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Novel Biomarker Approaches for Managing Patients With Cardiac Transplantation

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Abstract

Despite major advances in the medical care of patients following heart transplantation (HTx) and a steady increase in long-term survival, allograft surveillance is still based on endomyocardial biopsy, the gold standard since the 1970s. This invasive procedure calls for less burdening and more cost-effective approaches. In recent years, impressive progress has been made in utilizing blood-based biomarkers for the diagnosis and management of diseases in a variety of fields. Hence, a number of trials have been performed testing the usefulness of circulating molecules or other technical methods to overcome the need for surveillance myocardial biopsy in HTx patients. Here, we review current approaches and the state of research on novel biomarkers for the management of patients following heart transplantation.

Keywords

Rejection; Biomarker; Transplantation biology; Heart transplantation; MicroRNA; Survival

Introduction

Heart transplantation (HTx) is the only causative treatment for patients with advanced heart failure (HF). Major advancements in allograft protection such as improved immunosuppressive combination therapies have led to a steady increase in post-transplant survival from a median survival of 5 years in the early 1980s to almost 12 years as of current [24••]. However, acute rejection remains a major problem in transplant medicine and, even though acute cellular rejection episodes have dramatically decreased due to improved immunosuppressive regimens [32], cellular and especially noncellular rejection still poses a challenge for the clinician involved in the care of HTx recipients. The estimated incidence of at least one significant allograft rejection within the first year after HTx is between 13 and 30 % [24••].

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Compliance with Ethics Guidelines

Conflict of Interest Peter J. Kennel and P. Christian Schulze declare that they have no conflict of interest.

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Endomyocardial biopsy (EMB) is the current standard of care used to identify rejection after a HTx often with at least 13 scheduled biopsies within the first year after transplant. This invasive approach is burdening and cost-intensive: Aside from the risk of any invasive procedure, EMB carries a risk of pericardial tamponade and tricuspid regurgitation and the series of surveillance biopsies carries a financial burden of up to 2000 USD per procedure [23]. Each year, around 2300 patients receive a heart transplant in the USA [27], with the majority of them undergoing surveillance EMB for years after transplant.

Allograft rejection is diagnosed with endomyocardial biopsy specimen staining and is graded using the ISHLT classification. The main criterion for acute cellular rejection severity is the grade of mononuclear cell infiltration: no or only one focus of myocyte damage is classified as grade 1R, whereas multifocal myocyte damage equals grade 2R. Grade 3R is diagnosed when an infiltrate with diffuse myocyte damage, cellular edema, hemorrhage, or vasculitis is present [9]. Antibody-mediated rejection is assessed using immunofluorescence or immunoperoxidase staining against IgG, IgM, IgA, and complement deposition (C3d, C4d, C1q, as well as CD68 staining).

The long-term mortality rate after HTx is approximately 3.5 % per year after the first year post-procedure [24•]. The causes of death shift from primary graft failure and multiorgan failure as the primary causes of death within the first year after transplant towards malignancy, renal failure, and cardiac allograft vasculopathy later in the course of the post-transplant period. Whereas acute rejection can be well managed in the majority of cases, cumulative rejection episodes and chronic rejection are likely to contribute to long-term graft failure and graft vasculopathy.

The invasive and costly current gold standard of using myocardial biopsy for allograft surveillance calls for less burdening and more cost-effective approaches. In recent years, impressive progress has been made in utilizing blood-based biomarkers for the diagnosis and follow-up of diseases in a variety of fields.

Classical Cardiac Biomarkers—Troponin and Brain Natriuretic Peptide

A biomarker can be roughly defined as an objective indicator of a medical state which can be measured accurately and reproducibly [31]. The impact and usefulness of blood-based biomarkers in the cardiovascular sciences field can be illustrated by success stories such as troponin testing during acute coronary syndrome workup, brain natriuretic peptide (BNP) as a well-established marker for congestive HF [25], or LDL [1] as a thoroughly studied cardiovascular biomarker.

