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Advances and New Insights in Post-Transplant Care: From Sequencing to Imaging

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Advances and New Insights in Post-Transplant Care: From Sequencing to Imaging

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Abstract:	Purpose of review: Cardiac imaging and sequencing have greatly improved over the recent years. The goal of this review is to summarize these recent advances in cardiac imaging and sequencing, their application in heart-transplantation, and provide our perspective in how artificial intelligence provides a new paradigm for big data driven analysis in heart-transplant research. Recent findings: Cardiac imaging, particularly parametric mapping by cardiac MRI and global longitudinal strain by echocardiography, has improved our understanding of cardiac allograft rejection and prediction of adverse clinical outcomes. Independently, gene expression profiling and measurement of donor-derived cell free DNA have greatly improved risk stratification for acute rejection. More recently, data-driven phenotypic clustering using novel machine learning algorithms has been used to identify a distinct macrophage subset, associated with acute rejection. Summary: Developments in imaging and sequencing techniques in the application of heart-transplant research are improving rapidly and in parallel with improvements in analysis of these large datasets. The approach to heart-transplant research is in the transition of significant change as big data driven analysis identifies new mechanistic patterns that can be combined with traditional hypothesis testing.

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Purpose of review: Cardiac imaging and sequencing have greatly improved over the recent years. The goal of this review is to summarize these recent advances in cardiac imaging and sequencing, their application in heart-transplantation, and provide our perspective in how artificial intelligence provides a new paradigm for big data driven analysis in heart-transplant research.

Recent findings: Cardiac imaging, particularly parametric mapping by cardiac MRI and global longitudinal strain by echocardiography, has improved our understanding of cardiac allograft rejection and prediction of adverse clinical outcomes. Independently, gene expression profiling and measurement of donor-derived cell free DNA have greatly improved risk stratification for acute rejection. More recently, data-driven phenotypic clustering using novel machine learning algorithms has been used to identify a distinct macrophage subset, associated with acute rejection.

Summary: Developments in imaging and sequencing techniques in the application of heart-transplant research are improving rapidly and in parallel with improvements in analysis of these large datasets. The approach to heart-transplant research is in the transition of significant change as big data driven analysis identifies new mechanistic patterns that can be combined with traditional hypothesis testing.

INTRODUCTION

The morbidity and mortality of patients with heart-failure have improved considerably over recent years [1–3]. However, there remain patients who continue to progress in their heart-failure and ultimately require heart-transplantation for increased survival and improved quality of life [4, 5]. Remarkably, heart-transplant volumes have continued to grow in the past decade despite the apparent limited supply of donor hearts [5].

Heart-transplant care has gradually improved with an increase in median cardiac allograft longevity from 11 to 12.5 years over the last two decades [5]. Immune-mediated rejection however limits longer allograft survival and is defined as either acute or chronic rejection [6]. Acute rejection is currently diagnosed by endomyocardial biopsy, classified as either acute cellular rejection (ACR) or antibody mediated rejection (AMR), and typically occurs during the first year post-transplant. Chronic rejection refers to cardiac allograft vasculopathy (CAV), currently diagnosed by invasive angiography, and the median time to diagnosis is typically 10 years post-transplant. Overall, the trend has been towards less immunosuppression on the backbone of calcineurin-inhibitor based therapy with the goal of early steroid weaning within the first 6 months. The “less is more” strategy of immunosuppression is due to the recognition of the harm of too much immunosuppression, leading to deaths from fatal infections as well as malignancies [7].

The fields of cardiovascular imaging and sequencing have independently grown and novel techniques and applications are introduced in rapid succession each year [8, 9]. Their growth as well as our need for less invasive allograft surveillance has spurred the application of cardiovascular imaging and sequencing in post-transplant care. Thus, the purpose of this review is to introduce the reader to the current practice of post heart-transplant care, recent developments in cardiovascular imaging and sequencing in heart-transplantation, and future

perspectives of how artificial intelligence provides a new paradigm for big data driven analysis in transplant immunology.

CARDIAC IMAGING IN HEART-TRANSPLANTATION

Noninvasive imaging modalities such as echocardiography, nuclear imaging, coronary computed tomography angiography (CCTA), and cardiovascular magnetic resonance imaging (CMR) are available to assess the cardiac allograft (Table 1). Transthoracic echocardiography is used for initial assessment of the cardiac allograft structure and function, pericardial effusion and any valvular disease. Other imaging techniques also can be used depending on the specific heart-transplant related indication. As the first step, the heart-transplant cardiologist will determine whether the indication is for acute versus chronic rejection.

Acute Rejection

Heart-transplant patients presenting with a clinical concern for acute rejection have a limited time window for accurate diagnosis. The presence of hemodynamic compromise with acute rejection will limit the first-line test to an echocardiogram, often looking for a significant drop in left ventricular ejection fraction (LVEF), increase in myocardial wall thickness or diastolic indices that would raise the concern for acute rejection [10]. The current practice is to then confirm the diagnosis by histopathology obtained from an urgently performed endomyocardial biopsy [6].

