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Conjugating immunoassays to mass spectrometry: Solutions to contemporary challenges in clinical diagnostics

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Abstract
Developments in immunoassays and mass spectrometry have independently influenced diagnostic technology. However, both techniques possess unique strengths and limitations, which define their ability to meet evolving requirements for faster, more affordable and more accurate clinical tests. In response, hybrid techniques, which combine the accessibility and ease-of-use of immunoassays with the sensitivity, high throughput and multiplexing capabilities of mass spectrometry are continually being explored. Developments in antibody conjugation methodology have expanded the role of these biomolecules to applications outside of conventional colorimetric assays and histology. Furthermore, the range of different mass spectrometry ionisation and analysis technologies has enabled its successful adaptation as a detection method for numerous clinically relevant immunological assays. Several recent examples of combined mass spectrometry-immunoassay techniques demonstrate the potential of these methods as improved diagnostic tests for several important human diseases. The present challenges are to continue technological advancements in mass spectrometry instrumentation and develop improved bioconjugation methods, which can overcome their existing limitations and demonstrate the clinical significance of these hybrid approaches.

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1. Introduction

1.1. Biomarkers and clinical analytical techniques

The term biomarker can denote any biological molecule or combination of factors that indicate a particular biological state, which are often used to differentiate normal or abnormal processes or conditions [1]. Whereas new biomarkers are identified using untargeted, semi-quantitative (comparative) analytical approaches, to develop a viable clinical test there needs to be a reproducible method for their absolute quantitation. For implementation in clinical laboratories, analytical tests designed for diagnostic applications need to meet performance requirements with respect to their accuracy and predictive capabilities [2]. Diagnostic accuracy is determined based on a test’s abilities to positively identify individuals who have a condition and eliminate those who do not, or its sensitivity and specificity, respectively. A test’s predictive value is evaluated by calculating the proportions of correct diagnoses out of the total positive and total negative test results, or positive and negative predictive values, respectively [2]. In the context of biomarkers, these factors are dependent on the dynamic range, accuracy, and reproducibility of whichever analytical method is used to detect changes in their abundance.

Most common clinical laboratory tests make use of spectrophotometric and/or immunologic detection methods [3]. Of these, immunoassays, which take advantage of the highly selective interactions between specific immunoglobulins and their target antigens, are some of the most clinically relevant techniques [4,5]. Furthermore, the chemical composition of these large proteins enables a variety of strategies for modifying their structure with limited perturbation of their antigen-binding activity (discussed in detail in Section 2.2). This feature allows for immunoassays to be coupled to a range of different detection methods, including radiometric, fluorescent, colorimetric, chemiluminescent, non-labelled (light scattering) and electrochemical detection [4,6]. Detection can be further enhanced using enzymatic, polymerase chain reaction (PCR), liposome and nanomaterial-based signal amplification strategies [7–9].

In many instances, absolute levels of biomarkers in biological fluids or tissue biopsies are insufficient for determining a reliable
diagnosis, particularly for diseases characterised by complex changes in tissue morphology and localised changes in protein expression [10]. More relevant information can therefore be obtained by comparing the spatial distribution of biomarkers in normal and diseased specimens. Immunohistochemistry (IHC) involves the labelling of specific antigens in tissue sections with antibodies, which are then visualised using some combination of staining and imaging techniques. Owing to its simplicity, affordability and versatility, this technique is commonly employed in diagnostic pathology [10,11].

Developments in immunoassay and IHC technologies, such as automated enzyme-linked immunosorbent assays (ELISAs), microfluidics, lab-on-a-chip technologies, and computer-assisted image analysis, have resulted in significant reductions in analysis time and complexity, sample volumes and specialised equipment or expertise required [5,10]. However, many of these methods still utilise some form of spectrophotometric detection and are therefore limited in the number of analytes that can be detected in a single experiment due to overlaps in the emission ranges of different fluorophores and narrow dynamic range [12]. The development of immunoassays that utilise detection methods not constrained by the inherent limitations of spectrophotometric measurements has therefore become an important goal for modern diagnostic medicine [10,11].

