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Serological and pathophysiological aspects of ANCA associated vasculitis – a historical review

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Contributors: C.G.M. Kallenberg
F.J. van der Woude
A. Wiik
E. Csernok
N. Olschowka

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EDITORIAL

In 1982 an Australian group around D.J. Davies reported staining of neutrophil cytoplasm to be a characteristic diagnostic finding in 8 patients with a generalized illness associated with segmental necrotizing glomerulonephritis. This staining had not been previously described, and because the patients mainly came from the Murray River valley, the authors concluded that this condition was probably related to epidemic polyarthritis caused by the Ross River virus. Two years later, Hall and coworkers found the same staining in 4 patients with systemic vasculitis, all of whom had pulmonary symptoms. Van der Woude and colleagues were the first to suggest that ANCA yielding a diffuse granular cytoplasmic fluorescence pattern on ethanol-fixed neutrophils was a specific marker for Wegener's granulomatosis (WG) and a useful tool to assess disease activity.

We are proud to have been able to win so many European leaders in the field of ANCA to write for this journal: It starts with a review by Cees G.M. Kallenberg from Groningen, Netherlands, on the clinical characteristics of ANCA-associated vasculitis and Fokko van der Woude himself describes the first steps in ANCA detection on page 8. In their articles on page 10 and page 13, respectively, Alan Wiik from Copenhagen and Elena Csernok from Bad Bramstedt, Germany, describe the diagnosis of ANCA-associated vasculitides and different tests for the detection of ANCA that are routinely used today.

In spring 2003 Pharmacia Diagnostics launched four new ANCA assays: Varelisa PR3 and MPO ANCA, as well as the fully automated EliA PR3 and EliA MPO. PR3 and MPO ANCA on both Varelisa and on EliA are highly precise, observer-independent ANCA tests with a minimum of labour intensity and a maximum of flexibility for the laboratory. On page 14 some background data on the development of these assays is presented. Several studies from independent laboratories have been initiated to evaluate the performance of the assays in daily routine and to gain further knowledge on monitoring vasculitides with the help of ANCA. The results of these studies will be presented at an ANCA symposium organized by Pharmacia Diagnostics in September this year.

Enjoy reading,
Your Elias Journal editorial team.

Clinical characteristics of ANCA-associated vasculitis

C.G.M. Kallenberg

Department of Clinical Immunology, University Hospital Groningen,
P.O. Box 30.001, 9700 RB Groningen, The Netherlands

Introduction

The primary systemic vasculitides are a group of heterogeneous disorders of unknown etiology characterized by more or less wide-spread inflammation of the vessel wall. A clinical classification of the various disease entities within this group has been proposed by the American College of Rheumatology (ACR) and is based on the presence of particular clinical symptoms and histopathological findings [1]. These so-called ACR-criteria for the classification of vasculitis are widely used but have their drawbacks with regard to disease specificity and sensitivity. Therefore, more precise definitions for the primary vasculitides have been proposed by a group of experts in this field in 1993 [2]. These definitions for the nomenclature of the vasculitides are known as the Chapel Hill Consensus Conference definitions and are now widely used as diagnostic criteria although they were not intended as such. Based on these definitions new diagnostic and classification criteria have to be developed.

Within the spectrum of the primary vasculitides (table 1) renal involvement is common, particularly in the small-vessel vasculitides [3]. Immunopathologically, Henoch-Schönlein purpura (HSP) and cryoglobulinemic vasculitis are characterized by immune deposits which are considered to play a major and initiating role in the development of renal lesions. The remaining small-vessel vasculitides show paucity or absence of immune deposits. These pauci-immune vasculitides, that is Wegener's Granulomatosis (WG), Churg-Strauss Syndrome (CSS), microscopic polyangiitis (MPA) and its renal limited form, are strongly associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA), in particular antibodies directed to proteinase 3 (PR3-ANCA) and to myeloperoxidase (MPO-ANCA) [4,5]. In this review I will discuss the clinicopathological correlates of the ANCA-associated vasculitides. More specifically, I will

focus on three subjects. First, I will discuss differences in clinical presentation, histopathologic findings and possible immunopathologic mechanisms between PR3-ANCA and MPO-ANCA associated vasculitides. Secondly, I will review risk factors for the occurrence of relapses as these factors may shed light on the induction of those relapses. Finally, new treatment modalities will be dealt with. Collaborative studies by the European Vasculitis Study Group (EUVAS) have resulted in more evidence based approaches to these complicated and life threatening diseases.

PR3-ANCA- and MPO-ANCA associated vasculitis - a comparison

As stated, the idiopathic pauci-immune necrotizing small-vessel vasculitides are strongly associated with ANCA directed to either PR3 or MPO. It has been noted that PR3-ANCA are predominantly found in patients with WG and MPO-ANCA in patients with MPA, its renal limited form, that is idiopathic necrotizing crescentic glomerulonephritis (NCGN), or Churg-Strauss Syndrome (CSS) [4,6-9]. Nevertheless, patients with WG incidentally have MPO-ANCA and PR3-ANCA do occur in patients with MPA and idiopathic NCGN [6-9], table 2 [4]. These associations led to the question whether patients with PR3-ANCA differ from those with MPO-ANCA with respect to clinical presentation, histopathological findings and clinical outcome. In order to approach this question, Franssen et al. [10] retrospectively analyzed clinical features, pattern of pre-treatment renal function loss, renal morphology and outcome in a consecutive series of 46 patients with PR3-ANCA and 46 patients with MPO-ANCA. Patients with MPO-ANCA had a higher median age than patients with PR3-ANCA (63 and 56 years, respectively). The prevalence of renal involvement did not significantly differ between

PR3-ANCA- and MPO-ANCA positive patients (83% and 67%, respectively), but, prior to treatment, renal function deteriorated significantly faster in PR3-ANCA-compared to MPO-ANCA associated renal disease. Moreover, kidney biopsies from PR3-ANCA positive patients showed a higher activity index and a lower chronicity index than biopsies from patients with MPO-ANCA (fig. 1). However, although PR3-ANCA positive patients showed a more active renal disease, kidney survival did not differ between PR3-ANCA positive patients (73%) compared to MPO-ANCA positive patients (61%). The authors suggest that the more acute clinical presentation of patients with PR3-ANCA results in the earlier institution of (immunosuppressive) treatment explaining the comparable or even better renal outcome. These data are in agreement with a recent study from Hauer et al. [11]. They analyzed 173 patients with WG or MPA having undergone a renal biopsy. They also observed that glomerulosclerosis, interstitial fibrosis and tubular atrophy were more frequent and more extensive in MPO-ANCA- than in PR3-ANCA positive patients. They, in agreement with Franssen et al. [10], concluded that patients with MPO-ANCA associated MPA display a more chronic injury in their kidneys at the time of presentation, probably due to a delayed diagnosis in patients with MPA compared to patients with WG. The study of Hauer et al. was performed as part of collaborative studies of the European Vasculitis Study Group (EUVAS). A previous study from this group had standardized the evaluation of renal biopsies from patients with systemic vasculitis [12], and had shown that

Primary vasculitides (ref. 2)

Large vessel vasculitis

- Giant cell (temporal) arteritis
- Takayasu's arteritis

Medium-sized vessel vasculitis

- Polyarteritis nodosa
- Kawasaki disease

Small vessel vasculitis

- Wegener's granulomatosis*
- Churg-Strauss syndrome*
- Microscopic polyangiitis*
- Henoch-Schönlein purpura
- Essential cryoglobulinemic vasculitis
- Cutaneous leukocytoclastic angiitis

*associated with ANCA

Table 1

CLINICAL BACKGROUND

review of the biopsies using a quantitative scoring system resulted in higher inter- and intra-observer agreement as it requires that the observers score the tissue specimens systematically. Using this scoring system Bajema et al. [13] evaluated the predictive value of renal biopsy findings for renal outcome in ANCA-associated necrotizing glomerulonephritis. In a group of 157 patients they measured serum creatinine at the time of renal biopsy and one year later. In addition, they included the lowest serum creatinine level during follow-up as the optimum value of renal recovery. Interestingly, the percentage of normal glomeruli in the biopsy was the best predictor for renal recovery and outcome. Reversely, glomerular sclerosis, diffuse interstitial infiltrates, tubular necrosis and tubular atrophy were each associated with a worse recovery and outcome. This study, also, shows that the extent of chronicity in the renal biopsy at presentation is the major factor for renal outcome and not the activity of the glomerular lesions. The latter, apparently, are largely reversible once adequate immunosuppressive therapy has been instituted.

