Antineutrophil cytoplasmic antibodies (ANCA) – their role in pathogenesis, diagnosis, and treatment monitoring of ANCA-associated vasculitis

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Abstract

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) constitutes a group of rare diseases characterized by necrotizing inflammation of small blood vessels and the presence of ANCA. Increasing clinical and experimental evidences support their pathogenic role in AAV, but the exact mechanism is not fully understood. Recently, the important role of neutrophil extracellular traps (NETs) in pathogenesis of AAV is underlined. There is an indication that NETs can be a source for the formation of ANCA. The most common ANCA target antigens are myeloperoxidase (MPO) and proteinase 3 (PR3). Though the mechanism of action of ANCA is still under exploration, ANCA serology is being increasingly used for classification of AAV and revealed as kenner in defining various disease subsets associated with different genetic background, clinical features, treatment response, and prognosis. Controversy exists regarding the utility of serial measurements of ANCA in patients with AAV to monitor treatment and predict disease relapse.

Key words: *ANCA, vasculitis, pathogenesis, diagnosis, treatment monitoring.*

(Cent Eur J Immunol 2020; 45 (2): 218-227)

Introduction

The antineutrophil cytoplasmic autoantibodies (ANCA)-associated vasculitides (AAV) comprise a group of diseases characterized by necrotizing vasculitis of small vessels, with frequent involvement of the kidneys, lungs, lack or paucity of immune deposits in the vessel wall, and the presence – in the majority of cases – of autoantibodies to neutrophil constituents [1]. The consequence is ischemia and necrosis of organs supplied by these vessels. Clinically and pathologically, three forms are traditionally recognized such as granulomatosis with polyangiitis (GPA, formerly called Wegener's granulomatosis), which is distinguished by necrotizing granulomas on biopsy and frequent upper respiratory tract involvement, microscopic polyangiitis (MPA) mainly with kidney involvement, and eosinophilic granulomatosis with polyangiitis (EGPA, formerly called Churg-Strauss syndrome), which is much less common and distinguished by a prominent eosinophilia and allergic features [1].

Characteristic for AAV is the presence of ANCA. They were first reported in 1982 by Davies *et al.* [2] in serum from a few patients with segmental necrotizing glomeru-

lonephritis. The data was largely disregarded until Van der Woude *et al.* [3] described similar patterns of autoantibodies in patients with systemic vasculitis (GPA) in 1985. Then, myeolproxidase (MPO) and proteinase (PR3) were recognized as the major autoantigens accounting for the perinuclear (p-ANCA) and cytoplasmic (c-ANCA) patterns, respectively [4, 5]. ANCA in serum were originally detected by indirect immunofluorescence (IIF), and a positive IIF staining of only neutrophils without lymphocytes distinguishes ANCA from other types of autoantibodies. Currently, they are widely considered as a laboratory test biomarker in the diagnosis of patients suspected with vasculitis.

Although ANCA are characteristic feature for AAV, they not always occur in AAV patients: c-ANCA are detected in 95% of cases of new onset GPA, and p-ANCA in 80% of patients of new onset MPA, and only in 40% of EGPA [4-6]. Additionally, PR3-AAV clearly differs in its clinical presentation from MPO-AAV. Franssen *et al.* [7] observed that patients with PR3-ANCA have, in comparison with patients positive for MPO-ANCA, more organs involved, resulting in faster decline in renal function, more frequent relapses of the disease, and in particular, granulomatous nec-

rotizing inflammation in the airways. Recently, an emerging concept in AAV found a relationship between disease serotype and phenotype [8]. Since the manifestations, clinical course and relapse rates correlate closely with target antigen, with some clinicians using the terms PR3- or MPO-AAV rather than GPA, MPA, and EGPA [9].

The discovery of ANCA have revolutionized the diagnostic process of AAV, but beyond a diagnostic serological marker, clinical and experimental data support a pathogenic role for ANCA, which promote activation of primed neutrophils and monocytes and their adhesion to the endothelium, leading to subsequent tissue damage [10, 11]. Although various hypotheses with different kinds of triggers have been suggested concerning ANCA formation, none has been confirmed to date. In contrast to an established, although not fully understood, role of ANCA in pathogenesis of vasculitis, their role for monitoring disease activity and treatment results of patients with AAV remains still unclear. Rising titers of c-ANCA were shown to predict a relapse of disease [12], and pre-emptive treatment with cyclophosphamide and steroids based on rising titers of c-ANCA was able to prevent the occurrence of relapses [13]. Other authors, however, could not confirm a strong correlation between rising titers and ensuing relapses [14, 15]. In this review summary of current knowledge about the role of ANCA in pathogenesis, diagnosis and monitoring of treatment of AAV was presented.

ANCA and their role in pathogenesis of AAV

How ANCA can lead to the development of AAV

A breakthrough regarding the pathogenicity of ANCA was based on the discovery by Falk *et al.* [10] who proved that ANCA can activate primed neutrophils to produce reactive oxygen species (ROS) and the release of lytic enzymes. Antigens such as microbial components or complement stimulate ANCA autoantigen expression on the membrane surface of neutrophils [16, 17]. ANCA are thereby developed and released from B cells and activate neutrophils by binding to the ANCA autoantigens. Once activated, neutrophils attach to the endothelium of blood vessels, and release ROS and inflammatory cytokines leading to systemic vasculitis and multiple organ injuries [18]. The signaling pathways involved in priming are not fully known, but the activation of the p38 mitogen-activated protein kinase, extra signal regulated kinase, and phosphoinositol-3 kinase appear to be important steps in the translocation of ANCA antigens to the cell membrane [19, 20]. Although neutrophils play a key role in the pathogenesis of AAV, it has been demonstrated that other immune cells (e.g., T cells and monocytes) are also involved [21].

