Current Cardiovascular Risk Reports (2019) 13: 29
https://doi.org/10.1007/s12170-019-0626-9

GENETICS (A.T. OWENS AND N. REZA, SECTION EDITOR)

Genetic and Genomic Approaches to Predict Cardiac Allograft Rejection

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Published online: 29 August 2019

Abstract

Purpose of Review The goal of this paper is to review the current state of and ongoing developments in the detection and diagnosis of cardiac allograft rejection using genetic and genomic approaches.

Recent Findings The current strength of genetic and genomic methods is primarily in reducing the overall number of surveillance endomyocardial biopsies in otherwise low-risk patients. Tissue-based analyses of endomyocardial samples are beginning to identify a more objective phenotypic description of cardiac allograft rejection than traditional histologic evaluation; however, a true "gold" standard remains elusive.

Summary The current success of genetic and genomic techniques for identifying cardiac allograft rejection is in their high negative predictive value and ability to rule this condition out. Future testing platforms will build upon the success of AlloMap to hopefully improve the diagnostic accuracy and prediction of this technology. The molecular microscope technique may eventually lead the field towards a "gold" standard for cardiac allograft rejection; however, this technique is still under development.

Keywords Cardiac allograft rejection · Myocardial gene expression profiling · Donor-derived cell-free DNA · microRNA · Molecular microscope

Introduction

The rate of treated cardiac allograft rejection has been steadily declining over the past several decades with a first-year post-discharge rate of < 15% observed during 2010–2015 [1]. Despite improvements, acute rejection remains a critical clinical event following heart transplantation (HT). In addition to clinical and hemodynamic criteria, the endomyocardial biopsy (EMB) continues to be a pillar in the diagnosis of rejection despite being a low-yield test with non-trivial interobserver variability in histologic grading [2, 3] and sampling error that may occur during biopsy. Additionally, procedural complications including damage to the tricuspid valve and cardiac tamponade may occur [4]. That said, EMB remains an imperfect "gold" standard in the diagnosis of acute cellular rejection (ACR) and antibody-mediated rejection (AMR) and comes with a risk to patients. The need for an objective and minimally invasive test has led to significant developments in genetic and genomic approaches for the prediction and diagnosis of acute cardiac allograft rejection episodes [5–7]. In this paper, we will review the current state of genetic and genomic approaches for predicting acute cardiac allograft rejection and highlight ongoing studies as well as emerging methodological approaches.

Gene Expression Profiling

The first iteration platform for the non-invasive evaluation of cardiac allograft rejection is based on microarray gene expression profiling (GEP) of peripheral blood mononuclear cells (PBMC). This technique was built upon several decades of research [5, 8, 9] and culminated in the Cardiac Allograft Rejection Gene Expression Observational (CARGO) Study [10] with eventual Federal Drug Administration (FDA) approval of the AlloMap (CareDx, Brisbane, CA, USA) blood

This article is part of the Topical Collection on *Genetics*

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test in August 2008. We will briefly review below the pivotal trials in the development and refinement of this approach.

CARGO Trial Initiated in 2001, the CARGO study examined the clinical utility of gene expression testing using RNA extracted from peripheral blood leukocytes for the non-invasive screening of cardiac allograft rejection in heart transplant patients. The AlloMap test was specifically developed against a phenotypic reference of moderate-to-severe ACR as seen on histological evaluation. Therefore, utilization of the AlloMap test is predicated on a low likelihood of AMR in a given patient at a given point in time. Published in 2005, the CARGO study identified 11 genes, known to be involved in T cell and natural killer cell function, hematopoiesis, steroid responsiveness, and alloimmune recognition, that formed a reliable genetic fingerprint to rule out moderate-to-severe ACR (International Society for Heart Transplantation [ISHLT] grade ≥ 3A) versus no rejection (ISHLT grade 0). A scoring system was developed based on the GEP (range 0 to 40) and identified that patients > 1 year post-transplant with a score of < 30 ruled out ACR with a negative predictive value (NPV) of 99.6%. The positive predictive value (PPV) of this test is low and therefore not intended to "rule in" rejection.

