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REVIEW

Predicting and monitoring treatment response via 1-H NMR metabolomics of plasma, serum, and urine

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Abstract
The field of metabolomics is witnessing a rapid growth in its applications. Applications related to biomarker discovery for the purposes of disease diagnosis constitute a major part of that growth. Of equal interest is the ability to predict and assess treatment responses in various diseases. Obtaining such information before the start of treatment or early on during the treatment, without the need to perform invasive biopsy procedures, would greatly improve patient care and management. This would allow the physician to find alternative treatments and/or modify or stop ongoing treatment when no-response or adverse effects are observed, thereby sparing the patient from continuing with unnecessary and/or toxic treatments. The latter is especially important in the treatment of many cancers. The current literature on the applications of metabolomics to the monitoring of treatment is limited. Here we compile NMR-based metabolomics studies on plasma, serum, and urine geared towards such applications focusing on cancer, hepatotoxicity, neurological disorders, transplants (renal and liver), and sepsis. We discuss the challenges faced in these applications along with approaches to address these difficulties. Finally, insight into the future of this approach and how it can move closer to clinical utility is shared.

Keywords Metabolomics · NMR spectroscopy · Plasma · Serum · Treatment response · Urine

Background

The suffix *-omics* generally refers to fields of study in biology, such as genomics, proteomics, and transcriptomics. A more recent addition to this list is "metabolomics"—the study of the metabolome, representing the collection of metabolites present in a cell, tissue, or biological fluid (Kaddurah-Daouk et al. 2008). Metabolomics has an advantage over other *-omics* platforms because of its greater ability to predict phenotypic properties and disease risk (Chu et al. 2019).

Biopsies remain the gold standard for obtaining ultimate diagnostic and prognostic information on a patient. However, biopsies are invasive in nature and prone to sampling errors. Furthermore, performing repetitive biopsies to determine disease progression and/or assess treatment response is unfeasible. Obtaining such information from body fluids presents a non-invasive or minimally invasive alternative to the biopsy procedure. There are several body fluids that can be used for this purpose including plasma, serum, urine, cerebrospinal fluid (CSF), saliva, semen, amniotic fluid, etc. This review, however, will focus only on the use of plasma, serum and urine as these samples are the most commonly used ones. Advantages and disadvantages of using each sample will be discussed. Since these samples require different sample collection/preparation and data acquisition methods, they will be discussed separately.

The current metabolomics literature has focused primarily on diagnostic applications relying primarily on the change in the metabolome that serves as a marker for a particular disease (Zhang et al. 2012). However, the same concept can also be used to monitor the progression of the disease, the assessment of treatment response, and prediction of response with some modification of protocols. Beyond finding diagnostic information, it is equally important to be able to determine the prognosis of the disease and also assess treatment response over time (via analyzing body fluids collected at various selected time points over the course of the

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treatment). Assessing treatment response over time is essential as it can assist in optimizing and modifying the treatment in a more personalized fashion (Garcia-Simon et al. 2015; Sumner et al. 2010). For example, the treatment for tuberculosis is standardized in which every patient receives the same dosage of the medication for the same duration of time. Such standardization can lead to insufficient treatment in some or overtreatment in others. Although the literature on such applications is scant, an effort was made in this review article to put together the available information in a coherent manner.

The major instruments used to study metabolic profiles of biological fluids include NMR, LC–MS, and GC–MS (Kaddurah-Daouk et al. 2008). While mass spectrometry enjoys higher sensitivity, NMR spectroscopy has several advantages including: minimal sample preparation, high reproducibility, non-destructiveness of sample, and allowing quantitative measurement of metabolites (Huang et al. 2022). Hence, this review article will only focus on NMR-based metabolomics.

Blood plasma and serum analysis

Sample characteristics

Both serum and plasma are commonly used in metabolomics research. Overall, there is not much difference in the types of metabolites found in the two samples. However, the concentrations of most metabolites differ significantly between them. Plasma is obtained by adding anticoagulants to whole blood and then removing cells (platelets and both red and white blood cells) by centrifugation, while serum is obtained using the same protocol without anticoagulants so that fibrinogen and other clotting factors are also removed during the centrifugation step (see below).

The advantage of plasma is its quick processing time without the need to wait for blood clotting. The coagulation time (30–60 min) and room temperature during the handling introduce variables, and these changes have been shown to affect the analysis of serum metabolite components. In contrast, plasma is more suitable for metabolite analysis as it does not undergo the blood clotting process, and the results are easier to replicate. If serum is chosen as the sample, it is necessary to standardize the clotting time for all samples.

Detectable metabolites in blood plasma attach to albumin, a protein that is highly abundant in blood plasma and a main transporter of metabolites. Jupin et al. have found that the affluence of albumin in blood plasma has a negative effect on metabolite concentration (Jupin et al. 2014). The higher metabolite concentration in serum may be related to the volume displacement effect caused by the deproteinization in serum samples as a result of separation from the blood clot and absence of an anti-coagulant. This allows for better distribution of smaller molecular weight metabolites in serum resulting in higher concentrations.

Repeated measurements of 122 metabolites on 83 samples of plasma and serum (Yu et al. 2011) showed a high correlation between the first and second measurements with mean correlation coefficient (r) of 0.83 (plasma) and 0.80 (serum). Although the reproducibility for both samples was high, the difference in the correlation coefficients between the two samples was statistically significant ($p = 0.01$). Furthermore, the study showed that metabolite concentrations were generally higher in serum than in plasma. Out of 122 metabolites, 104 (85%) were significantly higher in serum and the average value of the relative difference over all metabolites was 11.7% higher in serum (Yu et al. 2011). However, other considerations such as hemolysis should also be taken into account as it affects serum more than plasma. A study done to compare the use of whole blood and serum for sepsis metabolomics found that the red blood cell hemolysis in serum resulted in lower metabolite concentrations (Stringer et al. 2015). The authors also noted that serum provides higher sensitivity than plasma when average metabolite concentrations were compared between subjects with different phenotypes. As shown in Table 1, 40 metabolites in serum were identified to have significantly different mean concentrations when comparing type 2 diabetes (T2D) patients vs. non-T2D individuals, while plasma only revealed 25 metabolites with statistically significant differences. Similar results were also obtained when comparisons were made between male and female subjects, and smokers vs. non-smokers, with serum always showing larger number of significantly different metabolites. The ability for serum samples to detect more metabolites is a major advantage over plasma for metabolomic studies. The reason for this higher sensitivity in serum samples is more likely due to the lack of proteins in serum that would otherwise affect metabolite concentrations.

Sample collection and preparation

Sample preparation is no less important and crucial than data acquisition. Proper preparation is needed to obtain optimal results and make accurate conclusions. The preparation of serum and blood plasma differ from each other in that an

Table 1 Number of metabolites showing statistically significant differences in plasma and serum (Yu et al. 2011)

	Plasma ($n = 377$)	Serum ($n = 377$)
T2D vs. non-T2D (51 vs. 326)	25	40
Male vs. Female (197 vs. 180)	62	69
Smoker vs. non-smoker (45 vs. 332)	4	6

anti-coagulant is added to plasma samples. Common anti-coagulants such as EDTA, heparin, or citrate are used for plasma collection (Yu et al. 2011). As for serum, the blood sample is commonly left to coagulate on ice for a maximum of 2 h, then the top layer (serum) is extracted from the blood clot. Both plasma and serum samples are centrifuged (1600 g for 15 min at 4 °C) before the addition of sodium azide – a chemical commonly added for preservation purposes. The addition of sodium azide does not result in any unwanted signal in the spectrum (^{1}H NMR) as it does not contain any hydrogens. Before the addition of solvent for 1-H NMR analysis, sera and plasma can be both stored at −80 °C for future analysis (Beckonert et al. 2007). When samples are ready for 1-H NMR analysis, they can be left at room temperature to thaw and 200 µL of serum or plasma is collected and mixed with 400 µL saline (0.9% NaCl in 10%D_2O). The supernatant is then centrifuged at 13400 g for 5 min. Only 550-µL aliquot of supernatant is needed for the NMR analysis. If further analysis is to be done later, the solution can be frozen at −40 °C until it is ready for another analysis (Teahan et al. 2006). Detailed protocols for plasma and serum metabolomics have been published by Nagana Gowda et al. (Gowda and Raftery 2019) and Emwas et al. (Emwas et al. 2025).

