The uses of mass spectrometry in translational medicine

Innovative MS hardware and software solutions enable cutting-edge omics research at a distinguished Dutch medical center.

By Rohan Thakur, PhD

Translational medicine involves the progression of research through to biomarker discovery and development, resulting in diagnostic assays for personalized healthcare, diagnosis, prognosis, and better understanding of the mechanisms of disease and drug action. Mass spectrometry (MS) is a popular technique used in omics research, which encompasses proteomics, glycomics, metabolomics, and genomics. Personalized healthcare considers individual differences among patient populations, with a key principle of identifying molecular biomarkers as primary drivers of patient management.

By tailoring medical treatment to the individual characteristics, needs, and preferences of each patient, clinicians can greatly reduce the number of patients receiving ineffective treatments. Next generation genome sequencing, MS, and imaging technologies are driving innovative biomarker and diagnostic developments. For example, improvements in MS have enabled proteomics experts to analyze intact proteins through top-down proteomics, to help identify post-translational modifications (PTMs) in patients and advance targeted proteomics. What is being discovered by researchers will have significant impact on clinical diagnostics and therapeutics in the future.

Translational omics at Radboudumc

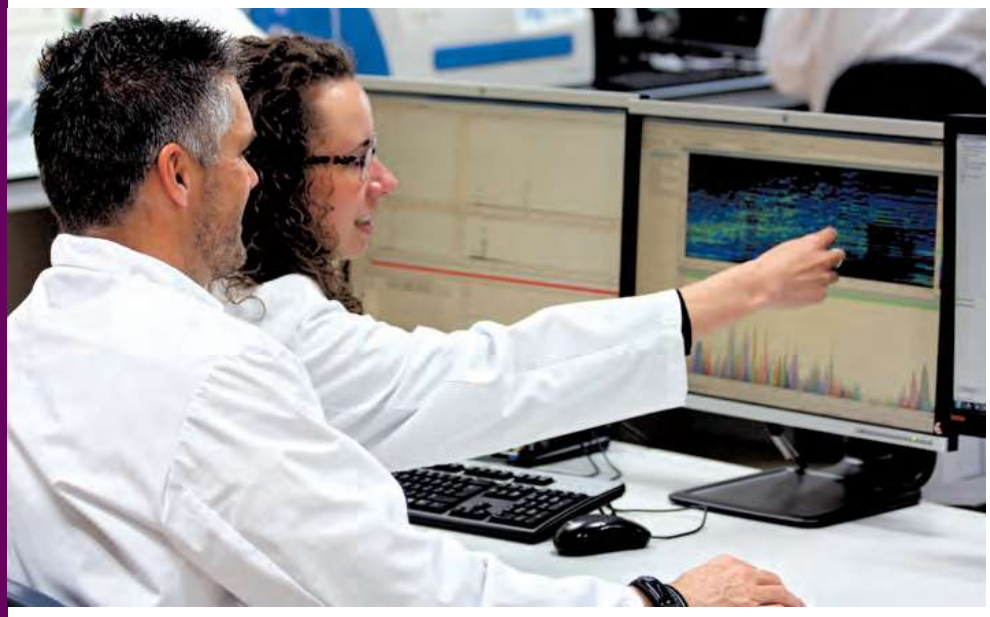
Radboud University Medical Center (Radboudumc) in Nijmegen, Netherlands, is a large medical school with approximately 11,000 employees. This institution contains 50 departments which collectively focus on personalized healthcare and whose core activities are centered on patient care, research, and education. Radboudumc's technology infrastructure is organized through 19 Technology Centers that span omics, imaging, and data analysis, among other disciplines, and are coordinated by Professor Alain van Gool. The Technology Centre for Mass Spectrometry includes the Translational Metabolic Laboratory (TML), which Professor van Gool heads. The TML is part of Radboudumc's larger Department of Laboratory Medicine, where it partners with four other clinical laboratory departments (Genetics, Pathology, Pharmacy, and Medical Microbiology) to form the Radboud Diagnostic Lab.

The TML specializes in the metabolic side of disease, focusing on proteins, metabolites. and enzyme functions. Metabolic data is combined with insights from genetic analysis to identify biomarkers and create novel personalized diagnostics that are run at TML under ISO15189 accreditation. The laboratory bases a large part of its technological approach on MS through integrated proteomics, glycomics, and metabolomics mass spectrometry platforms.

Glycoproteomics

PTM occurs during or after protein biosynthesis, with a wide variety of possible outcomes. For example, phosphorylation introduces a new phosphate group to the protein chain, a crucial event in cellular signal transduction. Glycosylation results in the attachment of a new carbohydrate molecule to a protein. Approximately 80 percent of all proteins are glycosylated, and many of them are major determinants of health and disease; around half of all human diseases are affected by glycosylated proteins. Protein glycosylation profiles can be changed following mutations in the genome, but also if the patient is challenged with a certain disease or infection. Such specific PTMs have a high potential as relevant disease biomarkers and can only be detected using MS.

"To be able to look at glycosylated proteins, one needs extremely high resolution hardware to distinguish the different types of carbohydrate chains (the glycosylations) with high accuracy, so there is no doubt over the nature of the glycosylation branch," explains van Gool. "Also, one needs to be able to annotate the different peaks with complementary software to automatically identify the glycosylation pattern."



