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Critical Care

REVIEW

Metabonomics and intensive care

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Abstract

This article is one of ten reviews selected from the Annual Update in Intensive Care and Emergency medicine 2016. Other selected articles can be found online at http://www.biomedcentral.com/collections/annualupdate2016. Further information about the Annual Update in Intensive Care and Emergency Medicine is available from http://www.springer.com/series/8901.

Background

Metabonomics is "the quantitative measurement over time of the metabolic responses of an individual or population to drug treatment or other intervention" [1], such as a disease process, and provides a 'top-down' integrated overview of the biochemistry in a complex system. The metabolic profile is determined by both host genetic and environmental factors [2]. As such, metabonomics has great potential for intensive care medicine, where patients are complex and understanding the relationship of host factors, disease and treatment effects is key to improving care. Approaches that focus on single or small sets of biomarkers may fail to capture this complexity, so metabonomics may have advantages for both understanding diseases and improving diagnostics and treatment monitoring.

Spectroscopic techniques, including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry, have been used to determine the global metabolic profiles of numerous types of biological samples. Most commonly, blood and urine are analyzed but any biological specimens, including tissue, cerebrospinal fluid or exhaled breath condensate, can be used [3–6]. Metabonomic methods have been used to evaluate numerous clinically-significant conditions including trauma [7, 8], acute kidney injury (AKI) and monitoring of dialysis [9–11], subarachnoid hemorrhage [12], and acute lung injury (ALI) [13].

The two broad analytical platforms, NMR and mass spectrometry, each have their own strengths and weaknesses and together give complementary information. Data can be acquired that either provides non-targeted global metabolic information, which is useful for initial biomarker discovery, or can be targeted to obtain detailed information on a specific class of metabolites or metabolic processes.

Nuclear magnetic resonance spectroscopy

NMR spectroscopy harnesses the magnetic properties of certain nuclei that possess spin, for example ^{1}H and ^{13}C. Commonly in metabonomics, ^{1}H or proton NMR is used. NMR spectrometers use superconductors to generate a strong magnetic field (Fig. 1). A spinning charge placed in such a magnetic field produces two spin states: one up, aligned with the magnetic field; and one down, aligned against the magnetic field. The energy difference between the two spin states is influenced by the local electron environment, which acts to shield the nucleus. When a sample containing these nuclei is excited with a radio frequency pulse, those nuclei in the lower energy spin state excite into the higher energy state and the subtle differences in the resonances generated can be used to give information regarding chemical structure. Resonances are reported in relation to a reference signal, such as 3-(trimethyl-silyl) propionic acid (TSP) or tetramethylsilane (TMS), and in order to account for magnetic fields of different strengths these values are given as chemical shifts in parts per million (ppm).

Chemical shifts are predictable based on the local electron shielding and give information about the structure of the molecule. The magnitude or intensity of NMR resonance signals is displayed along the vertical axis of a spectrum, and is proportional to the concentration of the sample.

Typically, ^{1}H NMR spectra of urine contain thousands of narrow, low molecular weight metabolites, whereas those from serum and plasma contain a mixture of low

