

Observed elevated donor-derived cell free DNA in orthotopic heart transplant recipients without clinical evidence of rejection

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Abstract

Donor-derived cell free DNA (dd-cfDNA) has rapidly become part of rejection surveillance following orthotopic heart transplantation. However, some patients show elevated dd-cfDNA without clinical evidence of rejection. With the aim to provide a clinical description of this subpopulation, we retrospectively analyzed 35 cardiac transplant recipients at our center who experienced elevated ($\geq 20\%$) dd-cfDNA in the absence of clinical rejection, out of a total 106 recipients who had dd-cfDNA results available during the first year. The median time to first elevated dd-cfDNA level was 46 days, and the highest dd-cfDNA recorded within 1 year was .31% [inter-quartile range, .23–.45]. Twenty-two (63%) patients experienced infections (cytomegalovirus (CMV) or other), and 16 (46%) presented with *de novo* donor-specific antibodies. Cluster analysis revealed four distinct groups characterized by (a) subclinical rejection with 50% CMV ($n = 16$), (b) non-CMV infections and the longest time to first elevated dd-cfDNA (187 days) ($n = 8$), (c) right ventricular dysfunction ($n = 6$), and (d) women who showed the youngest median age (45 years) and highest median dd-cfDNA (.50%) ($n = 5$). Continued prospective analysis is needed to determine if these observations warrant changes in patient management to optimize the utilization of this vital non-invasive graft surveillance tool.

KEYWORDS

antibody-mediated (ABMR), heart (allograft) function, dysfunction, rejection, acute, rejection

1 | INTRODUCTION

Monitoring graft function is a critical component of patient care following solid organ transplantation. Timely diagnosis of organ rejection and its prompt management are essential for long-term survival. The current gold standard for rejection surveillance in organ transplantation is endomyocardial biopsy with histologic assessment. This invasive technique bears a substantial risk of procedural complications and suffers

from inadequate tissue sampling, patient discomfort, high costs, as well as significant inter-observer variability in the histological assessment.¹

For the past decade, research has focused on the development of novel technologies for identification of acute allograft rejection based on genomics, transcriptomics, proteomics, and metabolomics.² An emerging area of research has been on donor-derived cell free DNA (dd-cfDNA). This technique utilizes single-nucleotide polymorphisms distributed across the genome to distinguish between donor and

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recipient DNA molecules. Specifically in acute cellular rejection (ACR) and antibody-mediated rejection (AMR), cell death in the allograft leads to increased levels of dd-cfDNA in the recipient's blood stream. Prior studies have found that dd-cfDNA is released from cells within the donor organ during episodes of significant injury.¹ Furthermore, that study also suggested that dd-cfDNA may begin to rise weeks to months prior to clinical or histological rejection.

AlloSure (CareDx, Brisbane, CA, USA) is a next generation sequencing assay that has targeted amplification methods, compared to previous shot-gun approaches. This assay has been validated to quantify a percentage of dd-cfDNA in transplant recipients without the need for donor or recipient genotyping.³ The test measures the proportion of total cell-free DNA that is derived from the donor and the recipient. The amount of dd-cfDNA is generally < .15% of the total cfDNA in the patient with a quiescent allograft. However, significantly higher amounts of dd-cfDNA are released from the injured allograft when there is rejection.³ The dd-cfDNA-based AlloSure test is currently approved for commercial use and is performed in combination with gene expression profiling (AlloMap) as a biomarker for proactive surveillance method in cardiac transplantation programs under the name HeartCre. The seminal multicenter trial leading up to this approval was the D-OAR study of 740 heart transplant recipients (ClinicalTrials.gov Identifier: NCT02178943).³ Our institution started using HeartCare in 2018 and has incorporated these assays as part of the surveillance protocol in 2019.

Yet publications from the seminal D-OAR also demonstrated the possibility that AlloSure scores rise in the absence of clinically proven rejection. We hypothesize that there are certain demographics and clinical conditions that are historically observed in the stable, but high-risk, patient population post-cardiac transplantation without signs of rejection but with elevated AlloSure values. We aimed to describe possible confounders that exist warranting the need for more intelligent use of this non-invasive surveillance technique.

