Review of dd-cfDNA in Kidney, Heart, and Lung Transplant

Vanderbilt 20th Annual Nurse Practitioner Symposium

October 14th, 2024

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Outline for Today's Session

Topic	Speaker	Time
Opening and Introductions/Grab Lunch	All	12:00- 12:10pm
Update Data on the Utilization of dd-cfDNA in Kidney transplantation	Chris Ensor, PharmD	12:10-12:25pm
SHORE Registry Data Update	Chris Ensor, PharmD	12:25-12:40pm
Extreme Molecular Injury in Lung transplant	Jennifer Gray, PharmD	12:40-12:55pm
Questions and Answer Session	All	12:55-1pm

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nature medicine

Article

https://doi.org/10.1038/s41591-024-03087-3

Cell-free DNA for the detection of kidney allograft rejection

Olivier Aubert[1,2], Cindy Ursule-Dufait[1], Romain Brousse[1], Juliette Gueguen[1], Maud Racapé[1], Marc Raynaud[1], Elisabet Van Loon[3], Angelica Pagliazzi[3], Edmund Huang[4], Stanley C. Jordan[4], Kenneth D. Chavin[5], Gaurav Gupta[6], Dhiren Kumar[6], Tarek Alhamad[7], Sanjiv Anand[8], Jorge Sanchez-Garcia[8], Basmah A. Abdalla[9], Julien Hogan[10], Rouba Garro[11], Darshana M. Dadhania[12], Pranjal Jain[13], Didier A. Mandelbrot[14], Maarten Naesens[3], Raja Dandamudi[15], Vikas R. Dharnidharka[15], Dany Anglicheau[1,2], Carmen Lefaucheur[1,16] & Alexandre Loupy[1,2]

Nature Medicine | Volume 30 | August 2024 | 2320–2327

Derrick C, Kidney Transplant Recipient

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Cell-Free DNA for the Detection of Kidney Allograft Rejection

The AlloSure Nature Medicine Publication (embargoed until Monday, June 3, 2024)

Reflective of real-world and contemporary kidney transplant cohorts

Study Design:

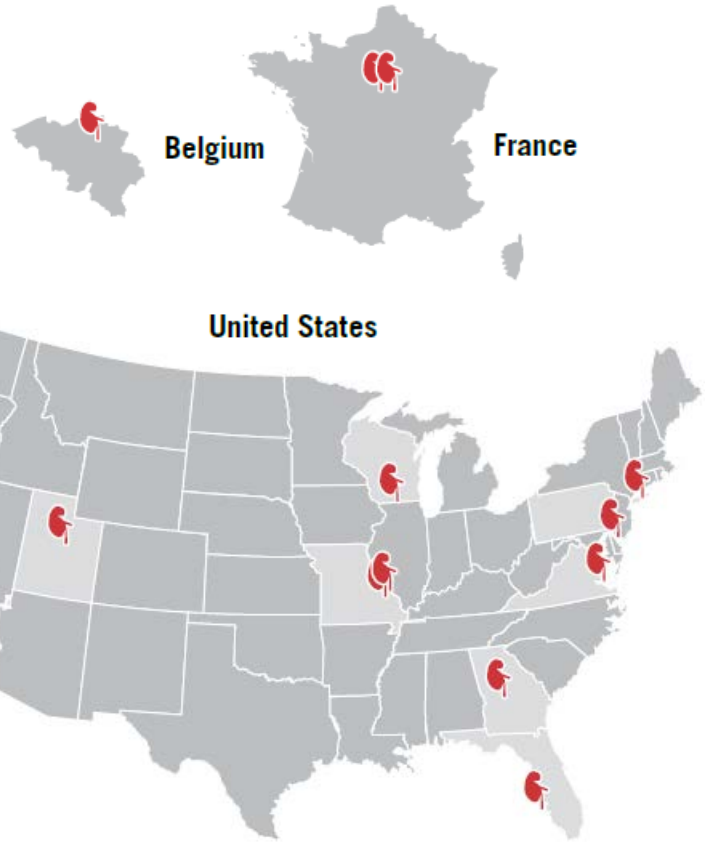
Multi-Center
14 Transplant Centers

International Number of Transplant Centers Participating:
US = 11 France = 2 Belgium = 1

Robust Patient Cohorts
2,882 Kidney Transplant Recipients (KTRs) with 3,732 Biopsy-Paired dd-cfDNA Assessments

Derivation Cohort	Validation Cohort
n=1,134 KTRs (France)	n=1,748 KTRs* (US & Belgium)

Short and Long-Term Outcomes
Outcomes Examined Within the First Year Post-Transplant and > 1 Year Post-Transplant



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Manuscript accepted: Aubert O, *et al*. Donor Derived Cell-Free DNA and Rejection of Kidney Allografts. *Nat Med.* 2024

Baseline Recipient Characteristics

Reflective of real-world and contemporary kidney transplant cohorts

	Derivation cohort (n=1,134)		External validation cohort (n= 1,748)	
	N		N	
Recipient characteristics				
Age (years), mean (SD)	1,134	55.22 (14.85)	1,745	45.87 (18.14)
Sex male, No. (%)	1,134	693 (61.11)	1,735	1,009 (58.16)
Cause of end stage renal disease	1,134		1,707	
Glomerulopathy, No. (%)		294 (25.93)		509 (29.82)
Polycystic kidney disease, No.(%)		176 (15.52)		191 (11.19)
Interstitial nephritis (%)		94 (8.29)		178 (10.43)
Diabetes, No. (%)		104 (9.17)		290 (16.99)
Vascular, No. (%)		93 (8.20)		235 (13.77)
Other, No. (%)		145 (12.79)		201 (11.78)
Unknown etiology, No (%)		228 (20.11)		103 (6.03)

Table 1: Baseline patient characteristics in the derivation and validation cohort

- **Time post-transplant to 1st dd-cfDNA-paired biopsy:**
  - **1 year (IQR 0.26 - 1.59)**
  - **0.85 years (IQR 0.26 - 2.05)**
- **Median dd-cfDNA**
  - **0.27% (IQR: 0.16 - 0.46)**
  - **0.4% (IQR: 0.19 - 1.2)**

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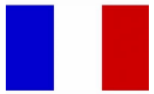
Aubert O, *et al*. Donor Derived Cell-Free DNA and Rejection of Kidney Allografts. *Nat Med.* 2024

dd-cfDNA Paired Biopsy Characteristics

Large number of biopsies and rejection cases



62% Protocol Biopsy, 38% For- Cause

Biopsy findings, No.(%)	1,415	
Active AMR		129 (9.12)
Chronic active AMR		42 (2.97)
Inactive AMR		11 (0.78)
Equivocal for diagnosis of AMR		5 (0.35)
Acute TCMR		15 (1.06)
Chronic active TCMR		19 (1.34)
Mixed rejection		17 (1.20)
Borderline lesions		19 (1.34)
Viral nephritis		20 (1.41)
Glomerulitis without rejection		30 (2.12)
FSGS		48 (3.39)
IF-TA		557 (39.36)
No specific lesions		503 (35.55)

Supplementary Table 1

+

26% Protocol Biopsy, 74% For- Cause

Biopsy findings, No.(%)	2,317	
AMR		352 (15.19)
TCMR		224 (9.67)
Mixed rejection		103 (4.45)
Borderline lesions		183 (7.90)
Viral nephritis		100 (4.32)
Glomerulitis without rejection		62 (2.68)
FSGS		32 (1.38)
IF-TA		624 (26.93)
No specific lesions		637 (27.49)

Supplementary Table 3

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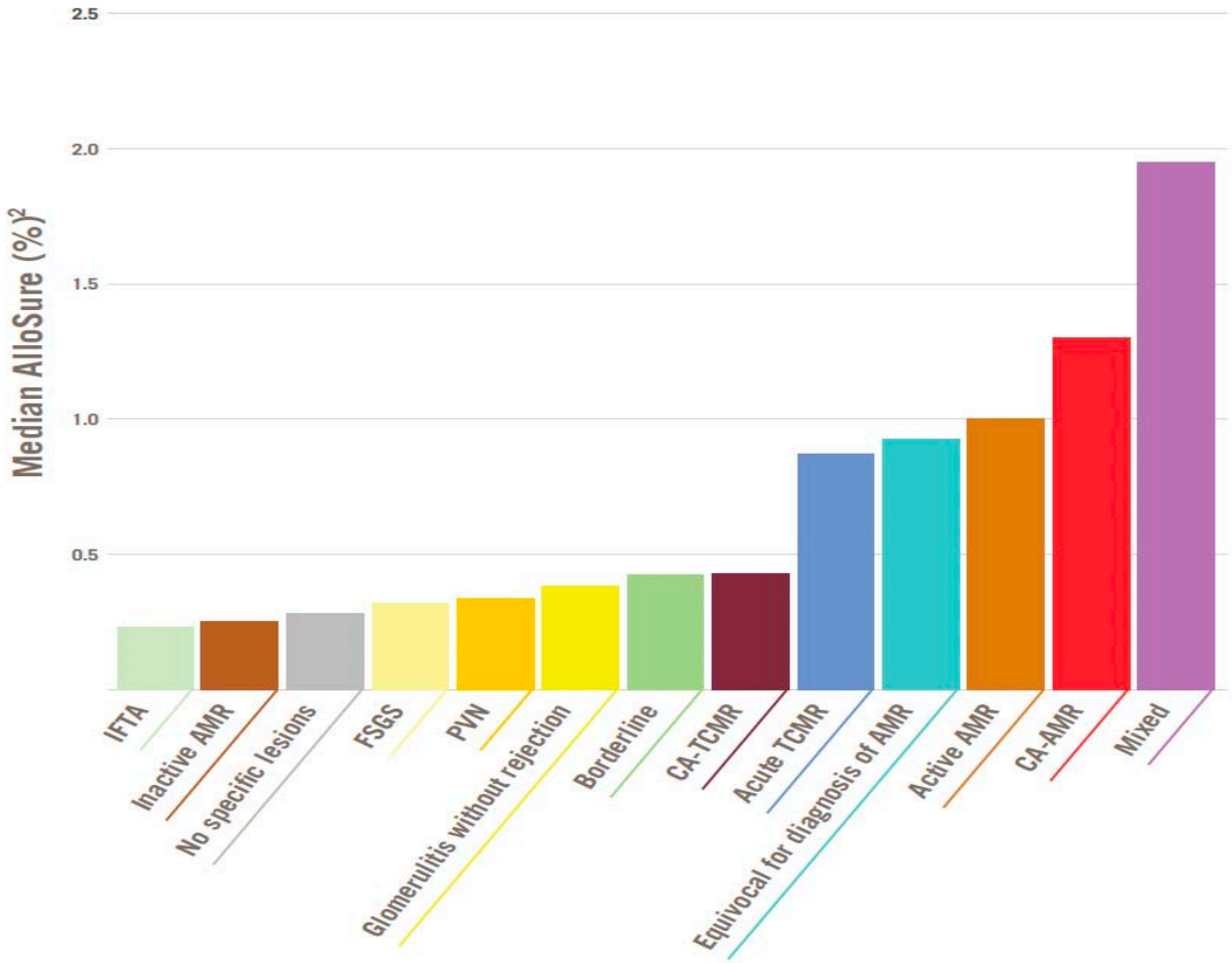
Manuscript accepted:
Aubert O, *et al*. Donor Derived Cell-Free DNA and Rejection of Kidney Allografts. *Nat Med.* 2024

