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STATE-OF-THE-ART PAPER

The End of Endomyocardial Biopsy?

A Practical Guide for Noninvasive Heart Transplant Rejection Surveillance

Luise Holzhauser, MD,[a] Ersilia M. DeFilippis, MD,[b] Andriana Nikolova, MD, PhD,[c] Mirnela Byku, MD, PhD,[d] Johanna P. Contreras, MD,[e] Teresa De Marco, MD,[f] Shelley Hall, MD,[g] Kiran K. Khush, MD, MAS,[h] Amanda R. Vest, MBBS, MPH[i]

HIGHLIGHTS

- Rejection surveillance using gene expression profiling and donor-derived cell-free DNA (dd-cfDNA) is noninferior to endomyocardial biopsy.
- Transitioning away from traditional biopsy surveillance raises many practical questions.
- In this paper, we provide guidance for the transition and early implementation process.
- The clinical value of dd-cfDNA may offer benefits beyond current surveillance strategies, pending future prospective studies.

ABSTRACT

Noninvasive heart transplant rejection surveillance using gene expression profiling (GEP) to monitor immune activation is widely used among heart transplant programs. With the new development of donor-derived cell-free DNA (dd-cfDNA) assays, more programs are transitioning to a predominantly noninvasive rejection surveillance protocol with a reduced frequency of endomyocardial biopsies. As a result, many practical questions arise that potentially delay implementation of these valuable new tools. The purpose of this review is to provide practical guidance for clinicians transitioning toward a less invasive acute rejection monitoring protocol after heart transplantation, and to answer 10 common questions about the GEP and dd-cfDNA assays. Evidence supporting GEP and dd-cfDNA testing is reviewed, as well as guidance on test interpretation and future directions. (J Am Coll Cardiol HF 2022;■:■–■) © 2022 Published by Elsevier on behalf of the American College of Cardiology Foundation.

Endomyocardial biopsy (EMB) has been the cornerstone of post-heart transplantation (HT) rejection surveillance since the 1970s.[1] However, EMB is limited by the associated adverse event profile, lack of portability, patient discomfort, low interobserver concordance of rejection grading, and low sensitivity for antibody-mediated rejection (AMR) detection.[2,3] Therefore, more accurate and

From the [a]Division of Cardiology, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [b]Division of Cardiology, Columbia University Irving Medical Center, New York, New York, USA; [c]Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA; [d]Department of Cardiology, University of North Carolina in Chapel Hill, North Carolina, USA; [e]Mount Sinai Hospital, New York, New York, USA; [f]Division of Cardiology, University of California, San Francisco, California, USA; [g]Baylor University Medical Center, Dallas, Texas, USA; [h]Division of Cardiovascular Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA; and the [i]Tufts Medical Center, Boston, Massachusetts, USA.

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ABBREVIATIONS AND ACRONYMS

ACR = acute cellular rejection

AMR = antibody-mediated rejection

CAV = cardiac allograft vasculopathy

cfDNA = cell-free DNA

dd-cfDNA = donor-derived cell-free DNA

dnDSA = de novo donor-specific antibody

DSA = donor-specific antibody

EMB = endomyocardial biopsy

GEP = gene expression profiling

HT = heart transplantation

ISHLT = International Society of Heart and Lung Transplantation

LVEF = left ventricular ejection fraction

less invasive methods of HT rejection surveillance have been sought for several decades, with blood-based assays now widely available in the United States. Many HT programs are adopting gene expression profiling (GEP) and quantification of donor-derived cell-free DNA (dd-cfDNA) into post-HT surveillance protocols to substantially reduce the frequency of EMBs for asymptomatic rejection screening. Given the low incidence of asymptomatic rejection, a sensitive surveillance strategy with a high negative predictive value (NPV) may minimize the number of EMBs post-HT. A contemporary surveillance strategy should also permit early detection of rejection, as compared with histopathological changes that represent advanced stages of immune activation. Blood-based assays also avoid the sampling errors and interobserver variability in grading that limit EMB performance.[3-5] This evolution in clinical practice offers benefits in terms of patient satisfaction and safety, as well as the potential for more timely and accurate rejection detection; however, a transition away from EMB-based surveillance may also prompt uncertainty and challenges for patients and clinicians alike. The purpose of this review is to provide practical guidance for clinicians transitioning toward a less invasive acute rejection monitoring protocol after HT, and to answer 10 common questions about the GEP and dd-cfDNA assays.

1. WHAT DOES THE DD-cfDNA ASSAY TEST, AND HOW DOES IT COMPARE WITH OTHER REJECTION MARKERS?

Cell-free DNA is released constantly during normal cell turnover, with a minute fraction normally originating from the heart. In HT recipients, the allograft releases dd-cfDNA that is distinguishable from recipient cfDNA. Although initial dd-cfDNA assays required donor and recipient genotyping, current commercial dd-cfDNA assays are based on next-generation sequencing that quantifies dd-cfDNA using single-nucleotide variations (SNV [formerly SNP]). dd-cfDNA is expressed as the percentage of dd-cfDNA within the total circulating cfDNA. Following development of the AlloSure dd-cfDNA assay (CareDx, Inc), the D-OAR (Utility of Donor-Derived Cell Free DNA in Association With Gene Expression Profiling) registry (N = 740; 26 HT centers) showed that increased dd-cfDNA correlates with EMB-detected rejection[6] (**Table 1**). D-OAR established dd-cfDNA as a specific marker of graft injury with also a high NPV of 97% for both acute cellular rejection (ACR) and AMR. The GRAfT (Genomic Research Alliance for Transplantation) investigators used a research-grade dd-cfDNA assay that detected early allograft injury at a median of 3.2 months before EMB-detected AMR. Recent preclinical developments suggest potential differentiation between ACR and AMR based on temporal trends, magnitude of dd-cfDNA elevation, fragment length, and the genomic composition of cfDNA fragments.[13]

The current commercially available tests include stand-alone dd-cfDNA assays (eg, Prospera Heart, Natera, and Viracor TRAC, Eurofins Viracor), as well as combination GEP and dd-cfDNA (HeartCare, combining the AlloMap GEP assay and the AlloSure dd-cfDNA assay, CareDx) (**Table 2**). The Viracor TRAC test has only been studied to date in kidney and liver transplant recipients, and Prospera Heart and Viracor TRAC are not approved by the Food and Drug Administration or Centers for Medicare and Medicaid Services. GEP quantifies the expression of 11 genes in peripheral blood mononuclear cells that are involved in lymphocyte activation, cell migration, T-cell priming, hematopoietic proliferation, steroid sensitivity, and platelet activation pathways that are altered during cellular rejection. An activation score (0 to 40) was created from the CARGO (Cardiac Allograft Rejection Gene Expression Observational) study to distinguish between moderate/severe ACR and quiescence.[7] Use of GEP in IMAGE (Invasive Monitoring Attenuation Through Gene Expression) reduced the number of EMBs performed without an increase in serious adverse effects,[8] and received a Class IIa indication in the 2010 International Society of Heart and Lung Transplantation (ISHLT) guidelines.[15]

Although GEP is reflective of the recipient's immune activation status and is designed to detect ACR with a high sensitivity, it was not developed to detect AMR and does not provide information on graft injury. The dd-cfDNA assay, therefore, provides complementary information to the GEP that can reflect myocardial damage and thus helps to detect AMR post-HT. Furthermore, as discussed in question 7, dd-cfDNA levels also increase with de novo donor-specific antibody (DSA) formation, further strengthening the link to AMR.[18,19]

