Q&A

What Is Wrong with Clinical Proteomics?

Moderators: Ashley Di Meo[1] and Eleftherios P. Diamandis[2,3,4*]
Experts: Henry Rodriguez,[5] Andrew N. Hoofnagle,[6] John Ioannidis,[7] and Mary Lopez[8]

Proteomics is defined as the large-scale study of proteins, particularly their structures and functions. Clinical proteomics aims to apply proteomic discoveries and technologies to patient care. One of the workhorses of proteomics is mass spectrometry (MS).[9] Over the last few years we have witnessed spectacular advances in MS-based proteomics; these advances now allow almost complete proteome identification of complex biological fluids, tissues, cells, etc. in a matter of hours. Recently, mass spectrometers have become faster and more sensitive, and they can generate tremendous amounts of information related to protein primary structure, posttranslational modifications, splice variants, mutants, etc. Higher sensitivity for detecting individual proteins in complex mixtures in still an issue with proteomics. New instruments are continually upgraded and new methods for sample preparation are emerging, allowing faster and more sensitive measurements of many analytes in complex biological mixtures. Such methods may require analyte enrichment steps by using antibodies or other binding reagents.

With all these advances, it was expected that MS-based proteomics could revolutionize medical sciences by providing insights into protein structure and function, but also, in translating some of these discoveries to the clinic. Particularly, it has been anticipated that novel disease biomarkers for diagnosis, monitoring, and prediction of therapy will be discovered and implemented into the clinic. The multiparametric nature of MS-based proteomics allows profiling of various biological fluids for clinical applications.

Despite a 15-year effort, no major disease biomarkers have been discovered by using MS-based proteomics.

Funding does not seem to be the problem since government bodies (including granting agencies such as the NIH) and diagnostics companies have already invested hundreds of millions of dollars toward this goal.

In this Q&A we discuss with 4 experts in the field why this is the case and what the future may be for clinical proteomics.

Why do you think there was so much anticipation that proteomics could deliver the next generation biomarkers in the clinic?

Henry Rodriguez: Following the initial draft of the human genome in late 2000 (genomics), there was much excitement to immediately move into its scientific counterpart (proteomics). The basis was 3-fold: first, basic biology had already shown that proteins are the workhorses of a cell, the machinery that provides most of the cell's functionality and makes up most of the structures of the cell, and thereby mediators of phenotype characteristics; second, researchers had for the first time a blueprint (the human genome) to infer the possible gene products derived from a genome; and third, biotech/pharma were developing drugs that either act by targeting proteins or are proteins themselves.

So coupling these items with the technological breakthroughs at the time in identifying vast amounts of proteins and their posttranslational modifications

[1] Graduate student, Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [2] Head, Division of Clinical Biochemistry, Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [3] Head, Clinical Biochemistry Section, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada; [4] Biochemist-in-Chief, Department of Clinical Biochemistry, University Health Network, Toronto, Ontario, Canada; [5] Office of Cancer Clinical Proteomics Research, Center for Strategic Scientific Initiatives, Office of the Director, National Cancer Institute, Bethesda, MD; [6] Head, Division of Clinical Chemistry, Director, Clinical Mass Spectrometry, Assistant Director, Clinical Immunology, Director, Nutrition and Obesity Research Center, Analytical Core, University of Washington, Seattle, WA; [7] Chair in Disease Prevention at Stanford University, Professor of Medicine and

Director of the Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA; [8] Director, BRIMS Center, Thermo Fisher Scientific, Cambridge, MA.
* Address correspondence to this author at: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 6th Floor, Rm. 6-201, Box 32, 60 Murray St., Toronto, Ontario, Canada M5T 3L9. Fax 416-619-5521; e-mail ediamandis@mtsinai.on.ca.
© 2014 American Association for Clinical Chemistry
Received March 27, 2014; accepted April 4, 2014.
[9] Nonstandard abbreviations: MS, mass spectrometry; FDA, Food and Drug Administration; PSA, prostate-specific antigen; MRM, multiple-reaction monitoring; CDx, companion diagnostics.