The strong evidence that MPO-ANCA are involved in AAV pathogenesis comes from studies using MPO-defi-

cient mice [22, 23]. Additionally, in a human case study, a newborn was reported who developed pulmonary-renal syndrome with placental transmission of MPA-ANCA [24]. In PR3-AAV, the pathogenic role of PR3-ANCA is not fully confirmed [25-28], but although no sufficient experimental model can be used, there are some clinical arguments confirming this relation. The first comes from some observations that the persistence of ANCA after induction of remission in patients with AAV is associated with relapse of the disease [29]. Another argument comes from two studies describing the efficacy of rituximab, a B-cell-depleting monoclonal antibody in patients with severe, active AAV [30, 31]. Both showed that rituximab was not inferior to cyclophosphamide treatment (regardless of the route of administration), and may be more effective in severe, with impending renal insufficiency, and relapsing disease [30, 31]. Although the results of B-cell depletion may be more extensive, its efficacy in AAV can be explained at least in part by its effect on ANCA production.

The mechanism of ANCA formation in AAV

There is considerable evidence that ANCA are involved in the pathogenesis of AAV, but the mechanism leading to their formation is unclear. Experimental [32] and epidemiological [33] studies have provided strong supportive evidence that infections may trigger exacerbations of AAV and may even be the main pathogenetic mechanism of ANCA formation. The presence of transient immunoglobulin M (IgM) PR3-ANCA with an acute respiratory manifestation of AAV also suggests a possible link between an infectious trigger and AAV disease activation [34].

One factor that has been linked to GPA and anti-PR3 antibodies is nasal carriage of *Staphylococcus aureus* (*S. aureus*). Stegeman *et al.* [29] first noted that 63% of patients with GPA were chronic nasal carriers of *S. aureus*, and that this carriage was associated with a higher relapse rate, with an adjusted relative risk of 7.16. Furthermore, it has been shown that a treatment with cotrimoxazole could prevent the occurrence of relapse [35]. The main influence of *S. aureus* on AAV activity is mainly attributed to its superantigens (SAgs), an extremely potent immunostimulatory molecules [36, 37], but so far, the results of clinical studies evaluating a critical role of SAgs for AAV activity have not been unequivocal [38, 39].

The mechanisms underlying the relationship between *S. aureus* and AAV remain unclear, but numerous theories have been postulated [16, 40]. Autoantigen complementary has been suggested as one of the mechanisms that can break a tolerance to ANCA antigens. According to this hypothesis, the initial immune response in patients with AAV is not directed towards the autoantigen, but rather to a peptide that is complementary to the autoantigen epitope. The complementary peptide immunogen could arise from antisense transcription of autoantigen gene or exist as mi-

crobial exogenous peptide that mimics the complementary antisense peptide. Antibodies to a complementary protein may induce anti-idiotypic antibodies that cross-react with the original protein. Another hypothesis is based on molecular mimicry. It has been theorized that similarities between antibodies to pathogens and epitopes of self-antigens may lead to cross-reactivity and mounting an autoimmune response. Such molecular mimicry may also be the primary mechanism in the development of focal necrotizing glomerulonephritis in patients with ANCA directed against human lysosome membrane protein (hLAMP-2) [16].

Recently, the important role of neutrophil extracellular traps (NETs) in the pathogenesis of AAV is underlined. There are evidences supported the concept that NETs can be a source for the formation of ANCA. NETs were first described in 2004 and were initially associated with antibacterial role in bacteria trapping [41]. These formations characterize a special type of neutrophil-related cell death named NETosis, which in neutrophils leads to a production of a meshwork of chromatin fibers associated with citrullinated histone H3 and antimicrobial proteins, including MPO and PR3. This meshwork forms extracellular nets that trap and kill microbial pathogens [16]. In 2009, a study demonstrated that NETs released after in vitro ANCA-induced neutrophil activation contained MPO and PR3 [42]. Another study showed that MPO and PR3 transfer from NETing neutrophils into dendritic cells, with subsequent generation of anti-MPO-ANCA and development of autoimmune vasculitis in animals [44]. Additionally, PR3 and elastase containing NETs have been detected in affected human glomeruli [42]. Results of recent studies showed that simultaneous stimulation of neutrophils by different NETosis-induced agents result in diminished formation of NETs, as compared to a single stimulation. This indicates that cells may possess an internal regulatory mechanism that prevent overgeneration of NETs among healthy people [44].

On the other hand, the relation between ANCA and NETs in clinical studies is not evident. Kraaij *et al.* [45] investigated NET formation in 99 patients with AAV. They found a significant excess of ex vivo NET formation in both MPO-ANCA- and PR3-ANCA-positive patients with AAV, as compared to healthy individuals. Excessive NET formation did not correlate with serum ANCA levels. Likewise, immunoglobulin G depletion had no effect on excessive NET formation in patients with AAV, indicating an ANCA-independent process. Next, they demonstrated that excessive NET formation was predominantly found during active disease, more so than during remission. Excessive NET formation was found in patients with AAV hospitalized for disease relapse, but not during severe infection. Authors concluded that excessive NET formation in AAV was independent of ANCA, and an excess of ex vivo NET formation was related to active clinical disease in patients with AAV and a marker of autoimmunity rather than infection.

ANCA and genetics

Recently investigated by genome-wide association study (GWAS) methods showed that the pathogenesis of AAV has a genetic component, and that the association of these genetic polymorphism is related to the ANCA type. PR3-AAV is strongly associated with HLA-DP (human leukocyte antigens-DP) and the genes encoding for α-1-antitrypsin (*SERPINA1* gene) and PR3 (*PRTN3* gene), while MPO-AAV is significantly associated with HLA-DQ [8, 46]. Whereas EGPA is associated with HLA-DRB1*04 and HLA-DRB4 [47, 48], it suggests a strong CD4+T lymphocyte activation, possibly triggered by allergens or antigens. Patients with AAV are also more likely to carry polymorphism in CTLA4 and PTPN22, which are thought to be "general" susceptibility factors associated with autoimmune disease. Ultimately, genetic studies indicated AAV as a polygenic disease and supported the pathogenicity of ANCA, with HLA and non-HLA genes variants predisposing to this condition [47]. Genetic associations suggest that AAV could be divided into several subsets beyond the classical phenotypic diagnoses of GPA, MPA, and EGPA. Particularly, some findings indicated that classifying patients according to ANCA status and specificity could be more pertinent in practice than phenotypic MPA or GPA diagnosis, since ANCA status might correspond better to differences in clinical presentations and outcomes [9]. However, this leads to various problems when considering the significant minority of patients with ANCA-negative disease. It would be of great interest to examine genetic associations in the ANCA-negative patients; however, numbers needed are likely to limit the ability to perform such study [47].

