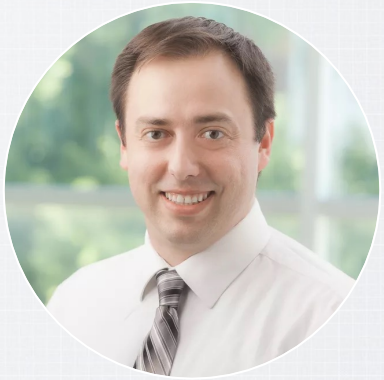
The Use of cf-DNA Testing for Rejection Surveillance in Heart Transplantation

TODAY'S PRESENTER



Marshall P. Hyden II

MD

Advanced Heart Failure and
Transplant Cardiology

UNMC
Nebraska
Medicine

Tuesday, April 23 2024, 2:00pm – 3:00pm ET

Continuing Education Information

Evaluations & Certificates

Nursing

The Organ Donation and Transplantation Alliance is offering **1.0 hours of continuing education credit** for this offering, approved by The California Board of Registered Nursing, Provider Number CEP17117. No partial credits will be awarded. CE credit will be issued upon request within 30 days post-webinar.

CEPTC

The Organ Donation and Transplantation Alliance will be offering **1.0 Category I CEPTC credits** from the American Board for Transplant Certification. Certified clinical transplant and procurement coordinators and certified clinical transplant nurses seeking CEPTC credit must complete the evaluation form within 30 days of the event.

Certificate of Attendance

Participants desiring CE's that are not being offered, should complete a certificate of attendance.

- Certificates should be claimed within 30 days of this webinar.
- We highly encourage you to provide us with your feedback through completion of the online evaluation tool.
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- You will receive a certificate via email upon completion of a certificate request or an evaluation
- Group leaders, please share the follow-up email with all group participants who attended the webinar.

Deanna Fenton

Senior Manager, Program Development and Operations

THE Alliance

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786-866-8730

Meet Our Moderator

Lecia Snell Kinen MSN, RN

Manager,
Heart Transplant Program

Children's NEBRASKA

Meet Our Presenter

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Noninvasive Surveillance of heart transplant rejection – cell free DNA

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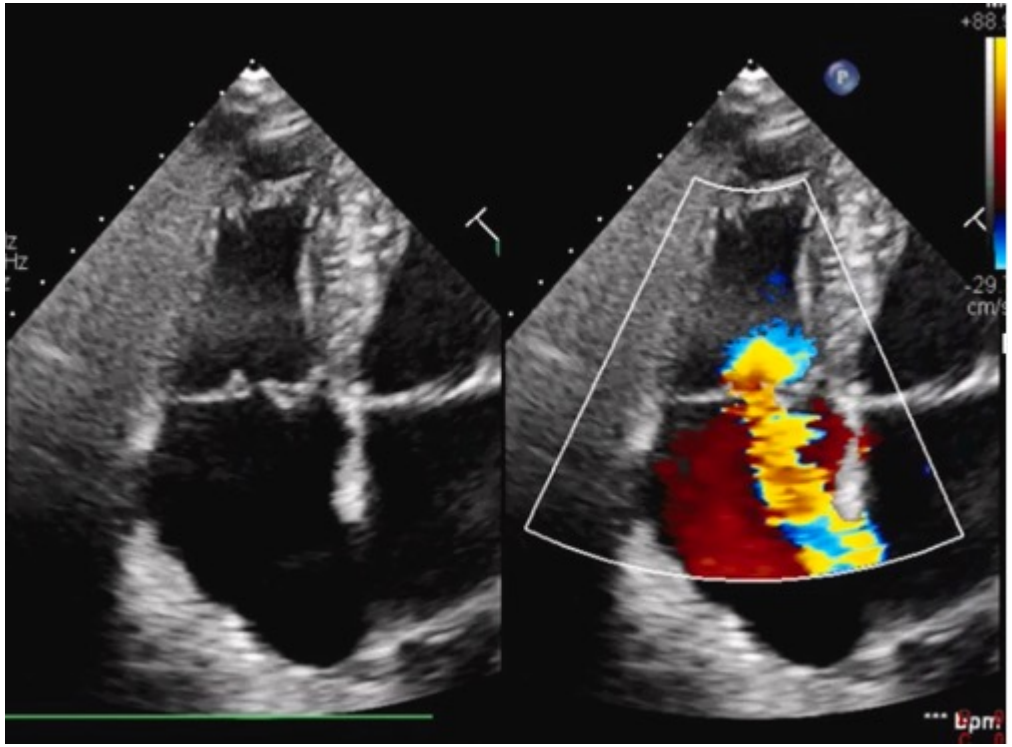
Monitoring for Acute Rejection of Heart transplant

- **Historically monitoring was performed via frequent clinic visits with echocardiogram and routine scheduled endomyocardial biopsy.**
  - With recurrent biopsies rates of complications, particularly tricuspid regurgitation were increased

- **Tricuspid regurgitation occurs due to disruption of the tricuspid valve apparatus during biopsy.**
  - With increased number of biopsies performed, likelihood of obtaining sample of scar tissue is increased leading to more passes of the bioptome and higher risk of complication.