2 | MATERIALS AND METHODS

This was a single-center retrospective study of heart transplant recipients at Baylor University Medical Center between February 2018 and November 2020. Patient data were reviewed for outcomes up to 1-year post-transplant with a study end date on February 1, 2021. Data collection was approved by the Institutional Review Board of Baylor Scott & White Research Institute under an umbrella protocol for retrospective research, and the use of written informed consent was waived. Patients with elevated AlloSure scores (defined as $\geq .20\%$)³ within 1 year post-transplant were included if they did not receive a dual-organ transplant or have biopsy-proven AMR ($> 0R$) or ACR ($\geq 2R$) assessed according to the most recent International Society of Heart and Lung Transplantation (ISHLT) definition.^{4,5} Although a .15% criterion has been used for AlloSure data more recently,⁶ we chose a more conservative cut-off value for the AlloSure test of .20% based on a recent study confirming this value in a secondary analysis of data from the D-OAR study.³

2.1 | Clinical assessments

Routine follow-up visits during the first-year post-transplant at our center occur weekly for 4 weeks, then every 2 weeks until the end of prednisone taper, or until 6 months in case of dual organ (heart/kidney) and maintained on prednisone, then monthly to the end of 1 year. Follow-up visits include assessment for viremia every 2 weeks for 6 months, then monthly through 1 year. Routine prophylaxis for CMV mismatch patient is valganciclovir 450 mg twice daily for 6 months. Routine assessment for rejection is done by endomyocardial biopsy at 2 weeks, 6 weeks, and 1 year post-transplant. The frequency of biopsies in our management protocol was decreased after the institution of HeartCare (which includes both AlloSure and AlloMap) as part of our standard of care which are performed monthly (or more frequently if deemed clinically necessary). A baseline right and left heart catheterization to include intravascular ultrasound (IVUS) for evaluation of donor-derived coronary artery disease (CAD) is performed at 6 weeks.

2.2 | Data elements

The following data elements were extracted by chart review from electronic health records: Patient age, body mass index (BMI) at transplant, sex, race, ethnicity, ischemic cardiomyopathy, prior use of mechanical circulatory support (MCS) devices, pre-transplant human leukocyte antigen (HLA) antibodies, donor-derived CAD at 6 weeks post-transplant, induction therapy, post-transplant HLA antibodies, abnormal central venous pressure (CVP ≥ 10 mm Hg), post-transplant right ventricular (RV) dysfunction, hepatitis C-positive recipient status, post-transplant infection (treated or a viral load > 137 copies/dl of cytomegalovirus (CMV) or non-CMV infection), highest AlloSure result within 1 year post-transplant, and time to first elevated ($\geq .20\%$) AlloSure from date of transplant. RV dysfunction was defined based on echocardiographic assessment defined as tricuspid annular plane systolic excursion (TAPSE) of < 1.7 cm, or with an S' of < 9 cm/s, or with an RV base dilated > 42 mm.⁷

2.3 | Statistical analysis

Patient characteristics were reported as the frequency (%) for nominal data and as the mean (standard deviation) or, if skewed, the median [quartiles] for continuous variables. Nominal features were ranked as the top 15 from most to least frequent.

To identify groupings of clinical characteristics observed in patients, we used unsupervised cluster analysis blinded to the AlloSure results. This analytical tool has the potential to provide insight into characteristics that are simultaneously present within subgroups of patients. Specifically, a partitioning around medoids clustering (PAM) technique was chosen, utilizing the DAISY algorithm. This method, a more robust version of K-means clustering, is appropriate for determining dissimilarities among patients based on a mixture of categorical and quantitative variables. The potential to identify distinct groups was

determined using the Hopkins statistic. The starting set of variables was selected based on clinical experience reflecting characteristics previously associated with rejection and graft dysfunction. The final set of variables were determined in a backwards stepwise selection approach maximizing the Hopkins statistic with a stopping criterion of $> .70$. The optimal number of clusters was selected using the elbow technique which aids in identifying the point where additional clusters have a smaller impact on reducing the within-cluster variability (**Supplemental Figure**). Quantitative variables were centered to mean = 0 and standard deviation = 1. As an exploratory step, cluster characteristics were compared using the Kruskal Wallis test or Fisher's Exact test as appropriate for each data type. All analyses were performed in R (version 4.0.2) with the PAM clustering implemented using the *pam* and *daisy* functions in the *cluster* package.

