Antineutrophil Cytoplasmic Autoantibodies: How Are They Detected and What Is Their Use for Diagnosis, Classification and Follow-up?

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Abstract Antineutrophil cytoplasmic antibodies (ANCA) are traditionally detected by an indirect immunofluorescence technique. According to the international consensus on ANCA testing, ANCA should also be tested by antigen-specific tests for myeloperoxidase-ANCA and proteinase 3-ANCA. The direct noncompetitive enzyme-linked immunosorbent assay (ELISA) used to be the method of choice. Nowadays, these assays are called "first-generation" assays. Second-generation tests (capture ELISA) or third-generation tests (anchor ELISA) are more sensitive and specific for ANCA testing. We postulate that ANCA as detected by these newer ANCA tests may replace the need to perform indirect immunofluorescence-based assays. For classification of patients, ANCA serotype seems more important than classifying patients according to their clinical subtype, since genetics, clinical manifestations and response to therapy are more related to ANCA serotype than to clinical subtype. Detection of ANCA to monitor disease activity is still a controversial issue. Treatment based on ANCA levels is at present only experimentally performed in those patients who are treated with B-cell depletion therapy with rituximab. Future studies are needed to establish whether this way of monitoring patients is warranted.

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Patients with systemic vasculitis affecting small- to medium-sized blood vessels can be classified as having either vasculitis that is associated with antineutrophil cytoplasmic autoantibodies (ANCA; ANCA-associated vasculitis—AAV), vasculitis that is associated with circulating cryoglobulins or vasculitis that is associated with IgA deposition in blood vessels (IgA vasculitis; Henoch–Schönlein purpura—HSP). Whereas in cryoglobulin-associated vasculitis and in HSP immune complexes can be detected both in the circulation and in the tissue lesions [1, 2], the lesions in AAV are considered to lack immune deposits and are hence labelled "pauci-immune". Indeed, in renal lesions in patients with AAV, immune deposits are, generally, not observed as detected by immunofluorescence (IF) testing of these tissues. More recently, however, it became clear that immune deposits as detected by electron microscopy may be present [3]. Furthermore, more than a decade ago, we demonstrated that in skin lesions in patients with AAV, immune deposits are not infrequently found [4]. So, it cannot be excluded that also in AAV, immune complex formation plays a role during (initial) lesion development [5].

A hallmark of AAV, however, is the detection of ANCA in the circulation. ANCA in AAV recognise two different antigens: proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA). Already soon after the discovery of ANCA in AAV, it became clear that patients with MPO-ANCA differ from patients with PR3-ANCA with respect to clinical manifestations, disease course and response to therapy. Whereas in patients with PR3-ANCA as well as in patients with MPO-ANCA, pauci-immune necrotizing crescentic glomerulonephritis and systemic vasculitis may occur,

patients differ with respect to extrarenal manifestations. Patients with PR3-ANCA have more often a generalized vasculitis involving many different organ systems and have typically necrotizing lesions in the respiratory tract. In contrast, patients with MPO-ANCA more often have asthma and polyposis nasi [6, 7]. Recently, it became clear that patients with PR3-ANCA and MPO-ANCA have a different genetic background [8] suggesting that a classification based on serotype of ANCA may be the preferred way to classify patients with vasculitis [9]. AAV, either PR3-ANCA associated or MPO-ANCA associated, comprises granulomatosis with polyangiitis (GPA, Wegener's), eosinophilic granulomatosis with polyangiitis (EGPA, Churg–Straus syndrome) and microscopic polyangiitis (MPA). Patients with PR3-ANCA-associated GPA clearly differ from patients with MPO-ANCA-associated GPA [10]. From this, it seems logic to classify patients in the future based on ANCA serotype and not on descriptive disease characteristics. However, also a new classification based on ANCA serotypes has disadvantages since it is clear that a substantial proportion of patients presenting with AAV do not have detectable ANCA in the circulation. This is especially true for patients with EGPA [11, 12] and for patients with localized GPA [13, 14]. Treatment strategies, however, do not yet differ between patients with either PR3-ANCA- and MPO-ANCA-associated AAV. In contrast, therapy is at present adjusted to disease severity and disease extent irrespective of the underlying diagnosis and/or ANCA serotype [15]. Treatment of AAV traditionally consists of cyclophosphamide and prednisolone. This form of therapy improved the outcome of AAV dramatically: at least 80–90 % of the patients survive at 1 year whereas only less than 20 % of the patients survived in the past without therapy. During the last two decades, attempts have been made to replace cyclophosphamide because of its severe toxic effects by other strategies [15]. At present, there is good evidence that cyclophosphamide may be replaced by B-cell depleting therapy (i.e. rituximab) [16].