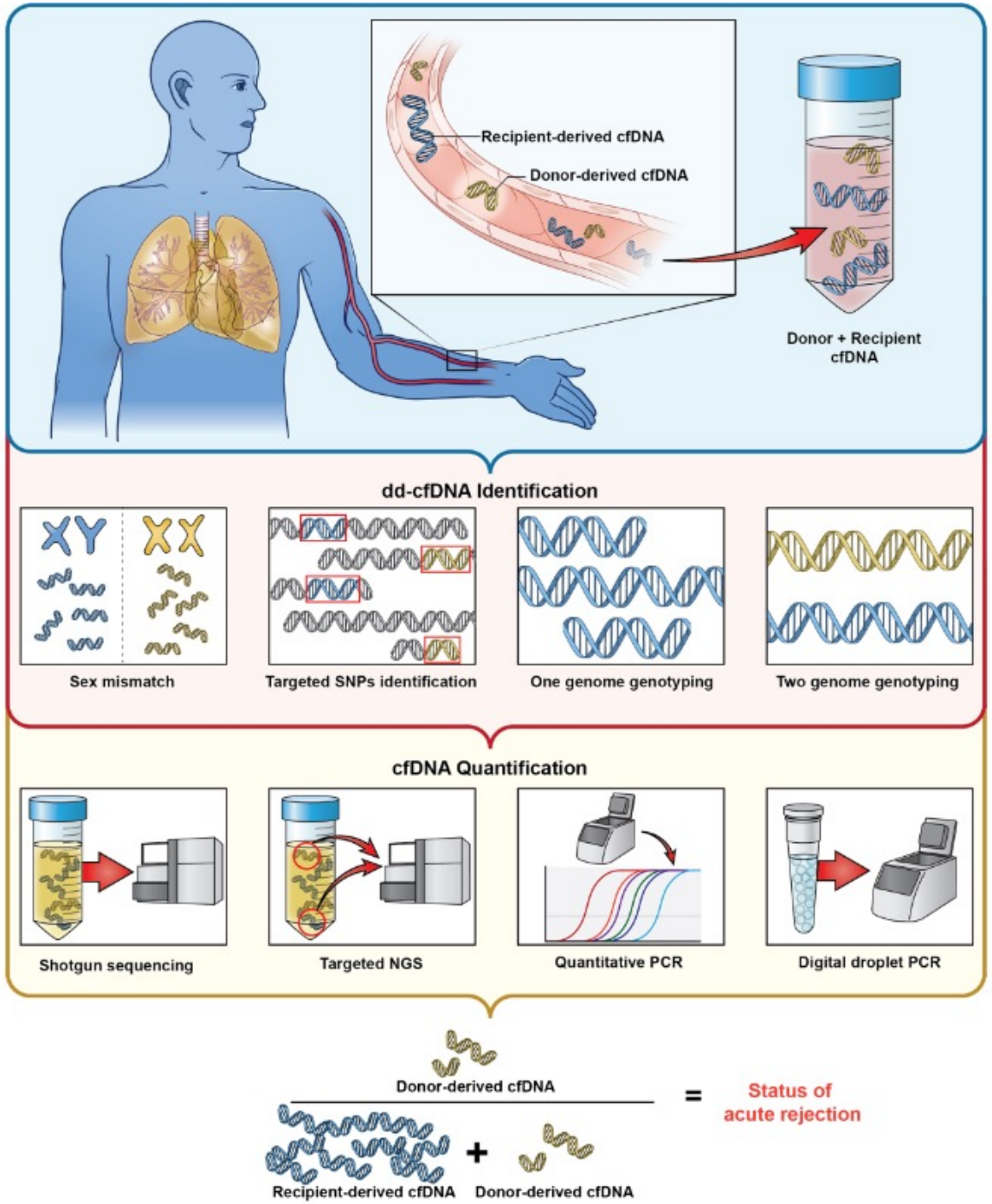
Monitoring for Acute Rejection of Heart transplant

- **Gene expression profiling on peripheral blood samples was developed which reduced scheduled biopsies. Using this technology, at Nebraska Medicine, biopsies were no longer scheduled after the first few months following transplant and were performed only for cause or when gene expression testing was positive.**
  - High rate of positive testing leading to additional screening biopsies, particularly when patient has acute viral infections

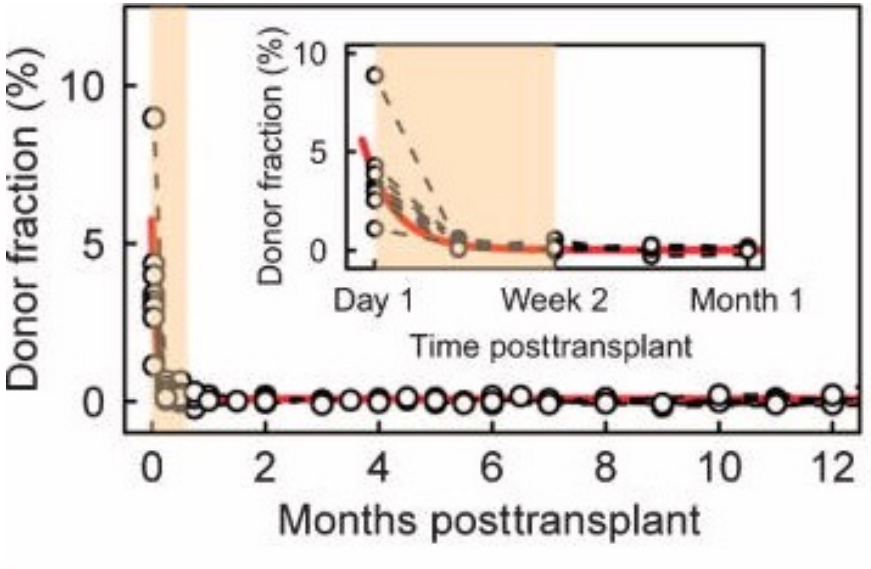
- **Donor derived cell free DNA testing allowed for further reduction in scheduled biopsies with significant reduction in false positive tests.**
  - Scheduled endomyocardial biopsies reduced to 5 total 9/1/2021 and recently further reduced to 3.

Donor derived cell free DNA

- When allograft cells undergo apoptosis or necrosis, they release fragments of double-stranded DNA into the circulation known as cell-free DNA
- The majority of these fragments are mononucleosomal with lengths of 150-180 base pairs
- The half life of cfDNA has been estimated to range from 16 minutes to 2.5 hours in the bloodstream
- These fragments can be identified, sequenced and a percentage of donor derived cell free DNA can be obtained.
- The percentage of donor derived cell free DNA highly correlates to acute rejection

