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Impact of Advanced Metabonomic Technologies on Surgical Interventions: A Comprehensive Review

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

Aim: This study aims to evaluate the impact and potential of advanced metabonomic technologies in enhancing surgical decision-making and patient outcomes, focusing on oncological and critical care settings. **Methods:** A comprehensive review of literature was conducted to gather and analyze data on the application of MAS-NMR spectroscopy, mass spectrometry imaging (MSI), and rapid evaporative ionization mass spectrometry (REIMS) in surgical contexts. Studies encompassing tumor margin assessment, intraoperative tissue characterization, and metabolic profiling in critical illness were reviewed to synthesize current findings and technological advancements. **Results:** MAS-NMR spectroscopy demonstrated high sensitivity in distinguishing between benign and malignant tissues, offering rapid and accurate intraoperative guidance. MSI provided histologically specific molecular profiles, enhancing surgical precision in tumor resections. REIMS enabled real-time tissue analysis during surgery, facilitating immediate decision-making based on molecular composition. These technologies showed promise in predicting patient outcomes, optimizing therapeutic strategies, and minimizing surgical complications. **Conclusion:** Advanced metabonomic technologies represent a paradigm shift in surgical practice, offering precise, real-time molecular insights that improve diagnostic accuracy and therapeutic efficacy. Integration of MAS-NMR spectroscopy, MSI, and REIMS enhances surgical decision-making in oncology and critical care, paving the way for personalized treatment approaches and improved patient outcomes.

Keywords: MAS-NMR spectroscopy, mass spectrometry imaging, rapid evaporative ionization mass spectrometry, surgical oncology, critical care, metabonomics.

Introduction:

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Molecular tools are becoming more and more important for illness classification, subtype distinction, and individual biological variation detection in clinical diagnosis, prognosis, and treatment selection (1). Significant improvements to healthcare are anticipated from the deployment of novel and stratified therapeutic techniques that have been improved through predictive modeling of deep biological data (genetic, metabolic, and physiological). Furthermore, these developments have the potential to significantly alter clinical settings' socioeconomic conditions, healthcare delivery, regulations, and research (1). Precision medicine has enormous potential in the areas of molecular epidemiology, prognostics, and clinical diagnostics, especially with metabolic phenotyping or metabotyping (2). Metabotypes are highly variable and regulated by a host's genetic makeup, lifestyle, nutrition, and gut microbiota. They can be identified by the makeup of biofluids, or tissues

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obtained in clinical settings (3, 4). As a result, metabotyping plays important roles in enabling patient classification in population-based disease-risk research and customized healthcare (4).

Deriving metabolic phenotypes from large epidemiological cohorts offers significant statistical power to find putative metabolic biomarkers for disease risk in a variety of populations, including metabolome-wide association studies where higher blood pressure predictors are found (3). The connections between genes and environment that shape metabotypes are similar to those that affect treatment response and illness risk in the general population. This highlights the biological and statistical significance of metabolic studies in a range of medical settings (4). In the past, doctors have used urine color, odor, and taste to infer diagnoses and treatments. This is an example of how metabolic phenotypes have been implicitly quantified and mapped (5). Modern spectroscopic methods, like mass spectrometry and nuclear magnetic resonance (NMR), allow for the thorough profiling of metabolites, transforming our capacity to examine metabolic processes on a multivariate level (1).

Various metabolic-analysis techniques are referred to by different terms. For example, metabolomics (6) describes a sample's metabolic makeup in terms of the existence and concentration of metabolites; the metabolome is the multivariate sum of these elements. There are over 500 histologically diverse cell types in the human body, each with a specialized function as well as distinct proteomes, metabolomes, and patterns of gene expression. Although cellular metabotypes can overlap in histological specimens, the lymphatic and vascular systems allow for spatial and temporal interaction. Humans therefore have about 500 dynamic cellular metabolomes, in addition to extracellular fluid compartments specific to each tissues and a variety of biological fluids that are secreted and excreted, all of which have different compositions from the surrounding cells. Metabotypes undergo dynamic modifications as a result of the various time periods across which disease processes and medical therapies develop. Since the late 1990s, complex systems' metabolic reactions to perturbations across time have been mapped using appropriate analytical and statistical approaches, a process known as "metabonomics" (7). Disease, dietary modifications, medication therapy, genetic modification, and other inputs are examples of such stressors. Specifically, these phenotypic alterations are addressed by metabonomics, which commonly analyzes bodily fluids like blood plasma or urine at the small-molecule metabolite level.

With over 26,000 and 10,400 Google Scholar results, respectively, at the time of writing, the terms metabolomics and metabonomics are commonly and interchangeably used. Over the past 30 years, metabolic profiling has been used in many different research domains, including animal toxicology, plant and food science, microbiology, and causes of illness. However, the greatest interest is currently focused on the clinical applications of metabolic profiling. High-throughput metabolic technologies are appealing because they may provide novel biomarkers for diagnosis and reveal the underlying causes of disease. The present review delves into important and recently developed fields of clinical metabotyping and their various uses in improving our comprehension of human illness processes.