The dd-cfDNA assay may also help to differentiate a false-positive vs true GEP elevation in the setting of ACR: if the GEP elevation truly indicates cellular rejection, dd-cfDNA would be expected to be elevated, given the high NPV of dd-cfDNA for acute rejection (**Figure 1**). A direct comparison study of a dd-

TABLE 1 Key Studies of Noninvasive Surveillance Methods Using GEP and dd-cfDNA

Study Name, Year of Publication	Study Design	Key Study Findings
GEP studies		
Cardiac Allograft Rejection Gene Expression Observational Study (CARGO), 2006[7]	• Prospective observational 8-center cohort study of HT recipients • 11-gene real-time PCR test was derived from a training set (n = 145 samples, 107 patients) • Linear discriminant analysis, converted into a GEP score (0-40) and validated prospectively in an independent set (n = 63 samples, 63 patients) • Study period began 2001	• GEP test differentiated moderate/severe rejection from quiescence ($P = 0.0018$) in the validation set, with agreement of 84% (95% CI: 66% to 94%) with grade ISHLT ≥3A rejection • Patients >1-year post-HT with GEP <30 (68% of the study population) were unlikely to have grade ≥3A rejection (NPV = 99.6%)
Invasive Monitoring Attenuation Through Gene Expression (IMAGE), 2010[8]	• Randomized trial of 602 low-risk patients assigned to a GEP vs EMB surveillance strategy starting 6 mo post-HT • Study period 2005-2009	• Over median 19 mo follow-up, GEP monitoring for ACR was noninferior to the EMB-based protocol for the primary composite endpoint of rejection with hemodynamic compromise, graft dysfunction, death, or retransplantation (15.5% vs 15.3%; HR: 1.04, 95% CI: 0.67-1.68)
Early Invasive Monitoring Attenuation Through Gene Expression (eIMAGE), 2015[9]	• Randomized trial of 60 patients assigned to a GEP vs EMB surveillance strategy from 55 d post-HT, conducted at a single center • Study period 2009-2011	• At 18 mo, GEP vs EMB monitoring for ACR had a similar 10% vs 17% primary composite endpoint of rejection with hemodynamic compromise, graft dysfunction, death or retransplantation ($P = 0.44$)
Cardiac Allograft Rejection Gene Expression Observational (CARGO II), 2016[10]	• Prospective observational 17-center European and U.S. cohort study of 499 HT recipients • Study period 2005-2009	• Validated the GEP assay for surveillance of EMB-diagnosed ACR • With GEP threshold ≥30 at ≥2-6 mo post-HT had a PPV = 2.6, NPV = 98.4, sens = 37.5, and spec = 73. GEP threshold >34 at >6 mo post-HT had a PPV = 4.3, NPV = 98.3, sens = 25, and spec = 88.8 • Based on 938 paired biopsies, the GEP test score AUC-ROC for detection of ≥3A rejection was 0.70 and 0.69 for ≥2-6 and >6 mo post-transplantation, respectively
Risk evaluation using gene expression screening to monitor for acute cellular rejection in heart transplant recipients (OAR), 2019[11]	• Prospective, observational, 35-center study of 1,504 patients at least 2 mo post-HT undergoing GEP surveillance • Study period 2013-2017	• Largest study conforming high NPV of GEP testing • At 2-6 mo, GEP score ≤30 NPV = 98.4% for significant rejection; >6 mo, GEP score ≤34 NPV of 98.5% • High survival rate at 1, 2, and 5 y post-transplant was 99%, 98%, and 94%, and low rate of rejection with GEP monitoring (≥2R) 2.0% from 2-6 mo and 2.2% >6 mo

Continued on the next page

cfDNA alone vs GEP plus dd-cfDNA strategy for HT rejection surveillance has not yet been performed, and furthermore, the Centers for Medicare and Medicaid Services-approved testing option, HeartCare, routinely provides both the dd-cfDNA and GEP values. Thus, it is not currently possible to quantify how much additional clinical information the GEP score provides beyond the dd-cfDNA assay. However, there are substantial variations in clinical responses across transplant programs to the scenario of an abnormal GEP plus normal dd-cfDNA, and many clinicians would be very interested in an analysis of the value added by GEP to the dd-cfDNA. An indirect answer to this question may be possible with the upcoming DETECT (Donor Derived Cell-free DNA to DETect REjection in Cardiac Transplantation; NCT05081739) clinical trial that will randomize HT recipients between a dd-cfDNA-guided vs an EMB-guided surveillance strategy, without use of GEP, for the primary endpoint of treated rejection with or without graft dysfunction, graft dysfunction, retransplantation, or death.

Prior research has indicated that other cardiac biomarkers including troponin T, troponin C, N-terminal pro-B-type natriuretic peptide (NT-proBNP) and C-reactive protein (CRP) are of limited value for diagnosing acute rejection, and the ISHLT 2010 guidelines recommend against their use for HT rejection screening.[15,20,21] High-sensitivity troponin C assays have recently shown a superior NPV to conventional troponin C assays for ACR detection,[22] but it is currently unknown whether adding other cardiac biomarkers to dd-cfDNA would improve performance characteristics. An additional biomarker available for solid organ transplantation monitoring is the immune cell function assay (ImmuKnow, Eurofins Viracor). This Food and Drug Administration-approved assay measures ATP release from activated peripheral lymphocytes and correlates with immune activation. Higher assay scores (≥525 ng/mL) are associated with greater rejection risk, whereas lower scores (≤225 ng/mL) are associated with higher infection risk, but there are no prospective outcomes data available in HT recipients at this time.[23,24]

2. WHAT CLINICAL SCENARIOS CAN AFFECT THE PERFORMANCE OF THE GEP OR DD-cfDNA TESTS?

GEP and dd-cfDNA are sensitive markers of immune activation and cellular injury, and can therefore be