Methods of ANCA Detection

ANCA are traditionally detected by an indirect immunofluorescence (IIF) technique [17]. For this, serum samples of patients are incubated with either total white blood cells or neutrophilic granulocytes that are fixed in ethanol. The substrate is then incubated with an anti-human IgG antibody which is conjugated with fluorescein isothiocyanate. Subsequently, in patients with AAV, either a cytoplasmic (C-ANCA) or perinuclear (P-ANCA) staining pattern can be observed when using fluorescence microscopy. In patients with vasculitis, a C-ANCA staining pattern is most often caused by PR3-ANCA, whereas a P-ANCA staining pattern is often the result of MPO-ANCA. MPO-ANCA cause the P-ANCA pattern due to an artefact during ethanol fixation since MPO (a strongly cationic enzyme) binds to the negatively charged nuclear membrane. This artifactual P-ANCA pattern can be avoided when neutrophils are fixed with formalin. In that case, MPO remains in the cytoplasmic granules and thus MPO-ANCA cause a C-ANCA pattern after formalin fixation. The IIF technique is subjective, labour intensive and requires expertise of the technicians involved. Automation is possible but not yet worldwide available [18].

According to the international consensus on ANCA testing, ANCA should be tested both by an indirect immunofluorescence test and subsequently by antigen-specific tests for MPO-ANCA and PR3-ANCA [19]. Antigen-specific tests of the first generation are direct noncompetitive enzyme-linked immunosorbent assays (ELISA). As in many other autoimmune diseases, the direct noncompetitive ELISA is the method of choice for detection of antigen-specific antibodies (Fig. 1) [17]. In this assay, the antigen is solid phase bound to the microtiter plates and unoccupied protein-binding sites on the carrier are blocked to prevent nonspecific binding of antibodies. This type of ELISA is referred to as "direct ELISA" and is considered a true quantitative assay for ANCA detection. When the reference standard for these ANCA tests is used, the laboratory is able to report the results in international units. During the last decade, multiple variants of the direct ELISA have become available. As carriers for the purified antigens, membranes may be used in dot-blot assays [20] and/or line immunoassays. Also, beads may be used as carrier in multiplex flow cytometry [21]. In the direct ELISA, the anti-human IgG reagent is, generally, conjugated with enzymes such as horseradish peroxidase or alkaline phosphatase. Otherwise the enzyme can be replaced by a fluorochrome for use in fluorescent enzyme immunoassays [22] or Luminex-based technology [21]. Finally, chemiluminescence technology [23] has become available and latex turbidimetry is currently developed for high-throughput testing of ANCA.

