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A modern heart transplant rejection surveillance protocol utilizing cell-free DNA: A single-center experience

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BACKGROUND: Endomyocardial biopsy (EMBx) is considered the gold standard for rejection monitoring after heart transplantation; however, it is invasive and histologic interpretation has limitations. Sensitive blood biomarkers, including donor-derived cell-free DNA (dd-cfDNA), have emerged to decrease EMBx frequency.

METHODS: We retrospectively reviewed data on 237 patients who underwent heart transplantation at our institution. Of these, 125 patients underwent monitoring using dd-cfDNA, combined with a fewer number of EMBx, and 112 patients underwent monitoring using EMBx only. We compared rates of rejection, graft dysfunction, and survival at 1 year.

RESULTS: Median age at time of transplant was 59.8 years, and 77.6% were men. In the dd-cfDNA group, there were significantly fewer episodes of EMBx defined acute cellular rejection (ACR) (2.5% vs 18.8%, $p < 0.001$) and treated ACR (4.2% vs 19.6%, $p = 0.001$). Comparatively, there were more EMBx defined antibody-mediated rejection (AMR) (5% vs 0.9%) and treated AMR (5% vs 2.7%) in the dd-cfDNA group. No significant differences were observed in graft dysfunction, presence of donor-specific antibodies, or survival at 1 year.

CONCLUSIONS: In conclusion, a modern rejection surveillance protocol utilizing noninvasive testing is safe, led to significantly fewer EMBx, fewer treated rejection episodes, and no difference in survival at 1 year. More AMR episodes identified via dd-cfDNA could lead the way for more accurate diagnostic and treatment decisions.

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Background

Endomyocardial biopsy (EMBx) is considered the gold standard for post orthotopic heart transplant (OHT) rejection surveillance since the 1970s.[1] The inherent limitations include the invasive nature of the procedure, patient discomfort, interobserver variability in histologic interpretation, and variable sensitivity for detection of antibody-

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mediated rejection (AMR).[2] Noninvasive surveillance strategies using blood biomarkers, including gene expression profile (GEP) and quantification of donor-derived cell-free DNA (dd-cfDNA), have emerged as complimentary tests in low-risk population with the purpose of decreasing EMBx frequency post OHT.

Cell-free DNA particles are constantly released from the body during normal cell turnover. The detection of the donor-derived portion (dd-cfDNA) can be achieved contemporarily using next-generation sequencing utilizing single-nucleotide variation technology without the need for both donor and recipient genotyping. Increased dd-cfDNA with a threshold of 0.2% was found to be a sensitive biomarker for both acute cellular rejection (ACR) and AMR, with a negative predictive value of 97% in D-OAR trial[3] (Utility of Donor-Derived Cell Free DNA in Association with Gene expression Profiling). Further data from the GRAfT study (Genomic Research Alliance for Transplantation) demonstrated that dd-cfDNA can detect early graft injury 3.2 months before EMBx detected AMR.[4]

Meanwhile, GEP quantifies the expression of 11 genes in peripheral blood associated with immune activation using quantitative polymerase chain reaction, microarray, or RNA sequencing. These genes are involved in ACR and can be quantified with a score between 0 and 40. The evidence behind GEP is derived from landmark studies, including CARGO (Cardiac Allograft Rejection Gene Expression Observational)[5] and IMAGE (Invasive Monitoring Attenuation Through Gene Expression),[6] giving it a class IIa recommendation in the 2022 International Society for Heart & Lung Transplantation (ISHLT) guidelines.[7] Since GEP was not developed to detect AMR, it is often complemented by dd-cfDNA quantification, which can reflect graft injury as a marker of AMR.

There exists substantial variability in the interpretation of abnormal tests across transplant programs, with different cut-offs for considering an abnormal test, and variable thresholds to trigger pursuing an EMBx to confirm and define a possible rejection. Furthermore, the response to an abnormal GEP and a normal dd-cfDNA is not standardized. In this study, we evaluate a contemporary protocol for graft rejection monitoring utilizing dd-cfDNA with a smaller number of EMBx, compared to conventional strategy using EMBx alone, with focus on outcomes including incidence of rejection and survival (graphical abstract).

Methods

This study was approved by the institutional review board at Baylor Scott and White Health, and is in compliance with the ISHLT ethical standards.

Patients

We identified 237 consecutive patients who underwent OHT at our institution and their data were retrospectively reviewed. Of these, 125 patients transplanted between 2019 and 2021 underwent graft monitoring via our institution protocol, utilizing dd-cfDNA with a fewer number of EMBx (total of 3 biopsies in the first year, and 4 for dual-organ transplants) (noninvasive group). Comparatively, 112 patients transplanted between 2017 and 2018 underwent conventional monitoring using EMBx only for total of 8 biopsies in the first year (EMBx group). These cut-off dates correlated with out institutional graft-monitoring policy change. Demographic data were collected using the electronic medical records system and included baseline characteristics, cytomegalovirus (CMV) status of both donor and recipient, virtual and retrospective crossmatch results, pretransplant human leukocyte antigens, and calculated panel reactive antibody (CPRA).