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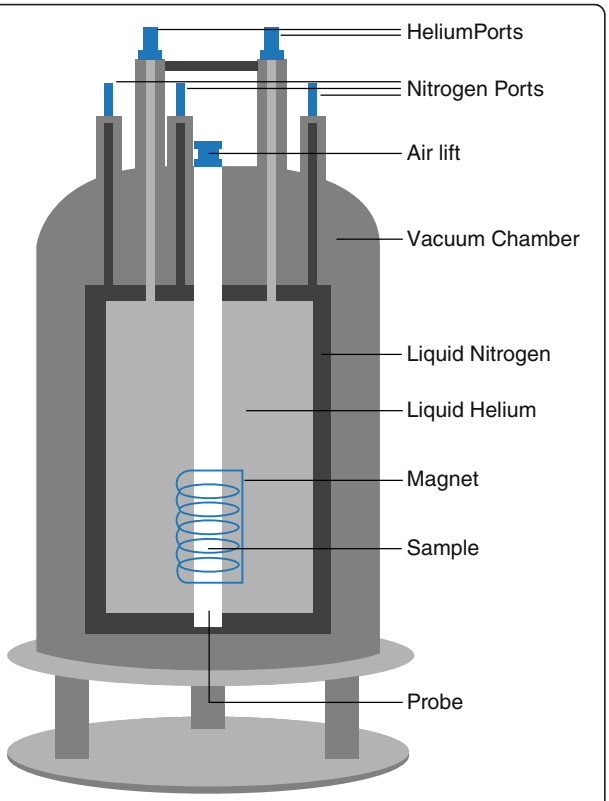

Fig. 1 Schematic diagram detailing the main components of a nuclear magnetic resonance (NMR) spectrometer

and high molecular weight compounds (Fig. 2). Experimental pulse sequences can be chosen to selectively suppress particular spectral features; for example, the Carr-Purcell-Meiboom-Gill (CPMG) sequence will suppress large molecular weight metabolites revealing those of a smaller weight. Common to all experiments is the need to suppress the large water peak and this is achieved with a solvent suppression pulse sequence.

For the purposes of metabonomics, ^{1}H NMR has several strengths. Little sample preparation is required, and the technique is relatively non-destructive, quantitative, and non-invasive. Data obtained from NMR experiments are reproducible [14] and robust. Concentrations of metabolites are detectable down to micromole/l concentrations and analysis is relatively quick, taking as little as 3–4 min per sample.

Mass spectrometry

Mass spectrometryis a technique that aims to identify metabolites within a sample based on the detection of the mass-charge ratio (m/z) of ions produced by the ionization of chemical compounds. Molecules in a sample are vaporized before being ionized by bombardment with either electrons or other ions. The molecule is thus broken into charged fragments which can be sorted based on their m/z ratio and detected by a device capable of detecting charged particles. Several techniques exist to separate and detect molecular fragments; an example is separation by accelerating ions and subjecting them to an electric or magnetic field (Fig. 3). The recorded data can be displayed as a spectrum of the relative abundances of the various ions with the same m/z ratios.

In order to improve mass separation, mass spectrometry is often coupled to chromatographic techniques. Such techniques include gas chromatography-mass spectrometry (GC-MS), where a gas chromatogram is used to separate molecules in gaseous phase before they are fed into the ion source. Liquid chromatography-mass spectrometry (LC-MS) and high performance liquid chromatography-mass spectrometry (HPLC-MS) similarly separate molecules in a sample in a liquid mobile phase using a liquid chromatogram with a combination of organic solvents prior to ionization.

Mass spectrometry-based platforms have the advantage of greater sensitivity compared to NMR; however, some substances, such as sugars and amino acids, are difficult to analyze with this method due to their polarity and lack of volatility [15]. Mass spectrometry requires reasonably extensive sample preparation and, with long chromatographic times, can take longer to process than NMR. Also, because of the need to vaporize and ionize the sample, mass spectrometry is a more destructive analytical technique than NMR.

Data analysis

Analytical techniques used in metabonomics generate data sets that are unlike those produced in many other scientific fields. Whereas there would often be many more subjects than variables, metabonomics generally produces thousands of variables, several of which may correlate, and many may not be normally distributed. These features pose problems for regular statistical methods so analysis is generally performed using multivariate statistics. Broadly speaking multivariate methods can be split into unsupervised tests, where no class information is supplied to the model, and supervised tests, designed to look for group separation based on class information. Unsupervised tests are good at finding natural clustering within the data sets and at identifying outliers. Supervised tests, on the other hand, look for variation between predefined groups or classes and are able to build predictive models.

