
Re: Serum Proteomic Patterns for Detection of Prostate Cancer

Petricoin et al. (1) proposed that assessment of serum proteomic patterns with mass spectrometry and combination of these patterns with a bioinformatic algorithm can detect prostate cancer with approximately 95% sensitivity and 70%–80% specificity. These figures seem favorable when compared with common biochemical testing for prostate cancer, which currently includes analysis of total prostate-specific antigen (PSA) and percent free PSA in serum. Similar approaches have been proposed by the same group for the diagnosis of ovarian and other cancers (2).

The authors speculate that the discriminatory proteomic patterns originate from proteins (including cytokines, chemokines, metabolites, or enzymatic cleavage products) released by the prostate or its microenvironment during blood percolation through the organ. However, a limitation of this technology, also discussed elsewhere (3), is that the authors offer no evidence in regard to the identity of their seven most discriminatory molecules. In a previous publication (4), the authors contend that the identity of these proteins is not important in the context of their method. However, the identity of such proteins, as well as their relative concentrations in serum, is of paramount importance for understanding why they offer discriminatory power for detecting prostate cancer and how they originated in the circulation. For example, to derive their algorithm, Petricoin et al. (1) used serum samples from patients with biopsy-proven prostate cancer and total PSA levels of more than 4 ng/mL and from patients with no evidence of disease and total PSA levels of less than 1 ng/mL. It would have been interesting to know whether the authors' method identified this internal control and highly discriminating molecule (i.e., PSA) as one of the discriminatory proteins in serum. Such a finding would confirm that their method is sensitive enough to detect proteins at a concentration level of 1 ng/mL or higher. Their method of extracting serum prior to analysis (i.e., by hydrophobic interaction with a C16 column),

which is not specific for any molecule, would favor the isolation of highly abundant serum proteins and the loss of low-abundance proteins. Furthermore, the current sensitivity of mass spectrometry would preclude identification of molecules present in serum at nanogram-per-milliliter levels, unless shown otherwise by the authors. Because PSA is present in the prostate and seminal plasma at milligram-per-milliliter levels, but only at 10^6 -fold lower levels in serum, it would seem unlikely that prostate-derived proteins would be detected efficiently in serum by the authors' method. This information would most likely lead to the conclusion that the discriminatory proteins identified in the serum of these patients are unlikely to come from prostate tissue. Instead, these proteins are probably epiphenomena of cancer, represented by highly abundant nonprostatic proteins—that is, they could be acute phase reactants. Such epiphenomena could lead to errors when used for diagnosis, especially in patients with diverse abnormalities, such as malnutrition, diabetes, inflammation, and infections.

Given the implications of the reported findings by Petricoin et al. (1), it will be important to identify the nature of the discriminatory proteins, their concentration in the circulation, and their tissue(s) of origin. Such identification would allow for a better understanding of their differential expression and association with prostate cancer.

ELEFTHERIOS P. DIAMANDIS

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Affiliations of author: E. P. Diamandis, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital and the Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada.

Correspondence to: Eleftherios P. Diamandis, M.D., Ph.D., F.R.C.P.C., Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario M5G 1X5, Canada (e-mail: ediamandis@mtsinai.on.ca).

RESPONSE

We agree with Dr. Diamandis that the identity of the proteins comprising the discriminatory ions within the diagnostic proteomic pattern can potentially lead to insights concerning their source and relationship to the underlying pathology. However, the clinical evaluation of proteomic patterns as a new diagnostic paradigm can, and must, proceed independently from the pursuit of the physiologic source and identity of these proteins. Indeed, the characterization and identification of prostate-specific antigen (PSA) and CA 125 (for protein identification only) has had little impact on their ongoing utility and widespread clinical use as diagnostic cancer biomarkers.

Subsequent to our initial report in *Lancet* (1), a number of research groups have confirmed the diagnostic potential of serum proteomic patterns, as applied to a variety of cancers (2–4) and to our own ongoing efforts (5).

We hypothesize that the ion amplitudes comprising the diagnostic patterns in matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) spectra are derived (directly or indirectly) from the molecular state of the tumor–host microenvironment, and that the proteomic pattern that emanates from this microenvironment may signal the presence of an early-stage lesion. Under this hypothesis, the discriminatory ions are likely to be metabolic products, enzymatic fragments, modified proteins, peptides, or cytokines that could be highly specific for the microenvironment of the lesion.

Any plan to identify such ions must take into consideration two important issues. First, the amplitude of a given ion peak in a MALDI-TOF spectra is a complex result of the ionization pattern in the context of the matrix and the population of proteins and protein-binding partners in the sample that influence the ionization of each other. Thus, a peak amplitude value at a given m/z value cannot be equated to the concentration of a molecule (of that molecular size) in a serum sample. Second, an ion peak with high amplitude does not necessarily equate to a more abundant protein

than one with a correspondingly low amplitude in the same spectral image; this concept is very important to understand.

Perhaps it is time to move beyond the presumption of the existence of a single cancer-specific biomarker. Because cancer cells are themselves deranged host cells, we may never find a “true” cancer-specific biomarker. Alternatively, the complex proteomic pattern of the tumor–host microenvironment may be unique and may constitute a biomarker amplification cascade. In fact, the most important biomarkers may be normal host proteins that are aberrantly clipped or reduced in abundance. A pattern analysis approach takes into consideration this loss or gain of ions within the spectra.

Depending on the identity of the discriminatory ion, it may or may not be desirable, or even feasible, to proceed directly to the development of a serum immunoassay for an individual biomarker. If the biomarker is the cleaved version of a larger protein, it may be difficult to generate antibodies that recognize the cleaved version but do not cross-react with the parent species.

Mass spectroscopy platforms of the future, coupled with heuristic pattern recognition algorithms, may become superior to immunoassays as clinical analyte sensors. Within seconds, mass spectroscopy can generate complex proteomic spectra from a small volume of blood—in effect, sensing the presence of hundreds to thousands of events simultaneously. Current mass spectroscopy platforms routinely achieve sensitivity in the femtomolar range and will become even more sensitive with improvements in the technology. As mass spectroscopy technology advances, it will be possible to obtain direct biomarker identification of diagnostic ions “on-the-fly.”

Toward this goal, we are exploring a wide variety of mass spectroscopy platforms and tools. Mathematically, it should be obvious that a pattern of multiple biomarkers will contain a higher level of discriminatory information compared with that of a single biomarker, particularly for large heterogeneous patient populations. We are currently undertaking clinical trials to explore and validate this concept as applied to a variety of diseases. As evidence of the growing acceptance of this concept,

large commercial laboratories have begun initiatives to explore mass spectrometry proteomic patterns for routine diagnosis (6).

EMANUEL F. PETRICOIN III
LANCE A. LIOTTA

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Affiliations of authors: E. F. Petricoin III (Office of the Director/Center for Biologics Evaluation and Research), Food and Drug Administration–National Cancer Institute (FDA–NCI) Clinical Proteomics Program, FDA, National Institutes of Health (NIH), Bethesda, MD; L. A. Liotta, Laboratory of Pathology, Center for Cancer Research, FDA–NCI Clinical Proteomics Program, NCI, NIH, Bethesda.

Correspondence to: Emanuel F. Petricoin III, Ph.D., Bldg. 29A, Rm. 2D12, 8800 Rockville Pike, Bethesda, MD 20892 (e-mail: petricoin@cber.fda.gov).