Franssen et al. [14] also evaluated their patients with PR3-ANCA- and MPO-ANCA associated vasculitis for extra renal involvement. They found that, at diagnosis, patients with PR3-ANCA had a higher vasculitis activity index than patients with MPO-ANCA. Moreover, the number of affected organs in the PR3-ANCA group was higher than that in the MPO-ANCA

group. Involvement of both kidneys and the respiratory tract was far more common in patients with PR3-ANCA (78.3% of 46 patients) than in patients with MPO-ANCA (23.9% of 46 patients). Renal-limited vasculitis occurred exclusively in patients with MPO-ANCA. At histopathological examination, granulomas were found in 41.3% of PR3-ANCA positive patients and in only 4.3% of MPO-ANCA positive patients. It should be mentioned that granulomas may vary histopathologically in appearance as shown by Bajema et al. [15] for renal granuloma formation.

Taken together, PR3-ANCA and MPO-ANCA are markers of different disease entities within the spectrum of primary small-vessel vasculitides. PR3-ANCA associated vasculitides are characterized by more wide-spread organ involvement, granuloma formation, and active renal lesions, whereas MPO-ANCA associated vasculitides are more diverse in presentation and show ongoing scarring in the kidney (table 3).

The pathophysiology of ANCA associated vasculitis is not fully known but both in vivo and in vitro experimental data suggest

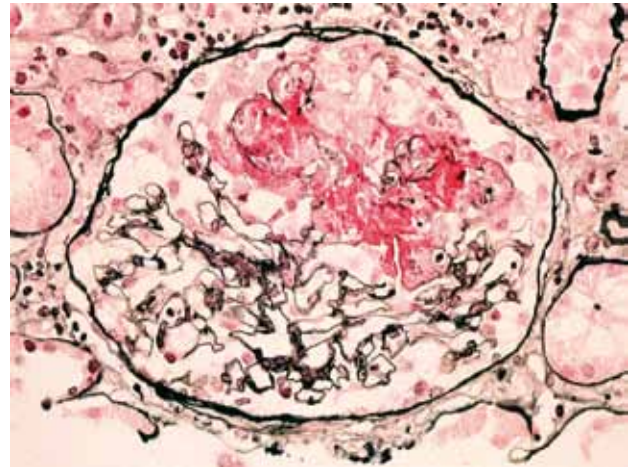


Figure 1. Necrotizing glomerulonephritis in a patient with PR3-ANCA positive Wegener's Granulomatosis.

that the autoantibodies are involved in disease expression [16,17]. In vitro studies have particularly focussed on the capacity of ANCA to activate neutrophils to the release of lysosomal enzymes and the production of reactive oxygen species [17]. In order to explain the observed difference in inflammatory activity of the lesions between PR3-ANCA- and MPO-ANCA positive vasculitis, Franssen et al. compared the neutrophil activating capacity of IgG fractions from newly diagnosed PR3-ANCA positive vasculitis patients with that of newly diagnosed MPO-ANCA positive vasculitis patients [18]. Indeed, PR3-ANCA positive IgG fractions induced more

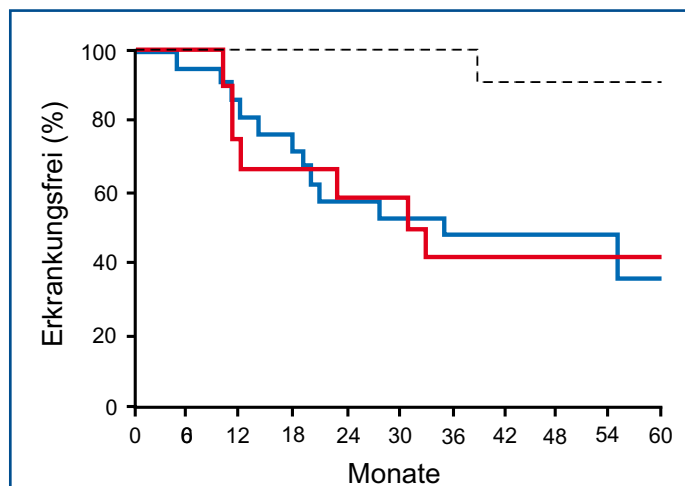


Figure 2. Disease free interval and c-ANCA status. Disease free interval according to the course of c-ANCA (n=54). The time of disease free interval was counted from the beginning of the most recent period of disease activity. Dashed line = c-ANCA negative (n=21); blue line = intermittently c-ANCA positive (n=21); red line = persistently c-ANCA positive (n=12). c-ANCA negative versus intermittently and persistently c-ANCA positive, $p < 0.001$.

From Stegeman et al., *Ann Intern Med* 1994;120:12-17.

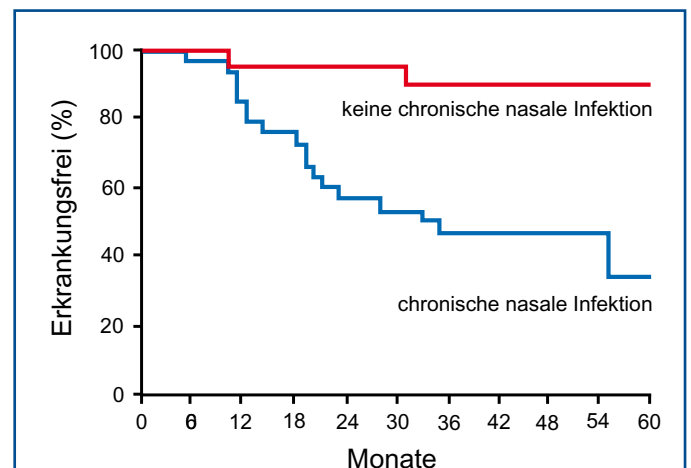


Figure 3. Disease free interval of 57 patients with Wegener's Granulomatosis grouped according to chronic nasal carriage of *Staphylococcus aureus* (n=36) or no chronic nasal carriage (n=21). The time of disease free interval was counted from the beginning of the most recent period of disease activity ($p < 0.001$).

From Stegeman et al., *Ann Intern Med* 1994;120:12-17.

oxygen radical release from donor neutrophils than MPO-ANCA positive IgG fractions, both when measuring the intracellular production of oxygen radicals by the oxidation of dihydrorhodamine to rhodamine and when assessing their extracellular release by the ferricytochrome C reduction assay. In addition, IgG fractions from PR3-ANCA positive patients generated more neutrophil degranulation of β -glucuronidase than IgG fractions from anti-MPO positive patients. These observations may, at least in part, explain the clinical and histopathological differences between PR3-ANCA- and MPO-ANCA positive patients with (renal) vasculitis.