### 1.2. Evolution of biomolecular and clinical mass spectrometry

Since its inception in the early 20th century, mass spectrometry (MS) has developed into an important tool for biomedical researchers and clinicians [13–15]. Soft ionisation methods, such as electrospray ionisation (ESI), matrix-assisted laser desorption/ionisation (MALDI) and chemical ionisation, enable ionisation of molecules with minimal fragmentation. These methods are therefore very useful for the MS analysis of intact biomolecules, with MALDI and ESI commonly utilised in both research and clinical settings [3,13,14,16]. Compared to spectrophotometric methods for detecting biomolecules, MS differentiates analytes based on the mass-to-charge ratio (m/z) of intact molecules and/or the characteristic products of their gas-phase fragmentation, and therefore provides high specificity and sensitivity and enables the detection of different isoforms [13,17]. The ability of MS to detect many different analytes simultaneously, or multiplex, is useful for analysing complex biological mixtures as entire proteomes, lipidomes, or metabolomes can be investigated for a single sample [13,18]. This technology has obvious applications in diagnostic medicine; hence, renewed enthusiasm for advancement in MS methodology is now aimed at developing clinically viable platforms [17,19].

Widespread adoption of MS in clinical laboratories did not occur until the 1980’s, after the limitations of immunoassays for illicit drug screening and detection of steroids became apparent. Subsequent acceptance of gas chromatography-mass spectrometry (GC-MS) for immunoassay validation in clinics lead to mainstream use of the more versatile liquid chromatography-MS (LC-MS) [17,18,20]. Despite its waning popularity due to the requirement for extensive sample preparation and often chemical derivatisation, GC-MS still has an important place in the clinical analysis of selected compounds such as excreted steroid metabolites [21,22].

The development of tandem MS (MS/MS), which enables the unambiguous assignment of biomolecules based on their unique fragmentation patterns, widened the boundaries of MS within the clinical laboratory to include protein and peptide biomarker detection, multi-analyte therapeutic drug monitoring, drug abuse screening, toxin analysis, endocrinology and screening for metabolic diseases [15,23]. The capabilities of this technology are exemplified by the now wide-spread adoption of MS/MS-based blood-spot screening for congenital metabolic diseases in newborns, which was previously limited to individual metabolites but can now detect in excess of 40 analytes simultaneously [24,25].

Another revolutionary development in clinical MS was the discovery of a method for identifying bacterial molecular fingerprints using MALDI, hence providing a faster and easier alternative to other time-consuming laboratory tests for identifying pathogenic microorganisms [17,26]. This demonstration of MALDI-MS as a clinically viable technique, which could be performed with limited sample preparation, was followed by US Food and Drug Administration (FDA)-approval of two MALDI systems for identifying gram-negative bacteria [3,17,27]. The multiplexing capabilities of MALDI have since been exploited to develop rapid PCR-based MS assays for screening of human coronaviruses, including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) viruses. In this study, the authors concluded that multiplexed analysis reduced false negative results and has the potential to detect novel viruses [28].
Until 1997, immunochemical detection still had a distinct advantage over MS: the capacity to determine the spatial distribution of proteins in tissues. The invention of an innovative new way to acquire MALDI spectra led to the establishment of a new research field: MS imaging (MSI). During MSI experiments, individual mass spectra are obtained sequentially across the surface of a biological specimen and converted into an intensity map showing the localisation of ions with specific m/z [29]. With the development of additional ionisation techniques, such as desorption electrospray ionisation (DESI) and secondary-ion mass spectrometry (SIMS), MSI can now be used for the analysis of proteins and protein complexes, small molecules, lipids, metabolites, oligonucleotides and sugars, many of which cannot be detected using immunochromical methods [30–33]. A form of laser ablation MS, rapid evaporative ionisation mass spectrometry (iKnife; Waters Corporation, Milford, US), has even been developed to assist surgeons during tumour removal by providing real-time analysis of patient tissue components to identify cancerous tissue margins [30].

1.3. Current challenges for the development of clinically viable MS methods

For analysis of biological samples, MS workflows generally include a sequence of sample preparation, separation and MS analysis procedures and their clinical viability is dependent on the complexity, cost and duration of each step [17,34]. Some inherent limitations to common MS technologies also have a significant influence on their effectiveness in clinical diagnostic applications.