Top-down proteomics data analysis with Professor van Gool at Radboud University Medical Center

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The TML group has been studying glycosylation mechanisms to elucidate which mutations in patients affect the mechanisms of glycosylation. They have translated this knowledge into diagnostic assays for intact glycoproteins and glycans. The glycosylation and high-throughput proteomics approaches were combined, resulting in a glycopeptide profiling workflow with very promising diagnostic potential. "We now can detect around 40,000 unique glycopeptides in a single MS scan. Our recent cohort analysis shows that such profiles can be used to cluster patients based on their unique profile with unparalleled specificity," comments van Gool.

He continues: "We invested heavily in data analysis pipelines last year, to cope with analyzing the data resulting from these workflows. Using these deep learning analysis pipelines, we can use the whole profile, about one million data points, to cluster patients into certain groups. If you can identify the defect or mutation in those clusters, that's a very quick diagnosis. The next step we're taking is to identify all those differential glycopeptides, to break it down to single biomarkers, which helps us to elucidate the mechanism of disease and develop novel personalized diagnostics."

Intact protein analysis

Technology has thus far restricted the analysis of intact proteins, but in recent years this has been developed, and top-down proteomics is gaining strength in laboratories such as that at Radboudumc for application in intact protein analysis, PTMs, and analysis of low- to medium-complexity samples. Researchers at the TML specialize in the development and application of top-down proteomics and protein complexes.

Van Gool explains: "We use instruments, supported by software, to look at a purified complex to identify proteoforms. We've been able to isolate different protein complexes in the mitochondrial energy chain in mice, and can semi-quantitate the different proteoforms of the complex components in different tissues at an intact protein level. This opens up an entirely new level of biology which is extremely interesting. One of our strategic objectives is to combine the analysis of PTMs and intact proteins to develop novel personalized diagnostics, as we recently did for the intact glycoprotein transferrin that we apply to diagnose specific subtypes of congenital disorders of glycosylation."

Harnessing genomics

The TML works closely with the Department of Genetics at Radboudumc, where, last year, 8,000 patients were genetically investigated in a diagnostic setting by whole exome sequencing. Beginning in 2018, the department is moving toward whole genome sequencing and will combine these efforts with the functional omics efforts at TML.

"Sequencing patients is a powerful approach toward a personalized genetic diagnosis, but after filtering a lot of variance away you may still have 10 to 20 variants remaining," van Gool comments. "You have to pick which one is the causal mutation. We support this with functional omics platforms to contextualize those mutations, so together we can make a thorough personalized diagnosis. This tandem approach with next-generation sequencing and mass spectrometry is extremely powerful."

Adding the extra layer of metabolomics is complex, but it helps scientists uncover which variant is associated with a certain clinical phenotype. "In the years to come, I think this approach will help us make better diagnoses—it really is a complementary technology," explains van Gool, "We're adding this next generation layer of functional omics to the whole genetic screening. We see this as a strategic way of moving forward, to really interpret patients holistically, not by single mutations. Ultimately we want to have functional omics pipelines, where genetics is followed by metabolomics in cases where we suspect a metabolomics difference. We also want to combine genetics with glycoproteomics where we suspect there is something happening in the patient."

A challenge and a solution

One of the key challenges facing personalized healthcare, and van Gool's laboratory, is the innovation gap in biomarker research and development. Thousands of novel candidate biomarkers are in discovery research, without proper follow-up experimentation to validate findings and to develop them to improved robust diagnostics. For example, approximately 2,000 new biomarkers for prostate cancer are published per year in peer-reviewed journals, and it takes one to three years to validate a biomarker and up to 10 years to develop a diagnostic assay.

To overcome such gaps, several recommendations can be made. One of these focuses on how researchers handle data. Good data stewardship applies the "FAIR" principles to all experiments. Professor van Gool explains how this should be implemented: "FAIR stands for Findable, Accessible, Interoperable, and Reusable. This is a very important principle which, if implemented properly, encourages the production of data which can be found and re-used by other scientists, using accurately documented methods. This way of working will certainly contribute to improving the reproducibility of scientific studies. It is a fundamental way of working in which we aim to share our data for the greater good of science, so that together we can do better science than we can by ourselves."

Future applications

The field of translational medicine and personalized healthcare is rapidly progressing, in line with the technologies used by proteomics, glycomics, metabolomics, and genomics laboratories. Since scientists realize that the full proteomics picture cannot be obtained from digested proteins, more laboratories are set to use the intact approach, using innovative top-down proteomics. Laboratories such as that of Professor van Gool bring such new technologies into the clinical setting, and use translational science to increase insights into disease mechanisms in service of the ultimate goal of improving personalized patient care.

Rohan Thakur, PhD, serves as Executive Vice President for **Bruker Daltonics**. He has more than 20 years' experience in mass spectrometry, including 14 years in MS development, and holds several patents in the field of ion optics. Prior to joining Bruker, he held positions as Director, Global Marketing, for mass spectrometry solutions at Thermo Fisher Scientific and as Director of Drug Discovery for a pharmaceutical contact research organization.