

Non-invasive Biomarkers in Monitoring Kidney Allograft Health

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Abbreviations

ABMR: Antibody-Mediated Rejection

DGF: Delayed Graft Function

DSA: Donor Specific Antibodies

EGFR: Estimated Glomerular Filtration Rate

IFTA: Interstitial Fibrosis/Tubular Atrophy

IRI: Ischaemia Reperfusion Injury

K-SORT: Kidney Solid Organ Response Test

L-FABP: Liver-type Fatty Acid-Binding Protein

NIH: National Institutes of Health

NGAL: Neutrophil Gelatinase-Associated Lipoprotein

TCMR: T-Cell Mediated Rejection

Abstract

Purpose of review:

A key aspect of post-transplant management is to identify and treat graft injury before it becomes irreversible. The gold-standard for detection is histology, but biopsy is uncomfortable for the patient and carries a risk of complications. Detection of changes at a molecular level may pre-empt histological injury, and thereby identify injury earlier.

Recent findings:

Indicators of immune system activation, such as candidate chemokines CXCL9 and CXCL10, and by-products of neutrophil activity, have been related to acute rejection and early allograft function. Transcriptomic studies of multiple-gene panels have identified candidate combinations that have proven very promising in risk-stratification and prediction of acute rejection, as well as diagnosis of both T-cell-mediated and antibody-mediated rejection. Serum and urine cell-free DNA is also a promising area of investigation, particularly in antibody-mediated rejection.

Summary:

Non-invasive, rapid and accurate tests for risk-prediction and diagnosis in renal transplant allografts are urgently required. The ideal candidate is one that can be measured in either urine or blood, is cheap, and is both sensitive and specific for the condition of interest. Numerous strategies have been proposed, with varying degrees of clinical and pre-clinical success. Few that meet the essential criteria have been evaluated, few have made it as far as clinical testing.

Keywords: kidney transplantation, graft rejection, biomarkers

Introduction

Histological analysis remains the gold standard method for assessing renal allograft health, either on an indication basis when renal function worsens, or a protocol basis at set time points after transplantation. Renal allograft biopsy has its risks, with a 1% incidence of major complications in large series (1). It is possible that changes at a molecular level may pre-empt histological injury, and thereby demonstrate acute rejection and other forms of injury earlier than invasive tests (2). Given the risks and discomfort associated with invasive biopsies using trucut needles, biomarkers of renal allograft health have been eagerly sought. Whilst serum creatinine is a safely sampled biomarker that is cheap to monitor, it is not specific and rises late in renal injury (3).

Screening tests must have certain characteristics in order to be clinically useful and justified: They must be safe and acceptable to the patient, they must detect a condition at a stage at which treatment can alter disease progression and they must detect abnormalities with a satisfactory accuracy and frequency to justify the risks and costs. With regards to biomarkers in particular, the National Institutes of Health (NIH) Biomarker Definition Working Group defines a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic responses, or pharmacological responses to a therapeutic intervention” (4).

In the case of renal transplantation, there are potentially three key phases during which biomarkers may be of help in making clinical decisions (**Error! Reference source not found.**). For the purposes of this review we will concentrate on those biomarkers with a potential role in monitoring the renal allograft after transplantation and focus on those with recent clinical results available.

Immune system activity

Evidence of increased immune system activity is one potential target for biomarkers of renal allograft injury. The T-cell chemoattractant chemokines CXCL9 and CXCL10 are upregulated during immunological activation, and have been evaluated as biomarkers of acute rejection in several studies (5). Recently CXCL9 underwent a relatively robust validation in clinical studies as both protein and mRNA (6). During the first 6 months after transplantation increasing levels of urinary CXCL9 can be seen up to 30 days before clinical signs of graft dysfunction (6). Whilst the positive predictive value was modest in this study (61-65%), the negative predictive value was much better, over 92%, so the absence of urinary CXCL9 is possibly more useful as a way of ruling out acute rejection in cases of graft dysfunction (6). This group also found that urinary levels of granzyme B were associated with T-Cell Mediated Rejection (TCMR) but did not improve the predictive value of CXCL9.

The longer-term follow up from this study has recently been published (CTOT-17) and did not find a relationship between early immune markers and 5-year graft loss or eGFR (7)**. The group found that a 30% or greater decline in Estimated Glomerular Filtration Rate (eGFR) between 6 and 24 months post-transplant was a better predictor of graft loss at 5 years (7)**. Recent studies of CXCL10 have shown association with TCMR in the first 12 months after transplant, eGFR decline and risk of allograft loss (8). The chemokine CCL2 was evaluated in the same study, and whilst it showed association with eGFR in the first 6 months, it was not related to eGFR at later time points (8). Serum CXCL10 has been tested in combination with 2 other chemokines (fractalkine/CX3CL1 and Interferon-gamma), with the combination of these results in the first week proving to be a better predictive test of acute rejection in the first month after transplantation (9)*. Urinary levels of the chemokine CXCL13 have also been found to be predictive of chronic allograft nephropathy and acute rejection in a recent, large clinical study (10). Interestingly in this study, steroid resistant acute rejection was associated with significantly higher levels of urinary CXCL13 and elevated levels in the first month after transplant were predictive of eGFR up to 6 months after transplant (10).

