The use of MS for the investigation of irritable bowel syndrome and inflammatory bowel disease

Currently, the diagnosis of bowel diseases such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) relies on invasive and expensive procedures. Identification of biomarker-based tests to aid diagnosis is an important area of research. Here we review the use of mass spectrometry in this search and discuss recent findings.

by Dr B. De Lacy Costello, Professor N. M. Ratcliffe and S. Shepherd

Inflammatory bowel disease (IBD) is an inflammatory autoimmune disease caused by an inappropriate response of the immune system to commensal gut microbes [1]. There are two types of IBD, ulcerative colitis (UC) and Crohn's disease (CD). UC affects the large bowel only, affecting variable lengths of the colon continuously from the rectum, primarily affecting the mucosa [Fig. 1]. CD can affect any part of the GI tract, and is a transmural disease [2]. Common symptoms of IBD are severe abdominal pain, defecation urgency and diarrhoea, which can contain blood.

Irritable bowel syndrome (IBS) is a functional disorder of the digestive tract. It is characterized by its symptoms, with no physiological changes in the GI tract. IBS can be diarrhoea predominant (IBS-D), constipation predominant (IBS-C) or symptoms can alternate between the two (IBS-A). Common symptoms include abdominal pain and cramps, bloating and flatulence, and unusual bowel habit. IBS has, as yet, no known cause. People with IBS show abnormal gut motility and hypersensitivity to pain in the GI tract. Stress and anxiety are known to cause changes in gut motility [3] with stress and anxiety being common symptoms of IBS. When under physical or psychological stress IBS patients showed increased gastro-intestinal sensitivity when compared to healthy controls [4]. Recently it has been thought that there may be changes in the gut microbiota in patients with IBS, the evidence being that IBS symptoms often occur after infective gastroenteritis or in patients in remission from IBD or diverticulitis. SIBO (small intestinal bowel overgrowth) has also been implicated in IBS and other function bowel disorders. One current hypothesis is that an altered microbiota activates the immune system within the mucosa, leading to an increase in epithelial permeability, causing dysregulation of the enteric nervous system [5]. Genome-wide association studies have successfully identified many genetic loci involved in susceptibility to IBD, and it is thought that genetic factors may also play a role in IBS [1].

Diagnosis of GI disease

IBS-D can present with symptoms similar to IBD and other non-functional bowel conditions. The diagnosis of IBS is often one of exclusion, where more serious bowel diseases, such as IBD or colon cancer which present with common symptoms, are ruled out. The current gold standard for diagnosis of IBD is endoscopic and histological testing; however, these investigations are both invasive and costly, and have associated risks. Of the patients referred for endoscopy few actually have organic bowel disease [6]. The costs associated with functional bowel disease are significant, with healthcare costs for IBS patients being significantly higher than non IBS controls [7].

There are currently no known biomarkers of IBS. There are various biomarkers that have potential in the differentiation of functional from inflammatory gastro-intestinal disease, but there is still a need to identify biomarkers and to develop quicker, lower cost and less invasive testing for diagnosis of gastro-intestinal disease.

Biomarkers such as lactoferrin, calprotectin, c-reactive protein (CRP) and erythrocyte sedimentation rate (ECR) have all been used to help distinguish functional from inflammatory bowel disorders and to diagnose IBD. Serological markers such as antibodies to bacterial and fungal antigens that can indicate an abnormal response to commensal microbes can also be useful in identifying IBD.

Fecal calprotectin and lactoferrin are protein biomarkers of inflammation. In 2010 a meta-analysis of six studies (n=670) in adults by Van Rheenen et al. [8] found that screening patients by testing fecal calprotectin levels would have reduced the number of endoscopies performed by 67%, although its diagnosis would have been delayed in 6% of patients. When taking a weighted mean of 19 studies including 1001 patients, where IBD patients were compared with controls of IBS and other colonic diseases, fecal lactoferrin has a sensitivity and specificity of 80% and 82%, respectively [9].

Although these biomarkers can be useful as part of the screening process when establishing a diagnosis [6, 8], there is currently no biomarker or test that can replace the need for endoscopic and histological investigations. Mass spectrometry techniques are at the forefront of research for biomarker prospecting for IBS/IBD.

Mass spectrometry

Mass spectrometry (MS) has the ability to identify numerous compounds in a single sample. It is also high throughput allowing rapid analysis of many samples, which is especially useful for large studies or for the diagnosis of many samples. The ability to obtain results quickly, usually in less than 1 hour makes it attractive for clinical use.