1.3.1. Matrix effects and ion suppression

Another significant issue for MS analysis of complex biological samples is ion suppression, which refers to the reduction in ionisation of target analytes because of interference from other components within the biological matrix, such as salts, detergents or other non-volatile compounds [34,35]. For ESI-based analyses, this problem is usually addressed during sample preparation and separation steps or additional enrichment steps, for example by using LC to separate non-volatile components [34,36].

Comparatively, sample preparation for MALDI-MS is a lot simpler as this ionisation method is more tolerant of biological sample components, such as buffers [3]. However, the absence of pre-analysis enrichment steps also makes it more difficult to detect low-abundance ions, as MALDI spectra are often dominated by signals from more concentrated, albeit less clinically significant, biomolecules, a phenomenon sometimes described using the analogy of a needle in a haystack [31].

For imaging experiments, MALDI also requires additional sample preparation steps to that of immunohistochemistry, such as enzymatic digestion or chemical release of proteins and glycans [13]. Some common tissue conservation techniques, such as paraformaldehyde fixation followed by long-term storage, can result in incompatibility with MSI analysis [33]. Furthermore, in most instances these experiments are limited to qualitative assessments of analyte spatial distribution due to the influence of tissue-specific ion suppression unless isotopically labelled internal standards or specialised MALDI matrices are used [37]. For these reasons, MALDI-MSI is far from replacing immunohistochemistry as a primary diagnostic technique; however, it has established an important place within clinical research as a tool for novel biomarker discovery, tumour classification and staging, and treatment monitoring [38].

1.3.2. Relative and absolute quantification of biomarkers using MS

For clinical applications, a major caveat of soft ionisation techniques, including ESI and MALDI, is that some analytes will ionise more efficiently than others, meaning that absolute concentrations cannot be determined based on ion counts alone. Absolute quantification therefore requires the use of structurally similar or isotope-labelled internal standards, which may not be commercially available or economically viable for large scale clinical testing [16,34]. For example, the analysis of complex protein digests would require labelled peptides for each target, making multiplexed experiments expensive if commercially synthesised standards are used. Proteomics experiments can also introduce additional variability in preanalytical steps, such as enzymatic digestion, so ideally make use of labelled intact proteins and therefore require the availability of recombinant protein expression systems [39–41].

Alternatively, various approaches to generating labelled standards using simple chemical labelling reagents are available and enable relative quantification between samples using MS. These methods utilise common amino acid modification strategies (Table 1) to label either proteins or peptides with stable isotopes, hetero-elements (e.g. halogens), MS-cleavable tags and/or affinity handles [42]. Significant examples include the biotinylated isotope-coded affinity tag (ICAT) reagents for labelling peptides, amine-directed isotope-coded protein labelling (ICPL), and the isobaric tandem mass tag (TMT) and isobaric tag for relative and absolute quantitation (iTRAQ) systems [42–44]. Despite their obvious clinical utility, approval of these workflow for routine testing is impeded by their inherent variability and the absence of effective bioinformatics and data analysis platforms for interpreting the complex data they generate [42].

Absolute quantification can be achieved in a more straightforward manner using inductively coupled plasma (ICP)-MS, which involves atomisation of molecules using extremely high temperatures (7000–10,000 K) to detect hetero-elements (any element other than C, N, O and H) [12,45,46]. This ionisation technique is highly sensitive, has a wide dynamic range, and produces signals that are directly proportional to the sample concentration of a given element, irrespective of the solvent, analyte and sample matrix [42]. Hence, the technique has been useful for quantitative analysis of biomolecules with naturally occurring trace for phosphoproteins, metalloproteins, and selenoproteins [12,30,45]. However, quantification of intact complex biomolecules, such as peptides and antibodies, is also possible through chemical or metabolic labelling with hetero-element-containing reagents [42,45]. This technology has important implications for the implementation of ICP-MS and other MS platforms in clinical setting and is discussed in more detail in Sections 2.3.2 and 2.4.1.