Factors involved in relapse of PR3-ANCA associated vasculitis

The ANCA-associated vasculitides are idiopathic disorders of unknown etiology. Immunosuppressive treatment results in remission in most of the cases. However, after reduction or withdrawal of immunosuppressives, the disease relapses in a substantial number of cases. For that reason maintenance treatment with immunosuppressives has been advocated, usually for a period of at least two years [19]. After that period, and even during maintenance treatment, patients may relapse. In WG, induction of remission is reached in at least 75% of patients with the combination of prednisone and cyclophosphamide, whereas 80% of patients relapse within 10 years despite maintenance treatment with (decreasing dosages of) cyclophosphamide [20]. Presently, it is not known which factors induce relapses. We do know, however, that certain factors are associated with an increased risk for relapses. This might, possibly, enable us to select those patients with a highly increased relapse rate for more intensive maintenance treatment with immunosuppressives. Which factors are associated with relapsing disease?

It has been noted by several groups that patients with PR3-ANCA relapse more frequently than patients with MPO-ANCA associated vasculitis [14,21,22]. The reason for this difference in relapse rate is not clear. In patients with PR3-ANCA persistence of (PR3-)ANCA after induction of remission is a risk factor for relapse. The initial study on ANCA in WG by van der Woude et al. [23] already pointed to a relation between disease activity and titers of ANCA. Longitudinal observations made by several groups [24-26] subsequently showed that relapses of WG were preceded by rises in ANCA titers, although these data

Disease entity	Sensitivity of	
	Anti-proteinase 3 %	Anti-myeloperoxidase %
Wegener's granulomatosis	85	10
Microscopic polyangiitis	45	45
Idiopathic crescentic glomerulonephritis	25	65
Churg-Strauss syndrome	10	60
Polyarteritis nodosa	5	15

Table 2. Disease associations of anti-proteinase 3 antibodies and anti-myeloperoxidase antibodies

were not confirmed by others [27]. In all these studies ANCA titers were determined by two-fold titration in the indirect immunofluorescence test (IIF), a method with inherent inaccuracies. More recently, Boomsma et al. [28] evaluated 100 patients with WG, in a prospective longitudinal study, for the relationship between PR3-ANCA levels, as measured by PR3-specific ELISA, and relapses of WG. They found that 34 out of 37 relapses (92%) were preceded by a rise in ANCA titer as detected by ELISA or IIF. However, 43% of patients who showed a rise in ANCA by IIF and 29% with a rise in PR3-ANCA by ELISA did not subsequently experience a relapse.

From these data it is clear that rising titers of PR3-ANCA are a strong risk factor for the subsequent occurrence of a relapse. It even has been suggested that rising titers may be used as a guideline for the institution of treatment in order to prevent relapses. Indeed, Cohen Tervaert et al. [29], in a pilot study, found that relapses of WG could be prevented by institution of immunosuppressive treatment with cyclophosphamide and prednisone once a significant rise in ANCA-titer had occurred. This small size study, however, did not prove if this approach was really beneficial to the patient as the amount of cyclophosphamide required to prevent relapses may be harmful to the patient in the long term due to its toxicity. Therefore, Boomsma et al. [30] recently performed a prospective study in which a group of 40 patients were assigned to pre-emptive therapy with azathioprine (9 months, decreasing dose) and prednisone (4.5 months, decreasing dose) or no pre-emptive therapy once a rise in ANCA titer had occurred. Disease free survival at 12 months was 89% and 73%, respectively. Furthermore, it proved that early relapses could be prevented with pre-emptive treat-

ment but that late relapses occurred in many cases after stopping preventive treatment. Thus, rising titers of ANCA are frequently followed by relapses but whether treatment should be instituted once a rise in titer has occurred, is still under discussion. Besides rising titers of ANCA, persistence of ANCA after induction of remission in WG also has been identified as a risk factor for an ensuing relapse [26,31,32], fig. 2, which suggests that long-term maintenance treatment should be instituted in patients who are persistently positive for ANCA after induction of remission.

A second factor relevant for relapse in WG is chronic nasal carriage of *Staphylococcus aureus*. Stegeman et al. [31] observed that 63% of patients with WG were chronic nasal carriers of *S.aureus* and that relapses occurred almost exclusively in carriers of *S.aureus* (adjusted relative risk of 7.16), fig. 3. In agreement with these data, maintenance treatment with trimethoprim-sulfamethoxazole resulted in a reduction of relapses in WG [33]. The mechanisms involved in relapse induction by nasal carriage of *S.aureus* are not clear as yet, but several hypotheses have been suggested and, partly, been tested. *S.aureus* derived superantigens may activate the immune system and induce disease re-activation [34]. Indeed, the presence of *S.aureus* carrying the toxic shock syndrome toxin-1 (tsst-1) superantigen has been associated with a significant risk for relapse (relative risk of 13.4) [35]. Besides superantigens, *S.aureus* derived cationic proteins may adhere to (glomerular) basement membranes, induce a subclinical vasculitis/glomerulonephritis which, in the presence of ANCA, develops into clinically overt disease [36].

Genetic risk factors have been implicated as well in the occurrence of relapses [37].

Organ involvement Statistical significance	PR3-ANCA (n=46)	MPO-ANCA (n=46)	Statistical
Kidney	38 (83%)	31 (67%)	n.s.
Upper respiratory tract	40 (87%)	13 (28%)	p< 0.01
Lower respiratory tract	24 (52%)	14 (30%)	n.s.
Eye	18 (39%)	6 (13%)	p<0.01
Musculoskeletal system	31 (67%)	23 (50%)	n.s.
Skin	10 (22%)	9 (20%)	n.s.
Nervous system	12 (26%)	3 (7%)	p=0.01

Table 3. Organ involvement in patients with PR3-ANCA vs. MPO-ANCA

Patients with polymorphic forms of Fc-gamma receptors that exhibit low affinity for certain IgG-subclasses were more prone to disease relapses of WG in the first 5 years after diagnosis [38]. In particular, patients homozygous for the R131 form of Fc-gamma receptor IIa and the F158 form of Fc-gamma receptor IIIa, which are both associated with decreased Fc-receptor-mediated clearance, had an increased incidence of relapses, possibly, because they are less able to clear *S.aureus* from their nasal cavity. Recently, membrane expression of proteinase 3, the target antigen of ANCA in WG, has been detected on resting neutrophils. Individuals differ in the extent of membrane proteinase 3 expression on resting circulating neutrophils. In patients with WG, high membrane expression of proteinase 3 was associated with a significantly increased risk for relapse [39]. So, increased membrane expression of proteinase 3 on resting neutrophils, assumed to be genetically determined, may have functional significance with respect to (ANCA-induced) neutrophil activation.