Elevated levels of AlloSure are highly associated with the presence and activity of all types of rejection



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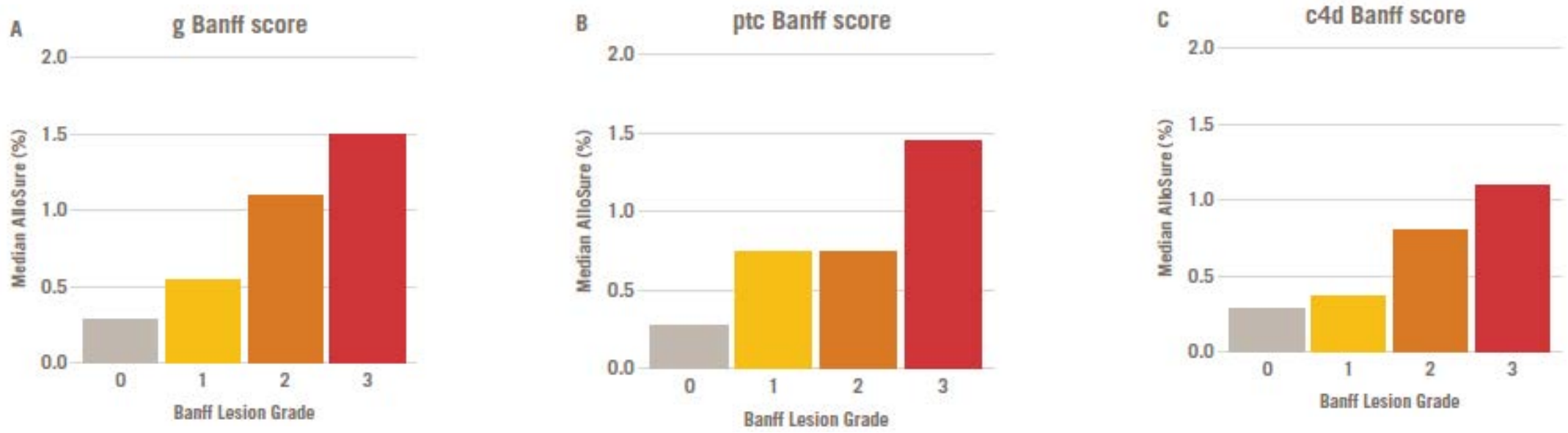
Manuscript accepted: Aubert O, *et al*. Donor Derived Cell-Free DNA and Rejection of Kidney Allografts. *Nat Med.* 2024

Elevated levels of AlloSure are highly associated with the severity of all types of rejection

ABMR Lesions



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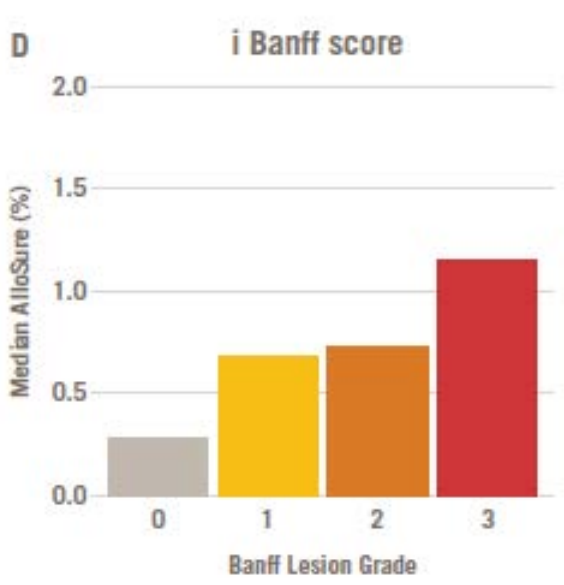
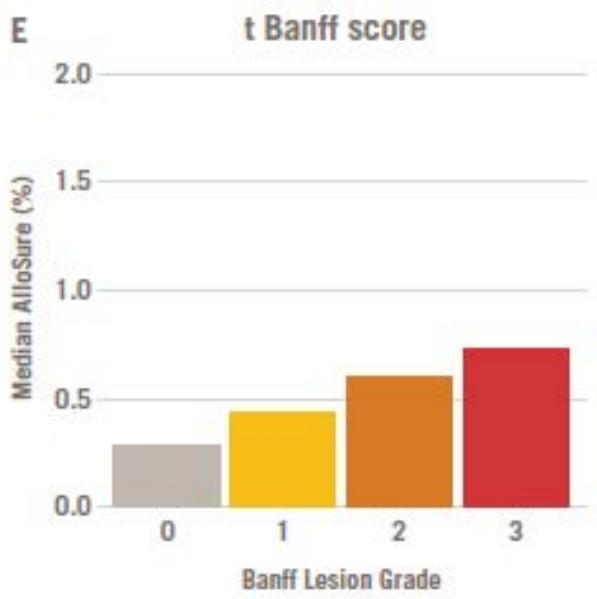
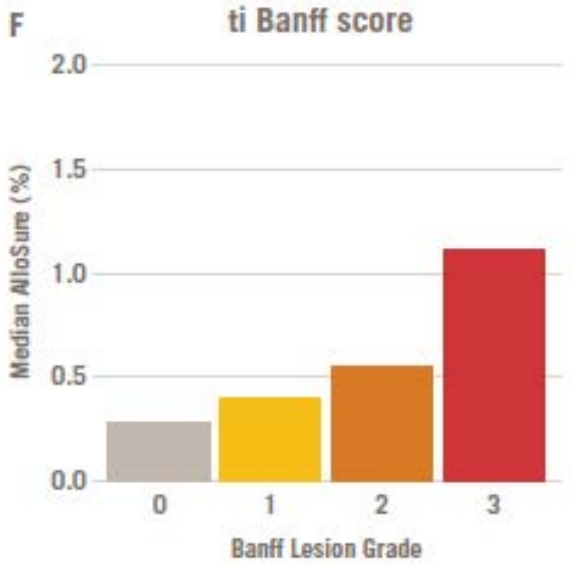
TMCR Lesions







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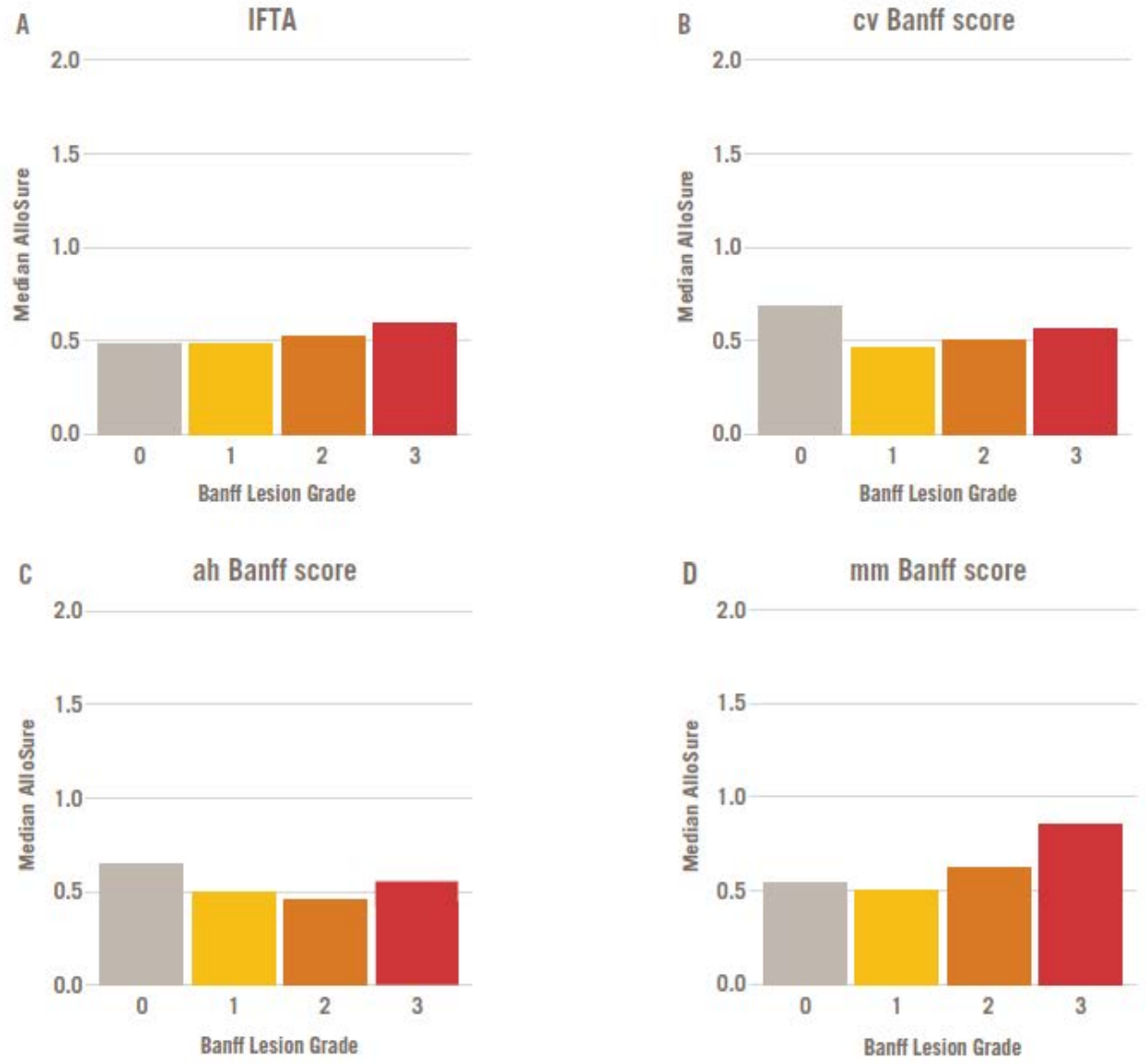
AlloSure levels were not elevated in chronic Banff lesions