Principal component analysis

Principal component analysis (PCA) is a common method of unsupervised multivariate analysis used in metabonomics. It is used to elucidate the covariance

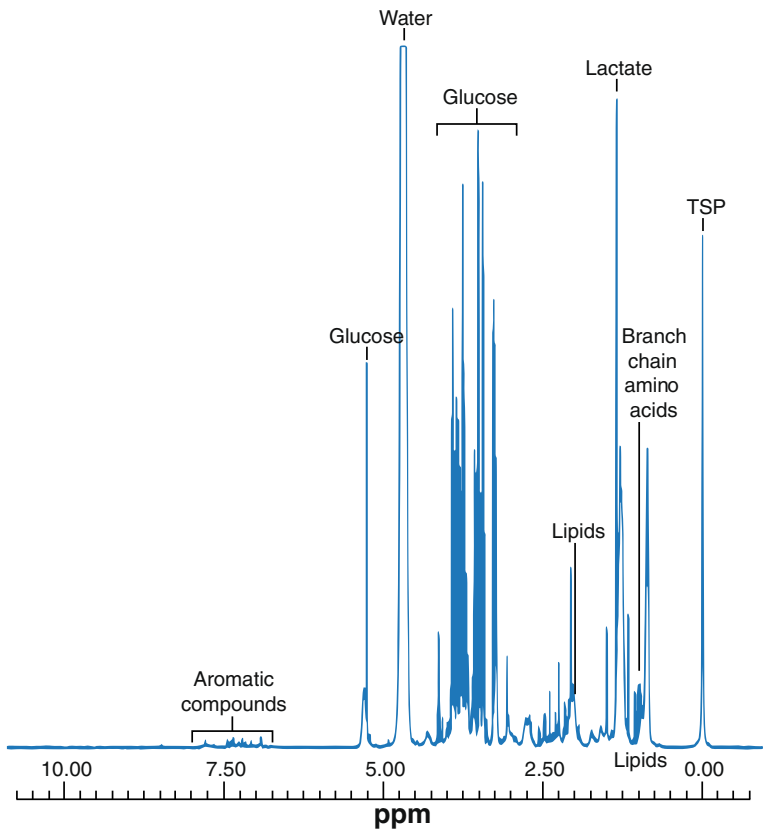

Fig. 2 Example of a ^{1}H nuclear magnetic resonance Carr-Purcell-Meiboom-Gill (NMR CPMG) spectrum of human serum. TSP: 3-(trimethyl-silyl) propionic acid

structure of the data set by representing the data along new axes based on the direction of the maximum variation, the principal components. The first principal component is the direction of greatest variation and the second principal component is that with the second largest value that is orthogonal to the first (Fig. 4). This method of analysis allows data reduction. Some components will contain very little variation and those with low magnitudes, which contain little information, are discarded. The data can then be re-displayed using the