New therapeutic modalities in ANCA-associated vasculitis

Standard treatment of the ANCA-associated vasculitides consists of oral cyclophosphamide (2-3 mg/kg daily) in combination with corticosteroids (1 mg prednisolone/kg daily). This regimen results in induction of remission in 75-90% of cases [19]. In severe, life-threatening cases at presentation, methylprednisolone (1 gr on 3 consecutive days) or plasma exchange generally is added. Results of a study comparing methylprednisolone and plasma exchange in efficacy (the so-called MEPEX-study) are underway, and already suggest that plasma exchange is more effective in cases that are dialysis dependent at presentation. Once

remission has been induced steroids are tapered but cyclophosphamide is continued in order to prevent relapses. This regimen is effective in controlling disease activity but is associated with the frequent occurrence of severe, often life-threatening side-effects [19]. Therefore, the European Vasculitis Study Group (EUVAS) has designed a number of collaborative therapeutic trials in order to find therapeutic regimens as effective but less toxic than cyclophosphamide (information on www.vasculitis.org). The first trial that has been completed compared cyclophosphamide with azathioprine for maintenance of remission. The results show that azathioprine is, indeed, as effective as cyclophosphamide in preventing relapses (16% relapses in both arms after 18 months) with a trend to fewer serious adverse events in the azathioprine arm [40]. For induction of remission in patients without kidney involvement (non-renal ANCA-associated vasculitis) methotrexate has been compared with cyclophosphamide [41]. All drugs were tapered and withdrawn by 12 months. Remission rates were comparable at 6 months (83% and 84%, respectively) but relapse rates were far higher in the methotrexate group. Various other studies are underway and results will become available in the next years.

Conclusion

PR3-ANCA- and MPO-ANCA-associated vasculitides differ in clinical and histopathological presentation, probably due to differences in (autoimmune) immunopathogenesis. PR3-ANCA associated disease relapses more frequently and factors influencing relapse rate have been defined. New therapeutic approaches, in part based on new insight in the immunopathogenesis of the vasculitides, are being tested and will, hopefully, lead to less toxic and more effective treatment of this group of life-threatening diseases.

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Serological and pathophysiological aspects of ANCA associated vasculitis – a historical review

Prof. Dr. F.J. van der Woude

Vth Medical University Clinic (Nephrology, Endocrinology, Rheumatology), Klinikum Mannheim, Germany

In the pathogenesis of Wegener's Granulomatosis (WG) and Microscopic Polyangiitis (MPA), both humoral and cellular immunity are likely to play a role. Traditionally, humoral immunity was considered to be more important. In the 1970s and 1980s, it was repeatedly stated by some authoritative workers in the field that Wegener's Granulomatosis is caused by tissue deposition of immune complexes. For example, the following statement was given in a review paper at that time: "The prevailing theory, for which there is now substantial evidence, is that most of the vasculitic syndromes are caused by, or closely associated with, deposition of immune complexes in blood vessel walls" [1]. This statement was partially based on experiments in which rabbits, which had been injected with bovine serum albumin during several weeks,

developed arteritis, glomerulonephritis and granulomata [2]. However, the lesions in this model did not resemble the lesions observed in Wegener's disease. In another paper from that time, circulating immune complexes were detected during active disease and they disappeared during induction of remission [3].

Because immune complexes were supposed to be of importance in the pathogenesis of the disease, I started to look for circulating immune complexes in the serum of Wegener patients in the early eighties. I used an indirect granulocyte phagocytosis test (IGPT) to detect immune complexes. In this test, granulocytes of a healthy volunteer are incubated for 1 h at 37° C with patient serum. After the cells have been washed, cytocentrifuge slides are prepared and fixed in acetone. Thereafter, immuno-

globulin containing inclusions in the cells are detected by indirect immunofluorescence. From looking at sera from patients with WG, it was clear that the immunoglobulin-containing inclusions in the granulocytes incubated with WG sera had a morphology different from what was seen with preformed immune complexes or immune complex containing sera from SLE patients. Using an ACA 34 column we could show that the activity in the IGPT with serum from a patient with active WG had the same molecular size as IgG. Moreover, using immunofluorescence with anti light chain sera, we could show that Fab2 fragments made from WG sera also reacted with the patient's own granulocytes. This was of course the formal proof that we were dealing with autoantibodies directed against granulocytes. Independently, the autoantibodies - now called ANCAs - had also been found by Allan Wiik at the Rigshospitalet in Copenhagen, Denmark, and by DJ Davies in the St Vincent's Hospital in Melbourne, Australia. Since the paper by Davies linked the phenomenon to IgA nephritis and Ross River virus, we were not aware of the fact that ANCAs had already been described in 1982 [4]. Dr Allan Wiik had known about the autoantibody for many years, and had developed a detection method, using ethanol fixed granulocytes, that is still the standard method today. In cooperation with colleagues from Groningen and Leiden

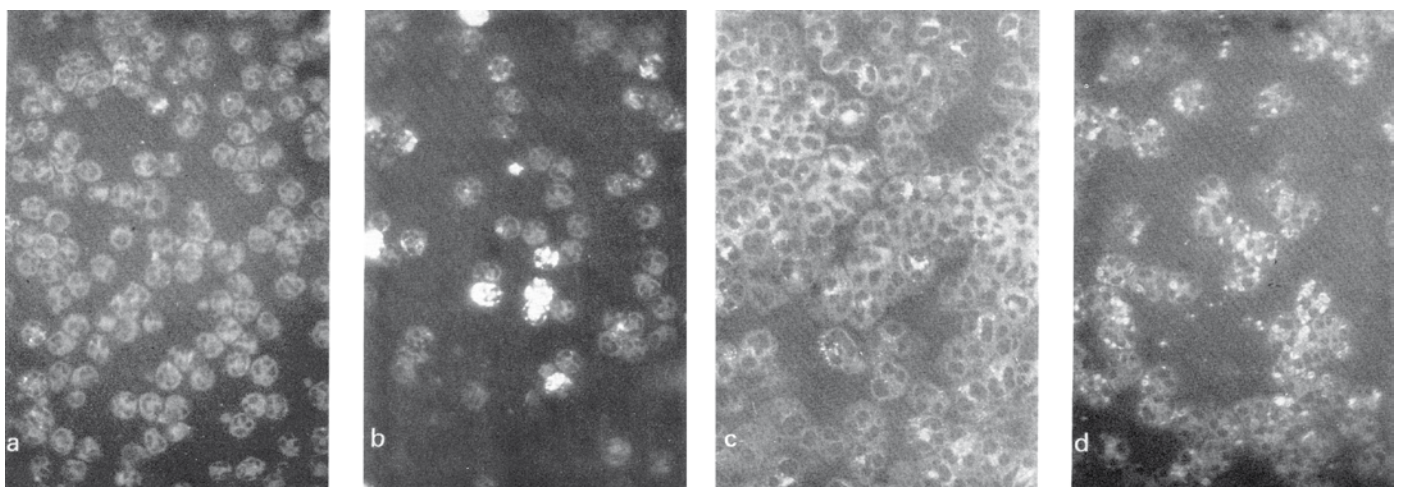


Fig 1. Morphology in the IGPT test (original magnification 252x): a. after incubation with a control serum. b. after incubation with an immune complex containing SLE serum. c. after incubation for 10 seconds with a WG serum. d. after incubation for 60 minutes with a WG serum, ring like structures are visible in the cell cytoplasm

in The Netherlands and from Copenhagen, Denmark, we published a paper in *The Lancet* describing autoantibodies against neutrophils and monocytes as a tool for diagnosis and marker of disease activity in Wegener's Granulomatosis [5].

During one of the ANCA workshops later on, the autoantibodies were renamed ANCA (Anti Neutrophil Cytoplasmic Antibodies) instead of ACPA (Anti Cytoplasmic Antibodies). We now know that these autoantibodies react with myeloid lysosomal enzymes (which are also present in monocytes). Whereas cANCA give a cytoplasmic staining on ethanol-fixed granulocytes, pANCA react in a perinuclear fashion. cANCA are directed against Proteinase 3 in most cases, pANCA, however, mainly react with myeloperoxidase.