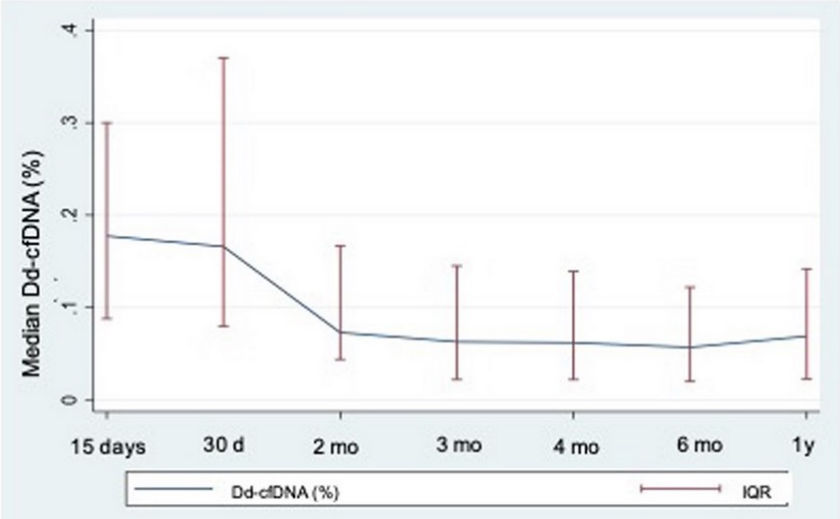


Keller M, Agbor-Enoh S. Donor-Derived Cell-Free DNA for Acute Rejection Monitoring in Heart and Lung Transplantation. Curr Transplant Rep. 2021;8(4):351-358. doi: 10.1007/s40472-021-00349-8. Epub 2021 Nov 5. PMID: 34754720; PMCID: PMC8570240.



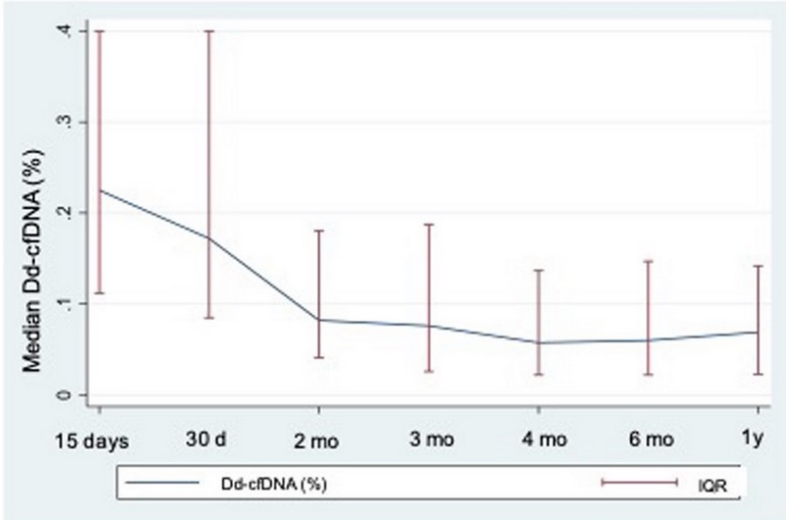
De Vlaminck I, Valantine HA, Snyder TM, Strehl C, Cohen G, Luikart H, Neff NF, Okamoto J, Bernstein D, Weisshaar D, Quake SR, Khush KK. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med. 2014 Jun 18;6(241):241ra77. doi: 10.1126/scitranslmed.3007803. PMID: 24944192; PMCID: PMC4326260.

Figure 1A. Donor-derived cell-free DNA kinetics in patients with 0R



1006 pairs of endomyocardial biopsy and ddcfDNA from 206 patients

Figure 1B. Donor-derived cell-free DNA kinetics in patients with 0-1R



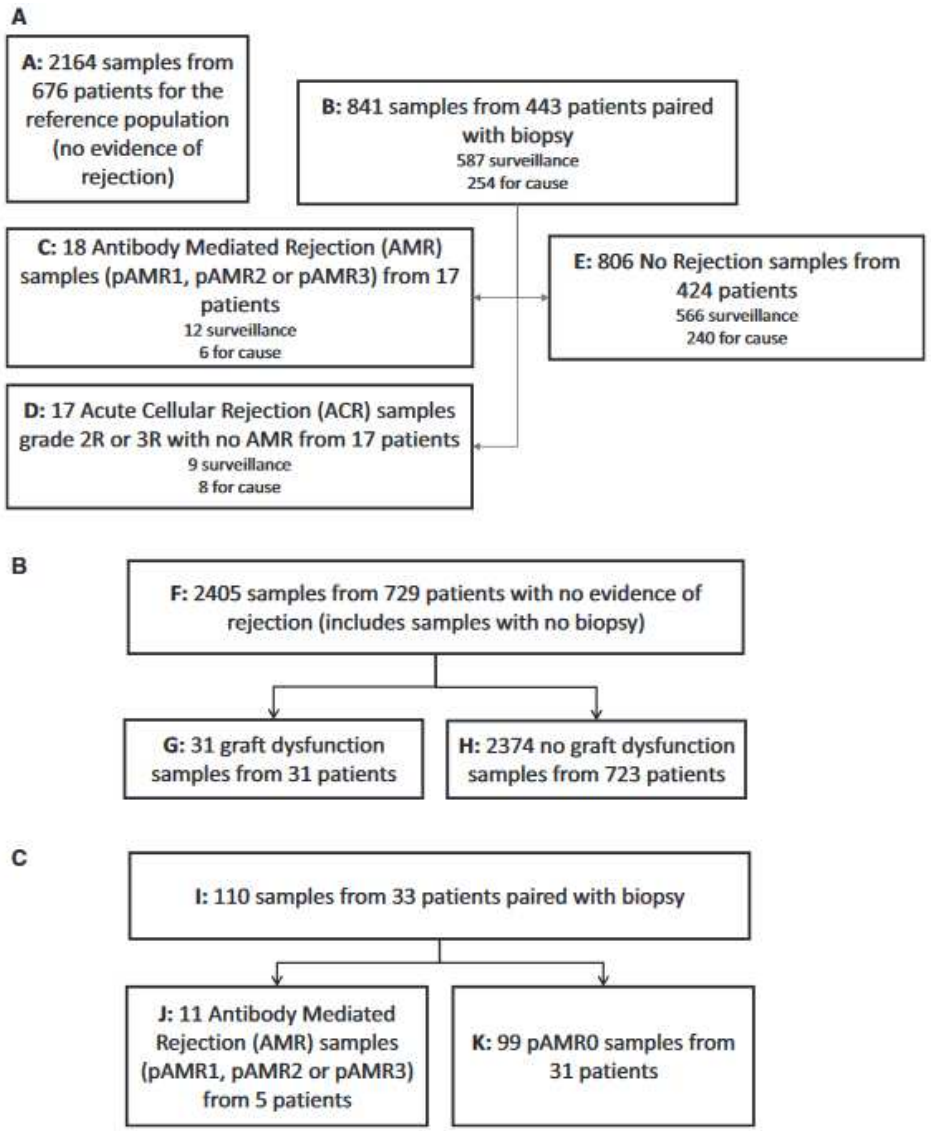
	Time point 1 (15 days)	Time point 2 (30 days)	Time point 3 (60 to 365 days)	p value* (Time point 1 vs 2)	p value** (Time point 1 vs 3)	p value*** (Time point 2 vs 3)
0R (n=52)	0.177 (0.088-0.3)	0.166 (0.08-0.37)	0.065 (0.03-0.144)	0.777	< 0.001	< 0.001
0-1R (n=962)	0.23 (0.11-0.4)	0.172 (0.085-0.4)	0.069 (0.03-0.16)	0.135	< 0.001	< 0.001