NMR data acquisition and analysis

Blood samples contain ~ 95% of water which is observed in a 1-H NMR spectrum unless water suppression is applied. If water suppression is not taken into account, a wide range of important metabolites will not be visible in the spectrum. There are many experimental techniques for water suppression which need to be performed for metabolomic analyses to retrieve interpretable 1-dimensional (1D) NMR spectra. Common techniques include NOEPR (1D NOESY pulse sequence with water presaturation), WET (water suppression enhanced through T1 effect), WATERGATE (water suppression by gradient tailored excitation), and excitation sculpting (ES) (Kruk et al. 2016). NOEPR is the most popular technique because it can obtain great water suppression with high reproducibility. The disadvantage of the WATERGATE technique is that it provides weak water suppression and lacks sensitivity (Aranı´bar et al. 2006). WET is a technique that uses a shorter pulse than a presaturation approach which abstains from baseline misrepresentation and can easily suppress other solvent signals if needed; however, WET has disadvantages including improper use which leads to false inferences (Barding et al. 2012). Water suppression by excitation sculpting has seen improvements with CW on-resonance saturation pulse or WGL (adiabatic frequency modulation pulse during relaxation delay) (Kruk et al. 2016). Excitation sculpting removes the water signal completely but also affects the resonance peaks around the water signal (Capati et al. 2017).

1D NMR spectra have problems including overlapping of peaks which can be fixed by applying two-dimensional (2D) NMR spectroscopy. Common 2D NMR techniques include COSY (homonuclear correlation spectroscopy), TOCSY (total correlation spectroscopy), STOCSY (statistical TOCSY), and 2D JRES NMR (2D J-resolved nuclear magnetic resonance spectroscopy). The advantages and disadvantages of using the different 2D NMR techniques are discussed in a review by Kruk et al. (Kruk et al. 2016). The major 2D NMR technique used in metabolomics is 2D JRES NMR because it keeps the benefits of 1D NMR while simultaneously spreading overlapping signals into 2D, reducing resonance congestion and increasing metabolite specificity (Ludwig and Viant 2010).

The quantification of metabolite concentration relies on the peak integrals in the NMR spectrum. Known concentrations of internal standards also known as reference compounds include: 3-trimethylsilylpropionic acid (TSP), 2,2-dimethyl-2silapentane-5-sulfonate sodium salt (DSS), or tetramethylsilane (TMS); which are comprised in the NMR spectrum for quantification purposes. Another advantage of using these reference compounds is that their chemical shift (set at 0 ppm) does not interfere with the analytes of interest in the sample (Beckonert et al. 2007). Data analysis of a serum and plasma NMR spectrum is quite difficult and almost impossible to do with the human eye. Pattern recognition-based systems are built to analyze, compare, and contrast the complex signals in the NMR spectrum using multivariate methods of analysis. The rational for the use of pattern recognition in metabolomics is for profiling metabolites in the spectra of different cohorts to determine the similarities and differences that exist between them.

The use of multivariate analysis (MA) that allows simultaneous observation and analysis of more than two statistical variables is recommended in metabolomics. Multivariate analysis includes multiple variance analysis, multiple regression analysis, factor analysis, principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), cluster analysis, and machine learning.

Principal component analysis is a popular and useful linear transformation technique used in numerous applications including gene expression analysis and metabolomics studies. In metabolomics, where datasets are often high-dimensional and complex, PCA serves as a valuable tool for dimensionality reduction and data visualization. By reducing the data to its principal components, PCA helps in simplifying the dataset while retaining important information, making it easier for machine learning algorithms to process and analyze the data effectively.

One of the key benefits of PCA analysis is its unsupervised nature which makes it more straightforward in terms of parameterization. Unsupervised here refers to PCA not requiring class label information. This means there is

relative simplicity of the algorithm so that it may be computed on the platform initially and the key parameter is the number of components directly related to how much of the variance one wishes to maintain in the reduced data. PCA is often used as a preliminary step in data analysis and preprocessing for other machine learning tasks and enables visualization of high-dimensional data. While PCA itself may have few parameters, the preprocessing steps on the data like normalization and scaling can significantly impact its performance and the interpretation of the results. PLS-DA is another classification method in metabolomics data analysis, which combines regression models with dimensionality reduction and discriminant analysis of regression results using certain discriminant thresholds. PLS-DA is more commonly used in metabolomics because of its ability to achieve better classification (Long 2013; Barker and Rayens 2003; Chen et al. 2022).

A typical workflow for NMR metabolomics is shown in Fig. 1 (Huang et al. 2022). As shown in the figure, the workflow is divided into four main phases: (a) pre-analytical including sample collection and sample preparation, (b) data generation which involves NMR spectra acquisition, spectral processing, and the generation of spectral bins and/or metabolite concentrations, (c) data analysis including preprocessing, multivariate and univariate analysis, and (d) biological interpretation.

Multivariate analysis of NMR data is crucial for metabolomic studies, such as monitoring treatment response, drug efficacy, toxicity levels from drugs, or determining conditions of recurrence of a disease after receiving complete remission. It may be worthwhile to add here that in the event that NMR data needs to be reused for a different application, there are best practices that need to be followed for data storage and management (Gouveia et al. 2024). The following section takes a closer look into how plasma and serum biofluids are used for metabolomic studies via 1-H NMR for the applications listed.

Applications

Prediction of treatment response in breast cancer

Breast cancer is very common among women where 1/8 women has the disease. It is a clinically heterogeneous disease, so the outcome of treatment such as neoadjuvant chemotherapy varies among individuals. According to Jones et al., less than 30% of breast cancer patients show complete response to neoadjuvant chemotherapy (Jones and Smith 2006). The main clinical assessment to determine if administration of this treatment is working is done using MRI. Unfortunately, this is only performed after the completion of the treatment, so patients with no response or partial response are unnecessarily being exposed to toxic drugs which may induce adverse effects. A study done by Wei et al. used metabolomics as an approach for predicting response to neoadjuvant chemotherapy for breast cancer (Wei et al. 2012). Serum samples were collected 45 min before chemotherapy treatment (4 three-week EC cycle). Samples were analyzed using 1-H 1D 500 MHz NMR and LC–MS. Out of the 28 breast cancer patients (age: 37–60) included in the study, 8 had complete response (pCR), 6 had no response (SD), and 14 had partial response (PR). A summary of the data is shown in Table 2. Threonine showed significance difference between pCR vs. SD and PR vs. SD. Isoleucine only showed significance difference between pCR vs. SD. Glutamine was significantly different between pCR vs. SD and PR vs. SD. Histidine only showed significance between pCR vs. PR. The study showed a clear distinction of metabolites between the three groups. Using these findings, it is possible to clinically assess the treatment success of neoadjuvant chemotherapy as well as reduce the exposure of toxicity to patients who would not benefit from the complete chemotherapy cycle. Figure 2 shows PLS score plots delineating those who showed complete response, partial response and no response.

Assessment of drug induced liver injury (DILI)

Drug induced liver injury (DILI) is very common especially from treatment with acetaminophen, but there is no major clinical assessment to quantify DILI risk. A study done by Kim et al. evaluated plasma and urine biomarkers from humans that were treated with acetaminophen (Kim et al. 2013). The aim of the study was to find biomarkers in urine and plasma that could lead to predicting hepatotoxicity caused by drugs such as acetaminophen. In the plasma 1-H NMR spectral data, the results showed ten endogenous plasma metabolites: lactate, glucose, 3-hydroxyisovalerate, isoleucine, acetylglycine, acetone, acetate, glutamine, ethanol, and isobutyrate, which all increased significantly after acetaminophen treatment. The urinalysis results displayed endogenous urine metabolites that also show significant differences before and after treatment. A complementary post-analysis of both urine and blood plasma can be very helpful in monitoring the hepatotoxicity levels caused by acetaminophen treatment. This information can help the physician in deciding whether to continue the treatment or stop it. Figure 3 shows the expected plasma metabolic pathway change as a result of treatment with acetaminophen. Enhanced levels of lactate and glucose are consistent with mitochondrial impairment, which can lead to an inability to use pyruvate in the citric acid cycle (Kim et al. 2013).

