

Metabolomics in monitoring kidney transplants

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Purpose of review

The success of any given kidney transplant is closely tied to the ability to monitor patients and responsively change their medications. Transplant monitoring is still, however, dependent on relatively old technologies: serum creatinine levels, urine output, blood pressure, blood glucose and histopathology of biopsy samples. These older technologies do not offer sufficient specificity, sensitivity, or accuracy to allow appropriate and timely interventions. Using the tools of genomics, proteomics and metabolomics new biomarkers are being found that may greatly improve transplant monitoring and significantly enhance graft survival. This review describes the basic principles of metabolomics and summarizes a number of recent developments in the use of metabolite biomarkers and metabolomics to monitor kidney transplants.

Recent findings

Changes in the concentration profiles of a number of small molecule metabolites found in either blood or urine can be used to localize organ damage, identify organs at risk of rejection, assess organs suffering from ischemia–reperfusion injury or identify organs that have been damaged by immunosuppressive drugs.

Summary

The application of metabolomics to kidney transplant monitoring is still very much in its infancy. Nevertheless, there are a number of easily measured metabolites in both urine and serum that can provide reliable indications of organ function, organ injury, and immunosuppressive drug toxicity. As the field matures, metabolomics may eventually lead to the development of rapid, inexpensive and noninvasive approaches to assist clinicians in monitoring kidney transplants.

Keywords

kidney transplant, metabolites, metabolomics, renal function

Abbreviations

CsA	cyclosporine A
CVD	cardiovascular disease
NMR	nuclear magnetic resonance
TMAO	trimethylamine-N-oxide

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Introduction

The first successful kidney transplant was performed more than 50 years ago [1]. In the intervening period, kidney transplantation has become the most successful and widespread organ transplant operation performed today. Kidney transplants now account for more than 60% of the 25 000 organ transplants performed annually in North America. This life-saving, life-transforming surgery would not be possible without carefully controlled immunosuppression. Prior to the development of modern immunosuppressive techniques, 1-year graft survival was less than 65% [2]. Thanks to the development of calcineurin inhibitors and other modern immunosuppressive therapies, 1-year postengraftment survival now approaches 90% [3]. Long-term organ survival, however, is not yet optimal. About 25% of all kidney transplants fail within 5 years after transplantation [4,5] while 10-year graft survival rates range from 33% for deceased donor kidneys to 67% for living donor grafts [4].

Transplants may fail for any number of reasons including preoperative organ stress, surgical complications, post-operative infection, acute rejection, or immunosuppressive nephrotoxicity. Organ loss is not the only concern. Transplant patients also face increased risks for developing diabetes, atherosclerosis, hyperlipidemia, hypertension, chronic viral infections (hepatitis B virus, cytomegalovirus or BK virus), bone disease and lymphoma [6,7]. Because of the ever-present risk of post-engraftment failure and other health complications, kidney transplant patients must be monitored closely, requiring regular checks on renal function, cardiac function, signs of infection and immunosuppressive drug toxicity.

The monitoring of transplants and transplant patients, however, is still dependent on somewhat older technologies: serum creatinine levels, total urine output, body temperature, blood pressure or blood glucose. In some cases, these simple clinical assays do not offer sufficient specificity, sensitivity, or accuracy to allow appropriate

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and timely interventions. As a result, costly follow-up biopsies and time-consuming histopathological measurements are typically required to make definitive diagnoses. Recent studies suggest that even these 'gold-standard' histology assays are not without their problems [8^{*}]. Given these limitations, more and more transplant specialists are looking to the emerging fields of genomics, proteomics and metabolomics to improve the current situation.

The hope is that these high-throughput 'omic' techniques could help identify combinations of biomarkers that might be used to inexpensively and noninvasively identify transplantation problems far earlier and far more robustly than is currently possible. Proteomic methods have already identified several urinary protein biomarkers that seem to robustly identify acute rejection and other renal disorders [9,10^{*}]. Similar collections of transcript biomarkers have also been identified from kidney biopsies using microarray experiments [11^{*}]. As we shall see later, however, small molecule metabolites may well prove to be the most useful biomarkers for monitoring kidney function and detecting adverse renal events. This is because the kidney is fundamentally a metabolic organ designed to concentrate or filter small molecule metabolites and small molecule toxins. Therefore one should expect changes in metabolite levels in blood or urine to be both more detectable and more reflective of kidney function than subtle changes to the kidney's proteome or transcriptome [12^{**}].

In this review we will look at how the measurement of metabolites or metabolic profiles can be used to monitor kidney function. Specifically we will cover three areas in which metabolite measurements or metabolomic studies are having an impact in renal transplantation. These include the application of metabolomics towards assessing ischemia–reperfusion injury; assessing immunosuppressive drug toxicity and monitoring transplant organ function and localizing organ damage. Before addressing these specific applications in kidney transplantation, however, it is perhaps worthwhile to briefly discuss the field of metabolomics.

