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Clinical Mass Spec Attains Critical Mass

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Clinical applications for mass spectrometry technology have exploded in recent years. Mass spectrometry analysis is often faster, cheaper, and more sensitive than other methods and is thus ideally suited for both diagnostics and therapeutic monitoring.

“Mass spectrometry is finally being accepted by microbiologists as a powerful analytical tool and is currently revolutionizing diagnostics,” says Haroun Shah, Ph.D., head of the proteomics research unit for Public Health England. “I think if you visit the smallest hospital laboratory in the U.K. today, you will find the technique either being used or being considered.”

Dr. Shah helped design the first dedicated linear matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) instrument used for clinical microbiology in 2000. In addition, he co-developed the mass spectrometry methods that are now used by clinical laboratories and biotechnology companies such as Bruker and bioMerieux.

In 2004, Dr. Shah and his group created the first microbial database for mass spectrometry, which was instrumental for applying the technique to clinical diagnoses. “Because of the huge diversity among microorganisms, you need to have a database that is very comprehensive to identify variants within a species,” explains Dr. Shah.

According to Dr. Shah, clinical microbiology labs have widely adopted MALDI-TOF because of the technique’s simplicity, speed, and accuracy. “Within minutes, you can train staff who have no experience at all to analyze samples,” remarks Dr. Shah. Blood culture samples that previously required overnight incubation can be analyzed within two to three hours, meaning diagnosis and treatment of infections can begin within the same day.

The technique does have limitations. “One of the drawbacks of the linear MALDI-TOF technology is that one relies entirely on mass spectral pattern matching, without knowing the exact identity of each mass ion,” observes Dr. Shah. This is problematic for identifying specific strains of bacteria. Dr. Shah says that the answer to this problem lies in adopting tandem mass spectrometry to identify amino acid signatures across strains.

According to Dr. Shah, future microbiology applications for mass spectrometry will involve mapping the proteome to identify antibiotic resistance or virulence markers, especially within high-risk pathogens.

Biomarkers in Breath

With a new technique called selected ion flow tube mass spectrometry (SIFT-MS), clinicians can acquire quantitative measurements of volatile compounds exhaled by patients in real time. Previous techniques using gas chromatography-mass spectrometry (GC-MS) required that samples be collected and transported to a machine—a process that could take an hour to complete.

David Smith, Ph.D., F.R.S., and Patrik Spanel, Ph.D., professors of chemical physics at Keele University, U.K., developed SIFT-MS. “The biggest and most exciting aspect is the detection in the lungs of a bacterium called *Pseudomonas aeruginosa*, which infects the airways and lungs of these poor people who suffer from this debilitating disease called cystic fibrosis,” informs Dr. Smith. Dr. Smith’s group previously determined that the *Pseudomonas aeruginosa* bacterium emits the chemical hydrogen cyanide.

The SIFT-MS technique is attractive because it is completely noninvasive. “No one objects to giving a breath sample—we have analyzed children as young as 1 year and people as old as 80 years using SIFT-MS,” says Dr. Smith. According to Dr. Smith, this makes the technique ideal for the early detection of bacterial infection that is so dangerous to cystic fibrosis patients: “If you can catch it early and eradicate it early with the typical antibiotics, then the prognosis is improved and lifetimes may be extended.”

The group is also using SIFT-MS to identify volatile compounds released by other bacterial and fungal infections as well as tumors in the lungs. Additionally, Dr. Smith and colleagues used SIFT-MS to discover that pentane levels are elevated in the breath of patients with inflammatory bowel disease.

One downside of SIFT-MS is the size of the apparatus. “The instrument is a bit large at the moment—about 100 kilograms,” admits Dr. Smith. “Everyone would like small, handheld instruments to detect particular biomarkers. That’s on the horizon, but it’s fairly distant at the moment.”

Measuring Metabolites

“The advantage of mass spectrometry is that it increases the number of chemical entities you can measure in biofluids, like plasma or urine, by two log order fold,” asserts Subramaniam Pennathur, M.D., associate professor of internal medicine and director of molecular phenotyping and metabolomics at the University of Michigan.

Dr. Pennathur and his colleagues have used both targeted and untargeted mass spectrometry methods to identify potential metabolite biomarkers for diabetes and kidney disease. “When I review blood work from my patients, typically I have maybe about 10 to 20 metabolites to base my clinical decisions upon,” notes Dr. Pennathur. “With the newer technologies that are available with untargeted metabolomics, you can come up with something as high as about 500 to even 700 named compounds, and several that are unnamed.”

The group is currently analyzing blood and urine samples that were collected from kidney disease patients 7 to 10 years ago to look for metabolites that correlate with disease progression. The hope is that these metabolites could be used to predict a patient’s outcome and may give insights into disease-causing mechanisms.

These potential mechanisms can then be explored in animal models. “We can get an inhibitor that turns off an enzyme that actually makes this metabolite, for example, and then see if it reverses progression of the disease,” details Dr. Pennathur. He adds that such mechanistic inquiries should eventually lead to new therapies.”

According to Dr. Pennathur, one limitation of untargeted mass spectrometry is that there is not a single platform that can analyze amino acids, carbohydrates, nucleic acids, alcohols, and lipids. The untargeted technique is also not very quantitative or reproducible. That is why Dr. Pennathur and his team use a combination of approaches—and mass spectrometers—to do their research: quadrupole time-of-flight (Q-TOF) for discovery studies and triple quadrupole and GC-MS for more targeted quantitative work.