There are the following arguments that ANCA may play a role in the pathogenesis of vasculitis:

1. ANCA are associated with the presence of disease.
2. There is an association of ANCA-associated vasculitis (AASV) with ANCA-antigen related genes.
3. ANCA have multiple effects on molecules and cells in vitro
4. ANCA may transmit disease in animal models.

It is beyond the scope of this paper to give an extensive review of these topics, so I will briefly touch on a few highlights only. It has been convincingly shown that ANCA tests can be standardized and can be used with a reasonable sensitivity and good specificity for the diagnosis of AASV [6]. The relationship between ANCA titres and disease activity is much more controversial. There is certainly a highly significant relationship between disease activity and ANCA-titres. However, active patients may be seronegative and patients with high ANCA-titres may be in prolonged remission [7]. Patients with other diseases (endocarditis) may have ANCA without vasculitis. The quantitative expression of PR 3 on the neutrophil membrane is genetically determined, the frequency of the mPR3 high phenotype is significantly increased in patients with

ANCA-associated vasculitis (85% versus 55% in healthy subjects) [8]. Alpha 1 antitrypsin is the natural inhibitor of PR3. Severely and medium deficient alpha-1-antitrypsin phenotypes are associated with anti-PR3-, but not with anti-MPO disease [9]. ANCA have multiple effects on molecules and cells in vitro (for review see [10]), and may inhibit the inactivation of PR3 by alpha-1-antitrypsin [11]. However, ANCA do not inhibit enzymatic Proteinase 3 activity against every substrate [12]. The effects of ANCA on granulocytes, monocytes, lymphocytes and endothelial cells have been described [10].

Recently, the interest in the pathogenicity of ANCA has increased due to an interesting new animal model. The group of Falk and Jennette in Chapel Hill immunized MPO Knock out mice with murine MPO. From these animals anti-MPO antibodies were transferred to RAG2 KO mice, these animals developed glomerulonephritis and vasculitis [13]. The results could be extended to wild type mice.

Due to the absence of immunoglobulins in the lesions and the presence of monocytes/macrophages and lymphocytes, it was recognized early that cellular immunity is probably also of importance in the pathogenesis of the disease [14]. An abnormal cellular immunity is invariably present in patients with ANCA-associated glomerulonephritis and vasculitis. There is a prominence of cell-mediated immunity effectors in "pauci-immune" glomerulonephritis. The disease is associated with MHC Class II genes and anti-T cell therapy is effective. Although there are no good experimental models of T-cell mediated ANCA-associated vasculitis, it is nevertheless most likely that both humoral and cellular effector arms of the immune response are involved in the disease process in AASV. It is interesting to see how more than 30 years after the discovery of ANCA, new and exciting data continue to increase the complexity of our hypothesis of the pathogenesis in AASV. It is hoped that this will allow us to develop new therapeutic concepts that will be more effective and have less side effects than the current standard therapy [15].

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Diagnosics of ANCA-associated Vasculitides

Allan Wiik

Department of Autoimmunology, Statens Serum Institute Copenhagen, Denmark.

It is a difficult clinical challenge to diagnose systemic vasculitides due to their very different forms of presentation. To diagnose them one needs to merge specialized clinical expertise from many fields of medicine and eminent laboratory medicine having detailed knowledge about differential diagnostics in autoimmune serology. The clinical aspects have been dealt with elsewhere in this issue of the journal, so the following will address the topic how diagnosis can be supported by high quality laboratory testing.

Introduction

Since the early days of autoimmune serology indirect immunofluorescence technique (IF) has been the preferred approach to search for autoantibodies that can bind to cells and tissue sections. After positive screening more detailed specificity of the antibody can be sought by use of other techniques.

When Calabresi et al. used IF to screen for autoantibodies to white cells in patients with spontaneous leukopenia in 1959 he found antibodies preferentially binding to neutrophils using peripheral blood smears as substrate [1]. The actual specificity of neutrophil-specific autoantibodies (NSA) for human neutrophils was evidenced by Faber et al. in 1964 when they described NSA appearing in serum of a patient with Felty's syndrome [2]. Later on such NSA were found to be frequent in many other chronic inflammatory conditions e.g. rheumatoid arthritis, ulcerative colitis, chronic active hepatitis etc. all being characterized by a prominent participation of neutrophils in the inflammation [reviewed in 3]. These antibodies clearly seemed to bind to neutrophil and monocyte nuclei but lack of suitable methodology at that time precluded target antigen determination. Later studies have indicated that at least some of these antibodies are directed to nuclear material located in the heterochromatin or in the nuclear membranes of myeloid cells (4), however, until more is known about these NSA it is proposed to call them by the neutral name NSA [4].

Using human leucocytes as substrate in 1973 we saw IgG antibodies binding to neutrophils with a clearly different pattern. These antibodies decorated granules in the cytoplasm of the cells, and the positive serum was found to derive from a patient with systemic Wegener's granulomatosis (WG) [5]. The common presence of this particular type of antibody in WG patients was not established until 1985 when this antibody was described to be prevalent both in Scandinavian [5] and in Dutch WG patients, the latter patient sera having been collected by Dr. van der Woude et al. in Groningen [6]. As described elsewhere in this issue of the journal the anti-neutrophil cytoplasmic antibodies (ANCA) found by the IF technique were identical to those revealed by the indirect phagocytosis test which had been researched in Groningen. ANCA directed to azurophil granule components are now known to be commonly found in necrotizing small vessel vasculitides (SVV) such as WG, microscopic polyangiitis (MPA), Churg-Straus syndrome and more limited forms of these diseases [7].

Indirect Immunofluorescence

Very soon after the publication of our results several groups of scientists started to use our IF method and worked out other methods to detect ANCA in patients with vasculitides, and it soon became apparent that results obtained in different groups were not comparable. Therefore, in 1988 33 investigators from around the world convened in Copenhagen for the First International Workshop on ANCA to share experiences and attempt to harmonize methods used for their detection. Several groups had brought sera as well as home-made IF slides showing some form of ANCA. After a practical exercise using these sera results were compared, and it was agreed to stay with one particular technique. Cardinal to this technique was the use of washed human buffy coat leucocytes, smeared or cytocentrifuged onto slides and then fixed with ethanol or acetone to give antibodies access to the inte-

rior of the cells. With this method it was possible to distinguish two binding patterns, one cytoplasmic granular and another perinuclear pattern. A detailed description of the agreed method was published soon after the workshop [8].

In the Second International Workshop on ANCA in 1989 in Noordwijkerhout in the Netherlands it was agreed to name these IF patterns *cytoplasmic* ANCA (C-ANCA) and *perinuclear* ANCA (P-ANCA), respectively [9], since both of these vasculitis-associated ANCA had been shown to target antigens in the azurophilic granules [10, 11]. The recognition of neutrophil/monocyte specificity of the antibodies is best secured by leaving all types of leucocytes in the buffy coat substrate as the differential staining of these cells compared to the negative lymphocytes and eosinophils in the slide is easily appreciated during fluorescence microscope reading. The addition of cross-linking agents such as formalin in a fixation mixture with acetone or ethanol [10] has never gained widespread popularity in laboratory routine, most likely since NSA reactions are now always followed up by testing for antigen specificity of the antibodies, but also because many neutrophil antigens do not retain their reactivity with antibodies after cross-linking. The vasculitis-associated ANCA predominantly target proteinase 3 (PR3) or myeloperoxidase (MPO) which both are contained in azurophilic granules of neutrophils and a subpopulation of granules of monocytes. Hence, the terms to be used for these ANCA was proposed to be PR3-ANCA and MPO-ANCA [9].

