Novel Biomarkers to Monitor and Predict Rejection in Kidney Transplantation

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Despite the substantial successes of kidney transplantation, this field continues to be hampered by the inability to monitor the intensity of the immunosuppressive regimens. As a result, chronic antibody-mediated rejection (under-immunosuppression), as well as drug-related toxicity, malignancies, and opportunistic infections (over-immunosuppression) continue to be the leading causes of allograft loss. In addition, counter to all the predictions, the vast improvement in the early acute rejection rate has not resulted in similar improvement in long-term allograft survival. Serum creatinine, the traditional marker to monitor kidney function, is highly unreliable in predicting renal injury of any kind. Protocol kidney biopsies, in addition to being invasive, suffer from poor inter-observer variability, sampling bias, and limited acute rejection prediction. Therefore, the majority of the above-mentioned complications are diagnosed after they have occurred. Ideally, the goal of post-transplant monitoring should be to predict and prevent such complications. Clearly, a reliable biomarker to predict acute rejection that is non-invasive and inexpensive is the most urgent need in the organ transplantation world, as long as the “holy grail,” transplant tolerance, remains elusive.

The earlier years of organ transplantation employed crude immunosuppressive regimens to prevent acute rejection but resulted in mortality as high as 50% within the first year because of severe infections. Improved understanding of the biology of rejection led to the development of more sophisticated drugs during the 1980s and 1990s, mainly targeting the T and B lymphocytes. To prevent toxicity, therapeutic drug level range was identified and monitored for some drugs such as cyclosporine and sirolimus. However, the pharmacokinetics of the drugs did not serve the clinician in understanding the pharmacodynamics of the drug in the individual patient, and over- or under-immunosuppression continued to remain a problem. Earlier, there was an interest in the role of quantitative immunoglobulins, specifically IgG, in the long-term management of the transplant recipient. Immunosuppression was slowly weaned as long as the IgG level was in the normal range. Indeed, development of hypogammaglobulinemia in immunosuppressed patients was identified as a serious risk for infectious diseases of all types. However, this method did not predict T cell behavior, which is critical in predicting acute rejection. The previous decade saw the introduction of more advanced techniques. Numerous methods were proposed, including DNA microarray analysis in kidney biopsy specimens, to detect gene expression profiles associated with rejection; measurement of urinary cell mRNA profiles; and the ELISPOT assay. A detailed discussion about these markers is beyond the scope of this article but one of these deserves special mention. A cell-mediated immune function assay, Immuknow (Cylex Inc., Columbus, MD), was introduced in 2002. This test detects induced ATP levels in the CD4+ T cells after an 18-hour incubation with a mitogenic phytohemagglutinin. The resulting ATP level estimated the T cell allostereactivity, which is calibrated to predict infection vs. rejection. This test initially gained widespread popularity, but subsequent studies showed conflicting results. While this test is still commercially available, its role in transplant patient management remains uncertain.

Two recently introduced novel genetic methods offer hope in bridging this gap. The TruGraf test (Transplant Genomics Inc., Mansfield, MA) uses peripheral blood samples to study gene expression patterns using microarray analysis (1). The test is based on the idea that gene expression patterns within the allograft are unique for normal functioning compared to renal injury, and this pattern is detectable in the peripheral blood. In this test, RNA is extracted, amplified, and hybridized to DNA microarrays. The pattern of hybridization, or “signature,” is then compared to a reference dataset using an algorithm to generate a qualitative result of “Transplant eXcellence” (TX) or “not-TX.” TX indicates immune quiescence, suggesting to the clinician that the patient is adequately immunosuppressed, and not-TX suggests that immunosuppression modification is warranted. For example, patients with previous TX status that changes to not-TX status following a reduction in immunosuppression might benefit from reversing the immunosuppression change or from a diagnostic kidney biopsy. TruGraf was tested in 105 transplant patients in four medical centers and had a 73% concordance with the final diagnoses made based on other clinical data. The sensitivity of this test was 81%, specificity 70%, positive predictive value (PPV) 47%, and negative predictive value (NPV) 92%, with a false negative rate of 19%. The test will likely become commercially available soon.

AllouSure (CareDx, Brisbane, CA) is a donor-derived cell-free DNA (dd-cfDNA) measurement technique in the peripheral blood (2). The premise of the test is that injury to the transplanted kidney leads to cell death and that escape of cell DNA into the peripheral blood can be measured. The test employs targeted amplification and sequencing of single-nucleotide polymorphisms (SNPs) to quantify donor and recipient DNA contributions in each patient’s blood sample. This is accomplished without requiring the donor or the recipient’s DNA genotyping information. The test utilizes a next-generation sequencing assay that employs 266 SNPs. This panel was selected based on allelic frequency across ancestral heritage groups, sequencing accuracy, and lack of linkage. These SNPs were also chosen not to have association with common genetic disorders to avoid reporting incidental findings. Based on several validation studies, the result of <1% dd-cfDNA is associated with high NPV and low PPV for acute rejection. A value of >1% is associated with risk of active rejection. It must be noted that another test utilizing dd-cf DNA developed by Natera (San Carlos, CA) is being offered for transplant patients.

Despite the excitement and promise of these tests, a few important points are to be noted:

1. The test results are qualitative and not quantitative. They do not provide information on the severity of rejection.
2. Positive test results do not provide all the information that clinicians require. More clinical data including a renal biopsy are often indicated.
3. The tests platforms utilizing microarrays and SNPs are FDA-approved, but the tests themselves need further validation by widespread clinical use before they become part of the standard of care.

Together, these tests offer a unique opportunity to monitor, predict, and manage acute rejection episodes in kidney transplant patients. If successful, they have the potential to bring about a long-awaited radical improvement in posttransplant outcomes.

References