Letter to the Editor

Niels Rasmussen, Jan Damoiseaux, Elena Csernok, Niels H.H. Heegaard, Bernhard Hellmich, Pieter van Paassen, Bo Baslund, Pieter Vermeersch, Daniel Blockmans, Jan-Willem Cohen Tervaert and Xavier Bossuyt*

Individual values of antineutrophil cytoplasmic antibodies do not correspond between antigen-specific assays

DOI 10.1515/cclm-2017-0362

Received May 5, 2017; accepted June 27, 2017

Keywords: antineutrophil cytoplasmic antibodies (ANCA); PR3-ANCA; MPO-ANCA.

To the Editor,

We have recently described the diagnostic performance of immunoassays for proteinase3 (PR3) and myeloperoxidase (MPO) antineutrophil cytoplasmic antibodies (ANCA) in patients with ANCA-associated vasculitides (AAV). These results were compared to the performance of indirect immunofluorescence (IIF) techniques [1] as well as to the individual performances of each immunoassay [2]. Based on receiver operating characteristics (ROC) curves and likelihood ratios (LRs) [3], we found a high degree of diagnostic precision for all of the immunoassays investigated. Moreover, the ROC curves and LR demonstrated very similar diagnostic performances. It would, therefore, appear that the immunoassays could be used interchangeably. However, this uniformity only applies to the general performance of the assays. A patient may be negative in one assay and positive in another assay.

Therefore, when there is a strong clinical suspicion of AAV, the use of another immunoassay is advised when the first test is negative [2].

Additionally, many clinicians use increases in ANCA values to identify a possible imminent relapse [4]. This practice is especially relevant for patients who have had previous renal involvement and/or alveolar hemorrhage [4].

To evaluate the risk of false information about relapse risk using ANCA values from different immunoassays, we have performed simple correlations of ANCA values between the eight immunoassay used in the original investigation [1, 2].

For this purpose, we used samples of patients with granulomatosis with polyangiitis (GPA) for correlation studies of PR3-ANCA assays and samples of patients with microscopic polyangiitis (MPA) for correlation studies of MPO-ANCA assays in order to obtain a maximum of homogeneity. After removing all censored data (i.e. values below the detection limit and values above the highest standard), we could include 74 patients for PR3-ANCA correlations (Figure 1) and 21 patients for MPO-ANCA correlations (Figure 2). Visual inspection reveals good correlations for some comparisons, but assay incompatibility for other comparisons. We then calculated Spearman rank

Q1: Please check and confirm the corresponding author affiliations

Q1 = OK

*Corresponding author: Xavier Bossuyt, Department of Laboratory Medicine, University Hospitals Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium, Phone: +32 16 347009, Fax: +32 16 34 79 31, E-mail: Xavier.Bossuyt@uzleuven.be; Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium; and Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium
 Niels Rasmussen and Niels H.H. Heegaard: Department of Autoimmunology and Biomarkers, Statens Serum Institute, Copenhagen, Denmark
 Jan Damoiseaux: Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, The Netherlands
 Elena Csernok and Bernhard Hellmich: Department of Rheumatology and Immunology, Medius Kliniken Kirchheim/Teck, University Tübingen, Germany

Pieter van Paassen: Department of Internal Medicine, Section Nephrology and Immunology, Maastricht University Medical Center, Maastricht, The Netherlands

Bo Baslund: Department of Rheumatology, Rigshospitalet, Copenhagen, Denmark

Pieter Vermeersch: Clinical Department of Laboratory Medicine, University Hospitals Leuven, 3000 Leuven, Belgium; and Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium

Daniel Blockmans: Clinical Department of General Internal Medicine, Research Department of Microbiology and Immunology, Laboratory of Clinical Infectious and Inflammatory Disorders, University Hospitals Leuven, Leuven, Belgium

Jan-Willem Cohen Tervaert: Maastricht University, Maastricht, The Netherlands

Q2: Please supply complete address (i.e., department, state, city, etc.) for the author Jan-Willem Cohen Tervaert

Figure 1: Correlations of individual values of PR3-ANCA in GPA patients ($n=74$) measured by eight different immunoassays. Spearman rank correlations (r_s) (lower left cells in inset) and p-values (upper right cells in inset) for significant linear correlations are included in the graph. The immunoassays included were from INOVA [QuantaLite (1) ELISA and QuantaFlash chemiluminescence (CLIA) (2)], Thermo Fisher fluoroenzyme immunoassay (FEIA) (EliA) (3), Bio-Rad bead-based assay (BioPlex 2200) (4), Euro-Diagnostica capture ELISA (5), Orgentec ELISA (6), Euroimmun ELISA (7), and Medipan (CytoBead assay) (8). The data were from reference [1] after removing censored data.

correlation coefficients for all possible comparisons. The best linear correlations ($r_s > 0.9$) were found between Euroimmun ELISA and INOVA QuantaLite ELISA and between EliA FEIA (fluoroenzyme immunoassay), Orgentec ELISA and Euro-Diagnostica ELISA. The worst correlations were found for comparisons with the bead-based Bio-Plex 2200 assay from Bio-Rad ($r_s < 0.8$ for 3/7 PR3-ANCA comparisons and 4/7 MPO-ANCA comparisons) and with the Medipan CytoBead assay ($r_s < 0.7$ for 7/7 MPO-ANCA comparisons).