A continuum of metabolic activities that contribute to the overall metabotype is produced by a number of interconnected metabolic networks that function across various body compartments. This covers the effects of medication, food, and gut microbial activity (8). By examining samples from these compartments, such as tissue or plasma—two of the most commonly used clinical diagnostic fluids (**Fig. 1**)—the local phenotypic expression of these network interactions can be documented. This technique produces a series of static snapshots of metabolic activity, which, unless there is an obvious metabolic disorder, can be difficult to interpret in isolation due to physiological variability. A longitudinal metabolic pattern or trajectory can be observed by gathering time series of samples from patients receiving diagnostic or prognostic evaluations, or at various stages of a disease process. This can yield more precise information about the location, degree, and possibly even the mechanism of damage (9). Reactions to therapy can be handled in the same way. In actuality, though, only a restricted range of tissue or fluid types are suitable for sampling. Comprehensive examination of these samples, even with sophisticated metabolic and spectroscopic techniques, produces only "islands of information" that reflect systemic activities influencing the extracellular environment (plasma and urine) or local activities (tissue or specialized biofluids) (**Fig. 1**). Building mathematical bridges between these islands to produce system-level models is one of the challenges of metabolism-based 'top-down' systems biology (10). Using 'bottom-up' systems-biology techniques, these models can then be utilized to produce biochemical or medicinal hypotheses for additional testing.

Samples of urine and plasma carry significantly distinct sets of information about different chemicals and pathways that represent many systemic timeframes. A snapshot of the metabolic system at the time of sampling is provided by plasma data, while long-term changes brought about by dietary or long-term interventions may also be seen. Urine, on the other hand, is time-averaged since it is collected and stored in the bladder.

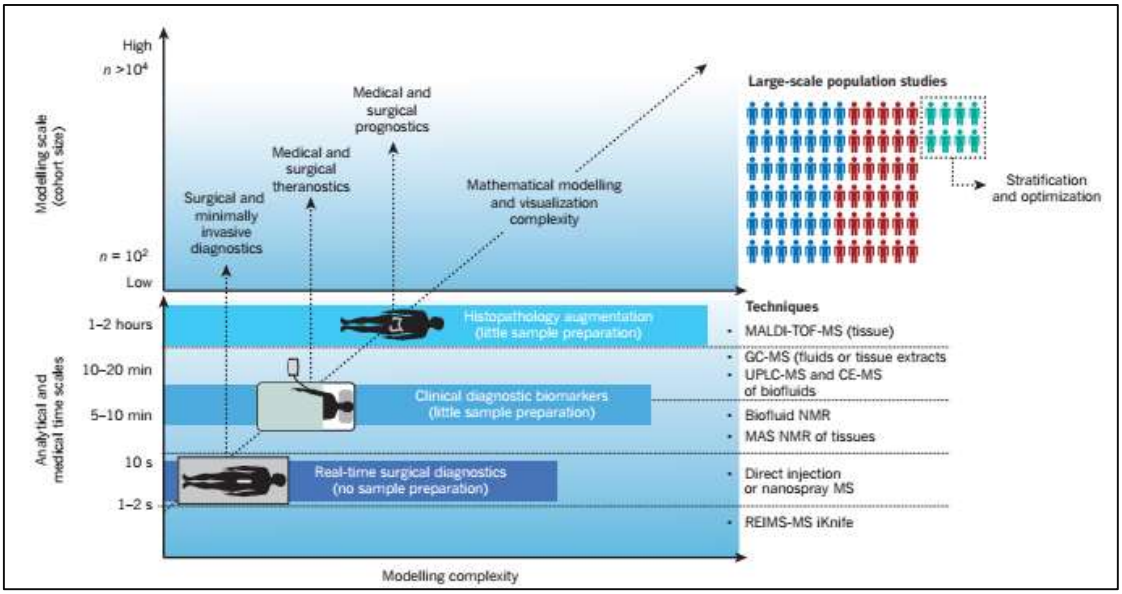

Fig. 1: Technology platforms and analytical timescales for patient journey phenotyping, diagnostic and prognostic biomarker discovery, and population disease-risk biomarker modelling (Nicholson, et al. 2012).

Furthermore, the requirements for sample preparation and analysis are dictated by intricate physicochemical interactions and variations in analytical matrix properties, which offer additional forms of dynamic diagnostic information not revealed by straightforward compositional analysis. Distinctly from mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy does not disturb these intricate disturbances in dynamic physicochemical interactions between molecules in biofluids. Though some biofluids (such semen) are extremely reactive post-collection due to inherent enzymatic activity, dynamic chemical characteristics have received less attention from a diagnostic standpoint than compositional biomarker analysis (11). Metabonomic and related techniques supplement other 'omics' technologies like transcriptomics, proteomics, metagenomics, and genomics in terms of biological data creation. Each addresses a distinct facet of cellular and systemic action, and they are all connected. In order to gain a more comprehensive understanding of human disease, systems-biology approaches seek to combine various datasets through the use of suitable multivariate statistical analysis and network modeling. Nevertheless, up until now, multiple omics screening has rarely proven practical in a clinical setting. A condition's whole biological picture cannot be obtained from a single instrument, metric, or platform. Every method produces theories that need to be thoroughly investigated and verified in real-world settings. One benefit of metabolic profiling is that it can be used for large-scale testing due to its comparatively low cost per assay or process. Commonly available samples, such plasma and urine, can yield valuable information in a therapeutic environment.