Troponin and BNP are well-established protein biomarkers extensively used by the cardiologist to assess for the presence and the degree of myocardial damage. Consequently, several studies have been conceived investigating on the usefulness of those classic cardiac biomarkers in HTx patients. A study in 95 heart transplant recipients demonstrated a strong correlation between the severity of rejection and serum troponin T levels and determined the sensitivity and specificity for the detection of significant graft rejection (ISHLT grade 3/4) to be 80 and 62 %, respectively, with a negative predictive value of 96 % [15]. In another study

in 145 transplant patients, Chance et al. concluded that troponin T testing, despite having a poor sensitivity in their study, has a convincing specificity in patients with normal renal function and can be used as an adjunct test in this setting [6]. However, other studies have found poor reliability in the use of troponin as a biomarker for transplant rejection [26], and in the most recent trials, which used high-sensitivity assays, results were still inconsistent [4, 28]. Despite the inconclusive findings concerning troponin in heart transplant patients, an increase of troponin levels in a patient with normal renal function is cause for concern and can be an early sign of allograft rejection. Similar findings have been described for BNP. Serum BNP levels are almost uniformly increased after HTx [21], and while a few studies found a predictive value of increased BNP levels during acute rejection episodes [18, 35], others did not [4, 3].

Novel Nonprotein Biomarkers

Circulating DNA and RNA as Biomarkers

Utilizing circulating ribonucleic acids as biomarkers promises several advantages: the specimen can be collected with a simple blood draw and DNA/RNA quantification is economical, fully automated, accurate, and reproducible, enabling this approach to be a favorable target for biomarker development. Consequently, gene expression profiling for cardiac allograft surveillance has been studied with promising results and is currently the only test that has reached market maturity with FDA approval in 2008. Allomap (marketed by CareDX Inc.) tests mRNA expression levels of 20 genes (11 rejection-related genes and 9 genes for normalization and quality control) in mononuclear cells and is “intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate to severe acute cellular rejection (ACR) at the time of testing in conjunction with the standard clinical assessment” [17]. Allomap was studied in two large registration trials, IMAGE (“Invasive Monitoring Attenuation through Gene Expression”) [29•] and CARGO (“Cardiac Allograft Rejection Gene Expression Observational Study”) [13•]. The initial CARGO trial defined several candidate genes for gene expression profiling, developed a one-digit scoring system to indicate the risk of severe graft rejection, and concluded that this approach detects the absence of moderate or severe rejection and can avoid a biopsy in certain clinical settings [13•]. In the subsequent IMAGE trial, 605 heart transplant patients, who had received a heart transplant 6 months to 5 years prior enrollment, were randomized to rejection surveillance using biopsy or gene expression analysis. The study found that patients who were monitored with Allomap had similar outcomes compared to the biopsy group, defined as similar 2-year cumulative rates of rejection with hemodynamic compromise, graft dysfunction, death, or retransplantation [29•]. Results from these trials and initial results from the confirmatory CARGO-II trial in Europe conclude that gene expression profiling (GEP) score instability [14], after a 1-year post-transplant stabilization period, and a score above 34 on the Allomap scoring system predict future events [10]. Gene expression profiling using the commercially available program is increasingly used and offered in the USA and Europe.

A new and rapidly evolving approach is the measurement of donor-derived cell-free DNA. First described in renal transplant cohorts, circulating donor-derived DNA is an attractive

target based on the assumption that the concentration of this prospective biomarker correlates with the grade of cardiomyocyte and/or endothelial [11•] damage. A recent study by Vlamincx et al. in adult and pediatric heart transplant recipients was designed to count the number of donor- and recipient-derived circulating cell-free DNA after SNP genotyping of donor and recipient [12•]. In their study, the authors found that this method was able to detect acute rejection with comparable sensitivity and specificity as the gold standard, myocardial biopsy. In another recent study using a targeted genotyping approach, Hildebrand et al. found that a cell-free donor-specific DNA (dscfDNA) fraction of less than 1 % of total cfDNA had a very strong negative predictive value [19•]. However, only a limited number of studies are available, and several key questions concerning this technique remain, such as long-term reliability of dscfDNA testing and skew effects due to mechanisms influencing recipient cfDNA levels [11•, 19•].