ANCA and their utility in the diagnostic process of AAV

ANCA as a preliminary diagnostic tool

ANCA have been recognized as an important diagnostic marker in patients with AAV. Therefore, patients presenting clinical features of systemic small-vessel vasculitis suspected of AAV are routinely tested for ANCA [49]. Moreover, target antigens of ANCA (PR3 and MPO) should be identified as they are helpful for reaching a diagnosis [50, 51]. On the other hand, ANCA are not a specific marker for AAV. Interestingly, detectable MPO- and PR3-ANCA but also ANCA directed against other autoantigens (such as lactoferrin) have been described in different kinds of non-vasculitis disorders with a controversial clinical relevance [52]. Furthermore, circulating autoantibodies against MPO and PR3 have been reported in healthy individuals and designated as "natural" autoantibodies [53]. Lower titre, lower avidity, and lack of IgG3 subclass of these autoantibodies suggest their non-pathogenic co-existence in serum, but whether ANCA are targeting these

epitopes remaining truly non-pathogenic at higher concentrations such as those observed in AAV, remains unclear [54]. As a result, an ANCA test should be performed only in the appropriate clinical settings to support diagnosis based on other disease observation [49].

The standard method for ANCA detection in serum is IIF on ethanol-fixed human neutrophils smeared onto glass slides [55]. A positive staining of ANCA can usually be described in three main patterns by IIF. First, a cytoplasmic pattern (c-ANCA) is detected as fluorescence staining in the cytoplasm of neutrophils and is mainly related to the presence of autoantibodies against PR3. The second pattern is perinuclear (p-ANCA) staining detected as fluorescence around the multi-lobed nucleus of neutrophils and is mostly caused by autoantibodies against MPO. The third method, atypical (a-ANCA) showing different patterns or a combination of both c-ANCA and p-ANCA patterns [56]. The overall sensitivity of IIF is approximately 67-78% and the specificity is nearly 93% [57]. Because both sensitivity and specificity of IIF remains unsatisfactory, a confirmatory test is needed. Enzyme-linked immunosorbent assay (ELISA) is one of the confirmatory tests for ANCA, which can identify both serum ANCA titers and their target antigens on neutrophils [56]. A combination of IIF and ELISA tests provides up to 92% of sensitivity and 99% of specificity [58]. Although the tests have extremely high sensitivity, ANCA are sometimes undetectable in patients with mild symptoms, especially those who present with granulomatous disease limited to the respiratory tract [59].

Based on the results obtained by the recent large European vasculitis study group (EUVAS) multicenter study [60-62], the novel guidelines for ANCA testing in vasculitis have been developed [63]. In brief, this new data set provides the evidence for using high-quality immunoassays for screening of patients suspected of AAV but screening each sample by both IIF and antigen specific immunoassays is not necessary. Where new onset vasculitis is suspected, the detection of ANCA required only tests for PR3- and MPO-ANCA, without categorical need for IIF. If both are negative, and there is still a strong suspicion of small vessel vasculitis, the use of other immunoassays and/or IIF, or a referral to an experienced laboratory is recommended [63]. The guidelines underlined that a positive PR3- and/or MPO-ANCA result contributes only to the diagnostic work-up for AAV, but is not diagnostic as such, and also negative ANCA tests (PR3 and MPO) cannot be the basis for AAV exclusion [63].

ANCA specificity as a basis of new phenotyping patients with AAV

Traditionally, three distinct AAV diseases have been distinguished based on clinical and pathological features: GPA, MPA, and EGPA [64]. In the last years, it became evident that ANCA specificity is associated with different genetic background, clinical features, treatment response, and prognosis, and it could be keener in defining different disease subsets than clinical diagnosis of GPA or MPA [65]. The basis of these findings are genetic data from GWAS and African American population pointing to genetic differences between PR3- and MPO-ANCA patients. This study showed that autoantigen specificities of PR3 and MPO correlate better with different HLA risk genes (PR3-ANCA with HLA-DP, MPO-ANCA with HLA-DQ) than with clinical and pathological phenotypes of GPA and MPA. Moreover, the association with genes encoding PR3 (*PRTN3*) and its inhibitor α-1-antitrypsin (*SERPINA1*) with PR3-ANCA disease and/or GPA additionally supports central pathogenetic role of autoantigens and their neutralizing counterparts [8].

Although few studies have indicated that ANCA subtype does not impact disease outcome [66, 67], accumulating evidences indicate that ANCA type better identifies AAV patients that the GPA/MPA classification [9, 68-73]. PR3-ANCA-positivity vasculitis is associated with destructive granulomatous inflammation, more upper airway involvement, more extensive extrarenal organ manifestation (on average four organs are involved), and a higher relapse risk. In contrast, MPO-ANCA-positivity vasculitis is frequent in kidney-limited disease with glomerular sclerosis and a worse renal prognosis. Patients with MPO-ANCA are more likely than patients with PR3-ANCA to have a profibrotic phenotype such as strong association with lung fibrosis. Among patients with clinical diagnosis of GPA, those positive for MPO-ANCA more frequently have limited disease without severe organ involvement, higher prevalence of subglottic stenosis (SGS), less frequent need for cyclophosphamide or rituximab therapy, and fewer relapses than patients who are PR3-ANCA-positive [9, 68-73]. Furthermore, PR3-ANCA patients respond better to rituximab for induction and maintenance of remission than cyclophosphamide/azathioprine, whereas these treatments were equally effective in MPO-ANCA patients [73, 74]. Finally, MPO-ANCA vasculitis is characterized by fewer relapses and is associated with poorer survival, as compared with relapsing PR3-ANCA vasculitis [9, 75, 76]. Detailed list of study results shows Table 1.