TABLE 1 Continued

Study Name, Year of Publication	Study Design	Key Study Findings
dd-cfDNA studies		
Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection, 2014[12]	• Prospective study on Genome Transplant Dynamics (GTD) using SNP genotyping • 65 adult and pediatric HT recipients with 565 longitudinally obtained plasma samples compared with EMB	• Established rapid clearance of dd-cfDNA after HT and a stable baseline allowing for implementation of a time-independent threshold for the diagnosis of AR when collected more than 2 wk after HT • dd-cfDNA detected both ACR and AMR, and levels correlated with severity • ROC analysis for detection of ACR (≥2R/3A or AMR) by dd-cfDNA revealed AUC of 0.83 (sens = 0.58, spec= 0.93 at a dd-cfDNA threshold level of 0.25%) • Findings indicate that dd-cfDNA could replace EMB
Donor-Derived Cell-Free DNA-Outcomes AlloMap Registry (D-OAR), 2019[6]	• Prospective observational cohort of 740 HT recipients from 26 U.S. centers aged ≥15 y and >55 d post-HT who were undergoing AlloMap GEP and AlloSure dd-cfDNA testing for surveillance, plus a single-center cohort of 33 patients at high risk for AMR • Study period 2014-2018	• Median dd-cfDNA was 0.07% in reference HT recipients (n = 2,164 samples) and 0.17% with acute rejection (n = 35 samples; $P = 0.005$) • At a 0.2% threshold, dd-cfDNA had a 44% sensitivity to detect rejection and a 97% NPV • In the cohort at risk for AMR (n = 110 samples from 33 patients), dd-cfDNA levels were elevated 3-fold in AMR compared with patients without AMR (99 samples; $P = 0.004$)
Genomic Research Alliance for Transplantation (GRAfT), 2021[13]	• Prospective observational cohort study of HT recipients aged ≥18 y from 5 U.S. centers • Aimed to validate the test characteristics of dd-cfDNA for acute rejection and to determine the ability to predict long-term outcomes including CAV, graft failure, and mortality • Dd-cfDNA measured by shotgun sequencing and included donor-recipient-paired genotyping to identify SNPs (research grade assay-not available clinically) • Study period 2015-ongoing	• Median dd-cfDNA levels were 0.34% vs 0.04%; $P < 0.006$ for ACR ≥ grade 2 vs ACR grade 1 • Median levels were 0.63% vs 0.02%; $P < 0.001$ for AMR 1 versus grade 0 rejection and 1.68% vs 0.63%; $P = 0.039$ for AMR ≥2 vs AMR 1 • dd-cfDNA levels rose as early as 120 d before acute rejection, and fragment length >100 bp was associated with AMR diagnosis
A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation (DEDUCE), 2022[14]	• Observational 2 center study with retrospective and prospective component • Using the clinically available Prospera dd-cfDNA test (Natera) • Study period 2017-2022	• 811 samples from 223 patients with dd-cfDNA testing and contemporaneous EMB with 49 EMBs showing AR in 35 patients • dd-cfDNA was significantly higher in AR (median 0.58%; IQR: 0.13%-1.68%) compared with non-AR (median 0.04%, IQR: 0.01%-0.11%, $P_c < 0.001$) • AUC-ROC of 0.86 (95% CI: 0.77-0.96) • For dd-cfDNA ≥0.15% 78.5% sens (95% CI: 60.7%-96.3%) and 76.9% spec (95% CI: 71.1%-82.7%) for AR • PPV 25.1% (95% CI: 18.8%-31.5%) and NPV 97.3% (95% CI: 95.1%-99.5%)
Surveillance HeartCare Outcomes Registry (SHORE) [NCT03695601]	• Prospective observational registry study, HT patients with HeartCare (AlloMap and AlloSure) monitoring initiated within 3 mo of HT compared with historical control group not monitored with HeartCare • Planned enrollment of 2,300 patients completed (plan to match with 1,150 historical controls) • Primary outcome: Percentage of patients alive at 1, 2, and 3 y post-HT • Study period 2018-2024	NA

ACR = acute cellular rejection; AMR = antibody-mediated rejection; AR = acute rejection; AUC = area under the curve; CAV = coronary allograft vasculopathy; dd-cfDNA = donor-derived cell-free DNA; EMB = endomyocardial biopsy; GEP = gene expression profiling; HT = heart transplantation; ISHLT = International Society of Heart and Lung Transplantation; NA = nothing available, NPV = negative predictive value; PPV = positive predictive value; PCR = polymerase chain reaction; ROC = receiver-operator characteristic; sens = sensitivity; SNP = single-nucleotide polymorphism; spec = specificity.

affected by scenarios other than acute rejection (**Table 3**). Thus, it is important to interpret these results within the clinical context to determine whether a confirmatory EMB is required.

There are situations where the results of the GEP and dd-cfDNA may be discordant, such as GEP false elevation during systemic inflammatory or infectious states, commonly including cytomegalovirus viremia.[25] An elevated GEP without corroborating evidence of allograft damage from the dd-cfDNA may trigger an EMB in some HT programs, whereas other programs may place their confidence in the high NPV of the dd-cfDNA test and chose not to biopsy in this scenario if they feel sufficiently confident that a non-ACR cause is responsible for the GEP elevation, and the patient is clinically well. These case-by-case decisions often incorporate additional data points such as symptoms or laboratory testing reflecting the

TABLE 2 Commercially-Available GEP and dd-cfDNA Assays

GEP/dd-cfDNA Test Name	Vendor	Availability/Approval	Key Research Studies	Practical Considerations	Notable Features
AlloMap	CareDx, Inc	Available in the U.S. FDA approved since 2008 for use ≥55 d post-HT in recipients ≥15 y CMS approved in the U.S.	CARGO[7] IMAGE[8] eIMAGE[9] CARGO II[10] OAR[11]	Minimum needle size for phlebotomy is 22G and collect blood in CPT tubes Avoid transporting sample in pneumatic tubing systems GEP samples need to be processed in a specialized laboratory within 3 h of blood draw Centrifuge and dry ice needed to maintain 6°-37°C	Remote phlebotomy offered by vendor Class IIa recommendation in ISHLT 2010 guidelines for the care of HT recipients[15] AlloMap results within 50-72 h
AlloSure heart (dd-cfDNA) HeartCare (GEP+dd-cfDNA)	CareDx, Inc	Available in the U.S. CMS approved as HeartCare (combined with GEP AlloMap test) ≥55 d post-HT in patients ≥15 y AlloSure alone is not FDA approved, classified as CAP/CLIA test	D-OAR[6] SHORE [NCT03695601]	Minimum needle size for phlebotomy is 22G and collect blood in Streck tubes. dd-cfDNA must be drawn before EMB or >24-48 h after to avoid false elevations from biopsy-induced myocardial trauma	Remote phlebotomy offered by vendor Plasma dd-cfDNA test performed at a single CareDx CLIA laboratory Analyzes 405 SNPs via NGS HeartCare results within 50-72 h
AlloSeq (dd-cfDNA)	CareDx, Inc	Available outside the U.S., in Europe and additional countries Registered and certified with CE IVD Not paired with GEP testing	NA	dd-cfDNA must be drawn before EMB or >24-48 h after to avoid false elevations from biopsy-induced myocardial trauma	Analyzes 202 SNPs via NGS at local/in-house laboratory (no shipment to CareDx required) Interpretation performed locally Plasma levels of dd-cfDNA and interpretation of results are identical to AlloSure
Prospera Heart (dd-cfDNA)	Natera, Inc	Available in the U.S. Not FDA or CMS approved, classified as CAP/CLIA test	DEDUCE[14] ProTECT [Prospera Test Evaluation in Cardiac Transplant;NCT05205551, enrolling] DETect [Donor-Derived Cell-free DNA to DETect REjection in Cardiac Transplantation; NCT05081739, planning] Trifecta-Heart [Trifecta-Heart cfDNA-MMDx Study; NCT04707872, enrolling] DEFINE [Development of Non-invasive Cell-free DNA to Supplant Invasive Biopsy in Heart Transplantation; NCT05309382, planning]	dd-cfDNA must be drawn before EMB or >24-48 h after to avoid false elevations from biopsy-induced myocardial trauma Use ≥21G needle and collect blood in Streck tubes	Analyzes 13,292 SNPs Prospera results in 48-72 h
Viracor TRAC (dd-cfDNA)	Eurofins Viracor, Inc	Available in the U.S. only, but the U.S.-based CAP/CLIA lab accepts samples from outside the U.S. Not FDA or CMS approved, classified as CAP/CLIA test	Studied in kidney[16] and liver[17] transplant recipients	dd-cfDNA must be drawn before EMB or >24-48 h after to avoid false elevations from biopsy-induced myocardial trauma Use 21G or 22G needle and collect blood in Streck tubes Ambient shipping temperature	Genotyping of recipient only, after which donor genotype is inferred using computational approaches TRAC results within 4-6 business days

CAP = Certified Authorization Professional; CE = Conformité Européenne; CLIA = Clinical Laboratory Improvement Amendment; CPT = Cell Preparation Tubes; CMS = Centers for Medicare and Medicaid Services; FDA = Federal Drug Administration; IVD = in vitro diagnostic; NGS = next-generation sequencing; other abbreviations as in Table 1.

potential for an active infection and should be individualized to the baseline patient risk for rejection and prior degree of concordance between GEP, dd-cfDNA, and EMB findings. In some cases, it may be appropriate to repeat the noninvasive testing before making a decision about the role of EMB in a patient who is otherwise well and considered unlikely to have ACR.