We compared outcomes between both groups including survival at 1 year, incidence of ACR (grade ≥ 2R) or AMR (pAMR ≥ 0), and graft dysfunction on surveillance echocardiograms (considered as left ventricular ejection fraction (LVEF) < 45%). The rejection outcomes were independently confirmed by authors A.S. and S.M. from the electronic medical record system using pathology report and review of slides as appropriate.

A subset analysis of these outcomes was then conducted between dd-cfDNA group patients at different cut-off values detailed below. For the purpose of creating a more homogenous sample, dual-organ transplant recipients were excluded from this subanalysis.

Surveillance protocol

In our institution, we monitor dd-cfDNA levels monthly during the first year. We utilize the HeartCare panel (CareDx, Inc.) which combines GEP testing (Allomap) and dd-cfDNA (Allosure) for surveillance of asymptomatic patients; however, GEP testing generally played little role in decision making. For dd-cfDNA testing, patients need to be at least 14 days post OHT, and testing is done prior to EMBx to avoid procedural induced elevation of values. EMBx are done at intervals of 2 weeks, 6 weeks, and 12 months. For patients receiving dual-organ transplant, an additional EMB is done at 6 months. Comparatively in the EMBx group, patients underwent EMBx at 2, 4, 6, and 8 weeks and at 3, 6, 9, and 12 months for a total of 8 biopsies within the first year.

We stratify patients based on dd-cfDNA results into low risk (< 0.2%), intermediate risk (0.2%-0.99%), and high risk (≥1%). These cut-offs were based on internal review of simultaneous HeartCare and usual EMBx testing over the period of 4 months, and identifying these 3 clusters of concordance. Results interpretation is summarized in Figure 1:

1) If dd-cfDNA result is low, no further workup is usually pursued.
2) If dd-cfDNA is intermediate, testing is usually repeated after 2 weeks. EMBx and further investigations maybe pursued based on clinical picture.
3) If dd-cfDNA is elevated, for the first time, then further investigations, including EMBx, human leukocyte

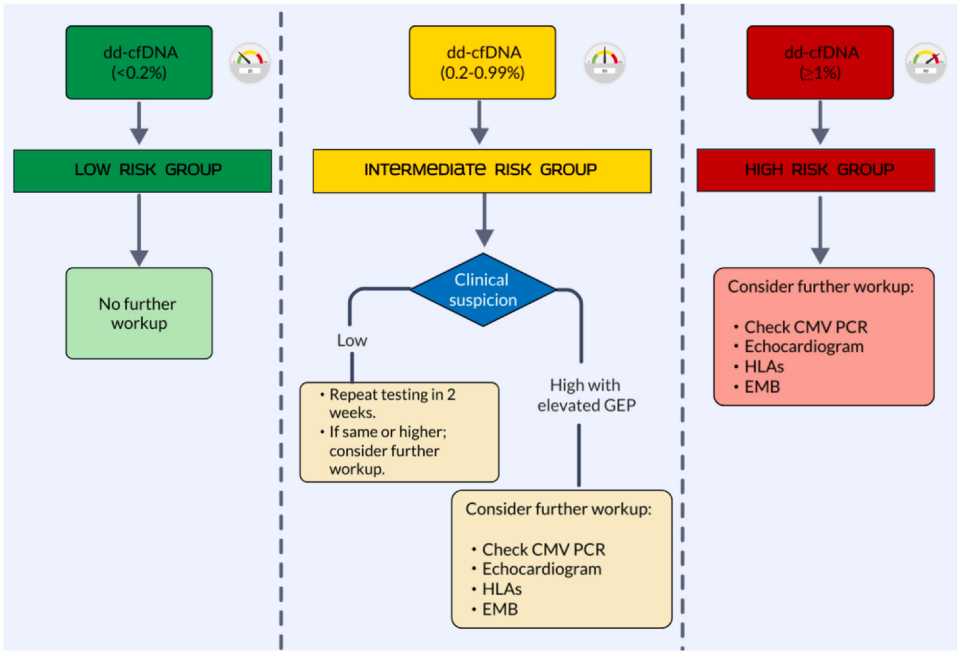

Figure 1 Rejection surveillance protocol using dd-cfDNA. CMV, cytomegalovirus; EMB, endomyocardial biopsy; dd-cfDNA, donor-derived cell-free DNA; GEP, gene expression profiling; HLA, human leukocyte antigens; PCR, polymerase chain reaction.

antigen testing, and CMV polymerase chain reaction, are usually done.

Of note, there were 5 patients in the noninvasive group who expired within 30 days and did not undergo dd-cfDNA testing. The cause of death in all of them was not rejection related. These patients were included in the analysis.