1.3.3. Accessibility of MS in the clinical laboratory

Most MS experiments require specialist knowledge of correct sample preparation methods and instrumentation, in addition to dedicated software programs to acquire, analyse and interpret data [47]. Hence, despite their significant advantages, establishing robust clinical MS methods is often more complicated, expensive and/or time-consuming compared to conventional approaches, such as immunohistochemistry and ELISAs [47]. Due to these limitations, MS technologies are still underutilised in clinical settings in favour of immunochromical methods [19].

2. Combined immunochemical and mass spectrometric approaches to biomolecule analysis

Approaches combining immunoassay methodology with MS detection have been developed in efforts to overcome some of the individual limitations of these techniques. However, owing to their relatively high molecular weight and substantial heterogeneity, direct MS analysis of antibody–antigen complexes poses additional challenges [48,49]. ESI of intact complexes requires samples to be
prepared in volatile aqueous buffers to preserve non-covalent interactions; these conditions favour formation of high m/z ions and salt adducts, which reduces ion transmission and mass accuracy, and therefore sensitivity and selectivity [49]. Similarly, MALDI analysis requires specialised matrices or chemical cross-linking to analyse intact complexes and generates singly charged, high m/z ions with poor mass resolution [48,49]. To address these issues, several novel methods for detecting biomarkers have been proposed that involve chemically modifying antibodies to facilitate their analysis using standard MS instrumentation.

2.1. Immunoaffinity-based solutions in quantitative proteomics

Given the limitations of MS for direct analysis of complex mixtures, the features of immunoassays that enable isolation of analytes from biological samples offer opportunities for adapting MS detection to clinical sample analysis by improving selectivity and sensitivity. Various conjugation methods are available for immobilising antibodies on different solid supports (covered in detail in Section 2.2.1). These combined immunoaffinity capture-MS methods, or MS immunoassays (MSIs), utilise immobilised antibodies to enrich target proteins from complex biological samples and thus improve detection limits for low-abundance analytes. Similar technology has also been adapted to develop functional assays of biomarkers and therefore providing important insight into pathological biomolecule interactions and treatment monitoring [50,51].

2.1.1. MS immunoassays

The high specificity of MS makes it possible to evaluate different protein isoforms and PTMs, which typically cannot be differentiated by antibodies alone [30,52,53]. This concept was first demonstrated with MALDI, for the detection of snake venom proteins in human whole blood after enrichment using antibodies immobilised on agarose beads [54]. The MSI has since been developed into a pipette tip-based commercial platform (MSIA D.A.R.T.S™; Thermo Fisher Scientific, Waltham, US) compatible with ESI systems, and various iterations have now been used to quantitatively clinically relevant protein isoforms involved in insulin resistance, Alzheimer’s disease, renal function, endocrine function, lung cancer, and cardiovascular disease, among others [52,53,55–58]. This combination of immunoaffinity capture and MS detection can provide substantial improvements in sensitivity and specificity by exploiting the enrichment capacities of antibodies, while eliminating issues related to non-specific binding by using direct measurement of unique antigen m/z’s [59]. MSIA workflows are also applicable to both bottom-up and top-down proteomics analyses, the latter of which can identify novel protein isoforms without prior knowledge of the complete amino acid sequence and PTMs, therefore providing more complete sequence coverage [60–62].

Alternatively, the stable isotope standards and capture by antibody methods (SISCAPA; SISCAPA Assay Technologies, Washington DC, USA) platform enables multiplexed analysis of pre-digested protein samples using peptide-reactive antibodies immobilised onto capillary columns, which are incorporated into an autosampler LC system. Samples are spiked with isotopically labelled peptide standards before on-line washing and enrichment, hence retaining the high-throughput and quantitative capabilities of LCMS proteomics workflows [63,64]. In analogous immuno-MALDI (iMALDI) workflows, peptides are eluted from the capture antibody using an acidic MALDI matrix solution directly onto the MALDI target plate [61,65]. For these peptide-centric assays, the process for generating biomarker-reactive antibodies and isotope-labelled standards is relatively simple, compared to protein-centric MSIs [61].