(which are not predicted nor detected from a genome), it's understandable why there was so much excitement to jump start this field. The question is "did researchers overpromise and overhype on what was attainable at the time?" What we do know is that the work that has been done with genome sequencing may turn out to have been simple by comparison with the work that is needed to understand proteins on a grand scale—while challenging, it is absolutely achievable.

Andrew N. Hoofnagle: Over the past 25 years, we have gradually realized that rare polymorphisms and epigenetic modifications in the human genome are difficult to detect and difficult to interpret. Perhaps more importantly, it is impossible from first principles to anticipate how specific genetic modifications will interact with other local or distant genetic modifications and the environment to alter the way proteins are expressed, localized, and posttranslationally regulated. As a result, the most desirable readout of the human genome is not an individual's genome itself, but instead it is the expression of the genome into proteins, the cellular localization of those proteins, and the posttranslational modifications of those proteins. This would reveal how the genome and the environment have worked together to generate the human condition.

The phenotype of a patient is really defined as the proteome and the metabolome of trillions of different cells. The genotype is only a part of what determines the phenotype. There was so much anticipation that proteomics would deliver novel biomarkers because it was assumed that the phenotype would be much more important than the genotype in assessing the state of the human organism at a particular moment in time, which would be more effectively used for diagnosis, prognosis, and therapeutic monitoring of disease.

John Ioannidis: Proteomics largely followed the same path of hyped promises that accompanied the advent of all omics technologies (including genomics, transcriptomics, gene expression profiling, metabolomics, pharmacogenomics, etc.). These promises fuelled mostly spurious and unfounded

expectations about personalized, individualized, precision medicine. Moreover, one special aspect for proteomics was that several of the biomarkers that had been successfully (or seemingly successfully) introduced to the clinic in the 20th century were proteins. So, it did make some sense that with improved ability to characterize not just single proteins and peptides, but the whole proteome, our diagnostic, prognostic, and predictive options would expand geometrically. This clearly did not come to pass.

Mary Lopez: The ideas and concepts were sound, but the problems were more challenging than anticipated. Proteins are so much more difficult to analyze than genes! Because genomics progressed so rapidly, it was expected that proteomics would rapidly follow. It has taken some time for the technology to catch up with the ideas, but we are reaching a time when the available tools do provide the requisite sensitivity and selectivity that is needed to discover and measure proteins with precision and accuracy.

Is the level of funding from international and national granting agencies and private organizations enough to develop clinical proteomics?

Henry Rodriguez: As a biomedical researcher by training, my interest is in advancing scientific knowledge to patient care. As anyone knows, this takes time and resources. So from the big picture, the issue of funding involves more than just the field of clinical proteomics. All disciplines of biomedical research are affected by low funding. This is ever so true today, where many of the most exciting possibilities stem from the convergence of several factors: innovative tools and technologies, generation of large publicly accessible data sets (big data) from omics-based research (for example, proteomics and genomics), and advances in computational power for extracting knowledge (basic and clinical) from those data sets (bioinformatics).

The reality is that there are no easy solutions, and dealing with tight budgets has been unavoidable. I think the key is not losing sight of the big picture—namely, to make scientific discoveries and increase the knowledge available to improve human health.

Andrew N. Hoofnagle: In any answer to this question we must recognize the importance of the definition of

"clinical proteomics." There are many definitions that I have heard and used. For example: (1) the quantitative measurement of multiple proteins in a biological sample that is related to human disease, (2) the identification and relative quantification of proteins in a biological sample that is related to human disease, and (3) the quantitative measurement of proteins in human samples in experiments that will directly lead to an improvement in the care of patients. As a clinical chemist, I obviously care most about the third definition in this noninclusive list. However, this is the most difficult area of study to be funded. Fortunately, the Clinical Proteomics Tumor Analysis Consortium, funded by the NIH, has broken this mold. While a substantial part of this program is devoted to a more complete understanding of tumor biology through in-depth proteomics, there is a substantial component devoted to the development of novel assays and technologies that will assist clinical researchers in accurately and precisely quantifying proteins in actual clinical specimens. I am hopeful that the tools that this program generates will be useful to clinical laboratories for immediate implementation. In this light, there is some support for what I consider the most important aspect of clinical proteomics, but it would be great to see more.

