Letters to the Editor

pmol/L to $1 \times 10^6$ pmol/L and tested with 2 different lots of the Combo SP device (lot nos. 8080021 and 9020102). The results showed a lot-to-lot difference in the effect of hCGβcf on the results for hCG-positive urine (Fig. 1). Lot 8080021 was unaffected by up to $1 \times 10^6$ pmol/L hCGβcf. On the other hand, lot 9020102 was inhibited by in a dose-dependent manner and at an hCGβcf concentration of $1 \times 10^6$ pmol/L produced a result that was nearly invisible (Fig. 1). These data demonstrate the same inhibition by hCGβcf, in a single lot of the Combo SP Rapid Test device, that was previously reported for the OSOM and ICON devices.

Why these devices exhibit such between-lot variation is unclear. The package insert states that the device uses 1 monoclonal antibody and 1 polyclonal antibody. It is possible that one lot used a new batch of polyclonal antibody with a more avid specificity for hCGβcf or that it used polyclonal antibody at a concentration that yielded a lower binding capacity for hCG.

We previously provided data showing that caution is warranted when hCG devices in which hCGβcf causes negative interference are used to test women who are pregnant beyond 5–8 weeks gestation, because false-negative results may occur (1). Our new data suggest that the same caution should be used when interpreting a negative result from any qualitative hCG-testing device. At this time, we know of no device that is free from the inhibition effect by hCGβcf. This observation has been reported to the manufacturer and to the US Food and Drug Administration.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Reference


Ann M. Gronowski2*
Michelle Powers2
Ulf H. Stenman3
Lori Ashby4
Mitchell G. Scott2

2 Department of Pathology and Immunology
Washington University School of Medicine
St. Louis, MO
3 Department of Clinical Chemistry
Helsinki University Central Hospital
Helsinki, Finland
4 Barnes-Jewish Hospital
St. Louis, MO

* Address correspondence to this author at:
Washington University School of Medicine
Department of Pathology and Immunology
Box 8118
660 S. Euclid Ave.
St. Louis, MO 63110
Fax 314-362-1461
E-mail gronowski@wustl.edu

Previously published online at DOI: 10.1373/clinchem.2009.133280

Use of Likelihood Ratios Can Improve the Clinical Usefulness of Enzyme Immunoassays for the Diagnosis of Small-Vessel Vasculitis

To the Editor:

Antineutrophil cytoplasmic antibodies (ANCAs)1 are associated with small-vessel vasculitis (SVV), such as Wegener granulomatosis, microscopic polyangiitis, and Churg–Strauss syndrome (1). Indirect immunofluorescence (IIF) analyses can distinguish 2 major patterns: cytoplasmic and perinuclear. The cytoplasmic ANCA pattern is typically associated with antibodies to proteinase 3 (PR3), whereas the perinuclear ANCA pattern is typically associated with antibodies to myeloperoxidase (MPO). PR3 ANCAs are predominantly seen in patients with Wegener granulomatosis, whereas MPO ANCAs are predominantly seen in patients with microscopic polyangiitis (1–3).

Studies that have addressed the clinical usefulness of specific enzyme immunoassays (EIAs) for PR3 and MPO for the diagnosis of SVV have used a single cutoff value. In this letter, we illustrate how the likelihood ratio (LR) for SVV depends on the titer of anti-PR3 or anti-MPO antibodies. Our calculations are based on a clinically well-defined group of 37 consecutive patients with newly diagnosed SVV (data collected over a 10-year period) and 285 consecutive control individuals with disease (data collected over 21 months) that a physician suspected to be a vasculitis. Patients suspected of or having

1 Nonstandard abbreviations: ANCA, antineutrophil cytoplasmic antibody; SVV, small-vessel vasculitis; IIF, indirect immunofluorescence; PR3, proteinase 3; MPO, myeloperoxidase; EIA, enzyme immunoassay; LR, likelihood ratio.
a diagnosis for a gastrointestinal problem (e.g., inflammatory bowel disease) were excluded. The characteristics of the included patients have previously been described (4). One patient with antiphospholipid syndrome who had PR3 and MPO ANCs was excluded because SVV could not be ruled out. Two of the control individuals with disease were excluded because no residual serum sample was available. IIF analysis was performed with ethanol-fixed human neutrophil preparations (INOVA Diagnostics). PR3 ANCs and MPO ANCs were measured in international units per milliliter with the Wieslab capture kit from Euro-Diagnostica, in units with the QUANTA Lite™ method from INOVA Diagnostics, and in units per milliliter with the EliA™ assay platform from Phadia. The recommended cutoffs were 5 IU/mL, 20 U, and 7 U/mL, respectively. Patients were considered to have tested positive if either the PR3 ANCA result or the MPO ANCA result was positive.

We calculated the likelihoods and the LRs (likelihood of the patients with disease divided by likelihood of the control individuals) for different test-result intervals for each of the EIAs and for IIF. Table 1 summarizes the results. For the Wieslab capture kit, the LRs were 0.17, 5.1, 20, and 123 for 0–5 IU/mL, 5–7 IU/mL, 7–100 IU/mL, and >100 IU/mL, respectively. For the INOVA assay, the LR for a negative test result was between 0.1 and 0.2 for both the EIAs and the IIF assay, indicating a substantial, but modest, difference in pretest-to-posttest probability. The use of the manufacturer’s proposed cutoff produces more false-negative results with the Inova assay. The LR for a negative test result was between 0.1 and 0.2 for both the EIAs and the IIF assay, indicating a substantial, but modest, difference in pretest-to-posttest probability. Use of the manufacturer’s proposed cutoff produces more false-negative results with the Inova assay. The LR for a negative test result was between 0.1 and 0.2 for both the EIAs and the IIF assay, indicating a substantial, but modest, difference in pretest-to-posttest probability.