Parametric mapping in CMR for diagnosis of acute rejection

More commonly, the heart-transplant cardiologist attempts to diagnose acute rejection episodes early prior to the onset of cardiac allograft dysfunction [11]. The current standard is repeated surveillance endomyocardial biopsies, typically monthly during the first year, for early histopathologic diagnosis. CMR shows the most promise to diagnose and/or predict acute

rejection because of its superior myocardial characterization compared to other noninvasive imaging modalities. CMR parameters include native T1 relaxation time (myocardial injury), T2 relaxation time (myocardial edema), myocardial strain, extracellular volume (ECV), late gadolinium enhancement (LGE) and also intracellular lifetime of water (cardiomyocyte hypertrophy) in addition to biventricular volumes, mass, and ejection fraction [12, 13]. T2 relaxation time and ECV appear to be the most consistent independent predictors of acute rejection [14–16]. Butler and co-authors show that T2 relaxation time greater than 59 milliseconds and increased right ventricular end-diastolic volume index (mean 89 ml/m^2 in patients with acute rejection) independently predicted treated acute rejection and when used together, showed a high negative predictive value of 98%. Interestingly, the authors observed a relatively high rate of biopsy-negative rejection of 42%. Though not the focus of this particular study, this finding in conjunction with observations from additional literature [11, 17–19] highlight the need for better understanding of these biopsy-negative rejection episodes that often lead to nonspecific cardiac allograft dysfunction.

LGE for the diagnosis of acute rejection

Though LGE is essential for other cardiovascular diseases [20], its utility in heart-transplantation has been limited because it is relatively prevalent in heart-transplant patients and is reported to range from 18 to 61% when quantified as a percentage of LV mass [14, 21–28]. The pattern of LGE are either infarct-typical, infarct-atypical, or often both patterns, and do not consistently correlate with the type of rejection [25, 26]. Thus, given the relatively high prevalence and nonspecificity of LGE in heart-transplant patients, LGE has not been convincingly shown to be diagnostic for either acute rejection or CAV.

Other noninvasive imaging modalities for detection of acute rejection

Other noninvasive imaging modalities have not demonstrated as much development or success as CMR for the diagnosis of acute rejection. Echocardiography has further improved with the use of strain and three-dimensional imaging but thus far has not been able to be consistently reproduced as strain imaging and analysis continues to evolve [10, 29, 30]. Molecular imaging with nuclear imaging and CMR shows promise in mechanistic evaluation of acute rejection, typically targeting and tracking lymphocytes and macrophages [31]. However, molecular imaging has not been able to transition into the clinical setting because of a lack of a clear-cut front runner for a molecular target as well as identification of the best molecular imaging modality [32].

Chronic Rejection

Chronic rejection in heart-transplantation classically refers to CAV [8]. CAV is commonly cited as the limiting factor in long-term cardiac allograft longevity with a median cardiac allograft survival of 12.5 years in the current era [5]. Thus, there continues to be a strong need for understanding the underlying mechanisms for CAV to improve cardiac allograft longevity [33]. Recognition and surveillance for CAV have improved since the standard nomenclature of CAV was first defined 10 years ago by the ISHLT [34]. The trend in the years that followed has been to identify and develop a noninvasive imaging modality that demonstrates similar sensitivity and accuracy compared to the current gold standard of invasive coronary angiography, often in conjunction with intravascular ultrasound (IVUS).

Echocardiography for the diagnosis of CAV

Echocardiography has also been evaluated by numerous investigators to diagnose CAV [10, 35, 36]. Decreased global longitudinal strain (GLS) by speckle-tracking echocardiography of greater than -16.5% independently correlated with severity of CAV [37]. The authors speculate that repetitive ischemia leads to impaired longitudinal myocardial function that can be quantified by speckle-tracking echocardiography, even at rest. However, just as in acute rejection, myocardial

deformation imaging by echocardiography is not yet established for routine surveillance for CAV. Currently, dobutamine stress echocardiography (DSE) is one of the noninvasive imaging modalities commonly used for CAV surveillance, particularly in patients greater than 3-5 years from their heart-transplantation with low suspicion for clinically significant CAV [6, 38]. DSE is favored over exercise stress echocardiography because of the advantage of being less affected by heart-rate limitations often seen in denervated cardiac allografts. Though initially thought to show good diagnostic performance for clinically significant CAV and correlate meaningfully with myocardial infarction and death, recent studies have cast doubt on the accuracy and prognostic value of a negative DSE [39, 40]. In general, our center's experience is consistent with pooled sensitivity of 60% and specificity of 86% for an area under the curve (AUC) of 0.73.