MicroRNAs (miRNAs) are short noncoding RNAs with regulatory function, ubiquitously expressed, and stable. Of note, miRNAs have tissue-specific expression patterns and are, therefore, potential candidates for myocardial biomarker development similar to tissue-specific protein biomarkers. The practicability and validity of this approach in cardiovascular disease have been demonstrated using miRNA deep-sequencing to characterize miRNA profiles unique for heart failure: Akat et al. found significant increases of muscle-specific miRNAs (myomirs) and cardiac-specific myomirs [2] in serum of HF patients. To date, only a few studies have investigated the feasibility of miRNA profiling in humans to detect cardiac allograft rejection. In an initial small study of ten patients with graft rejection, Dewi et al. screened 175 miRNAs known to be present in humans and identified 7 target miRNAs with increased circulating levels during acute rejection, which were subsequently confirmed by regular qPCR [33]. In another trial including 113 HTx recipients, divided into a test cohort and a validation cohort, the investigators assessed for serological expression levels of 14 miRNAs related to immune and inflammatory processes by regular qPCR. In this study, van Huyen et al. found 7 miRNAs to be able to discriminate between rejection and nonrejection [16•]. However, the miRNAs described in both of these studies were not overlapping, leaving the question for a rejection-specific miRNA unanswered. Moreover, since the studies cited above assessed for expression changes in only a selected subset of miRNAs, no results from comprehensive deep-sequencing studies are currently available.

Another major group of noncoding RNAs are long-noncoding RNAs (lncRNAs). This type of circulating ribonucleic acids comprises RNAs with more than 200 nucleotides. Several studies have shown a possible role of lncRNAs in a great variety of physiological as well as pathological processes. For example, a recent study revealed the complex interplay between mRNA, miRNA, and lncRNA in cardiac hypertrophy [34] and, together with other recently discovered functions of lncRNAs in cardiovascular disease suggesting their modulability, lncRNAs seem to be another target of interest for the development of a circulating biomarker. lncRNAs have been associated with pivotal processes for cardiac function such as cardiomyocyte apoptosis, contractility, and energy homeostasis. Of note, one study found a close association between the upregulation of one specific lncRNA and cardiovascular death after myocardial infarction, claiming a possible role for it as a biomarker to predict cardiovascular death in HF patients [22]. To date, the potential of lncRNA profiling in

cardiac transplant rejection has not been investigated; however, initial research undertaken in kidney transplant patients [7] has shown promising results.

Protein-Based Biomarkers

As in the exploration of ribonucleic acids as biomarkers, the search for protein-based biomarkers to indicate organ rejection has been initially explored in kidney transplant research. In recent years, several studies tried to identify circulating proteins with the potential to indicate acute graft rejection using mass spectrometry, bioinformatic [8•], or gel electrophoresis approaches [5]. Similar to the results from RNA deep-sequencing, mass spectrometry analysis of the proteome in rejection yielded an abundance of potential targets [5, 8•, 20], illustrating several basic obstacles in the development of a specific biomarker [30••]. Human biofluid proteomes show a great interindividual variability and are dominated by a few proteins contributing to the vast majority of the total proteome, leading to a high dynamic range: potential targets such as proteins related to immunological processes are often present only in ng/ml ranges. Furthermore, mechanisms such as posttranslational modification and proteolytic processes further increase the number of circulating proteins detected by sophisticated techniques such as mass spectrometry. To date, even though several proteins have been proposed to be useful in detecting acute allograft rejection, potential candidate proteins have yet to be validated and are still in an experimental stage.

Conclusions

Despite the major advances in HF therapy and the development of reliable ventricular assist devices, HTx remains the only causative treatment for patients with severe HF. Graft as well as overall survival has continuously increased since the introduction of HTx in 1967. However, endomyocardial biopsy which has been established as gold standard for graft surveillance in the 1970s is expensive, carries medical risks, and is burdening for patients. The development of a new, minimally invasive, and reliable test to detect cardiac allograft rejection is desired, and several approaches have been undertaken to replace EMB as a primary test. Among these, Allomap testing is the first clinically established test with biomarker characteristics, with several other concepts being currently evaluated, such as cell-free donor-specific DNA, long-noncoding and microRNA, or protein-based assays. The development of a specific, reliable, reproducible, and cost-effective test utilizing a single molecule or set of molecules is complicated by several technical obstacles, and with the exception of Allomap testing, no blood-based test has been established in clinical practice as of current.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Adhyaru BB, Jacobson TA. New cholesterol guidelines for the management of atherosclerotic cardiovascular disease risk: a comparison of the 2013 American College of Cardiology/American Heart Association Cholesterol Guidelines with the 2014 National Lipid Association

