

Mini Review

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Mass spectrometry in the clinical laboratory. A short journey through the contribution to the scientific literature by *CCLM*

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Abstract: Mass spectrometry (MS) has been a gold standard in the clinical laboratory for decades. Although historically refined to limited areas of study such as neonatal screening and steroid analysis, technological advancements in the field have resulted in MS becoming more powerful, versatile, and user-friendly than ever before. As such, the potential for the technique in clinical chemistry has exploded. The past two decades have seen advancements in biomarker detection for disease diagnostics, new methods for protein measurement, improved methodologies for reliable therapeutic drug monitoring, and novel technologies for automation and high throughput. Throughout this time, *Clinical Chemistry and Laboratory Medicine* has embraced the rapidly developing field of mass spectrometry, endeavoring to highlight the latest techniques and applications that have the potential to revolutionize clinical testing. This mini review will highlight a selection of these critical contributions to the field.

Keywords: GC-MS; LC-MS; mass spectrometry.

Introduction

Since its launch in 1963, *Clinical Chemistry and Laboratory Medicine* (*CCLM*), then known as *Zeitschrift für Klinische Chemie*, has driven the application of modern technologies and methods in laboratory medicine. Mass spectrometry (MS) has played a significant role in the clinical laboratory for

decades, providing a robust means to identify and quantify compounds in human biological samples. As analytical technologies have developed, the potential for MS in this field has expanded. This is evident in the increased use and discussion of mass spectrometry in *CCLM*, which has seen a 12-fold increase over the last 20 years of publications (based on a PubMed search of articles containing the phrase “mass spectrometry” from 1999 to 2001 and 2019–2021, search performed in August 2022, Figure 1).

Since the journal’s rebranding to *CCLM* in 1998, approximately 500 research articles have been published in the journal which discuss the topic of clinical MS. A proportion of these contributions to the field were highlighted in the 2020 special issue “Advancements in mass spectrometry as a tool for clinical analysis” [1, 2], the journal’s first dedicated issue to the use of mass spectrometry in clinical and laboratory medicine. The two-part special highlighted the latest trends and developments, covering the use of MS in therapeutic drug monitoring, dried blood spot analysis, protein measurement and MS-imaging, amongst other important fields in laboratory medicine (Figure 2). Indeed, one review article also included a basic introduction to mass spectrometry aimed at the non-expert to aid translation to clinical professionals [3]. Overall, the issue was a snapshot of the exciting and ever-expanding avenues of clinical research that can now be exploited through the use of mass spectrometry.

This mini review will explore a selection of areas of clinical chemistry in which *CCLM* has demonstrated the exciting potential for mass spectrometric techniques, through novel research, thoughtful reviews, and insightful discussion on what future technologies may yield and what developments are still required.

Automation and high-throughput analysis

A primary roadblock in the widespread use of mass spectrometry in clinical laboratories has been the limited

