Letter to the Editor

POINT: EPCA-2: A promising new serum biomarker for prostatic carcinoma?

In a recent paper [1], Leman et al. claim that they identified a new and highly promising prostate cancer biomarker, early prostate cancer antigen (EPCA)-2. They postulate that this biomarker performs with better sensitivity and specificity than the traditional prostate cancer biomarker, prostate-specific antigen (PSA). They have further shown that this marker can differentiate between localized and extracapsular disease and that it may be suitable for patient monitoring post-prostatectomy. Clearly, if this new biomarker is found to perform with similar efficiency in independent validation studies, it may have a major impact in reducing prostate cancer biopsies and in selecting patients who are suitable for individualized treatments.

Soon after its publication, the paper was picked-up by numerous national and international newspapers (e.g. see Baltimore Sun: http://www.baltimoresun.com/news/health/bal-te.prostate26apr26,0,7620752.story?coll=bal-home-headlines), as well as television stations (e.g. see CBS News: http://www.cbsnews.com/stories/2007/04/26/health/webmd/printable2731577.shtml) and sparked comments by prominent urologists and agencies, such as the National Cancer Institute and the American Urological Association. While the data have been widely characterized as “striking”, “remarkable”, “important”, etc., others were somewhat skeptical on its actual clinical value over and above PSA. However, the scientific validity of the work was not questioned. The authors, and others, have predicted that the test will be out in approximately 2 years and that it may replace PSA. A company is currently working towards commercialization.

This case is reminiscent of another highly publicized method surfaced-enhanced laser desorption ionization-time-of-flight-mass spectrometry (SELDI-TOF-MS) [2], which was subsequently found to represent, more or less, science fiction. A healthy debate on that technology [3–8] facilitated the understanding of its shortcomings. It also stimulated more research, which has conclusively shown that the original data were flawed by bioinformatic artifacts [6,9], biases in sample collection and processing [10,11], and other design biases [12]. Another related technology, which also attracted widespread attention [13], has also been criticized for similar biases and related problems [14].

The paper under discussion [1], upon careful scrutiny, appears to suffer from a number of shortcomings, as follows:

1. The target antigen (early prostate cancer antigen — EPCA2) is mentioned to be a nuclear protein, identified previously by proteomic analysis. The acronym EPCA is not an accepted gene name, as indicated by searches of the HUGO Gene Nomenclature Committee (www.gene.ucl.ac.uk/nomenclature) and other databases. To my knowledge, the gene encoding this protein has not been conclusively cloned or characterized.

2. It will be highly surprising to those who work with circulating cancer biomarkers if a nuclear antigen represents a good serological marker for early cancer diagnosis. Simply, if the mechanism of release into the circulation is cell death, the amounts expected in serum, originating from dying cancer cells, are unlikely to be of sufficient quantity to be easily and reliably measurable, especially with small, localized tumors.

3. Apparently, the primary EPCA antibody was raised against peptides, representing certain epitopes. The indirect ELISA described briefly in the paper is unlikely to operate consistently, if at all, for the following reasons:

The authors attempt to coat the EPCA-2 epitope by incubating serum, diluted 2-fold, in polystyrene microtiter plates. Based on their published procedure [1], the authors pipette approximately 4 mg of total protein per 50 μL of sample volume (assuming a total protein concentration in serum of 80 g/L). This is equivalent to approximately 4,000,000 ng of total protein. It has been previously established that the surface coated inside a microtiter well, with 100 μL of fluid, can afford a maximum binding of approximately 40 ng of protein [15]. A simple calculation, based on the author’s protocol, indicates that only 1 molecule for every 100,000 added molecules of protein will immobilize on the microtiter well (0.001% of the input protein). This is equivalent to practically nothing. Since most of the total protein added to the wells represents albumin and other high-abundance proteins, it is highly unlikely, or impossible, that any low-abundance proteins, present in serum, will immobilize to the microtiter plate. Consequently, with this kind of assay, the generated signals will likely be dependent not so much on a low-abundance protein antigen concentration in serum, but more on the variability of total protein (i.e. high-abundance proteins), lipids or salts. It is thus almost certain that such an assay will not perform well in detecting a low-abundance protein concentration in the circulation. The presented data may suffer from an unknown bias in sample collection or processing, as described by Ransohoff [12].

