National Academy of Clinical Biochemistry Guidelines: The Use of MALDI-TOF Mass Spectrometry Profiling to Diagnose Cancer

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Abbreviations: CID, collisional-induced dissociation; ESI, electrospray ionization; ELISA, enzyme-linked immunosorbent assay; FT-ICR MS, Fourier transform ion cyclotron resonance mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometer; m/z, mass-to-charge ratio; NCI, National Cancer Institute; SELDI, surface-enhanced laser desorption/ionization; TOF, time-of-flight

INTRODUCTION

Despite impressive scientific, medical and technological achievements over the past few decades, cancer is still a leading cause of death, largely because most cancer patients are diagnosed when disease is advanced. Accumulating evidence suggests that in the case of many cancers, early detection is associated with improved survival rates (1). Mass spectrometry has the potential to revolutionize cancer diagnostics by facilitating biomarker discovery, enabling tissue imaging and quantifying biomarker levels. This short review summarizes the principles of mass spectrometry as applied to cancer diagnostics and proposes recommendations for the use of this technique in clinical practice, based on currently published evidence and expert opinions.

PRINCIPLES OF DIAGNOSTIC MASS SPECTROMETRY

The typical mass spectrometer consists of an ion source, a mass analyzer that measures the mass-to-charge ratio (m/z) of the ionized analytes and a detector that registers the number of ions at each m/z value (2). A typical proteomic experiment with mass spectrometry consists of five stages: 1) separating the proteins present in the cell lysate, tissue or fluid by various fractionation or affinity selection techniques to reduce the complexity of the target proteome, 2) enzymatically degrading (usually with trypsin) the isolated proteins to peptides, 3) separating the peptides by techniques such as high performance liquid chromatography (HPLC) and directing the eluted fractions into an ion source (electrospray or MALDI) where they are converted into ionized species that enter the mass spectrometer, 4) taking a mass spectrum of the peptides eluting at each time point, and 5) identifying the protein fragments and parent proteins comprising the mass spectra by a variety of algorithmic approaches (3).

Mass spectrometric measurements are carried out in the gas phase of ionized species. Two commonly used techniques to volatize and ionize the proteins or peptides are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) (4-6). A variant of the latter is surface-enhanced laser desorption/ionization (SELDI) (Ciphergen, ProteinChip^{TM}) (7,8). The mass analyzer separates ionic species according to m/z ratio. Four basic types of mass analyzers are commonly used in proteomic research: the ion trap, time-of-flight (TOF), quadrupole and Fourier transform ion cyclotron resonance (FT-ICR), with a potential fifth variant being the new Orbitrap mass spectrometer (Thermo Electron, Inc). These basic types may be variously combined in hybrid instruments.

Protein identification is achieved through either peptide mass fingerprinting or peptide sequencing. In the former, peptide masses are compared with mass spectra of proteins listed in databases using appropriate software (9,10). Peptide sequencing is based on induction of

random cleavage of peptide bonds between adjacent amino acid residues, using approaches such as collision-induced dissociation (CID). The resulting ion series is analyzed by software (11-15) to determine the amino acid sequence.

APPLICATION OF MASS SPECTROMETRY IN CANCER DIAGNOSTICS

Mass spectrometry has been applied to cancer in a variety of contexts, including

- Diagnosis, prognosis, and management
- Biomarker discovery
- Diagnostic tissue imaging
- Biological studies related to mechanism of disease.

Mass spectrometry is considered to be particularly well suited to serve as a diagnostic or biomarker discovery tool in cancer, given emerging evidence that during cancer development, cancer cells and/or the surrounding microenvironment generate proteins and peptides of different type and in different concentrations than normal cells. These abnormal tissue distributions can be analyzed by imaging-based mass spectrometry and the patterns compared with controls to identify cancer-specific changes that may prove to be clinically useful. Should leakage to the circulation occur from the tumor-host microenvironment, then a multiplex of cancer-specific analytes may be detectable in the blood as well, leading to even more widespread clinical utility and convenience of testing (16-19). This concept is graphically illustrated in Figure 1.

The identification of cancer-specific protein patterns in blood by mass spectrometry was first demonstrated by Petricoin et al. (18) and Rai et al. (20) for ovarian cancer, Li et al. (21) for breast cancer and Adam et al. (22) for prostate cancer. Subsequently, many other investigators have used similar approaches to identify multiple markers and informative profiles for many other types of cancer, as summarized in Table 1 (18-51).

In almost every published paper, the profiles generated by MALDI-TOF MS have been shown to yield better diagnostic sensitivities and specificities than the established cancer biomarkers in current use. Because of this, the MALDI-TOF MS approaches have received extensive publicity since they promise to revolutionize early cancer detection, sub-classification, prognosis, prediction of therapeutic response, etc. However, the initial enthusiasm has been tempered somewhat by parallel reports that have identified potential problems with this approach and its clinical reliability (52-71). These issues are not unlike those facing the gene transcript profiling community (64). Future validation studies will determine how close this technology is for clinical use.

CURRENT ADVANCES AND EXISTING LIMITATIONS OF MALDI-TOF MASS SPECTROMETRY-BASED PROFILING FOR CANCER DETECTION

If MALDI-TOF profiling is to be successful in the transition from a research technique to a clinical diagnostic tool, then an extensive understanding of pre-analytical, analytical and post-analytical sources of variation must be realized and controlled (52-71). For example, the effect of sample storage and processing, sample type, patient selection and demographic variables (gender, age) on test outcome must be clearly established (72). Analytical performance must improve to the point where sensitivity, specificity and the dynamic range become comparable to those of established techniques such as ELISA. The reproducibility of protein patterns across different batches of chips (when SELDI-TOF is employed), different analysts, different sites and different instrumentation is still under investigation. Robustness of the methodology, in general, is of concern, as are issues related to bioinformatic artifacts, data over-fitting and bias arising from experimental design. However, a large number of these issues relate to inappropriate analysis of publicly available mass spectral data sets that were not meant to be compared. Recently, a large consortium of investigators has shown success at reproducibly obtaining mass spectral signatures, including diagnostically important ones, at multiple sites across time and instruments. This finding establishes a very positive result for those attempting to employ MALDI-TOF type approaches for protein fingerprinting based diagnostics (66).