Statistical analysis

Statistical analysis was performed using R version 4.2 (R Foundation for Statistical Computing[8]). Continuous variables are presented as means ± standard deviations or medians [quartile 1, quartile 3], if skewed. Categorical variables are reported as frequencies and percentages. Patient characteristics and outcomes were compared between the EMBx and dd-cfDNA cohorts using 2 sample *t*-tests and Chi-square tests (or Wilcoxon's Rank Sum test and Fisher's Exact test), as appropriate. Time-to-event outcomes, including survival, freedom from graft dysfunction, and the composite outcome of survival free from graft dysfunction, were evaluated using Kaplan-Meier analysis with log rank tests. Subset analysis of the patients in the dd-cfDNA group at different cut-offs of dd-cfDNA was performed. Outcomes were compared using Chi-square or Fisher's test and Kaplan-Meier analysis with log rank tests was conducted to evaluate difference in survival and graft dysfunction. Fisher's test was used to correlate CMV viremia with elevated dd-cfDNA results, and a logistic regression model was used to calculate odds ratios. All *p*-values were reported as 2-sided tests, with $p < 0.05$ considered statistically significant.

Results

Patient characteristics

Median age at the time of transplant was 59.9 years, and 77.6% of patients were men. There were more African American patients in the dd-cfDNA group compared to the EMBx group (29.6% vs 18.8%, $p = 0.045$), as well as more dual-organ transplants (heart and kidney (12.8% vs 5.4%, $p = 0.07$). There was no statistically significant difference in CMV status between both groups. Baseline characteristics are listed in Table 1.

With regards to immunological profile prior to transplant, there was no difference in CPRA between both groups (9% in dd-cfDNA group vs 11.5% in EMBx group, $p = 0.29$). More patients in the dd-cfDNA group had a positive virtual crossmatch (20% vs 12.5%, $p = 0.02$); however, there was no significant difference on retrospective crossmatch post OHT. Subsequently more patients received induction with antithymocyte globulin in the dd-cfDNA group (10.4% vs 2.7%, $p < 0.001$) (Table 2).

Implementation of dd-cfDNA testing

A total of 1,509 dd-cfDNA tests were recorded, and the median number of observations per patient was 13 (quartiles 11-15). The number of patients by highest observed dd-cfDNA value was 48 patients in the low group (40%), 56 patients in the intermediate group (47%), and 16 patients in the high group (13%).

Table 1 Baseline Characteristics

Patient characteristics	Overall ($n = 237$)	EMBx ($n = 112$)	dd-cfDNA ($n = 125$)	p-value
Age at OHT (years)	59.9 [53.6, 65.5]	60.5 [53.6, 65.3]	59.9 [53.7, 65.9]	0.77
Sex, male	184 (77.6%)	85 (75.9%)	99 (79.2%)	0.65
Race				**0.045***
American Indian or Alaska Native	2 (0.8%)	0 (0%)	2 (1.6%)	
Asian	5 (2.1%)	4 (3.6%)	1 (0.8%)	
Black or African American	58 (24.5%)	21 (18.8%)	37 (29.6%)	
Native Hawaiian or Other Pacific Islander	1 (0.4%)	0 (0%)	1 (0.8%)	
White	171 (72.2%)	87 (77.7%)	84 (67.2%)	
Hispanic/Latino ethnicity	20 (8.4%)	7 (6.2%)	13 (10.4%)	0.36
HF etiology				0.80
ICM	92 (39.3%)	45 (41.3%)	47 (37.6%)	
Mixed	2 (0.9%)	1 (0.9%)	1 (0.8%)	
NICM	140 (59.8%)	63 (57.8%)	77 (61.6%)	
LVEF pretransplant	15 [10, 25]	15 [10, 20]	18 [10, 25]	0.06
Diabetes mellitus	108 (45.6%)	55 (49.1%)	53 (42.4%)	0.37
Hypertension	145 (61.2%)	53 (47.3%)	92 (73.6%)	**< 0.001***
Pretransplant CKD				0.27
1	52 (22%)	23 (20.7%)	29 (23.2%)	
2	82 (34.7%)	42 (37.8%)	40 (32%)	
3A	55 (23.3%)	27 (24.3%)	28 (22.4%)	
3B	26 (11%)	11 (9.9%)	15 (12%)	
4	7 (3%)	5 (4.5%)	2 (1.6%)	
5	14 (5.9%)	3 (2.7%)	11 (8.8%)	
CMV status				0.80
D-/R-	25 (10.5%)	10 (8.9%)	15 (12%)	
D-/R+	39 (16.5%)	17 (15.2%)	22 (17.6%)	
D+/R-	48 (20.3%)	24 (21.4%)	24 (19.2%)	
D+/R+	125 (52.7%)	61 (54.5%)	64 (51.2%)	
Dual organ				0.07
Heart/kidney	22 (9.3%)	6 (5.4%)	16 (12.8%)	
Heart/liver	1 (0.4%)	0 (0%)	1 (0.8%)	
Heart/kidney/liver	1 (0.4%)	0 (0%)	1 (0.8%)	
No	213 (89.9%)	106 (94.6%)	107 (85.6%)	