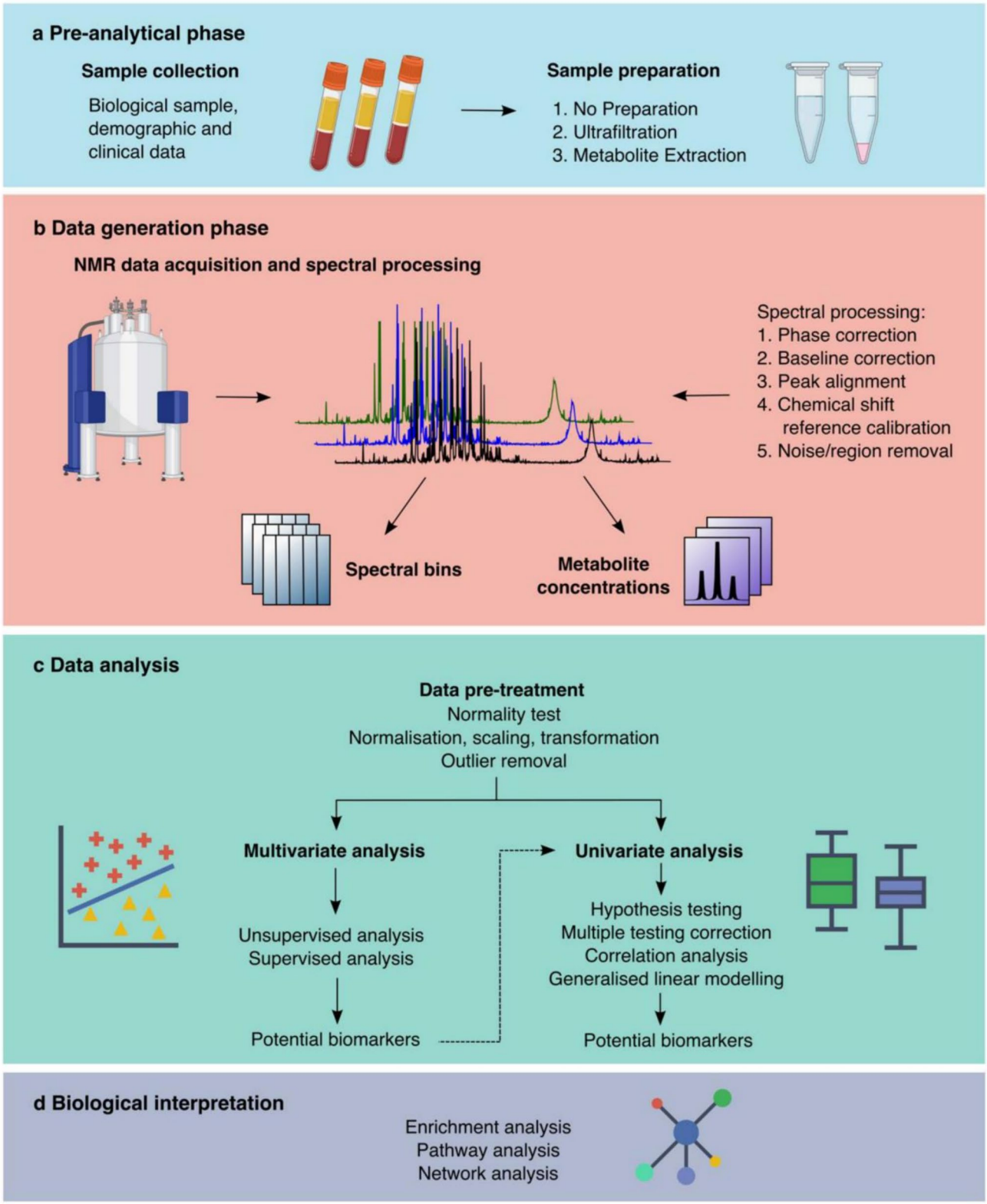

Fig. 1 A typical workflow for untargeted NMR metabolomics. The NMR metabolomics workflow is divided into four main phases: (**a**) pre-analytical including sample collection and sample preparation; (**b**) data generation which involves NMR spectra acquisition, spectral processing, and the generation of spectral bins and/or metabolite concentrations. (**c**) Data analysis is performed for both data types including pre-treatment, and multivariate and univariate analyses. The dashed arrow shows the optional integration of multivariate and univariate analyses. The fourth phase is (**d**) biological interpretation (Huang et al. 2022)

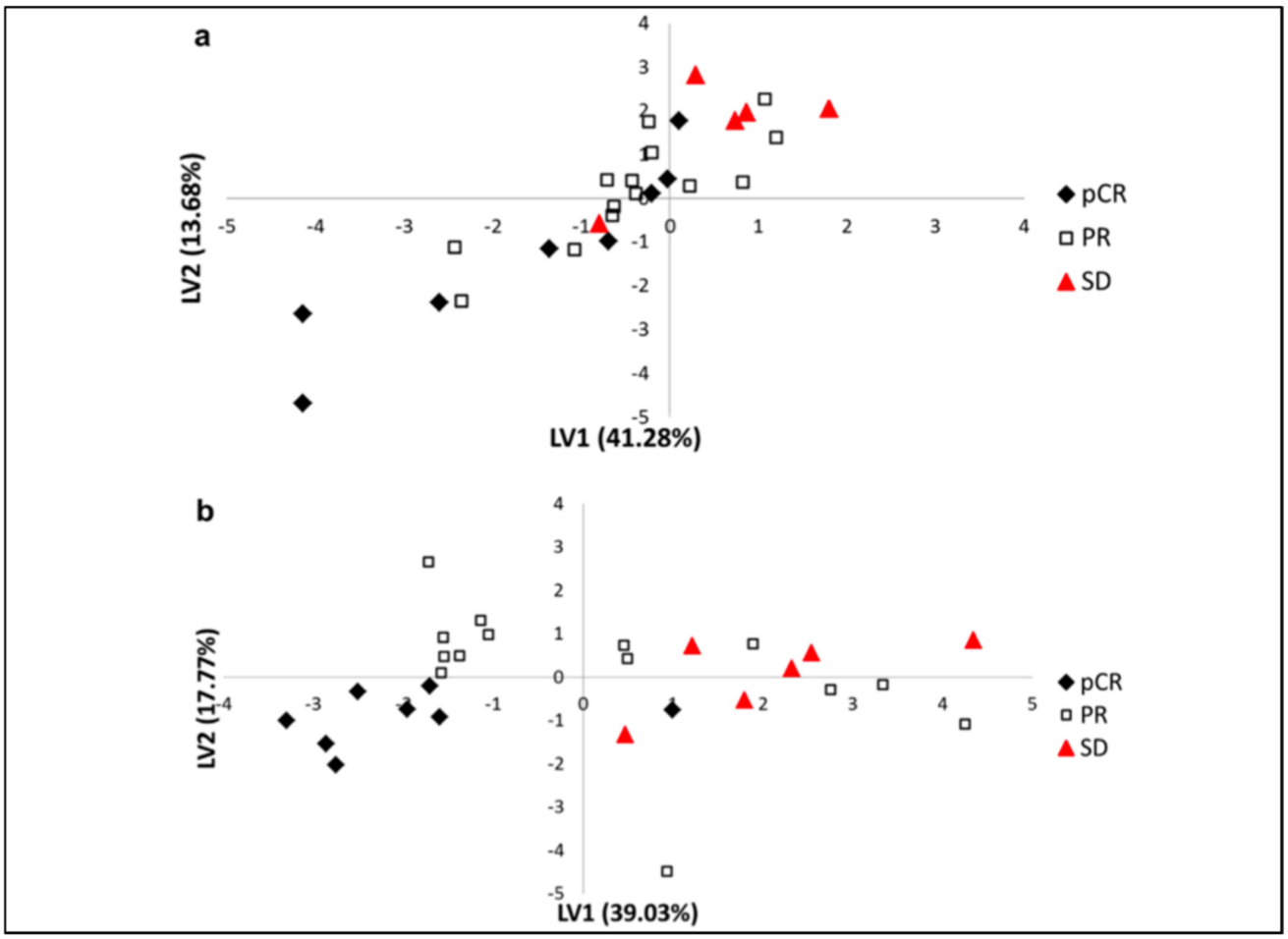

Fig. 2 PLS score plot based on (**a**) 27 metabolites detected by NMR and (**b**) 9 metabolites detected LC–MS for all the patients. In (a), one outlier was deleted. The LV label on the two axes stands for latent variable. pCR, pathologic complete response; PR, partial response; SD, stable disease (Wei et al. 2012)

Table 2 Summary of NMR metabolites showing significant differences with their respective *p*-values (Wei et al. 2012)

Chemical shift (δ)	Multiplicity	Assignment	(pcR vs. SD)	(pCR vs. PR)	(PR vs. SD)
4.24	m	Threonine	0.04	0.08	0.01
1.00	s	Isoleucine	0.04	0.10	0.20
2.09	m	Glutamine	0.01	0.30	0.02
7.07	s	Histidine	0.29	0.01	0.54

Assessment of treatment in neurological disorders

Schizophrenia is a severe psychiatric disorder that affects 1% of global population; however, little is known about the disease pathology. Current drug therapies target dopamine, serotonin, and glutamate systems. A study done by Cai et al. on plasma and urine samples using MS, UPLC-MS and 1-H NMR was able to detect and identify biomarkers that are related to schizophrenia and risperidone (RIP) treatment (Cai et al. 2012). The plasma metabolites: alanine and glycine, increased in the First-Episode Neuroleptic-Naïve Schizophrenia Patients (FENNS) after RIP treatment. Glucose levels in plasma samples of FENNS were lower than in the control cohort. The findings of plasma and urine biomarkers from 1-H NMR of FENNS patients after RIP treatment were also monitored with targeted tandem mass spectrometry.