In summary, the integration of molecular tools in clinical diagnosis, prognosis, and treatment selection is revolutionizing the healthcare landscape. The deployment of advanced therapeutic techniques, refined through predictive modeling of genetic, metabolic, and physiological data, is poised to enhance patient outcomes and transform healthcare delivery, regulations, and research. Precision medicine, especially through metabolic phenotyping or metabotyping, holds significant promise in clinical diagnostics and molecular epidemiology. Metabotyping, influenced by genetics, lifestyle, nutrition, and gut microbiota, allows for detailed patient classification and the identification of disease biomarkers across diverse populations. Modern spectroscopic techniques, such as NMR and mass spectrometry, have advanced our ability to profile metabolites, enabling a deeper understanding of metabolic processes. Metabolomics and metabonomics have become essential in various research fields, with significant clinical applications emerging. The ability to generate metabolic phenotypes from large epidemiological cohorts offers substantial statistical power, aiding in the identification of disease risk biomarkers and enhancing personalized healthcare. The dynamic nature of metabotypes, shaped by disease processes and medical treatments, necessitates the collection of longitudinal samples to capture comprehensive metabolic patterns. Despite the challenges of obtaining a complete biological picture from limited sample types, advanced techniques and systems-biology approaches strive to bridge the gaps between isolated data points. By integrating various 'omics' data, researchers can develop holistic models of human disease, paving the way for new diagnostic and therapeutic strategies. Ultimately, metabolic profiling stands out for its relatively low cost and feasibility for large-scale testing, making it a valuable tool in clinical settings. As we continue to explore the vast potential of metabolic analysis, its

role in advancing our understanding of human disease processes and improving patient care will undoubtedly expand.

Analytical Platforms:

The two most prominent analytical platforms in metabolic profiling are nuclear magnetic resonance (NMR) spectroscopy (12) and mass spectrometry (MS) (13). The basic principles of these techniques are illustrated in **Fig. 2.** Both methods produce spectral data, and by analyzing the positions and intensities of the peaks, one can determine the biochemical species present and their relative concentrations (12). Currently, no single platform offers a comprehensive analytical overview, as each has distinct strengths and limitations. Therefore, utilizing a combination of NMR and MS, which complement each other's analytical capabilities, is beneficial during the initial experimental phase. MS is highly regarded for its sensitivity and reliable metabolite identification, making it an excellent choice for detecting metabolites in complex biosamples. In MS, metabolite separation is achieved using either gas chromatography or liquid chromatography, with the choice depending on the chemical compound being analyzed (14). Gas chromatography is effective and sensitive, but it is not suitable for large and thermolabile compounds like sugar nucleotides or large oligosaccharides due to their limited volatility (15). In these cases, liquid chromatography is the preferred method. MS experiments can be conducted using targeted (15) or untargeted (16) approaches, depending on whether the goal is to profile and quantify predefined metabolites or to create a more comprehensive metabolic profile. However, MS has drawbacks, including time-consuming and expensive sample preparation steps, and it is destructive to samples. In contrast, NMR spectroscopy, while offering lower sensitivity, has several advantages, such as minimal sample preparation, well-established metabolite databases, and excellent reproducibility with minimal variability between machines. Modern high-throughput automated NMR platforms can analyze up to 500 samples per day, making NMR a highly attractive option for rapid and cost-effective sample analysis (17).

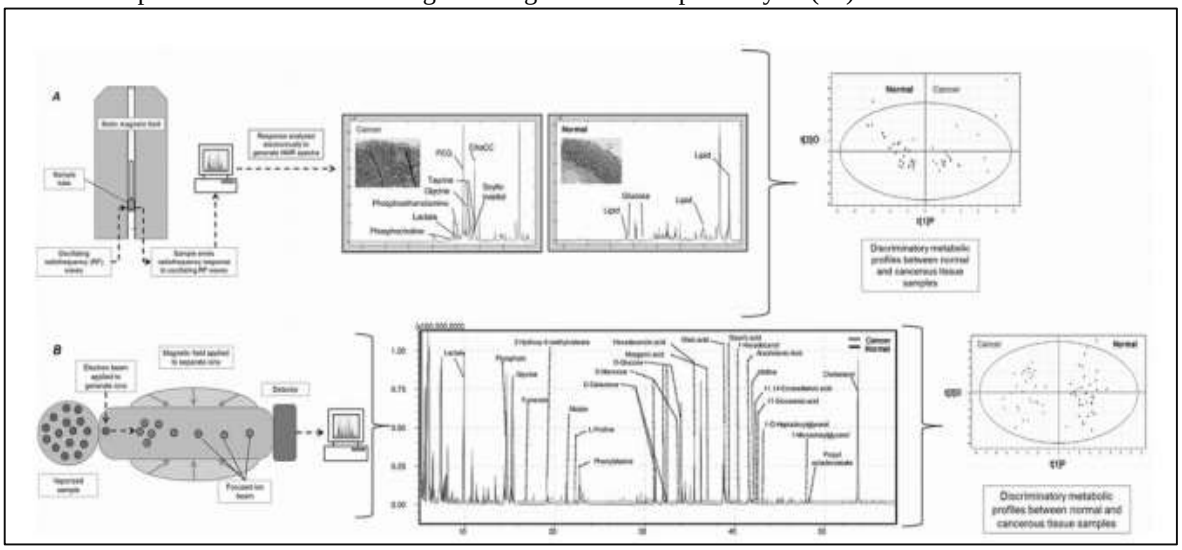

Fig. 2: Data analysis (Mirnezami, et al., 2012).

Data Analysis for Surgical Operations:

Large data sets are produced by metabonomic experiments, which calls for the employment of reliable data processing and analysis tools. In this context, principal component analysis (PCA) and partial least squares analysis (PLS) are two of the most often used statistical procedures. The multidimensional character of the data, where the sample size is frequently very small compared to the number of measured variables (metabolites in this case), is one of the fundamental obstacles in high-throughput "omics" profiling. By removing redundant data and lowering the dimensionality of the data set, PCA offers a mathematical technique for lowering the total number of variables in a sample. This makes it easier to identify the most important variables, or "principal components," while retaining as many of the sample's original characteristics as feasible (18). By mapping samples according to their biochemical similarity, this method creates scoring plots that can show data distribution, clustering, and the existence of outliers (**Fig. 3**). Another popular statistical technique that incorporates aspects of PCA and multivariate regression is partial least squares analysis. It is frequently used for discriminant analysis and is especially helpful when a quantitative relationship between two data sets is desired (19). Although a thorough analysis of these tactics is outside the purview of this review, a wealth of information may be found in the literature (18-24).