The second-generation tests are so-called capture ELISAs (Fig. 1). In these tests, a monoclonal antibody (MoAb) specific for MPO or PR3 is coated to the carrier and, next, the antigen preparation, i.e. either a crude extract or a purified antigen, is added. The antigen is captured by the MoAb, hence the name. In 1988, we firstly reported the use of capture ELISAs for ANCA detection [24]. It appeared that this assay was especially very sensitive to detect PR3-ANCA [25]. Furthermore, ANCA as detected by capture ELISA for PR3-ANCA, MPO-ANCA, and elastase ANCA appeared to be very specific for systemic vasculitis [25–27]. Theoretically, the advantage of the capture technology is better consolidation of the three-dimensional structure of the antigen and an additional purification of the antigen by the use of the capturing MoAb when compared with a direct
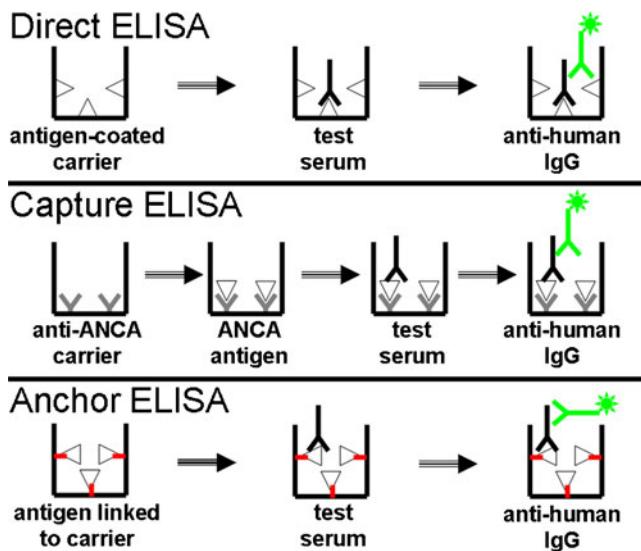
Fig. 1 PR3-ANCA and MPO-ANCA detection by antigen-specific assays. The purified antigen, either PR3 or MPO, is in the direct enzyme-linked immunosorbent assay (*ELISA*) directly coated to the carrier (2a) or in the capture ELISA via a capturing monoclonal antibody (2b), or in the anchor ELISA via a peptide linker (2c). Incubation with the patient serum and the final detection reagent is similar in all three assays

ELISA. For detection of PR3-ANCA, both the sensitivity and specificity are increased when the capture ELISA is compared with the direct ELISA [28]. However, for MPO-ANCA, capture ELISA seems to be more specific but not more sensitive for ANCA detection in AAV [29]. Importantly, high PR3-ANCA levels as measured by a capture ELISA but not high PR3-ANCA levels as measured by a direct ELISA are associated with decreased patient survival in patients with AAV with renal involvement [30]. Furthermore, PR3-ANCA as detected by capture ELISA is a better marker for disease reactivation than direct ELISA [21, 31–33]. Different MoAbs are currently being used for detection of PR3- or MPO-ANCA [34–36]. As antigen, in most cases, purified antigens are nowadays used. Since PR3 recognition by ANCA depends on the intact conformation of the antigen, its recognition by MoAbs can be affected. Lee et al. therefore developed a capturing system in which a MoAb to C-myc is used and where the antigen is recombinant PR3 that is C-myc tagged [37]. More recently, third-generation ANCA assays were introduced which are referred to as anchor ELISAs (Fig. 1). In these assays, the antigen is conjugated to a peptide linker which is used for coating to the carrier. The bridging molecule may be either undisclosed [38, 39] or a HIS tag [40]. Indeed, these anchor ELISAs show an increased sensitivity for PR3-ANCA when compared to direct ELISAs and probably also when compared to capture ELISAs [38–41].

Finally, for patients who present with rapidly progressive onset of renal and/or pulmonary manifestations, a quick test for ANCA is needed. In these patients it is important to have results available within 24 h. Because rapidly progressive glomerulonephritis and/or alveolar haemorrhage have a high pretest probability for the diagnosis of AAV, it may be sufficient to test only with antigen-specific assays for PR3-ANCA and MPO-ANCA and to omit the IIF test. Since the Goodpasture's syndrome is also within the differential diagnosis of the renal–pulmonary syndrome, it is advised to include additional testing for anti-GBM antibodies. Although nowadays most automates for autoantibody testing have random access and thereby enable rapid testing in the routine setting, there are also several rapid tests assays available for laboratories that have not yet automated their ANCA diagnostics [20, 42–44]. Generally, these tests are based on dot blots in which PR3 or MPO is directly coated to a carrier. Recently, for rapid screening, also a biochip technology assay has become available, in which the immunofluorescence test is combined with a dot blot test for PR3, MPO and the Goodpasture antigen [44]. Results obtained in the rapid tests are often qualitative only and should eventually be confirmed and quantified by the assays in use for routine testing.

