Published in final edited form as:
Curr Opin Organ Transplant. 2012 February ; 17(1): 26–32. doi:10.1097/MOT.0b013e32834ee402.

IMMUNOLOGIC MONITORING IN TRANSPLANTATION REVISITED

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Abstract

Purpose of review—Tailoring immunosuppressive drugs to the individual needs is crucial to improve long-term outcomes of organ transplant patients. The purpose of this review is to summarize data on promising biomarkers able to detect the risk of acute or chronic rejection and to discuss the potential issues for their implementation in the clinic.

Recent findings—Multiple publications indicate that circulating antibodies targeting HLA and non-HLA antigens, as well as donor-specific memory T cells are associated with accelerated graft failure. Other studies published within the year show that specific genomic and proteomic signatures obtained from urine, blood and graft tissue correlate with acute rejection in kidney and heart transplant patients.

Summary—Developing reliable biomarkers is crucial for individualizing therapy aimed at extending allograft survival and improving patient health. Emerging data indicate that monitoring assays, likely used in panels, have potential to be diagnostic and possibly predictive of long-term outcome. In addition to ongoing discovery efforts, progress in the field will require multicenter validation, assay standardization and commercialization so as to efficiently deliver reliable testing strategies to the practicing clinician.

Keywords

acute rejection; biomarker; ELISPOT; genomic; proteomics

Introduction

Improvements in transplant care have decreased morbidity, reduced acute rejection rates and modestly lengthened graft survival [1]. Nonetheless, long-term outcomes for transplant recipients are suboptimal and patients with failed transplants comprise an increasing percentage of candidates on transplant waiting lists [2]. While transplant care is generally performed by protocols, the recognition of the clinical, immunological and genetic heterogeneity that exists among donors and recipients raised the possibility that immunosuppression could be tailored to optimize outcome for each recipient. Development of individualized treatment strategies will require reliable, reproducible, cost effective, yet noninvasive biomarkers capable of assessing the risk of graft injury. Ideally, these biomarkers would guide immunosuppressant withdrawal in patients at minimal risk and direct specific therapeutic strategies to limit injury in patients at higher risk, together resulting in prolonged graft survival and improved patient health. The purpose of this review is to summarize recent progress in biomarker research relevant to transplantation.

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Biomarker fundamentals

Biomarkers can be defined as anatomic, biochemical, or molecular parameters that indicate, or are associated with a clinically significant alteration in physiology. The utility of a given biomarker is context-dependent. In the transplant setting, biomarkers could be used for: a) assessing organ quality pretransplant, b) predicting/diagnosing delayed graft function, c) predicting/diagnosing acute rejection, d) defining under- or over-immunosuppression, e) predicting/diagnosing chronic injury, and f) delineating functional tolerance. The reader is referred elsewhere for reviews on biomarkers and delayed graft function or immune tolerance [3–5].

A biomarker should be simple and inexpensive, requiring a noninvasively obtained sample (e.g. urine or blood), yet should be specific, sensitive and able to predict a clinically relevant endpoint before its occurrence. Biomarker discovery occurs via “biased” and “unbiased” approaches. Biased discovery starts from a basic science mechanism and tests whether the involved molecules or readouts can predict a clinical endpoint. Such biased approaches have the advantage of being supported by previous scientific findings but can be limited in scope. Unbiased approaches attempt to correlate molecular patterns derived from genomic, proteomic, or other large-scale screening assays with clinical phenotypes. While this strategy is hypothesis-generating rather than hypothesis-testing, it can result in discovery of novel biomarkers that can drive new mechanistic studies. For any biomarker to become a clinically useful test it should be prospectively validated in an independent, multicenter patient cohort. The assay should also be standardized such that any qualified laboratory can replicate the results, and ultimately commercialized for routine clinical use.