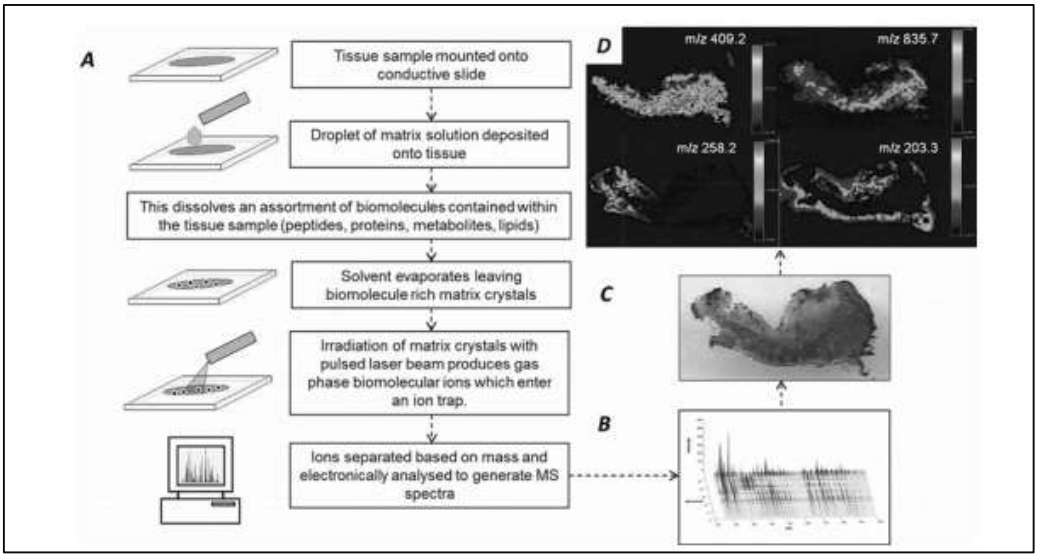

Fig. 3: MALDI-imaging Spectroscopy (Mirnezami, et al., 2012).

Clinical Applications:

Many advances in modeling methodologies, chemometrics, and biomarker discovery have resulted from the widespread use of metabotyping approaches in animal models to research disease, drug toxicity, and therapeutic action (25-28). A discernible trend towards clinical investigations has emerged with the maturity of these technologies and modeling platforms. This Review concentrates on particular issues that could have a big influence on clinical care in hospitals and translational medicine. Among these is the screening of people with known illnesses in order to find novel biomarkers for clinical categorization. We are now in a position to provide a systems-biology paradigm for challenging clinical issues that are frequently exacerbated by drastic changes in the gene-environment. The goal of early metabolic profiling research on clinical disorders was to find biomarkers, but they were constrained by small group sizes and technological issues. However, these findings opened the door for a wider application of metabotyping in the study of systemic disorders and the identification of prognostic and diagnostic biomarkers. The fundamentals of metabotyping techniques were well established by the 1980s, especially when it came to overt metabolic illnesses such as type 2 diabetes (29) and inborn errors of metabolism using urine and serum profiles generated from 1H-NMR spectroscopy (30). Early research indicated that after insulin withdrawal in individuals with type 2 diabetes, systemic biochemical information might be regained, such as elevated serum alanine and decreased branched-chain amino acids. Moreover, treatment optimization through alterations to plasma lipid and lipoprotein profiles could be monitored by metabolic profiling (29).

The area has broadened to encompass population-scale and epidemiological investigations (18) as well as consideration of diabetic consequences, such as vascular lesions that result in early mortality (30). Potential processes underlying type 2 diabetes that are conserved in rats, mice, and humans and are linked to nucleotide metabolism and regulation of N-methylnicotinamide have been shown by cross-species metabolic study (31). A panel of five branched-chain amino acids is predictive of type 2 diabetes (33) whereas metabolic dysregulation of lipid and amino acid metabolism predates the start of the disease in type 1 diabetes (32). Comprehensive reviews on the contributions of metabonomics and metabolomics have been conducted, with a focus on insulin resistance and types 1 and 2 diabetes (34).

Early studies on cancer research concentrated on extracts from tumors; 1H-NMR spectroscopy and pattern recognition techniques were useful in differentiating between various cancer tissue types (35). Findings that are clinically significant include the identification of putative cancer biomarkers in biofluids, such as plasma ovarian cancer signatures, which are marked by modifications in alanine, valine, and low-density lipoproteins as well as altered patterns of ceramides, lysophospholipids, and ketone bodies (36-37). Urinary hippurate and trigonelline levels, in addition to increased d-3-hydroxyisovalerate, α-hydroxyisobutyrate, and N-acetylglutamine, have been used to differentiate lung cancer patients from controls (38). Metabolite profiles have shown correlations between stearic acid and serum acylcarnitines and endometrial cancer (39), as well as acylcarnitines dysregulation in kidney cancer (40). When it comes to predicting early-stage tumors in colorectal cancer, serum metabolite profiles frequently perform better than traditional markers like carcinoembryonic antigen (41). Differentiating between early- and late-stage tumors appears to be a promising area of research for ovarian, breast, and renal cell carcinoma (42-43). Higher levels of plasma glucose, proline, lysine, phenylalanine, and N-acetylcysteine, as well as lower levels of lipids, are

predictive of micrometastases in patients with breast cancer, according to metabolic models that also forecast clinical outcomes for specific malignancies (44). Pre-treatment serum levels of glucose, glutamate, and phenylalanine in metastatic breast cancer predict treatment toxicity, overall survival, and time to progression for HER2-positive patients (45). A thorough evaluation of metabolic studies on cancer biomarkers and tumor growth has been conducted throughout the last ten years (46-48).

