Controversy

Taking anti-neutrophil cytoplasmic antibody (ANCA) testing beyond the limits

Fokko J. van der Woude

Vth Medical University Clinic (Nephrology, Endocrinology, Rheumatology), Faculty for Clinical Medicine at Mannheim, Karl-Ruprechts-Universität Heidelberg, Klinikum Mannheim, Mannheim, Germany

Keywords: ANCA

The application of anti-neutrophil cytoplasmic antibody (ANCA) testing has received much interest since ANCA were discovered [1] and since they were reported to be useful in both the diagnosis and monitoring of disease activity in Wegener’s granulomatosis (WG) [2]. Although there is little doubt that the recognition of the association between the presence of ANCA and active vasculitis has had a very positive influence on research on the pathogenesis and treatment of ANCA-associated diseases (reviewed by Savage [3]), there are reasons to worry about the application of this recently gained knowledge in clinical practice. In this comment the problems with the clinical application of ANCA tests are categorized for the sake of clarity as (i) standardization problems, (ii) difficulties with application of the test in the appropriate clinical context and (iii) the assumption that ANCA titres alone are reliable and sufficient to monitor disease activity and therapy during follow-up of patients.

Standardization

Standardization of ANCA testing is possible, but not easy. Ten years ago, the BCR office of the European Union (EU) sponsored a large international standardization project that looked at different antigen purification methods [4], the suitability of such purified antigens for solid-phase assays and standardization [5] and finally the clinical utility of the developed tests [6]. The main result of the project was that a properly standardized indirect immunofluorescence (IIF) test needs to be combined with a similarly properly standardized solid-phase assay to obtain results, which can safely be applied clinically [6]. Although an extensive discussion of the methodology is beyond the scope of this comment [7], some major points will be mentioned briefly. In performing the IIF test one tries to detect the presence of antibodies against autoantigens in the cytoplasm of human neutrophil granulocytes and monocytes. ANCA do not react with lymphocytes, and therefore the presence of lymphocytes on the test slides enables the observer to easily distinguish between the presence of antinuclear antibodies and ANCA. Most commercially available slides for IIF-ANCA screening lack these control cells. A second important source of error is the leucocyte isolation and fixation procedure; it is recommended to use ethanol-fixed leucocytes (acetone or formalin fixation leads to irreproducible results), which have not become activated during the isolation procedure. The EU/BCR study showed that reproducible results for IIF-ANCA testing could be obtained by using the recommended procedure. C-ANCA, P-ANCA and atypical ANCA patterns could reliably be identified; however, standardization of titres was not possible [5,6]. The specific antigenic targets of ANCA should then be identified in solid-phase assays. Proteinase-3 (PR3), a serine protease present in the azurophilic granules of neutrophils, is the major antigen associated with the C-ANCA fluorescence pattern. On the other hand, antibodies to multiple antigens in the cytoplasm of neutrophils may be responsible for the P-ANCA pattern. The principal P-ANCA antigen is myeloperoxidase (MPO), an enzyme also present in the azurophilic granules of neutrophils. Other antigens with P-ANCA reactivity include elastase, cathepsin G, azurocidin, lactoferrin, lysozyme and bactericidal/permeability-increasing protein (BPI). The results of the BCR/EU project clearly demonstrated the importance of proper standardization of antigen-specific solid-phase assays. It should be kept in mind that even under optimal conditions after several standardization rounds the inter-centre coefficient of variability still amounted to 15–35%. Standardized serum mixtures (obtained from Dr Allan Wiik,
Department of Autoimmunology, Statens Serum Institute, Copenhagen, Denmark), which can be used as a positive control in anti-PR3 and anti-MPO-assays, proved to be indispensable for obtaining reproducible results and clinically meaningful cut-off values. Again as for the IIF test, antibody titres could not be standardized. After completion of the EU/BCR study it has been claimed that high or increasing titres of ANCA detected with a capture ELISA [8] or with an ELISA specific for IgG3 [9], would be more specific for active disease. It should be realized that these assays have not been subjected to the same rigorous standardization procedures as the previous assays, and that these results could not be confirmed in a later study [10,11].

The diagnostic value of ANCA testing depends on the clinical setting

The utility of ANCA testing cannot be assessed without some basic understanding of Bayes’ theorem, which provides a means of calculating the post-test probability of disease if the pre-test probability, sensitivity and specificity are known. The first difficulty is that WG, microscopic polyangiitis, Churg–Strauss syndrome and pauci-immune glomerulonephritis are uncommon diseases. In addition, signs and symptoms present at onset may vary to a large extent. For example, glomerulonephritis, which occurs in ~80% of patients with WG during the disease course, is present in only ~20% of patients at initial presentation. The second difficulty is that there are no standardized criteria for diagnosing active disease. The Chapel Hill classification defines nomenclature [12], but no diagnostic criteria. The Birmingham Vasculitis Activity Score [13] is a quantified opinion-based impression of disease activity by the treating physician, which is very difficult to standardize. Bayes’ theorem predicts that when there is a low pre-test probability of WG, the post-test probability is too low in most clinical situations to justify the use of ANCA testing alone as a means of diagnosing WG [14]. This prediction has recently been tested in an elegant study by the Edinburgh group [15]. For the combined IIF–ELISA test the sensitivity or positive predictive value was not >0% in departments other than nephrology and rheumatology. They are right in arguing that the ANCA test is being widely applied with very poor return.

ANCA and disease activity

In the past it has been claimed that relapses in WG can be prevented by treatment based on ANCA titres [16]. In the study that led to this conclusion all ANCA titres from past and current sera were monthly tested in one assay. The study lasted 1 year. In a group of nine patients, treatment was based on 2-fold increases in ANCA titres. There were nine titre increases observed in this group, but no relapses. In a second group of 11 patients, which was treated according to clinical signs and symptoms, there were 11 titre increases and nine relapses. In the second group more immunosuppression had to be given, and more side effects were observed. The conclusion of this study was more or less supported by two subsequent publications [17,18]. However, far more studies with far more patients included [10,11,19–26] failed to confirm these findings, which is even more impressive if one realizes that there is a strong publication bias against negative results. Most studies find that although ANCA positivity is associated with relapse, discordance between ANCA titres and disease activity is not unusual. A negative ANCA assay can never be used as a basis for eliminating active vasculitis or glomerulonephritis from diagnostic consideration.

Conclusions

ANCA testing, if applied carefully, can be a valuable part of the diagnostic armamentarium.

Standardization is not easy and careless use of commercial assays without taking into account numerous relevant details may seriously jeopardize the reliability of the tests. For useful application of ANCA testing good clinical assessment of patients with multi-system disease must be emphasized. Guidelines for ANCA testing in clinical syndromes with a high index of suspicion of systemic vasculitis have been published [15]. As current standard induction therapy for active WG and microscopic polyangiitis is extremely toxic, the diagnosis must be firmly established. This sometimes requires repeated application of invasive procedures such as bronchoscopy, renal biopsy or other similarly unpleasant methods. A negative ANCA test can never be used to rule out active disease. A positive test or a rapid increase in ANCA titres may be useful to suggest the presence of active disease, but can never be used alone.

References

7. Wiik A. Anti-neutrophil cytoplasmic antibodies tests: which tests should be used in practice? Internal Med 2001; 40: 466–470