John Ioannidis: When it comes to funding, I can hardly think of any better investment for human societies than an investment in science and research, so my general statement is that funding for research in clinical proteomics could always be (much) higher. However, acknowledging realistic restrictions to how much money can be allocated to different research fields, I don't think that proteomics has been underfunded to-date. The efficiency of the investment could possibly be improved nevertheless. Much research in the field has not had clear translational orientation and realistic goals for addressing questions that would matter for clinical medicine and practice. There is still room for funding large-scale multicenter collaborations that would streamline not only early biomarker development, but also large-scale validation, testing for clinical utility and clinical implementation. Truly rigorous "clinical" proteomics have received little attention from funders.

Mary Lopez: Of course it never seems to be enough! In the US, the NIH budget has stayed pretty flat although the cost of doing science has gone up tremendously. This has resulted in fewer grants and much more competition. Also, it has resulted in less money being given to innovative or "blue sky" projects owing to the risk-averse atmosphere. One positive outcome of this situation is that academic and industry collaborations have increased, since they can be mutually beneficial and advance the science.

What do you see as the major obstacle for clinical proteomics to deliver the promised goods?

Henry Rodriguez: In biomedical research, there are 2 factors to consider— understanding of the underlying mechanisms of a disease (basic science) and its translation to patient care (clinical science). Proteomics has come a long way in the past decade. However, without the ability to reproduce data across independent labs, the rate of clearance of protein markers will remain stagnant. Several items have been postulated to be major barriers in clinical proteomics, and research groups such as the National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium have addressed most of them in an effort to standardize and harmonize analytical proteomics workflows among their investigators and the greater biomedical community at large.

Items that have been addressed include: technological variability within/across proteomic platforms; biospecimen collection QC; regulatory science; publicly available antibody reagents (renewable), assays, and data sets (open access); improved data analysis tools to derive wisdom from data sets; proper experimental study design; and multidisciplinary research teams. If proteomics is to be successfully introduced to clinical diagnostics, universally accepted metrics will be necessary at many steps along the way, to ensure that changes observed are attributable to biological states, not workflow variability. More importantly, communication among proteomic researchers, assay sponsors, the Food and Drug Administration (FDA), the Centers for Medicare and Medicaid Services, clinicians, and clinical chemists is critical in expediting the translation of clinical proteomics. Such an endeavor requires a long-term commitment of community-based approaches. I think with these in place, the promise will come.

Andrew N. Hoofnagle: Again, definitions are important. If the "promised goods" are novel biomarkers for the diagnosis, prognosis, and therapeutic management of disease, then the main obstacles are well-designed studies that ask the most important clinical questions. There is no dearth of novel biomarkers. But, biomarkers that have been validated in studies that have been carefully designed and carried out are rare.

Another major hurdle could be the expectations that we have for novel biomarkers. Take for example prostate-specific antigen (PSA). For many years, this has been the cornerstone in primary care to identify patients who should be referred to a urologist for prostate biopsy. Unfortunately, it is an assay that suffers

from low clinical specificity for prostate cancer and the US Preventative Service Task Force recently reported that general screening using this biomarker is not supported by outcomes in prospective clinical trials. Are we looking for the next PSA? If so, we should be careful.

If the hope of clinical proteomics is to turn a panel of less-than-optimal biomarkers into a terrific super-biomarker, we should also be careful because many of the logical biomarkers are likely to correlate rather strongly. Also, our ability to design prospective clinical studies to demonstrate that measuring a novel biomarker or panel of biomarkers is useful in improving outcomes (particularly an assay used for general screening) is generally stymied by the cost and logistics of such an endeavor.

However, if the promised goods are superior assays based on novel clinical proteomic technologies, then the promised goods are already here.