Enzyme-Immuno-Assays

Various types of solid phase methods based on radio-immuno-assay (RIA) or enzyme-immuno-assay (EIA) principles were introduced in the 1980-ies [reviewed in 7]. However, the lack of correlation between these assay results and IF titers and positivity made it necessary to make an effort to standardize the methods used. A European multicenter project – the EU-BCR study on ANCA assay standardization – lead by Dr. van der Woude and supported by the European Union Commission – was initiated. This included altogether 14 different vasculitis centres in Europe.

The BCR study initially looked at the performance of existing methods when used to reveal ANCA in a set of sera selected centrally, and it became apparent that IF by use of the agreed method [8] showed practically identical classification into C-ANCA and P-ANCA in the different laboratories, while titres were much less comparable. Solid phase methods gave very different results.

The use of purified native PR3 and MPO as antigens in EIA seemed to be clearly preferable to crude extracts from neutrophils or azurophilic granules. The next phase of the study concentrated on the influence of different PR3 purification methods on EIA results found in centrally chosen SVV sera. It was clear that methods had to be much better aligned to obtain similar results, and detailed recipes were made which prescribed precisely how to use the antigens, buffers and the one conjugate sent to every participant. This helped somewhat, but still results were unsatisfactory. When PR3 was coated onto microplates centrally the PR3-ANCA results became very comparable from one group to another, but at the same time it was apparent that the one native purified MPO preparation used throughout the study by all had to be coated locally just before use to obtain satisfactory results [12].

Clinical Usefulness

The final proof that the methods were clinically useful had to be shown by investigating sera from actual idiopathic SVV patients. It was a very satisfying experience to see that both IF and standardized EIA methods resulted in reproducible ANCA results and a reasonable sensitivity and diagnostic specificity. This was found both in recent onset cases of SVV and in renal-limited necrotizing glomerulonephritides [13]. The diagnoses of these recent onset SVV had been made without the support of ANCA. In a large material of sera from patients with well-established SVV diagnoses collected from all centres we found very comparable ANCA results. It is important to mention, however, that cut-off for positivity in the PR3-ANCA and MPO-ANCA assays had been chosen not towards healthy control persons (over 700 controls) but towards inflammatory disease controls (connective tissue diseases, secondary vasculitides, granulomatous diseases without vasculitis). By setting the cut-off for each EIA at a level around 90% specificity towards disease controls using ROC curves we saw rather few and mostly low level positives for the two specificities among disease controls. Nevertheless, the final and most important conclusion was that satisfactory separation of idiopathic SVV from other chronic inflammatory disease patients could only be achieved if IF-ANCA results were combined with EIA results. A C-ANCA combined with positive PR3-ANCA was thus 99% specific for SVV, and similarly, a P-ANCA combined with a positive MPO-ANCA was 99% specific for SVV [13]. Practically no SVV patient harboured both PR3-ANCA and MPO-ANCA. Purification methods did not influence PR3-ANCA results.

Later studies addressing these issues have strongly supported these conclusions [14], and now an international expert group has reached consensus as to how to test for and report ANCA results [15]. For reasons of safety it is also advised to term SVV-associated ANCA differently from the NSA found in many chronic inflammatory conditions such as rheumatoid arthritis and ulcerative colitis [4]. A general characteristic of these latter antibodies is a clear positivity by IF but negative or very low positivity in PR3-ANCA and MPO-ANCA EIA. The often mixed IF patterns seen with these NSA (sometimes called atypical ANCA or X-ANCA) are likely to be the result of antibody binding to multiple antigenic targets in the neutrophils [15]. It should be noted, that patients suffering from drug-induced syndromes (sometimes appearing as SVV) commonly have ANCA directed to both MPO and some other constituent of azurophil granules, e.g. elastase [16].

Other Methods

Already early on Dutch investigators used a slightly different method for EIA detection of ANCA. They used mouse monoclonal antibody to PR3, MPO or elastase as bottom coating on microplates and then attached the specific antigens by incubation with azurophil granule extract, thereby capturing the wanted antigen [11]. Though results might differ somewhat between those obtained by capture and those seen by direct EIA techniques there seemed to be little overall differences. Further development of particularly the PR3-ANCA EIA technique, however, has led to some improvement in PR3-ANCA detection by selecting a particular monoclonal capture antibody that seemingly does not recognize an epitope targeted by SVV-associated PR3-ANCA [17]. The advantages seen are: a closer correspondence between EIA levels and titres of C-ANCA, and a clearer rise in PR3-ANCA levels before or during clinical exacerbations of WG [17]. Recent studies using PR3 capture technique have indicated that not only PR3-ANCA but also PR3/PR3-ANCA immune complexes may be detected by such capture technique [18].

Studies from Canada lead to the elaboration of a radioimmunometric method based on the initial binding of a radiolabelled serine protease inhibitor, di-fluoro-phosphate to serine proteases in a crude extract from human leucocytes and a subsequent immunoprecipitation of IgG complexes using protein A or G, followed by identification of the radiolabelled serine protease using autoradiographic electrophoresis [19]. In daily practice this has now been modified, and ANCA that binds to radiolabelled proteins

are quantified after deposition on microfilters, since PR3 is the only important serine protease targeted by ANCA in idiopathic SVV.

The advantage of these two methods is thought to be recognition of better exposed and conformationally intact epitopes on the PR3 molecules [17, 19]. In addition, one may envisage that certain epitopes on the rather hydrophobic PR3 molecule may be hidden after the direct attachment of PR3 to plastic surfaces. This is avoided using any of the two new methods. For both methods, however, it will be important to look at their performance in large cohorts of inflammatory disease control patients.

New techniques to test for autoantibodies are constantly introduced, but their clinical value has not been rigorously tested on large populations of inflammatory disease patients. Some of the new technologies also test for ANCA.

OTHER AUTOANTIBODIES

Although ANCA are the main candidate antibodies to study in patients with idiopathic SVV it is important to remember that anti-glomerular membrane autoantibodies (anti-GBM) can be associated with very similar necrotizing SVV. These antibodies target the non-collagenous alpha 3 domains of collagen type IV, which is selectively expressed in basement membranes of glomeruli and lung tissues. Typical disease expressions are therefore lung and kidney capillaritis, often with bleeding in the form of haemoptysis and haematuria, which can also occur in ANCA-associated SVV [20]. In young patients with haemorrhagic renal-pulmonary syndrome anti-GBM are often the only autoantibodies found, whereas in older patients they can co-occur with ANCA, most often directed to MPO. The main message thus is to look for both ANCA and anti-GBM in renal-pulmonary syndromes [20].

CONCLUSIONS

Finding PR3- or MPO-ANCA and/or anti-GBM in a patient with clinical vasculitis is a strong support for a systemic SVV diagnosis, where the prognosis relies very much on early recognition and effective treatment. Close collaboration between experienced clinicians, histopathologists and clinical immunologists is mandatory for early recognition of these conditions. High levels of PR3-ANCA measured by capture technique or anti-GBM measured by direct EIA usually reflect disease activity although there may be exceptions.