ANCA as a Diagnostic Tool

ANCA has been used specifically for diagnosing necrotizing crescentic glomerulonephritis (NCGN), GPA, EGPA and MPA. Furthermore, ANCA testing is also sometimes used for other conditions such as cocaine-induced midline destructive disease, pseudomonas carriage in cystic fibrosis and/or as a diagnostic test for ulcerative colitis and/or primary sclerosing cholangitis. The different diseases will be discussed separately.

(a) NCGN

In 1990, we reported the occurrence of PR3 or MPO-ANCA in 32 consecutive patients with NCGN [25]. ANCA as determined by a capture ELISA was not found in patients with post-infectious CGN or other causes of CGN ($n=10$). Twenty-one patients tested positive for PR3-ANCA, and 11 patients had MPO-ANCA. Importantly, patients with NCGN that was secondary to biopsy-proven GPA all had PR3-ANCA whereas 75 % of the patients with NCGN without extrarenal manifestations (renal limited NCGN) had MPO-ANCA. More recently, we tested serum samples of 115 consecutive patients with biopsy-proven pauci-immune NCGN by direct, capture and anchor ELISA [40]. In these, 58 patients had PR3-ANCA whereas 57 patients had MPO-ANCA. Testing for PR3-ANCA using the different testing systems did not reveal major differences in this cohort of patients. It is suggested in Europe that in the northern part of Europe, PR3-ANCA is much more common

than in the southern part, whereas the opposite is true for MPO-ANCA [45]. Also in China and Japan, MPO-ANCA is much more prevalent [46, 47].

We found also a remarkable difference in the relative occurrence of both serotypes in our two patient cohorts with biopsy-proven NCGN [25, 40]. The first one [25] was from the north of The Netherlands and the second one from the south of the Netherlands [40]. The differences in our small country, the Netherlands, can probably not be related to geographical factors and suggest another environmental factor [48, 49]. Indeed, in the southern part but not in the northern part of The Netherlands mining industry used to be important. Since mining is associated with silica and since silica is especially associated with the occurrence of MPO-ANCA [48], we postulate that the difference in the two serotypes between the two cohorts may be due to mining.

Finally, patients with PR3- and MPO-ANCA-negative NCGN may test ANCA negative due to the use of direct ELISAs to detect PR3- or MPO-ANCA, and it has been postulated that ANCA negativity in these cases is actually the consequence of the use of ANCA tests of the first generation. Indeed, some of these cases are negative in the direct ELISA but positive in capture ELISA and/or anchor ELISA.

(b) GPA

In GPA that is associated with glomerulonephritis, ANCA is nearly always detected. In these cases, virtually all patients do have PR3-ANCA [25]. There is, however, a proportion of patients who do not have PR3-ANCA but have MPO-ANCA. Especially in China, it was found that 60 % of the patients with GPA had MPO-ANCA, whereas 38 % tested positive for PR3-ANCA [50]. In patients with early systemic or limited GPA, virtually all patients also test positive for ANCA, either PR3-ANCA or MPO-ANCA [51, 52]. In patients with loco-regional GPA, however, ANCA is clearly not detected in all patients [13, 14]. These loco-regional GPA patients may have either PR3-ANCA, elastase ANCA or ANCA of other specificities. At least one third of these patients, however, do test completely negative, both in the immunofluorescence test and in antigen-specific tests in which different antigens are used. Indeed, ANCA is not found in a substantial proportion of patients with chronic nasal obstruction, bloody rhinorrhea, chronic *Staphylococcus aureus* carriage and chronic ulcerative mucosal lesions in the nose. These patients, however, generally can be treated with maintenance cotrimoxazole therapy suggesting that these patients suffer from a mild loco-regional form of GPA. Although patients with subglottic trachea stenosis or pseudotumor of the orbit may test positive for ANCA, also some patients with these loco-regional manifestations of GPA may test negative for ANCA. Finally, patients with

multiple necrotic lung nodules may be ANCA negative. In these latter cases, however, an extremely careful search for other diagnosis such as tuberculosis or malignancy should be carried out.