- Recommendations for Patient-Centered Management of Dyslipidemia. *Cardiol Clin.* 2015;33:181–96. [PubMed: 25939292]
2. Akat KM, Moore-McGriff D, Morozov P, Brown M, Gogakos T, Correa Da Rosa J, et al. Comparative RNA-sequencing analysis of myocardial and circulating small RNAs in human heart failure and their utility as biomarkers. *Proc Natl Acad Sci U S A.* 2014;111: 11151–6. [PubMed: 25012294]
 3. Arnau-Vives MA, Almenar L, Hervas I, Osa A, Martinez-Dolz L, Rueda J, et al. Predictive value of brain natriuretic peptide in the diagnosis of heart transplant rejection. *J Heart Lung Transplant.* 2004;23:850–6. [PubMed: 15261180]
 4. Battes LC, Caliskan K, Rizopoulos D, Constantinescu AA, Robertus JL, Akkerhuis M, et al. Repeated measurements of NT-pro-B-type natriuretic peptide, troponin T or C-reactive protein do not predict future allograft rejection in heart transplant recipients. *Transplantation.* 2015;99:580–5. [PubMed: 25136844]
 5. Borozdenkova S, Westbrook JA, Patel V, Wait R, Bolad I, Burke MM, et al. Use of proteomics to discover novel markers of cardiac allograft rejection. *J Proteome Res.* 2004;3:282–8. [PubMed: 15113105]
 6. Chance JJ, Segal JB, Wallerson G, Kasper E, Hruban RH, Kickler TS, et al. Cardiac troponin T and C-reactive protein as markers of acute cardiac allograft rejection. *Clin Chim Acta.* 2001;312:31–9. [PubMed: 11580907]
 7. Chen W, Peng W, Huang J, Yu X, Tan K, Chen Y, et al. Microarray analysis of long non-coding RNA expression in human acute rejection biopsy samples following renal transplantation. *Mol Med Rep.* 2014;10:2210–6. [PubMed: 25198465]
 8. Cohen Freue GV, Meredith A, Smith D, Bergman A, Sasaki M, Lam KK, et al. Computational biomarker pipeline from discovery to clinical implementation: plasma proteomic biomarkers for cardiac transplantation. *PLoS Comput Biol.* 2013;9, e1002963. [PubMed: 23592955] • Bioinformatic approach to discover valid biomarkers.
 9. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant.* 2010;29:914–56. [PubMed: 20643330]
 10. Crespo-Leiro MG, Stypmann J, Vanhaecke J. Utility of Gene Expression Profiling Test (GEP) score instability to predict future clinical outcomes in heart transplant: results from the CARGO 2 European-Based Multicenter Trial. *J Heart Lung Transplant.* 2013;32(4):S113–4.
 11. Daly KP. Circulating donor-derived cell-free DNA: a true biomarker for cardiac allograft rejection? *Ann Transl Med.* 2015;3:47. [PubMed: 25861602] • Introduces the concept of dscfDNA.
 12. De Vlaminck I, Valentine HA, Snyder TM, Strehl C, Cohen G, Luikart H, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. *Sci Transl Med.* 2014;6, 241ra277. • dscfDNA trial.
 13. Deng MC, Eisen HJ, Mehra MR, Billingham M, Marboe CC, Berry G, et al. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. *Am J Transplant.* 2006;6:150–60. [PubMed: 16433769] • Allomap key trial.
 14. Deng MC, Elashoff B, Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, et al. Utility of gene expression profiling score variability to predict clinical events in heart transplant recipients. *Transplantation.* 2014.
 15. Dengler TJ, Zimmermann R, Braun K, Muller-Bardorff M, Zehelein J, Sack FU, et al. Elevated serum concentrations of cardiac troponin T in acute allograft rejection after human heart transplantation. *J Am Coll Cardiol.* 1998;32:405–12. [PubMed: 9708468]
 16. Duong Van Huyen JP, Tible M, Gay A, Guillemain R, Aubert O, Varnous S, et al. MicroRNAs as non-invasive biomarkers of heart transplant rejection. *Eur Heart J.* 2014;35:3194–202. [PubMed: 25176944] • miRNA trial.
 17. FDA USFDA. 510(k) Substantial equivalence determination decision summary - http://www.accessdata.fda.gov/cdrh_docs/reviews/K073482.pdf. 2008.
 18. Garrido IP, Pascual-Figal DA, Nicolas F, Gonzalez-Carrillo MJ, Manzano-Fernandez S, Sanchez-Mas J, et al. Usefulness of serial monitoring of B-type natriuretic peptide for the detection of acute rejection after heart transplantation. *Am J Cardiol.* 2009;103:1149–53. [PubMed: 19361605]

