Letters to the Editor

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References

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Potential Interference by Antineutrophil Cytoplasmic Autoantibodies in Myeloperoxidase Immunoassays

To the Editor:

Myeloperoxidase (MPO)1 has been identified as a potential marker of cardiovascular disease (1). As the clinical utility of new biomarkers is identified and commercial assays are developed, limitations on their use need to be identified and reported. Antineutrophil cytoplasmic autoantibodies (ANCAs) are known to exist in patients with diseases characterized by primary systemic vasculitis, such as Wegener granulomatosis, microscopic polyangiitis, and Churg–Strauss syndrome, and in those with idiopathic pauci-immune necrotizing crescentic glomerulonephritis, systemic lupus erythematosus, and rheumatoid arthritis (2). In particular, the immunofluorescence-staining pattern for perinuclear ANCAs (pANCAs) is associated with anti-MPO autoantibodies. pANCAs are most prevalent in microscopic polyangiitis (40%–80%), followed by Churg–Strauss syndrome (20%–30%) and Wegener granulomatosis (5%–20%).

Autoantibodies can interfere with assays when the autoantibodies bind to analyte epitopes that are similar to those of the monoclonal antibodies used in the immunoassay. Anti-MPO autoantibodies are likely to bind to MPO in the sample and block or partially mask the epitopes necessary for binding to the antibodies used to capture and detect MPO. We evaluated the effect of MPO autoantibodies on the performance of the Cardio MPO™ immunoassay (Prognostix), which has been cleared by the US Food and Drug Administration. We similarly evaluated an immunoassay under development for the Abbott ARCHITECT® instrument (Abbott Laboratories). Characteristics of these assays have been described previously (3, 4). The 2 assays use different monoclonal antibodies that appear to detect unique epitopes (unpublished data).

We collected samples from 40 healthy donors with informed consent after we obtained institutional review board approval for our protocol. We also obtained 189 surplus pANCA-positive, deidentified patient samples from ARUP Laboratories (the University of Utah institutional review board waived informed consent for these samples). We used native MPO antigen (Athens Research & Technology) for spiking experiments.

We tested the samples from healthy donors without added analyte with both assays and then repeated the experiments after spiking the samples with a known MPO amount. MPO concentrations in samples without added analyte ranged from 44–5550 pmol/L with the ARCHITECT assay and from 161–2353 pmol/L with the CardioMPO assay. The difference between the 2 assays in MPO concentrations for the unsiked samples was possibly due to a lack of standardization. Assignments of calibrator values, matrix effects, and differences in antibody specificity may also account for this difference. Similarly, the amounts of spiked MPO recovered varied, depending on the assay used. The ARCHITECT assay results were more consistent than the CardioMPO results. To account for the difference in recovery and lack of standardization seen in the samples from healthy individuals, we determined the mean recovery for each assay.

1 Nonstandard abbreviations: MPO, myeloperoxidase; ANCA, antineutrophil cytoplasmic autoantibody; pANCA, perinuclear ANCA.
We then used this value to calculate the expected recovery. Recoveries ranged from 89% to 111% with the ARCHITECT MPO assay and from 59% to 143% with the CardioMPO assay. A nonparametric statistical analysis showed no significant difference between the results obtained with the 2 assays (Wilcoxon rank sum test, \( P = 0.40 \); Fig. 1).

Patient samples were tested both without added analyte and with MPO spiked to the same concentration as the healthy donors. Median recoveries were 95% (range, 0%–114%) for the ARCHITECT assay and 67.0% (range, 1.0%–155%) for the CardioMPO assay (Fig. 1). The results obtained for the 2 assays were significantly different in a nonparametric analysis (Wilcoxon rank sum test, \( P < 0.0001 \)). Compared with the samples from healthy individuals, the patient samples with MPO autoantibodies showed decreased MPO recoveries, demonstrating the presence of autoantibody interference in the 2 assays. Recoveries were variable, both between samples and between assays. The fact that the samples with the lowest recovery values were not necessarily the same in the 2 assays demonstrates the heterogeneity and polyclonal nature of the autoantibodies present and their differing epitope specificities. There was no correlation between pANCA titer and percent recovery for either assay (data not shown); some of the lowest recovery values were seen in samples with lower titers.