TABLE 1 Baseline characteristics

Variable	Overall (n = 35)
Age (year)	62.1 [55.8, 65.7]
Sex, female	5 (14%)
Race, African American	8 (23%)
Ethnicity, Hispanic	8 (23%)
BMI (kg/m ²)	27.4 [24.5, 30.5]
Ischemic cardiomyopathy	16 (46%)
CMV match at transplantation	
Donor-/Recipient-	5 (14%)
Donor+/Recipient+	15 (43%)
Donor-/Recipient+	3 (9%)
Donor+/Recipient-	12 (34%)
Hepatitis C-positive recipient	6 (17%)
Prior MCS	7 (20%)
Durable MCS	4 (11%)
Impella	3 (9%)
CAD at 6 weeks post-transplant	
0	25 (71%)
1	2 (6%)
2	1 (3%)
Not assessed due to acute kidney injury	6 (17%)
Missing	1 (3%)
Induction	
None	30 (86%)
Basiliximab	3 (9%)
rATG	2 (6%)
Pre-transplant HLA antibodies	19 (54%)
cPRA%	3 [0, 31]
De novo DSA	16 (46%)
<4000 MFI	6 (17%)
>4000 MFI or C1Q positive	1 (3%)
>10 000 MFI	10 (29%)

TABLE 2 Patient characteristics post-transplant and AlloSure test results

Variable	Overall (n = 35)
Highest AlloSure score (%)	.31 [.23, .45]
Time to first elevated AlloSure (d)	46 [26, 170]
Time to highest elevated AlloSure (d)	174 [38, 243]
# of elevated AlloSure scores	2 [1, 3]
AlloMap at time of highest AlloSure	35 [31, 37]
HLA antibodies	3 (9%)
Highest BNP (pg/ml)	246 [156, 555]
ACR(1R)	26 (74%)
RV dysfunction	8 (23%)
Pericardial effusion	8 (23%)
CVP \geq 10 mm Hg	8 (23%)
Diastolic grade > 2	14 (40%)
Infection	22 (63%)
CMV	11 (31%)
Non-CMV	12 (34%)

3 | RESULTS

A total of 35 patients who experienced elevated dd-cfDNA in the absence of clinical rejection were included out of a 106 patients who had AlloSure results available within 1 year post transplant. Patients who did not meet the inclusion criteria are described as follows: 58 (55%) had normal dd-cfDNA without rejection, four (4%) had normal dd-cfDNA with rejection, seven (7%) had elevated dd-cfDNA with rejection, and two (2%) were dual organ recipients who had elevated dd-cfDNA results without rejection. Baseline characteristics are reported in **Table 1**. Of the cohort, seven patients had mechanical circulatory support (MCS) devices at the time of transplantation including four with durable MCS, and three patients with Impella in place. Post-transplant findings are summarized in **Table 2**. All patients in the cohort had normal systolic function. Diastolic stiffness frequently noted in the post-transplant patients was noted in 40% of the patients. In particular, 26 patients developed ACR(1R), eight patients developed RV dysfunction, and 22 presented with infections, including: CMV (31%), Epstein-Barr virus (6%), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (9%), BK virus (3%), cellulitis (3%), nocardia (3%), methicillin-resistant staphylococcus aureus (MRSA) (3%), coccidiomycosis (3%), staphylococcus warneri (3%), giardia (3%), and hepatitis B (6%). The first elevated dd-cfDNA test result was recorded at a median of 46 [IQR: 26, 170] days and highest elevated dd-cfDNA test at 174 [IQR: 38, 243] days. The median highest dd-cfDNA result recorded during the first-year post-transplant was .31% [IQR: .23-.45].