(c) EGPA

In 1990, we reported the occurrence of MPO-ANCA in EGPA [7, 26, 53]. In these early cases, MPO-ANCA-associated EGPA was reported to be a severe form of systemic vasculitis which was often associated with NCGN. Later on, studies reported that only a proportion of patients with EGPA tested positive [11]. Indeed in our recent report on 32 patients with EGPA, we detected MPO-ANCA in 41 % of our patients, whereas the remaining patients were ANCA negative [12]. Patients who test positive for MPO-ANCA have more often glomerulonephritis, whereas cardiac involvement is more often found in MPO-ANCA-negative cases [12]. In addition, patients with localized EGPA, i.e. chronic nasal obstruction with bloody rhinorrhea, nasal polyposis and mild eosinophilia, test generally negative for MPO-ANCA. Occasionally, however, patients with severe adult-onset asthma, nasal polyposis and eosinophilia without clear manifestations of systemic vasculitis do test positive for MPO-ANCA, suggesting that these cases have an early phase of EGPA.

(d) MPA

When patients with systemic vasculitis affecting small- to medium-sized blood vessels cannot be classified as having either GPA, EGPA, HSP, cryoglobulin-associated vasculitis and/or vasculitis secondary to infection and/or another systemic autoimmune disease such as rheumatoid arthritis, patients must be classified *per exclusionem* as suffering from MPA. Most of these patients test positive for MPO-ANCA or PR3-ANCA [54]. These patients generally present with NCGN. However, also cases with a more limited presentation are being diagnosed as MPA, e.g. with cutaneous vasculitis, pulmonary vasculitis or vasculitis affecting the peripheral nervous system ("mononeuritis multiplex"). Within the group of patients with MPA, patients who test positive for PR3-ANCA, generally, have more necrotizing lesions in the respiratory tract compared to patients with MPO-ANCA whereas patients with MPO-ANCA have more often either nasal polyposis or chronic bronchial obstruction [6]. Furthermore, eosinophilia is more often found in patients with MPO-ANCA-associated MPA. These findings suggest indeed that a classification based on ANCA serotype is a preferred way to classify these patients compared to the classic classification into either GPA, EGPA or MPA.

(e) Miscellaneous

Elastase ANCA as detected by capture ELISA is only very infrequently found in patients suspected of systemic

vasculitis [27]. Elastase ANCA, however, is a specific finding for systemic vasculitis since elastase ANCA is nearly never found in other conditions [27]. Elastase ANCA points to the occurrence of drug-induced vasculitis [55, 56]. In drug-induced vasculitis not only elastase ANCA can be found but often also MPO-ANCA, PR3-ANCA, and/or ANCA directed against other specificities. Indeed, in propylthiouracil-induced ANCA, the presence of ANCA directed against many different specificities is related to the occurrence of vasculitis whereas the presence of ANCA related to only one antigen is, generally, not related to clinical manifestations of vasculitis [57]. Furthermore, elastase ANCA is specifically found in cocaine-induced midline destructive disease [58]. In these patients chronic nasal obstruction, bloody rhinorrhea, mucosal ulceration of the nose and chronic *S. aureus* carriage is, generally, found. Remarkably, in some of these cases, also destruction of the palatum molle occurs. Cocaine not only induces chronic midline disease but also can be associated with a systemic form of necrotizing vasculitis in which either PR3-ANCA, MPO-ANCA and/or the combination of PR3-ANCA and MPO-ANCA can be found [59, 60].

ANCA directed against human lysosomal-associated membrane protein-2 (hLAMP-2-ANCA) can be found in patients with NCGN [61]. Both patients with MPO-ANCA and patients with PR3-ANCA-associated NCGN test positive for hLAMP-2-ANCA. Importantly, also sometimes, patients that have NCGN without the presence of either MPO-ANCA or PR3-ANCA test positive for hLAMP-2 ANCA. Recently, the specificity of hLAMP-2-ANCA, however, was discussed since hLAMP-2 ANCA was also observed in patients with HSP [62].