Diagnosing Men’s Cancers

“Biomarkers of men’s cancers have not historically received much attention,” says Rainer Cramer, Ph.D., professor of chemistry at the University of Reading, U.K. “But it’s picking up now, and it’s about time.”

“The original question for us was, ‘Can we define the margins of where the cancer starts and stops?’” relates Dr. Cramer. “Because that’s quite important for penile cancer patients, obviously—not to cut more than is needed.”

To begin to answer this question, Dr. Cramer’s group and collaborators used MALDI imaging to search for penile cancer biomarkers. Using the technique, the group was able to identify a specific biomarker, S100A4, that was found in the malignant tissue samples but not in the noncancerous tissue from two penile cancer patients. This was subsequently verified by immunohistochemistry in a large patient cohort.

Although MALDI imaging is less sensitive than some other forms of mass spectrometry, its ability to provide quantitative spatial information about specific molecules is very powerful. “You can go through the various different masses and see how the distribution of a specific molecule changes,” explains Dr. Cramer. “You can directly see if there is more or less in one or the other type of tissues. That’s very nice in terms of the visualization.”

Dr. Cramer is now studying how mass spectrometry analysis of biomarkers can be used for the early detection of male cancers as well as to determine the stage of cancer in a particular patient. “It’s very important to know if cancer is relatively localized or if it has already metastasized—if it has metastasized, the outcome is normally not that great,” remarks Dr. Cramer. “If you can catch it before and tell the surgeon what to do, that’s quite important.”

Therapeutic Monitoring

Caprion Proteomics is developing multiple reaction monitoring (MRM) assays that use triple quadrupole mass spectrometry coupled to liquid chromatography to quantify levels of specific proteins and post-translational modifications on these proteins in patients before and after drug treatment. According to the company’s CSO, Daniel Chelsky, Ph.D., “The power of the technology is the specificity of being able to look at any protein and any post-translational modification with multiplexing power—all without the use of any antibodies.”

Caprion uses triple quadrupole mass spectrometers for MRM because it allows for the selection of both the parent peptide and specific peptide fragments. “That double selection really reduces the background

dramatically. The sensitivity is high," insists Dr. Chelsky. "The confidence in your peptide ID is very, very high." Internal reference standards can also be used in this technique, which allows comparison between different analyses.

A Caprion client is currently using the technology to see if their protease inhibitor drug is effective in patients. "We can look at specific sites that are normally cleaved, and we can ask whether those sites are intact or if we are getting degradation products due to the action of the endogenous enzyme," explains Dr. Chelsky. "To do that with an antibody-based approach is really tough because you would need an antibody against all of the different fragments you're looking at."

The technique has a wide range of clinical applications, and Caprion has ongoing projects covering all major therapeutic areas—from oncology to CNS diseases to cardiovascular. "The common applications are cleavage sites, protease inhibitors, kinase inhibitors, and simply assessing protein levels," notes Dr. Chelsky.

This technology is not without limitations, however, especially for proteins with low expression levels, such as cytokines. "You can't see everything; it's not the answer in all situations," concedes Dr. Chelsky.

"If there is a specific target of interest, we have done antibody enrichment followed by doing mass spec," remarks Dr. Chelsky. "That's a very good way to go also."

Caprion has an additional nontargeted assay that uses Thermo Scientific's Q-Exactive mass spectrometer for sequencing peptide fragments that can then be compared against a database. "You come up with a very straightforward assessment of thousands of proteins in a single assay," says Dr. Chelsky.

Statistical software and knowledge about protein pathways allow researchers to then focus on a small group of proteins. One current application is the identification of colon cancer biomarkers in FFPE sections from tumors and adjacent normal tissue. According to Dr. Chelsky, the ultimate goal is to significantly increase the amount and quality of information available to pathologists, leading to more informed decisions for patient care.

Drs. Shah, Smith, Cramer, and Chelsky will be presenting their work at the SelectBIO conference, "Clinical Applications of Mass Spectrometry," which will be held in Barcelona, Spain on October 29–30.

New Factor II/V Genotyping Test

The FDA recently approved Agena Bioscience's Impact Dx system for the clinical diagnosis of Factor II and Factor V Leiden. Specifically, the genotyping test is indicated for use as an aid in the diagnosis of patients with suspected thrombophilia.

Using MALDI-TOF mass spectrometry, the MassARRAY's underlying technology for the Impact Dx, identifies specific DNA biomarkers with high specificity and sensitivity, according to a company official. Factor II and Factor V mutations are each well-defined single nucleotide polymorphisms, providing a definitive multiplexing proof of concept for the first FDA-approved indication using this technology, he added.

"There are other DNA-based platforms already available for Factor II and Factor V genotyping," explained

Rhett Affleck, Ph.D., vp of R&D at Agena. “The test performance is a positive percent agreement of >99.9% and a negative percent agreement of 99.3% for Factor II.

“Similarly, a positive percent agreement of 98.7% and a negative percent agreement of 99.4% were obtained for Factor V Leiden. The MassARRAY technology has advantages in cost per sample and throughput.”

Developing a new genotyping assay for a research-use-only MassARRAY system is not complex, he continued. “It takes a few days in total, simply requiring DNA primer design through Agena’s Assay Design Suite software and then waiting for oligos to ship from your preferred supplier. Agena also provides predesigned assay kits for research use only.”

Dozens of laboratories are currently running clinical research applications on the MassARRAY platform, said Dr. Affleck. Applications and assays span from heritable diseases like cystic fibrosis to genotyping applications in blood cells, solid tumors and blood cancers, panels for pharmacogenomics and applications in methylation and quantitative gene expression.