The prevalence of these autoantibodies in the general population is difficult to quantify because only a small portion of the population, typically patients with primary systemic vasculitis, are clinically evaluated. The overall incidence of primary systemic vasculitis has been reported to be approximately 10–20 per million in studies of primarily European populations (5). The prevalences per million population are approximately 5–12 for Wegener granulomatosis, 3–12 for microscopic polyangiitis, and 0.5–4 for Churg–Strauss syndrome. The prevalence of such autoantibodies in other populations has not been evaluated in detail, but small studies have indicated a higher prevalence of MPO ANCA–positive disease in some Asian populations (5). As testing for MPO becomes more widespread, however, the potential for false-negative values must become better appreciated, and the clinical presentation, in addition to test results for other biomarkers, must be taken into account when making medical decisions.

**Fig. 1.** Combined box-and-whisker and dot plots of recovery results for the ARCHITECT MPO and CardioMPO assays in MPO-spiking experiments. Results for samples from healthy individuals (Healthy) and pANCA-positive samples (ANCA) are at left and right, respectively. Data are summarized as medians, 25th and 75th percentiles (boxes), and 1.5 times the interquartile range (whiskers). \( P \) values are for the Wilcoxon rank sum test.

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Concerns Regarding Lipoprotein Particle Measurement by Ion Mobility Analysis

To the Editor:

We read with interest the report by Caulfield et al. (1) describing a new method for measuring lipoprotein particle concentrations by ion mobility (IM)1 analysis. There is growing evidence that the LDL particle concentration, as assessed by apolipoprotein B (apo B) or nuclear magnetic resonance (NMR) measurements, is a better discriminator of cardiovascular risk than the LDL cholesterol concentration, and measures of VLDL and HDL particles have also been suggested to have clinical advantages over analyses of their lipid counterparts. For these reasons, methods capable of quantifying lipoprotein particle concentrations accurately and efficiently are of considerable interest, and we congratulate the authors on their efforts.

As part of the validation of the IM method, Caulfield and colleagues present several plots that compare IM-measured particle concentrations with corresponding lipid or apolipoprotein measurements. The results of some of these comparisons do not agree with expectations and raise concerns in our minds about the accuracy of the measured particle concentrations. As the authors point out, only a moderate association is expected between VLDL, LDL, and HDL particle concentrations and the corresponding lipid measurements, given that the cholesterol and triglyceride contents of these lipoproteins are known to vary considerably from person to person; however, because there is one apo B molecule on every non-HDL particle (LDL, intermediate-density lipoprotein, VLDL), very good agreement is expected between plasma apo B concentrations and IM-measured non-HDL particle concentrations. The comparison data in Fig. 4C of the report by Caulfield et al. do indeed show a strong correlation (r = 0.92) between the 2 particle assays. What is not clear is why particle concentrations obtained with the IM method are much lower than concentrations of apo B particles (30%–40% at median concentrations) and why the relationship appears to be nonlinear. The authors attribute the discrepancies to the apo B assay overestimating particle concentrations rather than to the IM method underestimating them and suggest that limitations of the apo B immunoassay are the source of the problem. The implicit assumption is that the IM method correctly quantifies lipoprotein particles because it “directly counts the particles present.”

The accuracy claim is called into more serious question by data in the study of Caulfield et al. that indicate that HDL particle concentrations measured by IM are strikingly lower than expected. The median total HDL particle concentration reported by Caulfield et al. for healthy adults was approximately 3000 nmol/L, only about 3-fold higher than LDL particle concentrations. In contrast, the median HDL particle concentration measured by NMR spectroscopy in healthy adults in the Multi-Ethnic Study of Atherosclerosis was about 10 times greater, approximately 30 000 nmol/L (2). Are HDL particle concentrations measured by IM much too low, or are NMR values much too high? The answer is provided by data provided by Caulfield et al. that relate the concentrations of apolipoprotein A-I (apo A-I) to total HDL particles (see Fig. 3B in these authors’ online Data Supplement at http://www.clinchem.org/content/vol54/issue8). Apo A-I does not have a fixed stoichiometric relationship with HDL particles, unlike apo B with LDL particles. Rather, compositional studies have indicated that small, medium, and large HDL particles contain 2, 3, and 4 molecules of apo A-I per particle, respectively (3–5). Because the concentrations of small HDL particles are much greater than those of large HDL particles, an individual on average has approximately 2.5 apo A-I molecules per HDL particle. In Fig. 3B of their online Data Supplement, Caulfield et al. plot apo A-I concentration against the IM-measured total HDL concentration. An examination of these data indicates that an HDL particle concentration of 3000 nmol/L corresponds to an.

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1 Nonstandard abbreviations: IM, ion mobility; apo B, apolipoprotein B; NMR, nuclear magnetic resonance; apo A-I, apolipoprotein A-I.