Abbreviations: CKD, chronic kidney disease; CMV, cytomegalovirus; dd-cfDNA, donor-derived cell-free DNA; EMBx, endomyocardial biopsy; HF, heart failure; ICM, ischemic cardiomyopathy; LVEF, left ventricular ejection fraction; NICM, nonischemic cardiomyopathy; OHT, orthotopic heart transplant.
*Statistically significant p-value ($p < 0.05$).

Rejection outcomes

Overall, there were 27 patients (11.4%) who were treated for ACR, of which 24 (10.1%) had biopsy-proven ACR ≥ 2R by ISHLT criteria. Comparatively, there were 9 patients (3.8%) who were treated for AMR and 7 (3%) of them were biopsy supported. Comparing the dd-cfDNA group to the EMBx group, there were significantly fewer incidences of EMBx supported ACR (2.4% vs 18.8%, $p < 0.001$), as well as treated ACR (4% vs 19.6%, $p = 0.001$). Also noted were more EMBx supported AMR (4.8% vs 0.9%) and treated AMR (4.8% vs 2.7%), however these did not reach statistical significance. There was no difference in the presence of positive donor-specific antibodies (DSAs) with high mean fluorescence intensity (MFI) greater than 4,000 or positive C1Q between both groups. These results are summarized in Table 3.

Subsequent analysis between risk groups in the dd-cfDNA cohort was conducted comparing low (< 0.2%), intermediate (0.2%-0.99%), and high-risk (≥1%) groups. For the purpose of this analysis, dual-organ transplants were excluded. Biopsy-proven AMR occurred in 4/103 patients (4%) (2 high, 2 intermediate, and 0 in the low category, $p = 0.03$). AMR was treated in 4/103 patients (4%) of whom 3/4 were in the "high" category and 1/4 in the low category ($p = 0.008$). There was no EMBx-proven ACR, nor treated ACR in the "low" group. The percentage of DSAs with positive C1q was highest in the "high" group ($n = 5/14$, $p = 0.045$) (Table 4).

Two patients with biopsy-proven AMR in the intermediate risk group were not treated for AMR. One patient had improved DSAs on repeat studies and concerns for treating in the setting of CMV viremia. The second patient had stable low-level DSA and decrease in cfDNA to low risk on repeat testing. One patient in the high risk group with biopsy-proven AMR was not treated with formal AMR treatment given the patient was clinically doing well and team opted to optimize oral immunosuppression regimen. Among patients treated with AMR, there was only 1 patient in the low category who was treated, despite negative

Table 2 Immunological Characteristics.

Pretransplant immunology	Overall ($n = 237$)	EMBx ($n = 112$)	dd-cfDNA ($n = 125$)	p-value
Pre-OHT CPRA (%)	10 [0, 40]	11.5 [0, 38.5]	9 [0, 40]	0.29
RXCM				0.87
Negative	198 (83.5%)	95 (84.8%)	103 (82.4%)	
Prospective	7 (3.0%)	4 (3.6%)	3 (2.4%)	
T-/B+	14 (5.9%)	5 (4.5%)	9 (7.2%)	
T+/B-	6 (2.5%)	3 (2.7%)	3 (2.4%)	
T+/B+	12 (5.1%)	5 (4.5%)	7 (5.6%)	
VXCM				**0.02***
Negative	198 (83.5%)	98 (87.5%)	100 (80%)	
Positive - unknown breakdown	2 (0.8%)	2 (1.8%)	0 (0%)	
Prospective	3 (1.3%)	2 (1.8%)	1 (0.8%)	
T-/B+	23 (9.7%)	9 (8%)	14 (11.2%)	
T+/B+	11 (4.6%)	1 (0.9%)	10 (8%)	
Positive DSA on XM	58 (24.8%)	24 (22%)	34 (27.2%)	0.44
Induction				**< 0.001***
ATG	16 (6.8%)	3 (2.7%)	13 (10.4%)	
Basiliximab	34 (14.3%)	27 (24.1%)	7 (5.6%)	
None	187 (78.9%)	82 (73.2%)	105 (84%)	

Abbreviations: ATG, antithymocyte globulin; CPRA, calculated panel reactive antibody; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; EMBx, endomyocardial biopsy; OHT, orthotopic heart transplant; RXCM, retrospective crossmatch; VXCM, virtual crossmatch.
*Statistically significant p-value ($p < 0.05$).

Table 3 Outcomes.