ANCA antigen specificity revealed to be more strongly associated with AAV clinical presentation and prognosis than the former diagnosis of the GPA or MPA. Therefore, there is a new proposal to use ANCA serotypes to classify disease and provide diagnosis based on the presence of PR3- and/or MPO-ANCA. That ANCA serotyping distinguishes distinct classes of ANCA disease: PR3-ANCA AAV, MPO-ANCA AAV, and ANCA-negative AAV [9, 65]. The identification of these distinct patients' subsets is the big step forward in AAV research and may be a first step towards development of precision medicine for AAV, which may help to better individualize the treatment in future.

Table 1. The role of ANCA as biomarker for diagnosis and in the context of treatment response in AAV patients

Study	PR-3 AAV	MPO-AAV
Lyons *et al.* [8] (*n* = 1233/1454)	HLA-DP, *SERPINA1*, *PRTN3*	HLA-DQ
Lionaki *et al.* [9] (*n* = 502)	Higher relapse risk	Lower relapse risk
Cordova-Sanchez *et al.* [68] (*n* = 62)	Higher relapse risk	Lower relapse risk
Puechal *et al.* [69] (*n* = 59)	Higher relapse risk	Lower relapse risk
Schirmer *et al.* [70] (*n* = 315)	More than two organs involvement, more need for aggressive treatment (CYC/RTX), higher relapse risk	More limited disease (more prevalence of SG), less need for aggressive treatment (CYC/RTX), lower relapse risk
Solans-Laqué *et al.* [71] (*n* = 450)	More upper airway involvement, higher relapse risk, higher survival	More renal involvement and AH, lower relapse risk, lower survival
Chang *et al.* [72] (*n* = 455)	Milder renal involvement, higher relapse risk, more constitutional symptoms	More severe renal involvement, lower relapse risk, fewer constitutional symptoms
Unizony *et al.* [73] (*n* = 197) (NCT 00104299 post-results)	Better remission induction and maintenance with RTX than CYC/AZA	Similar remission induction and maintenance with RTX vs. CYC/AZA
Miloslavsky *et al.* [74] (*n* = 197) (NCT 00104299)	More uncontrolled disease, higher relapse risk, better remission with RTX than CYC/AZA	Less uncontrolled disease, lower relapse risk, similar remission with RTX vs. CYC/AZA
	Neither ANCA titers nor B cell counts predicted disease flare	
Berti *et al.* [75] (*n* = 58)	Higher survival	Lower survival
Morgan *et al.* [76] (*n* = 252) (NCT 00430105)	Higher relapse risk	Lower relapse risk
	Becoming ANCA negative at the time of switching to maintenance therapy is associated with a reduced relapse risk	

ANCA – antineutrophil cytoplasmic antibody, AAV – antineutrophil cytoplasmic antibody-associated vasculitis, PR3 – proteinase 3, MPO – myeloperoxidase, CYC – cyclophosphamide, AZA – azathioprine, RTX – rituximab, SG – subglottic stenosis

ANCA and their clinical utility in EGPA

The role of ANCA in differentiation of two phenotypes of EGPA was described already in 2005. Two large studies [77, 78] demonstrated that ANCA were positive in 38% of EGPA patients, and ANCA-positive patients more frequently had peripheral neuropathy, glomerulonephritis, and purpura (so-called "vasculitic phenotype"), whereas endo-myocardial involvement and lung infiltration dominated in the ANCA-negative subset (so-called "tissular phenotype"). Apart from the distinct clinical manifestation, these phenotypes are characterized by different clinical course of disease and prognosis: vasculitic phenotype is characterized by frequent relapses and better outcomes, while in tissular phenotype, the relapses are less frequent, but the outcomes are worse. Further studies confirmed the differences in clinical disease presentation based on ANCA status and indicated that ANCA-positive patients should be treated more aggressively [79].

Based on these observations, members of the European Respiratory Society CSS-Task Force recently prepared recommendations for the diagnosis of EGPA, including a definition of detailed criteria of vasculitis (or surrogates of vasculitis) in patients with asthma and blood eosinophils greater than 1.5 G/l [80]. They proposed that patients with asthma and blood eosinophils greater than 1.5 G/l without ANCA, vasculitis, or surrogates of vasculitis should be named as hypereosinophilic asthma with systemic manifestations (HASM), non-vasculitis. Supporting these proposals, Cottin *et al.* [81] published in 2017 their results of retrospective study of 157 patients' population with EGPA and found that ANCA alone were an insufficient element to categorize patients with vasculitis features (47% of patients with systemic vasculitis features had no ANCA, while 29% of patients with MPO-ANCA had no vasculitis features) and in their opinion, the terminology of polyangiitis appears to be a misnomer for 41% of patients in this series. They fully approved the suggested criteria to separate patients with a prominent vasculitis phenotype (named as "EGPA") from patients with predominant hypereosinophilic manifestations including asthma (named as "HASM"). In this regard, the proposed definition could serve as a basis for individualized future therapeutic intervention in EGPA.

ANCA and their utility in monitoring of AAV activity

Advances in therapy have transformed AAV from a frequently fatal disease into a chronic illness. Current treatments caused a remission in more than 80% of AAV patients, with a 5-year overall mortality rate of 10-15%

[82]. Although it is possible for most patients to achieve a remission with therapy, 38% of cases experience a disease relapse within 5 years despite an expert care [83]. There are few serologic predictors of relapse in AAV [84-86], but so far, no predictor has been finally shown to reliably guide therapeutic decision-making.

