Chapter

Mass Spectrometry in Clinical Laboratories

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Abstract

The analyses performed in clinical laboratories require a high level of precision, selectivity, and sensitivity. The rising number of therapeutic agents from both the field of small and large molecules and the increasing use of modern screening approaches have brought mass spectrometry into almost every clinical laboratory. The need to screen the patients and to follow the therapy's success can often be fulfilled only by the highly selective and sensitive targeted approach with mass spectrometry. With improving instrument design and miniaturization of the separation technologies, mass spectrometry is no longer an exotic analytical approach. The use of mass spectrometry is now not restricted to the use in a clinical laboratory, but it is used in operating rooms for instant and on-site helping the surgeons with defining the margin of the tissue to be extracted. In this manuscript, we describe the use of mass spectrometry for selected clinical applications and show the possible way of future applications.

Keywords: Clinical laboratory, antibiotics, newborn screening, mass spectrometry

1. Introduction

The use of mass spectrometry in the clinical laboratory has become a standard for analysis of different substances such as antibiotics, for newborn screening, detection of immune-suppressive drugs, or the analysis of therapeutic antibodies used for the treatment of different diseases.

The focus of the use of mass spectrometry in clinical settings is the analysis of clinical samples and monitoring levels of active compounds and their metabolites in patients' blood and urine samples. The high sensitivity and specificity of the mass spectrometer and the possibility to perform specific detection of target analytes by applying MRM/SRM (multiple reaction monitoring/selected reaction monitoring) enable a targeted and highly specific analytical approach. The methods developed need a separation method in front of the MS and several companies such as Chromsystems (https://www.chromsystems.com/), ThermoFisher Scientific (https://www.thermofisher.com/at/en/home/clinical/diagnostic-testing/clinical-chemistry-drug-toxicology-testing/therapeutic-drug-monitoring.html) or BioRad (www.bio-rad.com), to name just a few, have developed fully verified and certified analytical systems. The interested reader is encouraged to search the internet for additional providers and systems.

Applying chromatography and mass spectrometry has its primary values in relatively fast detection and measuring of multiple analytes in a single sample with high sensitivity and high selectivity. In clinical routine, the key challenge for identifying

and analyzing active compounds is having the sensitivity of the analytical system needed and required to detect and quantify low-concentration analytes.

One of the challenges for using the MS in a clinical laboratory was the low ion yield, which significantly hampered the development of clinical applications. However, the development of new analytical systems, especially of new ion inlets and ion funnel designs with the most widely used electrospray ionization (ESI) sources has significantly improved ion focusing and ion transfer, which, finally, resulted in the overall increased sensitivity.

The quality of electrospray is highly dependable on separation conditions, i.e. mobile phase, presence or absence of salts, flow speed, column's inner diameter, etc. In proteomics, the use of columns with 50 μm or 75 μm ID is state-of-the-art. However, the columns operated at a low flow rate of several hundreds of nanoliters/minute are still rare in clinical analysis although they can provide a significant increase in analysis's sensitivity.

However, currently, the use of nanoflow separation still cannot cope with the demand for high sample throughput in clinical applications. Currently, the closest compromise between sensitivity and throughput is the use of the microbore and capillary columns of 300 μm – 500 μm and 1 mm – 2 mm inner diameter.

A new and exciting application of mass spectrometry in the clinical environment is the use of "live-MS" during surgical operations. Further development of this approach will revolutionize the diagnostics and help surgeons in extracting e.g. tumors with higher accuracy and higher yield.

2. Clinical applications

2.1 Analysis of antibiotics

Antibiotics, either cytotoxic or cytostatic to the microorganisms, have been widely used to treat and prevent infectious diseases and allow the body's natural defenses to eliminate them. They usually have a role to inhibit the synthesis of proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or other specific actions [1]. Using the energy-dependent transport mechanisms in ribosomal sites, antibiotics target bacterial cell wall by attaching to them, which consequently results in inhibition of protein synthesis and subsequent cell death [2].