Outcomes within 1 year	Overall ($n = 237$)	EMBx ($n = 112$)	dd-cfDNA ($n = 125$)	p-value
Rejection				
EMBx-proven ACR	24 (10.1%)	21 (18.8%)	3 (2.4%)	**< 0.001***
Treated ACR	27 (11.4%)	22 (19.6%)	5 (4%)	**< 0.001***
EMBx-proven AMR	7 (3%)	1 (0.9%)	6 (4.8%)	0.12
Treated AMR	9 (3.8%)	3 (2.7%)	6 (4.8%)	0.50
Positive DSA	87 (36.7%)	41 (36.6%)	46 (36.8%)	1.00
DSAs ≥ 4,000 MFI	37 (15.6%)	20 (17.9%)	17 (13.6%)	0.47
C1Q positive DSA	32 (13.5%)	14 (12.5%)	18 (14.4%)	0.81
Survival and graft dysfunction				
Graft dysfunction (LVEF < 45%)	23 (9.7%)	14 (12.5%)	9 (7.2%)	0.25
Retransplant	0 (0%)	0 (0%)	0 (0%)	1.00
Survival	213 (89.9%)	99 (88.4%)	114 (91.2%)	0.62

Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; dd-cfDNA, donor-derived cell-free DNA; DSA, donor specific antibody; EMBx, endomyocardial biopsy; LVEF, left ventricular ejection fraction; MFI, mean fluorescence intensity.
*Statistically significant p-value ($p < 0.05$).

EMBx, due to persistently elevated DSA with high MFI and C1q positivity. Of note, dd-cfDNA was intermediate to high in 61 patients with no EMBx-proven or -treated rejection.

There were 1,509 observations used to correlate dd-cfDNA with rejection events within 1 month of the test. The model yielded a specificity of 85% and sensitivity of 65%. The negative predictive value of a "low" test result was 99%; however, the PPV of a "intermediate" or "high" result was only 5% (Table 5).

Survival outcomes

There was no difference in survival at 1 year between patients in the dd-cfDNA group ($n = 114/125$, 91.2%) and EMBx group ($n = 99/112$, 88.4%), log-rank $p = 0.33$. Figure 2 shows KM survival curves at 1 year and at 500 days post-transplant.

Subgroup analysis showed no difference in survival at 1 year between the low, intermediate, and high dd-cfDNA subgroups (Figure 3).

With regards to graft dysfunction on echocardiography, there was no significant difference between patients in the dd-cfDNA group ($n = 9/125$, 7.2%) and EMBx group ($n = 12/112$, 12.5%) $p = 0.15$ (Figure 4). On subgroup analysis of dd-cfDNA group, 7 patients (7%) had graft dysfunction, of whom, 4 had high dd-cfDNA results ($p = 0.008$) (Figure 5). At 1 year, there was no difference in event-free survival (graft dysfunction) (Figure 6).

Table 4 Comparison of Outcomes Between cfDNA Groups

Outcomes within 1 year	Overall ($n = 103$)	Low ($n = 46$)	Intermediate ($n = 43$)	High ($n = 14$)	p-value
Biopsy-proven AMR	4 (4%)	0 (0%)	2 (5%)	2 (14%)	**0.03***
Treated AMR	4 (4%)	1 (2%)	0 (0%)	3 (21%)	**0.008***
Biopsy-proven ACR	3 (3%)	0 (0%)	2 (5%)	1 (7%)	0.19
Treated ACR	5 (5%)	0 (0%)	2 (5%)	3 (21%)	**0.005***
DSAs with positive C1q	14 (14%)	4 (9%)	5 (12%)	5 (36%)	**0.045***
LVEF < 45%	7 (7%)	2 (4%)	1 (2%)	4 (29%)	**0.008***
Death	6 (7%)	2 (4%)	2 (5%)	2 (14%)	0.35

Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; cfDNA, cell-free DNA; DSA, donor-specific antibody; LVEF, left ventricular ejection fraction.

*Statistically significant p-value ($p < 0.05$).

Table 5 dd-cfDNA Observation Level

dd-cfDNA value	Rejection ± 1 month of dd-cfDNA		
	No	Yes	Total
dd-cfDNA			
Low (< 0.20%)	1,260 (85%)	7 (35%)	1,267
Intermediate (0.20%-1.00%)	207 (14%)	11 (55%)	218
High (≥1.00%)	22 (1%)	2 (10%)	24
Total	1,489	20	1,509

Abbreviations: dd-cfDNA, donor-derived cell-free DNA.

NPV: 1,260/1,267 = 99%; PPV: 13/242 = 5%; Sensitivity: 13/20 = 65%; Specificity: 1,260/1,489 = 85%.

Correlation with CMV

Twenty patients (17.8%) in the dd-cfDNA group developed CMV viremia defined as > 1,000 copies/ml on polymerase chain reaction. A logistic regression model utilizing 1,509 test results was used to correlate CMV viremia with an elevated dd-cfDNA result ≥0.2% within 1 month. CMV viremia was associated with 2-fold increased odds of having an elevated dd-cfDNA (OR 2.27 (95% CI: 1.1, 4.7), $p = 0.03$); however, this correlation was not significant including a random subject effect.