Neutrophil Gelatinase-Associated Lipoprotein (NGAL) is released from activated neutrophils and is elevated in patients with end-stage renal disease. It has also been studied in the setting of renal transplantation. Results from the large, multicentre CONTEXT trial showed that, whilst plasma NGAL on day 1 after transplantation was associated with DGF, it was less predictive than urine output and did not correlate with 12-month measured GFR (11)**. The same study also found no relation between other potential biomarkers and GFR, including liver-type fatty acid-binding protein (L-FABP), cystatin C, and YKL-4(11)**.

Another potential serum biomarker of immune activity is soluble CD30, a member of the tumour necrosis factor family that is expressed on active T- and B-cells. A recent clinical study showed that plasma CD30 at day 7 and 14 after transplantation had a moderate association with acute rejection but not graft loss, and may be particularly useful when following the normally expected decrease in levels post-transplant (12)

Transcriptomics

Transcriptomics allows assessment of the overall gene expression of cells. This can be used to investigate the total RNA expressed in a tissue at a given time by microarray or RNA sequencing. Many candidate gene studies, and some genome-wide studies have been conducted, but most associations with acute rejection are weak. Also, the relatively low incidence of acute rejection has

meant that large populations have not been available for study (13). The definition of acute rejection has changed over time, and in combination with the mechanistically different forms of rejection (ABMR, TCMR and mixed) making transcriptomic associations has proven difficult.

Results from the large multicentre CTOT-4 study using urine samples showed that a 3-gene signature for CD3E mRNA, CXCL10 mRNA and 18s rRNA could accurately diagnose TCMR in matched histology specimens (14). A real-time PCR test called the Kidney Solid Organ Response Test (K-SORT) has been tested in the prediction of acute rejection and risk stratification of renal transplant recipients (15). The initial 43-gene set represented a combination of significant genes in acute renal allograft rejection identified by whole genome micro-array analysis of biopsy and paired blood samples, this was subsequently refined to 17 genes (15). A correlation-based algorithm was developed to analyse gene-expression data and validated in 124 samples, where it predicted rejection up to 3 months prior to detection by biopsy (15). The sensitivity and specificity of the test in this study was high (92.3% and 93.5% respectively). However, tested alongside contemporaneous renal transplant biopsies, it does not discriminate between ABMR and TCMR. K-SORT still needs prospective clinical evaluation and has been included as part of the protocol in an ongoing trial of steroid avoidance in renal transplantation (SAILOR study, SUGBG-012011).

MicroRNA are non-coding RNA that inhibit the translation of their complementary mRNA, and thereby control gene expression. In the context of renal transplantation, microRNA have been shown to be regulated in acute TCMR, ABMR and Interstitial Fibrosis/Tubular Atrophy (IFTA). A recently published study evaluated specific microRNA candidates in a large patient cohort after renal transplant (16)*. Selected candidate microRNAs: miR-223-3p, miR-424-3p and miR-145-5p distinguished TCMR and ABMR from normal function, but not from other pathologies or each other (16)*. MiR-145-5p expression was significantly downregulated in IFTA patients, however the multiple causes contributing to IFTA after kidney transplantation is still a challenge. There was only one microRNA, miR-15b-5p, whose regulation in blood cells was different between patients with stable graft function, ABMR and TCMR (16)*. In a previous study by the same group a significantly different miR-15b-5p expression was observed, when comparing patients with ABMR and TCMR, but not when comparing ABMR patients to patients with stable graft function (17). This work showed that 5 candidate microRNA array was significantly downregulated in T-cell mediated vascular rejection and in combination they enhanced the sensitivity and specificity for this diagnosis (17). Circulating miRNA-148a has been recently tested and reduced levels significantly correlated with histological findings of IFTA (18). In this study serum from patients with histologically diagnosed IFTA was compared to that from recipients with stable graft function, and healthy individuals, with 97% sensitivity and 72% specificity (18).

Proteomics & Metabolomics

Proteomics is the study of the entire set of proteins that is produced by an organism or system, whilst metabolomics is the measurement of small molecules associated with cellular metabolism and function. Both the cellular proteome and metabolome are related to environmental interactions and can indicate the current state, health and activity of a tissue.