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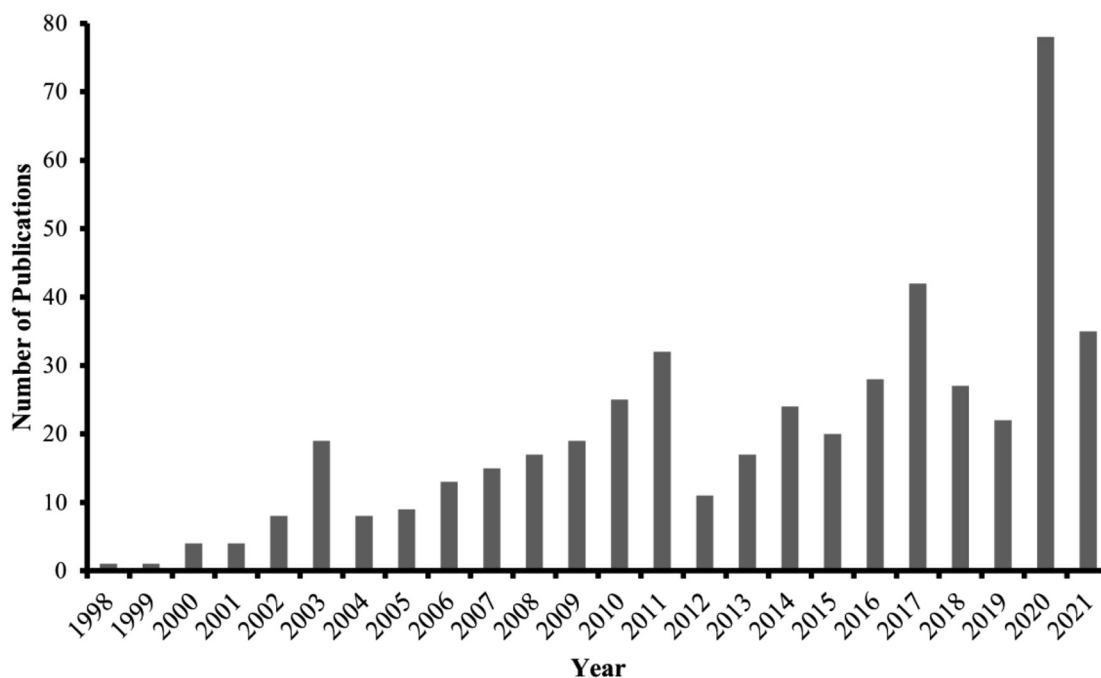


Figure 1: Number of publications mentioning the term “mass spectrometry” in *CCLM* from 1998 to 2021.

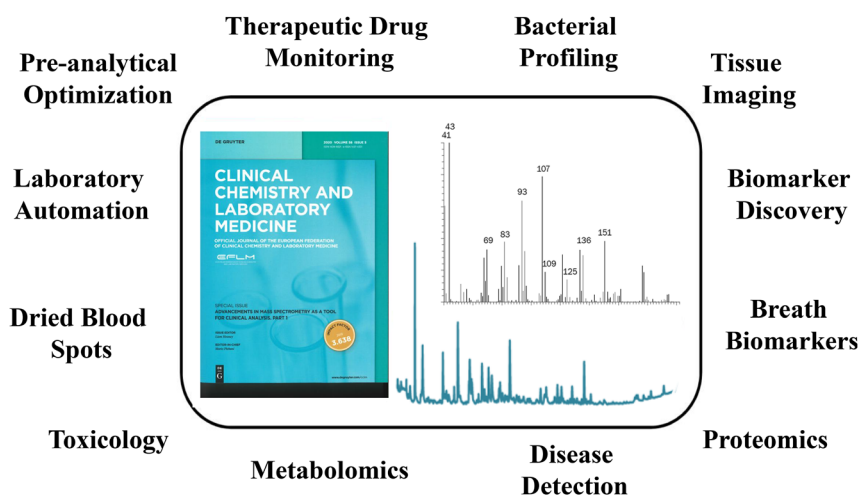


Figure 2: A graphical overview of topics published in the 2020 *CCLM* special issue focused on clinical mass spectrometry.

throughput of such techniques. The preparation of samples prior to analysis can often involve multiple extraction and cleanup steps, necessitating laborious manual processes. Furthermore, conventional hyphenated MS systems such as liquid and gas chromatography-mass spectrometry (LC-MS and GC-MS, respectively), can have analysis times of up to an hour per sample, severely limiting the number of samples that can be processed on a daily basis. In recent years, there has been a notable drive in the improvement of mass spectrometry techniques to reduce or even eliminate sample preparation, decrease chromatographic run times, and automate processes.