The dd-cfDNA result represents a fraction of dd-cfDNA to total cell-free DNA.[26] The assumption is that recipient noncardiac release of cfDNA remains stable; however, situations such as sepsis and malignancy can increase total cfDNA and thus erroneously lower the donor-derived percentage.[27-29] Alternatively, the dd-cfDNA could be falsely elevated due to myocardial ischemia or injury, including within 24 to 48 hours of an EMB procedure due to myocardial trauma (Table 3). Sex- and race-specific normal ranges have not been proposed, but it has been noted that dd-cfDNA percentage is higher for Black patients immediately after transplantation, as compared with non-Black patients (mean [SE]: 8.3% [1.3%] vs 3.2%

FIGURE 1 Possible Interpretation Strategies for Paired GEP and dd-cfDNA Testing

GEP	Normal	High	Normal	High
dd-cfDNA	Normal	Normal	High	High
Potential interpretation	Rejection unlikely	Consider immune activation from non-rejection conditions	Consider all etiologies of injury, including AMR, CAV, trauma	Highest PPV with strong likelihood of rejection
Frequency in D-OAR	56%	26%	11%	6%

Gene expression profiling (GEP) is considered normal if <30 in the first 2 to 6 months post-heart transplantation or <34 beyond 6 months; donor-derived cell-free DNA (dd-cfDNA) considered normal if <0.12%, with graft damage suggested if >0.2%.[6] AMR = antibody mediated rejection; CAV = coronary allograft vasculopathy; D-OAR = Donor-Derived Cell-Free DNA-Outcomes AlloMap Registry; PPV = positive predictive value.

[1.2%]; $P = 0.001$). The rate of decay in dd-cfDNA over the first week post-HT was equivalent between race groups, and values declined in both groups to a comparable plateau at 7 days post-HT (0.46% [0.03%] vs 0.45% [0.04%]; $P = 0.78$).[30]

3. HOW SHOULD TRANSPLANT PROGRAMS DEFINE THE THRESHOLDS FOR ABNORMAL GEP AND DD-cfDNA RESULTS, AND HOW SHOULD THEY RESPOND TO ABNORMAL VALUES?

There is no absolute level of GEP or dd-cfDNA that is definitively abnormal, and different transplant programs and research studies have used different levels depending on the surveillance strategy and patient population. Defining thresholds requires selecting an appropriate balance between test sensitivity and specificity. For example, the CARGO study initially used a predefined GEP threshold of ≥20, which yielded 80% agreement with biopsy grade ≥3A ACR and 59% agreement for biopsy grade 0. Increasing the threshold to ≥30 continued to maintain 80% agreement for ACR and 78% agreement for quiescence when used beyond 1 year post-HT.[7] At this GEP threshold of 30, their validation cohort showed positive predictive value (PPV) 6.8% and NPV 99.6%, with 68% of results being <30. Subsequent studies, including CARGO II and IMAGE, used a GEP threshold ≥34 at more than 6 months post-HT with

TABLE 3 Notes on GEP and dd-cfDNA Interpretation

Scenarios That Can Falsely Elevate GEP	Scenarios That Can Falsely Elevate dd-cfDNA
Infections (eg, cytomegalovirus, Epstein-Barr virus, varicella zoster virus, and others)	Myocardial ischemia or other graft injury (eg, cardiac allograft vasculopathy)
Systemic inflammatory state	Myocardial trauma or injury (eg, <24-48 h after endomyocardial biopsy, cardiac contusion)
Scenarios Where GEP Should Be Interpreted Cautiously	**Scenarios Where dd-cfDNA Should Be Interpreted Cautiously**
Within 30 d of packed red blood cell transfusions (unless leukocyte-depleted blood)	Multiorgan transplant, caused by release of cfDNA from the noncardiac graft
Steroid dose equivalent to more than 20 mg prednisone/day, or within 21 d of a steroid pulse	Recipients of bone marrow or stem cell transplant, caused by release of cfDNA from the bone marrow/stem cell donor
Low GEP score (eg, <10) likely reflects specimen processing error during centrifugation, whereas higher values may be normal in patients who are many years post-transplant caused by less immunosuppression	Pregnancy, caused by release of fetal cfDNA
The role of GEP in HT recipients with HIV or undergoing chemotherapy is not yet understood	Sepsis, systemic inflammation, HIV, or cancer: potential for falsely reduced dd-cfDNA, caused by increased cfDNA release from extracardiac locations or tumor cells

Abbreviations as in Table 1.

TABLE 4 Performance of GEP and dd-cfDNA Testing

Test Value	NPV	PPV	Sensitivity	Specificity
GEP, 0-6 mo post-HT[11]				
30	98.4	2.8	50	64.3
32	98.1	2.0	25	74.8
34	98.1	2.1	15	85.5
GEP, >6 mo post-HT[11]				
34	98.5	2.8	51	63.4
36	98.4	4.0	34.9	82.7
38	98.1	4.2	9.3	95.6
dd-cfDNA, >14 d post-HT[13]				
0.15%	97.4	7.8	55.9	71.5
0.20%	97.1	8.9	44	80
0.25%	96.7	8.8	32.4	85.5

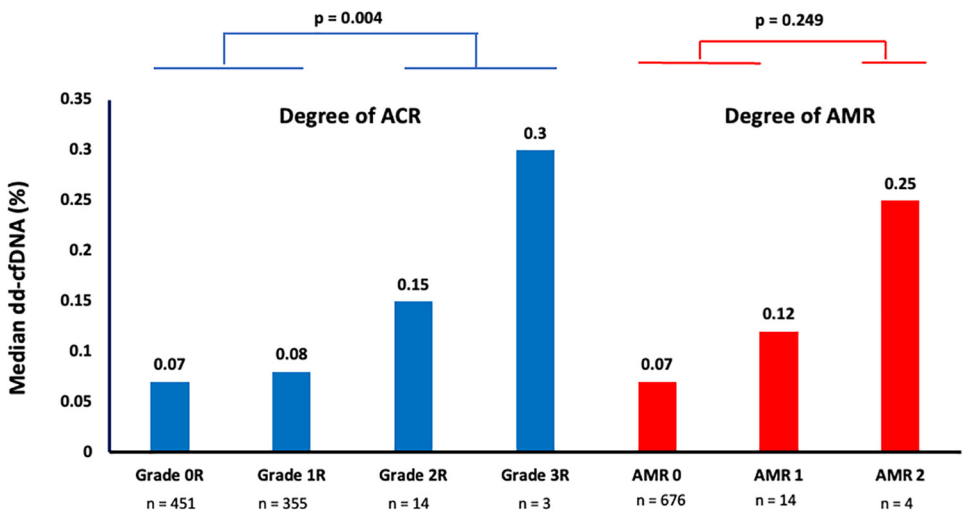
Values are n (%).
Abbreviations as in Table 1.