Another study done by Liu et al. monitored endogenous plasma metabolites for therapeutic response of depressed patients who were treated with the traditional Chinese medicine,

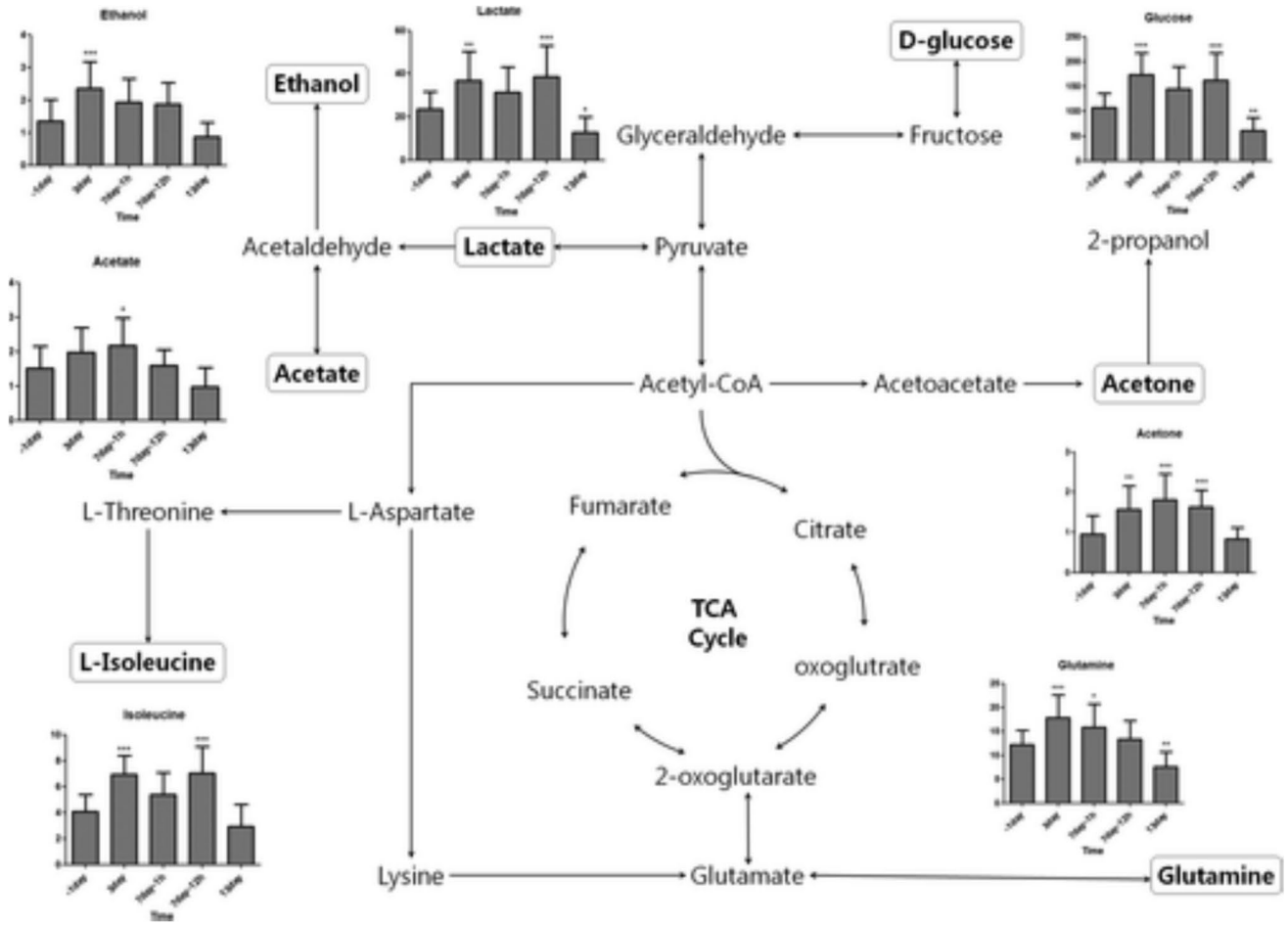

Fig. 3 Expected plasma metabolic pathway change due to acetaminophen treatment. Although TCA cycle components were not measured, increased plasma levels of lactate, acetone, and glutamine suggested that the TCA cycle might be downregulated by acetaminophen (Kim et al. 2013)

Xiaoyaosan (Liu et al. 2015). Depression is common mental disorder among individuals of all ages and Xiaoyaosan, an antidepressant, has been used to treat depression. This 1-H NMR study found that metabolites such as: trimethylamine oxide (TMAO), glutamine, and lactate were significantly greater in depressed patients than healthy controls and phenylalanine, valine, alanine, glycine, leucine, citrate, choline, lipids, and glucose were significantly lower. After the depressed patients were treated with Xiaoyaosan, metabolites including alanine, choline, TMAO, glutamine, lactate, and glucose returned to levels comparable to those of healthy individuals. The results show that these metabolites may be monitored to confirm therapeutic response of depressed patients treated with Xiaoyaosan.

Assessment of septic shock treatment

Unfortunately, sepsis therapy has a history of unsuccessful treatment in clinical trials due to heterogeneity of patients. The study of pharmacometabolomics has been an area that can be used as an approach to permit precise treatment for sepsis. Puskarich et al. collected serum samples before patients were treated with L-Carnitine and 24h and 48 h after treatment (Puskarich et al. 2015). A placebo-treated patient cohort was also included. The results showed that thirty-eight metabolites were detected in all samples, but only the concentrations of 3-hydroxybutyrate, acetoacetate, and 3-hydroxyisovalerate were significantly different when compared between pre-treatment and after treatment of survivors vs. non-survivors. Overall, this study was able to introduce pharmacometabolomics as an area of study to monitor treatment response and as a new approach for precision medicine.

Urine analysis

Sample characteristics

Although blood (plasma and/or serum) may be the most common sample analyzed in traditional clinical laboratories,

it suffers from poor patient compliance, due to the invasiveness of the procedure involved in its collection (Zhang et al. 2012). The use of urine for the same purposes provides an alternative as urine samples are obtained non-invasively and are readily available. Due to the ease of sample collection, these samples can be collected frequently for longitudinal and repetitive studies. Urine samples are also ideal to look for the end product of metabolic pathways and degradation products of administered drugs.

Sample collection and preparation

There are two alternative ways of collecting urine samples. The first method collects the urine samples over dry ice and freezes them at −70 °C for storage until the analysis of the samples (Sumner et al. 2010). The second method collects the samples at room temperature which are then aliquoted and stored at –70 °C to −80 °C for storage until the analysis of the samples (Liesenfeld et al. 2014; Ryu et al. 2019). As it is with other biological tests, some special considerations must be required to produce consistent and reliable data. For instance, samples for human urine analysis were discarded if the patient had used tobacco or alcohol products 24 h prior to the samples collection (Liesenfeld et al. 2014). The volume of the sample used in NMR studies is minimal, less than 1 mL. The typical 24-h urine output ranges from 800 to 2, 000 mL per day, assuming an average fluid intake of about 2 L. Urine samples should be collected midstream whenever possible to prevent contamination from the bacteria residing in the urinary tract (Emwas et al. 2016). Also, the patient characteristics should be matched with corresponding controls as much as possible to include age and gender for comparative analysis (Emwas et al. 2016). Fasting overnight prior to the collection of urine samples offers a more stable value for individual samples (Emwas et al. 2016). Furthermore, diet monitoring is warranted for certain metabolites that can be found in a diet that can potentially affect the end product of metabolism. In this case, the standardization of diet 24 h prior to the collection of samples is recommended (Emwas et al. 2016). A detailed protocol for urinary metabolomics has been published by Bezabeh et al. (Bezabeh et al. 2019).

Urine is one of the most convenient samples to analyze when it comes to biomarker identification and quantification using NMR. The instrument does not require a meticulous separation of samples prior to reading the signal from the analyte (Emwas et al. 2016). However, pH variation, water signal suppression, temperature control and the selection of an appropriate internal standard are some of the considerations one has to keep in mind to ensure reproducibility and optimize spectral quality (Emwas et al. 2016; Bharti and Roy 2012; Rist et al. 2013).

Variations in pH can cause significant chemical shift changes in urine samples (Emwas et al. 2016; Bharti and Roy 2012; Rist et al. 2013). This is due to the pH-associated changes in the respective metabolites. These changes can be remediated in a few different ways. First, the source of pH change can be eliminated (Rist et al. 2013). Collecting the samples over dry ice can cause chemical shift changes due to pH change which is caused by the carbon dioxide emitted from the dry ice. Second, a buffer can be added to keep the pH from changing. A concentrated (1.5 M) KH_2PO_4 can be added to keep the solution at its physiological pH of 7.0. Temperature also plays a key role in the sample preparation in terms of precision and reproducibility of NMR data. The sample should be cooled at 4 °C until the analysis to prevent deterioration. And if working with a frozen sample, it should be thawed to room temperature for 5 to 10 min prior to the analysis.