Proteomic approach

Although MS (with associated sample vaporisation methods) was originally limited to low molecular weight volatile compounds, in the last 2 decades advances in MS technology have enabled its use with high molecular weight compounds, changing the way proteins are analysed. The soft ionization techniques electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) allow for the analysis of proteins and other macromolecules [10]. The identification of proteins

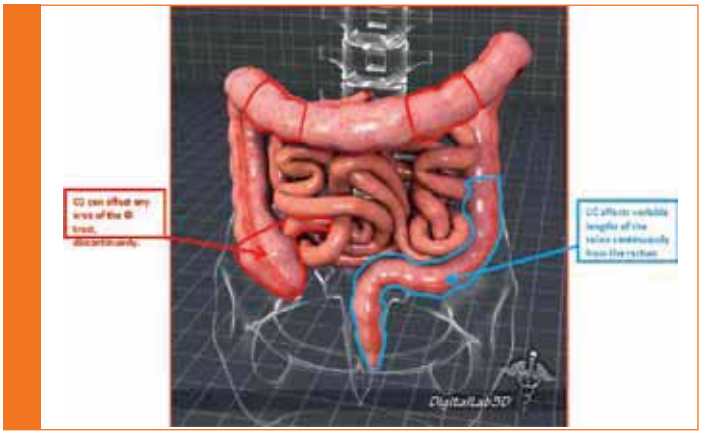

Figure 1. Diagram of the gut showing the areas where inflammatory disease is most likely to occur.

through peptide mass fingerprinting, or peptide sequencing using MS is more rapid than techniques such as *de novo* protein sequencing and data can be analysed automatically. MS can also be used to determine the abundance of a molecule in a sample [10].

Differential protein expression can identify different diseases, and can indicate the degree of the disease state, or be used to assess the effects of treatment – for example the response of IBD patients to anti-TNF alpha antibodies (infliximab) [11]. It also has applications in the identification of protein biomarkers.

In 2011 MALDI-MS was used by M'koma *et al.* for tissue analysis; through profiling of the proteome of the colonic submucosa they were able to distinguish UC from CD by comparing proteomic spectra. Definitive diagnosis of either UC or CD is important as people with UC also have an increased risk of colon cancer [12].

Goo *et al.* have investigated protein biomarkers for IBS. ESI with LC-MS was used on protein fragments from the urine of women with IBS. They found differences in some specific components of the urinary proteome, and demonstrated that there is a possibility for future biomarker studies for IBS [13].

There are still limitations to mass spectrometric protein analysis, for example the difficulty in detecting hydrophobic membrane proteins. However, it seems promising that, with the advances in mass spectrometry technology, there will be an increase in the discovery of protein biomarkers and key pathogenic factors of gastro–intestinal disease, and improved diagnosis and therapy.

Metabolomic approach

The metabolome is the set of small molecule metabolites found in a biological sample. Unlike proteomics, metabolomics can be a direct measure of production of compounds and activity of cells or systems in an organism. This can be especially useful when looking for disease biomarkers in IBS and other bowel diseases as it can be used to understand the environment of the GI tract, as well as factors such as digestion and absorption of dietary products and gut microbial activity [14], which are implicated in IBS pathogenesis.

Researchers have explored the use of various techniques incorporating MS on breath [15], urine [16] and stool [17] samples in search of metabolic biomarkers of bowel disease for non-invasive testing and many possible candidates have been identified.

The commonly used analytical techniques in metabolomics are GC-MS (gas chromatography-mass spectrometry) or LC-MS (liquid chromatography-mass spectrometry) and NMR (nuclear magnetic resonance) spectrometry. NMR has the advantage that there is no need to have the compounds in the vapour phase, although the limit of detection using NMR is much poorer than MS.

LC-MS metabolomic studies have been recently undertaken using urine to identify putative colon inflammation biomarkers [18]. The authors note that urinary biomarkers would be preferable to sampling intestinal tissue or blood as the collection of urine samples is non-invasive and multiple samples are more readily obtained.

The analysis of volatile organic compounds (VOCs) or metabolites (VOMs) is an emerging area of disease diagnosis. VOCs are small molecules that are readily analysed by GC-MS. Other commonly used methods of VOC detection are selected ion flow tube mass spectrometry (SIFT-MS) [Fig. 2], and the similar technique of PTR-MS (proton transfer MS).