area under the curve receiver-operating characteristics (AUC-ROC) for grade ≥3A (2R) rejection of 0.69 in CARGO II (Table 1).[10]

Many transplant programs have since adopted the same GEP thresholds to prompt an EMB as used in IMAGE and eIMAGE (Early Invasive Monitoring Attenuation Through Gene Expression): ≥30 during 2 to 6 months post-HT and ≥34 after 6 months (Table 4, Figure 1). GEP variability is also used by some programs, with the concept that greater within-patient variability of consecutive GEP scores may predict adverse clinical events. When defined as the SD of 4 GEP scores collected ≥315 days post-HT in CARGO II, the GEP variability AUC-ROC for composite events was 0.72 (95% CI: 0.6-0.8), with NPV for a variability score of 0.6 being 97% (95% CI: 91.4%-100.0%).[31] Despite GEP scores or GEP variability showing excellent NPV, the PPV is low due to the low prevalence of ACR. Therefore, GEP is an appropriate tool for a rule-out approach, particularly in asymptomatic patients at low risk for rejection, whereas EMB remains appropriate when a rule-in strategy is needed, including when signs, symptoms, or imaging concerning for rejection are present.

Similarly, thresholds of abnormality for dd-cfDNA are evolving and differ between programs depending upon patient risk for rejection, clinician comfort, and the desire to maximize either NPV or PPV. The median dd-cfDNA in D-OAR was 0.07% (IQR: 0.03%-0.14%, 2,164 samples) and a 0.2% threshold dd-cfDNA had a 44% sensitivity to detect rejection with 97% NPV (Table 1).[6] Median dd-cfDNA levels were 0.17% for both ACR (grade ≥2R) or AMR (grade ≥1) among combined surveillance and for-cause EMBs (Figure 2). In a parallel cohort incorporating patients at higher risk for AMR (panel reactive antibodies >10%,

FIGURE 2 Median dd-cfDNA Levels Associated With Either Surveillance or For-Cause Biopsies in D-OAR, Displayed by Rejection Type and Grade



Blue bars illustrate the grades of acute cellular rejection (ACR), and **red bars** indicate the grades of AMR in the main D-OAR registry cohort. Presence of ACR is defined as grade 2R and above, whereas presence of AMR is defined as grade 1 and above, and includes instances of mixed rejection. *P* values for comparison are: ACR grade 0 or 1R vs grade 2R or 3R = 0.004; AMR grade 0 vs grade 1 or 2 = 0.249. Figure adapted with permission from Khush et al.[6] Abbreviations as in Figure 1.

presence of DSAs, EMB-defined AMR, or EMB due to reduced ejection fraction), dd-cfDNA baseline levels were higher than in reference HT patients, likely reflecting low-level allograft damage from immune activation.[6] Patients with pathologic AMR 1 or 2 had higher median dd-cfDNA levels (0.50%) than those with pathologic AMR 0 (0.16%; $P = 0.004$).

The recently published DEDUCE study validated the newly clinically available Prospera dd-cfDNA test in 223 patients with 49 cases of biopsy-defined rejection. Using a dd-cfDNA threshold of ≥0.15%, sensitivity for ACR was 78.5% and NPV 97.3%.[14] In the GRAfT study, 168 of 923 total dd-cfDNA samples detected allograft injury as defined by dd-cfDNA ≥0.25%. However, more than three-quarters of allograft injury cases identified by dd-cfdNA testing were associated with a negative EMB (n = 135) with the majority of these "false-negative" EMBs being clinically relevant: 20.7% had concurrent allograft dysfunction (n = 28), 27.4% represented a dd-cfDNA rise preceding acute rejection (n = 46), and 16.7% preceded allograft dysfunction (n = 28) (**Table 1**).[13] Thus, the sensitivity of dd-cfDNA for early detection of rejection events has challenged the gold-standard status of the EMB.

Therefore, although absolute cutoffs of GEP and dd-cfDNA are routinely defined within programs to guide practice, it is important to individualize care to include the patients' risk profile, overall clinical picture, and trajectory of GEP and dd-cfDNA results over time. Patients with persistently low GEP scores and dd-cfDNA levels could be considered as lower risk for rejection and may be appropriate candidates for minimization of immunosuppression. However, there are no prospective data to indicate the safety or effectiveness in preventing malignancy and infection by using this approach. A suggested schema for interpreting and responding to GEP and dd-cfDNA results is presented in the **Central Illustration**.

4. HOW SHOULD A PROGRAM DECIDE POST-HT TIME POINTS FOR GEP AND DD-cfDNA MONITORING IN A SURVEILLANCE PROTOCOL?

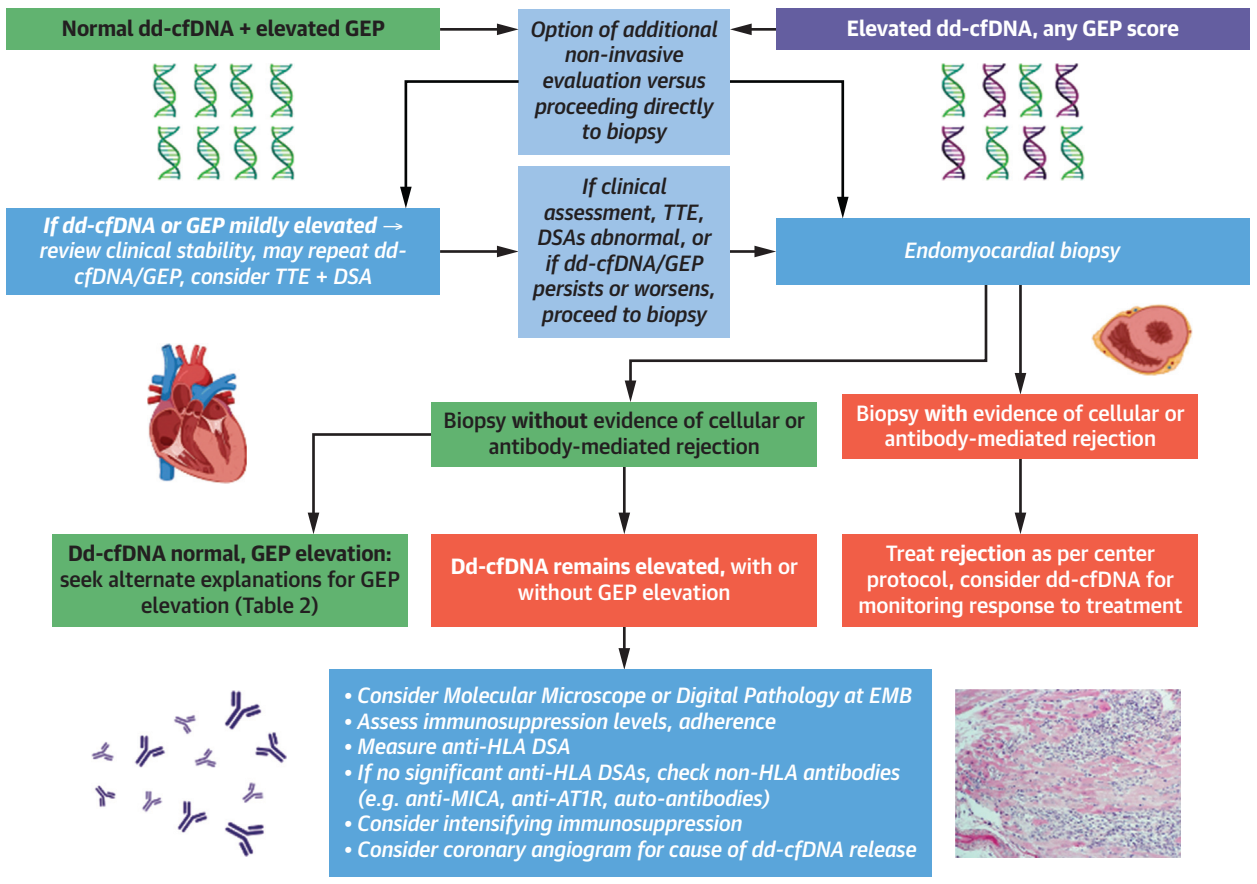
The performance of the GEP score can be affected by the administration of corticosteroid therapy, due to effects on expression of steroid-responsive genes, and thus the earliest opportunity for GEP use is 55 days post-HT so long as the prednisone dose is <20 mg/d. Stability of the dd-cfDNA assay has been demonstrated starting at approximately 14 days post-HT in D-OAR and GRAfT, and some centers start dd-cfDNA surveillance as early as 3 to 4 weeks post-transplantation. Centers utilizing the HeartCare combined GEP and dd-cfDNA test generally initiate testing after 55 days. Some centers pair the first GEP/dd-cfDNA with a routine EMB on the same day, and individual centers vary widely in their frequency of EMB vs noninvasive testing. The number of EMBs remaining within the first-year surveillance protocol depend upon factors such as the perceived rejection risk for individual patients and the overall population, the level of clinician comfort with noninvasive testing, and available testing resources (**Figure 3**).

