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Mini Review

Clinical Mass Spectrometry in the Bioinformatics Era: A Hitchhiker's Guide

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ABSTRACT

Mass spectrometry (MS) is a sensitive, specific and versatile analytical technique in the clinical laboratory that has recently undergone rapid development. From initial use in metabolic profiling, it has matured into applications including clinical toxicology assays, target hormone and metabolite quantitation, and more recently, rapid microbial identification and antimicrobial resistance detection by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). In this mini-review, we first succinctly outline the basics of clinical mass spectrometry. Examples of hard ionization (electron ionization) and soft ionization (electrospray ionization, MALDI) are presented to demonstrate their clinical applications. Next, a conceptual discourse on mass selection and determination is presented: quadrupole mass filter, time-of-flight mass spectrometer and the Orbitrap; and MS/MS (tandem-in-space, tandem-in-time and data acquisition), illustrated with clinical examples. Current applications in (1) bacterial and fungal identification, antimicrobial susceptibility testing and phylogenetic classification, (2) general unknown urine toxicology screening and expanded new-born metabolic screening and (3) clinical metabolic profiling by gas chromatography are outlined. Finally, major limitations of MS-based techniques, including the technical challenges of matrix effect and isobaric interference; and novel challenges in the post-genomic era, such as protein molecular variants, are critically discussed from the perspective of service laboratories. Computer technology and structural biology have played important roles in the maturation of this field. MS-based techniques have the potential to replace current analytical techniques, and existing expertise and instrument will undergo rapid evolution. Significant automation and adaptation to regulatory requirements are underway. Mass spectrometry is unleashing its potentials in clinical laboratories.

Contents

1. Introduction . . . 317
2. Basics of Mass Spectrometry . . . 317
 2.1. The ABCs of MS: The *Hards* and *Softs* of Ionization . . . 317
  2.1.1. Electron Ionization . . . 318
  2.1.2. Electrospray Ionization . . . 318
  2.1.3. Matrix-Assisted Laser Desorption Ionization. . . . 319
  2.1.4. Emerging Ionization Techniques . . . 319

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2.2. Until Detection: Ions Fly, Far and High . . . 319
2.2.1. The Quadrupole . . . 319
2.2.2. Time-of-Flight . . . 319
2.2.3. Ion Traps . . . 320
2.2.4. Orbitrap . . . 321
2.3. Thou Shalt Fly Again: MS in Tandem . . . 321
3. Clinical Mass Spectrometry . . . 321
3.1. Clinical Applications: I. Microbial Identification, Speciation, and Beyond . . . 321
3.1.1. Practical Aspects of MALDI-TOF MS Microbial Identification . . . 321
3.1.2. The Theoretical Basis of MALDI-TOF MS Microbial Identification . . . 323
3.1.3. Emerging Applications of MALDI-TOF MS in Clinical Microbiology . . . 323
3.1.4. The Future of MALDI-TOF MS in Clinical Microbiology . . . 323
3.2. Clinical Applications: II. Toxicology, Targeted Detection, Screening and Discovery . . . 324
3.2.1. Urine Toxicology Screening . . . 324
3.2.2. Expanded Newborn Screening: Chromatography-Free MS/MS in the Clinical Laboratory . . . 325
3.3. Clinical Applications: III. Metabolomics, Metabolites and Disease Markers . . . 326
3.3.1. Metabolomics in the Clinical Laboratory . . . 326
3.4. Automation in Clinical Mass Spectrometry . . . 326
3.5. Mass Spectrometry Bioinformatics in the Clinical Laboratory . . . 327
3.6. MS Avec Frontiers: The Limitations of MS and How to Solve Them . . . 327
3.6.1. Matrix Effect . . . 327
3.6.2. Isobaric Interference . . . 327
3.6.3. Protein Molecular Variants . . . 328
3.6.4. Is Mass Spectrometry the Right Tool for the Job? . . . 328
4. Conclusion . . . 328
Acknowledgement . . . 328
References . . . 328