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AlloSure was associated with rejection independent of standard of care parameters

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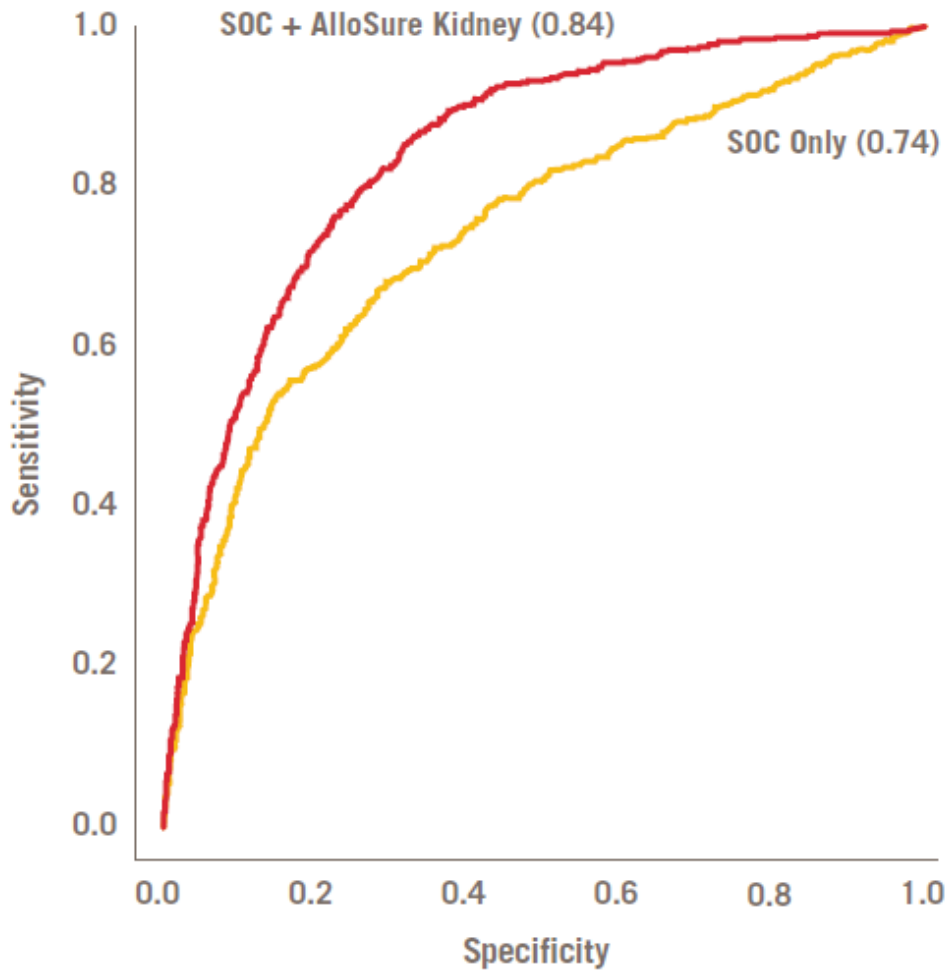
AlloSure enhances standard of care parameters for improved prediction of rejection



Validation cohort included 1,748 patients

	NPV	PPV	ROC AUC
Model with AlloSure and SOC parameters	0.885	0.591	0.842
Model with only SOC parameters	0.817	0.588	0.743
AlloSure Only	0.868	0.555	0.795

SOC Methods = eGFR, Proteinuria, change in Sr Cr., previous episode of rejection, and DSA

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Dd-cfDNA Kinetics Over Time Correlated with Clinical Scenarios

639 patients with 2 dd-cfDNA-paired biopsies (median 6.5 months [IQR 2.92-11.73] between biopsies)

- Dd-cfDNA **remained stable** in subjects with immune quiescence

Supplementary Table 9: Variation of circulating dd-cfDNA levels according to the 4 prototypical patient scenarios over time.

First dd-cfDNA evaluation (mean of the %)	Second allograft evaluation (mean of the %)	n	Delta dd-cfDNA	p-value*
Allograft immune quiescence				
0.56 ± 0.06%	**0.50 ± 0.04%**	386	**-0.06 ± 0.06%**	**0.3472**
De novo allograft rejection				
1.00 ± 0.16%	**2.01 ± 0.30%**	89	**+1.01 ± 0.29%**	**<0.0001**
Treated allograft rejection				
1.64 ± 0.27%	**0.77 ± 0.12%**	75	**-0.87 ± 0.26%**	**<0.0001**
Persisting allograft rejection after treatment				
2.26 ± 0.33%	**1.33 ± 0.17%**	89	**-0.93 ± 0.26%**	**0.0020**

*paired Wilcoxon test for the comparison of dd-cfDNA at the first and second evaluation

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Aubert, O., Ursule-Dufait, C., Brousse, R. *et al.* Cell-Free DNA for the detection of kidney allograft rejection. *Nat Med* (2024). https://doi.org/10.1038/s41591-024-03087-3

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- Dd-cfDNA **remained stable** in subjects with immune quiescence
- Dd-cfDNA **rose significantly** in subjects with de novo rejection
- Dd-cfDNA **declined significantly** in treated rejection and in patients with persistent rejection after treatment
- Subjects with persistent rejection continued to have elevated dd-cfDNA despite decline after treatment

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ORIGINAL CLINICAL SCIENCE

The Journal of Heart and Lung Transplantation

Surveillance with dual noninvasive testing for acute cellular rejection after heart transplantation: Outcomes from the Surveillance HeartCare Outcomes Registry

Kiran Khush, MD, MAS,[a] Shelley Hall, MD,[b] Andrew Kao, MD,[c] Nirav Raval, MD,[d] Ravi Dhingra, MD, MPH,[e] Palak Shah, MD, MS,[f] Lavanya Bellumkonda, MD,[g] Ashwin Ravichandran, MD, MPH,[h] Adrian Van Bakel, MD, PhD,[i] Nir Uriel, MD,[j] Snehal Patel, MD,[k] Sean Pinney, MD,[l] Eugene DePasquale, MD,[m] David A. Baran, MD,[n] Kevin Pinney, BSc,[o] Kris Oreschak, PhD,[p] Jeremy Kobulnik, MD, MHSc,[p] Ling Shen, PhD, MPH,[q] and Jeffrey Teuteberg, MD[a,1,2]

Khush et al., JHLT 2024 **DOI:** 10.1016/j.healun.2024.05.003

Sam D, Heart Transplant Recipient

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SHORE Registry

- Prospective, observational registry of heart transplant recipients in the United States monitored with GEP and dd-cfDNA
- 67 heart transplant centers
- 2732 patients enrolled
- Patients could be followed up to five-years post-transplant regardless of molecular or EMB surveillance schedule
- The first manuscript includes patients with complete EMB, DSA, ECHO, and angiographic data from date of transplant to end of follow-up