What is metabolomics?

Metabolomics is defined as the high-throughput measurement of large numbers of small molecule (<1500 Da) metabolites. Just as genes are part of the genome and proteins are part of the proteome, metabolites are part of the metabolome. The metabolome is the collection of all small molecule metabolites (endogenous or exogenous) that can be found in a cell, organ or organism. Metabolomics is a relatively new term, having been coined in 2000 [13]. Metabolomics is also known as metabonomics [14] or metabolic profiling [15]. Just like genomics and proteomics, metabolomics only

became possible with the advent of recent technological breakthroughs in small molecule separation and identification. These include the development of capillary electrophoresis and ultra-high pressure liquid chromatography (UPLC) systems for rapid compound separation, the invention of robust mass spectrometry instruments for precise mass determination, the development of high-resolution, high throughput nuclear magnetic resonance (NMR) spectrometers and the creation of new software tools to rapidly process spectral or chromatographic patterns [16^{**}]. With these hardware and software innovations it is now possible to identify and quantify not just one or two small molecules at a time, but literally dozens of small molecule metabolites in as little as a few minutes [16^{**},17–19]. Indeed, the high-throughput nature of metabolomics is what distinguishes it from more traditional small molecule fields such as clinical chemistry.

In clinical chemistry, most metabolites are typically identified and quantified using colorimetric chemical assays. In metabolomics, a large number of metabolites are measured using non-chemical, non-colorimetric methods such as gas chromatography–mass spectrometry, tandem mass spectrometry or NMR spectroscopy. Interestingly, in some versions of metabolomic analysis, the compounds are not identified; only their spectral patterns and intensities are recorded [17,18]. In other versions of metabolomic analysis, all (or most) of the compounds are identified and quantified [15,16^{**},19]. The former approach is based strongly on computer-aided pattern recognition and sophisticated statistical techniques. The latter approach relies on spectral curve-fitting and prior chemical or spectral knowledge. Both methods have their advantages and disadvantages, although there is a strong preference for absolute compound identification and quantification.

Just as genes and proteins are normally associated with specific pathways and processes, so too are metabolites. As might be expected, most of the small molecule metabolites measured by metabolomic methods are tied to generic metabolic processes (glycolysis, gluconogenesis, lipid metabolism) found in all living cells. Changes in the relative concentrations of certain 'universal' metabolites such as glucose, citrate, lactate, 2-oxoglutarate and others reflect changes in cell viability (apoptosis), levels of oxygenation (anoxia, ischemia, oxidative stress), local pH, general homeostasis and so on [5]. These molecules obviously can be quite informative of cell function or cell stress, and therefore organ function. Other kinds of metabolites are specifically associated with tissue remodeling, muscle atrophy and myofibrillar breakdown (methyl-histidine, creatine, taurine, glycine). Changes in the levels of these metabolites can provide important information about the extent of tissue repair or tissue

damage [14,17]. Some compounds, such as trimethylamine-N-oxide (TMAO), are actually used as buffers to stabilize serum proteins from the effects of accumulated waste products [20]. In other words, each metabolite has its own story to tell. The challenge for both the physician and the scientist is to figure out what that story is.

Metabolomics in assessing ischemia–reperfusion injury

Kidney transplantation is particularly traumatic to a healthy donor organ as it requires the removal of the organ from the host blood supply for a lengthy period of time (ischemia time). Obviously, the shorter the ischemia time the better the chance the organ has of recovering and functioning. Longer periods of ischemia are known to seriously damage most kidneys [21••]. In addition, tissues can also be damaged by the reoxygenation or reperfusion process. Reperfusion injury is a term used for the tissue damage caused when the blood supply returns to the transplanted organ after an extended period of ischemia. The absence of blood oxygenation creates a condition in which restored circulation results in inflammation and oxidative damage from the oxygen rather than restoration of normal function. This damage is caused by white blood cells, inflammatory proteins and free radicals flowing back into the tissue during the reperfusion process.

The identification of ischemia–reperfusion injury in newly transplanted kidneys is particularly challenging. Current methods rely on relatively simple and nonspecific measures such as serum creatinine, urine output and biopsies [21••]. As a result there has been a growing interest in the development of more reliable biomarkers and less invasive procedures, including metabolomic methods. To date most metabolomic studies on ischemia–reperfusion injury have focused on rat models [21••,22,23] although more recent investigations have been extended to humans [24•]. These NMR-based studies found that the extent of ischemia–reperfusion injury was correlated with elevated levels of citrate, dimethylamine, lactate and acetate in the urine. It was also noted that ischemia–reperfusion injury was highly correlated with increased levels of allantoin (50–100 times normal) and TMAO in the blood. Allantoin, which is an oxidative product of uric acid, is a common marker of oxidative cell stress. On the other hand, TMAO is a homeostatic ‘rescue’ compound that allows blood proteins to handle increased concentrations of urea and guanidine (both strong protein denaturants) that arise during renal failure or renal stress [20,25]. Additionally TMAO is known to be a marker of renal medullary injury. Surprisingly, it was determined that serum creatinine levels, which have long been used as an injury marker, did not correlate with the level of ischemia–reperfusion damage [21••]. Because of the

close similarity between rat and human metabolism, it is likely that the findings in these rat models will translate well to humans.