1. Introduction

Mass spectrometry (MS) is an analytical technique which measures the mass-to-charge ratio of ions generated from analytes [1], allowing the qualitative identification and quantitative determination of analytes. First put into functional forms by physicist Joseph J. Thomson (1856 – 1940) and chemist Francis W. Aston (1877 - 1945) [2–4], biochemical researchers and clinical scientists were relatively late adopters of the technique. In fact, when MS was first adopted by clinical laboratorians in the early 1970s, it was only an investigative tool for metabolic profiling in urine [5–8] and other body fluids [9–11]. The first clinical laboratory applications of MS were toxicology assays targeting therapeutic drugs, drugs of abuse and their metabolites [12–17]. With the rapid development of immunoassays in the 1970 to 1980s, first with radioactive labelling [18–20], then with enzymes [21] and fluorophores [22]; and the paradigm shift from phenotyping towards genotyping in the 1990s, largely facilitated by PCR [23], automated DNA sequencing technologies [24] and human and microbial genome projects [25,26], the many virtues of MS-based techniques were outshone. Coupled to the often prohibitive instrument cost, requirement for interpretative expertise and lack of substantial automation in many MS-based techniques, the application of MS in the clinical laboratory remained limited and, with due respect, often regarded as a reference or verification technique, for drugs, hormones [27–29] and one of the chemotaxonomic techniques for establishing microbial phylogeny [30,31].

While many of these applications remain pertinent to today's clinical laboratory - as detailed in the selected examples here – we, as practicing pathologists, have also been witnessing a disruptive change that is being brought about by MS-based techniques. We see manual or semi-automatic phenotyping of microorganisms vanish into the literal dark and hollow *Tunnel of TOF* and emerge as literal identities with quality scores from computer screens (MS-based microbial identification). We see the rainbow colours from a battery of tests, hinting at vague structural similarities among sugars, ketones or smaller organic molecules and inorganic ions, evaporate into *chromatographs* and condense as countless invisible mass spectra capable of enlightening the minimal structural differences. We see the glorious fleet of immunoassay analysers, intricately engineered and armed with multiple pipettes, reaction vessels and spectrophotometers to quantify analytes from small molecules of drugs to large and heterogenous glycopeptides, slowly tarnish as MS-based methods for specific, multi-target quantitation silently creep from research institutions to service-based laboratories.

In this review, we first outline the hardware and configurations of mass spectrometry, namely (1) ion source and ionization, (2) mass selection and determination and, perhaps of cardinal importance in current applications, (3) tandem mass spectrometry. Next, we illustrate the clinical laboratory application of each of the common MS configurations with specific examples in microbial identification, toxicology and clinical metabolomics, which are all made possible by the advances in bioinformatics in the past decades. Due to the wide-ranging characteristics of samples arising from the diverse applications, from crude body fluid and bacterial colonies to organic solvent extracts and purified peptides, the importance – or the lack thereof – of sample pre-treatment and analyte purification is also highlighted for the examples discussed. Finally, as researchers, end-users and advocates, we nevertheless critically discuss the known weaknesses and pitfalls with MS-based methods, which have sometimes led to incongruences with alternate contemporary modalities and offer some of our views and certain possible solutions.

2. Basics of Mass Spectrometry

2.1. The ABCs of MS: The Hards and Softs of Ionization

Mass spectrometry procedures begin with the generation of ions by different ionization techniques [1]. Ionization techniques can be classified into soft ionization techniques and hard ionization techniques, based on the amount of energy imparted to the analyte [32]. These techniques first opened the door to the study of small molecules, and subsequently biological macromolecules. The importance of soft ionisation methods, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), for the analysis of these macromolecules is engraved in the 2002 Nobel Prize in Chemistry, awarded jointly to John Fenn (1917 - 2010) and Tanaka Koichi (1959 -) [33–36]. As can be seen in the sections that follow, ESI and MALDI underlie