The top 15 patient characteristics by frequency are ranked in **Figure 1**. ACR(1R), accounting for 74% of the cohort, ranked highest. Infection was the second most common characteristic (63%) followed by pre-transplant HLA antibodies (54%) of which 46% were *de novo*

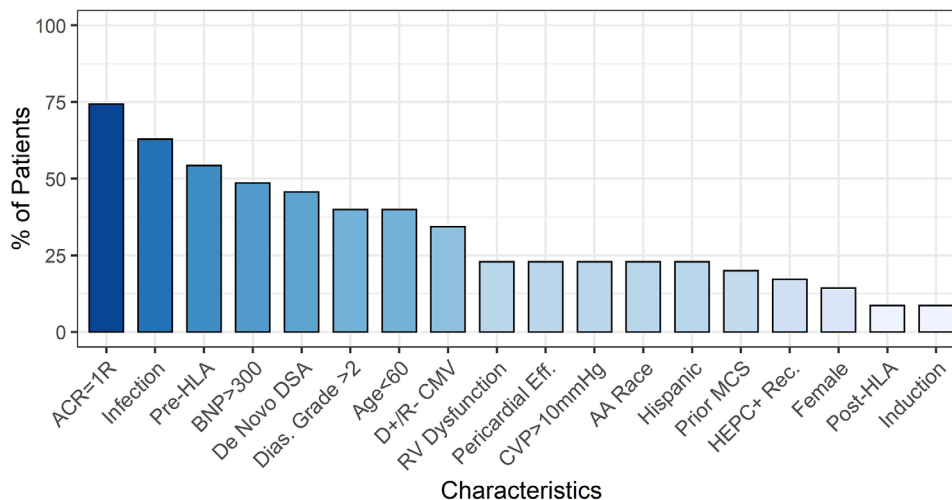


FIGURE 1 Ranking of presence of selected characteristics in the overall cohort ($N = 35$). HLA, human leukocyte antigen; DSA, donor-specific antibodies; BNP, B-type natriuretic peptide; Dias., diastolic; D+/R- CMV, donor-positive/recipient-negative cytomegalovirus match; RV, right ventricular; Eff., effusion; CVP, central venous pressure; AA, African American; MCS, mechanical circulatory support; HEPC+ Rec., hepatitis C-positive recipient

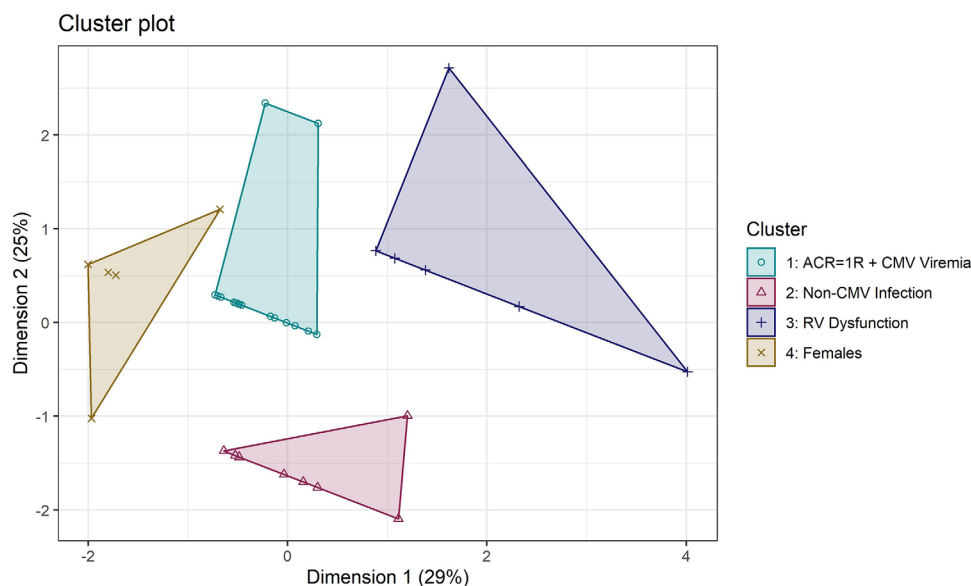


FIGURE 2 Scatterplot of clusters. Groupings are based on the 4 clusters derived from cluster analysis on sex, ACR(1R), highest BNP, post-transplant RV dysfunction, and infection. Raw data (individuals) used to derive the clusters are represented in the first two dimensions cumulatively explaining 54% of the total variance in the data

DSA. Elevated BNP ≥ 300 pg/ml were seen in 49% of patients, 40% of patients had a diastolic grade > 2 , 37% of the patients were younger than 60 years, and 34% of patients were a high-risk CMV donor positive/recipient negative mismatch at transplant. Additional features representing graft dysfunction such as pericardial effusions, abnormal CVP ≥ 10 mm Hg and post-transplant RV dysfunction occurred in 23% of the patients. African American race and Hispanic ethnicity also each occurred in 23% of the cohort whilst prior MCS was utilized in 20% of patients.

