Harmonization of antineutrophil cytoplasmic antibodies (ANCA) testing by reporting test result-specific likelihood ratios: position paper

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To the Editor,

Antineutrophil cytoplasmic antibodies (ANCA) are valuable laboratory markers to support the diagnosis of ANCA-associated vasculitis (AAV). High-quality immunoassays for proteinase-3 (PR3)-ANCA and myeloperoxidase (MPO)-
ANCA can be used to screen for patients suspected of having granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) [1].

Given the importance of ANCA testing for AAV, efforts have been undertaken to standardize ANCA measurements. Reference standards for PR3-ANCA and MPO-ANCA have become available under the auspices of the International Union of Immunological Societies (IUIS) and are used by several manufacturers for calibration of ANCA assays (EliA fluoroenzyme immunoassay from Thermo Fisher Scientific, capture ELISA from Svar Life Science, CytoBead assay from Medipan). More recently, the Institute for Reference Materials and Measurements (IRMM) released certified reference materials for MPO-ANCA and PR3-ANCA. An evaluation of the certified reference materials revealed that applying such materials aligns results between some assays but not between all assays [2]. Thus, despite the availability of reference materials, there remains a need to harmonize ANCA determinations.

**Figure 1:** Distribution of test results and test result-specific likelihood ratios (LR). Patients with antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) (n=251) and controls (n=924) were tested for ANCA by eight different assays.

*For each patient only the highest level of reactivity for either proteinase-3 (PR3)- or myeloperoxidase (MPO)-ANCA was included for analysis. The distribution of the test results obtained in controls and in AAV patients is given on the left Y-axis. The population distributions were determined by dividing the test results in 9–14 intervals; data points (fraction of controls or AAV patients) refer to the indicated mean value of each interval. The LRs are given on the right Y-axis. Estimation of the LR by calculating the slope between two points delineating overlapping intervals on the receiver operating characteristic (ROC) curve is indicated by the yellow dots. Estimation of test-result-specific LRs by Bézier curve [5, 6] is indicated by a green line. The red circles (and the associated X-axis values) indicate the assay-specific test results that are associated with an LR of 0.1, 1, 10 and 30 (right Y-axis). The cut-offs proposed by the manufacturers are indicated by arrows. *The assays include: QUANTA Lite ELISA and QUANTA Flash chemiluminescence assay (CLIA) from Inova Diagnostics; EliA PR3$^S$ and EliA MPO$^S$ fluorescence enzyme immunoassays (FEIA) from Thermo Fisher Scientific; MPO and PR3 multiplex immunoassay (MIA) on BioPlex 2200 from Bio-Rad Laboratories, Inc.; capture PR3- and MPO-ANCA ELISA from Svar Life Science; anti-PR3 hs ELISA and anti-MPO ELISA from Orgentec; anti-PR3-hn-hr ELISA and anti-MPO ELISA from Euroimmun AG; and CytoBead ANCA assays from Medipan/Generic Assays GmbH. The tests were performed according to the manufacturers’ instructions. The cut-offs proposed by the manufacturers are 20 Units for QUANTA Lite, 20 CU for QUANTA Flash, 3 IU/mL for EliA PR3$^S$ and 5 IU/mL for EliA MPO$^S$, 1 AI for BioPlex, 7 IU/mL for Svar Life Science, 10 U/mL for PR3 hs and 5 U/mL for MPO Orgentec, 20 U/mL for Euroimmun, and 5 IU/mL for CytoBead.
Here we recommend harmonizing clinical interpretation of ANCA test results by providing test result-specific likelihood ratios (LRs) [3]. We illustrate the approach using data from a previously described study in which 924 disease controls and 251 diagnostic samples from AAV patients (186 patients with GPA, 65 patients with MPA) were tested for PR3-ANCA and MPO-ANCA by eight different assays [3, 4]. Details on patients, controls and assays are available in [3, 4]. The study was in compliance with the World Medical Association Declaration of Helsinki.