The current limitations and promises of MALDI-TOF, particularly as applied to clinical practice and cancer diagnostics, are addressed more fully in several recently published reviews (17,19,52,54,67-71).

MALDI-TOF MASS SPECTROMETRY PROFILING: NACB RECOMMENDATIONS

Despite numerous publications describing impressive results of MALDI-TOF mass spectrometry as a diagnostic tool (Table 1), the level of published evidence, as described by Hayes et al. (73), is Level V (evidence from small pilot studies that estimate distribution of marker levels in sample population). According to the criteria of Pepe et al. (75), the stage of development of this technology as a biomarker tool is Phase 1 (preclinical exploratory studies). Based on this information, we have formulated the recommendations shown in Table 2. There is little question that MALDI-TOF MS approaches, either as a direct profiling tool, or using affinity enrichment front end components (e.g. Immuno-MS) will eventually become established in the diagnostic arena given its inherently attractive features, including analysis without the need for a labeling molecule, potentially perfect specificity, multiparametric analysis, high-throughput, very low sample volume requirements, and direct interface with computer algorithms. The major

limitations of the MALDI-TOF technology for MS profiling type work are, at present, a) the cross-platform reliability of the signatures generated; b) dramatic effects on final spectral composition from subtle changes in sample handling and processing; and c) analytical sensitivity, especially when the analyte is present in minute amounts in a highly complex mixture that includes high abundance molecules. However, new research has indicated that many low abundance proteins and low molecular weight analytes exist in a bound state in the serum, and are effectively amplified by carrier protein based sequestration (76-81). These low molecular weight analytes appear to have underpinned many past spectral fingerprints, thus indicating that many of these ions may be generated from low abundant analytes. A list of these low molecular weight carrier protein bound analytes have has recently been provided for early stage ovarian cancer patients (79), and the concept verified in an independent study with Alzheimer's disease detection (81). In that study, high-resolution MALDI-TOF serum proteomic profiling of Alzheimer disease samples reveals disease-specific, carrier-protein-bound mass signatures. These recent findings, together with other recent publications (24,29) describing truncated or fragments of proteins ("fragmentome") of the circulatory proteome, indicate that MALDI-TOF based approaches may be measuring analytes that are disease specific, of lower abundance than previously thought, and novel.

We conclude that, like all technologies that directly impact patient health, until extensive validation studies are performed, MALDI-TOF MS fingerprinting approaches should not be used as a diagnostic test for cancer in clinical practice. We recommend that investigators should perform such validation experiments following CAP/CLIA based codes of good practice and should provide data in a transparent form for full evaluation by the scientific community. We also recommend that in experiments in which MS fingerprinting is being employed, appropriate independent validation sets should be employed using inflammatory and benign controls along with high numbers of unaffected controls, since specificity will be an important determining factor of success in the clinic, especially for screening indications. Despite recent difficulties in extending research observations for genomic and proteomic profiling, the field is now evolving with a better understanding of potential sources of bias and instrument variances, as well as the requirements for developing good laboratory practices and standard operating procedures such that clinical adoption and validation could be achieved in the near term.

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Table 1. Mass spectrometry for cancer diagnosis and imaging

Application	Cancer type	References
Cancer Diagnosis		
	Nasopharyngeal	23
	Ovarian	18,20,24
	Breast	21,25-29
	Prostate	22,30-34
	Bladder	35,36
	Pancreatic	37-40
	Head and Neck	41,42
	Lung	43
	Colon	44
	Melanoma	45
	Hepatocellular	46
Tissue imaging		
	Gliomas	47
	Breast	48-50
	Lung	51

Table 2. NACB Recommendations for use of MALDI-TOF MS in Cancer Diagnostics

1. MALDI-TOF MS profiling in the realm of cancer diagnostics should currently be considered an investigational and research tool that, like all unvalidated methods, at this time is insufficiently reliable to be the basis of clinical decisions.
2. In order for MALDI-TOF MS profiling to become a clinically reliable tool, it must undergo validation according to the principles such as those described by Pepe et al (68) and avoid biases, as described by Ransohoff (67).
3. For MALDI-TOF MS profiling testing, validation of discriminatory peaks in the mass spectra should include statistically powered independent testing and validation sets that include large numbers of inflammatory controls, benign disorders and healthy controls, as well as other cancers. The degree of statistical powering of the validation studies should be carried out under methods such as those described by Pepe et al (68) as well as with consideration of the intended clinical use of the test itself.
4. Stability of bioinformatic algorithms should be evaluated using large numbers of samples, preferably from several institutions and countries.
5. Standardized protocols should be developed for sample collection, handling and processing.
6. Quality control and reference materials for MALDI-TOF MS must be developed and used more widely to monitor and improve method reliability.

Figure 1. Secretion of specific biomarkers into the blood circulation by tumors. Tumor-specific proteins may be actively secreted by tumor cells or released into the circulatory system by necrosis and apoptosis of these cells. Either of these conditions leads to an alteration of the serum protein profile. This may result in detectable differences based either on relative or unique signal intensities when comparing sera from normal and disease samples. [Figure reproduced from Reference 16 with permission from copyright owners]