Many clinical MS assays require the use of solid phase extraction (SPE) to concentrate and clean-up biological samples prior to analysis by MS. This is typically a manual process necessitating significant time and manpower, whilst introducing the potential for operator error. In recent years, technology has developed enabling the online coupling of SPE with the LC-MS instrumentation, reducing analysis time and requiring less manual sample preparation. An increased number of laboratories are now utilizing this technology. Savolainen et al. automated an online solid phase extraction LC-MS/MS method for the detection of serum testosterone, demonstrating a significantly faster

analysis time and higher precision due to the automation [4]. Similarly, Vogeser et al. used this approach for the rapid analysis of antimycotic drugs in plasma [5] and the detection of methylmalonic in urine [6]. The development of online extraction processes not only reduces manual labor time, thus freeing up analysts to perform other tasks, but also reduces the potential for human error.

The need to reduce sample preparation and analysis times is not the only limiting factor in the use of MS in clinical laboratories. In order for MS to become a regular fixture in such laboratories, there is a need for the development of automated systems that can be readily operated by laboratory technicians, including staff with little or no experience in MS. In 2020, Benton et al. demonstrated the first use of a fully automated clinical analysis system for the measurement of 25-hydroxy vitamin D in serum, which typically requires a laborious assay [7]. The Cascadion™ SM Clinical Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) was used in this study, an instrument designed to enable the simple implementation of LC-MS/MS capabilities into clinical laboratories. The Cascadion™ combines a liquid handling system for mixing and dispensing whole blood samples followed by centrifugation with direct transfer of the supernatant into the LC-MS/MS for analysis, providing an entirely automated analytical process. The system was installed in a hospital laboratory in which staff had no prior experience of LC-MS/MS. After a brief training period, laboratory staff were able to readily analyze 675 samples in a 24 h period, demonstrating the impressive throughput of the system even when operated by users with minimal experience. The following year, Hörber et al. also evaluated the potential power of the Cascadion™ system in clinical laboratories [8]. In this study, they demonstrated the first evaluation of a fully automated LC-MS/MS method for the analysis of immunosuppressive drugs in blood for therapeutic drug monitoring. As in the Benton study, the system was introduced into a hospital laboratory with no existing LC-MS capabilities or experience. The importance of these studies lies in the implementation of the research. They did not simply demonstrate new methods that could one day be useful in medical laboratories, but rather evaluated the incorporation of these novel systems into real-world clinical laboratories in which staff members are not necessarily highly trained mass spectrometrists. This translates MS from being a specialist instrument to a routine piece of the clinical laboratory puzzle.

Applications of mass spectrometry in the clinical laboratory are primarily GC-MS and LC-MS/MS methods. However over the past decade significant progress has

been made in the development of direct mass spectrometry techniques, eliminating the need for potentially time-consuming liquid chromatography methods. Ambient ionization mass spectrometry (AIMS) techniques enable the direct, preparation-free analysis of samples, resulting in the detection of target analytes in a matter of seconds. A recent study by Skaggs et al. used paper spray ionization mass spectrometry (PSI-MS) for the detection of triazoles, used to treat fungal diseases, in plasma samples [9]. PSI is a relatively new analytical technique which involves the application of the sample to a triangular piece of paper followed by the application of a high voltage, resulting in the near-instant ionization and detection of sample analytes. The method demonstrated reasonable agreement with a standard LC-MS/MS method, in addition to numerous advantages including reduced sample preparation, higher throughput, and lower solvent consumption, thus reducing costs and waste. To date, the use of AIMS in clinical work has been primarily confined to research, but that research has made it clear that AIMS has the potential to play a major role in improving the efficiency of clinical testing.

Therapeutic drug monitoring

Due to intra- and inter-individual pharmacokinetic variability, therapeutic drug monitoring is sometimes required to ensure drug dosages are both sufficient and safe. Furthermore, measuring drug levels in the body can also be used to ensure drug adherence. Mass spectrometry is an analytical technique ideally suited for this testing approach. Therapeutic drug monitoring is particularly important in the case of transplant patients to ensure appropriate levels of immunosuppressants to prevent organ rejection. As such, numerous efforts have been made to develop techniques for the robust and rapid measurement of relevant therapeutics in biological fluids.