Anti-HLA antibodies and transplant outcome

As a supplement to cross-match antibody testing, flow cytometry-based and Luminex-based assessments of serum binding to HLA-coated microparticles has revolutionized the measurements of anti-HLA antibodies in transplantation [6]. Emerging evidence from large cohorts of kidney transplant patients indicates that patients with serum anti-HLA antibodies, and particularly those with anti-donor HLA antibodies, have significantly worse outcomes [7,8]. As a result, pretransplant antibody specificities are routinely determined, and donor organs expressing those HLA alleles to which the patients’ sera react can be avoided (virtual cross-matching). Based on evidence from kidney [9,10] and heart transplant recipients [11] that de novo posttransplant anti-donor class II antibodies are associated with ongoing or incipient graft injury, some transplant centers are adopting posttransplant antibody monitoring. While these commercially available reagents have clinical value, there are several important issues that could confound conclusions. The repertoire of HLA antigens on the beads does not cover all alleles in the population, reagent lot inconsistencies, and technique and machine differences among testing sites introduce variability, and the threshold for defining a positive test remains controversial. Further optimization (ongoing, see www.ctot.org among others) will improve the utility of anti-HLA antibody testing as predictive biomarker. Nonetheless, without therapies capable of specifically eliminating alloantibodies and/or treating antibody-initiated injury the clinical impact of simply identifying patients with anti-donor HLA antibodies will be marginal.

Antibodies to non-HLA antigens and transplant outcome

Increasing evidence indicates that antibodies reactive to polymorphic non-HLA antigens (e.g. major-histocompatibility-complex class I-related chain A or MICA alleles) [12] could function as biomarkers for transplant outcome. While antibodies to MICA were shown in 2007 to be associated with an elevated risk of acute kidney rejection and lower 1-year graft survival [12], a relationship between anti-MICA antibodies and outcome was not observed

in heart transplant recipients [13]. This difference may be attributable to the higher expression of MICA antigens on renal [14] as compared to cardiac cells [13]. Further prospective validation and assay standardization will be required before anti-MICA antibody monitoring can be implemented routinely.

Several studies suggest that pre-existent and de novo autoimmune responses are associated with graft injury. Antibodies reactive to type V collagen strongly associate with and pre-date the development of bronchiolitis obliterans following lung transplantation [15]. In heart transplant recipients, others [16] and we [17] observed strong associations between anti-cardiac myosin immunity (among other cardiac antigens) and chronic allograft vasculopathy (CAV). Our studies showed that antibodies to cardiac myosin were associated with a 28-fold increase in the risk of developing CAV independent of anti-HLA antibodies [17]. Because anti-cardiac myosin immunity occurs commonly prior to transplantation as a cause or consequence of primary organ failure [18], measurements of anti-cardiac myosin antibodies could evolve into a pretransplant risk assessment tool.

Other 2011 papers highlight the potential utility of antibodies to non-HLA antigens as biomarkers for outcomes following kidney transplantation. A single center study demonstrated an association between de novo posttransplant anti-endothelial cell antibodies and recurrent transplant rejection resulting in allograft dysfunction [19]. The specific antigenic target remains to be determined. Others [20] and we [21] used protein microarray platforms as large-scale, nonbiased screening to discover autoantibodies associated with kidney transplant injury. The Sarwal group showed a relationship between serum antibodies reactive to a previously unrecognized, kidney expressed autoantigen and acute rejection [20]. Dinavahi *et al*. compared antibody repertoires in pre- and post-transplant sera from several cohorts of patients with or without transplant glomerulopathy (TG). The analysis showed, in test and validation cohorts, that reactivity to one intracellular antigen, peroxisomal-trans-2-enoyl-coA-reductase (PECR) was strongly associated with TG. Anti-PECR antibodies were also detectable pretransplant in patients destined to develop TG [21]. If confirmed in larger prospective analyses, these autoantibodies could become useful risk assessment tools.

Measurements of donor-reactive memory T cells and transplant outcomes

Among notable advances in transplantation immunology research is the recognition that a proportion of the alloreactive T cell repertoire derives from the memory pool [22], whose unique properties indicate that they may be detrimental to transplant outcome [23, 24], and consequently, measurements of memory alloimmunity could be used as biomarkers for posttransplant outcome [25]. Memory T cells and donor-reactive T cell memory are detectable by flow cytometry and cytokine ELISPOT. Single center studies showed that frequencies of donor-reactive memory cells are strong correlates of acute rejection, graft dysfunction/failure in kidney transplant recipients. Newer data indicate that those patients with the highest frequencies of pretransplant anti-donor T cells benefit from induction therapy [26], particularly rabbit anti-thymocyte globulin (rATG) which lowers anti-donor T cells [27] and enhances Treg [28], together likely improving graft function. Large-scale multicenter studies ongoing in Europe (www.transplant-tolerance.org.uk) and the US (www.ctot.org) are standardizing assay protocols, prospectively validating the utility of measuring T cell memory as a biomarker for graft injury in recipients of kidney or heart transplants, and testing whether measurements of T cell memory can guide therapeutic decision-making. While measurements of T cell memory are promising biomarkers in transplantation, the relatively high cost, assay complexity and labor intensity required for accurate performance represent barriers to commercialization. Creative industry collaborations and reimbursement strategies are needed to bring these tests to the clinic.