John Ioannidis: A major obstacle has been that the expectations of deliverables have been overrated. This does not allow thinking clearly and asking specifically what exactly can we realistically hope to get of these technologies. Developing tests with perfect diagnostic and/or predictive accuracy may be unattainable, but some modest, and potentially clinically meaningful, improvements in accuracy, efficiency, speed, or other aspects of the diagnostic spectrum should be feasible.

Once we set the expectations bar at the right level, remaining obstacles include the lack of large enough clinical studies, lack of standard, routinely adopted validation procedures, and unwillingness of investigators to perform proteomics research with a view for clinical testing and implementation. Much research in the field is happening with a view that an interesting observation and a related publication in a good journal are all that is needed.

Mary Lopez: The translation of newly discovered biomarkers into routine tests is now the major hurdle. Adoption of these methods in clinical laboratories will be accelerated if the methods and technology can be shown to be extremely robust and cost-efficient. New and innovative ways of reducing the costs per test are needed.

What are the gaps in current clinical assays that can be filled by clinical proteomics?

Henry Rodriguez: High production costs, long development time to generate high-quality immunoassay using antibodies, and limited multiplexing are gaps of conventional clinical assays (namely immunoassays). Because of the realization that panels of analytes will be needed to analyze healthy and/or disease conditions, there is a wake-up call within the clinical community regarding the need to develop more straightforward quantitative approaches that can be multiplexed. This is where clinical proteomic technologies are filling the gap.

Discovery proteomic experiments produce 1000s of protein biomarker candidate leads, but unfortunately, the majority of these will not have clinical utility. To "close this gap," laboratories have introduced a verification step that streamlines the process of moving candidates from discovery to validation by exploiting the sensitivity and specificity of targeted MS [namely, multiple-reaction monitoring (MRM)]. A turning point was in 2009 when MRM was demonstrated to be highly reproducible within and across laboratories and instrument platforms. The fact is that if one were to open up a catalog of immunoassay antibodies currently available, the list is simply far too small to tackle all the candidate leads being identified by laboratories. Verification using MRM solves this problem—economically and scientifically. It acts as a precise and quantitative multiplex filter ensuring that only the most credible candidates move forward to costly and time-consuming clinical validation studies, resulting in a more efficient product development for downstream in vitro diagnostics assays. Recent studies of targeted MRM have demonstrated its ability to multiplex up to 150 analytes. With immunoenrichment steps before MRM, the sensitivity of this approach is dramatically increased.

Andrew N. Hoofnagle: Current clinical assays suffer from many difficulties including (1) a lack of specificity, (2) interfering substances, (3) misleading results at extreme concentrations, and (4) poor interplatform concordance. If clinical proteomics is defined as the application of novel proteomics technologies to clinical samples, then we have already overcome many of the concerns with current clinical assays from a technological perspective. But, if (1) the human condition is defined by the juxtaposition and integration of the activities of multiple posttranslationally modified proteins, (2) the human condition is definable by clinical proteomics methodologies, and (3) human disease is directly defined by the human condition that is defined by clinical proteomics, then clinical proteomics can fill the gaping hole left by our current menu of clinical assays by providing a more complete survey of proteins in clinically relevant samples. That is the hope; we are still testing the waters of reality.

John Ioannidis: A major problem not only for proteomics, but for any new candidate technology that aims to change diagnostic and predictive practice, is that we have not mapped systematically what we know

and what we don't know about the currently used diagnostic and predictive tests in everyday healthcare. There are only a few hundred randomized trials for diagnostic tests in the entire medical literature, i.e., 200-fold fewer than randomized trials for drugs. We have repeatedly shown that reasonably good diagnostic performance (sensitivity and specificity) is indeed required for a test to be clinically successful, but it does not suffice. Most tests that have very good sensitivity and specificity are clinically useless—they are just adding cost or even harm to medical care.

A committed systematic effort is needed to create a map of diagnostic evidence and see what gaps we have across diverse conditions and diseases.