Cluster analysis identified four distinct groups in this cohort, as illustrated in **Figure 2**, which were characterized by ACR(1R), infections,

graft dysfunction, and sex. Characteristics for each of the four clusters are described in **Table 3** and depicted in **Figure 3**. The largest cluster, Cluster 1, consisting of 16 patients with ACR(1R), was also characterized by a high incidence of infections (69%) where eight patients had CMV and four had non-CMV infections. The median age was 63 years and 31% presented with *de novo* DSA. In this group of patients, the first elevated dd-cfDNA result was recorded at a median of 37 days post-transplant with the median highest dd-cfDNA result of .32% [IQR: .22–.39].

The second largest cluster, Cluster 2, consisted of eight recipients and had the highest incidence of non-CMV infections (62%) and

TABLE 3 Characteristics of the four clusters

Variable	Cluster 1: ACR 1R and CMV (n = 16)	Cluster 2: Non-CMV infection (n = 8)	Cluster 3: RV dysfunction (n = 6)	Cluster 4: women (n = 5)	P-value
Age (year)	63 [56, 67]	62 [60, 66]	62 [60, 64]	45 [41, 52]	.16
Sex, female	0 (0%)	0 (0%)	0 (0%)	5 (100%)	<.001
Race, African American	1 (6%)	1 (12%)	4 (67%)	2 (40%)	.012
BMI (kg/m ²)	27.1 [24.7, 31.1]	26.5 [24.5, 27.8]	30.7 [27.9, 33.6]	26.3 [23.2, 28.2]	.36
Ischemic cardiomyopathy	9 (56%)	4 (50%)	2 (33%)	1 (20%)	.54
Donor+/Recipient- CMV	6 (38%)	3 (38%)	2 (33%)	1 (20%)	.96
Hepatitis C-positive recipient	1 (6%)	3 (38%)	2 (33%)	0 (0%)	.11
Prior MCS	4 (25%)	1 (12%)	2 (33%)	0 (0%)	.62
De novo DSA	5 (31%)	4 (50%)	5 (83%)	2 (40%)	.20
Highest BNP (pg/ml)	195 [130, 452]	525 [239, 710]	491 [367, 1039]	132 [48, 186]	.02
ACR(1R)	16 (100%)	0 (0%)	6 (100%)	4 (80%)	<.001
CVP ≥10 mm Hg	3 (19%)	1 (12%)	2 (33%)	2 (40%)	.61
Post-transplant RV dysfunction	0 (0%)	1 (12%)	6 (100%)	1 (20%)	<.001
Pericardial Effusion	2 (12%)	3 (38%)	3 (50%)	0 (0%)	.12
Diastolic Grade > 2	6 (38%)	3 (38%)	3 (50%)	2 (40%)	.96
Post-HLA	2 (12%)	0 (0%)	1 (17%)	0 (0%)	.74
Infection	11 (69%)	5 (62%)	5 (83%)	1 (20%)	.21
CMV	8 (50%)	0 (0%)	3 (50%)	0 (0%)	.02
Non-CMV	4 (25%)	5 (62%)	2 (33%)	1 (20%)	.34
Highest AlloSure Score (%)	.32 [.22, .39]	.23 [.21, .33]	.26 [.24, .78]	.50 [.49, .63]	.046
Time to first elevated AlloSure (d)	37 [22, 133]	187 [21, 283]	91 [40, 206]	46 [32, 116]	.40
# of high AlloSure scores	2 [1, 3]	1 [1, 2]	1 [1, 4]	3 [3, 4]	.07
AlloMap at time of highest AlloSure	36 [34, 37]	31 [28, 34]	38 [36, 38]	34 [29, 36]	.21

highest BNP of 525pg/ml among the groups. None of these patients developed any rejection nor CMV viremia. Graft dysfunction recorded as an abnormal CVP ≥10 mm Hg (12%) and pericardial effusion (38%) occurred in a minority of these patients. One patient had RV dysfunction. The median age was 62 years and 50% presented with *de novo* DSA. The first elevated dd-cfDNA result was observed at a median of 6 months post-transplant and the median highest dd-cfDNA score was .23% [IQR: .21–.33].