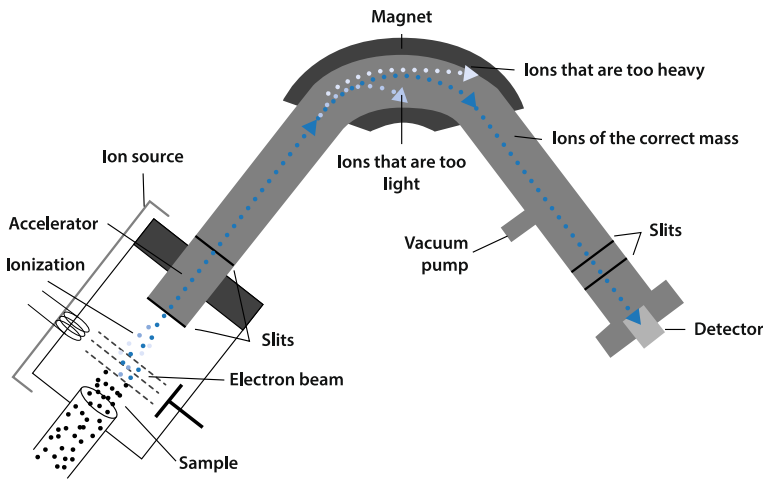

Fig. 3 A schematic diagram of the component parts of a mass spectrometer

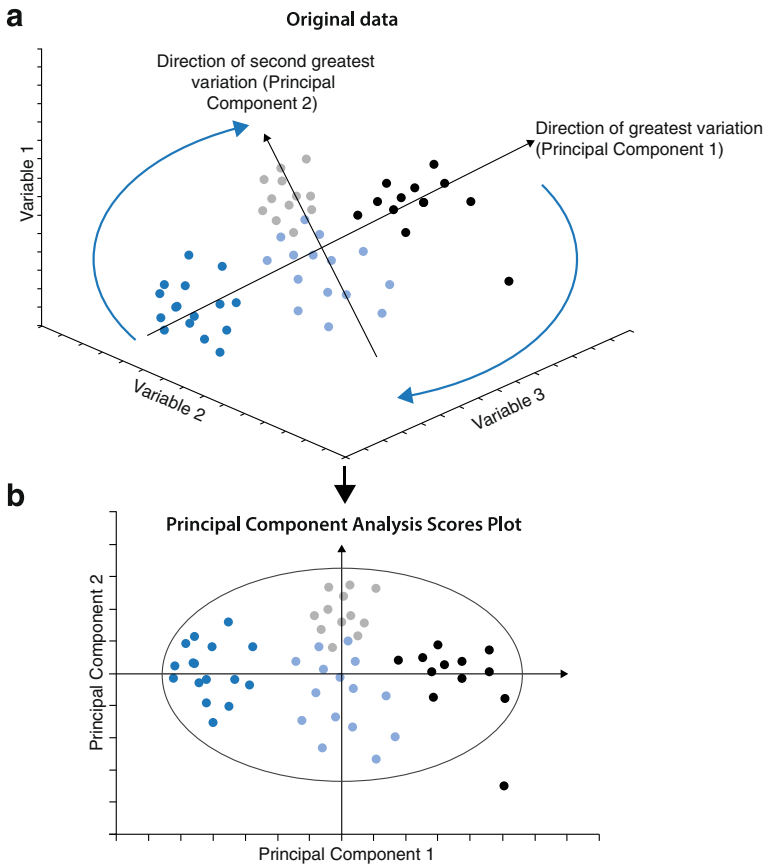

Fig. 4 Demonstration of how a multivariate set of data (**a**) is converted into a principal component analysis (PCA) scores plot (**b**) by detecting the directions of greatest variation and converting these into a new set of axes. The *circle* represents Hotelling's ellipse

principal components as a new set of axes, giving a PCA scores plot (Fig. 4).

An approximation to the Student's t-test, called the Hotelling's ellipse, can be projected onto the scores plot. This gives an indication of a 95 % confidence interval within which 95 % of observations should fall. Data points lying outside of this ellipse can be considered as outliers and can be examined in more detail.

Supervised analysis

Supervised multivariate analysis is aimed at finding the variation in the data matrix that explains predefined classifications. One of the underlying methods of supervised analysis is the partial least squares analysis (PLS). PLS determines the underlying relationship between two data matrices, one that contains the sample data and a second containing class information. This method finds the fewest variables that account for the differences in the class matrix. Overall, the goal is to predict cases and controls from metabolic data. Extensions of the PLS occur with orthogonal partial least squares (OPLS), which works in a similar fashion to PLS. However, in this method the variation in the data is divided into that which explains class separation and that which is orthogonal to it and does not explain class. For clinical studies, supervised analysis allows large metabonomic data sets to be reduced to variables that are important in separating cases and controls without losing predictive power.