Urinary mRNA as biomarkers for transplant rejection

Suthanthiran and colleagues established feasibility and utility for using quantitative PCR on urinary cell-, and peripheral blood mononuclear cell RNA as biomarkers for acute rejection following kidney transplantation (reviewed in [29]). This group reported in 2010 that urinary mRNA transcript levels for costimulatory molecules expressed on immune cells, OX40, OX40L, and PD-1, were strongly associated with acute rejection episodes in kidney transplant recipients with a sensitivity and specificity of >92% [30].

Expanding upon their previous single center studies, Suthanthiran reported preliminary findings from a multicenter trial at the 2011 American Transplant Congress (Philadelphia, PA, June 2011). These data indicate that serial measurements of urinary granzyme B (among other genes) detected incipient, biopsy-proven, acute rejection significantly prior to its clinical recognition [31]. This important result could permit early intervention thereby avoiding the need for biopsy and potentially limiting long term allograft injury.

In other work, van Ham and colleagues provided evidence that elevations in urinary granzyme A mRNA levels correlated specifically with subclinical cellular injury in kidney transplant [32]. Others showed that elevated expression of selected urinary microRNAs, a class of noncoding RNAs that posttranscriptionally control gene expression, may be associated with acute rejection [33].

Together, these results support using urinary gene expression profiling to non-invasively diagnose acute rejection and, possibly, to predict its occurrence. Future studies will be required to test whether treatment of patients with up-regulated urinary expression of these genes will prevent acute rejection and improve graft outcomes. Assay standardization (ongoing within CTOT consortia among other arenas), and collaborative commercialization strategies will be crucial to incorporate these assays into clinical practice.

Urinary chemokines and transplant outcomes

Following the documentation that chemokines are important modulators of transplant injury in animal models [34] several groups developed assays to measure human chemokines in urine of kidney transplant recipients. These studies [35] found increased urinary levels of CXCL9, CXCL10, and CXCL11 in patients with acute rejection, acute tubular injury, or BK virus nephropathy compared to patients with chronic rejection or stable function, and healthy individuals [36,37].

Extending these findings, data published in 2010 indicate that urinary chemokine measurements may predict late graft failure. In a single center study of 111 kidney transplant patients, 6 month urinary CCL2 levels were associated with the subsequent development of severe interstitial fibrosis/tubular atrophy (IFTA) and graft dysfunction at 24 months [38].

Ongoing prospective multicenter validations, along with newly initiated trials testing whether urinary chemokine levels can guide immunosuppressant withdrawal will better determine how these promising biomarkers will be useful clinically.

Serum proteins as biomarkers for transplant outcome

Because blood samples are considered noninvasive and are easily obtained, many groups have attempted to identify serum biomarkers capable for outcomes in transplant recipients. Multiple single center studies indicated associations between candidate serum markers and chronic allograft injury [39]. In 2010, research groups reported associations between serum myeloperoxidase and carbonyl proteins levels with an increased risk of acute rejection in

heart transplant patients [40], and elevated serum KL-6 values with an increased risk of bronchiolitis obliterans in lung transplant recipients [41]. While promising, these putative biomarkers require multicenter prospective validation.

One of the most extensively studied serum makers in transplantation is soluble CD30 (sCD30), a costimulatory molecule expressed by T cells upon activation, and released into the serum. While some data published in 2010 suggest that sCD30 could be used to assess the risk of acute rejection following kidney transplantation [42,43], other results do not support this conclusion [44,45]. Resolution of the conflicting findings will require larger-scale, prospective studies. One important caveat to interpreting such work and designing new studies is that immunosuppression regimens, including the use of induction therapy, can influence the utility of serum biomarkers [46].