Moreover, one could also use simulation models to evaluate whether the development of specific new diagnostic or predictive tests with specific performance would be worthwhile to pursue in different settings and indications. I am afraid that a lot of research currently is happening trying to develop diagnostic tests that, even if they are successfully developed and they are analytically perfect, would clearly have no potential use and would not improve patient outcomes or diminish the cost of healthcare.

Mary Lopez: The major gaps are directly related to selectivity, for the most part. Since the gold standard is the immunoassay or ELISA, any ELISA that has a high false-positive rate is a target for improvement. A good example for this is the much-maligned PSA test. Because PSA exists in so many different forms, it is nearly impossible to get a good selectivity with a standard ELISA. Identification of the variants at the sequence level is needed to accurately quantify the clinically relevant isoforms and reduce the false positives.

Are we ready to move beyond standard immunoassays and if so, why?

Henry Rodriguez: While it is safe to say that immunoassays are here to stay, they are specific cases (and surely more will come) for which their limitations can be complemented by MS-based assays. The fact of the matter is that immunoassays have been the most commonly used methods in clinical laboratory testing for proteins, and the FDA has extensive experience in the science and regulatory processes for standard-format immunoassays. Furthermore, their economics are well understood (cost per assay, start-up costs, and laboratory technician cost to run the assays). So while replacement of current immunoassays will likely require FDA approval, which will require instrument/platform analytical validation, there will be instances where converting to an MS-based assay will be necessary (either for technical or economical reasons). Scientifically,

there are circumstances where quantification of peptides using MRM will be preferred to traditional immunoassays for mutants, splice variants, and post-translational modification, as antibodies against these specific changes to the protein primary structures will be difficult to generate.

A good example of a technical (analytical) reason is thyroglobulin, where standard immunoassays are hindered by the interference of autoantibodies in 20% of the population, leading to false-negative results. To circumvent this issue, Hoofnagle and colleagues at ARUP Laboratories and Quest Diagnostics (*Clinical Chemistry*, 2008;54:1796–1804 and 2013;59:982–990) developed a peptide immuno-MRM MS assay with acceptable clinical diagnostic performance characteristics, which circumvents the interference of autoantibodies. In terms of economics, MS with its multiplex capability has the potential to drive down cost per assay and with automation platforms coming onboard, can greatly reduce their entry barrier into clinical laboratories. To facilitate the analytical validation of MS platforms, researchers from academia, industry and the National Cancer Institute and FDA published mock 510(k) submission documents in *Clinical Chemistry* in 2010 (56:165–171) on a multiplex immunoaffinity MS platform to educate the proteomics community on analytical evaluation requirements for multiplex assays to ensure the safety and effectiveness of these tests for their intended use.

Andrew N. Hoofnagle: Yes, we are ready. Immunoassays as a category are particularly problematic when analyzing human clinical samples. The field of clinical proteomics has spent time identifying reliable methods of calibration, sample handling, and analysis that put clinical chemists in a position to adopt the technology to improve patient care. It is not facile and talented laboratory technologists that are needed to ensure success. However, reference laboratories are already harnessing the power of clinical proteomics technologies and I am hopeful that other clinical laboratories will benefit from clinical proteomics technologies very soon.

John Ioannidis: In principle yes, as far as technology is concerned. However, standard immunoassays may be perfectly fine to use in many settings, and even preferable, if they have good accuracy and diagnostic/predictive performance and they can be widely performed by any laboratory at low cost. Newer and more sophisticated is not necessarily better.

Mary Lopez: Yes. Standard immunoassays suffer from a variety of pitfalls that typically result in false positives, as mentioned above. This can be due to a lack of spec-

ificity of the capture antibody or the heterogeneity of the target molecules or both. It is increasingly clear that most proteins exist in multiple forms due to truncations, posttranslational modifications, or single nucleotide polymorphisms. Many disease-associated proteins can exist in inactive and active forms and antibodies may capture these indiscriminately. Therefore, specific detection at the sequence level is required to accurately detect and quantify the disease-related isoforms. MS can provide this specificity, even when antibodies are used to enrich low-abundance targets.