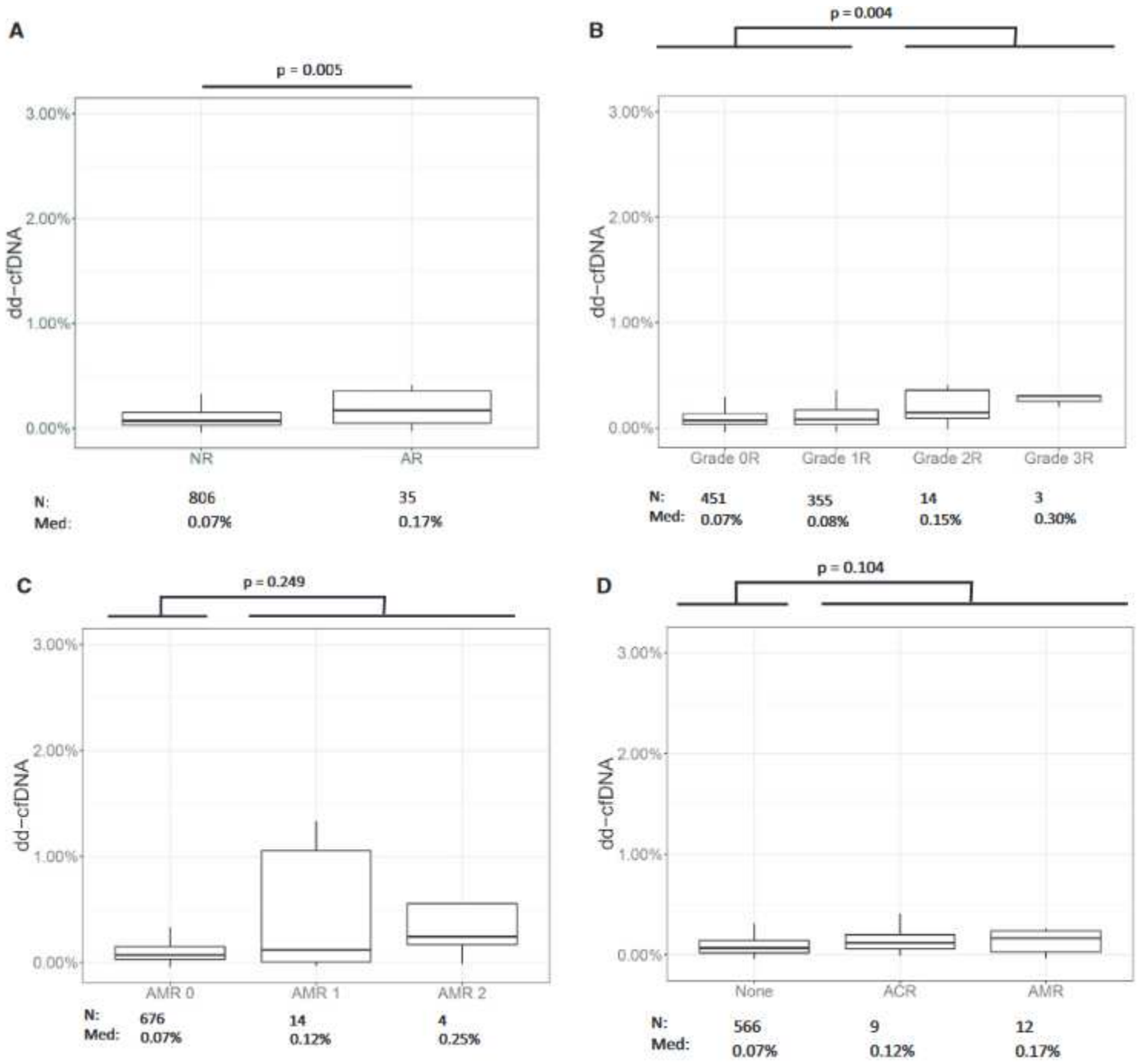
M Jimenez-Blanco Bravo, M Crespo Leiro, E Gonzalez Sardinero, M Gomez Bueno, R Lopez Villela, C Ortiz Bautista, M Farrero, A Maestro Benedicto, B Diaz Molina, C Diez Lopez, D Rangel Sousa, N Salterain, I Garrido Bravo, J Segovia Cubero, Donor-derived cell-free DNA kinetics in the first year post-transplant in patients without acute cellular rejection, *European Heart Journal*, Volume 44, Issue Supplement_2, November 2023, ehad655.1042, https://doi.org/10.1093/eurheartj/ehad655.1042

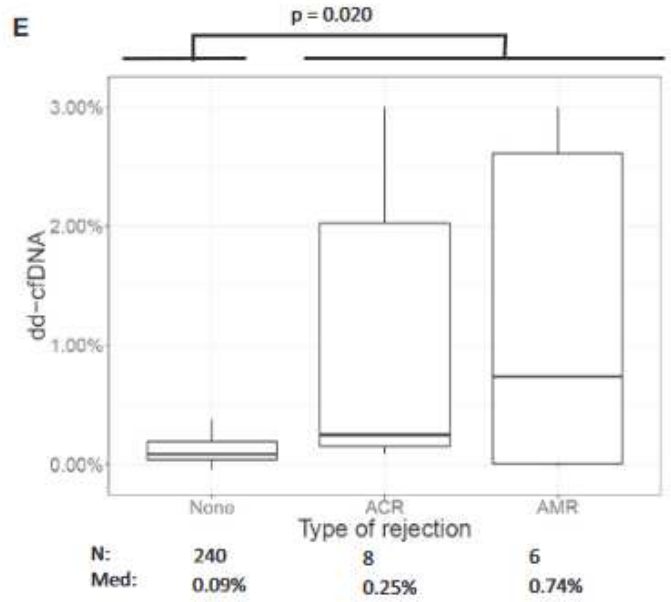
Nonivasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study