ANCA with specificity for lactoferrin can be found in a wide variety of chronic inflammatory disorders [63]. Within the patient population who suffer from diarrhoea, however, lactoferrin ANCA is specifically found in patients who suffer from inflammatory bowel disease. Teegen et al. found lactoferrin ANCA in 28 of 39 cases who suffer from ulcerative colitis [64]. In this latter study, Teegen et al. used lactoferrin-reconstituted granulocytes as substrate. Lactoferrin ANCA may not only be found in patients with ulcerative colitis but also in patients with primary sclerosing cholangitis, a disorder that is often associated with ulcerative colitis [65, 66].

Finally, ANCA with specificity for bactericidal permeability/increasing protein (BPI-ANCA) is also found in a wide variety of chronic inflammatory disorders. In patients with chronic lung diseases, e.g. cystic fibrosis, the occurrence of BPI-ANCA is associated with pseudomonas carriage and predicts poor outcome [67].

Importantly, different forms of systemic vasculitis are typically ANCA negative, i.e. HSP, cryoglobulin-associated vasculitis, isolated cutaneous leukocytoclastic vasculitis and (cutaneous) polyarteritis nodosa. In polyarteritis nodosa, the presence of ANCA indicates that the patient is actually suffering from MPA since ANCA in these cases indicates vasculitic involvement of small vessels as well [7]. Although MPO-ANCA and PR3-ANCA are highly specific for either NCGN, GPA, EGPA and/or MPA, it must be stressed that these ANCA serotypes are also found in patients who suffer from subacute bacterial endocarditis. These patients with endocarditis that test positive for ANCA often have also signs of small vessel vasculitis such as glomerulonephritis and/or cutaneous vasculitis [68].

Serial Measurement of ANCA Titers: a Useful Tool?

Many different studies demonstrated that patients with PR3-ANCA have a higher risk of relapse during follow-up than patients with MPO-ANCA [69]. Furthermore, it is clear that patients with GPA more often suffer from relapses than patients with MPA. Even within cohorts of patients classified as GPA or MPA, PR3-ANCA as compared to MPO-ANCA is associated with an increased risk of relapse [10]. During the last decade, it has been demonstrated in animal models of AAV that MPO-ANCA or PR3-ANCA in itself is sufficient to induce vasculitis and/or glomerulonephritis [70, 71]. Also, animal studies point out that additional factors may serve as a "second hit". Huugen et al. demonstrated that LPS aggravated the vasculitic process in MPO-ANCA-induced vasculitis in mice [72]. These findings suggest that ANCA persistence in patients may result in continuation of the vasculitic process. ANCA persistence, however, is often subclinical and becomes manifested only after additional factors. An infection therefore may result in the reoccurrence of the vasculitis. Indeed, several studies demonstrated that the persistence of ANCA is related to the occurrence of relapses in the future [73, 74]. The positive likelihood ratio, however, for persistence of ANCA during remission for subsequent disease relapse is only 1.97 (95 % CI 1.43, 2.70) as was recently estimated during a meta-analysis [74]. Interestingly, persistence of MPO-ANCA in patients during remission is not related to subsequent relapses but we found that patients with persistently high levels of MPO-ANCA are more prone to develop end-stage renal disease compared to those patients in which MPO-ANCA levels decreased during follow-up [75]. After induction therapy with cyclophosphamide, azathioprine maintenance therapy is started [76]. When maintenance therapy is started while the patient is ANCA positive, the risk to develop a relapse is more than two times higher than when the patient is ANCA negative at that time [69, 77]. Fortunately, a proportion of patients become ANCA negative during induction therapy and remain persistently ANCA negative. These patients are clearly at a lower risk of the development of a relapse [73,

77]. In many patients, however, ANCA reappear during tapering of the maintenance immunosuppressive therapy and/or after therapy has been stopped. These patients have an increased risk to develop relapses. Indeed, in more than 80 % of patients with AAV, relapses occur while ANCA tests are or have become positive again.