Are mass spectrometers the next clinical assay platform?

Henry Rodriguez: If an assay benefits patient care while being robust, reliable, automated, easy to operate, and cost-effective, it ultimately finds traction in clinical settings. That said, MS has been playing a pivotal role in a variety of scientific disciplines, and has long been a standard tool at public health laboratories. To me, the question is not whether MS will be the next clinical platform, but when targeted MS will be broadly adopted as a tool for clinical measurement of protein analytes, supplementing current immunoassays. It should be noted that there are no fundamental technical obstacles to its adoption in clinical laboratories (albeit not enough biomarkers), so its implementation is largely a matter of engineering.

It will take time for all clinical laboratories to be utilizing MS, but the rapid uptake of targeted MS and the interest by the clinical chemistry profession based upon publications in journals such as *Clinical Chemistry*, and growth of training webinars and other courses, makes a strong case for MS becoming an integral part of the clinical laboratory. The best way to view this is that MS assays are not a disruptive technology, but rather a complimentary method to immunoassays that has technological advantages for certain applications.

Andrew N. Hoofnagle: They already are. It is now time to put the calibration materials, robust assay reagents, reliable quality assurance programs, sensitive QC procedures, and trained staff in place to drastically change our ability to accurately and precisely characterize human disease one patient at a time.

John Ioannidis: This depends on a lot of local parameters that may modulate the extent to which laboratories performing routine tests can use MS reliably, as well as the cost, the training required, and the ease of adoption of the processes in routine practice for flow of information in the hospital and in the clinic. These are questions that can be addressed with late-stage translational research, translational stage 3 and 4 studies. Un-

til now, this type of research has received hardly any attention in proteomics.

Mary Lopez: MS is now, and will certainly continue to be, part of the next revolution in clinical assay platforms. MS answers a critical need for more specific tests. Not all tests will necessarily be migrated to the MS platforms but where immunoassays fail to deliver, MS will undoubtedly fill the gap.

Are you aware of any major successes of clinical proteomics in the clinic? Is diagnosis of microbial infections a good example?

Henry Rodriguez: Ask a patient whose diagnosis and treatment from a *Staphylococcus aureus* microbial infection benefited from clinical proteomics, and the answer is most likely YES. The first clinical MS system cleared by the FDA for rapid identification of disease-causing bacteria and yeast shows the great promise of MS for clinical proteomics. Such progress in clinical proteomics will pave the way for greater success in the future by accumulating knowledge and experience in the understanding and fulfillment of the validation criteria for multiplex MS-based assays.

Clinical proteomics should be viewed as a tool that further illuminates our understanding of the molecular mechanisms in a disease and helps identify the best medical care for a patient—whether diagnosis and/or treatment. And it is in the area of treatment that I think clinical proteomics will also have an impact. Therapeutic compounds are becoming more targeted to defined patient populations, and consequently the expectations for demonstrating benefit in these targeted groups are increasing. This is especially evident in the field of oncology, where there is a growing need for companion diagnostics (CDx), to identify patients with a specific biomarker that is predictive of response. For patients with cancer, for instance, those that are identified as nonresponsive can quickly move on to other, perhaps more effective therapies if they exist. While current CDx-approved tests involve FISH (fluorescence in situ hybridization), immunohistochemistry, PCR, or DNA/RNA next-generation sequencing, approximately a third of the drugs in clinical development are associated with some form of a genomic or proteomic marker—a 50% increase over the last 2 years. Considering that the pharmaceutical industry's daunting attrition rate of new drugs entering phase I are as high as 92%, it's fair to say that the incorporation of companion biomarkers (through clinical proteomics) as predictive tools in clinical trials will increasingly be viewed as enabling not only smarter decisions and resource investment, but also as potentially yielding new targeted medicines.