With the discovery of penicillin by Sir Alexander Fleming, a new, modern, chapter of innovation and antibiotics development began [3]. Today, there are different classes of antibiotics (**Table 1**) and they are widely used not only in human medicine but also in veterinary medicine and aquaculture [35]. However, antibiotics inadvertently released into the environment can cause a massive threat to the ecosystems and subsequently to human health. Consequently, they may accumulate in food and, which is much more worrying, antibiotic resistance of human pathogens might develop through the transfer of environmental bacteria genes (ARGs) [36–40]. Furthermore, sensitive individuals might experience allergic reactions triggered by antibiotic residues in food. Furthermore, the ingestion of subtherapeutic doses of antibiotics and uncontrolled use of antibiotics may initiate the development of drug-resistant strains of bacteria that initially appeared in hospitals only, where most antibiotics were being used [41].

In recent years, more and more scientific data and news reporting the misuse and the overuse of antibiotics [42, 43], the environment exposure pathways [44, 45], and the presence of antibiotic-resistant strains [46, 47] became available.

Over the years, numerous analytical methods have been developed and described to determine antibiotic residues in the environment and food.

Different classes of antibiotics	References
β-lactam	[4–6]
Sulfonamides	[7–9]
Aminoglycosides	[10–12]
Tetracyclines	[13, 14]
Chloramphenicol	[15, 16]
Macrolides	[17–19]
Glycopeptides	[20–22]
Oxazolidinones	[23, 24]
Ansamycins	[25, 26]
Quinolones	[27, 28]
Streptogramins	[29, 30]
Lipopeptides	[31, 32]
Antibiotic Resistance	[33, 34]

Table 1.
Overview of different classes of antibiotics and some related references describing their mass spectrometry analysis.

Chromatographic separation and detection of antibiotics and their metabolites using various detectors is the most widely used analytical approach for monitoring and determination.

The electrospray ionization technique has become the technique of choice in many areas of analyzing biologically relevant macromolecules [48]. The soft ionization MS techniques - matrix-assisted laser desorption/ionization (MALDI) and ESI [49, 50] proved to be the best approach for analysis due to the efficient ionization of polar antibiotics. Depending on the ionization mode applied, it has been shown that most antibiotics yield a better signal when positive ionization is used, with the most commonly formed protonated molecular ion $[M + H]^+$. Determination of analytes trace levels in complex biological matrices using the molecular ion generally is not enough selective due to the limited resolution of unit-mass MS instruments. Therefore, these obstacles are overcome by using modern equipment consisting of liquid chromatography (LC) MS instrumentation with tandem MS (MS/MS), which became the technique of choice in quantitative bioanalysis. Tandem MS capacities enhance selectivity and signal-to-noise ratio and provide essential structural information based on which it is possible to identify the structural conformation of analyzed samples. For these reasons, many laboratories use triple quadrupole (Q) MS/MS over ion trap (IT) MS instruments in routine practice for detection and analysis of antibiotics and other drug residues [51–55]. This advantage is reflected in its quantitative features regarding IT MS with its MS^n capabilities, which are highly beneficial for analysis of analyte's molecular structure and identification.

With technological advancement, instruments providing accurate-mass, high-resolution (HR) time-of-flight (TOF) MS, single TOF-MS, or hybrid instruments combined with a quadrupole (Q-TOF-MS) and the collision cell for MS/MS analysis became available. HR-MS has entered every day's practice of clinical laboratories as a viable alternative to traditional triple quadrupole mass spectrometer. The versatility of HR-MS (especially hybrid HR-MS) is reflected in increased selectivity by eliminating potential interferences originating from the matrix with remarkably similar mass-to-charge ratio (m/z) as of the measured analytes, but with a different structure.

Unlike IT and Q MS, TOF-MS is a pulsed, and a non-scanning MS. TOF-MS can acquire full spectral data, thus separates and detects ions of various m/z by measuring the time taken for the ions to travel through a field-free region. Therefore, these instruments are mostly combined with a fast LC separation if used for rapid non-targeted screening [56].