The lone human study on ischemia–reperfusion injury used metabolic profiling to identify the presence of significantly elevated serum levels of hypoxanthine and inosine (hypoxanthine nucleoside) following kidney reperfusion [24•]. Hypoxanthine and inosine are both well known markers of ischemia and oxidative damage. Both molecules are typically formed as breakdown products of ATP. Hypoxanthine can be converted to xanthine and then to uric acid via an enzyme called xanthine oxidoreductase. As an oxidase, this enzyme uses molecular oxygen as electron acceptor and generates a superoxide along with other reactive oxygen products, which upon reperfusion and re-oxygenation can lead to further oxidative tissue damage. While this study did not correlate the levels of ischemia–reperfusion injury or graft function with hypoxanthine levels, it does reaffirm that better markers for ischemia–reperfusion injury do exist. Collectively, these studies illustrate that metabolomic methods could significantly improve the monitoring of ischemia–reperfusion injury and further enhance our understanding of the effects of ischemia and reperfusion after kidney transplantation.

Metabolomics in assessing immunosuppressive drug toxicity

Kidney transplants would not be possible without immunosuppressive therapies. Immunosuppression for kidney transplant recipients, however, can also lead to nephrotoxicity as well as elevated risks for cardiovascular disease (CVD), diabetes and cancer. The detection and monitoring of these adverse drug effects are particularly challenging as relatively few tests exist for measuring immunosuppressive drug or drug metabolite levels and no single test exists for detecting the wide range of known adverse drug effects. Metabolomics may offer an answer to these problems. A key advantage of metabolomics over other ‘omic’ approaches is the fact that metabolomics is ideally suited for monitoring drugs and drug metabolites as well as for tracking the drug-induced changes to organ function and organ metabolism. This application is particularly important for the immunosuppressive drugs cyclosporine, sirolimus and tacrolimus. These potent drugs exhibit large inter-individual variability in their metabolism and a narrow therapeutic index.

Calcineurin inhibitors are metabolized by two cytochrome P450 variants known as CYP3A4 and CYP3A5. Polymorphisms in these enzymes leading to ‘ultrafast’ or ‘ultraslow’ metabolizers may have significant consequences for organ function and patient health [26•]. To help address this issue, metabolomic techniques (high

pressure liquid chromatography–mass spectrometry) have recently been developed to rapidly track serum concentrations of cyclosporine A (CsA) and five of its known metabolites among transplant recipients [27[•]]. Interestingly the concentration of one particular CsA metabolite, known as AM19, was found to correlate strongly with several inflammatory and atherosclerotic markers. These data suggest that adverse effects may be predicted and mitigated by using metabolomics to track certain CsA metabolite concentrations. Similar high-pressure liquid chromatography–mass spectrometry methods have also been used to develop effective blood assays to monitor the concentrations of the immunosuppressant mycophenolic acid and its metabolites [28[•]].

In addition to these drug-oriented metabolic profiling studies, several NMR and mass spectrometry-based metabolomic studies recently appeared that describe the consequences of CsA on endogenous metabolites [29,30^{••}]. These effects, which were assayed using rat models, include elevated levels of urinary glucose, acetate, trimethylamine and succinate along with reduced levels of urinary TMAO, kynurenate, xanthurenate, citrate and riboflavin [29]. A more recent study [30^{••}] focusing on serum instead of urinary metabolites found that both CsA and sirolimus led to elevated levels of glucose, hydroxybutyrate, creatine, creatinine, TMAO and cholesterol along with reduced concentrations of glutathione in the blood. These results are consistent with many of the calcineurin inhibitor complications seen in humans including diabetes (elevated glucose in urine and blood, elevated hydroxybutyrate), heightened risk of CVD (reduced riboflavin, elevated cholesterol), medullar damage (elevated serum TMAO and creatinine levels), increased incidence of kidney stones (low levels of citrate), proximal tubule damage (reduced kynurenate and xanthurenate) and general oxidative stress (high levels of acetate and succinate, reduced glutathione). Metabolomic studies in humans have shown similar CsA toxicity profiles including reduced citrate and increased oxalate levels [31[•]], increased cholesterol or LDL levels [32], increased malondialdehyde (a marker for oxidative stress) [33[•]] and glucose intolerance [34[•]]. In addition, human metabolic profiling studies focused on CsA and tacrolimus toxicity have shown increased levels of serum uric acid (a well known nephrotoxin) [35[•]–37[•]] as well as increased levels of homocysteine and other CVD risk markers [38].

