitals Leuven, Belgium] contributed newly diagnosed GPA (total n = 186) and MPA (total n = 65) patients and relevant diseased controls (total n = 924). The antigen-specific immunoassays evaluated (n = 8) were from 7 manufacturer's and included first-, second-, and third-generation assays applied on different technological platforms (ALBIA, CLIA, ELISA, and FEIA). For manual IIF, two different approaches were used. The original approach described in 1989 (Copenhagen) used an ethanol-fixed mixture of neutrophils and lymphocytes, whereas the other approach (Bad Bramstedt) used ethanol-fixed neutrophils in combination with additional tests on formalin-fixed neutrophils and HEp-2 cells to better discriminate between MPO-related P-ANCA and ANA reactivity (Wiik et al., 1993; Csernok and Moosig, 2014).

The results of the study revealed a large variability between IIF methods and variation in pattern assignment between IIF methods was observed (Csernok et al., 2016). The area under the curve (AUC; 95% CI) of the receiver operating characteristics (ROC) curve to discriminate AAV from controls considerably differed for the two IIF methods with AUC values of 0.925 (0.904–0.946) and 0.848 (0.821–0.876), respectively. By contrast, PR3- and MPO-ANCA by immunoassay had a somewhat higher diagnostic performance, with AUCs (95% CI) that only varied between 0.936 (0.912–0.960) and 0.959 (0.941–0.976), except for one immunoassay for which the AUC was 0.919 (0.892–0.945) (Fig. 1) (Damoiseaux et al., 2017). This study, which was performed on diagnostic samples obtained from patients without immunosuppressive treatment, did not reveal consistent differences between different assay generations and formats. It should be mentioned that there were some AAV patients that tested negative by both IIF and immunoassay or by either immunoassay or IIF. Thus, a diagnosis of AAV cannot be excluded for ANCA-negative patients.

In conclusion, the comparison of various ANCA detection methods showed that the diagnostic performance of IIF has been overtaken by that of PR3- and MPO-ANCA immunassays, and provided strong evidence for using high-quality antigen specific immunoassays for the detection of ANCA in patients suspected of necrotizing vasculitis, without the categorical need for IIF. Finally, these data challenged the role of IIF in the ANCA testing algorithm, and consequently, the earlier international consensus on ANCA detection utilizing dual IIF/antigen –specific immunoassay testing of each sample, was revised (Fig. 2). The aim of the revised consensus recommendations was to develop a more efficient test algorithm that provides a superior sensitivity and specificity for the diagnosis of AAV.

3. Revised 2017 international consensus on ANCA testing in small vessel vasculitis

The novel guidelines for ANCA testing in vasculitis have been developed based on the results obtained by the recent large EUVAS multicenter study (Csernok et al., 2016; Damoiseaux et al., 2017; Bossuyt et al., 2017a, 2017b). In summary, this new dataset provides the evidence for using high-quality immunoassays for the screening of patients suspected of AAV. Based on our data, a consensus was reached that screening each sample by both IIF and antigen specific immunoassays is not necessary. The detection of ANCA for the diagnosis of AAV required only tests for PR3- and MPO-ANCA, without the categorical need for IIF. Therefore, the ANCA detection algorithm currently used will be replaced by the new algorithm (Fig. 2).

We recognize that in some cases, dual PR3-/MPO-ANCA/IIF testing of a patient serum may be appropriate, despite negative antigen specific immunoassays results. For example, a patient with a high clinical index of suspicion for AAV and PR3- and MPO-ANCA negative results should be further tested by IIF. IIF results should than be interpreted in the specific clinical context of the patient in question.

The original document was prepared by EUVAS group of experts