Based on all the above-mentioned, LC–MS is an essential factor in the pharmaceutical industry and clinical laboratory due to the possibility of identifying impurities in synthetic products, characterize metabolites, and perform quantitative bioanalysis.

The following section of this chapter provides an overview of examples of mass spectrometry usage in clinical laboratories for detection and characterization of antibiotics in a different sample including pharmaceutical, blood (plasma, serum), environmental water samples (waste, surface, and drinking water) [57–62], animal and plants and products of animal and plant origin, etc. [63–65].

Depending on sample matrices such as muscle, liver, kidney, egg, milk, or honey, multiclass methods based on LC–MS or LC–MS/MS are used for the analysis of antibiotics residues [66–69]. The complexity of the methods depends also on the complexity of the sample preparation. Therefore, screening methods try to avoid complicated sample preparation such as solid-phase extraction (SPE) and the evaporation of the purified extract before the chromatographic separation whereas quantitative methods do not bypass this step [70]. Chico et al. [71] analyzed 39 analytes residues that belong to 5 families of antibiotics with different physicochemical properties which include sulfonamides (SAs), quinolones (Qs), tetracyclines (TCs), macrolides (MCs), and penicillins (PCs). To shorten the analysis time, their method set-up was based on ultra-high-pressure liquid chromatography (UHPLC), like in Yamaguchi et al. [72] and Tian et al. [73], combined with tandem mass spectrometry–MS/MS with ESI. Mass spectrometry parameters were determined and optimized by an infusion of standard solutions to accomplish the highest sensitivity. The singly protonated molecular ion was selected and used as the precursor ion for all compounds $[M + H]^+$, and the cone voltage was adjusted to its maximum signal at the first quadrupole of the mass spectrometer. The success of this method proved to be exceptional and, for that reason, was introduced as a method at the laboratory of Agència de la Salut Pública de Barcelona.

Several analytical methods are currently available to separately detect the fluoroquinolone and sulfonamide classes of antibiotics in manure, surface water, wastewater, and groundwater [74–76]. Haller et al. [77] focused on liquid–liquid extraction followed by LC–MS analysis of veterinary antibiotics (sulfonamides and trimethoprim), which are most commonly being leaked into the aquatic environment. Based on published LC–MS methods for sulfonamides separation on a reversed-phase chromatographic column, ammonium acetate buffered water and acetonitrile were used as mobile phases. The most successful baseline separation was achieved using the buffered mobile phases at pH 4.6, which enables more stable retention times and better peak shapes for almost all analyzed analytes due to their pKa. Analytes appear to be more hydrophobic and retain better on an RP HPLC column. Haller et al. [77] acquired SIM mass spectra of all samples (antibiotics and of the internal standard) in the full scan mode, using positive and negative electrospray ionization. Single-protonated $[M + H]^+$ or the $[M–H]^–$, and several (two to three) additional fragments that were generated through the in-source fragmentation, which is typical for single quadrupole mass spectrometer and that yielded the best signal-to-noise (S/N) ratios were selected for confirmation. The advantage of this method is multiple: a very simple extraction process was applied, thus sample preparation is faster, the method does not require tandem mass spectrometry, the

method is capable of detecting the investigated pharmaceuticals, to determine the half-lives of antibiotics in manure slurry, and to establish mass balances from antibiotic contents in medicinal feed to quantities.

Renew et al. [74] analyzed groups of antibiotics (fluoroquinolones, sulfonamides, and trimethoprim) simultaneously at sub micrograms per liter concentrations in wastewater effluents using readily available LC–MS techniques. Quantification and identification were performed by applying fluorescence detection and additionally confirmed by tandem LC–MS.