Overall, these results illustrate the potential of using metabolomics as a ‘one-stop’ shop for assessing immunosuppressive drug toxicity. Metabolomics appears to be flexible enough to allow for the noninvasive tracking of drug and drug metabolite levels (i.e. exogenous metabolites) as well as the noninvasive tracking of endogenous metabolite levels.

Metabolomics to assess organ function and localize organ damage

As has been remarked earlier, posttransplant monitoring of organ function is particularly important for identifying signs of renal dysfunction, for localizing organ damage or for detecting the early stages of acute rejection. Close monitoring can allow preemptive or corrective action to be implemented before the organ is irreparably damaged. Outside of serum creatinine measurements to assess gross organ function and protocol biopsies to help localize organ damage, however, relatively few alternative tests are being used. Given the demonstrated potential of metabolite measurements, this is somewhat surprising. Indeed, over the past 20 years more than 30 metabolomic papers have been published describing a plethora of urinary and serum biomarkers associated with posttransplantation function, renal dysfunction, acute rejection, subclinical rejection and localized organ damage. One feature common to almost all of these studies is the substantial (three to four-fold) increase seen in both urine and serum concentrations of TMAO [39,40]. As noted before, this metabolite is a homeostatic rescue compound that helps stabilize serum proteins from the effects of accumulated waste products. In addition to reports of elevated levels of TMAO, other organic amines (trimethylamine, dimethylamine) and amino acids (glycine, alanine) have also been found. Metabolomic studies of transplanted, dysfunctional or rejected kidneys have also been used to detect the presence of elevated (two to five-fold) serum levels of nephrotoxins such as hippuric acid and uric acid [36[•],41]. Kidney dysfunction is also associated with elevated serum levels of nitric oxide synthase inhibitors such as phenylacetic acid [42] and dimethylarginine [43], which lead to significantly reduced nitric oxide production [44]. Reduced nitric oxide levels are often correlated with hypertension and cardiovascular complications, both of which tend to further diminish kidney function. Damaged kidneys also appear to rapidly elevate serum and urinary levels of lactate, acetate, succinate, citrate and urea, which are generally considered to be markers of Krebs’s cycle (i.e. metabolic) distress, increased anaerobic metabolism and tubular acidosis [39,40]. The identification of these previously unidentified metabolic imbalances is leading to therapeutic and dietary interventions that appear to have some benefit [45,46].

Noninvasive (i.e. biopsy-free) approaches to localize organ damage are another area where metabolomic approaches may eventually find some clinical utility. A growing body of research is accumulating which shows that it is possible to correlate localized kidney damage with distinct metabolite patterns [47]. For example, using rat models and various site-specific nephrotoxins researchers have found that damage to the proximal straight tubules (via D-serine) is typically associated with

increased levels of lactate along with elevated levels of the amino acids tryptophan, phenylalanine, tyrosine, tyrosine and valine [48[•]]. Straight tubule injury is also manifested by reduced levels of methylsuccinic, sebamic and xanthurenic acid. Meanwhile damage to the proximal convoluted tubules (via gentamicin) is generally associated with elevated levels of urinary glucose and reduced levels of TMAO, xanthurenic acid and kynurenic acid [49[•]]. On the other hand it has been noted that renal papillary and medullary injury (via bromoethaneamide) is characterized by increased urinary concentrations of glutaric acid, creatine and adipic acid along with reduced levels of citrate, succinate, oxoglutarate and TMAO [17]. In contrast, renal cortical damage (via mercuric chloride) is associated with increased urinary glucose, alanine, valine, lactate, hippurate and decreased citrate, succinate and oxoglutarate [50]. While it may be some time before these animal model results can be translated to humans in the transplant clinic, the possibility of using simple metabolic profiles to noninvasively characterize the foci of organ damage is obviously quite appealing.

Conclusion

The application of metabolomics to kidney transplant monitoring is still very much in its infancy. It is now quite apparent that there are a number of metabolites that can be easily measured in both urine and serum and can provide reliable indications of organ function, organ injury, and immunosuppressive drug toxicity. Indeed the American Society of Nephrology has recently endorsed the development of core metabolomics facilities to facilitate the centralized processing of biologic materials [51[•]]. As the field advances, it is likely that more metabolite markers or more specific metabolic profiles will be discovered and clinically validated, allowing even more precise diagnostic determinations. While metabolomics clearly offers a number of exciting prospects, one must always remember that metabolites are only a small part of the biological picture. Understanding organ rejection, detecting certain kinds of organ injury or predicting the outcome of an organ transplant will always require the input from many disciplines and many technologies.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 665–666).

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