Water is the most abundant molecule in urine. The NMR peaks correspond to the concentration of certain molecules, and as such, water will have the highest peak in the NMR spectra if the signal is not suppressed adequately. There are many water suppression techniques currently available for urine NMR such as pre-saturation, excitation sculpting, WATERGATE, and NOESY. Different techniques demand specific requirements. For example, Bharti et al. do not recommend pre-saturation since it can partially affect the peaks of other signals present (Bharti and Roy 2012). On the contrary, Emwas et al. state that 1D NOESY pulse sequence with water pre-saturation is an effective technique to suppress the water signal if automated pulse width calibration is used (Emwas et al. 2016). Automated pulse width calibration significantly increases consistency, which is the major concern of pre-saturation because if it is done inconsistently, it will cause a detrimentally unfavorable alteration to the chemical shift.

Since metabolomics requires a quantitative analysis of metabolites, a careful selection of internal standard is critical in the quantification of the metabolites. Commonly, tetramethylsilane (TMS) is used in a typical NMR analysis. TMS is inexpensive and produces a distinctive peak. However, its highly volatile characteristics render it unsuitable for use as an internal standard (Bharti and Roy 2012). Furthermore, other widely used internal standards, such as trimethylsilyl propanoic acid (TSP) and 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) can be used but may interact with the analyte depending on its characteristics (Emwas et al. 2016; Bharti and Roy 2012). Deuterated 4,4-dimethyl- 4-silapentane-1-ammonium trifluoroacetate (DSA) is recommended by Bharti et al. due to its inactivity with biological molecules such as peptides, proteins, and lipids (Bharti and Roy 2012).

NMR data acquisition and analysis

For NMR data acquisition in urine samples, 1D NOESY pulse sequence with water presaturation, which is robust and commonly available on all spectrometers, is used. Typical parameters include relaxation delay of greater or equal to 5 s, 64 K time domain points, spectral width of 12–16 ppm, and 64–128 scans. Given the variability that arises due to different dilution conditions of urine, normalization of data is performed following acquisition. This can be done using different methods as described in Bezabeh et al. (Emwas et al. 2016).

In metabolomics, data is compared between control and treated or between pre- and post- treatment conditions. Both increases and decreases in the relative concentrations of metabolites in control and treated groups are analyzed to find correlations between the two. However, a plethora of signals are seen in the NMR spectra of urine samples which makes this process complex. Hence, characterization of biomarkers is a difficult manual task to accomplish. Thus, computer-generated self-correcting prediction model is run to determine the correlations between the two groups (Andrew Clayton et al. 2006). These data are again processed via PCA and partial least squares projection (PLS). PLS is a supervised method where the information for each sample group is inputted, whereas the PCA is an unsupervised method which sorts the data with its known grouping. Since there is usually a clear grouping between controls and treated, the PCA method is used in the development of biomarkers.

Applications

Assessment of colorectal cancer treatment

Liesenfeld et al. evaluated 199 urine samples from the colorectal patients prior to surgery, post-surgery, 6 months and 12 months after the surgery (Liesenfeld et al. 2014). The aim of the study was to compare the urine metabolomes of colorectal patients in different stages of treatment and to build a prediction model for prolonged prognosis. The study showed that the concentrations of the metabolites in the gut microbiome are significantly affected by surgery with these differences assumed to be caused by the tumor (Liesenfeld et al. 2014). The patient characteristics were carefully chosen to create similar conditions in each group. Uni- and multivariate analyses were performed using Metaboanalyst 2.0. Using a multivariate model containing 20 marker metabolites (AUCROC = 0.89; 95% CI:0.84-0.95) the authors were able to differentiate the metabolite profiles of colorectal cancer patients prior to surgery from those at any post-surgery timepoint.

Assessment of transplant status

With the advancement of stem cell and regenerative medicine, transplant technologies have developed tremendously. In the near future, a fully mechanized nanotechnology kidney will likely be in circulation which will have virtually no rejection. However, the rejection of transplants is still a big issue and a great field of study in biochemistry and immunology. The information which is needed for both of those disciplines can be obtained via NMR metabolomics. Current monitoring includes blood count, electrolyte monitoring, immunosuppressant quantification, ultrasound, physical symptoms and finally biopsy as a last resort. There are too many routine tests that provide too little information. There has been some NMR work related to liver and kidney transplants with the objective of replacing the routine tests and becoming a one-stop test before the biopsy.

Ornithine transcarbamylase deficiency (OTC) is the most common urea cycle defect. In a study by Legido-Quigley, hepatocyte transplantation was performed on a newborn with OTC (Legido-Quigley et al. 2009). Urine samples for NMR analysis were collected on 14 different days during the 350-day treatment period. The sample collection was performed on days 0, 2, 3, 4, 5, 6, 7, 10, 28, 35, 59, 98, 105 and 350 days after transplantation. PLS was used to evaluate the patterns. The analysis was able to differentiate the metabolites from pre- and post-transplantation. One of the notable changes was the adenine and adenosine trend. This presumably is due to mitochondrial dysfunction (Legido-Quigley et al. 2009).

Foxall et al. monitored the renal transplant functions of 33 patients who underwent primary renal allograft transplantation for 14 days via NMR of urine (Foxall et al. 1993). The result showed increased levels of trimethylamine-N-oxide (TMAO), dimethylamine (DMA), lactate, acetate, succinate, glycine, and alanine during episodes of graft dysfunction. They were able to accurately distinguish normal histology from acute or a combination of acute and chronic allograft rejection. Circulating concentrations of TMAO are elevated in chronic kidney disease and inversely associated with glomerular filtration rate (Hai et al. 2015).

Figure 4 shows spectral changes corresponding to each outcome. The mean concentrations of the metabolites detected in the two groups of patients (good graft function and graft dysfunction) are indicated in Table 3. Somorjai et al. also monitored renal transplant functions of 68 patients at regular intervals (Somorjai et al. 2002). The cohorts were separated into renal transplant patients with normal graft histology and those with acute rejection (using the Banff histopathological criteria). They were able to distinguish normal histology from allograft rejection based on urine NMR spectra and establish a prognosis of renal allograft rejection. In a study of subclinical acute rejection by Rush et al. (2000),

Table 3 ^{1}H NMR detectable metabolite concentrations (μM/mM creatinine) in the urine of two groups of renal transplant patients collected on day 7 post-renal transplantation

Metabolite μM/mM creatinine	Group 1 (N = 9) Good graft function	Group 2 (N = 10) Graft dysfunction
TMAO	91 ± 18	410 ± 102
DMA	146 ± 25	335 ± 93
Alanine	181 ± 51	224 ± 107
Lactate	352 ± 161	216 ± 54
Glycine	770 ± 180	623 ± 233
Hippurate	55 ± 13	123 ± 40

Values are means ± SE. $P \leq 0.025$, significantly different to group 1 with Student's unpaired t-test (Foxall et al. 1993)

urine samples were collected immediately prior to a protocol biopsy and subjected to NMR and IR. The spectra were able to reliably identify patients with normal allograft histology and detect subclinical rejection–a potential surrogate marker for chronic rejection.

Assessment of prognosis in septic shock

Sepsis is the main cause of death among hospitalized patients with a mortality rate of 36%. This condition is reversible with correct classification and interventions according to the classification. However, the assessment of severity is chronically inhibited due to high variability. Thus, a consistent biomarker is in need for accurate and expedient identification and classification. In a study by Garcia-Simon et al., urine samples were collected from 64 patients with severe sepsis or septic shock in the ICU for a 1-H NMR spectroscopic analysis (Garcia-Simon et al. 2015). Supervised analysis was performed on the processed spectra, and a predictive model for prognosis (30-days mortality/survival) of sepsis was constructed using partial least-squares discriminant analysis (PLS-DA). In addition, the prediction power of metabolomics data with respect to the Sequential Organ Failure Assessment (SOFA) score was compared. The internal cross-validation showed the robustness of the metabolic predictive model obtained and a better predictive ability in comparison with SOFA values. Amongst the biomarkers identified, glutamine plays a key role in prediction, since it is a primary energy source for many cells, and the decreased level indicates diminished body function. Figure 5 shows PLS-DA score plots discriminating survivors from non-survivors.

Conclusion

As seen in the applications above, metabolomic studies of plasma, serum and urine have the potential to provide useful metabolic information in predicting and/or monitoring treatment response. With regards to monitoring neoadjuvant chemotherapy using 1-H NMR Spectroscopy of serum, one can clinically assess how the patient is responding to the chemotherapy between cycles by observing the metabolites that show significant changes during the treatment (Wei et al. 2012). This can help reduce the patient's exposure to unnecessary toxicity and allow the physician to find an alternative treatment faster. Metabolomics can be useful in the future for developing targeted drugs and personalizing treatments (Palmnas and Vogel 2013). A severe side effect that accompanies the chemoprophylaxis interventions can be resolved more quickly by being able to stop the treatment at the precise time of recovery. More importantly, those who require further treatment or who do not respond to the conventional treatments can find out early enough so that they can opt out of the treatment and/or switch to an alternative intervention.