The median PR3-ANCA values were 35.6 U, 40.5 CU, 3.5 IU/mL, 1.9 AI, 11.7 IU/mL, 22.7 U/mL, 31.4 U/mL and 6.0 IU/mL for QuantaLite, QuantaFlash, EliA, Bio-Plex 2200, Euro-Diagnostica, Orgentec, Euroimmun, and CytoBead, respectively. The median MPO-ANCA values were

16.0 U, 25.1 CU, 6.1 IU/mL, 3.6 AI, 5.8 IU/mL, 5.5 U/mL, 29.6 U/mL and 2.4 IU/mL, respectively. We did not use an internal standard in an attempt to standardize the results. Such initiatives are currently ongoing using the new certified reference materials for MPO-ANCA [5] and PR3-ANCA.

In conclusion, in this study which was driven by the clinical relevant question on the comparability of PR3- and MPO-ANCA in vasculitis patients, we found that the majority of assays do not give comparable ANCA values. This is also true for the three assays (Medipan, Euro-Diagnostica and EliA) that were calibrated on the IUIS-CDC reference sera for PR3- and MPO-ANCA, respectively. It is therefore important that ANCA values followed over time for predicting relapse are measured by the same immunoassay.

Figure 2: Correlations of individual values of MPO-ANCA in MPA patients ($n = 21$) measured by eight different immunoassays. Spearman rank correlations (r_s) (lower left cells in inset) and p-values (upper right cells in inset) for significant linear correlations are included in the graph. The immunoassays included were from INOVA [QuantaLite (1) ELISA and QuantaFlash chemiluminescence (CLIA) (2)], Thermo Fisher fluorescence immunoassay (FEIA) (ELIA) (3), Bio-Rad bead-based assay (BioPlex 2200) (4), Euro-Diagnostica capture ELISA (5), Orgentec ELISA (6), Euroimmun ELISA (7), and Medipan (CytoBead assay) (8). The data were from reference [1] after removing censored data.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: Bio-Rad Laboratories Inc., Euro-Diagnostica AB, Euroimmun, Medipan/Generic assays GmbH, INOVA Diagnostics, Orgentec, and Thermo Fisher Scientific provided reagents to perform the study.

Employment or leadership: X.B. has received speaker fees from Thermo Fisher and INOVA, has been a consultant for INOVA and has received a research grant from Thermo Fisher. E.C. has received lecture fees from Bio-Rad Lab, Inova Diagnostics and Thermo Fisher.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and

interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Damoiseaux J, Csernok E, Rasmussen N, Moosig F, van Paassen P, Baslund B, et al. Detection of antineutrophil cytoplasmic antibodies (ANCA): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. *Ann Rheum Dis* 2016;76:647–53.
2. Bossuyt X, Rasmussen N, van Paassen P, Hellmich B, Baslund B, Vermeersch P, et al. Anti-neutrophil cytoplasmic antibodies (ANCA) by immuno-assay: a multicenter study to improve clinical interpretation of PR3- and MPO-ANCA. *Rheumatology (Oxford)* 2017. doi: 10.1093/rheumatology/kex170. [Epub ahead of print].

Q3:
Please check
and confirm
the author
name in
ref. 1

Q4:
Please
update ref. 2

Q4 = Epub
ahead of print

3. Vermeersch P, Blockmans D, Bossuyt X. Use of likelihood ratios can improve the clinical usefulness of enzyme immunoassays for the diagnosis of small-vessel vasculitis. *Clin Chem* 2009;55:1886–8.
4. Kemna MJ, van Paassen P, Damoiseaux JGMC, Cohen Tervaert JW. Maintaining remission in patients with granulomatosis with polyangiitis or microscopic polyangiitis: the role of ANCA. *Expert Opin Orphan Drugs* 2017;5:207–18.
5. Monogioudi E, Hutu DP, Martos G, Sheldon J, Schimmel H, Meroni PL, et al. Development of a certified reference material for myeloperoxidase-anti-neutrophil cytoplasmic autoantibodies (MPO-ANCA). *Clin Chim Acta* 2017;467:48–50.