CCTA for the diagnosis of CAV

CCTA has shown reasonable accuracy for the detection of CAV in heart-transplant patients [8, 41]. CCTA is particularly attractive because of its high spatial resolution and decreasing radiation exposure to patients, with the advent of 256- and 320-detector-row CT scanners and improved dose reduction techniques [42]. On patient-based analysis using 50% stenosis by invasive coronary angiography as the gold standard, the weighted mean sensitivity and specificity were 94% and 92%, respectively, and weighted mean positive and negative predictive values were 67% and 99%, respectively [43]. However, CCTA demonstrated a far lower negative predictive value of 50% for the diagnosis of CAV when compared with IVUS. High heart-rates were also a noted limitation, with a mean heart-rate of 85 beats/min despite beta-blockade. Despite this, occurrence of motion artifacts limiting interpretation was low and happened less than 1% of the time [44]. On the rare occasions that motion artifacts had limited interpretation, typically the right and left circumflex coronary arteries were affected whereas CAV detected was predominantly in the left anterior descending artery [41]. Thus, the current evidence suggests that CCTA is suitable for the diagnosis of clinically significant CAV while invasive coronary angiography with IVUS

should be used if the goal is early detection of CAV. The selection between these differing goals will depend on how the diagnosis of CAV would change the management of the heart-transplant patient [45].

Nuclear imaging and CMR for the diagnosis of CAV

Single photon emission computed tomography (SPECT) is one of the earliest noninvasive imaging modalities studied for the diagnosis of CAV [46]. The choice of the pharmacologic stress agent is institution dependent and the performance of dobutamine, dipyridamole and regadenoson has been shown to be similar and equally safe to administer [47, 48]. The diagnostic accuracy of SPECT however is not improved when compared to DSE and has the additional risk of radiation exposure [49].

Thus, positron emission tomography (PET) is suggested as the superior nuclear imaging modality because of its improved spatial resolution, accuracy and lower radiation dose [50]. Cardiac PET imaging provides additional quantitative parameters that can be particularly useful for evaluating a diffuse vasculopathic process in CAV, including rest/stress myocardial blood flow and myocardial flow reserve [51–53]. Chih and colleagues showed the utility in using myocardial flow reserve, stress myocardial blood flow and coronary vascular resistance determined by PET in risk-stratifying patients for CAV diagnosed invasively by IVUS with a sensitivity of 93% and specificity of 65% for 1 abnormal parameter. However, the calculated PET parameters showed modest correlation at best with corresponding invasive coronary physiologic parameters, including index of microcirculatory resistance. While PET imaging shows promise for noninvasive detection of CAV, it is less widespread and more expensive when compared to other noninvasive imaging modalities, limiting its universal applicability [54].

CMR has been evaluated by several groups for the diagnosis of CAV. Early studies evaluated LGE as a sign of myocardial injury mediated by CAV [26, 55]. However, high prevalence of LGE

in heart-transplant patients, as previously described, limits its specificity for CAV diagnosis. Though the prevalence of infarct-typical LGE does increase with severity of CAV, both infarct-typical and infarct-atypical patterns are seen in all grades of CAV and thus no consistent LGE pattern correlates specifically with CAV [56].

Stress perfusion imaging by CMR has gained increasing interest with recent studies showing better diagnostic accuracy for CAV compared to other noninvasive imaging modalities. Miller and colleagues demonstrated the accuracy of diagnosing both macrovascular and microvascular CAV using stress perfusion CMR and showed an impressive AUC of 0.89 for detection of moderate macrovascular CAV or microvascular disease [24]. Chih and co-authors also showed that myocardial perfusion accurately diagnosed macrovascular CAV when compared to IVUS using a myocardial perfusion reserve cut-off of 1.68, with a positive and negative predictive value of 86% and 100%, respectively [57]. Stress CMR has also consistently shown to be a robust predictor of microvascular CAV, a field within heart-transplantation that needs further study and has been limited by lack of accurate diagnostic tools [58–60]. At our institution, we have adapted the use of an artificial intelligence based approach for quantitative myocardial perfusion to further improve the quantification of myocardial blood flow and perfusion reserve, allowing for better diagnostic accuracy while improving workflow by reducing time spent for analysis [61, 62].