In the setting of the COVID-19 pandemic, many programs faced limitations in performing surveillance EMBs and modified their protocols to begin dd-cfDNA testing as early as 14 days post-HT. In some cases, this has accelerated the transition away from a traditional EMB-based surveillance protocol toward a less-invasive approach. Centers with the greatest experience in noninvasive post-HT rejection surveillance may fully transition patients with an acceptable baseline risk of rejection over to a GEP/dd-cfDNA surveillance rejection as soon as 8 weeks post-HT and reserve EMB only for patients with abnormal noninvasive testing, concerning signs/symptoms or imaging evidence of allograft dysfunction.

In addition to being a highly sensitive marker of early allograft injury,[6] circulating dd-cfDNA has a very short half-life of 30 minutes to several hours,[32] and can therefore be repeated within days in the appropriate clinical context for rejection monitoring.

Less is known about the stability of GEP and dd-cfDNA values after 2 years post-HT, although the SHORE registry (Surveillance HeartCare Outcomes Registry) is following patients out to 5 years and may be informative in this regard. It is ordinarily anticipated that the GEP score is higher in years 2 to 5 than it would be in the first 6 months post-HT, due to the lower intensity of immunosuppression further from the time of transplantation. There is currently no evidence base supporting the use of noninvasive surveillance tests for HT recipients beyond 5 years after transplantation, although some centers do use dd-cfDNA for detection of allograft injury in patients beyond 5 years post-HT on a case-by-case basis when signs, symptoms, or imaging raise concerns, even though the normal range in this time frame is undefined. For patients who are clinically stable without a planned change in immunosuppression, there is currently no role for routine rejection surveillance beyond 5 years post-HT.

CENTRAL ILLUSTRATION **Flowchart Proposing Interpretation of Noninvasive Surveillance With a Combination of Gene Expression Profiling and dd-cfDNA**



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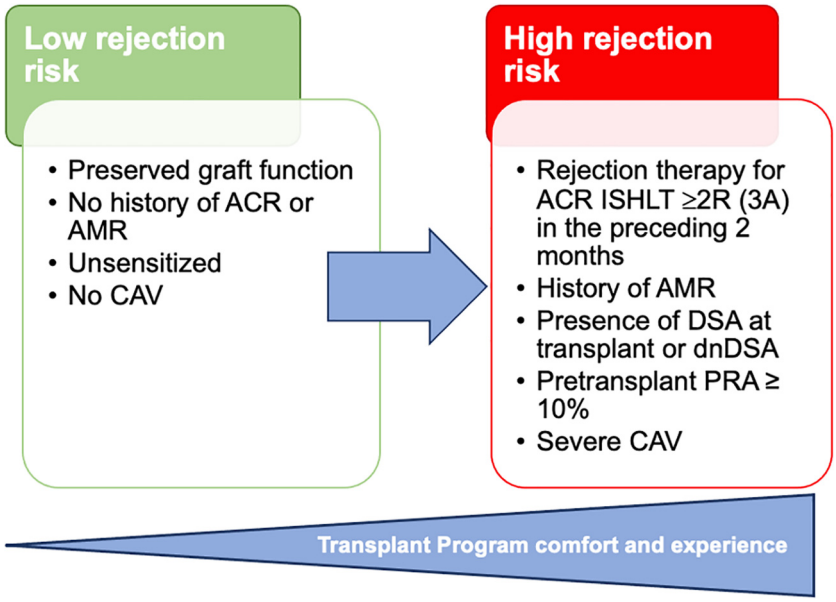
anti-AT1R = anti-angiotensin 1 receptor; anti-MICA = anti-major histocompatibility complex class I chain-related A; dd-cfDNA = donor-derived cell-free DNA; DSA = donor specific antibodies; EMB = endomyocardial biopsy; GEP = gene expression profiling; HLA = human leukocyte antigen; TTE = transthoracic echocardiogram. Created with BioRender.com.

5. DOES CARDIAC IMAGING NEED TO BE PERFORMED WITH EACH NON-BIOPSY SCREENING VISIT?

There is currently no consensus on whether cardiac imaging should be concurrently performed with each noninvasive rejection screening. In OAR, left ventricular ejection fraction (LVEF) did not add incremental value to the assessment of ACR. This may be explained by the fact that graft dysfunction is not only a reflection of hemodynamic ACR, but may also result from cardiac allograft vasculopathy (CAV), or primary graft dysfunction.[11] Furthermore, allograft dysfunction as a consequence of rejection is frequently a late manifestation, and the LVEF is a relatively insensitive measurement, given the influence of loading conditions.

In the GRAfT study, 81% of echocardiograms performed as part of clinical care had concurrent dd-cfDNA data. One-fifth of these echocardiograms showed allograft dysfunction, defined as a reduction of at least 5% in LVEF from the prior echocardiogram.[13] Corresponding dd-cfDNA levels correlated with severity of LV dysfunction. The dd-cfDNA assay is highly sensitive and may rise before a reduction in LVEF on echocardiography, although other imaging features such as ventricular wall thickening, new valvular dysfunction, and pericardial effusions may also add information about the presence of acute rejection. In IMAGE and eIMAGE, echocardiographic

FIGURE 3 Enrollment of Low vs High Rejection Risk Patients Based on Individual Program Comfort Level



dnDSA = de novo donor-specific antibodies; DSA = donor-specific antibodies; PRA = panel reactive antibodies; other abbreviations as in Figures 1 and 2.

assessments were done as part of standard of care without specific protocols.

