Mass Spectrometry-based Diagnostics: The Upcoming Revolution in Disease Detection

Advances in mass spectrometry-based diagnostics could ignite a revolution in the field of molecular medicine. This platform has the potential to become the practical clinical analyzer of the future for nucleic acids and proteins. Mass spectrometry-based diagnostics are an example of a "disruptive" or "nonlinear" technology *(1, 2)*. Such disruptive technologies are by their very nature polarizing, causing a dynamic dichotomy of excitement *(3)* as well as anxiety *(4)* in the clinical diagnostic community because this technology can potentially outperform traditional measurement and detection systems. In this issue of *Clinical Chemistry*, Bonk et al. *(5)* demonstrate the advantages of mass spectrometry compared with traditional electrophoretic methodologies. Bonk et al. used mass spectrometry as a "sensor" to detect the amplified product from a PCR amplification reaction. The ultimate clinical application for this study is the early detection of colon cancer, a topic of obvious public health importance.

Although major advances have been made in elucidating the genetic underpinnings of cancer, especially colorectal cancer, diagnostic methodologies for routine clinical detection and monitoring of important cancer genetic derangements have lagged behind. Microsatellite instability (MSI), caused by mismatch repair gene silencing, is predicted to be an important early event in cancer progression *(6–8)*.

In this issue, Bonk et al. *(5)* report on a study in which they use time-of-flight-based mass spectrometry to detect MSI. Unlike previous studies, in which chromatography, electrophoresis, and traditional DNA sequencing methodologies were used to detect the presence of MSI, these authors have chosen a technology that has many distinct advantages over these more traditional methods. As pointed out by the authors, these traditional methods are really not amenable to high-throughput diagnostics because they are low throughput, costly, and suffer from poor sensitivity. Mass spectrometry, on the other hand, is extremely rapid (the entire process can occur in less than 1 min), has tremendous cycle time (hundreds of samples can be analyzed sequentially, one after another without pause), and can achieve sensitivities in the femtomolar range *(9–11)*. After PCR amplification, Bonk et al. *(5)* used matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to directly detect the PCR products.

Mass spectrometry-based detection of surrogates for disease detection is a timely topic. Mass spectrometry has recently generated excitement (and anxiety) as a platform for protein-based biomarker profiling. The most reliable, sensitive, and widely available tests for the detection of cancer today are protein–ligand assays, such as ELISA systems. These tests are robust, linear, accurate, and have reasonable throughput. However, the use of an ELISA system to test for the presence of disease requires a single, meticulously validated analyte for detection as well as an extremely well-characterized, high-affinity antibody for detection of the analyte. This requires a tremendous amount of time and effort. Traditionally, the search for cancer-related biomarkers for early disease detection, aggressiveness, and therapeutic response has been a one-at-a-time approach looking for overexpressed proteins in blood that are shed into the circulation as a consequence of the disease process *(12–14)*. Unfortunately, this approach is laborious and time-consuming because there are potentially thousands of intact and cleaved proteins in the human serum proteome that would need to be identified, and antibodies would need to be developed, validated, and checked for specificity and sensitivity. Finding the single disease-related protein is like searching for a needle in a haystack, requiring the separation and identification of these entities one by one. Moreover, it is likely that discovery and use of the elusive single biomarkers for early detection of cancer will be nonexistent because clinical applications would be applied to a human population characterized by vast heterogeneity not only in their proteomes, but also in the underlying cancer genetics.

Within the past year, a new type of protein-based diagnostic paradigm has been described: proteomic pattern diagnostics *(15)*. With this approach, a single droplet of blood is placed on a specially treated surface that contains chemical bait molecules that bind with proteins in the blood. The surface is washed and subjected to MALDI-TOF analysis. The readout is a proteomic "barcode" pattern of the complex protein milieu that sticks to the surface. A typical low-resolution MALDI-TOF proteomic profile will have up to 15 500 data points that comprise the recordings of data between *m/z* 500 and 20 000, with higher resolution mass spectrometry instruments generating as many as 400 000 data points for *m/z* 500 to 12 000.

Proteomic pattern diagnostics use new types of pattern recognition systems to sift through this huge amount of data to find diagnostic patterns of protein expression. These artificial-intelligence-based bioinformatic systems are vigilant and powerful, and enable the analysis of these large, complex datastreams. These types of informatic algorithms have the special attribute to learn, adapt, and gain experience over time and are uniquely suited for proteomic data analysis because of the huge dimensionality of the proteome itself. The artificial intelligence tool learns, adapts, and gains experience through constant vigilant retraining—meaning that it can start to recognize a unique and new phenotype that the system had not been trained to recognize or even seen before. This is extremely important when one considers clinical applications involving the screening of hundreds of thousands of patients. In fact, it is possible to generate not just one, but multiple combinations of proteomic patterns with diagnostic potential *(16)*. With this approach, knowledge of

the underlying identities of the individual components of the pattern is not necessary for its use as a potential diagnostic and sentinel for the presence of the disease: the pattern itself is the diagnostic.

Mass spectrometry-based proteomic pattern diagnostics have been used for ovarian cancer detection, and the value of this paradigm has been confirmed in other diseases, including breast *(17)* and prostate cancer *(18, 19)*. What this means is that the unique tumor–host microenvironment can set off amplification cascades that may be specific to the disease process, and yet the signatures for the presence of cancer, even at its earliest stages, may be composed of untold numbers of combinations of slight but significant shifts in protein–protein interactions, protein folding, and protein abundances that are reflected in mass spectrometry-based protein profiles.