2.1.2. Surface plasmon resonance MS

Surface plasmon resonance (SPR) is another technique that has evolved from a method for studying antibody-antigen interactions into a clinically relevant quantitative tool for detecting biomarkers [49,51]. SPR measurements are often a product of a continuous flow of analyte across immobilised antibodies on a metal surface, which induces a change in the surface’s refractive index for each instance of analyte binding. This technique has the advantages of being able to determine absolute analyte concentrations and binding kinetics from very small volumes at low sample concentrations. However, the mode of detection prevents delineation of antibody-antigen complex stoichiometry, protein variants, complexes, and non-specific binding [49,50]. Coupling this technology with subsequent MS analysis has since overcome these limitations. Moreover, the development of SPR imaging now enables direct multiplexed MALDI analysis of SPR chip arrays, without the need for recovery of analytes from the SPR surface. This approach not only improves the dynamic range of MS analysis, but also significantly reduces sample
preparation time, making it useful for detecting a range of human biomarkers, in addition to providing insight into their higher-order structure and function [50].

2.2. Bioconjugation strategies and challenges

The broad range of reactive chemical groups on antibodies makes them amenable to various conjugation methods (Fig. 1 and Table 1) [66]. In addition to conventional methods for modifying amino acids via free amines and carboxyl groups, modifications at selectively reduced cysteine residues and glycosylation sites are commonly used for functionalising antibodies. Unlike modifications to lysine and acidic amino residues, these site-directed modifications are less likely to lead to loss of activity from changes to the antigen-binding (Fab) region [66,67]. Alternatively, antibodies can be engineered to incorporate non-natural amino acids to allow site-specific conjugations [67]. However, this approach requires additional resources and expertise, whereas several amine and carboxylate targeting reagents are commercially available and can be used to modify validated antibodies. The ongoing development of new bioconjugation chemistries will likely lead to more simple and effective conjugation strategies for modifying antibodies and thus make them more appropriate for routine use in clinical settings.

2.3. MS detection of labelled antibodies for immunoassays

Given the limitations of common MS instrumentation for analysing intact antibodies, there is a growing interest in developing conjugation reagents designed to improve their ionisation and detection. Many of these approaches utilise labelling reagents that are cleaved at some point during MS analysis to release a fragment of known m/z, which is detected as a proxy for the intact antibody-biomarker complex [59,75]. These fragments, or mass tags, can be designed to fall within optimum m/z ranges, facilitate ionisation and undergo signal amplification to overcome limitations in dynamic range, poor ionisation, stability and sensitivity. Altering the chemical structure of the mass tag can also be used to generate different m/z values and therefore enable multiplexed analysis using different antibodies in the same sample [76].

2.3.1. MALDI mass tags

MALDI instruments have been popular choices for demonstrating mass tag concepts, because the most common ionisation methods involve excitation of samples using an ultraviolet (UV) laser; mass tag release can therefore be triggered by UV-induced cleavage of photodegradable linkers covalently linked to antibodies (Fig. 2) [73,76]. An added benefit of this approach is that fixed charges can be incorporated into mass tags (or formed from their photodegradation products), enabling matrix-free laser desorption/ionisation (LDI) and thus eliminating matrix background signals [76]. Antibody mass tags have been effectively used for the development of MALDI/LDI-MS ELISA and microarray platforms. These early examples demonstrate the two most prominent mass tag design strategies: ortho-nitrobenzyl derivatives that can be incorporated into solid-phase peptide syntheses; and triphenylmethyl (trityl) tags, which fragment into cationic reporter ions, which can be detected in the absence of a MALDI matrix [73,77,78]. Comparatively more work has focussed on the application of these concepts for specific MSI, which is discussed in detail in Section 2.4.2.

2.3.2. ICP-MS and mass cytometry

Another type of MS instrumentation that has gained popularity in clinical settings is ICP-MS; the extremely high resolution, sensitivity and dynamic range of these instruments are desirable features when designing clinical assays. The development of new antibody conjugation reagents provided a way to apply ICP-MS for detecting fragile antibody molecules by labelling them with metal ions that provided unique isotopic signals in mass spectra, called mass cytometry (Fig. 3) [12,79]. Mass cytometry involves the detection of heteroatom labelled antibodies attached to specific antigens on or within individual cells in a suspension (a detailed explanation and protocol for the generation of heavy-metal-labelled antibodies for mass cytometry has been provided by Nolan and co-workers [69]). The detection of multiple markers enables separation of cells in complex biological mixtures such as blood, providing quantitative measurements of diagnostic cell populations (Fig. 4) [46].