Noninvasive imaging for prediction of clinical outcomes

Noninvasive imaging has also shown the ability to identify heart-transplant patients at high risk for major adverse cardiovascular events. This is in part due to diagnosis of more severe forms of immune-mediated rejection such as high-risk CAV disease [35, 49, 63–65]. In contrast, a normal CCTA exam portends a favorable prognosis with 93% free of significant CAV for a minimum of 3 years [66]. Additionally, vascular remodeling that can be readily visualized by CCTA may serve further insight into the pathogenesis of CAV and provide prognostic value [67]. Reduced GLS by

echocardiography (greater than -14%) also has shown to be predictive of all-cause mortality [68–70]. More recently, myocardial characterization by CMR has shown incremental prognostic value [22, 23, 27]. Although presence of LGE is often not specific to the type of rejection, the burden of LGE appears to increase the risk for major adverse cardiovascular events with a cut-off of 7.9% of myocardial mass [22, 27]. Additionally, elevation of T2 relaxation time (greater than 50.2 milliseconds) and ECV (greater than 29%) also predicted major adverse cardiovascular events beyond what could be attributed to acute rejection [23]. Further studies have shown elevated ECV correlates with prior repeated acute rejection episodes, suggesting that elevated ECV reflects accumulated damage and fibrosis over time [13, 71]. Reduced GLS, diastolic strain rate and myocardial perfusion reserve also predicted major adverse cardiovascular events [59, 72, 73]. In summary, CMR provides better prognostication compared to other noninvasive imaging modalities because of its superior myocardial characterization with T2 mapping, ECV, LGE, GLS and myocardial perfusion reserve.

SEQUENCING IN HEART-TRANSPLANTATION

Immune monitoring in transplantation

Evidence of immune-mediated rejection by histopathology, demonstrated by invasion and destruction of cardiomyocytes by lymphocytes, is a late finding. At the time of diagnosis, the rejection episode often has been ongoing for an undetermined time period, causing myocardial damage and fibrosis [13, 71]. The ImmuKnow assay was created to noninvasively monitor immune activity to detect a pattern of increased immune activation prior to an acute rejection episode (Table 2) [74, 75]. Specifically, the assay determines CD4+ T cell immune activity by measuring adenosine triphosphate production after stimulation with phytohemagglutinin. However, several studies have subsequently shown that ImmuKnow has not been predictive for rejection in the post-transplant period [76–79]. The limitations may be due to the nonspecific

phytohemagglutinin immune stimulus or perhaps the need for selection of a more specific T cell subset [80, 81].

Gene expression profiling of peripheral blood mononuclear cells (PBMC) is currently the only assay approved for immune surveillance in heart-transplant patients and has paved the way for reduction of surveillance endomyocardial biopsies and reduced immunosuppression [11]**.** This paradigm change to noninvasive surveillance for ACR, typically at 6 months post-transplant, is one of the most significant developments in heart-transplant management in the past 10 years. AlloMap is based on the analysis of PBMC RNA that uses a 20-gene expression signature to predict ACR in stable, low risk heart-transplant patients and obtained FDA approval for this use in 2008 [74]. The CARGO II and IMAGE trials showed that AlloMap testing can be successfully used as part of a noninvasive surveillance strategy as a rule-out test for ACR as early as 55 days post-transplant [11, 82]. These studies also confirmed the low incidence rate of ACR in the current era which contributes to the high NPV and limited PPV of the AlloMap test [82]. However, despite AlloMap's success, there are noted limitations with the assay. First, AlloMap has not been shown to detect nor predict AMR [74]. Second, the assay is limited to low risk heart-transplant patients who are asymptomatic with normal allograft function, on reduced corticosteroids (less than 20 mg/d), have not had a recent acute rejection episode, have not recently received hematopoietic growth factors or blood transfusions, and cannot be pregnant [83]. Third, AlloMap does not discriminate against immune activation due to ACR versus cytomegalovirus infection [84]. Thus, while AlloMap has significantly changed the landscape of heart-transplant care to more noninvasive monitoring, its limitations highlight the need for a more refined understanding of the immune response in acute rejection. In addition to AlloMap, we would be remiss if we did not also mention the importance of detecting donor specific antibodies for both diagnosis of AMR and its prognostic value, with its improved and more specific detection by the single antigen bead assay [85, 86].

Noninvasive surveillance of allograft injury

Further advancing noninvasive monitoring in heart-transplant patients, donor-derived cell free DNA (dd-cfDNA) has been more recently developed as a highly sensitive allograft injury marker [87–89]. Acute rejection causes cell death in the allograft and this leads to increased levels of dd-cfDNA that can be measured in the patient's peripheral blood. AlloSure is currently the most widely used commercial assay and uses 266 single-nucleotide polymorphisms to accurately quantify dd-cfDNA without the need for genotyping the recipient or donor [88]. In the DART study, AlloSure correlated with both T cell-mediated rejection or antibody-mediated rejection in 102 kidney-transplant recipients with an AUC of 0.74. Using a 1% dd-cfDNA cut-off, the authors showed positive and negative predictive values of 61% and 84%, respectively. In contrast to dd-cfDNA, serum creatinine did not identify acute rejection with an AUC of 0.5. Khush and colleagues similarly demonstrated good diagnostic performance for AlloSure in heart-transplant patients and at a 0.2% dd-cfDNA cut-off, the authors showed 44% sensitivity and 80% specificity and a positive and negative predictive value of 8.9% and 97.1%, respectively [87]. It is important to note the different cut-offs in heart- versus kidney-transplant patients, the reasons for which are not completely explained. Thus, dd-cfDNA further improves upon noninvasive surveillance of the allograft for acute rejection from gene expression profiling and is no longer limited to ACR and low-risk transplant patients.