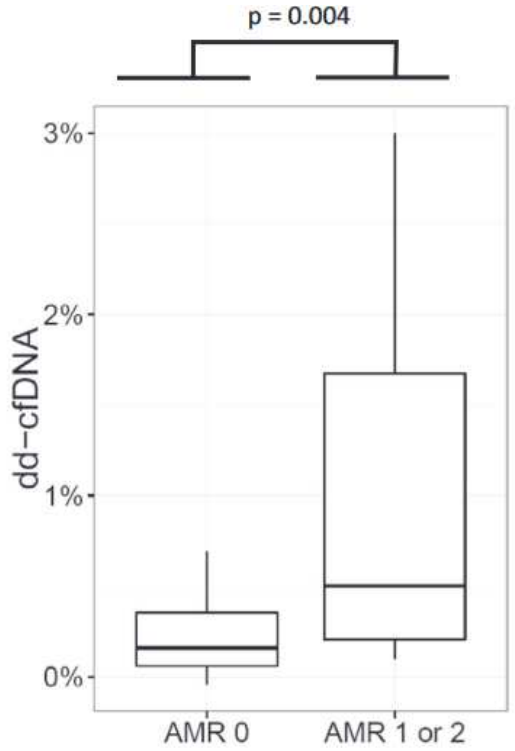
- Observational, prospective, multi-center registry to evaluate clinical outcomes in heart transplant recivpients
- Patients were heart transplant recipients 15 years or older and greater than 55 days post-transplant who were undergoing AlloMap gene expression profiling for rejection surveillance
- Blood specimens were collected for dd-cfDNA levels at each surveillance visit that occurred for AlloMap testing. Schedule determined by each center's standard of care. From 2016-2018 dd-CcfDNA was drawn only when there was clinical suspicion of rejection and a planned for-cause biopsy
- A parallel single center study was conducted at Cedars-Sinai Medical Center with inclusion criteria of pretransplant PRA greater than or equal to 10%, presences of post-transplant DSA or biopsy-proven AMR or for-cause biopsy due to reduced LVEF

Khush KK, Patel J, Pinney S, Kao A, Alharethi R, DePasquale E, Ewald G, Berman P, Kanwar M, Hiller D, Yee JP, Woodward RN, Hall S, Kobashigawa J. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. Am J Transplant. 2019 Oct;19(10):2889-2899. doi: 10.1111/ajt.15339. Epub 2019 Apr 8. PMID: 30835940; PMCID: PMC6790566.


A
A: 2164 samples from 676 patients for the reference population (no evidence of rejection)
B: 841 samples from 443 patients paired with biopsy
587 surveillance
254 for cause
C: 18 Antibody Mediated Rejection (AMR) samples (pAMR1, pAMR2 or pAMR3) from 17 patients
12 surveillance
6 for cause
E: 806 No Rejection samples from 424 patients
566 surveillance
240 for cause
D: 17 Acute Cellular Rejection (ACR) samples grade 2R or 3R with no AMR from 17 patients
9 surveillance
8 for cause
B
F: 2405 samples from 729 patients with no evidence of rejection (includes samples with no biopsy)
G: 31 graft dysfunction samples from 31 patients
H: 2374 no graft dysfunction samples from 723 patients
C
I: 110 samples from 33 patients paired with biopsy
J: 11 Antibody Mediated Rejection (AMR) samples (pAMR1, pAMR2 or pAMR3) from 5 patients
K: 99 pAMR0 samples from 31 patients


A
p = 0.005
3.00%
2.00%
1.00%
0.00%
dd−cfDNA
NR
AR
N: 806 35
Med: 0.07% 0.17%
B
p = 0.004
3.00%
2.00%
1.00%
0.00%
dd−cfDNA
Grade 0R
Grade 1R
Grade 2R
Grade 3R
N: 451 355 14 3
Med: 0.07% 0.08% 0.15% 0.30%
C
p = 0.249
3.00%
2.00%
1.00%
0.00%
dd−cfDNA
AMR 0
AMR 1
AMR 2
N: 676 14 4
Med: 0.07% 0.12% 0.25%
D
p = 0.104
3.00%
2.00%
1.00%
0.00%
dd−cfDNA
None
ACR
AMR
N: 566 9 12
Med: 0.07% 0.12% 0.17%





- Median dd-cfDNA significantly higher in patients with acute rejection 0.17% compared to patients without rejection 0.07%.
- ACR grade 1R had similar median level 0.08% to grade 0 biopsies

FIGURE 5 Donor-derived cell-free DNA (dd-cfDNA) correlates with antibody-mediated rejection (AMR) in the Cedars-Sinai study. Sample sizes and median dd-cfDNA levels for samples from patients with AMR grade pAMR0 and AMR grade ≥ pAMR1. Samples with pAMR1 or higher have elevated levels of dd-cfDNA

Cell-Free DNA to Detect Heart Allograft Acute Rejection

- Multicenter prospective cohort study
- 165 patients with median post-transplant follow-up of 17.7 months
- Endomyocardial biopsy performed on average 8 times per patient with average of 4.3 tissue fragments per biopsy
- Histopathology was compared to paired ddcfDNA starting 28 days post-transplant

Agbor-Enoh et al. Circulation. Cell-Free DNA to Detect Heart Allograft Acute Rejection, Volume: 143, Issue: 12, Pages: 1184-1197, DOI: (10.1161/CIRCULATIONAHA.120.049098)

Table 2. %ddcfDNA for Primary and Secondary End Points From Day 28 Onward (Table view)