IMAGE Trial The Invasive Monitoring Attenuation through Gene Expression (IMAGE) Study [11] was a randomized trial of 602 patients that were 6 months to 5 years post-heart transplant to a surveillance GEP versus routine EMB in conjunction with clinical and echocardiographic assessment of allograft function. The trial was a non-inferiority study with the composite primary endpoint of rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or retransplantation. At a median follow-up time of 19 months, there was no difference in outcomes, yet the GEP group underwent significantly fewer EMB per person-year (0.5 versus 3.0, $p < 0.001$). In contrast to the CARGO study, a GEP score cutoff of < 34 was used to classify low-risk patients not requiring EMB.

eIMAGE Trial As a follow-up to the IMAGE trial, Kobashigawa et al. [12] conducted a single-center trial of 60 heart transplant patients randomized to either GEP or EMB at 55 days post-transplant. A GEP cutoff score of ≥ 30 was utilized between 2 and 6 months and ≥ 34 following 6 months to trigger a follow-up or safety biopsy. The composite primary endpoint included death/retransplant, rejection with hemodynamic compromise, or graft dysfunction at 18 months post-transplant. There was no difference in the composite endpoint (10% versus 17%; log-rank $p = 0.44$). On average, the GEP group of patients underwent 1.4 biopsies per patient compared with 8.4 biopsies per patient in the EMB group with patient satisfaction higher in the GEP group. Prednisone wean was similarly successful in the majority of both GEP and EMB groups; authors therefore suggested using GEP testing to safely guide early corticosteroid weaning.

CARGO II CARGO II was a prospective, observational, European-based, multi-center follow-up study that analyzed the outcomes of 472 patients with 938 paired GEP-EMB samples that underwent routine GEP for rejection surveillance [13]. Similar to the eIMAGE trial, a GEP score cutoff of ≥ 30 was used for months 2 through 6 and ≥ 34 thereafter. The overall performance of surveillance GEP in the CARGO II trial was consistent with that seen in the original CARGO Trial with a NPV > 98% and low PPV of < 5%. An additional important finding from this study was that GEP scores have an early period of increase followed by stabilization that appears to track with corticosteroid weaning.

OAR Study The Outcomes AlloMap Registry (OAR) is an observational, prospective, multi-center study involving patients that were at least 55 days post-heart transplantation and above the age of 15. A recent analysis of this registry by Moayedi et al. [14•] reported on outcomes of 1504 patients managed with a GEP strategy. The authors found that among low-risk patients there was a low composite outcome of hemodynamically significant rejection, graft dysfunction, retransplantation, or death in the cohort; survival was 99% and 98% at 1 and 2 years, respectively. This analysis redemonstrated the finding that patients at low risk for ACR could successfully be managed with a GEP strategy and allow for a significant reduction in EMB frequency without a compromise in outcomes.

Serial Test Variability Beyond using a strict GEP cutoff of ≥ 30 at 2 to 6 months post-transplant and ≥ 34 after 6 months, the variability in serial GEP test results—defined as the standard deviation of 4 GEP scores obtained at least 315 days post-transplant—has prognostic significance. In a small case-controlled study, Crespo-Leiro et al. demonstrated a NPV of 97% for a GEP score variability of ≤ 0.6 [15]. The PPV was approximately 30% for a GEP variability ≥ 1.6, again identifying the need for additional confirmatory testing when rejection is suspected.

Limitations and Scope of This Method The ideal setting for a GEP surveillance strategy is in low-risk patients with no prior history of rejection or donor-specific antibodies, normal graft function on echocardiogram, and on a dose of prednisone below 20 mg at least 55 days following heart transplantation. While the GEP signature in the setting of cytomegalovirus has been shown to be distinct from that seen during an episode of ACR [16], overall GEP scores may be higher [14•, 17–19]. While the AlloMap test is not FDA-approved for dual-organ transplants, a recent retrospective analysis of 27 dual-organ transplant recipients (18 heart-kidney, 8 heart-liver, and 1

heart-lung) demonstrated similar temporal trends in GEP values, potentially opening the door to use of a GEP strategy in dual-organ recipients [19]. Similar findings were seen in a subset of 37 dual-organ recipients (30 heart-kidney, 7 heart-liver) from the OAR [14•].