Going beyond histopathology

Microarray technology provides high throughput measurement of thousands of RNA transcripts for bulk RNA gene-expression patterns and as a result, provides much more information than the 20-gene AlloMap panel [90]. Thus, microarray technology is particularly attractive to transplant physicians because of its potential to improve accuracy for diagnosis of acute rejection, reduce interobserver variability in the interpretation of histopathology and most importantly, to provide further insight into mechanisms responsible for rejection [91, 92]. Reeve and co-authors were one

of the earliest groups to demonstrate the possibility of creating a T cell-mediated rejection score in kidney-transplant patients using machine-learning with array-based data [93]. Subsequently, the MMDx-Kidney study group performed archetypal analysis using array-based data and generated six archetypes, with molecular archetype scores showing better prediction of allograft survival than histologic diagnoses with the worst prognosis in the fully developed and late-stage antibody-mediated rejection archetypes [94]. This study showed the potential of microarray technology to find additional phenotypes beyond what is currently diagnosed by histopathology. The MMDx molecular diagnostic system was also applied to endomyocardial biopsies and the INTERHEART study (clinicaltrials.gov, NCT 02670408) will soon provide a prospective assessment of the MMDx system [95]. However, these studies are still limited because they continue to compare the molecular scores and phenotypes to histopathology, a flawed gold standard [96]. Alluding to the near future, Reeve and co-authors suggest the possibility of going beyond histopathology for a better classification by molecular diagnostics to improve understanding of acute rejection pathology.

Data-driven immune phenotype clustering from array-based data provides additional biological information beyond standard histopathology. CIBERSORT is a significant step forward in bioinformatics that uses a leukocyte signature matrix to deconvolve immune cell subsets from array-based data [97]. In essence, the authors provide a powerful solution for performing digital cytometry from bulk tissue analysis [98]. Buscher and colleagues have further modified the leukocyte signature matrix, which was originally created to evaluate cancer tissue, and included additional leukocyte subtypes as well as cells that comprise the kidney compartment (*under review*). This allowed for cell type deconvolution from kidney-transplant biopsy analysis. Furthermore, predictive stochastic neighbor embedding tool for omics (PRESTO) was used for unsupervised machine learning to identify co-regulated networks [99]. CIBERSORT and PRESTO were subsequently used together for biopsy phenotype clustering (Fig 1). This bioinformatically

driven approach identified a unique M2 macrophage subtype which was confirmed to be involved in active rejection by immunostains that ultimately resulted in fibrosis. Thus, there is now a successful paradigm for a superior, big data driven molecular diagnostic approach that goes beyond histopathology to identify potential immune-mediated mechanisms.

Additionally, single cell RNA sequencing (RNA-Seq) shows promise to further uncover the complex interactions of different immune and parenchymal cells at a much higher depth than bulk transcriptional profiling [100]. Single cell RNA-Seq has greater potential to dissect complex tissue into multiple cellular subpopulations and can also identify rare cell types not possible with microarrays nor bulk RNA-Seq. This is a nascent field that has already shown the ability to uncover distinct immune cell subsets in atherosclerosis and in transplantation [9, 101–103]. Thus, we, as well as other labs, are currently utilizing single cell RNA-Seq with data-driven analysis to provide potential hypotheses for immune-mediated rejection mechanisms that can be taken back and tested in animal models.

ARTIFICIAL INTELLIGENCE IN HEART-TRANSPLANTATION

Artificial intelligence (AI) is a rapidly expanding branch of computer science that utilizes systems and algorithms to perform tasks that previously required human intelligence [104, 105]. Machine learning is a subset of AI in which algorithms are trained to perform tasks by learning patterns from data, instead of prespecified rules, and can be either supervised or unsupervised by researchers. In contrast to traditional data analysis methods, machine learning relies on a trial and error approach to optimize data predictive analysis [106, 107]. Applications of machine and deep learning, a subfield of machine learning that utilizes artificial neural networks, in medicine have grown at a dazzling pace and already are influencing and driving the fields of cardiovascular imaging, histopathology and analysis of sequencing data [108, 109].