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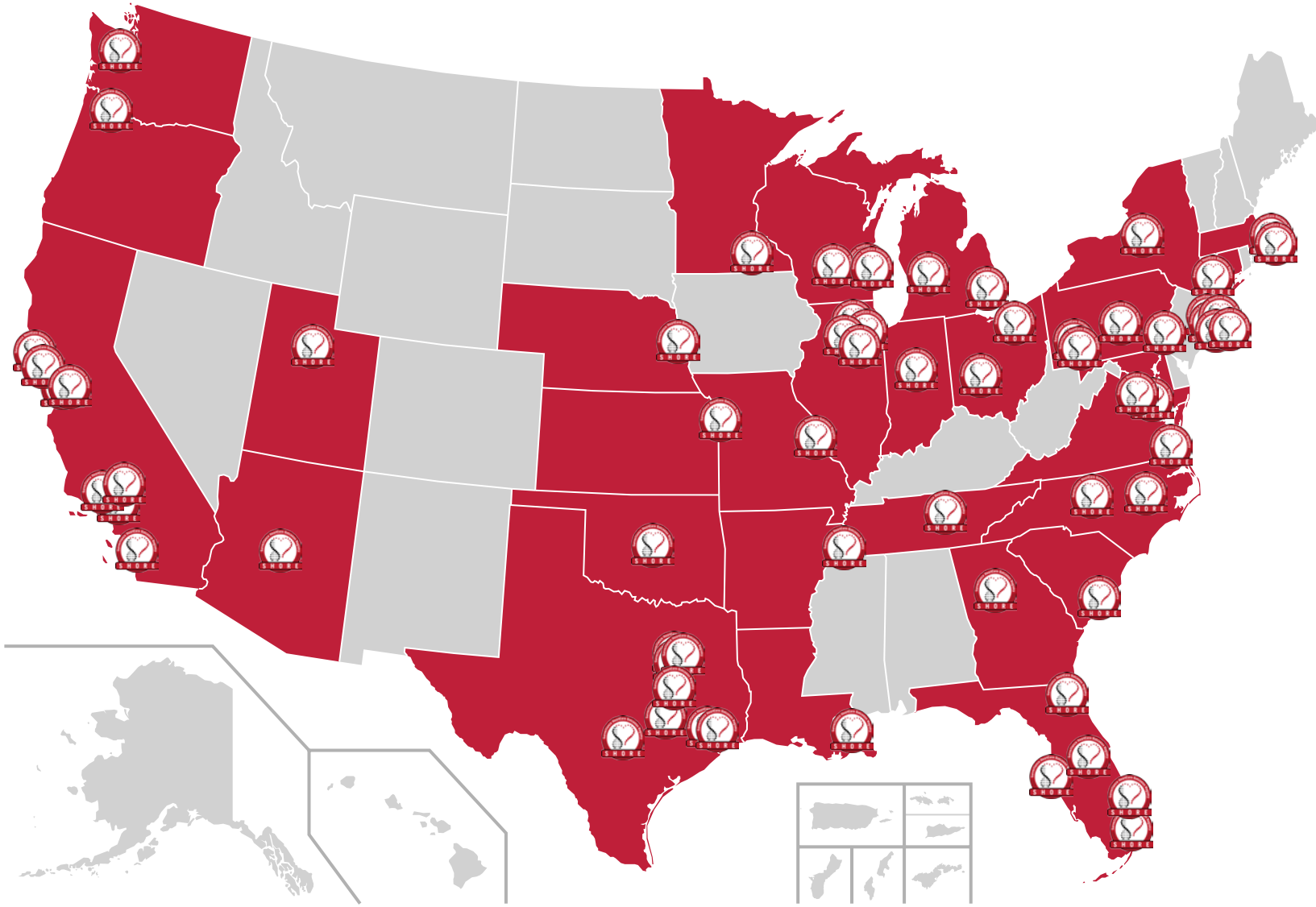
Khush et al., JHLT 2024 **DOI:** 10.1016/j.healun.2024.05.003

67 Centers Across the United States



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Khush et al., JHLT 2024 **DOI:** [10.1016/j.healun.2024.05.003](https://doi.org/10.1016/j.healun.2024.05.003)

Methods

- Inclusion criteria
  - Adult heart transplant recipients surviving to at least 55 days post-transplant
  - Transplanted between 1/1/17 and 12/31/22
  - At least one GEP or dd-cfDNA level available
  - Complete clinical data (all EMBs, DSAs, ECHOs, and angiograms) from transplant to last follow-up available
- Exclusion criteria
  - Pregnancy
  - Multi-organ transplant recipients (enrolled in SHORE, but excluded in manuscript 1)
  - Patients with no molecular test results available
  - Patients from the 15 sites without complete clinical data available
  - EMBs and molecular tests <55 days post-transplant were excluded from clinical validity/utility analyses, but were collected in SHORE

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Khush et al., JHLT 2024 **DOI:** 10.1016/j.healun.2024.05.003

Table 1: Demographics and Clinical Characteristics

	SHORE Enrolled Population N=2604	SHORE Study Population N=2077
Age at transplant	54 ± 12	54 ± 12
Race		
White	1717 (65.9%)	1401 (67.5%)
Black	562 (21.6%)	425 (20.5%)
Asian	74 (2.8%)	61 (2.9%)
Other	161 (6.2%)	130 (6.3%)
Unknown	90 (3.5%)	60 (2.9%)
Sex		
Male	1904 (73.1%)	1531 (73.7%)
Female	700 (26.9%)	546 (26.3%)
Reason for transplant		
Non-ischemic CM	1330 (51.1%)	1052 (50.6%)
Ischemic CM	712 (27.3%)	575 (27.7%)
Re-transplant	22 (0.8%)	19 (0.9%)
Other	540 (20.7%)	431 (20.8%)
Induction therapy (Yes)	770 (29.6%)	583 (28.1%)
Sensitized at transplant (PRA≥10%)	425 (16.3%)	333 (16.0%)
Pre-transplant MCS		
None	1118 (42.9%)	888 (42.8%)
LVAD	808 (31.0%)	654 (31.5%)
tMCS	632 (24.3%)	494 (23.8%)
Other/unknown	46 (1.8%)	41 (2.0%)
CMV serology status		
D-/R-	587 (22.5%)	486 (23.4%)
D-/R+	354 (13.6%)	297 (14.3%)
D+/R-	800 (30.7%)	655 (31.5%)
D+/R+	611 (23.5%)	485 (23.4%)
Unknown	252 (9.7%)	154 (7.4%)

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Khush et al., JHLT 2024 *DOI: 10.1016/j.healun.2024.05.003*

Table 1: Demographics and Clinical Characteristics

	SHORE Enrolled Population N=2604	SHORE Study Population N=2077
Donor Age	33 ± 11	33 ± 11
Donor Sex		
Male	1652 (63.4%)	1340 (64.5%)
Female	671 (25.8%)	535 (25.8%)
Unknown	281 (10.8%)	202 (9.7%)
Donor Race		
White	1397 (53.6%)	1120 (53.9%)
Black	366 (14.1%)	292 (14.1%)
Asian	35 (1.3%)	31 (1.5%)
Other	332 (12.7%)	265 (12.8%)
Unknown	474 (18.2%)	369 (17.8%)
# of GEP tests per patient post-transplant[a]		
Median (IQR)	11 (8-15)	12 (8-15)
# dd-cfDNA tests per patient post-transplant[a]		
Median (IQR)	12 (8-15)	12 (8-16)
Time to first GEP/dd-cfDNA post-transplant (months)		
Median (IQR)	2.0 (1.1-3.9)	2.0 (1.1-3.9)
Length of post-transplant follow-up (months)[a]		
Median (IQR)	39 (31-48)	40 (32-50)
Year Transplant Performed		
2017	45 (1.7%)	40 (1.9%)
2018	172 (6.6%)	149 (7.2%)
2019	653 (25.1%)	550 (26.5%)
2020	952 (36.6%)	763 (36.7%)
2021	779 (29.9%)	574 (27.6%)
2022	3 (0.1%)	1 (0.0%)

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Khush et al., JHLT 2024 *DOI: 10.1016/j.healun.2024.05.003*

Table 2: Clinical Outcomes

Clinical Outcomes	Total Eligible (N=2077)
N (%) of patients experienced rejection	627 (30.2%)
ACR only	350 (16.9%)
AMR only	185 (8.9%)
ACR and AMR	92 (4.4%)
Donor Specific Antibodies	
Yes	644 (31.0%)
No	1319 (63.5%)
Missing	114 (5.5%)
Donor Specific Antibody Class at First Positive Result	
Class I Positive Only	168 (8.1%)
Class II Positive Only	386 (18.6%)
Class I and Class II Positive	90 (4.3%)
Graft Dysfunction at one-year post-transplant	
Patients at risk at one-year post-transplant	1852
n (%)	62 (3.3%)
LVEF at one-year post-transplant	
mean ± SD	61 ± 6%
Graft Dysfunction at two-years post-transplant	
Patients at risk at two-year post-transplant	1782
n (%)	49 (2.7%)
LVEF at two-years post-transplant	
mean ± SD	61 ± 6%
Percentage alive at one-year post-transplant	97.9%
Percentage alive at two-years post-transplant	94.9%

EMB Results	
Total # of EMBs	**N=23729**
Acute Cellular Rejection Grade	
0R	13384 (56.4%)
1R	9447 (39.8%)
2R	622 (2.6%)
3R	29 (0.1%)
Inadequate tissue/No Grade	247 (1.0%)
Antibody Mediated Rejection Grade	
pAMR0	20377 (85.9%)
pAMR1	535 (2.3%)
pAMR2	160 (0.7%)
pAMR3	0 (0.0%)
Not performed	2657 (11.2%)