In the third cluster of six patients, features of graft dysfunction were present in a majority of the patients. RV dysfunction occurred in all patients in this group, and pericardial effusions in 50% with the second highest median BNP of 491 pg/ml. The median age was 62 years, 67% identified as African American, 83% presented with *de novo* DSA, 100% with ACR(1R), and 83% with infections. The first elevated AlloSure result was recorded at a median of 91 days post-transplant with the median highest AlloSure score of .26% [IQR: .24–.78].

Female recipients (n = 5) were identified as a separate cluster, four of whom developed ACR(1R) and one non-CMV infection. In this cluster, the median age of 45 years and median BNP of 132 pg/ml were both the lowest among all groups. Of the five females, four had children, two

were multiparous, two presented with DSAs at transplant and cPRA% was ≤2%. Women had the highest median AlloSure score of .50% [IQR .49–.63], and the median time to the first elevated AlloSure result was 46 days.

4 | DISCUSSION

This study is the largest-to-date single-center retrospective analysis to determine non-rejection variables that have been observed with elevated dd-cfDNA results, spanning a period of 2 years. As an early adopter of HeartCare, combined with our standard of care protocol, we were able to analyze 35 patients with elevated results in the absence of acute rejection out of 106 patients with HeartCare (including AlloSure and AlloMap) data available. Based on cluster analysis, we found clinical characteristics to distinguish groups of patients with elevated dd-cfDNA result to include ACR(1R) with CMV viremia, non-CMV infection, and RV dysfunction with infections and *de novo* DSA, and women. To our knowledge, this is the first study to describe these observations in patients following orthotopic heart transplantation.

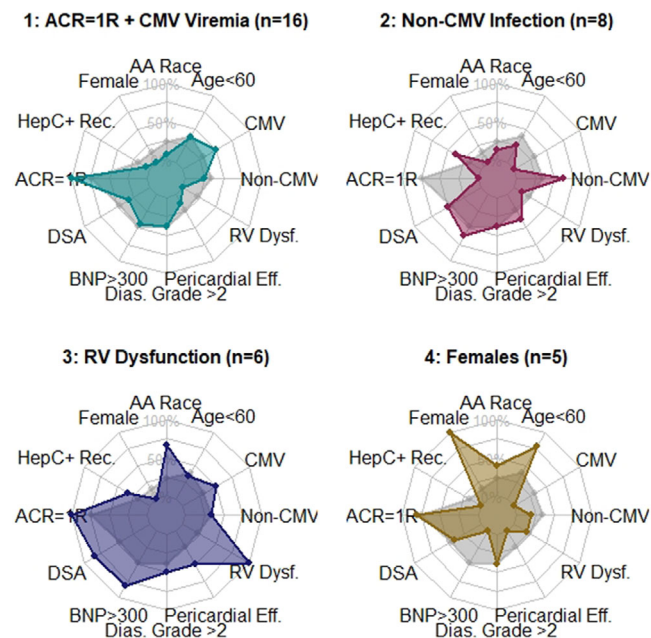


FIGURE 3 Characteristics of the four clusters relative to the overall cohort. The relative frequency of each characteristic is presented on a scale from 0% at the center to 100% on the outer edge of each radar chart. Each cluster is overlaid on the overall cohort in gray. AA, African American; HEPC+ Rec., hepatitis C-positive recipient; DSA, donor-specific antibodies; BNP, B-type natriuretic peptide; Dias., diastolic; Eff., effusion; RV Dysf., right ventricular dysfunction; CMV, cytomegalovirus viremia; Non-CMV, non-CMV infection