While there are other metabolomics platforms that can be used for the applications presented above, this review's focus was on NMR spectroscopy. Some strengths of this technique include the ability to do the analysis with a small volume sample, the ability to recover the sample, and the reproducibility of the data. Lastly, NMR spectroscopy requires minimal sample preparation thus making the approach easily adaptable in a clinical setting.

One of the weaknesses of NMR has been its sensitivity compared to techniques such as mass spectrometry with MS's detection limit reaching down to nanomolar whereas the NMR barely reaches the micromolar. The continued increase in field strength (currently reaching up to 1 GHz), the use of cryoprobes, and advances in electronics have helped improve the sensitivity of NMR. Some techniques such as fast pulsing and T2 relaxation attenuation can also be useful in this regard (Lee et al. 2014). Together with the advancement of electronics, data analysis methods have also evolved considerably over the last few years and have become more powerful, allowing maximum use of the available data. While mass spectrometry can be both targeted and non-targeted analysis, NMR is generally used in non-targeted mode with a few selective methods (Emwas 2015). To enhance its assessment's sensitivity and specificity, NMR can be used in combination with other modalities such as mass spectrometry and IR spectroscopy (Spratlin et al. 2009; Gowda et al. 2009). By doing so, Somorjai et al. showed that the overall diagnostic accuracy significantly increased when both NMR and IR data were combined compared to that obtained using a single modality (Somorjai et al. 2002). Additionally, methods like isotopic enrichment and hyperpolarization have been introduced to enhance sensitivity. For instance, dynamic nuclear polarization (DNP) boosts signals by transferring polarization from electron spins to nuclear spins, making it possible to detect low-abundance metabolites, while parahydrogen-induced polarization (PHIP) enhances signals

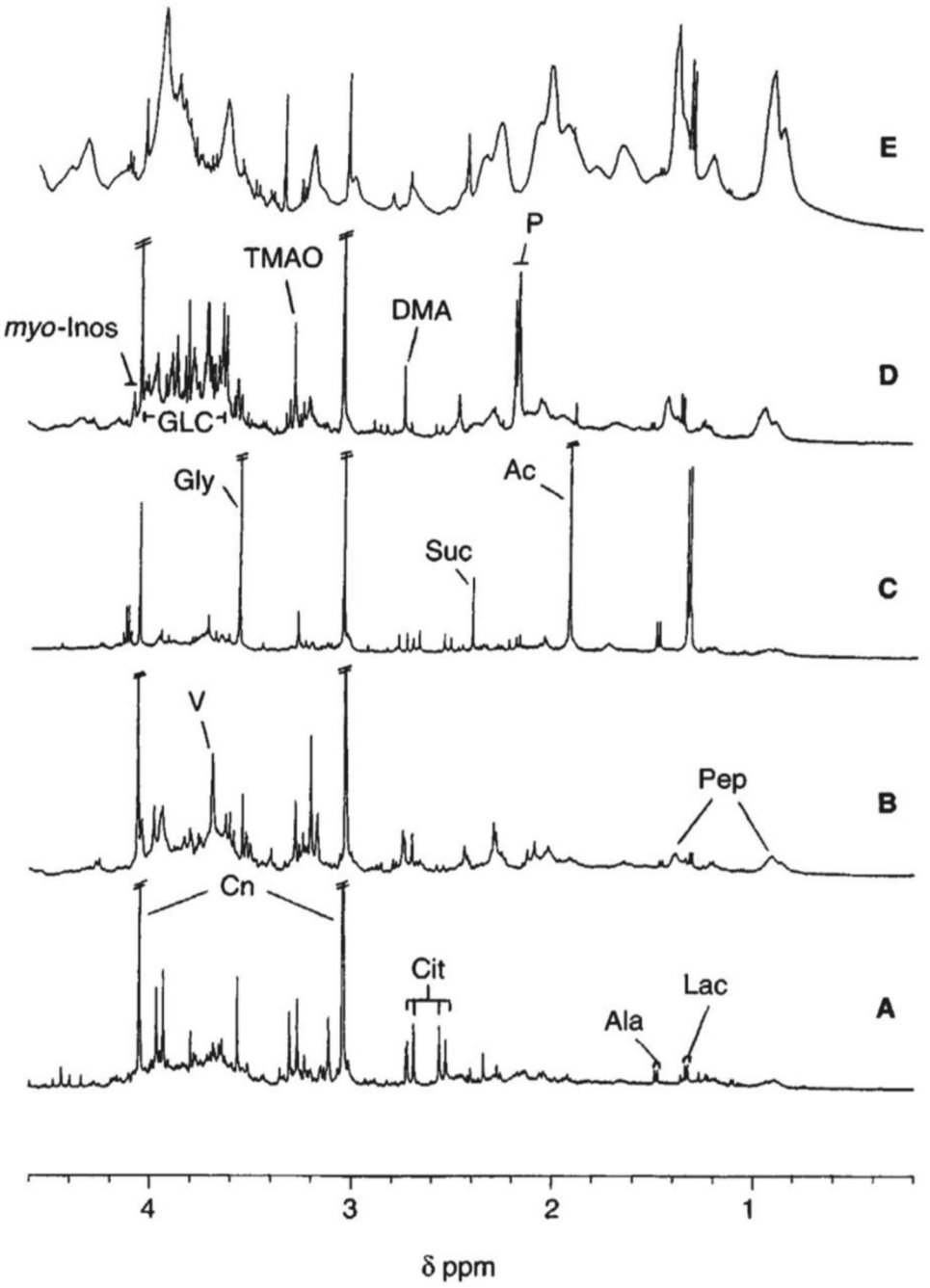

Fig. 4 Partial 500-MHz single-pulse 1-H NMR spectra of normal human urine (**A**) and urine collected from 4 patients on day 3 post-renal transplantation showing immediate functioning graft (**B**), urinary tract infection (**C**), renal tubular ischemia (**D**), and non-functioning graft (**E**). Ac, acetate; Ala, alanine; Cit, citrate; Cn, creatinine; DMA, dimethylamine; GLC, glucose; Gly, glycine; Lac, lactate; myo-inos, myo-inositol; P, acetaminophen (paracetamol) metabolites; Pep, peptides; Suc, succinate; TMAO, trimethylamine-N-oxide; V, CsA drug vehicle (Foxall et al. 1993)

by transferring nuclear spin polarization from parahydrogen to other nuclei, and signal amplification by reversible exchange (SABRE), a hyperpolarization technique that transfers spin polarization from para-hydrogen to target molecules via a reversible exchange process with a catalyst (Peng et al. 2024; Nagana Gowda and Raftery 2023).

Another approach that can be used to enhance the assessment power of the technique is to look at multiple samples (e.g., urine, blood) from the same patient. The combined and complementary information from multiple samples should give a more complete picture of what is happening in the patient in response to a given treatment. Zheng et al. proposed a new method of fusing the data of serum and urine together for evaluating prostate cancer (Zheng et al. 2017). The results show that the fusion of the two data sets improved performance and classification power.

Data processing pipelines have improved allowing researchers to automate or semi-automate workflows for higher throughput and reproducibility. For one-dimensional (1D) 1H NMR spectra, many proprietary programs, including Chenomx NMRSuite, B.I. QUANT, known for bioanalysis studies, and FoodScreener which is optimal for