The utility of ANCA status for monitoring AAV activity and guidance of treatment decisions is widely studied, but results are still controversial. Some studies demonstrated a relationship between an increase in levels of PR3-ANCA and the occurrence of relapses. Boomsma *et al.* [87] in a study on 100 GPA patients with PR3-ANCA observed that 26 out of 33 relapses were preceded by a rise in PR3-ANCA measured by ELISA (sensitivity, 79%), whereas 12 of 38 increases were not followed by a relapse (specificity, 68%). In turn, Stegeman *et al.* [29] showed that persistence of ANCA presents a relative risk of 9.0 for a relapse. In their retrospective study, Han *et al.* [88] reported similar results, and they stated that serial measurement of ANCA titers in patients with GPA during remission can help to predict relapses and pre-emptive increase in immunosuppression following fourfold titer reduced the risk of relapses. On the other hand, there are studies reporting that changes in serial PR3-ANCA titers not always correlated with disease activity. Kerr *et al.* [14] demonstrated that changes in serial ANCA titer temporally correlated with a change in disease status in only 64% of patients with AAV. Furthermore, an increase in the c-ANCA titer preceded clinical exacerbation of disease in only 24% of patients with remission or low-grade, smoldering disease. In turn, Thai *et al.* [89] observed that although ANCA were positive during most systemic relapses or residual disease, no strict clinical-immunological correspondence was observed for 25% of their patients. Authors concluded that GPA management cannot be based on ANCA levels alone. Whereas, Girard *et al.* [90] reported in their small prospective study that relapses were more frequent when ANCA remained positive or reappeared, the mean time between inclusion and relapse did not differ between the patients who became ANCA-negative and those who were persistently ANCA-positive, and the mean time to ANCA disappearance was similar for the patients who relapsed and those who did not. They underlined that although usually the progression of AAV was more frequent when ANCA remained positive or reappeared, discordance between ANCA and disease activity was not unusual. Finally, a meta-analysis of nine studies concluded that the value of serial measurements of ANCA in patients in remission is limited and neither the increase nor the persistence of ANCA in patients who achieved remission is highly predictive of relapse [91], therefore targeted determination of ANCA levels may be useful only if a relapse is clinically suspected [92]. Fussner *et al.* [93] confirmed these results, showing that an increase in PR3-ANCA levels is not an overly sensitive and specific predictor of a subsequent relapse in patients with GPA.

Although there is a relevance for ANCA monitoring as a marker of disease activity, there is only 50% ANCA elevation, which ended in relapses, and around half of relapses occur even in the absence of rises in ANCA titer [94]. Therefore, physicians monitoring ANCA are sometimes confused by the results and they do not know what they should do. The outcomes of prospective studies are helpful in these situations. According to them, PR3-ANCA are associated with higher risk of relapse, while patients with MPO-ANCA relapse less frequently and might benefit in the future from shorter term drug administration with less ANCA monitoring. The PR3-ANCA at diagnosis and 12 months persistence after starting induction were considered as parameters significantly influencing relapse occurrence [83, 89, 95].

Typically, high circulating titres of ANCA are seen during active AAV, with subsequent falls during therapy. These observations may support a pathogenic role of ANCA in AAV but on the other hand, ANCA are not detectable in approximately 20% of cases [96]. Moreover, clinical benefit following rituximab therapy is often seen before a significant reduction in ANCA titre [97] and has also been reported in ANCA-negative cases [98]. Additionally, although rituximab efficacy against AAV activity appeared to significantly correspond with the ANCA profile (and CD19 levels) of treated patients [99], the results of a prospective trial (MAINRISTAN2) showed that the attempt to adjust the infusion schedule to ANCA titers (and/or the presence of circulating CD19) was unreliable for relapse prevention [100]. These facts suggest the possibility that ANCA can be some phenomenon accompanying but not necessarily driving the disease. Thus, although a significant improvement has been made in our understanding about the role of ANCA in AAV, their final role in treatment monitoring and therapeutic decision-making needs further investigations.

Conclusions

In conclusion, ANCA have been recognized as an important biomarker to aid diagnosis in patients with AAV, a life-threatening small vessel vasculitis characterized by the presence of ANCA, and renal, lung and upper respiratory tract involvement. Most probably, they are involved in the pathogenesis of AAV, but the exact mechanism is not clear. The etiology and pathogenesis of this complex diseases appear to be multifactorial and involves the interplay of initiating and predisposing environmental and genetic factors, loss of immune tolerance, and mediation of acute injury. The big step forward in AAV research was undoubtedly the sub-classification and phenotyping of these complex diseases, associated with different genetic background, clinical features, treatment response, and

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prognosis in terms of relapse rate and survival rate. Identification of these distinct patients' subsets may be a first step towards development of precision medicine for AAV, which may be helping to better individualize the treatment in the future. Though ANCA can be used to assess prognosis in patients with AAV, the utility of their serial measurement to predict disease relapse remains controversial. Thus, therapeutic decision to increase immunosuppression should not be based solely on the levels of ANCA titers.

The authors declare no conflict of interest.