Following Chico et al. SPE followed by LC–MS analysis is utilized by this method. Hirsch et al. [78] and Hartig et al. [79] developed LC–MS techniques for sulfonamides detection. The application of this method allowed preliminary determination of the occurrence of these antibiotics in municipal wastewater treatment plants. Usually, normal phase chromatography (NPC), which implied that the use of a polar stationary phase, was used for the LC separation. However, the NP stationary phases usually show large heterogeneity, which was also observed in this experiment as a consequence of peak tailing and non-linear retention factors with varying analyte concentrations [80]. Different solvents were used to accomplish elution in NPC, from non-polar organic to some variants like the use of isohydric solvents [81, 82]. Some obstacles like lack of retention of highly hydrophilic compounds with ionizable functional groups have been exceeded by ion-exchange chromatography [83] or ion pairing on reversed-phase (RP) columns [84]. However, for those analytes with high hydrophilicity the problem has been overcome using hydrophilic interaction chromatography (HILIC). In contrast to the RP LC, the gradient elution in HILIC starts with a low-polarity, low acquoeus organic solvent and elutes polar analytes by increasing the polar content. In addition, in HILIC, ion pair reagents are not required, and the separation system can be easily coupled to MS, especially in the ESI mode [85].

A large topic opens when it comes to antibiotic treatment, as well as establishing resistance to them. MALDI-TOF [33, 86] technique is an ionization technique that allows the analysis of biomolecules and is used to monitor antibiotic treatment as well as rapid detection of antibiotic resistance. The feasibility of MALDI-TOF MS identification of bacterial colonies from solid media has been evaluated on a wide range of clinically relevant bacterial strains as well as yeast isolates.

MALDI-TOF MS whole-cell extracts identification represents a new method for obtaining a characteristic bacterial fingerprint, which allows for distinction of microorganisms based on different genera, species, and from different strains of the same species. The advantages of using this method are numerous: identification can be achieved in a short time after culture isolation, sensitivity is high, ability to detect microorganisms is not limited to prespecified targets, mass spectra obtained for unknown microorganisms are compared with reference database to achieve the identification. Therefore, MALDI-TOF MS represents a reliable method for rapid bacteria and fungi identification in a clinical setting.

The biggest global challenge due to growth rates of multi-drug-resistant microorganisms, especially in hospital settings, introduces new analytical methods not only for prevention and treatment but also for the detection and determination of antibiotic-resistant species. Numerous MALDI-TOF MS-based methods have been recommended for the rapid detection of antibiotic-resistance in bacterial pathogens isolated from bloodstream infections as well as for detection of antimicrobial-resistance in pathogenic fungi. Methods based on an assessment of β-lactamase activity, biomarkers detection responsible for drug-resistance, and/or non-susceptibility, and the comparison of bacteria proteomic profiles incubated with or without antimicrobial drugs, are the most widely studied [33].

2.2 Newborn screening-amino acid analysis

The newborn screening (NBS) program was developed for early diagnosis of asymptomatic newborns at risk for rare diseases such as inborn errors of metabolism (IEM). However, meanwhile, the screening program includes all newborn independently of the risk. IEM is a serious, degenerative, chronic disease with painful and unpredictable clinical manifestations varying from apparent clinical state or obfuscated with other diseases' symptoms to differing degrees of mental retardation and physical disability [87]. These diseases often result in disturbed levels of amino acids or acylcarnitines, which are used as diagnostic markers for IEM. Many problems correlated to irregular amino acid metabolism generate abnormal ammonia concentrations, resulting in an increased turnover of amino acids for energy production or an indicator of alterations in urea cycle metabolism [88]. In the 1960s, the first NBS for the most frequent aminoacidopathia, which is phenylketonuria (PKU), (Guthrie & Susi, 1963), was developed using a dried blood spot (DBS) [89]. It was established to detect PKU and enable early treatment and prevent neurodevelopmental problems if untreated. From the newborn screening perspective, time is a vital factor in the disease etiology. Without screening, many disorders cannot be recognized on time and untreated patients can exhibit serious symptoms of the disease and end up in a coma or even face death. Children diagnosed on time and with adequate treatment are functional, have reducing sequelae or at least substantially lessening organ damages, and may live normal life [90].