The usefulness of measuring ANCA titers to predict disease activities and/or to guide therapy is very controversial. Absolute titers correlate only weakly with disease severity. Rising titers of PR3-ANCA occurring during clinical remission predicts relapses in patients with GPA [78, 79]. In our original prospective study over 16 months involving 35 patients with GPA, we observed 17 relapses. All 17 relapses were preceded by a fourfold rise of ANCA as detected by the IIF technique [78]. In our subsequent prospective study, we followed 58 patients with GPA for a period of 2 years [79]. In this period, 20 patients had a fourfold rise of ANCA. Eleven patients were not pre-emptively treated, and in 6 of those patients a relapse occurred within a period of 6 months after the ANCA rise. During later follow-up, after more than 12 months, an additional three patients suffered from a relapse. In contrast, in those patients that were pre-emptively treated with a 9-month course of cyclophosphamide in combination with a 3-month course of prednisolone, none suffered a relapse. Importantly, in these studies, the close relation between ANCA and relapses occurred in patients that were treated with cyclophosphamide induction and maintenance therapy [78, 79]. The close relation between ANCA and relapses, however, could not be found in other cohorts in which patients were treated with other forms of maintenance therapy consisting of azathioprine, MTX and/or other drugs [80, 81]. Importantly, in a prospective study, we could demonstrate that a 9-month course of azathioprine and a 4.5-month course of prednisolone was able to postpone but not prevent relapses [10]. Recently, it was suggested that during maintenance therapy with B-cell depletion using rituximab, a close correlation between ANCA and relapses is present similar as observed during maintenance therapy with cyclophosphamide [16, 82]. More studies, however, are needed to confirm this latter finding.

For MPO-ANCA it has also been demonstrated that relapses generally occur after an ANCA rise [26, 80, 83]. Large prospective studies with respect to MPO-ANCA are, however, lacking. The largest study in the literature on this subject was performed by Terier et al. [83]. In contrast to our studies [78–80], this study was unblinded and not prospective. Results, however, were encouraging since they observed that relapses were associated with an increase in MPO-ANCA level in 10 out of 11 patients. Importantly, up to 60 % of cases of relapse occurred within 12 months after reappearance of MPO-ANCA [83].

In a meta-analysis performed on 9 out of 41 articles in which the relation between ANCA and relapses was studied, it was concluded that the positive likelihood ratio for a rise of ANCA (either c-ANCA/PR3-ANCA or p-ANCA/MPO-ANCA) during remission on a subsequent relapse of disease was 2.84 (95 % CI 1.65–4.90) [74]. In addition, it was found that the absence of an ANCA rise does not exclude disease reactivation since the negative likelihood ratio of an ANCA rise for a subsequent relapse was only 0.49 (95 % CI 0.27, 0.87) [74]. So, the risk of a relapse after a well-documented ANCA rise is substantial but clearly not 100 %. Furthermore, relapses may occur without a preceding ANCA rise.

Most of these studies are based on ANCA detection as performed using either the IIF technique or an ELISA technique of the first generation. The predictive value of an ANCA rise as detected by tests using the second or third generation of ANCA tests is slightly better for predicting an ensuing relapse. However, it is clear that more studies in this respect are needed. Furthermore, additional studies regarding epitopes [84, 85], IgG subclasses [80], avidity [86], affinity [87] and/or sialylation [88] of ANCA are needed to be tested in prospective studies.

Conclusion

ANCA are extremely useful tests for the diagnosis of primary small vessel vasculitides, collectively referred to as AAV. At present, there is international consensus that ANCA should be detected by a combination of IIF and antigen-specific assays, i.e. detection of PR3- and MPO-ANCA. Recently, new developments have influenced the detection of ANCA and it can be postulated that ANCA as detected by second- and/or third-generation ANCA tests may replace the need to perform IIF as a screening assay. For classification of patients, ANCA serotype, i.e. PR3-ANCA or MPO-ANCA, may be more important than classifying patients according to their clinical subtype, i.e. GPA, MPA or EGPA, since genetics, clinical manifestations and response to therapy seem to be more related to ANCA serotype than to clinical subtype. The detection of ANCA to monitor disease activity is still a controversial issue. Treatment based on ANCA levels is at present only experimentally performed in those patients who are treated with B-cell depletion therapy with rituximab. Future studies are needed to establish whether this way of monitoring patients is warranted.

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