Previous studies suggest that dd-cfDNA is less reliable in ACR yet more reliably discriminated in AMR based on data in renal transplant recipients.⁸ Huang et al. describe in renal transplant recipient dd-cfDNA of greater than or equal to .74% yielded a sensitivity of 100% and specificity of 71.8% for AMR but did not discriminate for ACR. Additionally, serial increase in percentage levels of dd-cfDNA of up to 61% outside the “acceptable range” have been reported in stable renal recipients.⁹ These increases were deemed due to biological variations and not associated with either clinical or histologically proven renal graft failure. Recently, a prospective cohort validation study from the GRAFT investigators assessed the utility of dd-cfDNA concentration for detection of rejection in cardiac transplant recipients.¹⁰ They showed ACR had a sensitivity of 79% and specificity of 85% versus AMR having a sensitivity of 88% and specificity of 85% at .25% dd-cfDNA threshold.¹⁰ The authors noted that differences in AMR and ACR and dd-cfDNA concentration likely are secondary to differences in pathological mechanisms. AMR is associated with donor-specific antibodies resulting in microvascular damage and cellular DNA release. ACR, on the other hand, is associated with localized lymphocytic injury often within the interstitium and perivascular tissue spaces and hence may not cause the degree of dd-cfDNA as AMR.

In the field of lung transplantation, dd-cfDNA has been shown to correlate with biopsy-confirmed or treated ACR and chronic lung allograft dysfunction.⁷ However, dd-cfDNA did not correlate with allo-

graft infection, including those with CMV. Additionally, there has been an observed elevation of dd-cfDNA for patients who tested positive for CMV.⁸ Interestingly, BK viremia has been observed to increase dd-cfDNA levels in patients without rejection after kidney transplantation.¹¹

Specific to cardiac transplantation, the D-OAR study described 740 heart transplant patients that yielded 2447 samples for analysis. Of these, 847 were paired with endomyocardial biopsy.³ There were 17 cases of ACR (grade $\geq 2R$) and 18 cases of AMR. In this trial acute rejection was defined as ACR/AMR $\geq 2R$. Of note, the D-OAR study excluded 1R cases for the paired biopsy analysis but the authors described ACR grade 1R having a similar median level (.08%) to grade 0 biopsies (.07%), whereas ACR 2R (moderate) had a median dd-cfDNA level of .15%, and ACR 3R (severe) had a median dd-cfDNA level of .30%. Based on the D-OAR results, a cut-off of .20% was established for significantly elevated AlloSure values.

Patients with no histologic evidence of rejection had a median dd-cfDNA level of .07%, whereas acute rejection patients had a median level of .17%. The area under the curve in ROC analysis for identifying rejection was .64, or a sensitivity of 44% and specificity of 80% with a NPV of 97.1% and PPV of 8.9%. The high NPV indicates high confidence when the AlloSure test is low for non-rejection. However, it is imperative to note that the low incidence of acute rejection (< 5% of samples) plays a role in the high NPV, and that 56% of acute rejection cases were incorrectly labeled as non-rejection as reflected in the low sensitivity. It is well known that in samples with high AlloSure scores, the low PPV gives low confidence in the accuracy of these results. None of the patients in our cohort developed clinically significant rejection. A recent study from the GRAFT investigators showed that even though cfDNA is cleared relatively quickly with a half-life of 30 min, with ongoing pathological process we can see slow uptick in dd-cfDNA weeks prior to an episode of acute rejection and graft dysfunction.¹⁰ Fortunately, we have not had a patient develop graft dysfunction even after the conclusion of our analysis. It would be of interest to determine if our patients will experience an episode of acute rejection based on histologic and symptom evidence. We plan to follow these patients longitudinally. Additionally, in these patient scenarios, further development of the current assay and understanding confounders that increases dd-cfDNA outside of rejection might lead to better discrimination of acute rejection.