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Comparison of different tests for detection of antineutrophil cytoplasmic antibodies

Elena Csernok

Department of Rheumatology, University of Schleswig-Holstein, Campus Lübeck and Rheumaklinik Bad Bramstedt, Germany

The spectrum of vasculitides that includes Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), Churg Strauss syndrome (CSS), and primary pauci-immune necrotizing and crescentic glomerulonephritis is associated with circulating antineutrophil cytoplasmic antibodies (ANCA) (so called ANCA-associated vasculitides). Only two types of ANCA, cytoplasmic C-ANCA/PR3-ANCA and perinuclear P-ANCA/MPO-ANCA, account for this association and they are highly specific markers for these vasculitides. Other neutrophil proteins have been identified as targets for ANCA, but the detection of antibodies with other specificities has not proved to be diagnostically helpful.

ANCA testing related to small vessel vasculitides has been used for about two decades, but the measurement of ANCA is still an imperfect science. The question now is what is the most appropriate strategy for testing for ANCA, meeting the criteria of accuracy, reproducibility, and cost-effectiveness. For routine detection of ANCA, indirect immunofluorescence technique (IFT) and/or antigen-specific direct ELISA (PR3- and MPO-ANCA) are the common screening methods. It has already been demonstrated that by inclusion of a standardized antigen-specific ELISA the value of the IFT can be greatly increased [1]. Recently, an international group of ANCA-researchers has published a consensus statement on ANCA testing [2]. These guidelines demand that in case of positive IFT-testing for ANCA, an ELISA test is obligatory as a minimum requirement. Many hospital/referral laboratories, however, only use commercially available ELISA kits to detect ANCA. The number of companies producing ANCA ELISA kits has increased rapidly even though the diagnostic efficiency of these assays is not well documented. Because there is no agreed international standard, these kits employ arbitrary units whose correlation

with titers obtained by IFT remains unknown. In a recent study we compared the sensitivity, specificity and predictive values of various commercial direct ANCA ELISA kits with those of IFT and our in-house PR3- and MPO-ANCA capture ELISAs in patients with clinically and histologically established WG and MPA. This study was designed to improve the usual day-to-day practice of the clinical laboratories and to increase the confidence of clinicians in the results provided by these laboratories. When ordering and interpreting an ANCA test, the clinician must be familiar with the specific assay being used to measure ANCA and with the differences between it and the various other commercial available ANCA assays. The major conclusions which have been drawn from this study are the following: 1) The performances of commercial direct ANCA ELISAs vary significantly from each other and, in some cases, from that of IFT ANCA assays; there are significant differences in sensitivity, specificity and predictive value among available commercial ELISA kits. 2) Only one out of 11 commercial direct ELISA kits, in-house PR3-ANCA capture ELISA, exhibited sensitivity and specificity for WG comparable to those obtained with IFT [3].

Another method for quantifying PR3-ANCA may prove to be superior to direct ELISA namely capture ELISA. In capture ELISA, the plate is precoated with a monoclonal antibody to capture the antigen. Some data suggest an advantage over direct ELISA [4; 5; 6; 7] but the diagnostic value of different capture ELISA is not yet evaluated and there exists no agreed standard on available capture tests. All the capture ELISAs are not standardized and these assays cannot be extrapolated to clinical practice. However, the evaluation of capture ELISA technique for ANCA determination is the aim of a new EUVAS study. In this respect, we first assessed whether

we need to standardize the capture ELISA by comparing the diagnostic performance of three different in-house assays (IFT, direct and capture ELISA) for WG in 6 reference laboratories (Bad Bramstedt, Boston, Groningen, Lund, Maastricht, Rochester). The specificities of the assays were related to rheumatoid arthritis sera and normal human sera. The diagnostic performance of each test was estimated by receiver operating characteristic curve analysis and sensitivity and specificity in detection of ANCA/PR3-ANCA were calculated for the respective methods. We report here the first results obtained in this study. Interestingly, the capture ELISA results showed a significant correlation between all participating centres. The interlaboratory variation in the capture ELISA was surprisingly small considering the differences in antigen-preparations, "catching" anti-PR3 antibodies, substrates, and conjugates. Based on our preliminary data, we conclude that capture ELISA seems to be the superior method of PR3-ANCA detection in Wegener's granulomatosis, which was confirmed in the majority of the participating laboratories. Thus, in patients with WG, the capture ELISA could be the preferred method to detect PR3-ANCA and should be used in conjunction with a compatible clinical picture and histological evidence. However, the diagnostic specificity and clinical utility of the capture PR3-ANCA ELISA as a screening or confirmatory test for certain types of vasculitis remains to be investigated. Furthermore, testing for ANCA by capture ELISA may occasionally yield false-positive results as PR3-ANCA from some patients may recognize an epitope on PR3 that is occupied by the capturing monoclonal antibody. Currently, a longitudinal study of the relationship between direct and capture PR3-ANCA ELISA scores and the clinical activity of vasculitis is in progress.

Today the most appropriate way to detect the presence of vasculitis-associated ANCA is by using both IFT and PR3- and MPO-ANCA ELISA. The answer to problems in ANCA detection is to concentrate on basic skills (proper use of IFT) and ELISA (careful use of commercial assay). Serum standards for PR3- and MPO-ANCA will be available within the next year (provided by A. Wiik, Copenhagen) and these may provide better tools for alignment of ANCA results worldwide. Furthermore, in order to optimise ANCA testing appropriate educational workshops should be held.

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The new era of ANCA testing: Varelisa PR3/MPO and EliA PR3/MPO

Nina Olschowka

Introduction

Detection of anti-neutrophil cytoplasmic antibodies (ANCA) is a well-established diagnostic test for evaluating patients suspected of having an autoimmune small vessel vasculitis such as Wegener's granulomatosis (WG) or microscopic polyangiitis (MPA). Only two types of ANCA, anti-proteinase 3 (PR3) and anti-myeloperoxidase (MPO) antibodies, account for this association and they are highly specific for these vasculitides. ANCA testing has acquired an unusually strong position in the process of diagnosing and monitoring primary systemic small vessel vasculitides, although their association with these conditions is a relatively recent discovery. Unfortunately, the appropriate interpretation of ANCA test results is complicated by the variability of ANCA detection methods and their lack of standardization.

Indirect immunofluorescence (IIF) microscopy using ethanol-fixed human neutrophils as substrate remains the *de facto* standard method for ANCA testing, despite being labour intensive and highly dependent on the experience of the laboratory personnel.

In contrast, enzyme linked immunosorbent assays (ELISA) for the detection of PR3- and MPO-ANCA are disease specific, observer independent and give quantitative results [1].

Varelisa PR3 and Varelisa MPO: the new generation of ANCA ELISAs

Varelisa PR3 and MPO ANCA were totally revised in 2002 to obtain clinically evaluated ELISAs with a maximum of clinical sensitivity and specificity. The rework included a new purification procedure for PR3, resulting in a higher immunological reactivity of the antigen. Furthermore the improved blocking reagent dramatically decreases the risk of unspecific binding, which is particularly important in ANCA testing. The new Varelisa ANCA assays thus represent state-of-the-art ELISAs for the detec-

tion of PR3 and MPO antibodies and increase your security in reporting reliable results.

EliA PR3 and EliA MPO: the first fully automated ANCA tests world-wide

In July 2003 we introduced the first fully automated ANCA method world-wide, EliA PR3 and EliA MPO. EliA is an integrated platform for automated autoimmunity testing. It applies the principles of microplate coating to single polystyrene wells which are automatically dispensed and processed in the UniCAP 100 instrument. Up to 46 different tests can be assayed in one run and up to 500 patients can be analysed per week and instrument. For higher throughput needs typical clusters of 2 – 4 instruments can be operated through the UniCAP software module. Mainframe connection is implemented.