Thirty years ago, the first report was published using tandem mass spectrometry (TMS) [91] for analyzing multiple acylcarnitines and amino acids on a single blood spot. The following development of TMS had been introduced as combined with fast atom bombardment (FAB) and electrospray ionization (ESI) [92–96] recently with high-resolution liquid or gas chromatography (LC, GC) respectively [97], and direct analysis in real-time [98] mass spectrometry for newborn screening purposes. According to multiple authors [99–103], blood spot extracts are analyzed by FIA coupled to triple quadrupole (TQ) TMS. Although TQ instruments possess robustness and sensitivity, these instruments also experience monoisotopic interferences with naturally occurring 13C isotopologues, in-source fragmentation interferences, and low mass resolving power, which leads to difficulties separating isobaric compounds with identical quantifying product ions.

The possibility of multiple disorders detection in a single blood spot shortly after birth increased with new technologies in mass spectrometry. TMS is the most widely used instrument for the detection and analysis of amino acids in the DBS and represents one of the most important advancements in the neonatal screening approach [104, 105]. **Figure 1** shows a general scheme of DMB sample preparation for MS analysis.

Analysis of specific amino acids proved to be adequate indicator for the presence of certain disorders in newborns. By measuring fluctuations and disturbances in amino acid metabolism, a diverse group of disorders can be identified and confirmed [107]. Disorders that affect the metabolism of amino acids include PKU, tyrosinemia type I (TYR I), maple syrup urine disease (MSUD), homocystinuria (HCY), argininosuccinic aciduria (ASA), and citrullinemia (CIT) (**Table 2**). These disorders are autosomal recessive and can be confirmed by analyzing amino acid concentrations in body fluids. Because of more than 500 confirmed disorders detected, the use of TMS for clinical screening in a newborn is the method of choice for a few million newborn screenings worldwide [90, 106, 112, 114–116].

When it comes to the operation mode of the instrument, TMS can be operated in different modes such as neutral loss scanning, precursor ion scanning, and multiple reaction monitoring. When neutral loss scanning mode is used, all

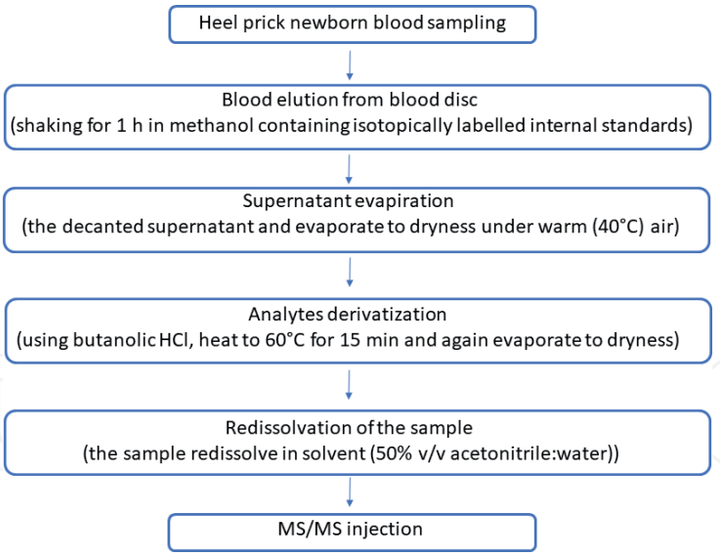

Figure 1.
Example of DMB sample preparation steps for MS analysis [106].