Deep learning has great potential to help shape the future of cardiovascular imaging and has already shown the ability to be used for object classification [105], improving image acquisition and workflow [110], automating analysis [109], enhancing image quality [111, 112], and risk prediction [108]. However, in our opinion, the greatest application of machine and deep learning will be the determination of new pathophysiologic findings, not evident to the imaging expert, that can be taken back to benchside research to better understand the mechanisms of disease. This is already being applied in CMR for detection of microvascular dysfunction in various cardiovascular diseases that was previously not identifiable by the usual qualitative review [61, 62, 113]. Thus, deep learning will continue to grow to inform us of new insights that can be further tested by hypothesis-driven research [114, 115].

Machine learning has also already begun to shape the fields of histopathologic interpretation and analysis of sequencing data to provide consistent and more accurate histopathologic interpretation [116–118] and identify gene expression patterns that would not be recognized with classic analysis [119, 120]. Buscher and colleagues have employed the use of two novel machine learning techniques, CIBERSORT and PRESTO, to identify a M2 macrophage subtype from a unique immune cell signature that is involved in active forms of acute rejection. This data-driven immune phenotype clustering is especially powerful because it demonstrates a successful paradigm for the use of machine learning in transplant immunology to identify mechanistic hypotheses that can be tested in animal models. Furthermore, this approach will be even more important in the analysis of large datasets derived from single cell RNA-seq.

Additionally, use of AI for development of accurate risk prediction models in solid organ transplantation is particularly important because of the limited allograft longevity and need to identify transplant patients at high-risk for allograft failure. This has been successfully used for prediction of kidney allograft failure with the creation of the iBox score from independent determinants that include time from transplant, current kidney functioning based on standard

laboratory measurement, donor specific antibodies and histopathologic findings [121]. More recently, in heart-transplantation, Loupy and colleagues created four CAV trajectory profiles using unsupervised latent class mixed models that accurately predicted the development of CAV and all-cause mortality using six donor and recipient (within the first year) characteristics [122]. This model already has the potential to reduce early and repeated invasive coronary angiography to heart-transplant patients at highest risk, specifically those in trajectories 3 and 4. As data collection and our understanding of allograft rejection improves, this will also continue to improve risk prediction models which will accurately guide transplant physicians in their care for their patients.

Despite the variety of successes in AI and machine learning, there remain limitations in their applications including need for larger and more accurate datasets, demonstration of reproducibility, ability to understand the derived models and trust the results, working towards uniformity and agreement on evaluation metrics, and most importantly, guidance of these models by clinicians to assure meaningful clinical impact [104, 123, 124]. Some of these limitations will be addressed by AI. For instance, as it creates improved and more consistent analysis of cardiac imaging, histopathology and sequencing, AI will then provide itself with more accurate datasets to improve its predictive models. Most importantly, collaboration between clinicians and AI specialists will be essential in the application of AI in medicine.

CONCLUSION

With limitations in animal models of allograft rejection that either did not accurately model rejection in humans or did not produce findings that translated clinically [125], our understanding of cardiac allograft rejection has not been able to progress significantly. However, as cardiovascular imaging and sequencing have made tremendous gains in recent years, identifying potential mechanisms for allograft rejection is now a reality that was long overdue. Furthermore, the large datasets

created by both imaging and sequencing can be leveraged by AI to recognize novel patterns that we have not been able to identify with traditional methods (Fig. 1). Thus, the field of transplantation is on the brink of a true paradigm shift that combines big data driven analysis with traditional hypothesis testing to overcome our previous limitations in understanding allograft rejection. Though we still have far to go, there is a clearer path towards better understanding of transplant immunology and ultimately increasing cardiac allograft survival for our patients.

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FIGURE LEGEND

Figure 1. This figure conceptualizes the future direction of heart-transplant research, combining data from imaging and sequencing and leveraged by artificial intelligence to identify new mechanisms that can be tested in animal models. (a) An example of cardiac MRI imaging (3 chamber view) with 3D cine acquisition. (b) RNA microarray data from biopsy tissue of kidney allografts with cell type deconvolution by CIBERSORT (courtesy of Dr. Buscher, manuscript under review). (c) An example of object classification using convolutional neural networks to accurately identify different abdominal organs and their laterality. (d) Gene network 4 (courtesy of Dr. Buscher) derived from PRESTO analysis of array-based data from biopsy tissue of kidney allografts. Network 4 is involved in acute rejection and shows co-regulated networks of extracellular matrix and fibrosis-related pathways. When used with CIBERSORT, Buscher and colleagues identified fibroblasts and a distinct M2 macrophage subset that appear to be involved in early transplant fibrosis.