The EliA platform provides autoimmune diagnostics under standardized conditions, thus excluding the human and the environmental factors, which leads to a very high precision with an inter-run CV below 5%.

The unique EliA calibration system can be used to evaluate the results of all analytes available. Once the 6 point standard curve is determined and stored it can be used for four weeks. This makes immediate quantitative testing of only one or a few samples possible and thus adds remarkably to the efficiency of your laboratory.

Excellent clinical performance

Evaluation of commercial tests mostly is done with blood donor samples and a rather small number of positive sera (defined positive through other methods). In contrast, for the evaluation of EliA and Varelisa ANCA tests we used an unusually high number of *clinically* well defined sera of patients with ANCA-associated vasculitides (WG, MPA, Churg-Strauss syndrome, necrotizing crescentic glomerulonephritis, all together 181 sera) and with

ANCA	Varelisa PR3 ANCA	Varelisa MPO
Sensitivity in %	61.8	50.6
Specificity in %	98.1	96.5
Positive predictive value in %	70.7	87.5
Negative predictive value in %	92.1	92.1
Efficiency in %	91.5	89.9

Table 1: Clinical sensitivity and specificity of Varelisa PR3 and MPO ANCA

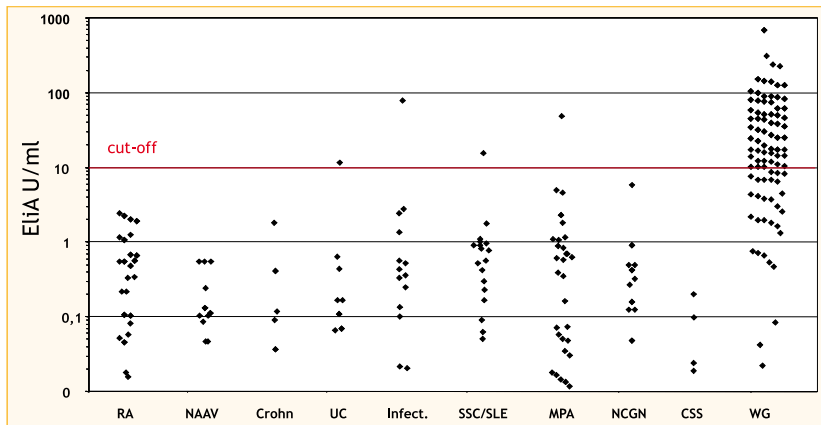


Figure 1: Discrimination of EliA PR3 between WG and various other diseases. Explanation of abbreviations see figure 2.

non-ANCA-associated diseases (rheumatoid arthritis, non-ANCA-associated vasculitides, inflammatory bowel disease, etc.). Additionally we tested over 400 blood donor samples.

Sensitivity and specificity data of Varelisa PR3 ANCA and Varelisa MPO ANCA for this special serum panel are shown in table 1.

Figure 1 shows the discrimination of EliA PR3 between Wegener's granulomatosis and disease controls. Four disease control sera were positive. However, all four sera showed positive results in different reference assays, as well. The number of positive disease controls is even lower than described in the literature.

The sensitivity of EliA PR3 for WG with the serum panel used was 63%, the specificity was 98%. The apparently quite low value for sensitivity is possibly due to the high number of samples from patients under treatment having inactive WG. Different commercial PR3 assays showed a lower sensitivity with the sera panel used.

Figure 2 shows the discrimination of EliA MPO between small vessel vasculitis and disease

controls. Two disease control sera were slightly positive (both rheumatoid arthritis). The sensitivity of EliA MPO for MPA was 58%, 50% for necrotizing crescentic glomerulonephritis, and 36% for Churg Strauss syndrome. The specificity was 98%.

In the meantime several studies of independent laboratories were performed and first data confirm the excellent clinical performance of EliA PR3 and EliA MPO.

Qualitative results

Although the diagnostic value of PR3- and MPO-ANCA test is well established, the usefulness of measuring ANCA titres in assessing disease activity and in guiding therapy is somewhat controversial. Absolute titres correlate only roughly with disease severity. There is, however, evidence that rising titres of PR3-ANCA during clinical remission often can predict relapses in patients with Wegener's granulomatosis, whereas falling titres are generally associated with a low risk of relapse [2-5]. In a recent study, Han et al. obtained evidence that rises of MPO-ANCA titres are probably also predictive

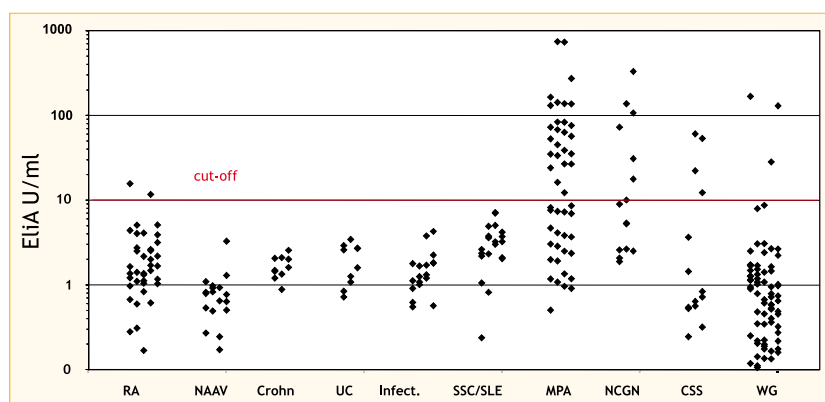


Figure 2: Discrimination of EliA MPO between ANCA-related vasculitides and various other diseases.

Abbreviations: RA = rheumatoid arthritis, NAAV = non-ANCA-associated vasculitides, Crohn = Crohn disease, UC = ulcerative colitis, Infect. = Infections (HIV and HCV), SSC = systemic sclerosis, SLE = systemic lupus erythematosus, MPA = microscopic polyangiitis, NCGN = necrotizing crescentic glomerulonephritis, CSS = Churg-Strauss syndrome, WG = Wegener's granulomatosis

of relapses, but they clearly claim, that titre changes only can be followed-up by the use of ELISAs [6].

With the 6 point calibration curve in EliA and in Varelisa and the exceptionally broad measuring range of EliA PR3 and EliA MPO these tests are optimal tools for an exact quantification of results and monitoring of patients.

Summary

Varelisa PR3 and Varelisa MPO are a new, clinically evaluated generation of ELISA tests for the detection of PR3 and MPO ANCA. EliA PR3 and EliA MPO are fully automated ANCA tests with all advantages automation has, such as increased efficiency, precision for consistent results, and ease of operation.

Both Varelisa and EliA PR3 and MPO were evaluated with a large number of clinically well defined sera. First independent studies using EliA ANCA show, that the clinical performance is exceptionally good.

All together PR3 and MPO ANCA on Varelisa as well as on EliA represent highly precise, observer independent ANCA tests with a minimum of labour intensity and a maximum of flexibility for the laboratory.

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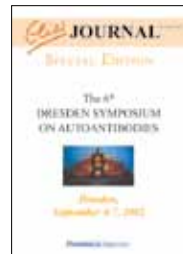
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Phadia GmbH, Munzinger Str. 7, D-79111 Freiburg Germany, Tel: +49 761 47-805-0, Fax: +49 761 47-805-120, autoimmunity@phadia.com, www.phadia.com

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