The field of metabotyping has also made significant advances in basic cardiac research (49), cardiovascular event prediction in coronary artery disease risk persons (50), and pathology origins knowledge (51), including environmental and non-infectious microbiological disease triggers. Serum and urine have traditionally been the main focus of diagnostic techniques, however gas chromatography for volatile components in exhaled breath shows promise for characterizing lung diseases. The composition of breath condensate can be used to differentiate between asthma and allergic rhinitis in children (52), and the presence of ketones, methyl-branched alkanes, and alcohols in exhaled breath can be used to determine the stage of chronic obstructive pulmonary disease (53). In order to offer supplementary information on systemic dysfunction, metabolic profiling examines many compartments and fluids. Lung disease signatures are detected in urine and serum. Differential urine TCA cycle intermediates are present in both stable and unstable asthma, and chronic obstructive pulmonary disease is associated with lower serum lipoprotein levels and higher levels of glutamine, 3-methylhistidine, and branched-chain amino acids (54-55).

Human metabolic traits and pathological mechanisms are significantly influenced by gut microbiota activity. Metabolic profiling describes the functional characteristics of the gut microbiome; deviations are associated with a range of illnesses, including diabetes, obesity, autoimmune diseases, and neuropsychiatric disorders (56-58). Urine, plasma, and fecal samples are used in inflammatory bowel disease metabotyping to describe metabolic implications and discover chemical alterations resulting from gut microbiota (59-61). In these circumstances, altered microbiota are indicative of changes in energy balance, and the activities of the gut microbiota impact the metabolic phenotypes of the host by means of intricate signaling pathways that link several organs, such as the immune system, liver, and brain (62-63). These axes include gut microbe-generated chemicals that impact host adipogenesis and CNS signaling, as well as bile-acid dependent signaling (64-65). Different urine metabolite phenotypes in autistic children indicate altered amino acid and nicotinic acid levels as well as gut-microbial metabolite excretion (66-67). Preterm delivery is associated with an increased risk of metabolic syndrome and end-stage renal failure, as early microbiome-host signaling interactions leave long-lasting metabolic impacts. Preterm and full-term people can be distinguished from one another by analyzing the adult microbial degradation products (68). Drug development and treatment approaches that target the microbiome, host metabolic pathways, and immunological signaling may be based on these metabolic signaling axes (69-71). In order to better understand complicated microbial-host interactions, future research on the microbiome will depend on the successful implementation of metabotyping, which offers more druggable targets than the human genome.

Applications in Cancer Research:

The idea of a modified metabolism in cancer was first introduced in the 1920s by Warburg (72), who showed that even in the presence of enough oxygen, cancer cells can eat large amounts of glucose and convert it to lactate (aerobic glycolysis). At the expense of glucose reserves, this elevated glycolytic activity produces a lot of adenosine triphosphate (ATP), which is used as energy. Numerous investigations have proven the Warburg effect, which is this phenomena. It is now commonly acknowledged that cancer cells have a reduced oxidative phosphorylation and an improved glycolytic pathway in comparison to healthy cells (73-75). This idea is supported by a recent study by Ong et al. (76), which found that a characteristic metabolic feature of colorectal cancer tissue is elevated glucose uptake in cancer cells. Premalignant polyp cells were found to exhibit the Warburg effect as well, which suggests that this metabolic change takes place prior to the hypoxic and angiogenic events that are known to be elements of the adenoma-carcinoma sequence (77-78). According to the authors, there could be a "glycolytic switch" that activates prior to the "hypoxic" and "angiogenic" switches. This could have significant consequences for the early detection of colorectal cancer (76).

Yakoub et al (79) additional investigation revealed aberrant metabolic markers in the histologically bland esophagus tissue next to malignant tumors. According to their theories, malignancies develop in regions with changed metabolic phenotypes and undergo a biochemical change prior to the development of any morphological signs of malignancy (79). These results demonstrate the potential utility of metabolic interrogation in early cancer diagnosis and offer crucial insights into the energy requirements needed for cells with malignant potential to complete the process of neoplastic transformation. Furthermore, the fact that a variety of tumor forms are dependent on glycolysis offers an alluring path for therapeutic intervention. With intriguing results, a number of organizations have assessed the potential of glycolytic pathway enzyme inhibitors as novel anti-

cancer agents (80-85). Glycolysis is insufficient to meet the high energy needs of cancer cells in the growth phase. Numerous additional pathways, such as tricarboxylic acid (TCA) cycle activity (86-87), lipid metabolism (88), protein metabolism (88), and nucleotide biosynthesis (88), have also been linked by metabolic studies to carcinogenesis. Changes in these and other metabolic processes seem to give cancer cells a survival advantage through altering the environment around tumor cells, cellular energetics, and cancer-cell signaling (88). Examining these metabolic profiles offers opportunities for the identification of novel biological targets based on cancer-specific metabolic features and for the future development of cancer biomarkers (89-93).