To assess the predictive capacity of a model, cross validation can be carried out. A number of methods exist to do this but a commonly utilized approach is to leave out every n^{th} row in the data matrix and build a model based on the remaining data. The remaining data can then be predicted by the model and the results compared to the expected outcome. This process can then be repeated until all of the data has been left out once. After cross validation it is possible to derive two descriptive metrics for the models. The first is known as the R^2 and explains the amount of variation between the classification groups that is explained by the model. This value ranges from 0 to 1.0 with values approaching 1.0 explaining almost all of the variation in the model and lower values suggesting that much of the variation in the

data is irrelevant or noise. The second value is the Q^2, which represents the predictive capacity of the model, again ranging from 0 to 1.0. The expected values of both R^2 and Q^2 are dependent on the type of data being analyzed but in general should ideally be no more than 0.2 apart; for biological systems a Q^2 of 0.4 represents a reasonable predictive accuracy. However, the ideal way to test a model is to challenge it with a completely new set of data from a validation cohort of samples that have not been used to generate the model in the first instance.

Metabonomics and intensive care

Metabonomics work relevant to critical care has mainly focused on sepsis and infection. A range of studies has been carried out attempting to use metabonomic techniques to explore infection. Cells, animals and human subjects have been used, with both NMR and mass spectroscopy, allowing over 500 metabolites and pathways to be implicated in infective processes.

Several animal models have been used, testing different biofluids including blood [16–20], broncheoalveolar (BAL) fluid [16] and lymph [18] as well as tissue, such as lung [16, 17], liver [17, 21], kidney [22] and spleen [17, 22]. Infections as diffuse as cerebral malaria [22], influenza [23], tuberculosis [17], peritonitis [16, 18, 24] and *Escherichia coli* sepsis [20] have been investigated. Metabolites including amino acids, those involved in energy and carbohydrate metabolism, fatty acids and those associated with mitochondrial dysfunction have all been identified in these models.

In human subjects, several clinical infections have been subject to metabonomic investigation. A number of studies has been carried out examining urinary tract infection using NMR of urine samples [25–29] with an attempt to identify specific causative organisms, potentially allowing for rapid diagnosis and targeted treatment. Other specific infections investigated with metabonomics have included cerebrospinal fluid analysis to distinguish various forms of meningitis and ventriculitis [30] and sepsis from various causes in both adults [31–34] and children [35, 36].

Pneumonia remains a common cause for admission to critical care units and a small amount of work has been carried out investigating this condition using metabonomics. Animal studies have found elevated lipoproteins, triglycerides, unsaturated and polyunsaturated fatty acids, ω-3 fatty acids, lactate, 3-hydroxybutyrate and creatinine and reduced glucose, choline, phosphocholine and glycerophosphocholine levels in the plasma of rats infected with *Klebsiella pneumoniae* compared to controls [37]. Mice with pneumonia caused by *Staphylococcus aureus* or *Streptococcus pneumoniae* have been separated from control animals based on urine metabolic profiles [38].

Human studies have focused on community-acquired pneumonia (CAP). A study using mass spectroscopy analysis of plasma from children in Gambia with pneumonia found elevated uric acid, hypoxanthine and glutamic acid and reduced tryptophan and adenosine diphosphate levels in infected individuals [39]. Another study looking specifically at patients with *S. pneumoniae* pneumonia found 33 urinary metabolites used to separate cases from controls including citrate, succinate, 1-methylnicotinamide, several amino acids, glucose, lactate, acetone, carnitine, acetylcarnitine, hypoxanthine and acetate, most of which were increased in those with infection [40]. This study also aimed to address several potential confounding factors associated with this type of investigation by comparing cases to several control groups, such as those with other types of lung disease, those with other types of pneumonia and those with other acute illnesses. In almost all cases, metabolic profiling was able to distinguish cases of pneumonia from controls.