Andrew N. Hoofnagle: The identification of bacteria and yeast by MALDI-TOF MS in the clinical laboratory is a huge step forward for clinical MS. However, calling MALDI-TOF microbial identification "clinical proteomics" may be misguided. It is obvious that some small proteins are captured in the spectrum of samples from the MALDI target, but it is the pattern of the mass spectrum that matters most. The identification of the proteins and lipids in the spectrum has never been paramount to the success of the platform. I would therefore consider MALDI-TOF a microbial identification tool, not a proteomics platform. Determining the difference between 2 species of bacteria is often like telling the difference between the 2 sides of the Grand Canyon from a hot air balloon. Yet, MALDI-TOF mass spectrometers cannot separate many related species of bacteria that are associated with severely different outcomes. Further, we must keep in mind that the difference between women with stage 1 ovarian cancer and normal controls is much less, more akin to distinguishing 2 sides of a small creek in the woods from a hot air balloon, and, with MALDI-TOF MS, it is unlikely that we will be able to distinguish the 2.

John Ioannidis: At the risk of being called a pessimist, I can't think of something that I would call a major success of clinical proteomics in the clinic to-date. For claiming a major success, I would like at a minimum to have some documentation in sufficiently large randomized trials that adoption and implementation of a proteomics technology improved major clinical outcomes for patients.

Earlier, more accurate diagnosis of bloodstream and other infections by MALDI-TOF MS is very promising, and these methods have already been adopted by many hospitals and laboratories. At some point, I would like to see randomized trials performed that would address what we really gain by this and other contester techniques of early diagnosis in clinical terms. For example, it sounds great that one could identify the microbial species earlier (up to 24 h before traditional methods) even if this is not accompanied by direct evidence on the antimicrobial susceptibility profile. However, how does this early information translate in terms of intermediate outcomes (e.g., duration of hospital stay, adverse events related to antibiotic treatment, adverse events in general, or successful treatment of infection) and hard clinical outcomes (death, major clinical events)? These questions require randomized controlled trials to be answered reliably. Moreover, as several different technologies may eventually compete for earlier diagnosis, one would have to perform head-to-head trials to see which one is the best.

Mary Lopez: One striking example is in the diagnosis of amyloidosis. In 2009, researchers at the Mayo Clinic developed an MS-based test with increased sensitivity and specificity compared to the previously used histopathological tests. Since its development, the assay has been widely adopted because it addresses many problems inherent to histopathology, including high background staining that interferes with subtyping.

Are there technological or other advances around the corner that could bring clinical proteomics closer to the clinic?

Henry Rodriguez: Two key areas that come to mind are analytical advances (hardware and software) and the clinical convergence of genomics with proteomics. The recent FDA 510(k) de novo clearance of 2 microbial screening assays based on MS technologies (BioMérieux and Bruker) and another solid-phase array for viral detection (BioArray Solutions) are milestones in the evolution of tomorrow's clinical laboratory tests. With the mock 510(k) documents available to the public on a multiplex immunoaffinity MS platform and a multiplex array–based platform (*Clinical Chemistry*, 2010;56:237–243), immuno-MRM MS assays and standardization of assay performance ± antibody qualification will be picked up by clinical laboratories using these MS platforms in the near future. Improvements in targeted MS-based proteomic approaches, ranging from automated sample processing to data acquisition, and greater sensitivity with lower load volumes, are resulting in higher throughput and highly multiplexed and sensitive targeted MS analyses, thus providing a viable future for MS-based measurements in clinical laboratories. Just as our genomics colleagues are employing multiplex DNA in clinical research, targeted multiplex proteomics may soon become a reality. Additional technological advances currently in the research space include assays based on single-cell proteomics (e.g., the CyTOF mass cytometer), MagArray (magnetic multiplex protein array), and NAPPA (nucleic acid programmable protein array) that will become more prominent on the horizon once the technologies can produce reproducible data across laboratories and become automated, and not stay as one lab's specialty.