There are already several FDA approved tests using volatiles from breath. These include testing for heart transplant rejection, hemoglobin breakdown in children and measurement of hydrogen or methane to diagnose GI lactose or fructose malabsorption. The measurement of breath hydrogen has also been used to diagnose SIBO. Recent work by Španěl *et al.* using SIFT-MS quantified the breath pentane concentration of study subjects using the reaction of O^{2+} with pentane. It was found that patients with CD and UC had significantly elevated breath pentane levels compared to healthy controls [15].

Figure 2. Photo of a SIFT mass spectrometer analysing VOCs in the breath of a volunteer in real time. Reproduced with kind permission of Patrik Španěl.

Testing for fecal biomarkers of bowel disease is facile as samples are easily obtained and have been in contact with the gastro intestinal tract. The changes in the odour of feces and flatus reported in many bowel conditions are due to changes in the VOC profile. This altered VOC profile could lead to identification of biomarkers of disease state. A recent pilot study carried out by Ahmed et al. using GC-MS on fecal samples from IBD and IBS patients identified a key set of VOMs which were able to distinguish IBS-D from Active IBD with a sensitivity of 96% and a specificity of 80% [19].

Conclusions

MS techniques show promise for the identification of biomarkers of various GI disease states, which have the potential to reduce invasive testing, improve patient care and reduce healthcare costs.

Instrumentation is still expensive and relatively large, limiting its use in hospital settings and particularly limiting its use for near-patient testing. Also biomarker discovery is still in its infancy and much remains to be clarified in relation to the significance of markers to disease and the underlying metabolic pathways.

However, work to reduce the size and cost of mass spectrometers is well advanced and would open up the possibility of instruments being deployed for point-of-care detection and monitoring of diseases including IBS and IBD.

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The terminology of mass spectrometry

GC-MS: Gas chromatography-mass spectrometry This is the gold standard method for prospecting for volatile biomarkers. The first part, GC, separates the volatile compounds using a flow of gas (mobile phase) through a long coated silica column. The coating (stationary phase) retains different volatiles according to their boiling point or polarity etc. The second part, the mass spectral detector, fragments the separated molecules into positive ions and their mass to charge ratio is identified. They are then matched to an extensive library of mass spectral fragmentations to aid structural identification.

LC-MS: Liquid chromatography-mass spectrometry This is similar to GC-MS except that a solvent rather than gas is used as the mobile phase

SIFT-MS: Selective ion flow transfer-mass spectrometry This method permits real time analyses of known volatile compounds and can be used readily to analyse the headspace of breath, urine and stool. Unlike the GC- or LC-MS there is no chromatography step so is extremely fast and gives quantitative analyses. The reaction of the analyte with precursor ions (H3O+, NO+, O2+) forms product ions. Detection of the precursor and product ions of interest is used to quantify the absolute concentration of a particular VOC in the sample using the known reaction kinetics.

PTR-MS: Proton transfer reaction mass spectrometry A PTR-MS instrument consists of an ion source directly connected to a drift tube (in contrast to SIFT-MS no mass filter is interconnected) and an analyzing system (quadrupole mass analyser or time-of-flight mass spectrometer).

ESI: Electrospray ionization Liquid solvent containing the analyte is aerosolised by electrospray, a high voltage is applied to the liquid causing it to aerosolise into charged droplets. As the solvent evaporation occurs, the droplet shrinks until it reaches the point that the surface tension can no longer sustain the charge (the Rayleigh limit) at which point a 'Coulombic explosion' occurs and the droplet is ripped apart. This produces smaller droplets. The process is repeated with the smaller droplets until 'naked' charged analyte molecules are formed. This process is useful for molecules such as proteins as it is a fairly gentle method if ionisation which doesn't disrupt the non-covalent bonds in the molecule.

MALDI: Matrix assisted laser desorption The analyte is embedded in a matrix, a laser beam is then focussed on the matrix which absorbs light of the frequency of the laser, this causes excitation of the molecules and desorption of matrix-analyte ions from the matrix. The matrix molecules then evaporate away leaving free analyte ions.

Peptide mass fingerprinting Proteins are digested into their constituent peptide fragments (the protein fingerprint), the proteins are then identified by matching the mass obtained for the fragments to the mass of known fragments using a database.