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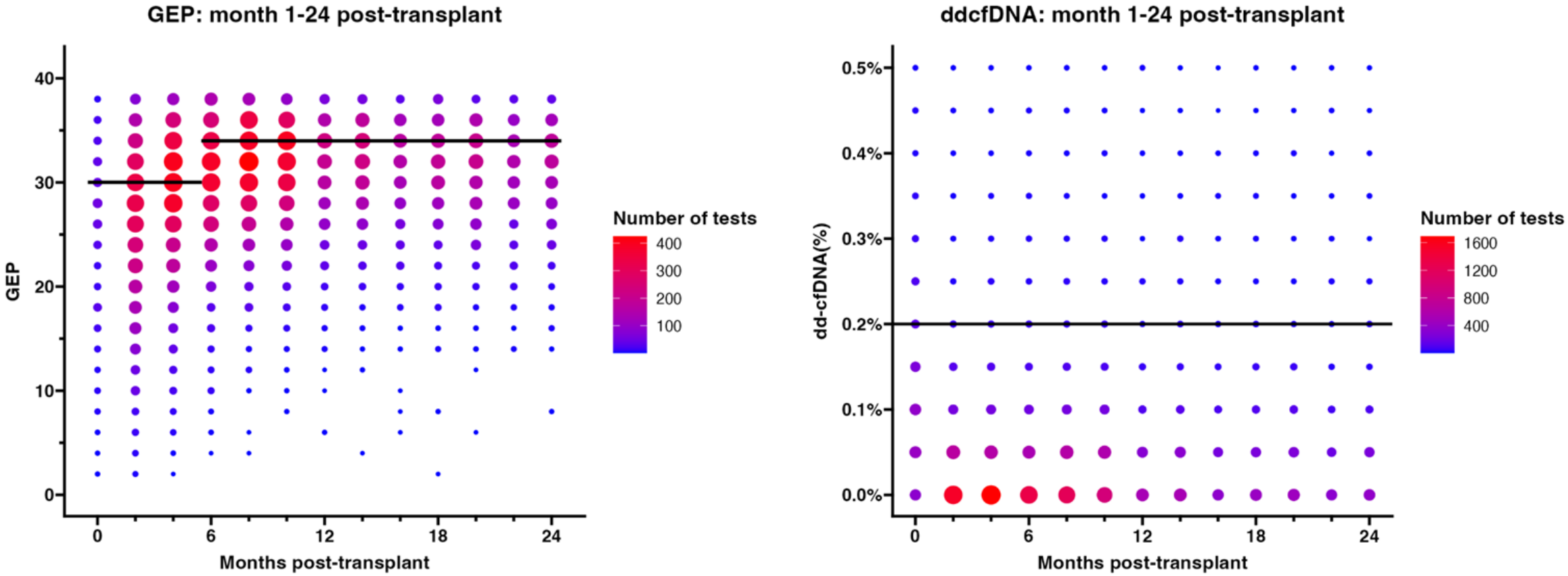
Khush et al., JHLT 2024 *DOI: 10.1016/j.healun.2024.05.003*

Figure 2: Distribution of GEP/ddcfDNA results by time post-transplant



Solid lines represent the threshold for positive testing for each molecular test.

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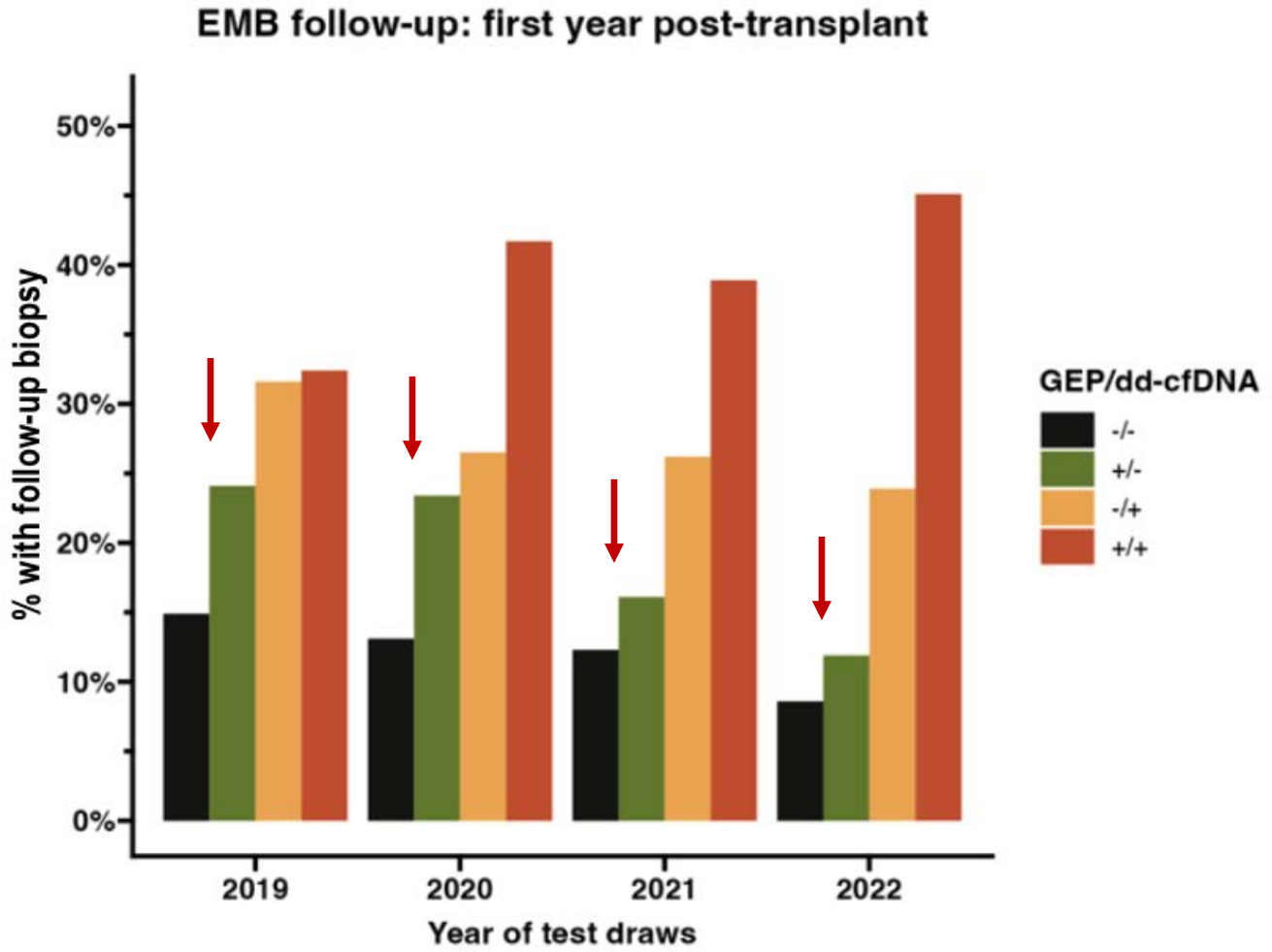
Figure 4: HeartCare Clinical Utility: First Year Post-Transplant EMB By Year



Includes biopsies performed 3-14 days after GEP/dd-cfDNA draw

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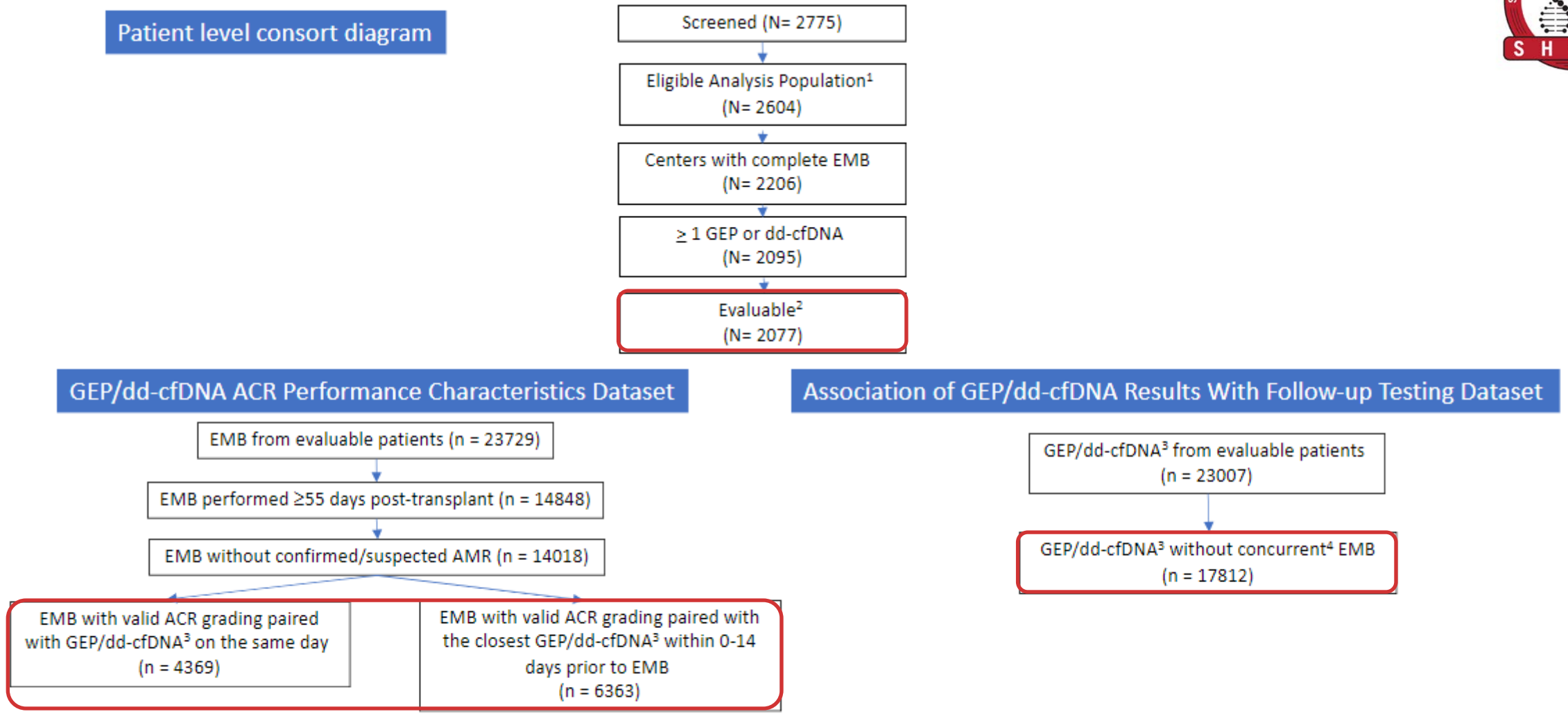
Khush et al., JHLT 2024 ***DOI:*** *10.1016/j.healun.2024.05.003*

Figure 1: Consort Diagram



[1] Eligible study population consisted enrolled eligible heart-only transplant patients from 1/1/2017 – 12/31/2022.