Disorders	Marker(s)	Method	References
Classic phenylketonuria (PKU)	Phe, Tyr	LC–MS/MS	[108]
Tyrosinemia type I	SA, Tyr	LC–MS/MS	[109]
Marple syrup urine disease (MSUD)	Leu, Ile, Val	LC–MS/MS	[110]
Homocystinuria (HCY)	Met	MALDI-TOF MS	[111]
Argininosuccinic aciduria (ASA)	Asa, Cit	HPLC–MS/MS	[112]
Citrullinemia (type I and II) (CIT)	Cit	ESI-MS/MS	[113]

Phe (phenylalanine), Tyr (tyrosine), SA (succinylacetone), Leu (leucine), Ile (isoleucine), Val (valine), Met (methionine), Asa (argininosuccinic acid), Cit (citrulline).

Table 2.
Exemplary overview of the parameters used in newborn screening and the technology applied.

precursors undergoing the loss of a common, neutral, fragment such as water, ammonia, or a phosphate-group are being detected and can be used for further experiments. The neutral loss method is applied for the detection of amino acids due to the neutral loss of m/z 46, which is being shared by many amino acids during fragmentation [117].

Acylcarnitines are detected by this method, as they produce a characteristic fragment ion of 85 m/z. On the other hand, other amino acids, e.g. arginine, ornithine, and citrulline, split off other fragments during collision-induced dissociation. Due to their basic functional group that fragments easily, the most common loss is a combination of butyl formate and ammonia [105, 116–119]. Therefore, for the detection of all amino acids, acylcarnitines, and other biological compounds in NBS, it is safer and better to use the multiple-reaction-monitoring mode for data acquisition (MRM).

To detect the compound of interest, this method requires individual mass transition optimization to achieve the highest selectivity and sensitivity for both amino acids and acylcarnitines, and only selected amino acids can be measured quantitatively and selectively [116]. It is crucial to emphasize the difference between "screening" and quantification in TMS analysis of amino acids. For the diagnosis of PKU, determination of phenylalanine/tyrosine (Phe/Tyr) is of higher importance than a precise measurement of only phenylalanine [120, 121].

For transient neonatal hypertyrosinemia, an elevated level of tyrosine is usually detected by TMS. To differentiate, the diagnosis of TYR I can be established by detecting the presence of succinylacetone in serum or urine [122]. It is important to emphasize that the high concentration of tyrosine is not always a companion of TYR I [123]. Allard et al. developed a method for verifying TYR1 by using succinylacetone as a determination marker (SUAC) in DBS [124]. Some data reported that this method is unmistakably sensitive and specific, while other reports pointed out that false-positive results were also obtained [125]. Many screening programs for homocystinuria have combined determination of methionine (Met) as a primary marker, methionine, and phenylalanine ratio (Met/Phe), and the total homocysteine (tHcy) as a second-tier marker in DBS [126, 127].

Bartl et al. incorporated the LC–MS/MS analysis as a potential first step in screening clinically symptomatic high-risk populations for the two types of HCY and severe B-vitamin deficiencies. In several IEMs, increased reactive oxygen species (ROS) causes pathophysiological oxidative damage that, in the case of HCY, excess Hcy directly supports ROS formation in the form of O_2^-, hydrogen radical, or H_2O_2 [128]. Elevated Hcy concentration is deemed a risk factor for neurodegenerative diseases inducing neurological dysfunction via oxidative stress [129]. Mild to moderate increases in Hcy levels have been associated with both vascular dementia and Alzheimer's disease (AD) [130, 131] and with a possible increased risk of developing Parkinson's disease at a later age [132–134].

Many cases on the diagnosis of PKU [135], MSUD [136], and HCY [126] in newborn blood spots using amino acid analysis by FAB TMS were also reported.