In order to test my concerns, the authors are invited to develop a PSA assay based on direct antigen coating from serum and report their results, in comparison to a regular PSA ELISA. My
prediction is that such a PSA assay design will fail miserably. I have personal experience in developing and using ELISAs for over 20 years and I am not aware of any commercial ELISA working this way. In fact, this assay does not qualify as an ELISA (enzyme-linked immunosorbent assay) since there is no immunosorbent used.

(4) The inadequacy of the developed assay can be further revealed by examining carefully the reported data. For example, the primary data used for calculating diagnostic sensitivities, specificities, etc., are presented in Table 1. EPCA concentration is approximately 20 ng/mL in serum of healthy men and women, while patients with prostate cancer exhibit approximately double this amount (40 ng/mL). However, in Fig. 3 of the manuscript, the levels in healthy men and women are below 1 ng/mL, while in patients with prostate cancer, the levels are increased by approximately 2-fold (∼2 ng/mL). The authors mention that the data of Fig. 3 were derived from an “optimized” assay, versus the data of Table 1, which were apparently derived from an “unoptimized” assay. There is a 15-fold difference between the values derived from the “optimized” and “unoptimized” assays. The major discrepancy was attributed to some background signal. In my opinion, the authors should have derived all their data with a single assay in its optimized format.

(5) The notion that the new assay performs well in patients before and after radical prostatectomy (Table 3) is not very convincing. PSA is reduced dramatically post-radical prostatectomy (>20-fold change in all patients), while EPCA-2 is only slightly reduced by 2- to 3-fold. For patient monitoring, then, PSA should be the marker of choice.

(6) An assay that can discriminate efficiently between organ-confined (OC) and non-organ confined (NOC) disease could be an important tool for making therapeutic decisions. However, EPCA-2 falls short of this prediction since the overlap between patients with OC and NOC disease is tremendous (Fig. 1). The finding shows some statistical significance but its clinical value should be trivial or non-existent.

Research aiming towards identifying new and improved prostate and other cancer biomarkers is very important. The Early Detection Research Network (EDRN) of the National Cancer Institute [http://edrn.nci.nih.gov/] is supporting discovery and validation of biomarkers for various types of cancers. Similar efforts are supported by the Ontario Cancer Biomarker Network [www.ocbn.ca]. PSA is an excellent prostate cancer marker whose clinical value for diagnosis and management has been well-established [16]. The recognized shortcomings of PSA have prompted some interviewed clinicians to declare that “PSA has already been beaten” by the new test, EPCA-2 (see link to CBS News above). These careless statements should be viewed with caution.

Recent history is telling us that spectacular reports disseminated by the media on new diagnostics, such as the Nuclear Magnetic Resonance (NMR) fiasco of the 80s [17] and the more recent revelations of proteomics and peptidomics for early cancer detection, did not withstand validation. Especially, with this new test, it should be clear to those who understand the principles of immunological analysis that the assay used is incapable of generating the reported data. I hope that EDRN, and other organizations, will soon blindly validate this technology and establish its merits and pitfalls. Already, EDRN has completed a carefully designed validation study on SELDI-TOF diagnosis of prostate cancer with totally negative results (announced at the recent EDRN workshop in Pittsburgh, PA, on September 18–20, 2006).

One major limitation of our current system is that seemingly spectacular results are highly publicized to the media but their subsequent failures are not. Nor have the journals that publish these data shown any willingness to ask the original authors to retract false information [I have such experience with the journal Lancet, which originally published the SELDI-TOF technology (2)].

Recently, both others and I have published on the quality of the scientific literature [18–21] and suggested ways to improve it. One proposal is to re-evaluate, after 2 to 5 years, key papers that describe promising and high-impact diagnostic and therapeutic modalities. For example, a 5-year outlook of the highly publicized Lancet paper [2] indicates failure to reach the clinic, despite promises to the opposite. Since there is no formal retraction, the paper continues to be cited.

Authors of high-visibility papers should be aware that, by publicizing their data to the media, they bear the burden of defending them in the future. In the field of biomarkers, we now have organizations such as EDRN and OCBN, which have the means to validate spectacular reports in a short period of time. Let us agree on the rules of the game: Once the methods are blindly validated, the data should be published, so that the scientific literature keeps a record on what is working and what is not.

Recently, the same authors have published on two, apparently highly promising colon cancer-specific biomarkers [22]. Since the methodologies are the same, it can be concluded that these biomarkers should also be considered with caution, until an independent evaluation is performed and published.

References


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