Intact Tissue Profiling:

The difficulty of non-destructively profiling cancer tissue specimens was a major obstacle until recently. This restriction has been overcome by developments in mass spectrometry (MS) and nuclear magnetic resonance (NMR) technologies, which have resulted in the creation of analytical platforms for intact tissue, such as matrix-assisted laser desorption/ionization (MALDI) MS (94) and high-resolution magic-angle spinning NMR (HR MAS-NMR)51. For surgical oncologists, high-resolution magic-angle spinning NMR (HR MAS-NMR) provides data on a feasible time scale and allows for extremely quick, non-destructive tissue metabolic profiling (10). Chan et al. recently analyzed the matched healthy and cancerous colonic mucosa from (31) individuals, showing that HR MAS-NMR could quickly and accurately distinguish between healthy and malignant tissue (10). Since the validity of frozen sections has been called into question on multiple occasions, it is expected that this technology will soon take the role of frozen sections for intraoperative tissue evaluation (95-97). The first "next-generation" micro-NMR apparatus capable of ultra-fast, thorough biopsy sample analysis (98) is the product of recent collaboration work at Harvard Medical School and Massachusetts Institute of Technology, demonstrating the translational utility of this method. This group's recent publication showed that, in contrast to 84% accuracy obtained with traditional immunohistochemistry, micro-NMR analysis of a variety of intra-abdominal malignancies may accurately diagnose cancer with 96% accuracy. Furthermore, a conclusive diagnosis using traditional immunohistochemistry often takes three days, but micro-NMR just takes an hour (98).

Matrix-assisted laser ionization and desorption Target-specific reagents, including antibodies, are no longer necessary thanks to MS, a new technology that allows for the direct, non-destructive, and non-targeted collection of a tissue section's metabolic profile (52). The term "molecular histology" (99) refers to the visual depiction of the spatial distribution of biomolecules in a tissue section that can be produced by combining this approach with imaging MS (MALDI-imaging) (**Fig. 3**). MALDI-imaging can concurrently disclose the distribution of hundreds of biomolecules, in contrast to conventional immunohistochemistry, which only shows the distribution of a few peptides or proteins (99). The neoplastic tissue from ovarian (98-100), oral (101), and metastatic colon cancers (101) has been profiled using this technology, and it is expected that MALDI-imaging will be a major component of future cancer evaluation efforts. Furthermore, MALDI-imaging might present a fresh approach to chemoresponsiveness characterization based on metabolic profiling. The location and relative content of oxaliplatin and its metabolite derivatives in renal tissue following intraoperative chemotherapeutic treatment were evaluated in a recent work by Bouslimani et al. (102) using a rat model. This type of metabolic phenotyping will support gene-expression-based profiling techniques and help to more robustly stratify patients according to their likelihood of responding to therapy and the best drug to use.

Application in Surgical Sepsis:

The therapy of sepsis in surgical patients presents a formidable challenge that necessitates the integration of critical care measures with surgical intervention, hence expounding upon the already intricate pathway for sepsis management. Patient outcomes have improved over the past 30 years as a result of the use of evidence-based management algorithms and prediction severity scores (103-104). Surgical sepsis still has a substantial death rate, though, ranging from 30% to 40% (65). Although the exact biomolecular mechanisms causing sepsis are unknown, host responses that are known to occur include elevated oxidative stress (105), dysfunctional mitochondria (106), altered gene expression (107), and endothelial dysfunction (108). According to recent research, basic metabolic changes have a crucial role in the onset and treatment of sepsis (109-111). Stringer et al. (112) used H-NMR to assess plasma metabolite patterns in sepsis-induced acute lung damage (ALI). According to this study, there is a correlation between the need for a ventilator and acute physiology scores as well as changed levels of glutathione, adenosine, and myoinositol in ALI (112). The scientists concluded that using this method could produce metabolite-based profiles that would help define ALI and group patients depending on how their cases are anticipated to progress clinically.

An NMR-based metabolic profile approach was published by Mao et al. (113) for the assessment of trauma patients who are severely unwell. Serum samples from 26 patients with multiple organ dysfunction syndrome (MODS), patients with systemic inflammatory response syndrome (SIRS), and healthy controls were examined. The study identified significant changes in amino acid and

carbohydrate metabolism throughout the SIRS phase, with a shift towards disordered fat metabolism signaling the development of MODS (113). It also created metabolite-based models corresponding with clinical status. According to the authors, NMR-based profiling is a useful tool for assessing how trauma patients' serious illnesses develop over time. Nosocomial infections are linked to higher hospital expenses, decreased health-related quality of life, and increased mortality in surgical critical illness (113). They also often aggravate the illness. Pathogen identification and typing using traditional culture-dependent approaches is challenging, time-consuming, and has low sensitivity (114). Furthermore, there are therapeutic problems associated with the rising prevalence of drug-resistant pathogenic strains (115). It has been demonstrated that various infections can cause different host metabolic reactions in biofluids that are easily accessible, such serum and urine (116). For instance, lipopolysaccharide signatures unique to a certain species can be identified and quantified using MS-based profiling, which eliminates the need for culture (117) and offers quick, precise diagnostic results. In addition to providing novel insights into pathogen-host interactions, these culture-independent metabolic profiles can provide metabolic phenotypes specific to a given species, which can be used to inform targeted therapy, monitoring, and diagnosis (74). Pathogen invasion upsets the typical symbiotic interaction between the host and native gut microbial communities, as recent research has confirmed (118). By utilizing pre-, post-, and synbiotics to resynchronize the host-microbiota axis, individualized commensal modulation methods can be generated by analyzing this interplay through the use of new metagenomics techniques (119).

These exploratory investigations have significant ramifications for the prognostic assessment of sepsis in the future. Sepsis may be effectively characterized by metabolic phenotyping of surgical critical illness, which considers distinct metabolic data points. By using these data points, treatment plans might be more individually tailored, and innovative targeted therapeutics aimed at restoring host metabolism to pre-sepsis levels could be created. In the future, it might be able to identify "patients at risk," individuals who appear metabolically susceptible to organ malfunction and/or sepsis later on. This could reveal which patients require particular therapies or medications in order to "super-optimize" their metabolism in preparation for surgery.