Work specifically within critical care has focused on the outcomes of patients with CAP and sepsis [41]. Analysis of plasma found higher levels of bile acids, steroid hormone metabolites, markers of oxidative stress and nucleic acid metabolites in non-survivors; however, the statistical models based on these differences had only modest sensitivity with an area under the receiver operating curve of 0.67. Other work in ICU patients has looked at predisposition to sepsis following trauma. Using NMR of plasma samples from 21 trauma patients, valine, citrate, aspartate, allantoin and hydroxybutyrate were identified as associated with the future development of sepsis [42]. Looking at adults with established sepsis on the intensive care unit (ICU), glycerophospholipids and acetylcarnitines were elevated in 33 septic patients when compared to 30 others with non-infective systemic inflammation [34]. In other investigations, metabonomic techniques have been used to try to predict mortality in 37 ICU patients with sepsis [33] and have looked at sepsis in 137 children from different age groups admitted to critical care [36]. In an attempt to explore mortality in adult ICU patients [43], plasma was analyzed with mass spectroscopy and the results showed that 31 metabolites were associated with mortality, most of which were elevated in those who died. As with the other studies, these covered a range of metabolites including lipids, carbohydrates and amino acids. Only six metabolites were greater in those who survived and these were all involved in the lipid metabolism pathway. A metabonomic study in sepsis-induced lung injury [13] compared 13 patients with ALI or acute respiratory distress syndrome (ARDS) to six healthy controls and found differences in plasma levels of glutathione, adenosine, phosphatidylserine and sphingomyelin. In another study using LC-MS of BAL fluid, several lipid metabolites

and amino acids were increased and a component of surfactant decreased in those with ARDS compared to healthy controls [44].

The studies outlined above demonstrate that, although only a small number of studies have been conducted in intensive care patients to date, common to all are the finding that a large number of metabolites and metabolic pathways are deranged in critical illness. These range from energy and lipid metabolism to amino acid and steroid hormone synthesis, most of which are not currently routinely measured. Not only are a range of pathways involved in critical illness but specific elements of these may be up- or down-regulated in different contexts, even within different individuals with similar diseases. At present, it is still too early to draw firm conclusions regarding the role of metabonomics in diagnosis, prognostication or monitoring of treatment effect within ICU patients. However, from the limited work done already, the ability of metabonomics to monitor such a diverse range of markers makes it an attractive approach for biomarker discovery and for understanding the subgroups or phenotypes of patients admitted to critical care. The ability of metabonomics to simultaneously measure several metabolites from a range of metabolic processes allows some understanding to be gained, not only about the impact of critical illness on individual pathways, but the interaction of many metabolic processes during illness. A further understanding of these complex interactions may aid in the identification of phenotypes of patients that are currently not clinically apparent and that may respond to treatments differently, previously termed stratified medicine but now referred to as precision medicine. Further metabonomic research within critical care should focus on addressing current challenges, such as monitoring treatment effect with the early identification of non-responders, identifying phenotypes of sepsis and ARDS that may respond differently to treatment or ventilation strategies and assisting in making challenging diagnoses such as early identification of ventilator-associated pneumonia (VAP).

Conclusion

Metabonomics is a relatively new scientific discipline that aims to explore the changes in global metabolic profiles in response to exogenous influences such as disease states or treatments as well as host factors. Broadly two analytical methods are employed, NMR and mass spectroscopy, to measure a vast array of metabolites that require specialist multivariate statistics for analysis. So far only a limited amount of work has been carried out in intensive care patients. However, from these studies, perturbation in a number of metabolic pathways has been implicated in critical illness. The ability to explore several metabolic processes simultaneously, and their interactions, is an exciting prospect for intensive care medicine. The complexity of this group of patients and the growing understanding that subgroups of patients may require tailored treatments suggests that metabolic profiling or phenotyping may help to improve diagnostics or target treatment strategies in clinical trials and clinical practice.

Competing interests
The authors report no competing interests in relation to this article.

Authors' contributions
Both authors drafted, read and approved the final manuscript.

Declarations
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