References

1. Jennette JC, Falk RJ (1997): Small vessel vasculitis. N Engl J Med 37: 1512-1523.
2. Davies DJ, Moran JE, Niall JF, Ryan GB (1982): Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbowirus aetiology? Br Med J 285: 606.
3. Van der Woude FJ, Rasmussen N, Lobatto LH, et al. (1985): Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1: 425-429.
4. Falk RJ, Jennette JC (1988): Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Engl J Med 318: 1651-1657.
5. Niles JL, McCluskey RT, Ahmad MF, Arnaout MA (1989): Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. Blood 74: 1888-1893.
6. Sinico RA, Di Toma L, Maggiore U, et al. (2005): Prevalence and clinical significance of antineutrophil cytoplasmic antibodies in Churg-Strauss syndrome. Arthritis Rheum 52: 2926-2935.
7. Franssen CFM, Stegeman CA, Kallenberg CGM, et al. (2000): Antiproteinase 3 – and antimyeloperoxidase-associated vasculitis. Kidney Int 57: 2195-2206.
8. Lyons PA, Rayner TF, Trivedi S, et al. (2012): Genetically distinct subsets within ANCA-associated vasculitis. N Eng J Med 367: 214-223.
9. Lionaki S, Blyth ER, Hogan SL, et al. (2012): Classification of antineutrophil cytoplasmic autoantibody vasculitides: the role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or proteinase 3 in disease recognition and prognosis. Arthritis Rheum 64: 3452-3462.
10. Falk RJ, Terrell RS, Charles LA, Jennette JC (1990): Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. Proc Natl Acad Sci 87: 4115-4119.
11. Nolan SL, Kalia N, Nash GB, et al. (2008): Mechanisms of ANCA-mediated leukocyte-endothelial cell interactions *in vivo*. J Am Soc Nephrol 19: 973-984.
12. Cohen Tervaert JW, van der Woude FJ, Fauci AS, et al. (1989): Association between active Wegener's granulomatosis and anticytoplasmic antibodies. Arch Intern Med 149: 2461-2465.
13. Cohen Tervaert JW, Huitema MG, Hene RJ, et al. (1990): Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. Lancet 336: 709-711.
14. Kerr GR, Fleischer TA, et al. (1993): Limited prognostic value of changes in antineutrophil cytoplasmic antibody titer in patients with Wegener's granulomatosis. Arthritis Rheum 36: 365-371.
15. Nowack R, Grab I, Flores-Suarez LF, et al. (2001): ANCA titres, even of IgG subclasses, and soluble CD14 fail to predict relapses in patients with ANCA-associated vasculitis. Nephrol Dial Transplant 16: 1631-1637.
16. Konstantinov KN, Ulff-Moller CJ, Tzamaloukas AH (2015): Infections and antineutrophil cytoplasmic antibodies: Triggering mechanisms. Autoimmun Rev 14: 201-203.
17. Jarrot P-A, Kaplanski G (2016): Pathogenesis of ANCA-associated vasculitis: An update. Autoimmun Rev 15: 704-713.
18. Dumoitier N, Terrier B, London J, et al. (2015): Implication of B lymphocytes in the pathogenesis of ANCA-associated vasculitides. Autoimmun Rev 14: 996-1004.
19. Mavropoulos A, Orfanidou T, Liaskos C, et al. (2013): p38 mitogen-activated protein kinase (p38 MAPK)-mediated autoimmunity: Lessons to learn from ANCA vasculitis and pemphigus vulgaris. Autoimmun Rev 12: 580-590.
20. Hao J, Meng LQ, Xu PC, et al. (2012): p38MAPK, ERK and PI3K signaling pathways are involved in C5a-primed neutrophils for ANCA-mediated activation. PLoS One 7: e38317.
21. McKinney EF, Willcocks LC, Broecker V, Smith KGC (2014): The immunopathology of ANCA-associated vasculitis. Semin Immunopathol 36: 461-478.
22. Xiao H, Heeringa P, Hu P, et al. (2002): Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest 110: 955-963.
23. Little MA, Smyth CL, Yadav R, et al. (2005): Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interaction *in vivo*. Blood 106: 2050-2058.
24. Schlieben DJ, Korbert SM, Kimura RE, et al. (2005): Pulmonary-renal syndrome in a newborn with placental transmission of ANCAs. Am J Kidney Dis 45: 758-761.
25. Rarok AA, Limburg PC, Kallenberg CG (2003): Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. J Leukoc Biol 74: 3-15.
26. Pfister H, Ollert M, Frohlich LF, et al. (2004): Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic *in vivo*. Blood 104: 1411-1418.
27. Van der Geld YM, Hellmark T, Selga D, Heeringa P (2007): Rats and mice immunized with chimeric human/mouse proteinase 3 produce autoantibodies to mouse Pr3 and rat granulocytes. Ann Rheum Dis 66: 1679-1682.
28. Tomer Y, Gilburd B, Blank M, et al. (1995): Characterization of biologically active antineutrophil cytoplasmic antibodies induced in mice. Pathogenetic role in experimental vasculitis. Arthritis Rheum 38: 1375-1381.
29. Stegeman CA, Cohen Tervaert JW, Sluiter WJ, et al. (1994): Association of nasal carriage of *Staphylococcus aureus* and higher relapse in Wegener's granulomatosis. Ann Intern Med 120: 12-17.
30. Stone JH, Merkel PA, Spiera R, et al. (2010): Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 363: 221-232.
31. Jones RB, Cohen Tervaert JW, Hauser T, et al. (2010): Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med 363: 211-220.

32. Herringa P, Little MA (2011): *In vivo* approaches to investigate ANCA-associated vasculitis: Lessons and limitations. Arthritis Res Ther 13: 204.

33. Chen M, Kallenberg CGM (2010): The environment, geoepidemiology and ANCA-associated vasculitides. Autoimmun Rev 9: A293-298.

34. Clain JM, Hummel AM, Stone JH, et al. (2017): Immunoglobulin (Ig)M antibodies to proteinase 3 in granulomatois with polyangiitis and microscopic polyangiitis. Clin Exp Immunol 188: 174-181.

35. Zycinska K, Wardyn KA, Zielonka TM, et al. (2008): Chronic crusting, nasal carriage of *Staphylococcus aureus* and relapse rate in pulmonary Wegener's granulomatosis. J Physiol Pharmacol 59 (Suppl 6): 825-831.

36. Popa ER, Cohen Tervaert JW (2003): The relation between *Staphyloccus aureus* and Wegener's Granulomatosis: current knowledge and future directions. Intern Med 42: 771-780.

37. Burian M, Grumann D, Holtfreter S, et al. (2012): Expression of staphylococcal superantigens during nasal colonization is not sufficient to induce a systemic neutralizing antibody response in humans. Eur J Clin Microbiol Infect Dis 2012; 31: 251-256.

38. Herlyn K, Buckert F, Gross WL, Reinhold-Keller E (2014): Doubled prevalence rates of ANCA-associated vasculitides and giant cell arteritis between 1994 and 2006 in northern Germany. Rheumatology (Oxford) 53: 882-889.

39. Fijolek J, Wiatr E, Petroniec V, et al. (2018): The presence of staphylococcal superantigens in nasal swabs and correlation with activity of granulomatosis with polyangiitis in own material. Clin Exp Rheumatol 36 (Suppl 111): 40-45.

40. Oliveira DBG (2018): Linked help from bcterial proteins drives autoantibody production in small vessel vasculitis. Med Hypotheses 112: 24-26.

41. Brinkmann V, Reichard U, Goosmann C, et al. (2004): Neutrophil extracellular traps kill bacteria. Science 303: 1532-1535.

42. Kessenbrock K, Krumbholz M, Schonermarck U, et al. (2009): Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med 15: 623-625.

43. Sangaletti S, Tripodo C, Chiodoni C, et al. (2012): Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. Blood 120: 3007-3018.

44. Pruchniak MP, Demkow U (2019): Potent NETosis inducers do not show synergistic effects *in vitro*. Centr Eur J Immunol 44: 51-58.

45. Kraaij T, Kamerling SWA, Van Dam LS, et al. (2018): Excessive neutrophil extracellular trap formation in ANCA-associated vasculitis is independent of ANCA. Kidney Int 94: 139-149.