Vogeser and Spöhrer developed the first automated LC-MS/MS method for the direct processing of large numbers of whole blood samples, with a focus on the detection and quantification of immunosuppressants [10]. The robust and cost-effective method enabled the rapid analysis of 70 samples per day, minimizing hands-on operator time in the process. Another study conducted repeated blood sampling of kidney transplant patients over an 8-h period using an intravenous microdialysis method, comparing the performance of an LC-MS method with a point-of-care optical immunosensor chip [11]. The study showed the combined approach of testing novel point-of-

care devices with robust LC-MS methods to work towards the development of a continuous real-time monitoring system. Therapeutic drug monitoring methods can also be applied to evaluate levels of antibiotics in patients, with appropriate dosages of these drugs essential for the treatment of critically ill patients with infections. Zander et al. developed a reliable 2D-UHPLC-MS/MS method for the detection of several common antibiotics in serum [12]. The method employed a semi-automated sample preparation process to reduce hands-on time for the analyst and enabled the targeted simultaneous analysis of antibiotics of different classes.

A particular focus of these studies has been the development of high-throughput techniques for therapeutic drug monitoring, in part due to the need for drug level measurements as close to real-time as possible. In the event that a patient has received an inappropriate dose of a drug, either due to medical error or patient-specific pharmacokinetic variations, the timely detection of such incorrect dosages is of the utmost importance. The vast number of studies in this area of research highlights the drive to develop faster and simpler techniques for drug monitoring.

Dried blood spots

Clinical mass spectrometry methods are readily applied to a variety of sample types, however dried blood spots (DBS) are amongst the most common, particularly in neonatal screening. DBS are collected by applying a small fingerprick (or heel prick for newborn babies) of patient blood to a card or filter paper, allowing the sample to be quickly collected, transported, and stored until needed for analysis.

Wiesinger et al. evaluated the suitability of high-resolution accurate mass MS (HRAM-MS) for the detection of hemoglobinopathies and β -thalassemias in DBS as an alternative to other well-established methods [13]. The study demonstrated HRAM to be an advantageous alternative with minimal sample preparation and a rapid analysis time of 2 min per sample. One study performed a small-scale inter-laboratory comparison of an assay for the detection of Lyso-Gb3 in DBS, an analyte used in the diagnosis of Fabry disease [14]. The results showed significant disagreement between different laboratories, highlighting the need for clinicians to exercise caution when making decisions based on certain DBS tests from multiple locations. Polo et al. developed a novel LC-MS/MS method for the detection of DBS lysosphingolipids (lysoSLs), which are potential biomarkers for several genetic diseases [15]. LysoSL detection is typically performed using plasma samples, however

by extracting DBS and performing LC-MS/MS, comparable results to the gold standard plasma technique could be achieved. This offers an advantageous alternative approach to testing for certain rare diseases.

Despite a long history of DBS use in a clinical setting, significant efforts are still focused on not only the expansion of testing to different diseases, but also evaluating weaknesses in existing assays and improving robustness. Zhuang et al. provided a critical evaluation of DBS studies and methodologies [16], with a particular focus on multi-omics studies and the advantages and weaknesses of different stages of DBS collection, storage and analysis. Some studies have included evaluations of the effects of different temperature and storage periods on the stability of specific analytes in DBS [17, 18]. Such studies aim to establish the appropriate pre-analytical conditions for samples prior to analysis and set standards to ensure accurate quantification of analytes. Winter et al. determined the effects of common contaminants on stored DBS (such as baby cleaning products), demonstrating that the presence of many contaminants significantly altered target analyte concentrations, potentially leading to false positives or negatives [19]. Finally, Veenhoff et al. developed a web-based app to evaluate the quality of a newly collected DBS [20]. DBS samples will be rejected at the point of analysis if they do not meet certain criteria relating to the size of the spot and how it is collected on the sampling card. By developing software capable of establishing 'rejects' at the point of collection, the operator can simply collect additional samples and eliminate further delays. As an alternative to DBS sampling, volumetric absorptive micro sampling (VAMS) has also been considered as a low-volume, minimally invasive technique that can be performed in the clinic or at the home of the patient. Canisius et al. evaluated the use of VAMS for the measurement of anti-epileptic drugs (AEDs) in whole blood, validating the method against a standard LC-MS/MS method [21]. It was demonstrated that VAMS could be a viable alternative approach, with the method demonstrating good correlation with results obtained by DBS sampling for 16 different AEDs.