Phenotyping Journeys:

With the development of metabotyping techniques, the notion of the patient journey in diagnosis and treatment has undergone substantial change. These techniques facilitate the development of improved diagnostic biomarker profiles at every phase of the patient's therapeutic experience, supporting differential diagnosis and therapy response evaluation. This is further enhanced by pharmacometabonomic techniques, which generate predictive models of therapy outcomes based on pre-intervention profiles of biofluids such as plasma or urine. For example, investigations using NMR spectroscopy have successfully predicted drug metabolism and toxicity based on urine profile in humans and experimental models. Metabolic profile methods have demonstrated potential in improving sepsis detection and predicting outcomes in trauma patients based on specific blood biomarkers in clinical settings, particularly critical care, where prompt decisions are vital. This capacity may lower mortality and healthcare expenses linked to unfavorable outcomes in addition to enhancing clinical decision-making. Moreover, current diagnostic and prognostic tools frequently lack the precision required for modern surgical precision in surgical practice, which necessitates highly customized therapeutic approaches. Surgical choices are usually made on the basis of risk assessments utilizing retrospective models or conventional biomarkers, which may not account for the intricacy of each patient's reaction or external factors like dietary plans and operating medications. Metabonomic profiling integration into surgical practice holds potential to overcome these issues. Through more in-depth understanding of patient-specific metabolic reactions during surgery, this method may enable more individualized interventions and better postoperative results. A phenotypically augmented clinical trial approach, which incorporates molecular data to supplement clinical trials, may greatly advance our knowledge of responder and non-responder phenotypes in a range of disease conditions. All things considered, the integration of metabotyping throughout the patient journey—from diagnosis through treatment and surgical procedures—holds the potential to completely transform clinical practice by offering more individualized and efficient treatment plans founded on molecular insights (120-130).

The evolving field of metabonomics is revolutionizing surgical practice by providing real-time insights into patient metabolism during surgical procedures. This approach not only helps in predicting outcomes and assessing risks but also aids in understanding the complex interplay between metabolic changes and surgical interventions (**Fig. 4**).

Key Points and Applications of Metabonomics in Surgery:

1. **Enhanced Surgical Decision-Making**: Metabonomic profiling allows for the objective quantification of environmental influences on patient outcomes during surgery. This includes monitoring changes

in metabolite levels that may indicate early clinical deterioration or predict adverse outcomes post-surgery.

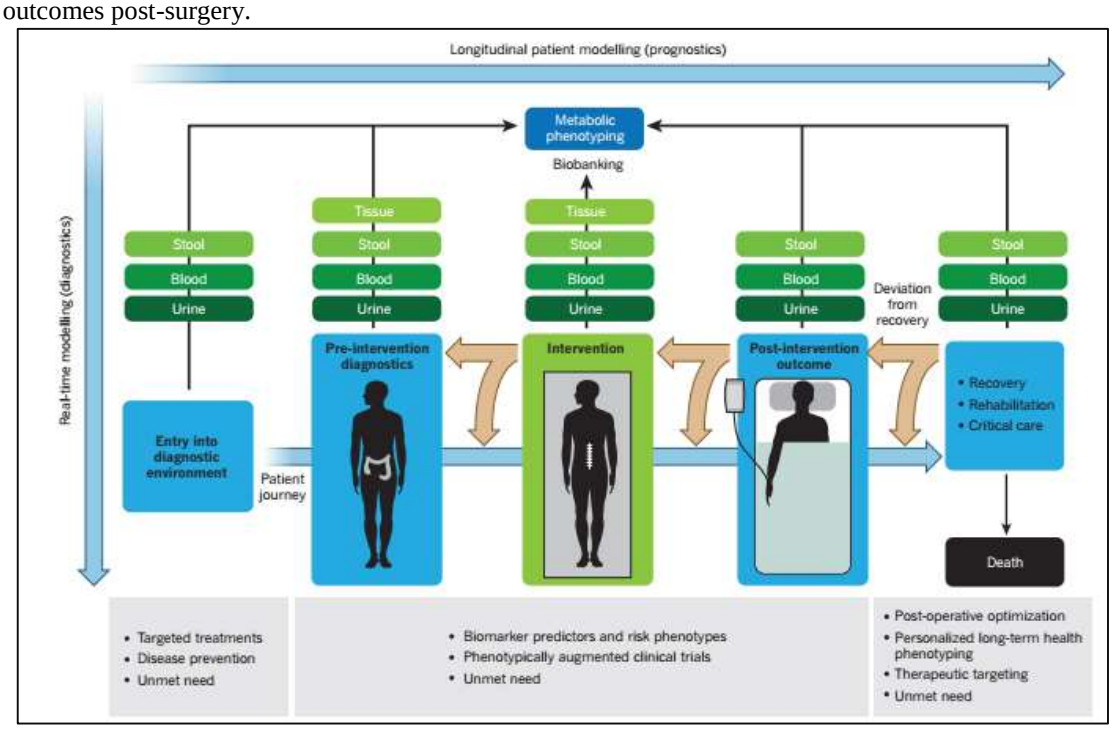

Fig. 4: Phenotyping Journy (Nicholson, et al. 2012).