[2] Evaluable patients in this analysis were defined as eligible study population from centers where complete EMB data were collected and with at least 1 GEP or dd-cfDNA testing. 22 patients were excluded from this analysis due to the inability to access the electronic medical record.

[3] GEP/dd-cfDNA consisted of same day GEP and dd-cfDNA tests.

[4] Concurrent EMB was defined as EMB performed within 0-2 days after the GEP/dd-cfDNA.

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Table 4: Proportion of GEP/dd-cfDNA Test Results Paired with EMB-Proven ACR

GEP/ddcfDNA Result	# of GEP/ddcfDNA Paired with EMB[a]	# of EMB with ACR	% of EMBs with ACR (95% CI)	# of Grade 2R	# of Grade 3R
-/-	3130	46	1.5% (1.1%, 2.0%)	45	1
+/-	2207	43	1.9% (1.4%, 2.6%)	42	1
-/+	461	20	→ 4.3% (2.8%, 6.6%)	20	0
+/+	565	52	→ 9.2% (7.1%, 11.9%)	49	3

[a] Paired GEP/dd-cfDNA and biopsy: GEP/dd-cfDNA drawn 0-14 days prior to EMB with valid ACR gradings.

GEP+ = ≥30 (0-6 months) ≥34 >6 months)
ddcfDNA+ = ≥0.20%

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Interpreting Discordant Results

Result	Likelihood of ACR	Frequency of Result
GEP-/ddcfDNA-	1.4%	49%
GEP+/ddcfDNA- ***(except subset below)***	1.6%	32%
GEP+/ddcfDNA- ***(ddcfDNA = 0.12-0.19 & ↑ from prior)***	→ ***5.8%***	***3.1%***
GEP-/ddcfDNA+ ***(except subset below)***	**3.5%**	**6.5%**
GEP-/ddcfDNA+ ***(GEP within 2 of threshold & ddcfDNA ↑ ≥ 0.2% from prior)***	→ ***10%***	***0.9%***
GEP+/ddcfDNA+	→ **8.9%**	**8.8%**

SHORE, internal data

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HeartCare Performance Characteristics

ACR Performance Characteristics; ***AMR Excluded***			
Molecular Test Result	**Sensitivity**	**Specificity**	**LR+**
GEP+/ddcfDNA+	32.3% (25.6%, 39.9%)	**91.7% (91.0%, 92.4%)**	**3.90 (3.08, 4.96)**
GEP+ alone	59.0% (51.3%, 66.3%)	56.8% (55.6%, 58.1%)	1.37 (1.20, 1.56)
ddcfDNA+ alone	44.7% (37.3%, 52.4%)	84.6% (83.7%, 85.5%)	2.91 (2.43, 3.49)

ACR Performance Characteristics; ***AMR Included***			
Molecular Test Result	**Sensitivity**	**Specificity**	**LR+**
GEP+/ddcfDNA+	32.0% (25.4%, 39.3%)	**91.0% (90.3%, 91.7%)**	**3.55 (2.81, 4.49)**
GEP+ alone	58.6% (51.0%, 65.7%)	56.6% (55.4%, 57.8%)	1.35 (1.19, 1.54)
ddcfDNA+ alone	46.2% (38.8%, 53.7%)	83.3% (82.3%, 84.2%)	2.76 (2.32, 3.27)

Includes paired GEP/dd-cfDNA and biopsy, defined as GEP/dd-cfDNA drawn 0-14 days prior to EMB with valid ACR gradings.

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Khush et al., JHLT 2024 **DOI:** 10.1016/j.healun.2024.05.003

ISHLT2024

44th Annual Meeting & Scientific Sessions

Heart Transplant Outcomes In The Contemporary Era: Results From The SHORE Registry

Originally Presented by Kiran Khush, MD, MAS
Professor of Medicine
Stanford University School of Medicine

The International Society for Heart & Lung Transplantation

Methods

- Descriptive statistics used to present incidence of clinical events at 6-, 12- and 24-months post-transplant
- **GEP+** was defined as a **value ≥30 (0-6 months)** or ≥34 (>6 months)
- **dd-cfDNA+** was defined as a **value ≥0.20%** at any time post-transplant
- For molecular testing analyses, patients were categorized based on month 2-6 molecular test results:
  - GEP-/ddcfDNA-: all molecular test results were negative
  - GEP+/ddcfDNA-: at least one GEP+ and all dd-cfDNA were negative
  - GEP-/ddcfDNA+: at least one dd-cfDNA positive, but no dual positive results
  - GEP+/ddcfDNA+: at least one simultaneous GEP+/dd-cfDNA+ result
- Relationship between GEP/dd-cfDNA and outcomes were assessed from 6-months to 2-years post-transplant

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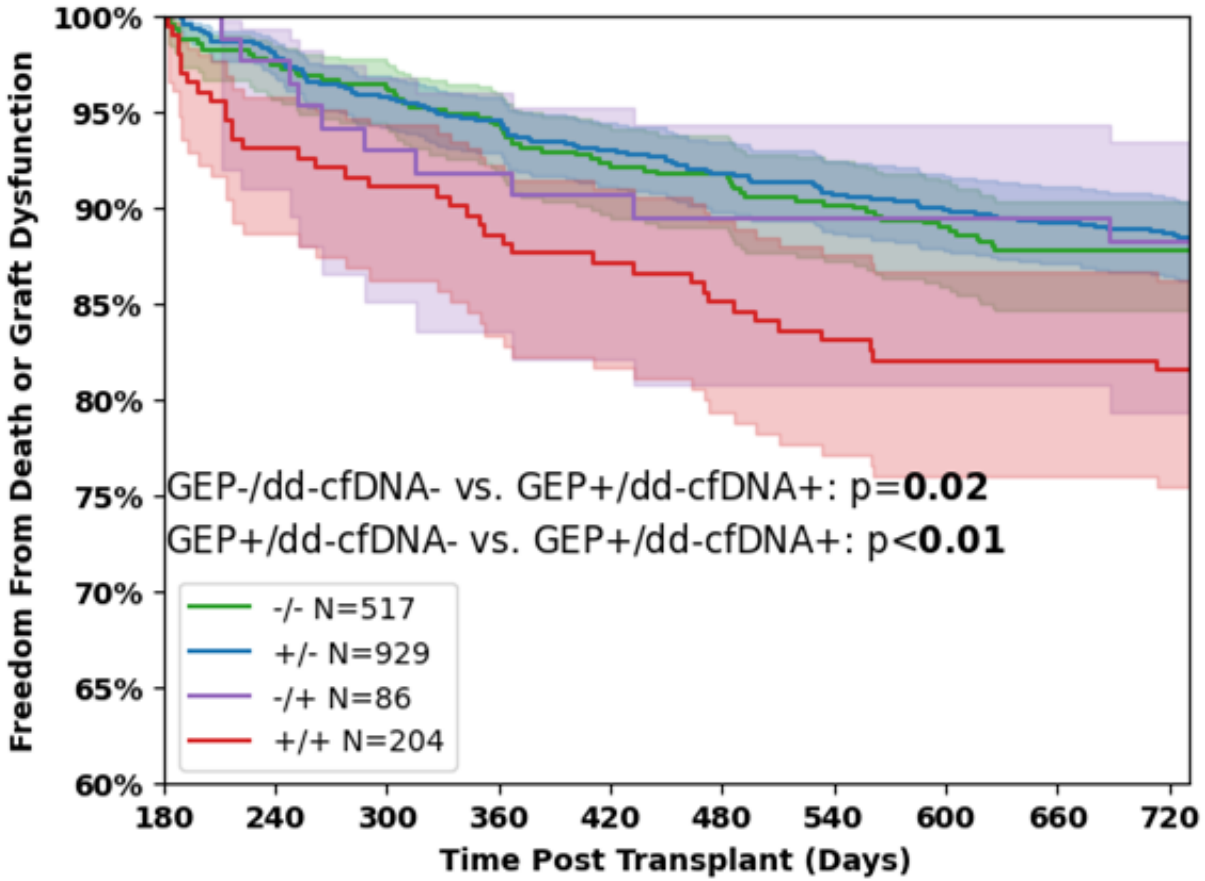
Khush et al., Oral Presentation at ISHLT 2024

2-year Death or Graft Dysfunction by Dual Molecular Testing Result in the First 6 Months



-/- N=517								
At risk	517	517	510	492	472	459	449	442
Censored	0	0	0	6	9	10	12	13
Events	0	0	7	19	36	48	56	62
+/- N=929								
At risk	929	929	920	884	850	828	812	798
Censored	0	0	2	6	17	22	25	30
Events	0	0	7	39	62	79	92	101
-/+ N=86								
At risk	86	86	86	80	77	76	76	75
Censored	0	0	0	0	1	1	1	1
Events	0	0	0	6	8	9	9	10
+/+ N=204								
At risk	204	204	196	184	176	166	161	160
Censored	0	0	0	2	3	6	7	8
Events	0	0	8	18	25	32	36	36