Discussion

In our study, we present our noninvasive-based surveillance protocol which we found to be safe, led to fewer EMBx, without missing clinically relevant immunologic events, and with no difference in survival at 1 year.

An ideal surveillance strategy should permit early detection of graft rejection as compared with the current standard of care, offer feasibility of testing, avoid sampling errors and inter-observer variability, as well as have standard results. The most recent practice guideline from the ISHLT reports on the promising role of GEP and dd-cfDNA testing in allograft surveillance,[7] and gives a class IIa recommendation to use GEP in low-risk patients between 2 months and 5 years, albeit there is no specific guidance on the interpretation of these results in different clinical scenarios, especially with discrepancy with dd-cfDNA test results.

The median age in our patient population was 59 years at time of transplantation, slightly higher than patients in the GRAfT study (53 years) and CARGO II (50.8 years). Older patients > 55 years are generally considered to be at higher risk for rejection, malignancy and infection,[9] although contemporary studies have shown comparable survival at 5 years.[10]

In our experience, an abnormal GEP test on its own did not influence treatment decision, as it is affected by administration of prednisone in the immediate post-transplant period, it tends to increase with time,[11] and can be falsely

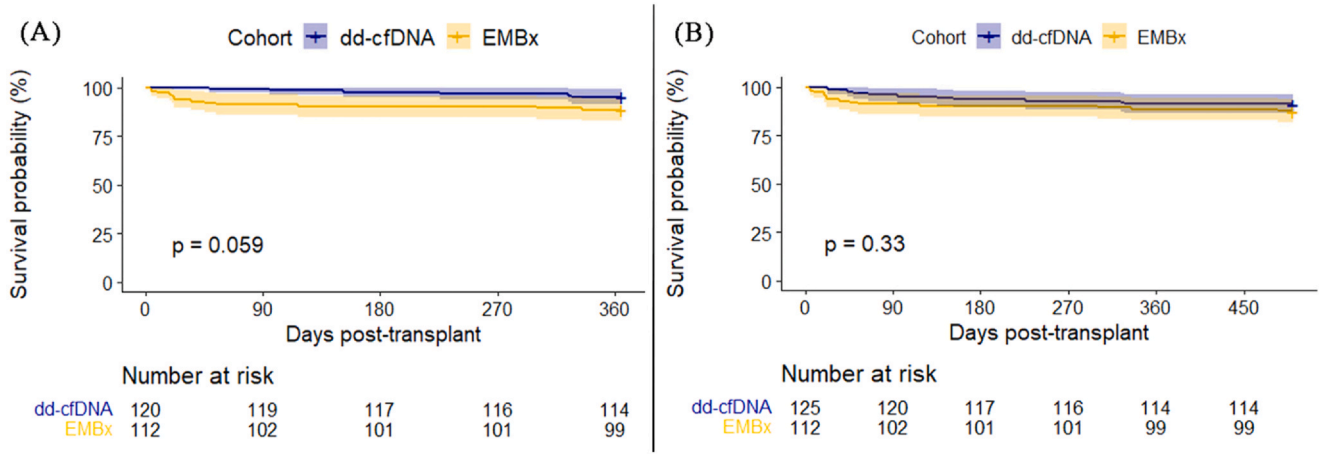

Figure 2 Kaplan-Meier curve depicting survival between both groups at (A) 1 year, (B) 500 days.

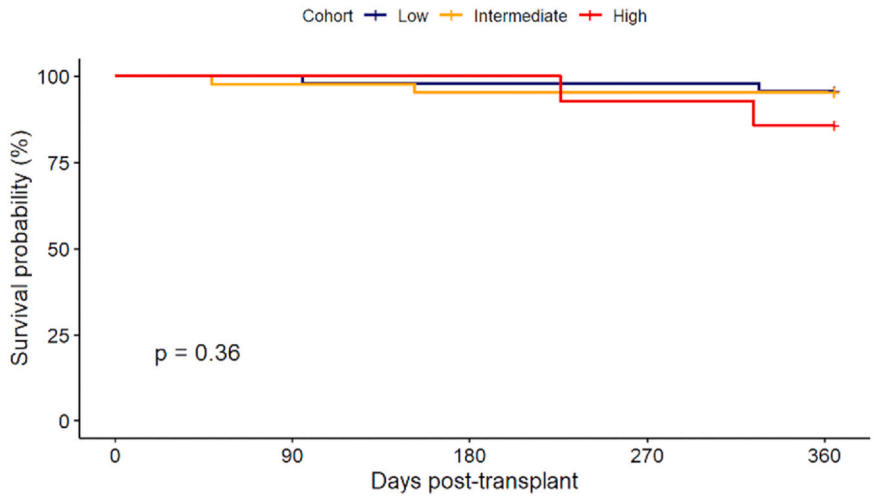

Figure 3 Kaplan-Meier curve depicting survival difference between categories of dd-cfDNA.