In terms of the convergence of genomics with proteomics, I see a lot of potential in this area, albeit currently still in the research space. For example, from a clinical relevance perspective, not every indication will benefit from the increased information by next-generation sequencing, as there are limited therapeutic interventions. Concomitantly, sequencing data using next-generation sequencing does not provide a holistic clinical perspective, and as a result, other types of test-

ing such as proteomics will become ever more relevant. In my view, advances in the understanding of linkages between genotypic markers, proteomic markers, and disease will make such simultaneous "proteogenomic" clinical laboratory tests relevant and actionable, increasing their use and overall impact on patient care.

Andrew N. Hoofnagle: The ability to enrich proteins or tryptic peptides before MS will be essential for the successful translation of clinical proteomics technologies. The development of useful reagents is warranted.

John Ioannidis: I doubt that it is more and more technological, "wet lab" advances that we need at the moment to bring clinical proteomics closer to the clinic. We have had tens of thousands of papers focusing on phase 0 and 1 translational research. What we need is to test the best candidate technologies in real life, with serious clinical and implementation research.

Mary Lopez: The increasing sensitivity and resolution of mass spectrometers coupled with new and more efficient sample preparation methods are increasingly facilitating accurate quantification of even low-abundance disease biomarkers. Once these methods are optimized for automation and robust high throughput, they will be increasingly adopted into routine environments because of the added value they deliver with respect to greatly increased specificity.

Can you envision the role of clinical proteomics in clinical medicine 10 years from now?

Henry Rodriguez: Of course. In the 1960s, immunoassays were used routinely to measure small molecules. Just as these methods have been replaced with MS and protein/antibody array assays where today millions are run annually in clinical laboratories worldwide (the majority by targeted MS), one can envision their further expansion into larger molecules (proteins), despite current instrumentation costs and knowledgeable personnel to run the instruments and analyze the data. Personally, I'm agnostic to technology. Whether it's a multiplex MS instrument or a multiplex protein/antibody array instrument, I just hope one of them does it accurately and quickly while being cost-effective.

Looking ahead, it is useful to step back and provide realistic timelines in terms of bringing new technologies to market and discovering and developing new protein biomarkers that will drive the use of such technologies in clinical laboratories, such as from new knowledge gained from proteogenomic network and pathway studies. If one were to use the pharmaceutical industry drug development pipeline as a comparator,

some interesting realities come to the forefront. Current projections estimate that it takes more than $1 billion and between 10 and 15 years to bring a new drug to market. To take it one step further, others have noted that once that estimate is adjusted for current failure rates and inflation, the estimate becomes $4 to $11 billion in research dollars spent for every drug that is approved. While there are many variables to these numbers, they are stunning.

I think the reason there is a heavy focus on timelines in clinical proteomics is because at the beginning there was maybe too much advertising. But if you look at other fields such as transcriptomics, there currently are not so many tests that are applied in the clinical field. As a community, we need to set realistic expectations to encourage others (basic researchers, clinicians, clinical chemists, and patient advocates) in moving the science forward. This type of symbiotic partnership will quickly accelerate the development and deployment of the next-generation clinical proteomics-based assays. It is my hope that new technologies and a better understanding of biology will deliver better care to a patient.

Andrew N. Hoofnagle: Yes: automated immunoassays will be replaced with MS assays in certain patients that require a more specific assay to help diagnose, prognose, or manage disease.

John Ioannidis: I don't envision spectacular successes in the next 10 years. However, I can see the possibility of having several applications, where MS or other proteomic technologies may have focused indications, with some incremental benefits in terms of accuracy, rapidity of early diagnosis, or both. I am less optimistic about the prospect of clinically meaningful improvements in predictive ability for common diseases or in outcomes in treated patients. To achieve whatever translational advances can be achieved, we need a shift to support well-designed, collaborative late-translational efforts. Otherwise, it is possible that 10 years from now and after a few hundred thousands of papers in proteomics, we will still have no well-documented major applications.

Mary Lopez: One possible scenario is the existence of clinical analyzers for routine measurement of disease-related protein markers and panels. The automated and routine application of tests for diagnostic and specific biomarker panels will make possible a more personalized approach to medicine. This technology will provide added benefit to genomic tests that cannot provide information on clinically important protein isoforms and variants.

Q&A