A recently published case-control study of urinary proteomics showed a high sensitivity for ABMR (100%), albeit with low specificity (75%), which may identify patients for biopsy (19). In this study the addition of the urine proteomic marker CKD273, improved the detection of patients with ABMR. Most of the sequenced peptides were fragments of collagen. A second study in paediatric transplantation identified urinary metabolite profiles that are associated with IFTA and degree of glomerular sclerosis, also showing that urinary metabolite profiles are associated with eGFR (20)*. This is the first study demonstrating the ability to identify the stage of histological severity of chronic allograft damage, using a urinary metabolomic profile. Interestingly in this study both percentage glomerular sclerosis and IFTA severity showed a weak but significant correlation with measured eGFR (20)*.

Cell-free DNA

Cell-free DNA (cfDNA) is released during apoptosis and several groups have investigated its use as a marker of renal allograft injury (21)*. Donor-derived cfDNA (dd-cfDNA) can be distinguished from recipient cfDNA even in the absence of a known donor tissue type using computational biology, meaning that just a recipient blood sample is required (21)*. Levels of donor-derived cfDNA peak with initial ischaemia reperfusion injury, more so with deceased renal donation than live donation, falling rapidly to baseline in both situations(22). Elevated levels of dd-cfDNA have been reported in recipients at the time of acute rejection and in patients developing donor specific antibodies (DSA) (23, 24). In one study of 102 renal transplants, cfDNA could predict ABMR with good negative predictive value (96%) but with a smaller positive predictive value (44%)(23). The measurement of dd-cfDNA can also improve the diagnostic accuracy of serum DSA in diagnosis of active ABMR (25)*. The positive predictive value of cfDNA to detect active ABMR in DSA-positive patients was 81%, the negative predictive value was 83%, whilst the positive predictive value for DSA positivity alone was 48% (25)*. Elevated cfDNA is not specific to acute rejection; elevated levels can be seen in BK Nephropathy, urinary infections, acute tubular necrosis and calcineurin toxicity (21). No published

studies have yet related cfDNA to long term outcomes of renal transplantation. In liver transplantation cfDNA allowed for earlier and more sensitive discrimination of acute rejection compared with conventional serum liver function tests (26).

Conclusion

Non-invasive and accurate tests for early identification of renal allografts injury are urgently required. The ideal candidate is one that can be measured without risk of complication, that is cheap, provides a rapid result, and is both sensitive and specific for the condition of interest. Numerous strategies have been proposed, with varying degrees of clinical and pre-clinical success and few that meet the essential criteria have been evaluated, some have made it as far as clinical testing. The kSORT real-time PCR test, in combination with the algorithm, kSAS, accurately indicates risk and incidence of acute rejection based on the expression of 17 genes, independent of patient age. There is good evidence for cfDNA as an injury biomarker and the majority of studies show a relationship with acute rejection, particularly with more severe grades of acute rejection and ABMR. However, cfDNA is associated with other causes of allograft injury and at the moment has not been refined to a specific test. From current publications the negative predictive value is higher than the positive predictive value and therefore cfDNA may be most suited to excluding acute rejection rather than diagnosing it. No studies have yet validated a threshold in an external population, or the impact of prospective monitoring on clinical outcomes.

Candidate biomarkers with a reasonable relation to transplant health have by and large a greater negative predictive value than positive predictive value. This makes them of better use for ruling out, rather than confirming a diagnosis of, acute rejection for example. Many tests are not specific for the cause of injury and therefore cannot specifically target treatment of the underlying cause. At the current point in time, therefore, many of these tests are perhaps of most use in identifying 'at-risk' patients in whom further investigation, such as invasive biopsy, are warranted.

Individual biomarkers, such as lone chemokines, have been tested with good results, but the most promising area in terms of potential specificity is likely to be combination tests reliant on more than one gene, whether as mRNA, micro-RNA or proteomic analysis. The challenge of inter-individual variation remains. The possibility of a "common rejection module" of 11 genes across heart, liver, lung and kidney allografts has been raised, with some evidence that similarities in gene expression support an analogous immune activity in the rejection of any foreign tissue (27).

In conclusion several serum and urine biomarkers have shown promise in the detection of renal allograft injury. Their detection may precede histological injury and even clinical manifestation, making early detection and treatment a possibility. It may also be possible to use biomarkers as a marker of tolerance and overall immunosuppression but prospective and comparative trials are required.

Key points

1. candidate chemokines CXCL9 and CXCL10, and by-products of neutrophil activity, have been related to acute rejection and early allograft function.
2. Transcriptomic studies of multiple-gene panels have identified candidate combinations that have proven very promising in risk-stratification and prediction of acute rejection.
3. Serum and urine cell-free DNA is also a promising area of investigation, particularly in antibody-mediated rejection.
4. The majority of existing biomarkers have greater sensitivity than specificity for causes of graft injury, making them most useful for guiding further investigation to determine the specific cause of injury

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Conflicts of interest

The authors have no conflicts of interest to declare.

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