Even the earliest example of an ICP-MS-based immunoassay for thyroid stimulating hormone showed improved sensitivity, compared to radioimmunoassay results for the same clinical samples [72]. More recent examples have explored the use of different labelling methods and signal amplification strategies, such as conjugated nanoparticles or metal-chelating polymers, to develop sensitive multi-parameter sandwich ELISAs and microarrays [46,80]. This multiplexing ability and compatible antibody labelling strategies of ICP-MS ELISAs also make this detection platform ideally suited for improving the diagnostic capabilities of existing flow cytometry technology [12,46]. The most recent commercially available mass cytometers (CyTOF3; Fluidigm Corporation, South San Francisco, US) can detect up to 40 markers per cell, compared to typically less than 20 for fluorescence-based flow cytometry [30,46,81].

2.4. Targeted MSI and IHC-MSI

The continued clinical utilisation of IHC and other imaging techniques emphasises the importance of spatial information in modern diagnostic pathology [10]. Although this technique has evolved to complement a wide range of imaging platforms, the exploration of IHC as an adjunct to MSI, or antibody-based targeted MSI, only began recently [33,79]. Development of ICP-MS and MALDI-MS into imaging platforms has led to the exploration of several targeted MSI methods, which are analogous to mass cytometry and MALDI/LDI mass tag strategies, respectively. However, these methods provide additional spatial information, which would prove indispensable if MS detection were to replace traditional antibody detection methods in clinical settings.
2.4.1. Imaging mass cytometry

Imaging mass cytometry (IMC) applies the principles of mass cytometry in combination with a form of laser ablation ionisation to create raster images of metal ions from antibody conjugates (Fig. 5) [79]. The technology was originally used to demonstrate the potential of multiplexed IMC experiments as a revolutionary quantitative biomarker detection tool for breast cancer with subcellular spatial resolution [82]. This study was followed by an exploration of patient responses to trastuzumab treatment for breast cancer using IMC, which proves that this technique can also serve as an important prognostic tool, which could be used to inform treatment regimens [83]. Since, IMC has been applied to biomarker detection for an extensive range of human diseases, with a focus on applications involving immune cells, including the study tumour-immune cell interactions, autoimmune diseases and immunophenotyping [69].

2.4.2. Targeted MALDI/LDI imaging

Targeted MALDI/LDI-MSI employs similar principles to those of mass tag-based immunoassays, whereby low m/z fragments are
**Fig. 4.** Mass cytometry imaging of various antigens in normal human prostate tissue. Reprinted with permission from Ref. [79].

**Fig. 5.** Targeted matrix-assisted laser desorption/ionisation mass spectroscopy (MALDI-MS) has been used to localise carboxypeptidase D protein in rat brain tissue sections. MALDI spectra of fluorescein isothiocyanate (FITC)-labelled (a) and mass-tagged (b) secondary antibodies corresponding to MALDI-MS (c), photographic (d) and fluorescence (e,f) images showing colocalisation of the mass tag photocleavage product (P-PC) m/z with anti-carboxypeptidase D (CPD) antibody binding. Reprinted with permission from Ref. [84].
cleaved from labelled antibodies by a scanning MALDI laser, thus enabling localisation of antibody binding sites on tissue sections (Fig. 5) [33,70,84,85]. Because MALDI/LDI methods can detect non-metal mass tags, the predicted costs of generating a wide range of antibody conjugates could be significantly lower compared to IMC. However, a present limitation to their wider application is the requirement for individual chemical syntheses for each mass tag of different m/z [33,68]. An effective solution is to incorporate photocleavable groups into peptide mass tags, which can then be easily modified by changing the amino acid or nucleotide sequence of the cleaved fragment. This tactic has been demonstrated using both solid-phase and automated peptide synthesis for duplexed imaging of ovarian cancer biomarkers [78,84].