Screening of a large number of disorders was established when Rashed et al. [137] used ESI for analyzing butyl esters of amino acids and acylcarnitines. Consequently, clinical laboratories around the world use this automated sample insertion and data analysis method for a newborn screening procedure to detect and analyze selected amino acids and acylcarnitines [138]. Chace et al. [139] first described the use of TMS for MSUD NBS and recommended the determination of total leucine (Xle) in combination with a total leucine and phenylalanine ratio (Xle/Phe, respectively) for improved detection. In the following studies, recommendations for MSUD detection was based on an elevated Xle or leucine (Leu) [112, 140–147]. Some studies reported that Val is also required for referral [148–150] while others did report Val, but without the cut-off value [139, 143, 151]. Other studies also included the Xle/alanine (Ala) ratio [151]. In a long 11-years-long study in the Netherlands, MSUD NBS was measured in almost two million newborns using TMS, and MSUD was confirmed for 4 patients and 118 false-positive referrals. The authors recommended Xle/Phe ratio as a promising additional marker ratio to their MSUD NBS strategy and advised consideration of method implementation in the Dutch NBS program [138].

Although sensitive, the newborn screening does have some limitations, and therefore, particular caution is required to the common symptoms that may indicate a metabolic disorder. Its goal is to prevent morbidity and mortality through the early detection of metabolic disorders. A significant number of these disorders may present in the neonatal period; therefore, the need for a newborn screening technique is rising. Tandem mass spectrometry has emerged rapidly in previous years as a crucial multiplex testing technique for biochemical genetics analysis and newborn screening and the number of possible disorders that may be included for NBS has exponentially increased.

2.3 On-site mass spectrometry in OP-room

The continuous increase in the prevalence of cancer requires continuous innovation of both diagnostics and treatment. One of the crucial steps in cancer therapy

is as complete as possible surgical removal of the tumor from the surrounding healthy tissue. This so-called negative margin assessment is of critical importance for complete tumor removal and for achieving tumor remission and improve the overall survival rate of patients.

Surgical on-site decision-making could be enhanced with devices and different methods that give an instant and adequate biochemical information about the multiple biopsies or continuous sampling during surgery. Different MS platforms have shown to be able to provide and substantial impact in surgical decision-making process in different points during clinical workflow. To achieve this goal, surgeons would greatly benefit from using mass spectroscopy during the actual operation is going and having immediate information about the resected tumor. This would significantly increase the rate of successfully and almost completely removed tumors and reduce the risk of tumor recurrence. One of the main requirements, or a minimum requirement, for surgery, is that the selected technique delivers fast and accurate information on unprocessed samples and that the ionization is performed as ambient ionization thus eliminating the need for suction and minimizing the use of other solvents than sterile water. DESI (desorption electrospray ionization) was the first technology to be used for the offline analysis of resected tissue. For DESI, a spray of charged solvents is directed onto the tissue's surface and secondary droplets containing the analytes are desorbed and sampled by the MS. Based on this approach, Eberlin et al. [152, 153] developed the MasSpec Pen for intraoperative MS analyses and rapid diagnostics of cancer.

One example is the use of the MasSpec Pen (MS Pen) for diagnostics of ovarian cancer [154] published by Sans et al. Ovarian cancer is a highly lethal disease that is very often diagnosed very late and it is the fifth leading cause of deaths among women [155, 156]. Furthermore, as with other cancers, accurate diagnosis is of extreme importance for the selection of the treatment and development of precision medicine approach and personalized medicine and therapy. For ovarian cancer, two therapy scenarios are possible: a) cytoreductive surgery before chemotherapy and b) surgery upon chemotherapy for tumors that cannot be fully resected. The timing for the cytoreductive surgery is of great importance and in both cases, it is very important to differentiate the tumor from the healthy tissue with high precision. Identification of a tumor can also be very difficult in cases where scarring or some other fibrous tissue is present and, sometimes, healthy tissue is removed, which should be avoided. Unlike iKnife, the MS Pen uses a water droplet to extract molecules from the tissue [157] and transfer it to the ion source. The full process is very fast, it needs no derivatization or other kinds of sample preparation and the acting surgeon gets an instant result based on a database search, which can help to properly identify the resected tissue and enable better determination of the resection margin. Sans et al. [154] have described the use of MassSpecPen for rapid diagnosis of ovarian cancer. The authors analyzed tissue samples from the tissue bank or from prospectively collected samples from endometriosis surgeries to establish the database needed. The authors analyzed the presence of small metabolites such as glycerophosphoinositol, glycerophosphoserine, glutathione, and glycerophospholipid. It was found that normal ovarian tissue was characterized by presence of ascorbate and some other small metabolites with a relatively high abundance in comparison to cancer samples.