Our observational study highlights several salient points. Higher AlloSure values were noted in patients with ACR(1R) with evidence of CMV viremia in the largest cluster of patients from this cohort. This may suggest that patients with ACR(1R) or patients with pre- or post-transplant HLA antibodies may eventually have some form of injury that is detectable by elevated AlloSure scores without developing clinical rejection during the first year. Additionally, prior studies have noted elevated CMV levels with subclinical rejection.^{12,13} It is possible that our observational finding can be explained by graft injury leading to release of dd-cfDNA in the setting of ACR 1R along with CMV viremia.

The second cluster noted was in patients that had evidence of non-CMV infections including SARS-CoV-2, Epstein-Barr virus and

hepatitis B infection. Perhaps like the findings of Bloom et. al¹⁰ evidence of non-CMV infection can lead to elevated dd-cfDNA. Unfortunately, in our study the sample size is too small to separate these viruses for clustering separately. Further studies are needed to evaluate the degree of orthotopic cardiac graft injury in the setting of significant viral load of the viruses noted in these results.

The third cluster was formed by female recipients who as a cohort did not show any evidence of viremia but 80% had ACR(1R) with 40% of these patients were noted to have *de novo* DSA. Women in general have a higher level of sensitization, especially with history of multiparity, and have higher rates of rejection.³ No prior evidence of higher AlloSure values in women has been noted in studies of renal or lung transplant recipients.³

The last cluster of interest was characterized by RV dysfunction. As many as 23% of patients with elevated AlloSure values had RV dysfunction. There are a few studies showing prognostic utility of RV assessment of the graft which showed independent association with incident rejection, CAV and death. Of note, 100% of patients in this cluster had ACR 1R as well. Given evidence of RV dysfunction in prior studies in patients with rejection, it is possible that graft injury markers are higher in patients with persistent RV dysfunction.

The task to precisely define heart allograft rejection and the decision to treat in the absence of a diagnostic endomyocardial biopsies, the traditional “gold standard”, is becoming a common dilemma. The paradigm is clearly shifting from the single diagnostic modality of histologic evidence to a multifaceted approach utilizing dd-cfDNA at the forefront as a tool of surveillance and early recognition of acute rejection. Our study identifies elevated dd-cfDNA levels in patients without evidence of clinical or histologic rejection otherwise. To our knowledge, we have described the first set of potential cohorts of patients where abnormal values were seen in the cardiac transplant population without evidence of rejection. However, with our small sample size and single center analysis, our findings are hypothesis generating and warrant further investigation.

4.1 | Limitations

There are several limitations to the study. First, as inherent to any retrospective analysis from a single center with a relatively small sample size, it is unknown if the characteristics analyzed are generalizable. Due to the retrospective nature of this study, associations between dd-cfDNA results and infectious complications cannot be studied, since the samples were not drawn concurrently. Despite this being the largest sample reported to date, a multi-center trial with programs that utilize similar surveillance modalities and protocols is warranted. Additionally, it is possible that elevated AlloSure values are connected to factors not evaluated in the study. However, this study reflects our clinical experience with a large number of variables that has not been described to date. Finally, there is well known subjective variability in pathology interpretation that cannot be accounted for in a retrospective study; our cohort could reflect subclinical rejection at the time of the collected elevated AlloSure scores.

4.2 | Conclusion

This analysis sets an initial and critical foundation for determining clinical characteristics in patients with elevation of dd-cfDNA following heart transplantation without clinical rejection. This analysis brings up several ideas for future research in this rapidly evolving field of non-invasive monitoring of graft rejection. Comparison of dd-cfDNA and histology assessment with molecular microscope (intra-graft mRNA transcripts) is another aspect that our team is currently evaluating. Further prospective multicenter studies are sorely needed to ascertain if confounding clinical characteristics exist thus facilitating the patient guided care and the improved specificity of this vital non-invasive graft surveillance tool.

DISCLOSURES

This research received grant support from CareDx, Inc. Two of the authors have relationships with CareDx: S.A.H. is a consultant and a member of the advisory board and speaker bureau; S.A.C. is on the speaker bureau.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

None.

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SUPPORTING INFORMATION

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How to cite this article: Afzal A, Alam A, van Zyl JS, et al.

Observed elevated donor-derived cell free DNA in orthotopic heart transplant recipients without clinical evidence of rejection. *Clin Transplant*. 2022;36:e14549.

<https://doi.org/10.1111/ctr.14549>