Variability of Lipoprotein-Associated Phospholipase A2 Measurements

To the Editor:

We read with interest the recent report in this journal by Khuseyinova and colleagues regarding the variability of serial lipoprotein-associated phospholipase A2 (Lp-PLA2) measurements in postmyocardial infarction patients (1). Because Lp-PLA2 plays a role in inflammation but, unlike other inflammatory proteins, is not an acute-phase reactant (2), nonspecificity issues associated with acute-phase reactants may be averted. Indeed, recent reviews verify that several epidemiologic studies have demonstrated a strong association between Lp-PLA2 and ischemic events (2–4). Thus, as indicated by Khuseyinova and colleagues, Lp-PLA2 appears to be a promising emerging biomarker for coronary heart disease and stroke. We applaud the efforts of this group to evaluate the performance of Lp-PLA2 measurements.

With all assay development, multiple iterations of assays are often developed before a given analyte is ready to be measured on a widespread basis, and Lp-PLA2 is a good example. It is important to keep these multiple iterations separate because they tend to differ. We too have had consistent results with the second-generation assay, the one reported by Khuseyinova and colleagues. This second-generation assay is no longer commercially available, however, and a third-generation assay has replaced it. We have completed extensive evaluations of both the second- and third-generation assay methods for Lp-PLA2. In our hands, the third-generation assay behaved quite differently from the second, with much greater variability and lot-to-lot variation, and values that were very different from those obtained with the second-generation assay for some patient samples. A comparison of the 2 iterations in 476 patient samples yielded the following correlation data: $3^{rdGen} = (0.557 \times 2^{ndGen}) + 67.0; R^2 = 0.712$. As a result of analytic problems and our inability to get the third generation assay to perform to reasonable specifications, we delayed and actually stopped reporting values obtained with the third-generation assay. In particular, results obtained while performing the assay in a manual format did not match values obtained using a Triturus® automated immunoassay analyzer. Thus, both the method and instrumentation used to perform the method should be considered during method validation. Although these issues were ultimately resolved and we did institute the third-generation assay in a manual format, we still monitor this assay very carefully and go through several steps to verify the performance of each new lot of reagents.

A fourth-generation assay has recently been cleared by the FDA for use and we have evaluated it in our laboratory. This automated method is more user friendly and has performance characteristics more similar to and perhaps even better than those of the second-generation assay. We found the automated method to be linear down to 30 μg/L ($y = 1.052x + 9.6, R^2 = 0.998$). Interassay CVs ranged from 6.6% to 10.4% for 6 samples over the concentration range of 94–441 μg/L ($n = 11$ replicates per sample). Discrepancies were observed when we compared the automated and third-generation assays ($\text{automated} = (1.20 \times 3^{rdGen}) + 3.4; R^2 = 0.4808; n = 65$), further demonstrating differences that may be observed from one iteration to the next. We are optimistic about the long-term success of the automated method, but areas of fine tuning are needed before it is ready for wider dissemination. The important conclusion to draw from this situation is that from the research and the clinical perspectives, care must be exercised in documenting what iteration of a given assay is being used, especially when comparing values over time. We do include a comment about this concern in all of our reports.

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References


Joseph P. McConnell*
Allan S. Jaffe
Department of Laboratory Medicine and Pathology and Division of Cardiovascular Diseases
Mayo Clinic
Rochester, MN
Depletion of abundant proteins from plasma and serum is an important initial step in many biomarker discovery platforms. Decreasing the concentrations of highly abundant proteins (e.g., albumin, IgG, and antitrypsin) facilitates the use of contemporary proteomics technologies, such as gel electrophoresis and mass spectrometry, for detection and identification of low-abundant proteins. Furthermore, decreasing abundant protein concentrations may also improve immunoprecipitation recovery efficiencies for targeted low-abundant species by decreasing nonspecific binding (i.e., shielding the antigen-binding domain) to the antibody and/or solid supports. A notable pitfall to depletion strategies is the potential for unintentionally removing low-abundant plasma or serum proteins. These low-abundant species may be bound specifically or nonspecifically to the depletion ligand, depletion target protein (e.g., carrier proteins), or the solid support(s). Thus, it is important to critically evaluate the effectiveness of abundant plasma protein depletion for enhancing the study of low-abundant protein biomarker(s).