Table 1. Noninvasive cardiac imaging modalities for evaluating heart-transplant patients

	Advantages	Disadvantages
Acute Rejection		
Echocardiography Key References: # [30]	• Readily accessible • Test of choice in the setting of hemodynamic compromise • First-line imaging modality to assess allograft function	• Insensitive in detecting acute rejection • Often unable to differentiate allograft dysfunction from acute rejection and CAV
CMR Key References: # [14–16]	• Contrast enhancement with gadolinium allows tissue characterization to detect myocardial injury, scarring and edema • T2 relaxation time and ECV most consistent independent predictors of acute rejection	• LGE is a nonspecific finding in heart-transplant patients • Contrast use limited with poor renal function • Cannot perform on hemodynamically unstable patients • Irregular rhythms such as atrial fibrillation significantly limit image quality
Cardiac Allograft Vasculopathy (CAV)		
Echocardiography Key References: # [35, 39]	• DSE is widely available and commonly used • Negative studies thought to have good prognostic value from older literature • Can be combined with GLS to improve sensitivity	• Recent literature casts doubt on accuracy of DSE with sensitivity as low as 28% to detect clinically significant CAV

CMR Key References: # [24, 26, 57, 59]	• Superior myocardial characterization compared to other imaging modalities • Addition of stress myocardial perfusion imaging allows for sensitive detection of macrovascular and microvascular CAV	• LGE is a nonspecific finding in heart-transplant patients • Contrast use limited in severe renal dysfunction
CCTA Key References: # [41, 43]	• High spatial resolution allows for sensitive diagnosis of CAV and a high negative predictive value • Decreasing radiation exposure, equal to or less than invasive coronary angiography, with newer hardware and software improvements	• High heart rates often seen in heart-transplant patients can introduce motion artifacts • Spatial resolution still limited in detection of microvascular CAV • Relatively contraindicated in patients with renal dysfunction
Nuclear Imaging Key References: # [51–53]	• Pharmacologic SPECT imaging is widely available and commonly used • PET provides quantitative myocardial perfusion parameters for sensitive detection of microvascular CAV • Renal dysfunction is not a limitation for nuclear imaging	• SPECT has limited accuracy in detection of CAV • PET is expensive and has limited availability • Radiation exposure is highest with SPECT compared to other imaging modalities while exposure with PET is similar to invasive coronary angiography
Prediction of Clinical Outcomes		
Echocardiography Key References: # [8, 10, 68, 69]	• LVEF is a strong predictor for outcomes • GLS can be predictive of all-cause mortality	• Decreased LVEF is a late finding • Standardization of deformation imaging is load dependent and continues to evolve. Precise normal values are not established in heart-transplant patients

CMR Key References: # [21–23, 27, 73]	• LGE burden, increased T2 relaxation time and ECV predict major adverse cardiovascular events beyond what could be attributed to acute rejection • Other MRI parameters including GLS, diastolic strain rate and myocardial perfusion reserve also are predictive of major adverse cardiovascular events	• Abnormal CMR parameters often do not convey mechanistic process causing allograft dysfunction • Contrast use limited in severe renal dysfunction
CCTA Key References: # [66, 67]	• Normal CCTA exam demonstrates decreased likelihood of developing CAV in 3 years • Vascular remodeling can be evaluated and quantified	• High heart rates often seen in heart-transplant patients can introduce motion artifacts • Relatively contraindicated in patients with renal dysfunction
Nuclear Imaging Key References: # [63–65]	• Myocardial flow reserve and blood flow quantified by PET can predict major adverse cardiovascular events • Not limited by renal dysfunction	• Optimal parameters cut-offs for PET perfusion not standardly defined and validated • Cost of PET and limited availability prevent further widespread use

CAV, cardiac allograft vasculopathy; CMR, cardiovascular magnetic resonance imaging; CCTA, coronary computed tomography angiogram; DSE, dobutamine stress echocardiography; ECV, extracellular volume; GLS, global longitudinal strain; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; PET, positron emission tomography; SPECT, single photon emission tomography

