Antineutrophil cytoplasmic antibodies: reporting and diagnostic strategies

It was with great interest that we read the correspondence of Mahler and Fritzler\(^1\) on our recent European Vasculitis Study Group (EUVAS)\(^2\) study describing the performance of immunoassays for antineutrophil cytoplasmic antibodies (ANCA) in patients with ANCA-associated vasculitides (AAV).\(^3\) In their letter, Mahler and Fritzler raise some interesting points, mainly related to (i) test result interpretation and (ii) diagnostic strategies. Besides, they pointed out that in the EUVAS study only two indirect immunofluorescence (IIF) assays were included, a commercial assay from Inova Diagnostics and a ‘home-made’ assay. They suggested to perform studies with more than two IIF ANCA tests and especially to include assays that are most commonly used in diagnostic laboratories. In a concomitant publication, we presented data on two additional commercial IIF assays, one from Euroimmun and one from Medipan.\(^\#\) Accordingly, we postulate that we included the most commonly used IIF assays. For example, IIF ANCA assays from Inova and Euroimmun are used by, respectively, 50% and 23% of the participants of the UK National External Quality Assessment Service (NEQAS) ANCA scheme (report September 2016). Our studies consistently showed that test characteristics of IIFs were highly variable between assays. We envisaged that this variability was dependent on the substrates used and methods applied for ANCA IIF testing, that is, the use of only ethanol-fixed neutrophils versus the combination of ethanol-fixed and formalin-fixed neutrophils and HEp2 cells.\(^4\) Moreover, we also found that the overall performance of high-quality immunoassays was at least as good as the performance of IIF methods, even when applied on modern automated systems. These observations lead us to conclude that the current international guidelines on ANCA testing\(^5\) should be revised. A large Russian vasculitis centre has already abandoned IIF for ANCA testing several years ago\(^5\) and in Japan, immunoassays are used for the diagnosis of AAV without IIF in most cases (Y Arimura, personal communication).

As we foresee that proteinase-3 (PR3)-ANCA and myeloperoxidase (MPO)-ANCA will be increasingly used to screen for ANCA-associated vasculitides (AAV)\(^6\), submitted for publication). We highly appreciate the need for improved interpretation of test results that takes into account antibody levels. This, together with the fact that there are three times more controls that are single positive by IIF than by the two immunoassays, argues for combining two high-quality immunoassays rather than for combining immunoassay with IIF. Our data also show that combining different tests is mainly useful in case of low antibody levels by immunoassay (associated with a low likelihood ratio for disease) and much less useful for high antibody levels, as such results are associated with a high likelihood ratio for disease. This again illustrates the need for improved interpretation of test results that takes into account antibody levels.

Mahler and Fritzler\(^1\) suggested to combine immunoassays with IIF by referring to ANAs testing, in which combining IIF with immunoassays adds value.\(^7\) In ANA testing, IIF can pick up antibodies to relevant antigens that are not picked up by immunoassays. Such antibodies can be of high titre and are found in patients with systemic lupus erythematosus or systemic sclerosis.\(^8\) However, in AAV PR3 and MPO are the main autoantigens and there is no need for IIF to detect antibodies to autoantigens other than MPO and PR3. Moreover, in patients with AAV, there is high concordance of antibody detection between immunoassays and between immunoassays and IIF. Seronegative patients are usually negative by immunoassays and by IIF. Pertinent to this, it should be pointed out that ANCA testing is only an adjunct for the diagnosis of AAV: clinicopathological features determine the diagnosis.

The EUVAS study focused on AAV and did not address ANCA testing for gastrointestinal diseases. As previously suggested by us\(^9\) and by Mahler and Fritzler,\(^1\) laboratories should differentiate between test requisitions for AAV versus other inflammatory conditions such as inflammatory bowel disease or autoimmune hepatitis. However, the clinical relevance of ANCA testing in non-AAV conditions is limited, as illustrated by the fact that ANCA test results are not incorporated in the respective diagnostic criteria.\(^10-14\)
Taken together, the data of the EUVAS study and additional data on test interpretation and testing strategies discussed above are a basis for a new international consensus on ANCA testing, which is currently in preparation. A strategy primarily based on antigen-specific assays seems to be supported by clinical practice in some laboratories, but we consider it mandatory that such strategy is validated in a prospective study, potentially including a wider array of ANCA tests.

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Competing interests XB has been a consultant to Inova Diagnostics.

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Table 1 Likelihood ratios (with 95% CIs) for the cut-off point proposed by the manufacturer and for a combination of tests are given.

<table>
<thead>
<tr>
<th>Test Combinations</th>
<th>AAV (n)</th>
<th>Control (n)</th>
<th>Likelihood ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantaFlash (−)</td>
<td>29</td>
<td>899</td>
<td>0.12</td>
<td>0.08 to 0.17</td>
</tr>
<tr>
<td>Euroimmun (−)</td>
<td>27</td>
<td>894</td>
<td>0.11</td>
<td>0.08 to 0.16</td>
</tr>
<tr>
<td>QuantaFlash (−) IIF (−)</td>
<td>23</td>
<td>854</td>
<td>0.10</td>
<td>0.07 to 0.15</td>
</tr>
<tr>
<td>QuantaFlash (−) IIF (+)</td>
<td>6</td>
<td>39</td>
<td>0.57</td>
<td>0.24 to 1.32</td>
</tr>
<tr>
<td>QuantaFlash (−) Euroimmun (−)</td>
<td>23</td>
<td>880</td>
<td>0.09</td>
<td>0.06 to 0.14</td>
</tr>
<tr>
<td>QuantaFlash (−) Euroimmun (+)</td>
<td>6</td>
<td>13</td>
<td>1.70</td>
<td>0.65 to 4.42</td>
</tr>
<tr>
<td>QuantaFlash (−) Euroimmun (−)</td>
<td>4</td>
<td>14</td>
<td>1.05</td>
<td>0.35 to 3.17</td>
</tr>
<tr>
<td>QuantaFlash (−) Euroimmun (+)</td>
<td>218</td>
<td>17</td>
<td>47</td>
<td>29 to 75</td>
</tr>
<tr>
<td>Total</td>
<td>251</td>
<td>924</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The number of patients and controls with a particular test result or combination of test result are given as well. The highest level of reactivity from the PR3-ANCA and MPO-ANCA determinations was selected for analysis. Data are from ref.2.

The AUC of the Inova QuantaFlash PR3-ANCA and MPO-ANCA assay for AAV was 0.925 (95% CI 0.909 to 0.940). The AUC of combining Quantaflash with an IIF ANCA assay was 0.94 (95% CI 0.925 to 0.953), which was not significantly different from the AUC of performing only Quantaflash (p=0.088) (method of Hanley and McNeil, MedCalc). The AUC of combining QuantaFlash with an immunoassay for MPO- and PR3-ANCA from Euroimmun on all samples was 0.943 (95% CI 0.928 to 0.955), which was significantly different from the AUC of Quantaflash alone (p=0.01) (method of Hanley and McNeil, MedCalc).

AAV, ANCA-associated vasculitides; ANCA, antineutrophil cytoplasmic antibodies; MPO, myeloperoxidase.

Figure 1 Test results for antineutrophil cytoplasmic antibodies (ANCA) by QuantaFlash (Inova) and by ELISA (Euroimmun). The highest level of reactivity from the PR3-ANCA and myeloperoxidase (MPO)-ANCA determinations was selected for analysis. Cut-off point proposed by the manufacturer is 20 U/mL of CU for both assays. Data are from ref.2.
REFERENCES


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