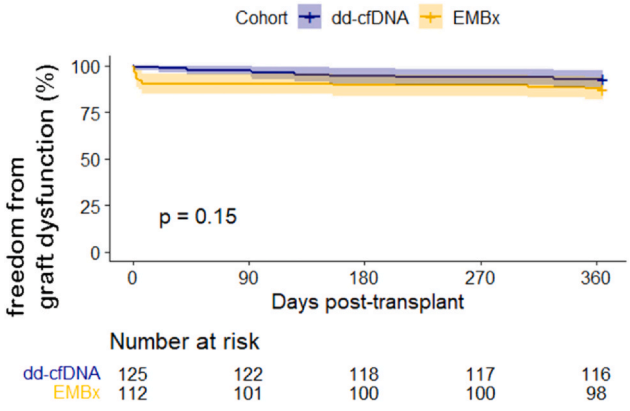

Figure 4 Kaplan-Meier curve comparing difference of graft dysfunction between 2 study groups.

elevated during systemic inflammation or infectious states, particularly CMV.[12,13] In our algorithm, we usually do not pursue additional workup if dd-cfDNA is low owing to the high NPV; however, further testing is done in the intermediate to high levels. In case of discrepancy between GEP and dd-cfDNA, particularly with a negative dd-cfDNA, repeat testing within 2 weeks is usually done to monitor the trend prior to proceeding to EMBx. DD-cfDNA has a very short half-life of 30 minutes to several hours[14] and can therefore be repeated within days if needed. We feel that this is safe and avoids the need for an invasive test, and may improve patient satisfaction.

In our study, more patients in the dd-cfDNA group had a positive virtual crossmatch which may have accounted for increased percentage of AMR episodes. The higher percentage of AMR episodes is also related to lower overall number of biopsies in the dd-cfDNA group, as these were mostly guided by an abnormal cfDNA results. Comparatively, there were remarkably less ACR compared to conventional monitoring group. This is likely related to larger number of biopsies in the EMB group which can sometimes lead to overdiagnosis and unnecessary treatments. With these lower rates of ACR, there is a possibility of subclinical rejection that may have been missed by not doing more frequent EMBx; however, this did not translate into meaningful clinical difference with regards to survival or graft dysfunction.

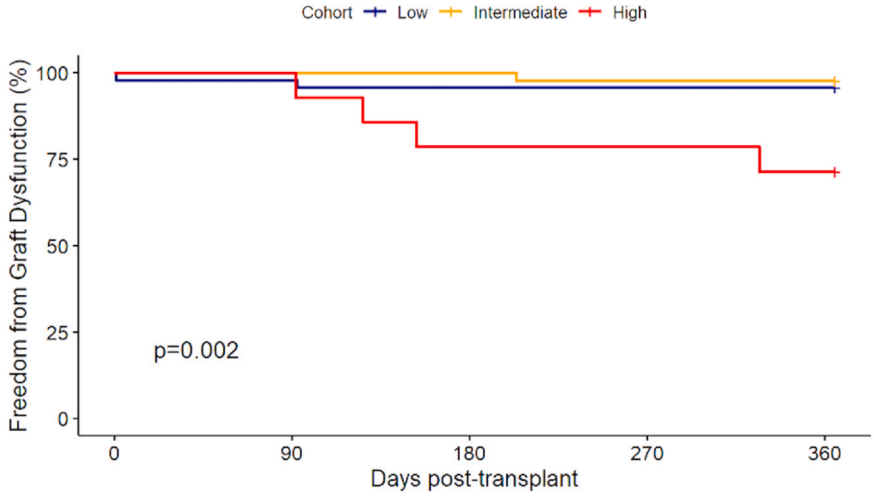

Figure 5 Kaplan-Meier curve comparing freedom from graft dysfunction between the 3 categories of dd-cfDNA.

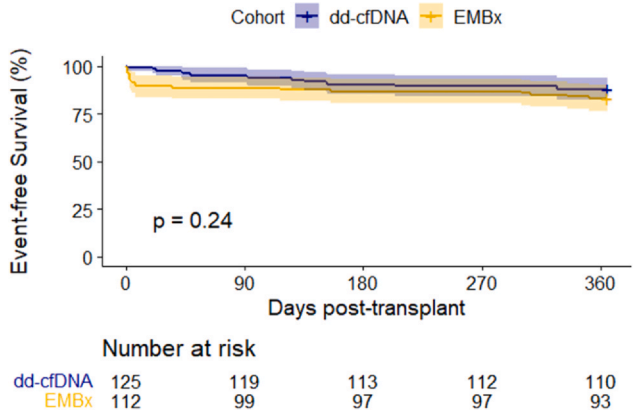

Figure 6 Kaplan-Meier curve comparing freedom from mortality and graft dysfunction between both groups.