Alternatively, notable examples of targeted LDI-MSI have focused on signal-enhancement strategies, such as the utilisation of multiple mass tags conjugated to avidin. These conjugates enabled labelling of biotinylated antibodies to breast cancer antigens with a higher number of tags without disrupting antigen-binding activity [70]. In this system, horseradish peroxidase and alkaline phosphatase-conjugated streptavidin/avidin can also be used for dual light microscopy and MS imaging because these enzymes catalyse formation of both coloured precipitates and amplified m/z signals from conventional IHC substrates [88,70]. Dendrimer-based mass tags for amplifying LDI signals from an activity-based probe, which binds to a specific enzyme receptor, have also been reported. Although this amplification strategy has yet to be demonstrated for antibodies; however, the authors pre-empted the imminent investigation of click chemistry as a tool for mass tag conjugation and amplification [33,86].

2.5. Challenges and outlook for labelled antibodies in clinical MS

More recent examples of immunoassays with MS detection have aimed to develop low-cost, transportable platforms that can be used for point-of-care diagnostic tests. For example, fluorescent mass-tagged aptamers and conductive chips have been utilised for multiplexed sensitive detection of cancer antigens using ESI-MS [87]. Other examples make use of simple mass tags that hydrolyse under mild alkaline conditions have been used to detect malaria and cancer biomarkers via a degradable paper-based device and nano-ESI-MS [88]. Paper spray MS (PSMS) methods such as this have notable clinical utility as they can be used to analyse small volumes, such as pin-prick blood samples, using handheld mass spectrometers [89].

Meanwhile, significant advancements in MALDI-MSI instrumentation, such as improved spatial resolution for cellular and sub-cellular localisation, will hopefully lead to the eventual matching of MSI capabilities with that of fluorescence microscopy [90]. The coupling of MALDI sources to high resolution mass analysers and additional separation techniques, such as ion mobility, has enabled localisation of proteins with improved selectivity, which will likely be a useful feature for targeted multiplexed imaging applications [91,92]. A range of additional ambient ionisation techniques, which require minimal sample preparation and have already been utilised for cell and plasma-based immunoassays, allude to new possibilities for MSI as a means of rapidly detecting biomarkers in clinical samples [87,92,93].

While these new developments offer promising solutions as detection methods for multiplexed immunoassays and IHC, such platforms are still limited by the cost and availability of commercially available labelling reagents, which often necessitates individual custom syntheses [33,87,94]. And like conventional immunoassays, each antibody must be carefully validated and to ensure specificity for target antigens and appropriate sensitivity for the given detection method [95]. Traditional detection methods will therefore continue have an important role in the validation of potential antibody panels for MS biomarker detection. Importantly, much of the existing literature is focussed on method development and the demonstration of novel techniques, with fewer examples applying them to broader clinical applications or aiming to establish standardised workflows [33,96,97]. As such, there is a need for larger translational and preclinical studies to establish practical and reproducible ways of utilizing these approaches as viable diagnostic tools.

3. Summary

Experienced pathologists can easily diagnose many important human diseases using basic histological techniques [31]. Hence, the development of MS methodology for clinical applications should be focused on conditions for which accurate clinical assessment requires greater specificity and/or multiplexed analysis. The potential for MS technology to detect biomolecules that are not amenable to immunohistochemical detection and analyse multiple biomarkers simultaneously makes it an ideal platform for diagnosing multifactorial diseases that have proven challenging using conventional approaches. However, the inherent limitations of common MS detection methods for complex biological samples preclude their wider clinical adoption. Hyphenated immunochmical-MS approaches have offered promising solutions to some of these limitations, such as increased sensitivity, specificity, dynamic range, in addition to facilitating affordable and quantitative multiplexed analyses.

There is a growing emphasis on delivering personalised medicine, which acknowledges the multitude of confounding factors-genetic, environmental or otherwise—that can influence patients’ individual risks of developing a disease and responses to treatment [98]. Diagnostic tests that integrate different technologies and evaluate multiple biomarkers will be required to facilitate improved clinical outcomes for a range of human diseases [99]. Of these, multiplexed MSIA technologies offer the potential for improved diagnostic performance, as such tests will not be reliant on a single marker or proteoform and therefore more effective for assessing diseases with high patient-to-patient variability. Likewise, the simultaneous quantification and localisation of low-abundance biomarkers, which is difficult using IHC methods based on staining intensities or cell counts, is practically feasible using MSI and is increasingly recognised as an important tool in clinical research [30,100].

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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