19. Hidestrand M, Tomita-Mitchell A, Hidestrand PM, Oliphant A, Goetsch M, Stamm K, et al. Highly sensitive noninvasive cardiac transplant rejection monitoring using targeted quantification of donor-specific cell-free deoxyribonucleic acid. *J Am Coll Cardiol*. 2014;63:1224–6. [PubMed: 24140666] • dscfDNA trial.
20. Kienzl K, Sarg B, Golderer G, Obrist P, Werner ER, Werner-Felmayer G, et al. Proteomic profiling of acute cardiac allograft rejection. *Transplantation*. 2009;88:553–60. [PubMed: 19696639]
21. Kirchhoff W, Gradaus R, Stypmann J, Deng MC, Tian TD, Scheld HH, et al. Vasoactive peptides during long-term follow-up of patients after cardiac transplantation. *J Heart Lung Transplant*. 2004;23:284–8. [PubMed: 15019637]
22. Kumarswamy R, Bauters C, Volkmann I, Maury F, Fetisch J, Holzmann A, et al. Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. *Circ Res*. 2014;114:1569–75. [PubMed: 24663402]
23. Lampert BC, Teuteberg JJ, Shullo MA, Holtz J, Smith KJ. Cost-effectiveness of routine surveillance endomyocardial biopsy after 12 months post-heart transplantation. *Circ Heart Fail*. 2014;7:807–13. [PubMed: 25104042]
24. Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, et al. The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report—2014; focus theme: retransplantation. *J Heart Lung Transplant* 2014;33:996–1008. [PubMed: 25242124] •• Statistics for cardiac allograft survival and incidence of rejection episodes.
25. Maisel A, Mueller C, Adams K Jr, Anker SD, Aspromonte N, Cleland JG, et al. State of the art: using natriuretic peptide levels in clinical practice. *Eur J Heart Fail*. 2008;10:824–39. [PubMed: 18760965]
26. Mullen JC, Bentley MJ, Scherr KD, Chorney SG, Burton NI, Tymchak WJ, et al. Troponin T and I are not reliable markers of cardiac transplant rejection. *Eur J Cardiothorac Surg*. 2002;22: 233–7. [PubMed: 12142191]
27. OPTN. Organ Procurement Transplantation Network - <http://optn.transplant.hrsa.gov/> 2014.
28. Patel PC, Hill DA, Ayers CR, Lavingia B, Kaiser P, Dyer AK, et al. High-sensitivity cardiac troponin I assay to screen for acute rejection in patients with heart transplant. *Circ Heart Fail*. 2014;7:463–9. [PubMed: 24733367]
29. Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Deng MC, Cappola TP, et al. Gene-expression profiling for rejection surveillance after cardiac transplantation. *N Engl J Med*. 2010;362:1890–900. [PubMed: 20413602] • Allomap key trial.
30. Qian WJ, Jacobs JM, Liu T, Camp 2nd DG, Smith RD. Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. *Mol Cell Proteomics*. 2006;5:1727–44. [PubMed: 16887931] •• Illustrates challenges and pitfalls in the development for protein biomarkers.
31. Strimbu KandTavel JA. What are biomarkers? *Curr Opin HIV AIDS*. 2010;5:463–6. [PubMed: 20978388]
32. Subherwal S, Kobashigawa JA, Cogert G, Patel J, Espejo M, Oeser B. Incidence of acute cellular rejection and non-cellular rejection in cardiac transplantation. *Transplant Proc*. 2004;36: 3171–2. [PubMed: 15686721]
33. Sukma Dewi I, Torngren K, Gidlof O, Kornhall B, Ohman J. Altered serum miRNA profiles during acute rejection after heart transplantation: potential for non-invasive allograft surveillance. *J Heart Lung Transplant*. 2013;32:463–6. [PubMed: 23384889]
34. Wang K, Liu F, Zhou LY, Long B, Yuan SM, Wang Y, et al. The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. *Circ Res*. 2014;114:1377–88. [PubMed: 24557880]
35. Wu AH, Johnson ML, Aaronson KD, Gordon D, Dyke DB, Koelling TM. Brain natriuretic peptide predicts serious cardiac allograft rejection independent of hemodynamic measurements. *J Heart Lung Transplant*. 2005;24:52–7. [PubMed: 15653379]