Protein analysis

The quantification of protein levels in clinical analysis has traditionally been performed using immunoassays and turbidimetric measurements. However many conventional assays can be non-specific, prone to interference from contaminants, and targeted to individual analytes [22]. As such, mass spectrometry has the potential to be an

attractive alternative technique for clinical protein analysis, as demonstrated by numerous studies over the past few years. The implementation of new technology for clinical applications requires robust testing and validation, and there is a clear drive in the clinical mass spectrometry community to achieve this. Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF/MS) is one such technique that has been considered as a potential tool in clinical protein analysis.

In 2005, Bons et al. produced a thorough review on protein profiling as a diagnostic tool for clinical laboratories, with a particular focus on the potential of SELDI-MS for serum protein profiling [23]. They collated studies that have evaluated the effects of numerous steps throughout the analytical workflow, including pre-analytical considerations, sample processing steps, and data interpretation. Aivado et al. discussed the potential for SELDI-MS to be used for routine protein analysis, but highlighted areas of the analysis that must be first validated [24]. They adapted the ProteinChip™ (Ciphergen Biosystems, Fremont, CA, USA) process to an automated liquid-handling robotic system, incorporating a 192-well bioprocessor. Using this system, they investigated several critical factors including reproducibility, sensitivity, inter-assay variation, and the effects of different experimental parameters on measurements, ultimately establishing some suggestions for best practice when performing protein analysis with SELDI-MS. A few years later, SELDI was evaluated for the purpose of saliva protein analysis [25]. Saliva is an attractive sample matrix for clinical analysis, primarily due to the low invasiveness of the collection, making it a potentially ideal sample type for biomarker detection and diagnostics. The study evaluated several crucial factors including the impact of storage time, sample preparation, and freeze/thaw cycles on the protein content of saliva, and furthermore examined inter-individual differences in the saliva proteome, demonstrating significant sex-specific differences. In 2011, Liu et al. published a fascinating study on the use of SELDI-MS to identify biomarkers for tuberculosis (TB) diagnostics [26]. In studying the serum protein profiles of healthy individuals, TB patients, and people with other lung diseases, they were able to detect 30 protein features that were related to TB. Given further work to identify these components and further validate the method, the study demonstrates the exciting potential for SELDI-MS as a tool for diagnostics.

Other mass spectrometry-based methods have also been evaluated for their potential use as alternative tools in clinical protein analysis. Jiang et al. developed an inductively coupled plasma-mass spectrometry (ICP-MS) method combined with an element-tagged immunoassay [27]. The

technique was applied to the analysis of carcinoembryonic antigen, an important biomarker of several types of cancer, particularly colorectal cancer. Encouragingly, the assay performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, highlighting another potential player in clinical mass spectrometry for the detection of cancer biomarkers. The same year, Cradic et al. developed and validated a LC-HRAM-MS method for the quantification of Vedolizumab, a therapeutic monoclonal antibody used for the treatment of Crohn's disease and ulcerative colitis [28]. This middle-up protein subunit detection and quantification approach demonstrated the suitability of this technique for Vedolizumab detection, meeting the required pre-defined criteria, and also highlighted the potential to extend the approach to other proteins. Finally, Israr et al. published a detailed review of the use of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) in clinical laboratories, with a heavy focus on peptide and protein analysis [29]. The work detailed the fundamental principles of MALDI-MS, the current clinical applications of the technique, and opinions on the future potential for MALDI-MS, particularly in disease diagnostics, therapeutic drug monitoring, and tissue imaging.