6. HOW DOES THE TIME COURSE AND DEGREE OF DD-cfDNA ELEVATION DIFFER BETWEEN ACR AND AMR?

The current commercially available assays for dd-cfDNA measurement are not able to distinguish the type of rejection, nor are they able to differentiate rejection from other causes of allograft injury. In D-OAR, the prevalence of biopsy-defined rejection was low (4.2%; 35/841 samples) with no statistically significant difference in the median dd-cfDNA levels between ACR and AMR. Conversely, AMR and ACR displayed unique and dichotomous dd-cfDNA patterns in the GRAfT study, where AMR exhibited greater dd-cfDNA elevation than ACR at comparable histological grades,[13] and dd-cfDNA was also higher for AMR than ACR in DEDUCE (A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation).[14] AMR also differed from ACR in terms of the genomic composition of the DNA fragments, with AMR being associated with a greater percentage of short fragments and higher guanosine-cytosine content than ACR.[13] However, the GRAfT study used a research-grade assay, and it is uncertain whether future clinically available dd-cfDNA assays will be able to differentiate between acute rejection phenotypes.

Several studies have now demonstrated that dd-cfDNA elevation precedes the histopathological diagnosis of both AMR and ACR, as well as allograft dysfunction, by a median of 3 to 5 months.[13,14] Therefore, in clinical practice, dd-cfDNA elevation provides a unique opportunity for intercepting the injury process before the onset of overt EMB-defined rejection or graft dysfunction, which could be particularly beneficial for surveillance of high-risk patients. When dd-cfDNA is elevated in the setting of a negative biopsy, it should trigger careful monitoring of immunosuppression trough levels and medication adherence, assessment of allograft function and DSAs, and supplemental diagnostic techniques, as outlined in the **Central Illustration**.

Using dd-cfDNA to guide immunosuppression could be a future application, although evidence from prospective clinical studies is lacking. In a small retrospective analysis of 17 individuals from the CARGO II trial who underwent treatment for biopsy-confirmed ACR 2R or 3R, a trend was observed for dd-cfDNA decrease in response to therapy.[33]

Further studies are needed to refine our understanding of how each individual antirejection therapy impacts cfDNA levels. Cytolytic therapies such as ATG lead to recipient T- and B-cell lysis with concomitant

release of recipient DNA into the blood stream. This event could theoretically lead to dilution of the dd-cfDNA content, thus altering the detected donor/recipient ratio. Similarly, plasmapheresis can dialyze small DNA fragments. The impact of these therapies on the kinetics of dd-cfDNA needs to be established before routine use for monitoring the response to rejection treatment.

7. CAN DD-cfDNA BE USED TO RISK STRATIFY PATIENTS WITH DE NOVO DSA FORMATION?

Despite the development of de novo DSA (dnDSA) in approximately one-third of HT recipients, only 54% of these patients will develop EMB-detected AMR.[34] The management of dnDSAs in the presence of a bland EMB and stable graft function presents a treatment dilemma, although molecular profiling of the EMB sample may help to delineate the pathological role of a DSA.[35] It is likely that the immune system-mediated allograft injury process incurred by pathological DSA begins in the microvasculature as an indolent process in many patients, not detectable as overt AMR on EMB. This raises the question of whether dd-cfDNA can detect allograft injury from pathological DSAs and could thereby guide treatment decisions.

Two recent small retrospective studies demonstrated that rising dd-cfDNA levels precede and predict the development of dnDSAs, and could identify associated graft injury.[18,19] In an analysis of 613 samples from the SHORE registry with available DSA levels and negative paired EMB, dd-cfDNA levels >0.15% were associated with a 4-fold greater likelihood of subsequent dnDSA detection within the first post-HT year.[18] An additional analysis of 67 patients showed that patients with dnDSA had higher dd-cfDNA levels compared with those with preformed DSAs and those without DSAs.[19] There is no current consensus on whether a dd-cfDNA elevation in the presence of dnDSAs and normal allograft function should trigger treatment, or what level of dd-cfDNA should be acted upon. Therapeutic decisions are complicated by the lack of data indicating improved outcomes after dnDSA treatment. It is also unclear whether dd-cfDNA helps to identify allograft injury associated with non-human leukocyte antigen (HLA) antibodies.

8. WHAT CAN DD-cfDNA TELL US ABOUT GRAFT HEALTH BEYOND THE PRESENCE OF REJECTION?

dd-cfDNA reflects any form of allograft injury and is not specific to rejection. Thus, dd-cfDNA has also been applied in graft health in a broader sense. Both the lack of a decay in dd-cfDNA levels early post-transplantation as well as elevated levels over time have been associated with adverse outcomes in preliminary studies, including mortality, retransplantation, and graft dysfunction.[36-38]

Two small single-center pilot studies raised the possibility that dd-cfDNA is elevated in CAV, possibly reflecting endothelial injury and ischemia.[39,40] The association between CAV and higher dd-cfDNA in patients without rejection was also observed in DEDUCE.[14] However, 1 study from Spain using different dd-cfDNA detection methods and thresholds did not confirm the association between dd-cfDNA and CAV, questioning its potential role as a CAV biomarker.[41] These divergent findings underscore the need for larger prospective studies to define the role of dd-cfDNA in screening for cardiac allograft dysfunction that is not caused by acute rejection.

In the case of elevated dd-cfDNA levels in the absence of detected rejection, lack of DSA, and normal graft function, most centers will choose to monitor patients more closely and assure therapeutic and optimized immunosuppression levels. There is currently no clear evidence for titration of immunosuppression in this clinical scenario; however a few very experienced centers might augment immunosuppression intensity, either temporarily or long term, in response to elevated dd-cfDNA or lack of decay. However, it is currently unknown whether the relationship between elevated dd-cfDNA and clinical events in the absence of rejection is because dd-cfDNA is a biomarker of other pathological processes, or whether the EMB lacks sensitivity for detection of clinically consequential rejection. The upcoming clinical trial MOSAIC (HeartCare Immunooptimization in Cardiac Allografts; NCT05459181) does not address the question of intensifying immunosuppression based on dd-cfDNA levels but rather is anticipated to determine whether dd-cfDNA guidance can optimize weaning of immunosuppression intensity and improve subsequent clinical outcomes. Future studies are needed to answer the question of whether dd-cfDNA elevation with and without dnDSA formation should trigger intensification of immunosuppression.

9. WHAT LOGISTICAL CHALLENGES MAY TRANSPLANT PROGRAMS ENCOUNTER WHEN ADOPTING GEP/ dd-cfDNA SCREENING?

The transition from a traditional biopsy-based approach to a less-invasive screening protocol is a significant undertaking that requires in-depth understanding of an individual program's culture,

patient population, and center-specific resources. An important first step is education of all team members on the use of GEP and dd-cfDNA monitoring and interpretation. Several vendor-specific details on phlebotomy timing, and sample collection and processing, are important to consider and may influence decisions on the choice of testing platform. All dd-cfDNA samples need to be collected before EMB, or >24 to 48 hours afterwards, to avoid false elevations from EMB-induced myocardial trauma. The GEP assay must be processed in a specialized laboratory within 3 hours of sample collection and cannot be transported via the hospital tubing system. **Table 2** summarizes assay availability and practical considerations for using the AlloMap GEP and various commercially available dd-cfDNA assays. In the United States, GEP/dd-cfDNA is currently only reimbursable for outpatient use, but options for compassionate use in inpatients are available. The cost-effectiveness of noninvasive testing as compared with traditional biopsy schedules has not yet been analyzed and will be difficult to study, given differences per country, type of insurance, and reimbursement contracts.