Mass spectrometry platforms, already capable of reporting tens of thousands of events in less than a few minutes from a microliter of blood, are advancing rapidly in speed, throughput, sensitivity, and "on-the-fly" protein identification. Semiquantitative MSI profiling, as described in this issue, represents an additional new and exciting component of the repertoire of mass spectrometry's diagnostic potential.

The coupling of advances in mass spectrometry with adaptive and vigilant bioinformatic pattern recognition tools may dramatically change how disease is detected and monitored. The result will be a rich source of information to aid the clinician in patient management. On the basis of these nonlinear technologic advances, the clinical diagnostic landscape is now shifting dramatically. Like it or not, we may be moving beyond existing immunoassay-based (for proteins) and electrophoretic (nucleic acid) approaches. Rapid, low-cost mass spectrometry-based alternatives with much higher clinical sensitivity and specificity for the detection and monitoring of disease may eventually dominate clinical diagnostics. The utility and validity of this vision will be answered over the next several months to few years. Clinical trials for these diagnostic tests are ongoing for early detection of cancer, individualization of therapy, and monitoring of relapse and drug-induced toxicity. As a demonstration of the commercial interest in the expansion of mass spectrometry-based diagnostics *(20)*, large reference laboratories have begun serious evaluation of the eventual implementation of proteomic pattern diagnostics in their routine practice *(21)*.

References

1. Coile RC Jr. The innovator's dilemma: disruptive technologies. Russ Coiles Health Trends 2000;12:2–4.
2. Christensen CM, Bohmer R, Kenagy J. Will disruptive innovations cure health care? Harv Bus Rev 2000;78:102–12,199.
3. Petricoin EF, Zoon KC, Kohn EC, Barrett JC, Liotta LA. Clinical proteomics: translating benchside promise into bedside reality. Nat Rev Drug Discov 2002;1:683–95.
4. Diamandis EP. Proteomic patterns in serum and identification of ovarian cancer [Letter]. Lancet 2002;360:170.
5. Bonk T, Humeny A, Gebert J, Sutter C, von Knebel Doeberitz M, Becker C-M. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based detection of microsatellite instabilities in coding DNA sequences: a novel approach to identify DNA-mismatch repair-deficient cancer cells. Clin Chem 2003;49:552–61.
6. Jass JR, Whitehall VL, Young J, Leggett BA. Emerging concepts in colorectal neoplasia. Gastroenterology 2002;123:862–76.
7. Haydon AM, Jass JR. Emerging pathways in colorectal-cancer development. Lancet Oncol 2002;3:83–8.
8. Starostik P, Muller-Hermelink HK. Diagnosis of microsatellite instability-positive colorectal cancer. Expert Rev Mol Diagn 2001;1:71–80.
9. McDonald WH, Yates JR 3rd. Shotgun proteomics and biomarker discovery. Dis Markers 2002;18:99–105.
10. Bonk T, Humeny A. MALDI-TOF-MS analysis of protein and DNA. Neuroscientist 2001;7:6–12.
11. Gygi SP, Aebersold R. Mass spectrometry and proteomics. Curr Opin Chem Biol 2000;4:489–94.
12. Adam BL, Vlahou A, Semmes OJ, Wright GL Jr. Proteomic approaches to biomarker discovery in prostate and bladder cancers. Proteomics 2001;1:1264–70.
13. Carter D, Douglass JF, Cornellison CD, Retter MW, Johnson JC, Bennington AA, et al. Purification and characterization of the mammaglobin/lipophilin B complex, a promising diagnostic marker for breast cancer. Biochemistry 2002;41:6714–22.
14. Rosty C, Christa L, Kuzdzal S, Baldwin WM, Zahurak ML, Carnot F, et al. Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. Cancer Res 2002;62:1868–75.
15. Petricoin EF III, Ardekani AM, Hitt BA, Levine P, Fusaro VA, Steinberg S, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet 2002;359:572–7.
16. Petricoin EF III, Mills GB, Kohn ES, Liotta L. Proteomic patterns in serum and identification of ovarian cancer [Reply]. Lancet 2002;360:170–1.
17. Zhang LJ, Rosenzweig J, Wang YY, Chan DW. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. Clin Chem 2002;48:1296–304.
18. Petricoin EF III, Ornstein DK, Paweletz CP, Ardekani A, Hackett PS, Hitt BA, et al. Serum proteomic patterns for detection of prostate cancer. J Natl Cancer Inst 2002;94:1576–8.
19. Adam B-L, Qu Y, David JW, Ward MD, Clements MA, Cazares LH. Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. Cancer Res 2002;62:3609–14.
20. Clarke S. Tandem mass spectrometry: the tool of choice for diagnosing inborn errors of metabolism? Br J Biomed Sci 2002;59:42–6.
21. Correlogic Systems. Correlogic Systems licenses ovarian cancer diagnostic test to Quest Diagnostics and LabCorp [Press Release]. http://www.correlogic.com/questlabcorp_final.htm (accessed January 2003).

Emanuel F. Petricoin[1*]
Lance A. Liotta[2]

[1] NCI/FDA Clinical Proteomics Program
Office of the Director
Center for Biologics Evaluation and Research
Food and Drug Administration
Bethesda, MD 20892

[2] NCI/FDA Clinical Proteomics Program
Laboratory of Pathology
Center for Cancer Research
National Cancer Institute
Bethesda, MD 20892

*Author for correspondence.