Exhaled breath

The use of exhaled breath and exhaled breath condensate (EBC) as clinical samples present significant advantages over typical testing matrices. The collection of a breath sample is a substantially less invasive procedure for the patient, simply requiring the patient to breathe into a collection device (such as a Tedlar bag or breathing mask), rather than being subjected to invasive intravenous blood draws or having to collect urine samples. The premise of exhaled breath analysis in clinical testing lies on the fact that small, volatile, molecules pass from the pulmonary blood supply into the alveoli and are then ejected from the body during exhalation. These molecules may be directly (or indirectly) related to metabolic processes associated with disease. As such an increased number of clinical mass spectrometry studies are focusing on the use of breath as a potential alternative for the detection of biomarkers of clinical interest, though the analysis of exhaled breath has been largely confined to the realms of research as opposed to real clinical testing.

Exhaled breath and EBC have been the focus of several studies in *CCLM* in recent years. The use of exhaled breath for the detection of cancer biomarkers is of particular interest. Ligor et al. used GC-MS to perform a pilot screen on exhaled breath of 65 lung cancer patients and 31 healthy

individuals [30]. A total of 80 VOCs were unique to lung cancer patients, with eight of those being selected as showing diagnostic utility. A later study used the same technique to study the breath of a slightly larger cohort of lung cancer patients in addition to VOCs produced by lung cancer tissues, interestingly identifying two of the same lung cancer biomarkers as the Ligor study (1-propanol and 2-butanone) [31]. Although a primary focus of exhaled breath analysis is for disease diagnostics, there are also potential applications in clinical and forensic toxicology. As the use of cannabinoids becomes more common and, in some places, legal, there is a need for the development of rapid and robust clinical tests to measure cannabinoids such as tetrahydrocannabinol (THC), particularly in relation to drug users driving under the influence. A recent study developed and validated a method to detect THC and related molecules in the exhaled breath of participants shortly after smoking cannabis [32]. Samples were captured using the Sensabues exhaled breath sampling device and subsequently extracted and analyzed by LC-MS, ultimately demonstrating the potential to detect THC in exhaled breath.

In 2013, Ahmadzai et al. provided a comprehensive review on the current state of exhaled breath condensate research [33]. The primary focus of the review was on the limitations of existing EBC analysis techniques, factors affecting method robustness, and the need for standardization of procedures before EBC analysis could be employed in hospital laboratories. Furthermore, the article provided an extensive overview of prior EBC research and common biomarkers of interest that can be detected using this sample matrix. In an earlier study, Kurova et al. highlighted the lack of standardization in EBC proteomics and performed a comparison of different methods to establish best practice for protein extraction and analysis [34]. The study provided a baseline for the healthy EBC proteome and discussed the advantages and pitfalls of different approaches.

The analysis of samples derived from exhaled breath is not yet widely performed in laboratory medicine, despite a great deal of research conducted to develop methods for breath analysis and demonstrate clinical applications. Nonetheless, there is a clear drive to develop the techniques further to be able to one day translate this analysis to the clinic, offering the potential to vastly advance clinical diagnostics.

Conclusions

In recent years, *CCLM* has driven the introduction and implementation of mass spectrometry in the clinical

laboratory. Through insightful discussions on the future of MS in clinical testing, introducing new technological advances, and highlighting novel applications, the current and potential role of MS in the clinical laboratory is evident. Research has focused on the reduction in analysis times to increase sample throughput, the automation of processes to reduce hands-on analyst time, and the development of techniques that can be readily used by the non-expert to make mass spectrometry more readily accepted by traditional clinical laboratories. In all, this research paves the way for modern MS technologies to be truly incorporated into medical laboratories, advancing our clinical capabilities. It is with excitement that we await the next generation of MS-based clinical research provided to the readership of this highly respected journal.

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