We have been actively developing a targeted biomarker discovery platform for characterizing the circulating forms of B-type natriuretic peptide (BNP) that includes protein depletion strategies, immunoprecipitation, gel electrophoresis, isotope dilution (absolute quantification), and nanoflow liquid chromatography coupled to high-performance hybrid Fourier transform mass spectrometry. BNP has emerged as a very important biomarker for the clinical diagnosis and management of heart failure. Despite the increasing clinical use of BNP, very little is known about the secretion, post-translational processing, and receptor binding of BNP at the molecular level. This gap in knowledge between the diagnostic and prognostic utility of BNP and the basic understanding of the molecular form(s) and function(s) of BNP is presently hindering the advancement of heart failure research. Increasing evidence in our laboratory and others reviewed by Goetze and Liang has shown that BNP circulates in heterogeneous forms that interfere with the commercially available BNP tests and potentially affect the pathophysiology of heart failure. These heterogeneous forms could have significant diagnostic, prognostic, and therapeutic value for the management of heart failure. The present obstacle to identifying circulating forms of BNP is the detection-limit gap that exists between the circulating concentrations of BNP, recovery efficiencies from plasma, and the high-performance hybrid Fourier transform mass spectrometry method. We sought to establish whether BNP-32 bound specifically or nonspecifically to the 6 most abundant proteins in plasma.

The general experimental setup in this study involved tracking radiolabeled BNP-32 (125I-[Tyr]-BNP-32; specific activity = 1322.66 Ci/mmol: Phoenix Pharmaceuticals) in human plasma (single individual samples collected from donors who gave informed consent and were deidentified in accordance with the Mayo Clinic’s Institutional Review Board) through the Multiple Affinity Removal System (MARS) (4.6 × 100 mm MARS 6, Agilent Technologies). Unbound (%B = 0) and bound (%B = 100) fractions were collected from the MARS 6 eluent and the 125I-[Tyr]-BNP-32 in each fraction was measured (1480 Wizard 3 Gamma Counter, Spectrofuge) as shown in Fig. 1. In the first experiment, 123 μL of plasma was combined with 123 μL of an 8 μg/L stock solution of 125I-[Tyr]-BNP-32 [in 110 mmol/L PBS (0.22 g NaH2PO4 + 1.280 g Na2HPO4 + 0.40 g NaCl + 0.01 g NaN3 in 100 mL deionized H2O; pH adjusted to 7.4 with NaOH) and 154 mmol/L NaCl] at 37 °C, then 25 μL aliquots of the 125I-[Tyr]-BNP-32/plasma sample were taken at 0, 1, and 2 h, diluted with 75 μL buffer A (Agilent Technologies; more information on buffer A can be found at http://www.chem.agilent.com/en-US/Support/FAQs/Proteomics/MARS/Pages/KB000818.aspx), filtered, and injected. The purpose of this experiment was to determine the effects of 125I-[Tyr]-BNP-32 incubation time in plasma on its binding to high-abundant proteins. Results for the 3 time intervals sampled showed that 98% of the 125I-[Tyr]-BNP-32 was eluted in each of the 3 unbound fractions (Fig. 1). The data clearly indicate that 125I-[Tyr]-BNP-32 does not bind to any of the top 6 most abundant proteins in plasma (albumin, IgG, antitrypsin, IgA, transferrin, and haptoglobin). A second set of experiments was performed to determine the reproducibility of the 125I-[Tyr]-BNP-32 recoveries shown in Fig. 1. The results show that 125I-[Tyr]-BNP-32 does not...