The spectrum of dd-cfDNA results can also be a helpful predictor for adverse events. We use a value > 1% to denote high risk population who were found to have a higher biopsy-proven AMR and ACR, while low risk patients with a score of < 0.2% had no ACR or biopsy-proven AMR, giving this test an excellent negative predictive value of 99%. Only one patient in the low-risk group was treated for AMR, however this was a biopsy negative rejection event in which the patient was treated due to persistently elevated DSA with high MFI and C1q positivity. In cases of intermediate values, GEP may play a role in further stratifying these patients into high or low risk groups.

There are currently no proposed sex- or race-specific normal ranges for interpretation of dd-cfDNA results. In a subanalysis of patients in the GRAfT study, Black patients had a higher dd-cfDNA percentage immediately after transplant compared with non-Black patients (8.3 vs 3.2%, $p = 0.001$); however, the rate of decay in dd-cfDNA over the first week was equivalent and plateaued at 7 days post OHT (0.46% vs 0.45%, $p = 0.78$).[15]

With regards to survival, there was no difference between the noninvasive group and the traditional EMBx group.

Allograft dysfunction on echocardiography is usually an insensitive marker for rejection as it is a late manifestation, can be affected by reader variability and loading conditions. In the GRAfT study, dd-cfDNA levels correlated with severity of left ventricular dysfunction, defined as a reduction of at least 5% in LVEF from the prior echocardiogram.[4] In our results, there were no difference between the dd-cfDNA group and the EMBx group in the rate of graft dysfunction; however, similar to the GRAfT study, patients with higher dd-cfDNA results were more likely to have graft dysfunction at 1 year. The dd-cfDNA assay is highly sensitive and may rise before a reduction in LVEF on echocardiography is seen.

The development of de novo DSAs is common in patients post OHT; however, many of these patients will not develop EMBx-detected AMR, and the decision to treat these patients is highly variable among transplant programs.[16] In an analysis of 613 samples from the SHORE registry with available DSA levels and negative EMBx, dd-cfDNA levels > 0.15% were associated with a 4-fold greater likelihood of subsequent DSA detection within the first year post OHT.[17] In our patients, there was no difference in the development of de novo DSAs between the noninvasive group and the traditional EMBx group; however, the percentage of DSAs with high MFI was highest in the "high risk" group ($n = 5/14$, $p = 0.08$), as well as positive C1q ($n = 5/14$, $p = 0.045$).

Future considerations

The definition of low risk patients is largely driven from the initial GEP studies that excluded patients with graft dysfunction, severe cardiac allograft vasculopathy, or therapy for rejection.[6] In patients with persistently low levels of GEP and dd-cfDNA, it is reasonable to consider them at low risk, and they may be candidate for lowering immunosuppression targets with longer follow-up.

Whether combining GEP and dd-cfDNA testing is superior to either test alone remains elusive. In our experience, we rely mainly on dd-cfDNA testing with less emphasis on GEP. In a retrospective review by Henricksen et al, 159 patients were monitored using either GEP alone or paired testing At 1 year, there was no difference in survival between both groups.[18] The upcoming DETECT clinical trial (Donor Derived Cell-free DNA to DETect Rejection in Cardiac Transplantation; NCT05081739) will shed light on whether the addition of GEP can influence clinical decisions.

Limitations

This study is limited by its retrospective nature and being conducted in a single center. Patients in the noninvasive groups were transplanted more recently compared to the EMBx group, which may have influenced the results. Despite having more available data, the follow-up in the EMBx group was censored at 1.35 years to stay similar to the noninvasive group to avoid bias. The detection of graft dysfunction on echocardiogram was determined based on echo reports and not via standardized approach, which may have been subject to reader assessment. The relationship between dd-cfDNA and cardiac allograft vasculopathy was not assessed given limited number of observations. Finally, this was not a randomized trial, and patient demographics were different between the 2 groups.

Conclusion

A noninvasive protocol for rejection surveillance is safe, led to significantly fewer EMBx, fewer treated rejection episodes, and no difference in survival or graft dysfunction at 1 year. More AMR episodes identified via dd-cfDNA could lead the way for more accurate diagnostic and treatment decision pathways in the future. As technology

improves and registries continue to recruit patients, implementation of this technology into guidelines and transplant program protocols is foreseen.

Author contributions

Data collection was performed by authors A.S., S.M., V.D. Statistical analysis was done via author J.V.Z. Final review and editing by senior author S.H. All authors participated in manuscript writing and arrangement and have reviewed the manuscript in its entirety prior to submission.

Data availability

The data that support the findings of this study are available from the senior author [S.H.] upon reasonable request.

Disclosure statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr Shelley Hall is a consultant for CareDx and Natera. Other authors have no disclosures to report. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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