Tissue-Based GEP While AlloMap uses GEP of PBMC to rule out ACR in low-risk patients, Loupy et al. [20•] recently published on EMB-based measurements of gene expression, in contrast to PBMC-based GEP, that can accurately classify AMR. This study found that gene transcripts representing natural killer cells, endothelial cells, macrophages, and interferon gamma were upregulated in pathological AMR. While the method explored by Loupy et al. is a tissue-based platform and requires an endomyocardial specimen; it opens the door to a more objective, molecular-based diagnostic assessment of rejection and may pave the way for non-invasive assessment of AMR down the road.

Cell-Free DNA

Detection and quantification of donor-derived cell-free DNA (dd-cfDNA) in the bloodstream of transplant recipients has become a feasible method [21–24] for the non-invasive assessment of donor allograft function. This technique relies on the measurement of donor allograft cell breakdown and subsequent quantification of dd-cfDNA in relation to recipient-derived cell-free DNA. A study by Snyder et al. [21] laid the groundwork for this approach by demonstrating a clear rise and fall of quantifiable dd-cfDNA leading up to, during, and then following a biopsy-proven rejection episode. Notably, this technique was shown by Vlaminck et al. [22] to discriminate both ACR and AMR. The results of their 3-year prospective trial demonstrated a receiver operating characteristic (ROC) area under the curve (AUC) of 0.83 (sensitivity of 0.58 and specificity of 0.93) at classifying moderate-to-severe ACR or AMR [22]. While initial research using this strategy required genotyping of both recipient and donor DNA, recent methodological advancements relying on differences in single nucleotide polymorphisms have made this a clinically practical monitoring strategy [23, 24]. The AlloSure (CareDx, Brisbane, CA, USA) assay, which uses the dd-cfDNA technique described above, was approved by the Federal Drug Administration (FDA) for use in renal transplant patients following publication of the "Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study" [25]. While the AlloSure assay is not yet FDA approved in heart transplantation, a recent study by Khush and colleagues [26•] demonstrated excellent performance of the AlloSure Heart assay in a large, prospective, multi-center, clinical validation study of 740 heart transplant recipients finding an NPV of 97% for detecting acute rejection, including both ACR and AMR.

Combining AlloMap and AlloSure testing platforms, the Surveillance HeartCare® Outcomes Registry (SHORE) is an observational registry designed to assess the clinical utility of the HeartCare platform [27]. Both assays offer a unique window into the allograft rejection process as discussed above and may be useful for the detection of both ACR and AMR. SHORE is a prospective, observational study with an estimated enrollment of 1600 patients and expected completion in June 2027.

MicroRNA

MicroRNAs or miRNAs are small non-coding RNA sequences present in blood and tissue that help regulate gene expression [28–30]. MiRNAs have been found to have an important role in immune function, including B cell and T cell differentiation, and play an important role in allograft rejection as well as the induction of immunologic tolerance [31, 32]. As such, unique miRNA signatures in the peripheral blood of heart transplant recipients may allow for the non-invasive surveillance and diagnosis of acute rejection [33–35]. Van Huyen et al. [33] identified several specific miRNA simultaneously upregulated in serum and myocardial tissue samples in patients with biopsy-proven ACR and AMR. In this study, discrimination of acute rejection using four upregulated miRNAs (miR-10a, miR-31, miR-92a, and miR-155) was excellent (AUC > 0.9). These findings were confirmed in an external validation data set [33]. In another study, a different set of microRNA was found to be upregulated in patients with biopsy-proven ACR as compared with controls [34]. Using the Prevention of Organ Failure (PROOF) Centre of Excellence (Vancouver, Canada) cohort to externally validate these findings, two specific miRNAs (miR-142-3p and miR-101-3p) had fairly reliable discrimination (ROC AUC = 0.78) for the detection of ACR. C-reactive protein (CRP) levels, a proxy for inflammation, did not differ in patients with acute versus non-acute rejection, and further, there was no correlation between miRNA levels and CRP [34]. A study by Singh et al. [36] demonstrated differential expression of circulating miRNAs in patients with chronic rejection in the form of cardiac allograft vasculopathy (CAV). The authors found that two miRNAs known to be associated with maintaining vascular health (miR-126-5p and miR-92a-3p) could reliably discriminate between patients with and without CAV (c-statistic = 0.8) [36]. Establishing robust correlations between intragraft and circulating miRNAs and their association with acute and chronic rejection signifies an important step towards the development of a truly non-invasive testing platform while also offering important mechanistic insights into the rejection process.

