# Clinical mass spectrometry in heart disease

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The presence of mass spectrometry (MS) in the clinical setting is not a new feature, with the use of gas chromatography-MS (GC-MS) for toxicology assays reported as far back as the 1970s.[1] GC-MS is a popular technique amongst analytical chemists due to the comprehensive chemical libraries that have been generated, and the ability to utilise these across systems with the use of tools such as the *n*-alkane retention index.[2] Although there is no questioning the qualities of GC-MS as an analytical technique, it owes to troublesome analysis for clinical samples. Bio-fluid metabolites are required to enter the system in a volatile state and therefore complicated preparation involving derivatisation must be performed. On the other hand, liquid chromatography-MS (LC-MS) allows for a much simpler sample preparation of bio-fluid matrices. LC-MS was a slow mover in the clinical setting, with many of the early instrumentation seen as too cumbersome and required experienced and skilled workers to achieve the desirable results. Advances in technologies have allowed increased user versatility and many of the modern instruments now afford a *plug and play* style interface.

Hyphenated mass spectrometric techniques offer researchers the ability to develop assays that can analyse multiple target molecules in one analytical run - an exciting prospect to a clinician in the view of reducing costs and lead time for results. In addition, the development of multiple analyser platforms, such as a triple quadrupole mass spectrometer (TQ), permits more selective filtering of molecules, leading to the ability to detect biomarkers at increasingly lower concentrations. Another feature of the TQ set up is the ability to improve analyte identification through tandem mass spectrometry, commonly known as MS/MS. Information acquired on the intact molecule and its fragmentation pattern, after being subject to collision-inducing energies, is repeatable and thus aids in identity confirmation. These advances in LC-MS/MS have led to the analysis of small molecules that would have previously been undetectable and allow new biomarkers to be unearthed.

Early uses of LC-MS in a clinical setting were to measure acyl carnitines in new born screening,[3] providing a sensitive method to diagnose deficiencies in fatty acid oxidation. As the years have progressed, although a mass spectrometer has a large financial layout, costs have reduced and high-throughput, multiplex assays can be developed, driving the increasing popularity of MS. Additionally to targeted assays, recent advances in both instrumentation and statistical softwares have shifted the focus of biomarker prospecting towards the global measurement of analytes. Wide-scope, untargeted analyses carrying the *omics* tag have grown in interest and it is now common to see publications that employ metabolomics (small molecules) or proteomics (proteins and peptides) as a tool for novel biomarker discovery. With ever-increasing clinical research laboratories utilising the *omics* approach and software packages improving computing power, multi-centre studies are producing large sample sets that allow progressively more reliable definitions to be made. HUPO, the Human Proteome Organisation, has expressed an emphasis on the development of an all-access database with cross-compatible formats to allow proteomics researchers to share data and produce a detailed library of the human proteome.[4]

For this editorial, in the instance of cardiovascular disease, we will focus on two recent mass spectrometric biomarker applications in heart failure. These are trimethylamine N-oxide (TMAO), which falls in to the small molecule section and is a metabolite formed in the liver by oxidation of trimethylamine, a gut microbe-generated molecule;[5] and b-type natriuretic peptide (BNP), a peptide biomarker present in individuals presented with, or at risk of heart failure.[6] To begin, a group of researchers at the Cleveland Clinic published an article in Nature in 2011 detailing that plasma TMAO levels showed association with patients suffering from coronary heart disease.[7] Further research on this biomarker reported it possessed pro-atherosclerotic properties,[8] and prolonged the hypertensive effect of angiotensin II.[9] When analysed in a subset of chronic heart failure patients, elevated levels of TMAO were independently predictive of all-cause mortality within 5 years.[10] TMAO is commonly analysed using stable isotope dilution-LC-MS/MS with multiple reaction monitoring (MRM). In MRM, the instrument is tailored to filter a selective list of ions, perform fragmentation on them and monitor all reactions in parallel. This approach allows high levels of sensitivity and the use of a stable isotope internal standard vastly improves reproducibility. These analyses are fast, easy to prepare and robust, and therefore lend themselves well to use in a clinical environment.

Another application of MS used in biomarker identification is matrix-assisted laser desorption ionisation-MS, or MALDI-MS. MALDI-MS allows the accurate mass measurement of intact protein structures and has been used in heart failure to measure the degradation products of BNP.[11] A downside to using this analytical method is that often complicated, lengthy procedures are required to concentrate the molecule of interest. For the example of BNP, we recently reported an immunocapture preparation method that allows sufficient pre-concentration of BNP to analysable levels.[12] The raw materials for these types of preparation methods are often costly, notably for the purchase of suitable antibodies, and analysis itself can be a time-consuming process. We are certain that future developments in MS will allow for these processes to be reduced in complexity and, therefore, streamlined. Although there is a list of candidate biomarkers for diagnosis and prognosis of heart failure, TMAO and BNP are the only molecules that have shown repeated research interest using MS-based techniques. Although BNP is measured in the medical setting using an immunoassay, MS-based analyses are yet to be established as routine procedures in the clinical laboratory.

So, the question to be asked is if there is a space for MS analyses in the clinical laboratory, for the assessment of heart failure, or other cardiovascular conditions? Our opinion is that of yes, of course, there is. Research in to TMAO and BNP look promising, but new metabolomic and proteomic experiments are regularly being reported and it is likely that they will produce new, and more sensitive biomarkers for cardiovascular diseases. However, the establishment of new routinely measured clinical biomarkers can be a slow process. Clinical decisions require reliable results and, therefore, the introduction of new techniques and technologies requires a level of trust and confidence to be built. A recent scientific statement released

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by the American Heart Association discusses several hurdles that must be overcome to achieve a more secure footing of MS in cardiovascular science.[13] A main issue raised is the requirement to improve the communication between the public, medical and research communities, as with the aforementioned HUPO proteomic standards initiative. Furthermore, the need to improve throughput and reliability of assays, standardisation of protocols and platforms and implement more widely accepted sample preparation is another note of concern, with many research groups suggesting their own developed methods. Importantly, the need for training of MS skills in researchers and clinical laboratory technicians without analytical chemistry backgrounds is paramount. An interesting point mentioned in the statement is the value that miniaturisation will add to the implementation of MS into a clinical setting. Recent developments of miniature mass spectrometers with direct ambient ionisation[14] show promise to be an exciting addition to clinical MS in the coming decades. It is without doubt in our mind that with continued effort from focussed research groups we can break the mould of more traditional assays, and implement our powerful MS-based analyses into the routine clinical environment. Perhaps in the future we will be discussing the next new technology that can out-perform clinical mass spectrometry? We personally believe that when MS is adapted in to routine screening, certainly with heart failure, it will be here to stay.

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