GEP+ = ≥30; **dd-cfDNA-** = ≥0.20%

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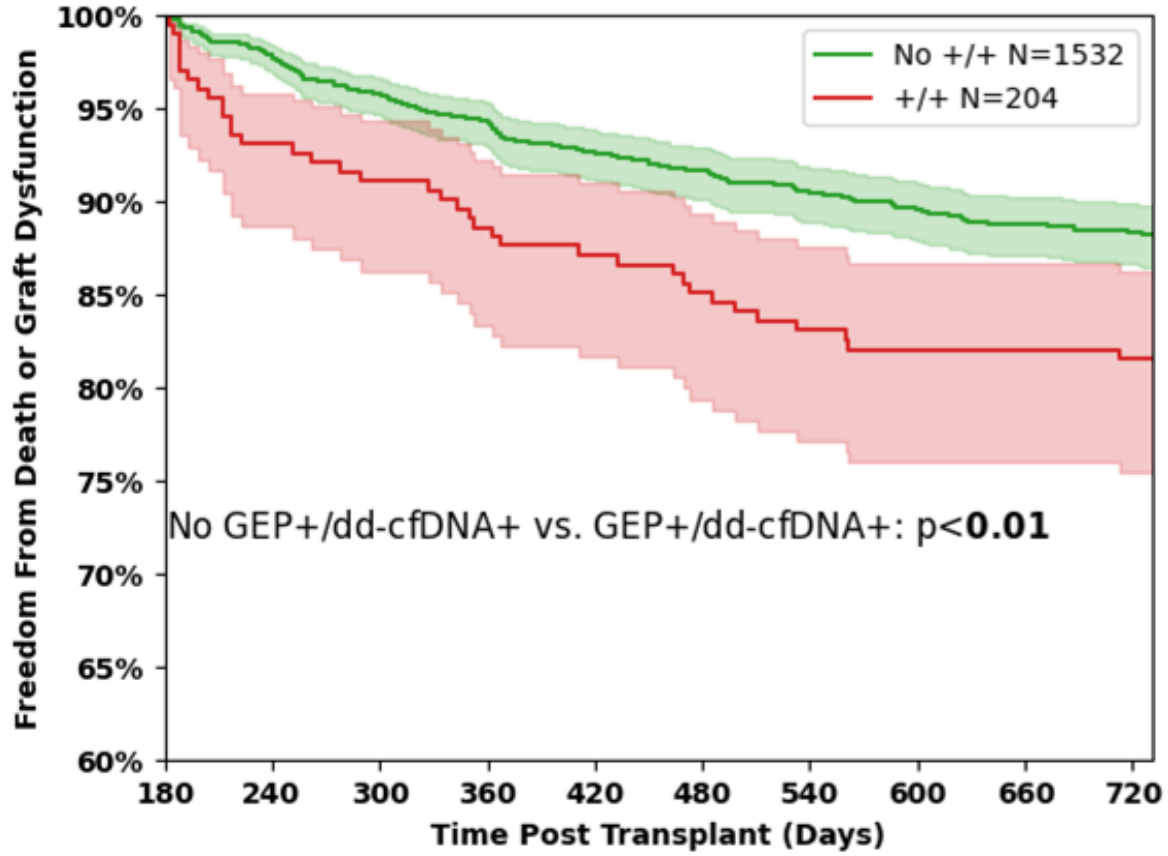
2-year Death or Graft Dysfunction: GEP+/dd-cfDNA+ vs. No Dual Positive Result in the First 6 Months

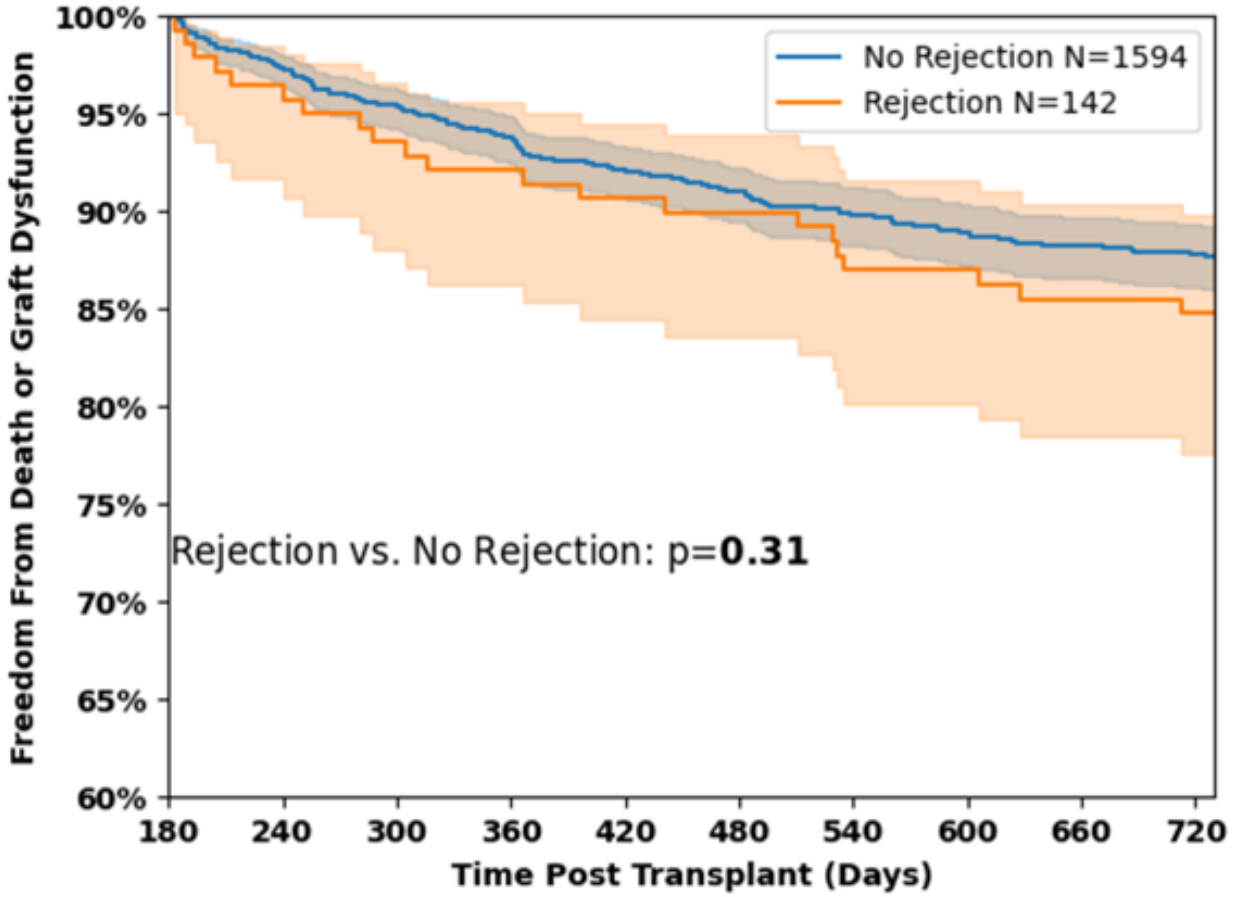


No +/+ N=1532								
At risk	1532	1532	1516	1456	1399	1363	1337	1315
Censored	0	0	2	12	27	33	38	44
Events	0	0	14	64	106	136	157	173
+/+ N=204								
At risk	204	204	196	184	176	166	161	160
Censored	0	0	0	2	3	6	7	8
Events	0	0	8	18	25	32	36	36

GEP+ = ≥30; **dd-cfDNA-** = ≥0.20%

Khush et al., Oral Presentation at ISHLT 2024

2-year Death or Graft Dysfunction: By Rejection Status, 2-6-Months Post-Transplant



Rejection N=1594								
At risk	1594	1594	1574	1510	1450	1407	1380	1359
Censored	0	0	1	11	26	33	39	46
Events	0	0	19	73	118	154	175	189
Rejection N=142								
At risk	142	142	138	130	125	122	118	116
Censored	0	0	1	3	4	6	6	6
Events	0	0	3	9	13	14	18	20

Rejection = ACR and/or AMR 2-6 months post-transplant
No rejection = No ACR or AMR 2-6 months post-transplant

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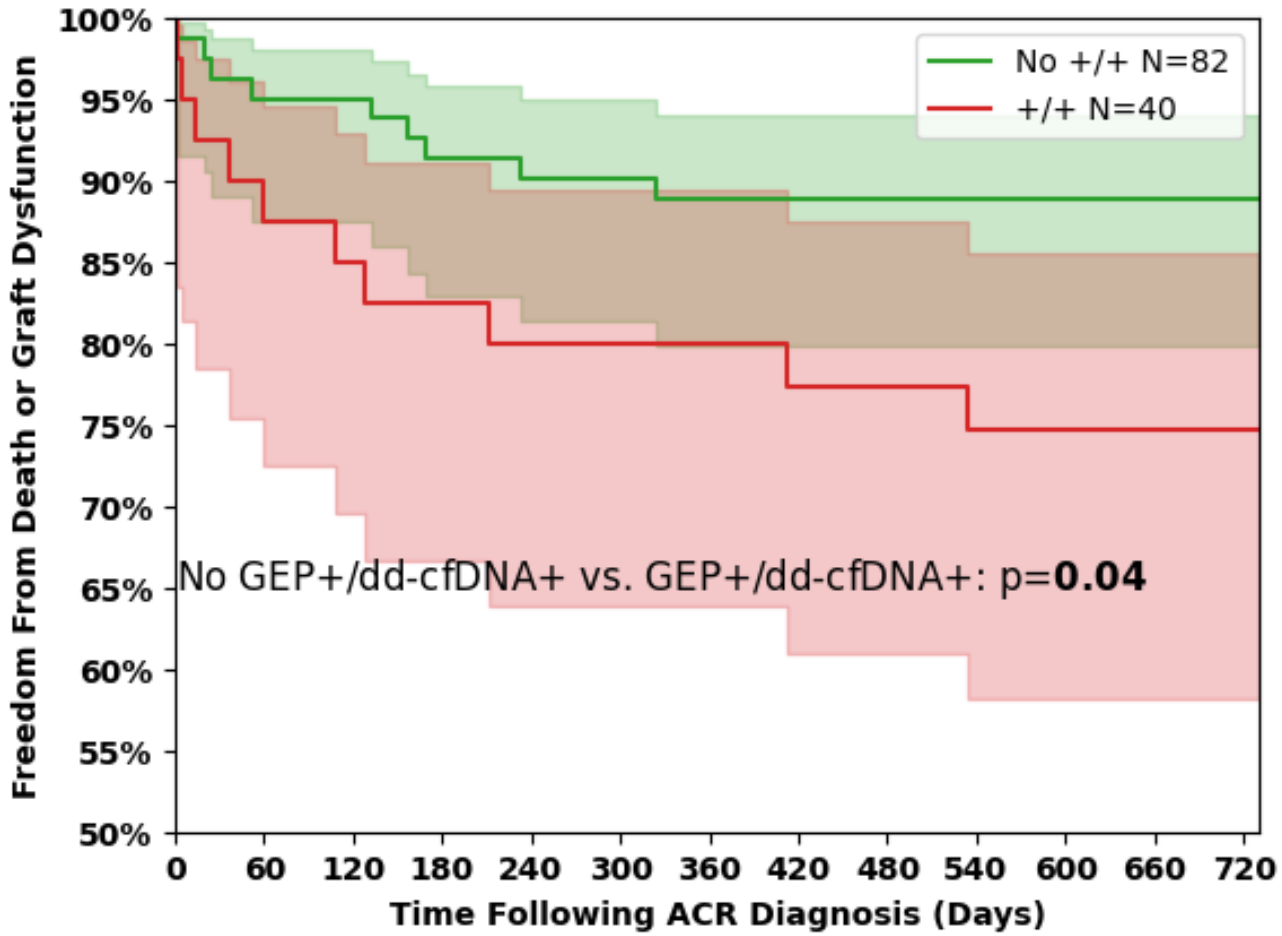
2-year post-ACR Death or Graft Dysfunction by Dual Testing Results