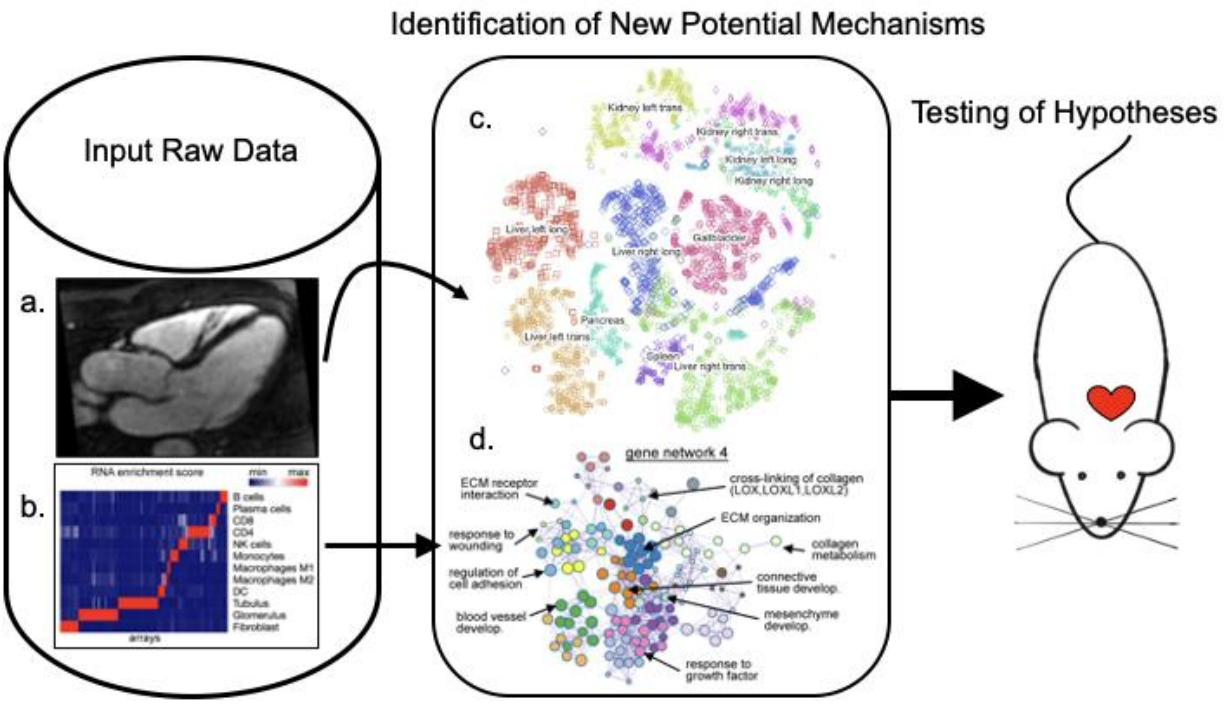
Table 2. Types of sequencing for detection of acute rejection in heart-transplant patients

	Advantages	Disadvantages
Immune Monitoring		
Immuknow Key References: # [77, 78]	• Noninvasive • Can identify patients at higher risk for infection	• Limited ability to predict acute rejection
Gene expression profiling Key References: # [11, 74, 82]	• Noninvasive • FDA approved for surveillance of ACR in heart-transplant patients • High negative predictive value for ACR	• Limited to low risk patients • Not sensitive for detecting AMR • Low positive predictive value for ACR • CMV viremia also can produce a positive result
Donor specific antibody Key References: # [85, 86, 126]	• Noninvasive • Recommended to check 1, 3, 6, and 12 month and then annually by ISHLT • Presence of anti-HLA antibody associated with rejection, CAV and poor allograft survival	• Non-HLA antibodies also implicated in AMR • Mean fluorescence intensity cut-offs differ between institutions
Allograft Injury Detection		
Donor-derived cell free DNA Key References: # [87, 88]	• Noninvasive • Highly sensitive for ACR and AMR • Complementary to gene expression profiling	• Different cut-offs depending on type of allograft • Not established for multi-organ transplant patients • Cannot differentiate between AMR and ACR
Molecular Diagnostic Techniques		

Microarray technology Key References: # [91, 92, 94]	• Potential to reduce wide interobserver variability seen with histopathology • Additional archetypes beyond current histopathologic definitions can be found	• Has not identified new mechanisms that may cause acute rejection • Still compared to the gold standard of histopathology • Quality control, discrepancy in array studies, and difficulty in detecting small changes from rare cell populations remain limitations
Machine-learning methods with array-based and bulk RNA sequencing data Key references: # [97–99]	• Big data driven molecular diagnostic approach can identify new immune-mediated mechanisms not identified previously with traditional methods	• Nascent field, not widely established, and has not demonstrated reproducibility • Derivation of accurate algorithms limited by need for large input data with adequate variety of pathology
Single cell RNA sequencing Key references: # [100, 102, 103]	• New and powerful technology with exponential use in recent years • Dissects complex tissue with ability to detect rare cell populations	• Currently cost-prohibitive • Not a high throughput method • Variances in techniques across different institutions

ACR, acute cellular rejection; AMR, antibody mediated rejection; CAV, cardiac allograft vasculopathy; FDA, Food and Drug Administration; RNA, ribonucleic acid

Figure 1.


Identification of New Potential Mechanisms
Input Raw Data
a.
b.
RNA enrichment score
min
max
B cells
Plasma cells
CD8
CD4
NK cells
Monocytes
Macrophages M1
Macrophages M2
DC
Tubulus
Glomerulus
Fibroblast
arrays
c.
Kidney left trans
Kidney right trans
Kidney left long
Kidney right long
Liver left long
Liver right long
Gallbladder
Pancreas
Liver left trans
Spleen
Liver right trans
d.
gene network 4
ECM receptor interaction
cross-linking of collagen (LOX,LOXL1,LOXL2)
ECM organization
response to wounding
collagen metabolism
regulation of cell adhesion
connective tissue develop.
blood vessel develop.
mesenchyme develop.
response to growth factor
Testing of Hypotheses