Clinical end point	Number of events	Subjects with events	Median %ddcfDNA	%ddcfDNA interquartile range (%)	*P* value
Controls (ACR 0, ACR 1, AMR 0)	1072	165	0.03	0.01–0.14	—
Acute rejection	49	31	0.38	0.31–0.83	<0.001*
ACR					
Grade 0	618	165	0.02	0.01–0.13	—
Grade 1	454	165	0.04	0.01–0.17	0.023†
Grade ≥2	28	21	0.34	0.28–0.72	<0.001†
AMR					
Grade 0	1072	165	0.03	0.01–0.14	—
Grade 1	14	9	0.63	0.34–0.77	<0.001‡
Grade ≥2	11	9	1.68	0.49–2.79	<0.001‡
Allograft dysfunction					
None	866	165	0.02	0.01–0.12	—
Mild	168	83	0.06	0.01–0.27	0.068§
Moderate	62	49	0.19	0.01–0.60	0.018§
Severe	38	28	0.32	0.05–0.47	<0.001§

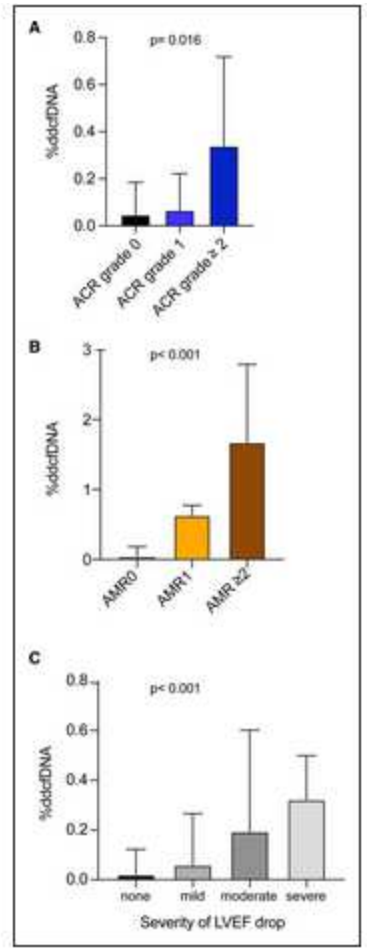
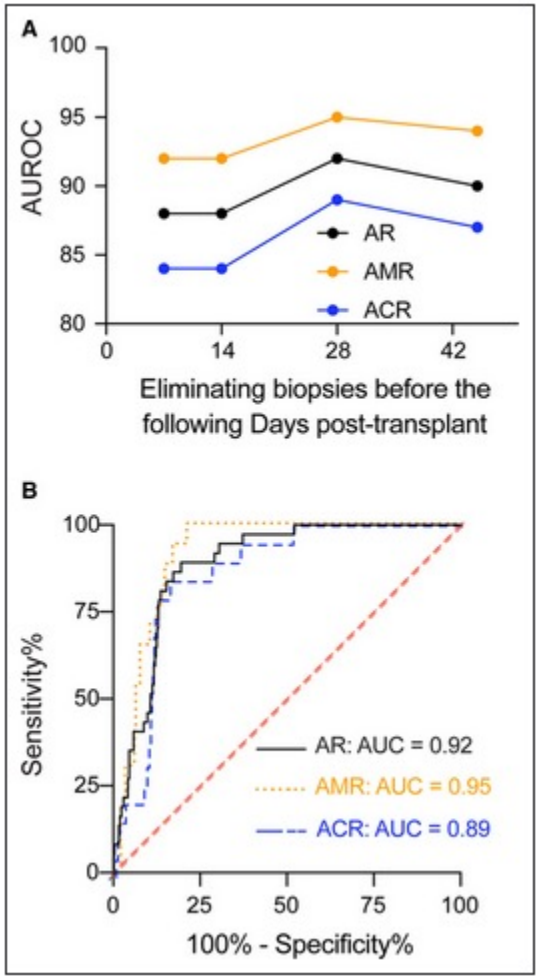


Figure 3. Correlation %ddcfDNA measures with biopsy-graded rejection and allograft dysfunction by echocardiography. A, %ddcfDNA in relation to severity of acute cellular rejection (ACR) by histopathologic interpretation of endomyocardial biopsy. Grade 0 rejection includes both ACR grade 0 and AMR 0. *P* value obtained by generalized estimating equation approach comparing all categories. **B**, %ddcfDNA in relation to severity of antibody-mediated rejection by histopathologic interpretation of the endomyocardial biopsy. Grade 0 rejection includes both ACR grades 0 or 1 and AMR 0. *P* value obtained by generalized estimating equation approach comparing all categories. **C**, %ddcfDNA in relation to severity of allograft dysfunction measured by echocardiography. Allograft dysfunction was defined as a reduction of left ventricular ejection fraction (LVEF) by ≥5%, and further stratified by severity on the basis of the magnitude of LVEF decline as no (<5%), mild (5% to <10%), moderate (≥10% to <15%), or severe (≥15%) allograft dysfunction. *P* value obtained by generalized estimating equation approach comparing all categories. %ddcfDNA indicates percentage of donor-derived cell-free DNA.

Variable	Sensitivity (%)			Specificity (%)			Area under the receiver operator characteristics curve
%ddcfDNA threshold	0.1	0.25	0.5	0.1	0.25	0.5	(95% CI)
Acute rejection diagnosis							
Acute rejection	95	81	41	68	85	92	0.92 (0.88–0.95)
Antibody-mediated rejection	100	88	65	68	85	92	0.95 (0.90–0.99)
Acute cellular rejection	89	79	21	68	85	92	0.89 (0.83–0.95)

Variable	%ddcfDNA, n (%)	
	≥0.25%	<0.25%
Biopsy + histopathology		
Positive	33 (19.6)	6 (0.8)
Negative	135 (80.4)	745 (99.2)
Total	168 (100)	751 (100)