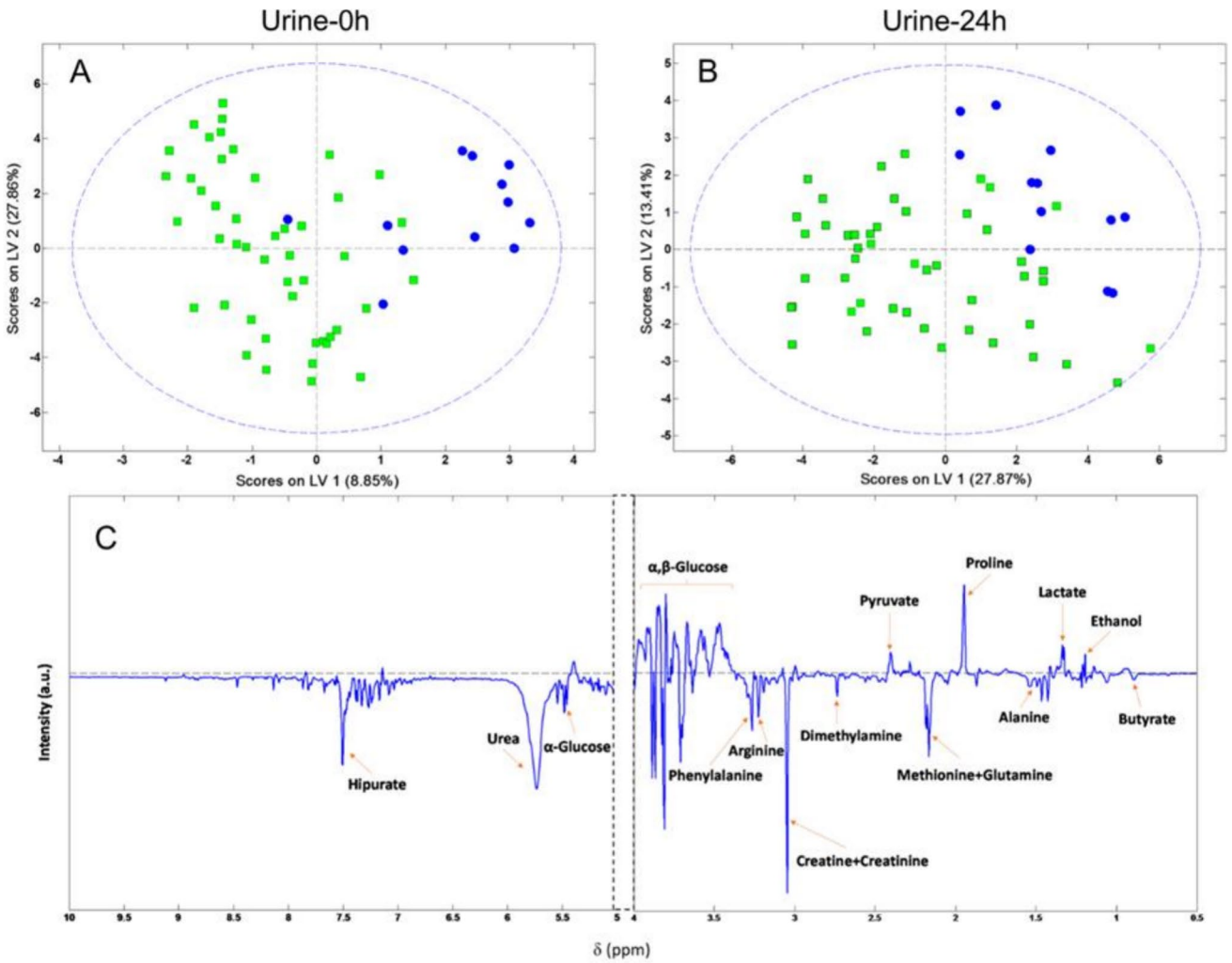

Fig. 5 PLS-DA score plot for discrimination between survivors (open squares) and non-survivor patients (black circles). (**A**) PLS-DA score plot constructed with Urine-0 h samples obtained at the admission to the ICU. (**B**) PLS-DA score plot constructed with Urine-24 h samples obtained at 24 h after admission to the ICU. (**C**) Loading plot of the PLS-DA score plot constructed with Urine-0 h samples (Garcia-Simon et al. 2015)

food screening, have become available. Additionally, freely available academic softwares have been developed including Batman, AQuA, ASICS, ASICS 2.0, and rDolphin (Wishart et al. 2022).

Many areas of NMR-based metabolomics workflow have improved from sample preparation, data acquisition, data processing, and interpretation. However, further development of NMR metabolomics may involve hyphenated instruments including Ultra High-Performance Liquid Chromatography (UHPLC) coupled with Mass Spectrometry to provide a more comprehensive understanding of the metabolome as well as accelerate data throughput.

The need for standardization is critical if metabolomics-based methods are to have a role in the clinics. To this end, several groups are working on standardizing protocols and making recommendations for such analyses. These recommendations are specific to each sample type and include sample collection, sample storage, sample preparation, NMR data acquisition pulse sequence and parameters, data preprocessing, and complete multivariate analysis methods (Emwas et al. 2025; Bezabeh et al. 2019; Gonzalez-Dominguez et al. 2020). With standardized protocols in place, it will make it easier for investigators to share, compare and combine data across institutions, thus creating larger and more robust databases and classifiers ensuring reliable and accurate results.

While NMR-based metabolomics has a strong potential in predicting and monitoring treatment response as shown in the above applications, there are still some challenges that need to be overcome before this can be adopted in a clinical setting. Letertre et al. have discussed some of these methodological considerations (Letertre et al. 2021). Accessibility

to a high-field NMR spectrometer for routine clinical test could pose a challenge in some places. However, benchtop NMR spectrometers that use permanent magnets and are more affordable could be used in some applications, despite their lower magnetic field strengths (Letertre et al. 2021). Lastly, the issues of quality assurance and quality control in metabolomics have been discussed in a review article by Long et. al. (Long et al. 2020). These are important requirements at each stage of the analysis for the technique's clinical success.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

Andrew Clayton T, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton et al (2006) Pharmaco-metabonomic phenotyping and personalized drug treatment. Nature 440(7087):1073–1077. https://doi.org/10.1038/nature04648

Aranı´bar N, Ott K, Roongta V, Mueller L (2006) Metabolomic analysis using optimized NMR and statistical methods. Anal Biochem 355(1):62–70. https://doi.org/10.1016/j.ab.2006.04.014

Barding GA, Salditos R, Larive CK (2012) Quantitative NMR for bioanalysis and metabolomics. Anal Bioanal Chem 404(4):1165–1179. https://doi.org/10.1007/s00216-012-6188-z

Barker M, Rayens W (2003) Partial least squares for discrimination. J Chemometr 17(3):166–173. https://doi.org/10.1002/cem.785

Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, Nicholson JK (2007) Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2(11):2692–2703. https://doi.org/10.1038/nprot.2007.376

Bezabeh T, Capati A, Ijare OB (2019) NMR-based urinary metabolomics applications. NMR-Based Metabolomics Methods in Molecular Biology, pp 215–229. https://doi.org/10.1007/978-1-4939-9690-2_13

Bharti SK, Roy R (2012) Quantitative 1H NMR spectroscopy. TrAC Trends Anal Chem 35:5–26. https://doi.org/10.1016/j.trac.2012.02.007

Cai H, Li H, Yan X, Sun B, Zhang Q, Yan M et al (2012) Metabolomic analysis of biochemical changes in the plasma and urine of first-episode neuroleptic-naïve schizophrenia patients after treatment with risperidone. J Proteome Res 11(8):4338–4350. https://doi.org/10.1021/pr300459d

Capati A, Ijare OB, Bezabeh T (2017) Diagnostic applications of nuclear magnetic resonance–based urinary metabolomics. Magn Reson Insights. https://doi.org/10.1177/1178623x17694346

Chen Y, Li EM, Xu LY (2022) Guide to metabolomics analysis: a bioinformatics workflow. Metabolites 12:357. https://doi.org/10.3390/metabo12040357

Chu S, Huang M, Kelly R, Benedetti E, Siddiqui J, Zeleznik O et al (2019) Integration of metabolomic and other omics data in population-based study designs: an epidemiological perspective. Metabolites 9(6):117. https://doi.org/10.3390/metabo9060117

Emwas AHM (2015) The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research. Metabonomics :161–193. https://doi.org/10.1007/978-1-4939-2377-9_13

Emwas AH, Roy R, McKay RT, Ryan D, Brennan L, Tenori L et al (2016) Recommendations and standardization of biomarker quantification using NMR-based metabolomics with particular focus on urinary analysis. J Proteome Res 15(2):360–373. https://doi.org/10.1021/acs.jproteome.5b00885

Emwas AH, Zacharias HU, Alborghetti MR, Gowda GAN, Raftery D, McKay RT et al (2025) Recommendations for sample selection, collection and preparation for NMR-based metabolomics studies of blood. Metabolomics 21(3):66. https://doi.org/10.1007/s11306-025-02259-7

Foxall PJD, Mellotte GJ, Bending MR, Lindon JC, Nicholson JK (1993) NMR spectroscopy as a novel approach to the monitoring of renal transplant function. Kidney Int 43(1):234–245. https://doi.org/10.1038/ki.1993.37

Garcia-Simon M, Morales JM, Modesto-Alapont V, Gonzalez-Marrachelli V, Vento-Rehues R, Jorda-Miñana A et al (2015) Prognosis biomarkers of severe sepsis and septic shock by 1-H NMR urine metabolomics in the intensive care unit. PLoS ONE 10(11):e0140993. https://doi.org/10.1371/journal.pone.0140993

Gonzalez-Dominguez R, Gonzalez-Dominguez A, Sayago A, Fernandez-Recamales A (2020) Recommendations and best practices for standardizing the pre-analytical processing of blood and urine samples in metabolomics. Metabolites 10:229. https://doi.org/10.3390/metabo10060229

Gouveia GJ, Head T, Cheng LL, Clendinen CS, Cort JR (2024) Perspective: use and reuse of NMR-based metabolomics data: what works and what remains challenging. Metabolomics 20(2):41. https://doi.org/10.1007/s11306-024-02090-6

Gowda GAN, Raftery D (2019) Analysis of plasma, serum, and whole blood metabolites using 1H NMR spectroscopy. NMR-Based Metabolomics Methods in Molecular Biology, pp 17–34. https://doi.org/10.1007/978-1-4939-9690-2_2

