

sured LDL (3.3%). Thus the CV of measured components should be lower to obtain an acceptable CV for calculated LDL and thus reach the Six Sigma goal. Otherwise, we must measure the serum LDL concentration by chemical methods.

In conclusion, when a QA process is implemented in the clinical laboratory, application of that process only to measured tests is inadequate. Because of their higher CVs, results of calculated tests have lower precision than those of measured tests. Thus, Six Sigma world-class quality may be difficult to attain for calculated tests.

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Validation of Breast Cancer Biomarkers Identified by Mass Spectrometry

To the Editor:

Li et al. (1) should be congratulated for a valiant effort to validate 3 previously identified serum breast cancer biomarkers by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS).

Because there is considerable controversy on the value of this technology for cancer diagnostics (2–11), it is important to comment on validation studies aiming to reproduce previously published data. Among 3 previously reported biomarkers, BC1, BC2, and BC3, one of these (BC1) was not confirmed, as it was previously shown to be decreased in breast cancer, whereas in the validation study by Li et al. (1), it was increased.

The other 2 candidate biomarkers, BC2 and BC3, were positively identified, by tandem MS, as complement C3a lacking its C-terminal arginine (C3a_{desArg}). BC2 was also identified as a truncated form of C3a_{desArg}.

In my opinion, the data presented in Fig. 4 of the article by Li et al. (1), showing the relative intensities of BC2 and BC3 in various groups of patients, are rather disappointing. For BC2, there is no difference between patients with benign breast diseases and patients with invasive carcinomas, although an increase was seen in ductal carcinoma in situ (DCIS). For BC3, there was no difference among patients with benign disease, DCIS, or invasive carcinomas.

The remaining question concerns the possible value of complement C3a_{desArg} and its fragment as candidate breast cancer biomarkers. The data provided by the authors (1) confirm my previous predictions that SELDI-TOF-identified biomarkers represent high-abundance proteins (in this case, C3, present in serum at concentrations of ~1.2 g/L) that are produced mostly by the liver (3–6). The proteolytic processing of peptides in the circulation by amino- and carboxypeptidases is well known, and it should not be surprising that the identified molecules represent modified and/or truncated forms of C3a.

I have previously speculated that a large number of SELDI-TOF-identified candidate biomarkers are acute-phase reactants (3–6). C3, in accordance with my previous predictions, is also an acute-phase reactant whose serum concentration is increased or decreased in a wide variety of clinical conditions (12).

I conclude that the positive identification of previously described candidate serum biomarkers, BC2 and BC3, confirms my previous predictions that these are high-abundance proteins produced by the liver and that they represent nonspecific biomarkers of acute-phase reaction. Their performance as breast cancer biomarkers, as assessed by SELDI immunoassay, is not impressive and likely of questionable clinical value.

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Some of the authors of the article cited above respond:

To the Editor:

We thank Dr. Diamandis for his comments and wish to comment on some of the issues that he raised.

First, we want to clarify that it was not the intention of the original discovery paper (1) or of the recent validation paper (2) to determine the suitability of "this [SELDI] technology for cancer diagnostics". We used surface-enhanced laser desorption/ionization (SELDI) technology for biomarker discovery and validation.

We do not disagree with Dr. Diamandis' statement and prediction that candidate biomarkers discovered in serum or plasma by SELDI (and by many other high-throughput proteomics profiling technologies) tend to be high-abundance proteins. We believe, however, that this situation reflects not only the low sensitivity of the current mass spectrometers and other detection technologies, but also the lack of sample preparation (depletion, fractionation) technologies that are applicable for high-throughput analysis with high reproducibility (low CV in both mass accuracy and relative protein expression measurement).

The letter by Dr. Diamandis did not provide any actual data to discount the findings in our 2 papers; the questions he raised centered on the more general issues of whether such abundant proteins can truly be disease-associated biomarkers and whether they can be clinically relevant.

Proteolytic processing of peptides in circulation is a well-known phe-

nomenon, and many of the protein fragments are from abundant proteins that are less likely to be directly secreted from the actual tumor sites. It is also possible, however, that cancer cells and/or the host immune system may produce proteases and other enzymes that are tumor specific and may produce specific fragmentation and cleavage of common proteins, including abundant proteins. An increasing body of evidence, from our own group (3,4) and from others (5,6), supports such possibilities. The fact that many known serum/plasma biomarkers are abundant proteins or their derivatives could be caused by limitations of the current technology. Alternatively, such biomarkers could be the "amplified" signal of a disease process that otherwise might not be easily detected at an early stage. A tumor biomarker is evaluated by its relevance to the disease; it is not a prerequisite for it to be a direct product of the tumor.

Cancers are heterogeneous diseases with multiple subphenotypes, each following a distinct pathway. With possibly a few exceptions, it is unlikely that a single marker will provide acceptable diagnostic sensitivity and specificity for any of these complex diseases. It is our firm belief that multiple biomarkers, each with clinically verifiable relevance to the disease, combined through statistically sound approaches will offer better diagnostic performance than any individual marker. It is possible that some biomarkers, such as BC2 and BC3, may not be super performers on their own, but provide value in combination with others.

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Pretreatment of Serum with Penicillamine: Effects on Capillary Electrophoresis Patterns and on Immunonephelometric Measurement of Immunoglobulins

To the Editor:

Capillary zone electrophoresis (CZE) performed on "clinical" analyzers effectively detects monoclonal components (MCs) (1-4) in human serum. Some small MCs (5), and a few large IgM MCs, escape CZE detection, but the latter are detected in the same assay after pretreatment of serum with thiol reagents (6-8). We investigated the effects of pretreatment with penicillamine (PA) on the CZE pattern and on measurements of immunoglobulins (IgA, IgG, and IgM) in a variety of sera.

We selected 204 serum samples, including 79 samples with either a physiologic CZE pattern and immunoglobulins or with polyclonal hypergammaglobulinemia (IgA, IgG, or IgM); 24 samples with IgA MCs or IgG MCs; and 101 samples with IgM MCs. Any MC initially detected either by CZE or by agarose gel electrophoresis (AGE) was confirmed