46. Merkel PA, Xie G, Monach PA, et al. (2016): Identification of functional and expression polymorphisms associated with risk for anti-neutrophil cytoplasmic autoantibody-associated vasculitis. Arthritis Rheumatol 69: 1054-1066.

47. Salama A (2018): Genetics and pathogenesis of small-vessel vasculitis. Best Pract Res Clin Rheumatol 32: 21-30.

48. Vaglio A, Martorana D, Maggiore U, et al. (2007): HLA-DRB4 as a genetic risk factors for Churg-Strauss syndrome. Arthritis Rheum 56: 3159-3166.

49. Suwanochte S, Rachayon M, Rodsaward P, et al. (2018): Anti-neutrophil cytoplasmic antibodies and their clinical significance. Clin Rheumatol 37: 875-884.

50. Savige J, Trevisin M, Pollock W (2018): Testing and reporting antineutrophil cytoplasmic antibodies (ANCA) in treated vasculitis and non-vasculitic disease. J Immunol Methods 458: 1-7.

51. Csernok E, Mahrhold J, Hellmich B (2018): Anti-neutrophil cytoplasm antibodies (ANCA): recent methodological advance – lead to new consensus recommendations for ANCA detection. J Immunol Methods 456: 1-6.

52. Ooi CJ, Lim BL, Cheong WK, et al. (2000): Antineutrophil cytoplasmic antibodies (ANCAs) in patients with inflammatory bowel disease show no correlation with proteinase 3, lactoferrin, myeloperoxidase, elastase, cathepsin G and lysozome: A Singapore study. Ann Acad Med Singapore 29: 704-707.

53. Cui Z, Zhao MH, Segelmark M, Hellmark T (2010): Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. Kidney Int 78: 590-597.

54. Xu PC, Cui Z, Chen M, et al. (2011): Comparison of characteristics of natural autoantibodies against myeloperoxidase and anti-myeloperoxidase autoantibodies from patients with microscopic polyangiitis. Rheumatology (Oxford) 50: 1236-1243.

55. Savige J, Gillis D, Benson E, et al. (1999): International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). Am J Clin Pathol 111: 507-513.

56. Csernok E, Bossuyt X (2018): Investigations in systemic vasculitis. The role of the laboratory. Best Pract Res Clin Rheumatol 32: 52-62.

57. Stone JH, Talor M, Stebbing J, et al. (2000): Test characteristics of immunofluorescence and ELISA tests in 865 consecutive patients with possible ANCA-associated conditions. Arthritis Care Res 13: 424-434.

58. Feng Z, Liu P, Li Z, Siu B (2008): Clinical relevance of anti-PR3 capture ELISA in diagnosing Wegener's granulomatosis. J Clin Lab Anal 22: 73-76.

59. Hellmich B, Flossmann O, Gross WL, et al. (2007): EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: Focus on anti-neutrophil cytoplasm antibody-associated vasculitis. Ann Rheum Dis 66: 605-617.

60. Csernok E, Damoiseaux J, Rasmussen N, et al. (2016): Evaluation of automated multiparametric indirect immunofluorescence assays to detect anti-neutrophil cytoplasmic antibodies (ANCA) in granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). Autoimmun Rev 15: 736-741.

61. Damoiseaux J, Csernok E, Rasmussen N, et al. (2017): Detection of antineutrophil cytoplasmic antibodies (ANCAs): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. Ann Rheum Dis 76: 647-653.

62. Bossuyt X, Rasmussen N, van Paassen P, et al. (2017): A multicentre study to improve clinical interpretation of proteinase-3 and myeloperoxidase anti-neutrophil cytoplasmic atibodies. Rheumatology (Oxford) 56: 1533-1541.

63. Bossuyt X, Cohen Tervaert JW, Arimura Y, et al. (2017): Revised 2017 international consensus on testing of ANCA in small vessel vasculitis. Nat Rev Rheumatol 13: 683-692.

64. Jennette JC, Falk RJ, Bacon PA, et al. (2013): 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 65: 1-11.

65. Cornec D, Cornec-Le Gall E, Fervenza FC, Specks U (2016): ANCA-associated vasculitis-clinical utility of using ANCA specificity to classify patients. Nat Rev Rheumatol 12: 570-579.

66. Miloslavsky EM, Lu N, Unizony S, et al. (2016): Myeloperoxi-dase-antineutrophil cytoplasmic antibody (ANCA)-positive and ANCA-negative patients with granulomatosis with polyangiitis (Wegener's): Distinct patient subsets. Arthritis Rheumatol 68: 2945-2952.

67. Heijl C, Mohammad AJ, Westman K, Hoglund P (2017): Long-term patients survival in a Swedish population-based cohort of patients with ANCA-associated vasculitis. RMD Open 3: e000435.

68. Cordova-Sanchez BM, Mejia-Vilet JM, Morales-Buenrostro LE, et al. (2016): Clinical presentation and outcome prediction of clinical, serological, and histopthological classification schemes in ANCA-associated vasculitis with renal involvement. Clin Rheumatol 35: 1805-1816.

69. Puechal X, Pagnoux C, Perrodeau E, et al. (2016): Long-term outcomes among participants in the WEGENT trial of remission-maintenance therapy for granulomatosis with polyangiitis (Wegener's) or microscopic polyangiitis. Arthritis Rheumatol 68: 690-701.

70. Schirmer JH, Wright MN, Herrmann K, et al. (2016): Myeloperoxydase-antineutrophil cytoplasmic antibody (ANCA)-positive granulomatosis with polyangiitis (Wegener's). Is a clinically distinct subset of ANCA-associated vasculitis? A retrospective analysis of 315 patients from a German Vasculitis Referral Center. Arthritis Rheumatol 68: 2953-2963.

71. Solans-Laqué R, Fraile G, Rodriguez-Carballeira M, et al. (2017): Clinical characteristics and outcome of Spanish patients with ANCA-associated vasculitides: impact of the vasculitis type, ANCA specificity, and treatment on mortality and morbidity. Medicine (Baltimore) 96: e6083.