The iKnife was developed with the same purpose as the MassSpec Pen but it relies on ionizing analytes in the smoke plum that is generated during electro cauterization of the tissue during the surgery [158]. Unlike the MassSpec Pen, iKnife is preferably used to identify lipids in the smoke plum. By comparing the mass spectra of the sample generated during the surgery and the database that was established earlier, the surgeon sees the result instantly on the screen and can make decisions

about further procedure. St. John et al. described the use of iKnife for the identification of breast pathology for breast cancer surgery [159]. The aerosol produced by the monopolar hand piece used in surgery was aspirated and analytes therein were ionized in the mass spectrometer's ion source. Generated data were used to identify the tissue by applying multivariate analysis. The method proved to be able to identify the substances within a very short time range of 1.8 seconds. Here, the spectral differences that arise between the two operational modes of the electrosurgical knife – the "cut" and the "clog" – were combined to create a multivariate statistical model and to allow for using both modes during the surgery.

A further application where the iKnife was applied is ex-vivo use for diagnosis of cervical disease. The specimen obtained by cervical punch biopsy can either bee snap frozen and used for confirmation of the conventional histology analysis or it can be analyzed immediately upon sampling. Tzafetas et al. [158] was showed that the application of this technology enabled identification of lipids that characterize cancer, the normal tissue, and samples affected by HPV.

MALDI mass spectrometry has already proved efficient for analyzing microorganism and for the offline imaging (MSI) tissue analyses. It is the MSI that represent an encouraging tool to support histopathology analyses and the decision-making processes. MALDI MSI captures the entire spectrum of biomolecules, including specific biomarkers, providing enhanced discriminating power over the visual inspection of tissue and placing it as a proper assisting method in diagnosis procedure.

With the progress of ambient mass spectrometry techniques, such DESI, MS became a powerful methodology for characterizing lipids within tumor specimens. The DESI MS analysis can be performed with minimal sample preparation and it provides molecular information from tissue samples rapidly. This qualifies the DESI and MALDI methods as a diagnostic method in the OP room. In addition to tumor classification, defining tumor subtypes, and identifying tumor grade, this method also provides necrotic tumor tissue identification, an indicator of high-grade malignancy, and can help distinguish necrotic tumor tissue from viable tumor regions [159–162].

3. Conclusion

The use of MS in clinical laboratories worldwide increasing, and, as a result, substantial improvements in assay performance are occurring rapidly in many areas such as toxicology, endocrinology, and biochemical genetics. Numerous types of mass spectrometers are being used for the characterization of small molecules such as drugs of abuse, steroids, amines, amino and organic acids, as well as larger compounds such as proteins and ribosomal RNA.

The development of MS technologies has pushed clinical MS toward the analysis of peptides and proteins for diagnostic examination. However, the quantitative analysis of proteins by MS is still a challenging area of laboratory medicine, which faces many challenges before being fit for a routine application. Also, MS contributes to the quality of the many test results (standardization of assays for steroids, lipids, hemoglobin A1c, etc.), and is used as a standard method in all US states for newborn screening. Furthermore, it is important to address that nearly every institution sends tests to the reference laboratories which frequently perform these tests using MS. With the improved functionality that benefits novel front-end modifications and computational abilities, MS can now be used for nontraditional clinical analyses, including clinical microbiology applications for bacteria differentiation and in surgical operating rooms.

We did not address the role of MALDI imaging technology for application in pathology, but it is one of the fastest-growing application of mass spectrometry in clinical settings and the growth is only impeded by a lack of fast and easy to use software packages for fast identification of analytes others than peptides or lipids.

Conflict of interest

The authors declare no conflict of interest.

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