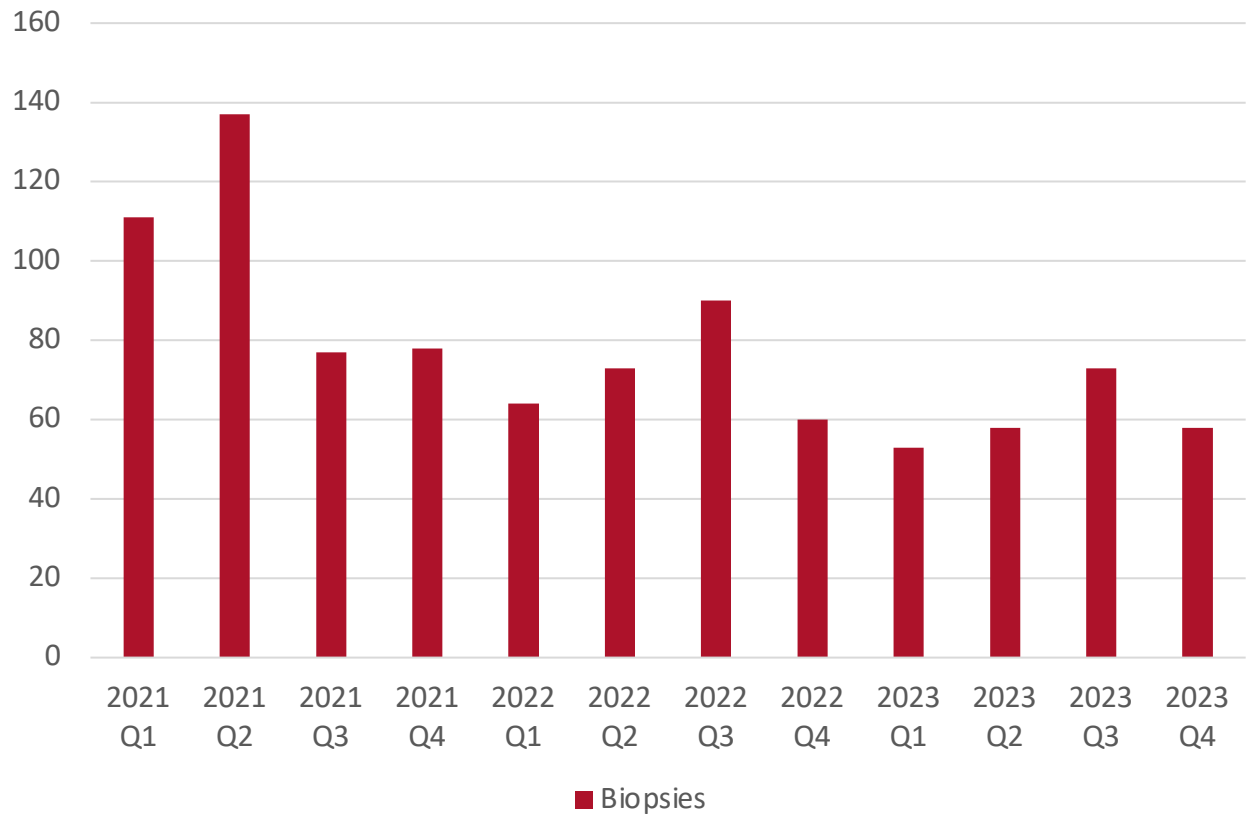

A
100
95
90
85
80
AUROC
AR
AMR
ACR
0
14
28
42
Eliminating biopsies before the following Days post-transplant
B
100
75
50
25
0
Sensitivity%
AR: AUC = 0.92
AMR: AUC = 0.95
ACR: AUC = 0.89
0
25
50
75
100
100% - Specificity%

Monitoring for Acute Rejection of Heart transplant

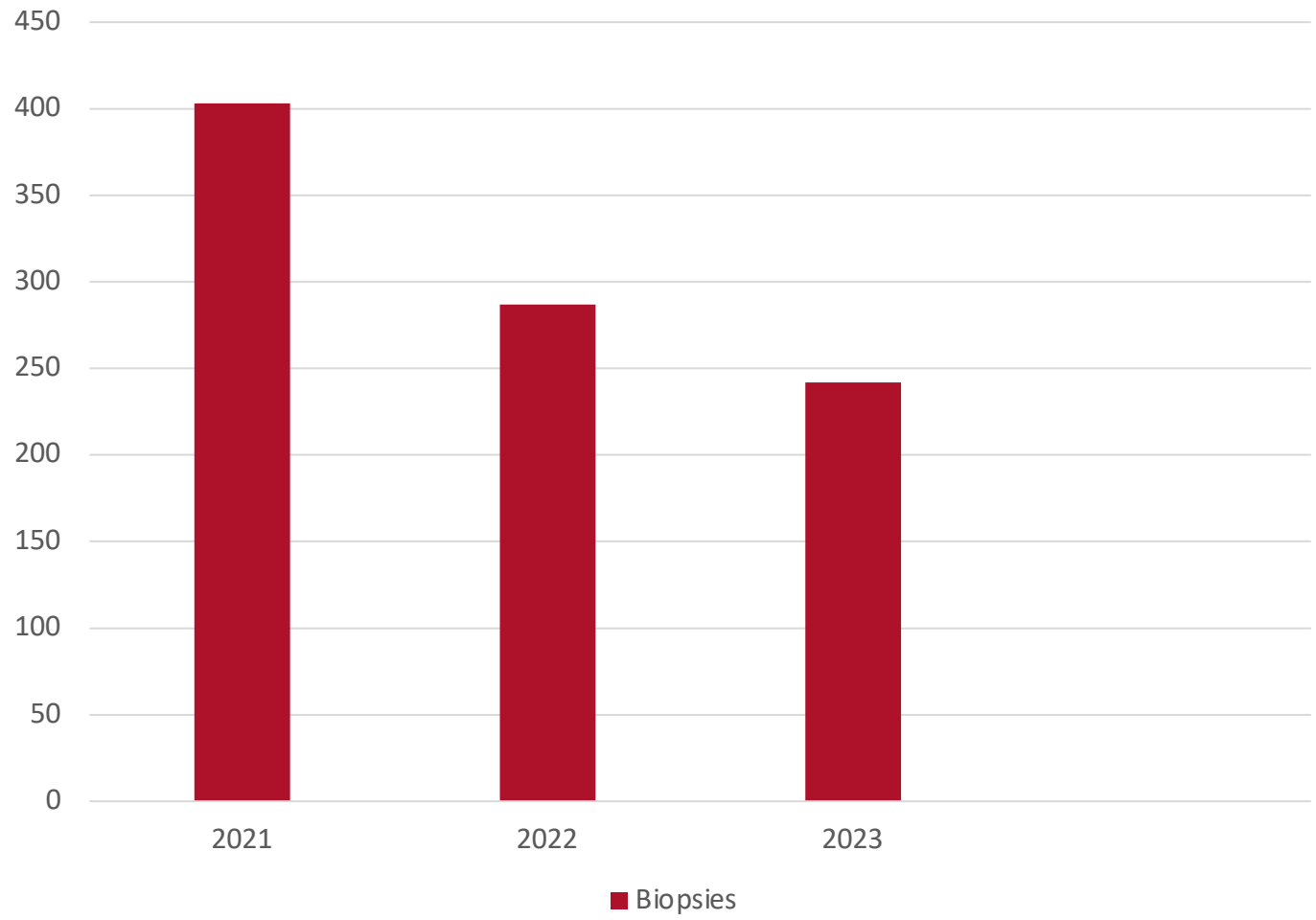
- **Donor derived cell free DNA testing allowed for further reduction in scheduled biopsies with significant reduction in false positive tests.**
  - Scheduled endomyocardial biopsies reduced to 5 total 9/1/2021 and recently further reduced to 3.