In conclusion, we have illustrated how the use of LRs for different test-result intervals can improve the clinical usefulness of EIA testing for SVV. Clinical laboratories might consider providing likelihood ratios for test-result intervals to improve clinical interpretation.

Table 1. Overview of the LRs as a function of antibody concentration for 3 different EIAs (Wieslab capture kit from Euro-Diagnostica, QUANTA Lite™ from INOVA Diagnostics, and EliA™ from Phadia) and one IIF assay (INOVA).

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Cutoff proposed by manufacturer</th>
<th>Test-result interval</th>
<th>LR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euro-Diagnostica (EIA)</td>
<td>5 IU/mL</td>
<td>0–5 IU/mL</td>
<td>0.17 (0.08–0.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5–7 IU/mL</td>
<td>5.1 (0.89–29.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7–100 IU/mL</td>
<td>20 (7.6–53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100 IU/mL</td>
<td>123 (16.8–903)</td>
</tr>
<tr>
<td>Inova (EIA)</td>
<td>20 U</td>
<td>0–14 U</td>
<td>0.14 (0.06–0.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14–20 U</td>
<td>7.7 (1.1–53.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–100 U</td>
<td>58 (13.8–243)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100 U</td>
<td>116 (14.7–850)</td>
</tr>
<tr>
<td>Phadia (EIA)</td>
<td>7 U/mL</td>
<td>0–7 U/mL</td>
<td>0.14 (0.06–0.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7–20 U/mL</td>
<td>5.1 (0.89–29.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–100 U/mL</td>
<td>100 (13.5–744)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;100 U/mL</td>
<td>131 (17.9–956)</td>
</tr>
<tr>
<td>Inova (IIF)</td>
<td>1:40 Negative</td>
<td></td>
<td>0.13 (0.05–0.33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:40</td>
<td>0.8 (0.19–3.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:80–1:320</td>
<td>8 (4.2–16.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;1:320</td>
<td>10 (5.3–19.0)</td>
</tr>
</tbody>
</table>

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: X. Bossuyt, IMMCO Diagnostics.
Stock Ownership: None declared.
Letters to the Editor

Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Other: X. Bossuyt, support from Phadia and INOVA Diagnostics to participate in scientific meetings.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: We thank Euro-Diagnostica, INOVA Diagnostics, and Phadia for providing reagents for this study.

References

Pieter Vermeersch2
Daniel Blockmans3
Xavier Bossuyt2

2 Laboratory Medicine, Immunology
3 General Internal Medicine
University Hospitals Leuven
Leuven, Belgium

* Address correspondence to this author at: Laboratory Medicine, Immunology University Hospitals Leuven Herestraat 49 B-3000 Leuven, Belgium Fax +00-32-13-347042 E-mail xavier.bossuyt@uz.kuleuven.ac.be

Increased Plasma Lipoprotein(a) Found in Large-Artery Atherosclerotic, but Not Small-Artery Occlusive, Stroke

To the Editor:

We previously reported that plasma lipoprotein(a) [Lp(a)]1 is an independent risk factor for coronary artery disease, abdominal aortic aneurysm, peripheral arterial disease, and ischemic stroke (1). Although the exact pathophysiologic role of Lp(a) has not been definitively established, both atherogenic and thrombogenic mechanisms have been proposed. In this study, we measured plasma Lp(a) in ischemic stroke patients who had been subdivided into etiologic subtypes with the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification to determine if stroke subtype is a cofounder of the Lp(a) association.

Ischemic stroke patients (n = 245) and arterial disease–free and stroke-free controls (n = 435) were recruited from the Otago region of New Zealand as previously reported (1). Stroke patients were placed into TOAST classification subtypes by means of a computer algorithm developed by Goldstein and colleagues (2). The TOAST subgroups were large-artery atherosclerosis (LAA), cardioembolism, small-artery occlusion (SAO), undetermined due to 2 or more causes (multiple etiologies) (ME), or undetermined etiology due to negative or incomplete evaluation. Inclusion criteria for controls were an age ≥52 years and no history of ischemic heart disease or stroke, including transient ischemic attack [Questionnaire for Verifying Stroke-Free Status (QVSFS) score = 0]. Plasma lipoprotein and demographic risk factors were assessed as previously described (1). The study was approved by the local human ethics committee, and all study participants gave written informed consent before being recruited into this study. The χ2 test and ANOVA were used to assess nominal and normally distributed variables, respectively. We used the Mann–Whitney U-test to assess Lp(a) because of the nongaussian distribution of this variable. Logistic regression models were used to stratify Lp(a) by control population quartiles. Odds ratios were calculated for the fourth quartile (>50 nmol/L) vs the first quartile (<4.5 nmol/L). Multiple logistic regression was used to identify statistically significant interactive effects of variables on susceptibility to ischemic stroke.

Demographic factors associated with ischemic stroke are shown in Table 1; statistically significant confounders were included in subsequent multivariable logistic regression models. Plasma Lp(a) concentrations were significantly higher in the total-stroke group than in vascular disease–free controls (P < 0.03, Table 1). Lp(a) was differentially associated with TOAST stroke subtypes. Most notably, SAO strokes were not associated with Lp(a), and the association with the all-strokes group was largely attributable to increased concentrations in the “atherothrombotic” stroke subtypes (LAA and ME). Overall, 86% of ME patients had LAA as one of their identified pathologies. The ME cases with LAA were grouped with the LAA TOAST subgroup to form a patient population designated as having an “atherothrombotic” stroke. Compared with stroke-free controls, atherothrombotic cases had an odds ratio of 3.47 (95% CI, 1.26–9.60; P < 0.02) and an ad-