Proteomic patterns to identify ovarian cancer: 3 years on

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Approximately 3 years ago, Petricoin III and coworkers published a highly provocative paper in the *Lancet* describing a novel method for early ovarian cancer diagnosis that had an overall sensitivity of 100% and a specificity of 95% [1]. Since then, the sensitivity and specificity of this method has improved to a perfect 100% [2]. The implications of such a method for the future of ovarian cancer diagnosis and treatment are enormous, since early ovarian cancer is highly curable, while late ovarian cancer is highly lethal. Published criticism of this technology has been minimal and mainly restricted to this author [3–6]. The same method was also applied for diagnosing prostate, breast, bladder, nasopharyngeal, pancreatic and various other cancers. This technology has already been reviewed and was declared as one of the most important medical advances of our times [7,8].

The eventual acceptance of any new breakthrough in clinical practice requires rigorous scientific confirmation and analytical validation, replication by other laboratories and careful clinical validation. Below, some of these issues as related to this technology are summarized.

Scientific confirmation & analytical validation

Careful examination of this technology reveals many shortcomings and inconsistencies. For example, a review of the relevant literature for prostate cancer diagnosis identified five reports from four different groups [5,6]. None of the four groups agree with each other as to which are the most informative discriminatory peaks. The same comments apply to two reports for ovarian carcinoma [5,6]. Importantly, in their latest report on ovarian cancer, Conrads and coworkers proposed four combinations of discriminatory peaks that can afford 100% specificity and sensitivity [2], but none of the peaks are the same with those initially identified in the original *Lancet* paper [1]. Also, known discriminatory molecules, such as the classical prostate and ovarian cancer biomarkers prostate-specific antigen (PSA) and cancer antigen 125 (CA125), have not been identified in published reports, suggesting that this method is not sensitive at low analyte concentrations. Among the molecules that have been positively identified by this technology as potential new cancer biomarkers, most, if not all, were found to be produced not by cancer cells but by the liver, and many are acute-phase reactants present in serum at huge concentrations (g/l), as predicted earlier by this author [3]. Some of the molecules identified as potential biomarkers by this technology were discovered more than 30 years ago but have not been used in clinical practice since they represent cancer epiphenomena or acute-phase reactants, thus lacking specificity [5,6]. Surprisingly, many parameters that could significantly affect the proteomic patterns in serum, such as details of sample collection, processing and storage, patient demographics and habits (e.g., gender, age, ethnicity, exercise, menopausal status, nutritional preferences, drug ingestion and noncancer diseases), have not been studied in detail, as is customary with novel diagnostic techniques. These and other arguments presented elsewhere in more detail suggest that this method requires careful analytical validation before the data presented in the literature becomes convincing [5,6].

Bioinformatic inconsistencies of the method have now been unraveled, following reanalysis of the original *Lancet* mass spectrometry data

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by two separate groups. Sorace and Zhan identified peaks that contributed decisively to the discrimination between normal and cancer patients with mass/charge (m/z) ratios that are below 500, a region that falls into the noise of the method [9]. These authors pointed to the possibility that there must have been a significant nonbiologic experimental bias between cancer and control groups, casting questions on the validity of the discriminatory peaks with m/z ratios greater then 2000. Essentially the same conclusions were reached by Baggerly and coworkers, who were also able to discriminate normals from cancer patients with peaks with m/z ratios less than 500 [10].

In my original commentary on this technology, it was proposed to the authors to positively identify the nature of their five discriminatory peaks at m/z ratios of 534, 989, 2111, 2251 and 2465, in order to understand the origin of these molecules, their relative abundance and possible biological connection to ovarian cancer [3]. Three years later, the identity of these molecules remains elusive. In their latest contribution, the authors do not reveal the identity of their discriminatory peaks either [2]. My prediction is that these peaks represent high-abundance molecules, many of them being acute-phase reactant proteins.

Reproduction of the method

The original method and data published in the *Lancet* have not been reproduced by other laboratories, based on published reports. The only other published report on ovarian cancer diagnosis with this method uses different proteomic chips and reports different discriminatory peaks, not overlapping at all with those of Petricoin III and coworkers [6]. Petricoin III and coworkers now ascertain that the original instrumentation used to develop their method is obsolete and does not have optimal resolution. They are now using instruments with higher resolution and mass accuracy that are also capable of positively identifying potential discriminatory peaks [2]. They also report that the original Ciphergen Biosystems, proteomic chips used to develop the *Lancet* method have now been discontinued. Their new data are based on different chips [2]. This information implies that it is now not possible to reproduce the original *Lancet* report. Petricoin III and coworkers now claim that they have sequenced thousands of circulating low molecular weight peptides which, they postulate, may represent a treasure trove for future diagnostics [11]. However, they did not report the identity of the five discriminatory peaks reported in the original *Lancet* publication or in their latest contribution [2]. Consequently, the *Lancet* report should be of questionable validity until the identity of the five discriminatory peaks is revealed and the method and results are reproduced by others. In general, the experience of other investigators regarding reproducibility and long-term robustness of the method has been unsatisfactory, as discussed in detail by Rogers and coworkers for renal carcinoma [12].