72. Chang D-Y, Li Z-Y, Chen M, Zhao M-H (2019): Myeloperoxidase-ANCA-positive granulomatosis with polyangiitis is a distinct subset of ANCA-associated vasculitis: a retrospective analysis of 455 patients from a single center in China. Semin Arthritis Rheum 48: 701-706.

73. Unizony S, Villarreal M, Miloslavsky EM, et al. (2016): Clinical outcomes of treatment of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis based on ANCA type. Ann Rheum 75: 1166-1169.

74. Miloslavsky EM, Specks U, Merkel PA, et al. (2013): Clinical outcomes of remission induction therapy for severe antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 65: 2441-2449.

75. Berti A, Cornec D, Crowson CS, et al. (2017): The epidemiology of ANCA- associated vasculitis in olmsted county, Minnesota (USA): a 20 year population-based study. Arthritis Rheumatol 69: 2338-2350.

76. Morgan MD, Szeto M, Walsh M, et al. (2017): Negative anti-neutrophil cytoplasm antibody at switch to maintenance therapy is associated with a reduced risk of relapse. Arthritis Res Ther 19: 129.

77. Sinico RA, Di Toma L, Maggiore U, et al. (2005): Prevalence and clinical significance of antineutrophil cytoplasmic antibodies in Churg-Strauss syndrome. Arthritis Rheum 52: 2926-2935.

78. Sable-Fourtassou R, Cohen P, Mahr A, et al. (2005): Antineutrophil cytoplasmic antibodies and the Churg-Strauss syndrome. Ann Intern Med 143: 632-638.

79. Sokolowska B, Szczeklik W, Wludarczyk A, et al. (2014): 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 centre. Clin Exp Rheumatol 32 (Suppl 82): 41-47.

80. Cordier J-F, Cottin V, Guillevin L, et al. (2013): Eosinophilic granulomatosis with polyangiitis (Churg-Strauss). Presse Med 42: 507-510.

81. Cottin V, Bel E, Bottero P, et al. (2017): Revisiting the systemic vasculitis in eosinophilic granulomatosis with polyangiitis (Churg-Strauss). A study of 157 patients by the Groupe d'Etudes et de Recherche sur les Maldies Orphelines Pulmonaires and the European Respiratory Society Task force on eosinophilic granulomatosis with polyangiitis (Churg-Strauss). Autoimmun Rev 16: 1-9.

82. Guillevin L (2017): Maintenance treatment of ANCA-associated vasculitides. Clin Exp Rheumatol 35 (Suppl 103): 199-201.

83. Walsh M, Flossmann O, Berden A, et al. (2012): Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 64: 542-548.

84. Suzuki K, Nagao T, Itabashi M, et al. (2014): A novel autoantibody against moesin in the serum of patients with MPO-ANCA-associated vasculitis. Nephrol Dial Transplant 29: 1168-1177.

85. Ahn SS, Jung SM, Song JJ, et al. (2018): Neutrophil to lymphocyte ratio at diagnosis can estimate vasculitis activity and poor prognosis in patients with ANCA-associated vasculitis: a retrospective study. BMC Nephrol 19: 187.

86. Park HJ, Jung SM, Song JJ, et al. (2018): Platelet to lymphocyte ratio is associated with current activity of ANCA-associated vasculitis at diagnosis: a retrospective monocentric study. Rheumatol Int 38: 1865-1871.

87. Boomsma MM, Stegeman CA, van der Leij MJ, et al. (2000): Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: A prospective study. Arthritis Rheum 43: 2025-2033.

88. Han WK, Choi HK, Roth RM, et al. (2003): Serial ANCA titers: Useful tool for prevention of relapses in ANCA-associated vasculitis. Kidney Int 63: 1079-1085.

89. Thai L-H, Charles P, Resche-Rigon M, et al. (2014): Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis (Wegener's) relapses? Results of a retrospective study on 126 patients. Autoimmun Rev 13: 313-318.

90. Girard T, Mahr A, Noel LH, et al. (2001): Are antineutrophil cytoplasmic antibodies a marker predictive of relapse in Wegener's granulomatosis? A prospective study. Rheumatology 40: 147-151.

91. Tomasson G, Grayson PC, Mahr AD, et al. (2012): Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis-a meta-analysis. Rheumatology (Oxford) 51: 100-109.

92. Verstockt B, Bossuyt X, Vanderschueren S, Blockmans D (2015): There is no benefit in routinely monitoring ANCA titers in patients with granulomatosis with polyangiitis. Clin Exp Rheumatol 33 (Suppl 89): 72-76.

93. Fussner LA, Hummel AM, Schroeder DR, et al. (2016): Factors determining the clinical utility of serial measurements of ANCA targeting proteinase 3. Arthritis Rheum 68: 1700-1710.

94. Aggarwal A, Sharma A, Rathi M, et al. (2015): Antineutrophil cytoplasmic antibodies (ANCA): Role in disease pathogenesis, diagnosis, and monitoring ANCA associated vasculitis. Indian J Rheumatol 10: 48-53.

95. Slot MC, Tervaert JWC, Boomsma MM, Stegeman CA (2004): Positive classic antineutrophil cytoplasmic antibody (c-ANCA) titer at switch to azathioprine therapy associated with relapse in proteinase 3-related vasculitis. Arthritis Rheum 51: 269-273.

96. Langford C (2012): Clinical features and diagnosis of small-vessel vasculitis. Clev Clin J Med 79 (Suppl 3): 3-7.

97. Jones RB, Ferraro AJ, Chaudhry AN, et al. (2009): A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 60: 2156-2168.

98. Khan A, Lawson CA, Quinn MA, et al. (2010): Successful treatment of ANCA-negative Wegener's granulomatosis with rituximab. Int J Rheumatol 2010: 846063.

99. Stone JH, Merkel PA, Spiera R, et al. (2010): RAVE-ITN Research Group. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 363: 221-232

100. Charles P, Terrier B, Perrodeau E, et al. (2018): Comparison of individually tailored versus fixed-schedule rituximab regimen to maintain ANCA-associated vasculitis remission: Results of a multicentre, randomised controlled, phase III trial (MAINRITSAN2). Ann Rheum Dis 77: 1143-1149.