Molecular Microscope

Following the development of the Molecular Microscope Diagnostic (MMDx; Transcriptome Sciences, Inc., Edmonton, Alberta, Canada) system for use in kidney transplant patients [37], Halloran et al. developed a similar microarray platform for use in heart transplantation for the detection of both ACR and AMR [38]. In essence, the MMDx platform provides a gene expression analysis of an EMB sample by looking at specific messenger RNA (mRNA) transcripts dubbed rejection-associated transcripts (RATs) and ascribes a probability of rejection by comparison of expression profiles to a reference set. The motivation for this technique is to move towards a more objective, molecular-based, grading system of EMB samples. The translation of this technique from kidney to heart transplantation is built on the observation of similarities in molecular disturbances seen in both the heart and kidney during rejection episodes [39, 40]. While not a non-invasive technique, this platform does offer a refined, transcription-level assessment of endomyocardial tissue samples and provide insights beyond standard histological evaluation. For example, Loupy et al. [20•], using this molecular-based analysis of EMB samples, demonstrated that pAMR1 (H+) has a similar molecular profile, characterized by natural killer cell transcripts, endothelial cell/angiogenesis genes, interferon gamma–inducible genes, and monocyte genes, to pAMR2/3, but not pAMR1 (I+). This finding has implications for clinical decision-making as many transplant cardiologists will only treat ISHLT grade 2 rejection or higher. Moreover, a tissue-based molecular assessment may offer mechanistic insight into the rejection process. In a recent study, MMDx reliably differentiated myocardial injury from rejection on the basis of unique molecular signatures of the variance in transcript expression [41]. With this in mind, the ability to differentiate molecular injury from molecular rejection patterns may reduce misdiagnosis of rejection using histology alone.

Lastly, the ongoing "Diagnostic and Therapeutic Applications of Microarrays in Heart Transplantation, a Multicenter Study (INTERHEART)" will provide a prospective assessment of the MMDx system and has an estimated enrollment of 900 patients and anticipated completion in December 2020 [42].

Conclusion

The diagnosis of acute cardiac allograft rejection continues to rely heavily on the subjective and highly variable histological assessment of EMB specimens. Techniques such as the MMDx hope to offer a more quantifiable and objective assessments than histology alone. While past research efforts (i.e., AlloMap) have successfully reduced the number of unnecessary surveillance biopsies, current tissue-based GEP methods are focused on the critical step of first establishing reliably objective, molecular-based (i.e., miRNA, mRNA) phenotypic criteria for acute rejection. The fact that there is still limited understanding of the acute rejection process and that there is no true "gold" standard makes this task exceedingly hard. Nevertheless, with the ongoing genetic and genomic exploration in both tissue-based and serum-based platforms, the field will continue progressing towards a more precise and more accurate diagnosis of acute rejection.

Compliance with Ethical Standards

Conflict of Interest Aaron Wolfson declares he has no conflict of interest.

Jon Kobashigawa has received grants from Novartis, Alexion, Sanofi-Genzyme, and Molecular microscope.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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