Genomic analyses and transplant outcomes

Genomic analyses of RNA obtained from peripheral blood cells and tissue represent nonbiased approaches used to uncover mechanisms of allograft injury and to discover biomarkers associated with outcomes [47,48]. New data published in 2010 indicated that kidney biopsy gene expression profiles provide prognostic information beyond clinical and histological phenotyping. From 105 “for-cause” biopsies obtained >1 year posttransplantation, and using independent test and validation cohorts, Einecke et al. identified a molecular risk score that accurately predicted late graft loss [49]. In other work, microarray data obtained from graft biopsies in heart and kidney transplant patients with acute rejection identified 45 upregulated genes [50], 3 of which were confirmed in an independent validation set. Analyses of peripheral blood from 77 kidney transplant recipients performed by Salomon and colleagues identified several unique signatures of mRNA transcript (and protein) biomarkers with high predictive accuracies for IFTA [51]. In another intriguing report [52], investigators used state-of-the-art molecular techniques to detect circulating, cell-free, donor DNA in serum from heart transplant recipients, and found that higher levels were associated with allograft damage. Specificity for rejection as well as the complexities of the assay may limit the use of this strategy as a clinically acceptable biomarker.

Together with previously published work, the emerging data indicate that transcription profiling of blood and/or graft tissue can potentially provide useful prognostic information. Prospective validation, lowering costs and simplifying assays will be required before the genomic assays reliably guide decision-making to improve transplant outcomes.

Proteomics and transplant outcome

The rapid evolution of proteomic technologies [surface-enhanced laser desorption/ionization with time-of-flight mass spectrometry (SELDI-TOF-MS), liquid chromatography mass spectrometry (LC-MS) and matrix-assisted laser desorption/ionization (MALDI)] has facilitated nonbiased analyses of serum, urine and allograft proteomes in patients with various disease processes, including transplantation (reviewed in [53]). Progress in this field includes a case-control discovery study in which the investigators examined patterns of plasma proteins during early kidney graft rejection [54]. They identified three proteins (titin, kininogen-1, and lipopolysaccharide-binding protein) which accurately discriminated patients with and without rejection. Nakorchevsky and colleagues performed a proteomic analysis of graft tissue in patients with IFTA. Among many findings, their analyses showed that alternative pathway complement, but not classical pathway complement generally associated with antibody-initiated injury, was preferentially expressed in grafts with IFTA [55]. Along with previous studies reported by others, the emerging data indicate that proteomic analysis can provide mechanistic insight and could identify unique biomarkers

associated with transplant outcome. Multicenter validation, and assay standardization/ commercialization remain barriers to clinical implementation.

ATP release as a measure of immune function

The ImmuKnow© assay is an FDA approved test that measures mitogen-induced ATP production by CD4+ T cells, and is touted as a functional measure of immunosuppression. Observational studies, including a 2010, single center series of ~300 heart transplant patients [56], have suggested that patients with assay results falling below the normal range are at elevated risk for infectious complications. While investigators have hypothesized that high levels of ATP release (presumptively under-immunosuppressed) would be associated with an increased risk of acute rejection, observational studies have provided inconclusive findings in this regard [57–59]. Preliminary data reported in abstract form from 101 liver transplant recipients indicated that titrating immunosuppressive therapy according to ATP levels reduces the infectious risk compared to standard monitoring [60]. If confirmed, this evidence would support the use of ATP measurements in the care of transplant patients.

Conclusion

Developing reliable biomarkers is crucial for individualizing therapy aimed at extending allograft survival. Emerging data indicate that many assays, likely used in panels rather than single assays, have potential to be diagnostic and predictive of long-term outcome. In addition to ongoing discovery efforts, progress in the field will require multicenter validation, assay standardization and commercialization so as to efficiently deliver reliable testing strategies to the practicing clinician.

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Key points

- Defining surrogate endpoints is instrumental to test biomarkers and new treatments in organ transplantation
- Anti-HLA and non-HLA antibodies, measures of donor specific memory, as well as urinary mRNA profiles and proteins represent promising biomarkers for acute injury and long-term graft outcomes
- Genomic signatures at the mRNA (transcriptome) level and proteomic signatures obtained from blood, urine and or graft tissue have the potential to provide prognostic information for transplant outcome
- Prospective multicenter validation, assay standardization, and commercialization are required before any biomarker can be integrated into clinical practice and thereby guide therapeutic decision-making