10. WHICH HEART TRANSPLANT RECIPIENTS MAY BE APPROPRIATE FOR A NONINVASIVE SURVEILLANCE PROTOCOL?

Most data and clinical experience with noninvasive rejection screening exists in low rejection risk cohorts. Currently, the definition of low risk remains largely based on the initial GEP studies that excluded patients with graft dysfunction, severe CAV, rejection therapy for ISHLT ≥2R ACR in the preceding 2 months, history of AMR, presence of DSA, prednisone doses of >20 mg/day, hematopoietic growth factors or blood transfusion during the previous 30 days, or pregnancy.[8,9] However, the evolution of dd-cfDNA assays that are highly sensitive for early detection of graft injury may potentially expand the pool of transplant patients for noninvasive surveillance.

Thus, with the combination of GEP/dd-cfDNA assays, transplant programs could conceivably perform noninvasive surveillance for every stable transplant patient, except for patients with multiple sources of nonrecipient cfDNA, such as multiorgan transplant or bone marrow recipients, and pregnant patients. An individual program's comfort level with patient selection and the institutional definition of high risk will vary. Programs could choose to commence their noninvasive screening protocols with lower-risk patients, and subsequently incorporate higher-risk patients as their experience and comfort level grows (**Figure 3**). Regardless of an individual patient's risk, it is important to note that the GEP and dd-cfDNA assays are intended for use as screening tests in stable transplant recipients.[8,9] Patients presenting with signs or symptoms concerning for rejection, and/or evidence of graft dysfunction, should still receive a for-cause EMB, regardless of participation in a noninvasive screening protocol.

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, use of the GEP platform to assess immunologic quiescence in combination with the high NPV of dd-cfDNA to detect allograft injury offers a robust strategy for surveillance of ACR and AMR post-HT. Center-specific screening protocols vary widely depending upon the rejection risk of their HT recipient population, the level of comfort with noninvasive screening, and the availability and cost of invasive and noninvasive testing resources. It is anticipated that these evolving screening tools will offer clinicians and patients the opportunity to achieve more personalized medical care post-HT, with lower rates of procedure-related complications and greater patient comfort and satisfaction.

Meanwhile, the for-cause EMB maintains an important role in post-HT practice, especially with novel adjunctive techniques that refine histopathological diagnoses, including intragraft mRNA transcript profiling and digital pathology. Myocardial mRNA transcription analysis, referred to as the "molecular microscope," appears to facilitate differentiation between quiescence, cellular rejection, antibody rejection, and graft injury (eg, caused by ischemia), which can be useful to resolve the cause of elevated dd-cfDNA and/or graft dysfunction when the initial EMB is unrevealing (**Central Illustration**).[35,42] Digital pathology aims to overcome the high interobserver variability of standard EMB histopathological interpretation by using computational histological analysis as an approach to standardize feature extraction.[43] These augmented techniques for the interpretation of EMB samples also offer research opportunities for studying associations between dd-cfDNA and EMB results and may clarify the pathophysiology in scenarios where dd-cfDNA is persistently elevated without a concurrent histopathological diagnosis of rejection.

After introducing a less-invasive HT surveillance protocol, centers should monitor and update their protocols based upon center-specific outcomes,

evolving literature, and society guidance. To fully inform future practice and society guidelines, the next research steps could include a prospective randomized trial comparing post-HT outcomes with traditional EMB vs noninvasive surveillance strategies (DETECT; NCT05081739, is anticipated), as well as investigating the added diagnostic value of dd-cfDNA fragment composition/length. The clinical utility of dd-cfDNA fragment length for discerning AMR from ACR is incompletely understood, and circulating micro-RNAs have also recently been shown to effectively distinguish between AMR and ACR.[44] There may be scope for these assays to guide minimization of immunosuppression and prevent longer-term development of malignancy or infection events. It could be of value to develop a multimodal score that utilizes several noninvasive surveillance techniques, such as dd-cfDNA, GEP, immune cell function assay (ImmuKnow), micro-RNAs, and imaging metrics, to optimize event prediction. Future strategies that allow dd-cfDNA to be used in the setting of multiorgan transplantation are also of strong clinical relevance.

Table 5 provides a summary of these key points on noninvasive monitoring and future directions. In conclusion, the evolving field of noninvasive HT allograft surveillance offers tangible opportunities to improve detection and timely treatment of acute rejection, personalization of immunosuppressive therapy, and longer-term improvements in HT outcomes and quality of life.

TABLE 5 Summary Key Points

1. The AlloSure (CareDx) assay is a commercially available dd-cfDNA assay that has been tested and validated for acute rejection surveillance in multicenter studies. The performance of the Prospera (Natera) assay was recently reported in a 2-center study.
2. Using a threshold value of 0.20%, AlloSure has >97% NPV for rejection diagnosis, with modest sensitivity and PPV. Hence, the best clinical application of dd-cfDNA is to rule out acute rejection.
3. Based on large multicenter studies, dd-cfDNA assays can be used as early as 14 days post-HT and have been shown to maintain stability up to 2 years post-HT. GEP is not approved for use until 55 days post-HT. Future studies are needed to define the utility of these tests for the assessment of allograft health beyond 2 years post-HT.
4. dd-cfDNA levels are not thought to be affected by corticosteroid dose and inflammation, but can be affected by other systemic processes, as described in Table 3.
5. The low sensitivity and PPV of the dd-cfDNA assays for rejection diagnosis are likely reflective of the tests' ability to capture allograft injury in general, even in very early stages: dd-cfDNA elevation can precede pathological rejection diagnosis by 3 months.
6. When dd-cfDNA levels are elevated, pathological confirmation via endomyocardial biopsy can also determine the type of graft injury/acute rejection that is present, which will then guide treatment strategies.
7. In the event of dd-cfDNA elevation with a paired EMB that is negative for rejection, refer to the Central Illustration for possible reasons and next steps in patient evaluation.
8. Preliminary studies have shown elevated dd-cfDNA levels concurrent with development of de novo DSAs after transplant, even in the absence of biopsy-defined antibody mediated rejection.
9. Future studies are needed to elucidate the complementary role of other noninvasive biomarkers. Those include gene expression profiling (AlloMap), immune monitoring assays (Immune Cell Function), intragraft mRNA transcripts (Molecular Microscope, Nanostring), digital pathology, exosomes, and microRNAs.

dd-cfDNA = donor-derived cell-free DNA; DSA = donor-specific antibody; EMB = endomyocardial biopsy; GEP = gene expression profiling; HT = heart transplantation; NPV = negative predictive value; PPV = positive predictive value.

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Dr Holzhauser has been a speaker for CareDx. Dr Byku has been a consultant for Abbott. Dr De Marco has received grant support for the SHORE study from CareDx; and has been a consultant for Natera. Dr Hall has been a consultant/advisor for CareDx, Natera, Abbott, Abiomed, and Medtronic. Dr Khush has been a scientific advisor, speaker, consultant, research grant recipient for CareDx. Dr Vest has been the site principal investigator for the SHORE study for CareDx. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Amanda Vest, Tufts Medical Center, 800 Washington Street, Boston, Massachusetts 02111, USA. E-mail: avest@tuftsmedicalcenter.org.

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