Biopsies Performed By Calendar Quarter

New policy officially adopted 9/1/22



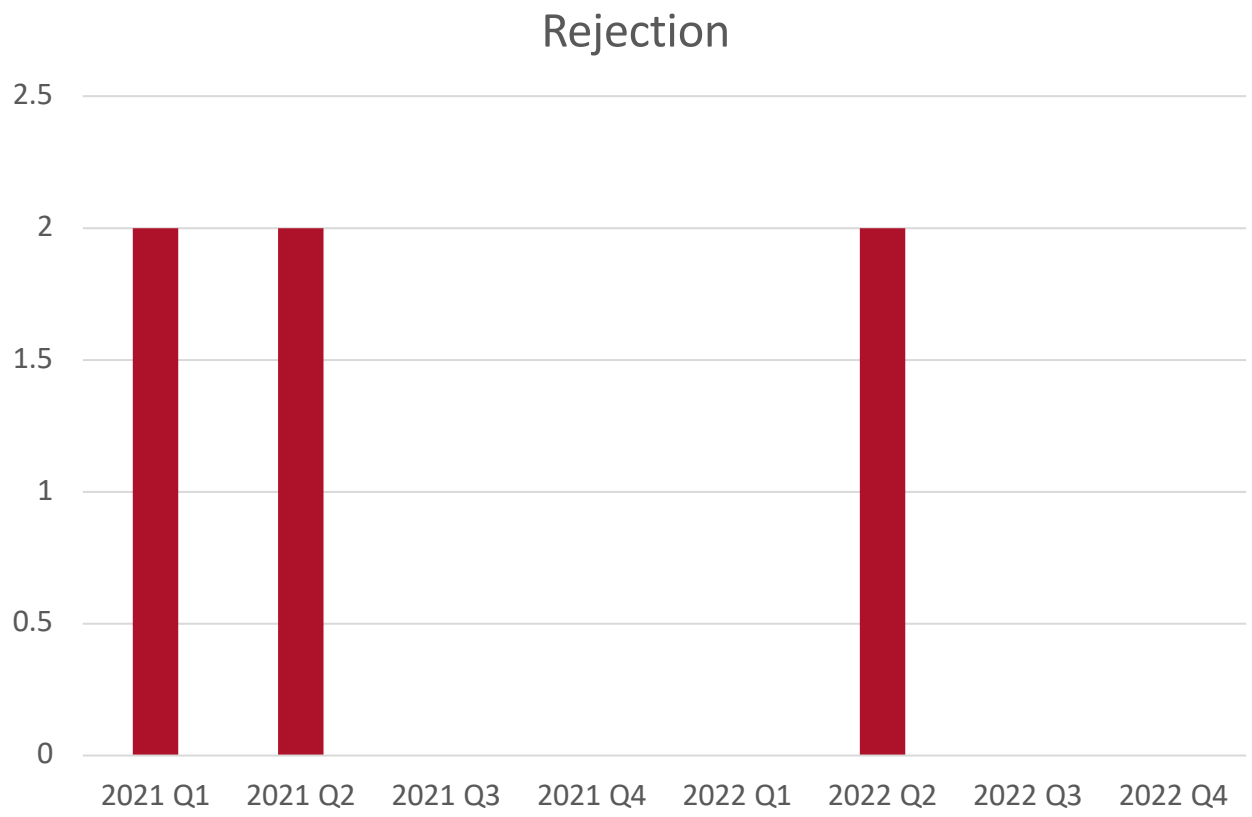
Heart Transplant
of Biopsies by Year



1-year Post Heart Transplant Rejection Rates

TCMR Grade > 1R or AMR C4D +

Key Take-Away
1 year rejection rates remained low



MMDX

- Molecular Microscope Diagnostic System uses gene expression profiling by measuring mRNA transcript levels in endomyocardial biopsy tissue.

- Endomyocardial biopsy performed per protocol and sent to pathology for histopathological evaluation. Additional samples obtained for MMDX and sent to outside lab.

Comparison	Rejection surveillance results			
MMDx vs. EMBx	MMDx	EMBx		
		No Rejection	*Rejection*	*Total*
	No Rejection	167	5	172
	Rejection	32	24	56
	Total	199	29	228
MMDx vs. dd-cfDNA	*MMDx*	dd-cfDNA		
		No Rejection	*Rejection*	*Total*
	No Rejection	90	38	128
	Rejection	12	36	48
	Total	102	74	176
dd-cfDNA vs. EMBx	EMBx	dd-cfDNA		
		No Rejection	*Rejection*	*Total*
	No Rejection	101	52	153
	Rejection	1	22	23
	Total	102	74	176

*Evolving the surveillance and workup of heart transplant rejection: A real-world analysis of the Molecular Microscope Diagnostic SystemAmit Alam, Johanna Van Zyl, Gregory Paul Milligan, Staci Michelle McKean, Raksha Patel, Shelley Anne Hall ,American Journal of Transplantation,*Volume 22 Issue 10 Pages 2443-2450 (October 2022)

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Q&A
QUESTIONS & ANSWERS