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Clinical validation

Petricoin III and coworkers are now pursuing a clinical validation of the ovarian cancer diagnostic method by collecting data suitable for a US Food and Drug Administration (FDA) submission. However, they are now concentrating not on early ovarian cancer diagnosis but rather on detecting ovarian cancer relapse. Clearly, the clinical value of detecting early relapse is far inferior in comparison with early ovarian cancer diagnosis since, as it is widely known, there are no effective salvage therapies for relapsing ovarian carcinoma. Despite current availability of alternative and relatively efficient methods for early ovarian cancer relapse detection (imaging techniques and serial CA125 analysis), these modalities are not contributing much to extending ovarian cancer survival or quality of life. It is of interest to examine why the focus of the method now appears to have changed from early diagnosis to detection of recurrence.

In the meantime, the Early Detection Research Network (EDRN) of the National Cancer Institute has embraced this new technology as one of the most promising approaches for early ovarian and other cancer diagnosis and has initiated an effort to standardize the methodology in various biomarker validation laboratories. These centers are using the original instrumentation proposed by Petricoin III and coworkers, which is now considered suboptimal [2]. It is thus likely that the efforts of EDRN may become obsolete before this evaluation is completed.

In a report in The New York Times on February 3rd, 2004, it was announced that Correlogic Systems (MD, USA), which developed the bioinformatic algorithm of the *Lancet* paper, has licenced the ovarian cancer test (known as OvaCheck™) to Quest Diagnostics (NJ, USA) and LabCorp. (NC, USA), the largest private laboratories in the USA, for commercial use. The test will be offered to women at high risk, despite no FDA approval. The test has been advertised to gynecologic oncologists at the 2004 annual meeting on *Women's Cancer*, organized by the Society of Gynecologic Oncologist (USA). It is unclear what a woman would do if the test is positive. The risk is that without knowing the positive predictive value of the test, many women may be unnecessarily subjected to potentially harmful follow-up procedures. Fortunately, the intervention of the FDA and a cautionary note of the Society of Gynecologic Oncologists has put the test on hold, at least for now [101].

Concluding remarks

Approximately 3 years ago, the hopes of finding novel ways for early ovarian cancer diagnosis were heightened by a paper published in the *Lancet* [1]. Since then, the method and data have not been reproduced; the method has not been clinically validated and was found to have methodologic and bioinformatic flaws that cast serious questions on its validity [5,6,9,10]. As published, the method is now practically impossible to reproduce or validate due to methodologic and instrumental changes. The identity of the original discriminatory peaks remains elusive.

Efforts to approve a revised method by the FDA are now focusing on detection of recurrence rather than on early diagnosis. Financial interests are hastily attempting to bring the test to patients, despite absence of systematic clinical validation and lack of knowledge on the effects of many biologic, technical and bioinformatic parameters on the final result. In the author's opinion, the application of this method to patients outside of controlled clinical trials is still premature and may lead to the identification of many false positives. This in turn could lead to serious and unnecessary investigations in large numbers of women. Despite the promise of sophisticated technologies such as proteomics and mass spectrometry to aid in the better diagnosis of cancer and other diseases, no testing, regardless of how sophisticated or technologically advanced, should be offered to patients without thorough clinical validation. Until this method is reproduced and validated by well-designed clinical trials, it should not be offered to the public. Essentially, the same recommendations have been published on February 7th, 2004, by the Society of Gynecologic Oncologists [101].

As far as the new developments are concerned, these are welcome and appear to be impressive [2]. However, there is a need to identify the proteins/peptides represented by the discriminatory peaks in order to understand their connection to ovarian cancer. Furthermore, everything said about the validation of the original *Lancet* report also applies to the new method as well. It should not take long to judge if this method will survive the test of time.

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Website

101 Society of Gynecology Oncologists statement regarding OvaCheck™. www.sgo.org/policy/position_statement.cfm (Viewed August 2004)

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