Gowda GAN, Ijare OB, Shanaiah N, Bezabeh T (2009) Combining nuclear magnetic resonance spectroscopy and mass spectrometry in biomarker discovery. Biomark Med 3(3):307–322. https://doi.org/10.2217/bmm.09.22

Hai X, Landeras V, Dobre M, DeOreo P, Meyer TW, Hostetter TH (2015) Mechanism of prominent trimethyl oxide (TMAO) accumulation in hemodialysis patients. PLoS ONE 10(12):e0143731. https://doi.org/10.1371/journal.pone.0143731

Huang K, Thomas N, Gooley PR, Armstrong CW (2022) Systematic review of NMR-based metabolomics practices in human disease research. Metabolites 12(10):963. https://doi.org/10.3390/metabo12100963

Jones RL, Smith IE (2006) Neoadjuvant treatment for early-stage breast cancer: opportunities to assess tumour response. Lancet Oncol 7(10):869–874. https://doi.org/10.1016/s1470-2045(06)70906-8

Jupin M, Michiels P, Girard F, Spraul M, Wijmenga S (2014) NMR metabolomics profiling of blood plasma mimics shows that medium- and long-chain fatty acids differently release metabolites from human serum albumin. J Magn Reson 239:34–43. https://doi.org/10.1016/j.jmr.2013.11.019

Kaddurah-Daouk R, Kristal BS, Weinshilboum RM (2008) Metabolomics: a global biochemical approach to drug response and

disease. Annu Rev Pharmacol Toxicol 48(1):653–683. https://doi.org/10.1146/annurev.pharmtox.48.113006.094715
Kim JW, Ryu SH, Kim S, Lee HW, Lim M, Seong SJ et al (2013) Pattern recognition analysis for hepatotoxicity induced by acetaminophen using plasma and urinary 1H NMR-based metabolomics in humans. Anal Chem 85(23):11326–11334. https://doi.org/10.1021/ac402390q
Kruk J, Doskocz M, Jodłowska E, Zacharzewska A, Łakomiec J, Czaja K, Kujawski J (2016) NMR techniques in metabolomic studies: a quick overview on examples of utilization. Appl Magn Reson 48(1):1–21. https://doi.org/10.1007/s00723-016-0846-9
Lee JH, Okuno Y, Cavagnero S (2014) Sensitivity enhancement in solution NMR: emerging ideas and new frontiers. J Magn Reson 241:18–31. https://doi.org/10.1016/j.jmr.2014.01.005
Legido-Quigley C, Cloarec O, Parker DA, Murphy GM, Holmes E, Lindon JC et al (2009) First example of hepatocyte transplantation to alleviate ornithine transcarbamylase deficiency, monitored by NMR-based metabonomics. Bioanalysis 1(9):1527–1535. https://doi.org/10.4155/bio.09.112
Letertre MPM, Giraudeau P, de Tullio P (2021) Nuclear magnetic resonance spectroscopy in clinical metabolomics and personalized medicine: current challenges and perspectives. Front Mol Biosci 8:698337. https://doi.org/10.3389/fmolb.2021.698337
Liesenfeld DB, Habermann N, Toth R, Owen RW, Frei E, Böhm J et al (2014) Changes in urinary metabolic profiles of colorectal cancer patients enrolled in a prospective cohort study (ColoCare). Metabolomics 11(4):998–1012. https://doi.org/10.1007/s11306-014-0758-3
Liu C, Wu Y, Feng G, Gao X, Zhou Y, Hou W et al (2015) Plasma-metabolite-biomarkers for the therapeutic response in depressed patients by the traditional Chinese medicine formula Xiaoyaosan: a 1H NMR-based metabolomics approach. J Affect Disord 185:156–163. https://doi.org/10.1016/j.jad.2015.05.00513
Long FH (2013) Multivariate analysis for metabolomics and proteomics data. Proteomic Metab Approach Biomark Discov 299–311. https://doi.org/10.1016/b978-0-12-394446-7.00019-4
Long NP, Nghi TD, Kang YP, Anh NH, Kim HM, Park SK, Kwon SW (2020) Toward a standardized strategy of clinical metabolomics for the advancement of precision medicine. Metabolites 10:51. https://doi.org/10.3390/metabo10020051
Ludwig C, Viant MR (2010) Two-dimensional J-resolved NMR spectroscopy: review of a key methodology in the metabolomics toolbox. Phytochem Anal 21(1):22–32. https://doi.org/10.1002/pca.1186
Nagana Gowda GA, Raftery D (2023) NMR metabolomics methods for investigating disease. Anal Chem 95(1):83–99. https://doi.org/10.1021/acs.analchem.2c04606
Palmnas M, Vogel H (2013) The future of NMR metabolomics in cancer therapy: towards personalizing treatment and developing targeted drugs? Metabolites 3(2):373–396. https://doi.org/10.3390/metabo3020373
Peng Y, Zhang Z, He L, Li C, Liu M (2024) NMR spectroscopy for metabolomics in the living system: recent progress and future challenges. Anal Bioanal Chem 416(9):2319–2334. https://doi.org/10.1007/s00216-024-05137-8
Puskarich MA, Finkel MA, Karnovsky A, Jones AE, Trexel J, Harris BN, Stringer KA (2015) Pharmacometabolomics of L-carnitine treatment response phenotypes in patients with septic shock. Ann Am Thorac Soc 12(1):46–56. https://doi.org/10.1513/annalsats.201409-415oc
Rist MJ, Muhle-Goll C, Görling B, Bub A, Heissler S, Watzl B, Luy B (2013) Influence of freezing and storage procedure on human urine samples in NMR-based metabolomics. Metabolites 3(2):243–258. https://doi.org/10.3390/metabo3020243
Rush D, Somorjai R, Deslauriers R, Shaw, Anthony S, Jeffery J, Nickerson P (2000) Subclinical rejection – a potential surrogate marker for chronic rejection – may be diagnosed by protocol biopsy or urine spectroscopy. Ann Transplant 5(2):44–49
Ryu SH, Lee JD, Kim JW, Kim S, Kim S, Kim K-B (2019) 1H NMR toxicometabolomics following cisplatin-induced nephrotoxicity in male rats. J Toxicol Sci 44(1):57–71. https://doi.org/10.2131/jts.44.57
Somorjai RL, Dolenko B, Nikulin A, Nickerson P, Rush D, Shaw A et al (2002) Distinguishing normal from rejecting renal allografts: application of a three—stage classification strategy to MR and IR spectra of urine. Vib Spectrosc 28(1):97–102. https://doi.org/10.1016/S0924-2031(01)00148-5
Spratlin JL, Serkova NJ, Eckhardt SG (2009) Clinical applications of metabolomics in oncology: a review. Clin Cancer Res 15(2):431–440. https://doi.org/10.1158/1078-0432.CCR-08-1059
Stringer KA, Younger JG, Mchugh C, Yeomans L, Finkel MA, Puskarich MA et al (2015) Whole blood reveals more metabolic detail of the human metabolome than serum as measured by 1H-NMR spectroscopy. Shock 44(3):200–208. https://doi.org/10.1097/shk.0000000000000406
Sumner SJ, Burgess JP, Snyder RW, Popp JA, Fennell TR (2010) Metabolomics of urine for the assessment of microvesicular lipid accumulation in the liver following isoniazid exposure. Metabolomics 6(2):238–249. https://doi.org/10.1007/s11306-010-0197-8
Teahan O, Gamble S, Holmes E, Waxman J, Nicholson JK, Bevan C, Keun HC (2006) Impact of analytical bias in metabonomic studies of human blood serum and plasma. Anal Chem 78(13):4307–4318. https://doi.org/10.1021/ac051972y
Wei S, Liu L, Zhang J, Bowers J, Gowda GN, Seeger H et al (2012) Metabolomics approach for predicting response to neoadjuvant chemotherapy for breast cancer. Mol Oncol 7(3):297–307. https://doi.org/10.1016/j.molonc.2012.10.003
Wishart DS, Cheng LL, Copié V, Edison AS, Eghbalnia HR, Hoch JC, ... Uchimiya M (2022) NMR and metabolomics—a roadmap for the future. Metabolites 12(8):678. https://doi.org/10.3390/metabo12080678
Yu Z, Kastenmüller G, He Y, Belcredi P, Möller G, Prehn C, . . . Wang-Sattler R (2011) Differences between human plasma and serum metabolite profiles. PLoS ONE 6(7). https://doi.org/10.1371/journal.pone.0021230
Zhang A, Sun H, Wu X, Wang X (2012) Urine metabolomics. Clin Chim Acta 414:65–69. https://doi.org/10.1016/J.CCA.2012.08.016
Zheng H, Cai A, Zhou Q, Xu P, Zhao L, Li C et al (2017) Analytical chimica acta optimal preprocessing of serum and urine metabolomic data fusion for staging prostate cancer through design of experiment. Anal Chim Acta 1–8. https://doi.org/10.1016/j.aca.2017.09.019