Figure 1 shows the distribution of pooled PR3- and MPO-ANCA test results obtained in disease controls and in diagnostic samples from AAV patients as well as the test result-specific LRs for eight different assays. The test result-specific LRs were estimated by locally estimating the derivative of the receiver operating characteristic (ROC) curve (see legend to Figure 1) and by applying Bézier curves [5, 6]. For each patient (disease controls and GPA/MPA patients) only the highest level of reactivity for either PR3- or MPO-ANCA was included for analysis. The feasibility of this approach is illustrated in supplementary material in which it is shown that the cutoff values proposed by the manufacturer and the specificities at these cut-off values were comparable for PR-ANCA and MPO-ANCA. For all assays, test result-specific LRs unmistakably increased with increasing antibody levels. The test results corresponding to an LR of 0.1, 1, 10 and 30 are denoted in Figure 1 and are recapitulated and summarized in Figure 2, Panel A. For (almost) all assays, the LR associated with the cutoff value proposed by the manufacturer was slightly higher than 1 and <10 (Figure 2, Panel A). Depending on the assay, 52–66% of AAV patients had a test result with an LR >30 and 14–27% of AAV patients had a test result with an LR between 10 and 30 (Figure 2, Panel B). The IRMM and IUIS PR3-ANCA reference materials had an associated LR that was >30 with all assays (Figure 2, Panel C). The MPO-ANCA reference materials had an associated LR >30 with all but one assays (Figure 2, Panel C).

The LR is the fraction of patients with a particular test result divided by the fraction of controls with the same test result. For example, a test result with an LR of 10 indicates
that this test result is 10 times more likely to be found in patients with the disease than in (disease) controls and a test result with an LR of 0.1 is 10 times less likely to be found in patients with the disease than in (disease) controls. A test result with an LR of 1 indicates that this test result is equally likely to be found in (disease) controls as in patients with the disease. Knowledge of the LR improves the clinical interpretation of a test result and allows to estimate the post-test probability of disease \[
\text{post-test odds} = \text{pre-test odds} \times \text{LR}\].

Current immunoassays for ANCA classically apply a single cut-off point with a dichotomous interpretation (positive/negative). Here we propose to employ test result-specific LRs to align test result interpretation across assays and manufacturers and to convey clinical information intrinsic to the antibody level. This is achieved by communicating the test results associated with an LR of 0.1, 1, 10 and 30, as suggested in Figure 1 and supplementary material Exhibit 2, Panel A, or by reporting LRs for test result intervals, as previously reported [3]. These recommendations conform with the revised international 2017 consensus on ANCA testing [3] and are supported by the European Federation of Laboratory Medicine (EFLM) task force on guidelines for autoimmune testing, European Autoimmune Standardization Initiative (EASI), by the European Consensus Finding Study Group on autoantibodies (ECFSG), also known as the European League against Rheumatism (EULAR) autoantibody study group, and by in-vitro diagnostic companies offering assays for ANCA testing that participated in the European Vasculitis Study Group (EUVAS) study [4].

**Key message**

Clinical interpretation of ANCA test results can be harmonized by providing test result-specific likelihood ratios.

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speaker fees from Thermo Fisher Scientific, Euroimmun, and Inova. BH has received a speaker fee from Orgentec, EC has received lecture fees from Bio-Rad Lab, Medipan/Generic Assays, Inova Diagnostics, Orgentec and Thermo Fisher. JR has been a consultant for Thermo Fisher Scientific. WF has received speaker fees from Thermo Fisher Scientific and Inova, has been a consultant for Thermo Fisher Scientific and Inova. MM is employed by Inova Diagnostics, WS received personal fees from Euroimmun, NO received personal fees from Thermo Fisher Scientific, PH and UL are employed by Orgentec, DR received personal fees from Medipan and Generic Assays, EB and RW are employed by BioRad. PV is a senior clinical investigator of the Fund for Scientific Research – Flanders. WF has received speaker fees from Thermo Fisher and is president of the Schweizerischer Verband der Diagnostikindustrie (SVDI).

**Ethical approval:** Research involving human subjects complied with the World Medical Association Declaration of Helsinki (as revised in 2013).

**Data availability:** Data are available upon request to the corresponding author.

**References**


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