2. **Predictive Biomarkers**: Studies using mass spectrometry and NMR spectroscopy have identified specific metabolites predictive of outcomes in cardiac surgery and trauma. For instance, short-chain acylcarnitines and ketone-related metabolites have been associated with poor operative outcomes, demonstrating the potential for personalized risk assessment.
3. **Transplant Surgery**: Metabonomics plays a critical role in transplant surgery by predicting graft failure, assessing organ toxicity, and evaluating hypoxic injury. This includes applications in kidney, liver, and gut transplantation, where rapid molecular diagnostics are essential for assessing graft suitability and survival.
4. **Microbiome Analysis**: Metabonomic techniques are also advancing our understanding of the gut microbiome's role in surgical recovery. They help in detecting shifts in microbiota composition post-surgery, such as those observed after bariatric procedures, which may influence long-term health outcomes and metabolic profiles.
5. **Aging and Surgical Considerations**: Metabonomic studies highlight age-related changes in gut microbiome function, influencing surgical outcomes in older patients. This underscores the need for tailored pre-operative nutritional strategies that consider metabonomic measures of gut health to mitigate surgical morbidity.
6. **Future Directions**: Integrating metabonomic technologies into surgical practice promises to enhance perioperative care by providing real-time functional insights into the human-microbiome axis. This approach is particularly crucial for vulnerable patient groups, such as older adults, where understanding metabolic responses can significantly impact surgical outcomes. In conclusion, metabonomics represents a promising avenue for personalized medicine in surgery, offering a deeper understanding of metabolic responses to surgical interventions and aiding in the development of targeted therapeutic strategies for improved patient care (131-135).

Metabonomics is transforming oncological surgery by providing advanced tools for real-time, precision-based diagnostics and intervention guidance. Here's how various metabonomic technologies are enhancing surgical outcomes, particularly in the context of oncology:

Applications of Metabonomics in Oncological Surgery:

1. **Magic-Angle-Spinning (MAS) NMR Spectroscopy**:
   - **Application**: MAS-NMR spectroscopy enables rapid differentiation between benign and malignant tissues with high

sensitivity and specificity. It has been extensively utilized in brain tumors, prostate cancer, and other malignancies.
    - **Benefits**: Provides detailed biochemical profiles that enhance MRI-based tumor characterization and can be performed within a short time frame (10–20 minutes), making it suitable for intraoperative settings.
2. **Mass Spectrometry Imaging (MSI)**:
    - **Application**: MSI, including matrix-assisted laser desorption/ionization (MALDI) imaging, offers molecular fingerprinting of tissues at a histologically specific level.
    - **Benefits**: While MSI is slower than MAS-NMR, it provides instantaneous tissue identification and can be used for interactive surgical guidance. It eliminates inter-operator variability in histological data and offers potential for automated analysis.
3. **Rapid Evaporative Ionization Mass Spectrometry (REIMS)**:
    - **Application**: Developed for in situ analysis during surgery, REIMS ionizes tissue molecules generated by thermal surgical instruments (e.g., electrosurgery).
    - **Benefits**: REIMS provides real-time, descriptive data comparable to traditional histopathology, with rapid feedback to surgeons (less than 0.9 seconds). It has been successful in identifying various cancers and metastases with high concordance rates with classical histology.

Advantages and Clinical Implications:

- **Precision in Margin Assessment**: Metabonomic techniques such as MAS-NMR and REIMS offer objective, real-time assessment of tumor margins during surgery. This helps reduce the need for re-excision by providing more accurate clearance assessments.
- **Enhanced Surgical Decision-Making**: By integrating with surgical instruments, REIMS allows for immediate tissue characterization, aiding in precise excision and minimizing damage to healthy tissue.
- **Future Directions**: Continued advancements in metabonomic technologies aim to improve spatial resolution and automation capabilities, further enhancing their utility in surgical oncology. This includes broader applications in neurosurgery, gastrointestinal surgery, and beyond.

In conclusion, metabonomics represents a pivotal advancement in oncological surgery, offering rapid, precise, and objective tools that augment traditional surgical techniques. These technologies not only improve surgical outcomes but also pave the way for personalized treatment strategies tailored to individual patient needs (136-139).

Conclusion:

In conclusion, the integration of advanced metabonomic technologies into surgical practice marks a transformative leap towards precision medicine in oncology and critical care. Metabonomics, encompassing techniques such as MAS-NMR spectroscopy, mass spectrometry imaging (MSI), and rapid evaporative ionization mass spectrometry (REIMS), empowers surgeons with real-time, molecular-level insights that redefine diagnostic accuracy and therapeutic decision-making. MAS-NMR spectroscopy emerges as a cornerstone in intraoperative tumor assessment, offering rapid differentiation between benign and malignant tissues with high sensitivity. Its ability to provide detailed biochemical profiles aids in precise tumor margin evaluation, reducing the need for costly and invasive re-excision procedures. Similarly, MSI, particularly MALDI imaging, enables histologically specific molecular mapping of tissues, revolutionizing the visualization of tumor boundaries and guiding surgical interventions with unprecedented accuracy. Although MSI entails longer analysis times, its capability to provide instant tissue identification and potential for automated analysis underscores its role in enhancing surgical outcomes. REIMS represents a groundbreaking approach by integrating mass spectrometry with surgical instruments to provide real-time tissue characterization during operations. This technique's ability to swiftly analyze tissue composition aids in on-the-spot decision-making, ensuring optimal excision margins and minimizing collateral damage to healthy tissue. Its successful application in identifying various cancers and metastases underscores its potential to replace conventional histopathology with more precise, immediate results. The implications of these metabonomic technologies extend beyond oncological surgery, with applications in trauma management, transplant surgery, and beyond. By bridging the gap between molecular biology and clinical practice, metabonomics not only enhances diagnostic precision but also lays the foundation for personalized treatment strategies tailored to individual patient profiles. Looking forward, ongoing advancements in spatial resolution, automation, and integration with surgical workflows promise to further refine these technologies' utility.

As they continue to evolve, metabonomic approaches are poised to redefine the standards of care in surgical oncology, offering clinicians unprecedented tools to improve patient outcomes and quality of life.

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