- **GEP+/dd-cfDNA+ vs. No Dual Positive Results**

No +/+ N=82								
At risk	82	78	75	73	72	72	72	72
Censored	0	0	0	1	1	1	1	1
Events	0	4	7	8	9	9	9	9
+/+ N=40								
At risk	40	35	33	32	31	30	29	29
Censored	0	0	0	0	1	1	1	1
Events	0	5	7	8	8	9	10	10

GEP+ = ≥30 (0-6 months) ≥34 >6 months)
dd-cfDNA+ = ≥0.20%

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Khush et al., Oral Presentation at ISHLT 2024

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Extreme elevations of donor-derived cell-free DNA increases the risk of chronic lung allograft dysfunction and death, even without clinical manifestations of disease

Michael B. Keller[1,2,3,4], David Newman[5], Muhtadi Alnababteh[1,2,3], Lucia Ponor[2,6], Pali Shah[2,4], Joby Matthews[2,4], Hyesik Kong[1,2], Temesgen Andargie[1,2], Woojin Park[1,2], Ananth Charya[7], Helen Luikart[8,9], Shambhu Aryal[2,10], Steven D. Nathan[2,10], Jonathan B. Orens[2,4], Kiran K. Khush[8], Moon Jang[1,2], Sean Agbor-Enoh[1,2,4]

J Heart Lung Transplant. 2024 May 3:S1053-2498(24)01644-9.

Kristin J, Stem Cell and Double Lung Recipient

Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

PURPOSE

- Previous work has demonstrated a cohort of patients who experience extreme elevations in dd-cfDNA in the upper quartile range of all patients with acute rejection (> 5%), which we hereby define as extreme molecular injury (EMI).
  - EMI develops even in the absence of clinical signs of acute rejection or infection.
  - The long-term consequences associated with these episodes of EMI are unknown.

- **The aim of this study was to define the cumulative incidence of EMI in lung transplant recipients and to test the hypothesis that episodes of EMI are associated with an increased risk of severe CLAD and death.**

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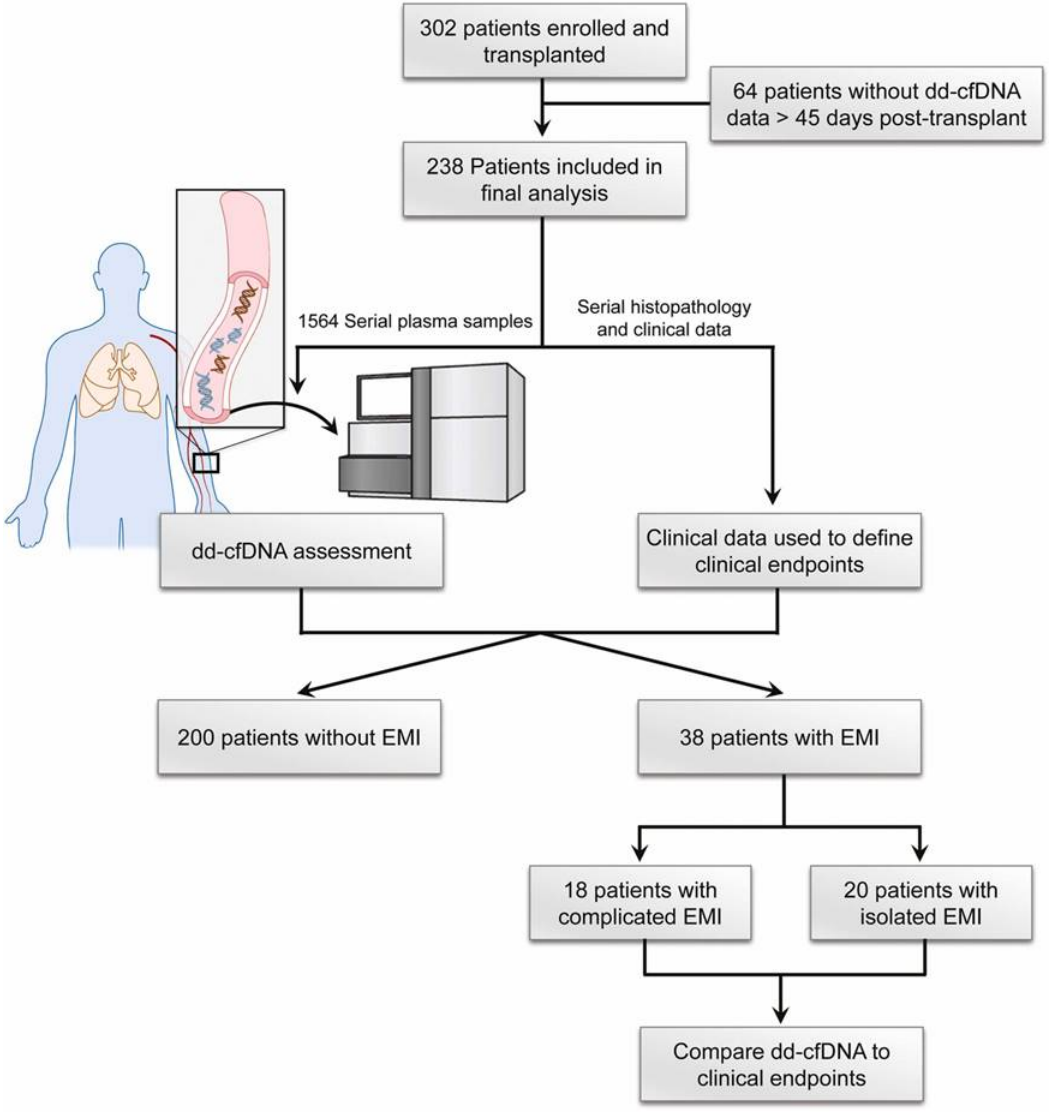
P-K-00138 rev 1 effective 2024.5| J Heart Lung Transplant. 2024 May 3:S1053-2498(24)01644-9.

Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

METHODS | Multicenter, prospective, observational study

- Adult lung transplant recipients in two prospective cohort studies
  - Genome Research Alliance for Transplantation (GRAfT) between July 2015 – Oct 2020
    - Johns Hopkins Hospital,
    - Inova Fairfax Hospital, and
    - University of Maryland Medical Center
  - Genome Transplant Dynamics (GTD) between Dec 2010 – Dec 2012
    - Stanford University Hospital



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Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

METHODS | Extreme Molecular Injury (EMI) Definitions

- Serial plasma samples were collected for dd-cfDNA measurement by shotgun sequencing.

- Extreme molecular injury (EMI) was defined as a dd-cfDNA above the third quartile of levels observed for acute rejection (dd-cfDNA level of ≥ 5% occurring after 45 days post-transplant)
  - Categorized as **Secondary** if associated with co-existing acute rejection, infection or PFT decline
  - Categorized as **Primary** if not associated to these conditions

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RESULTS | Table 1. Demographic Characteristics

	Overall N = 238	Patients without EMI N = 200	Patients with EMI N = 38	p-Value
Characteristic				
Age (years)	55.35 (13.85)	55.06 (12.20)	46.66 (18.06)	<0.001
BMI (kg/m2)	25.18 (4.70)	25.40 (4.60)	24.03 (5.06)	0.105
Lung Allocation Score at Transplant	50.17 (19.02)	50.70 (19.30)	47.47 (17.47)	0.352
Female Sex	110 (47.2%)	89 (45.6%)	21 (55.3%)	0.275
Race *White* *African American* *Other*	 192 (80.7%) 35 (14.7%) 11 (4.6%)	 160 (80.0%) 31 (15.5%) 9 (4.5%)	 32 (84.2%) 4 (10.5%) 2 (5.3%)	0.694
Double Lung Transplant	173 (72.7%)	142 (71.0%)	31 (81.6%)	0.320
Native Lung Disease *Interstitial Lung Disease* *Cystic Fibrosis* *Chronic Obstructive Pulmonary Disease* *Idiopathic Pulmonary Hypertension* *Other*	 113 (47.5%) 34 (14.2%) 42 (17.7%) 7 (2.9%) 42 (17.7%)	 101 (50.5%) 18 (9.0%) 38 (19.0%) 6 (3.0%) 37 (18.5%)	 12 (31.6%) 16 (42.1%) 4 (10.5%) 1 (2.6%) 5 (13.2%)	<0.001

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Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

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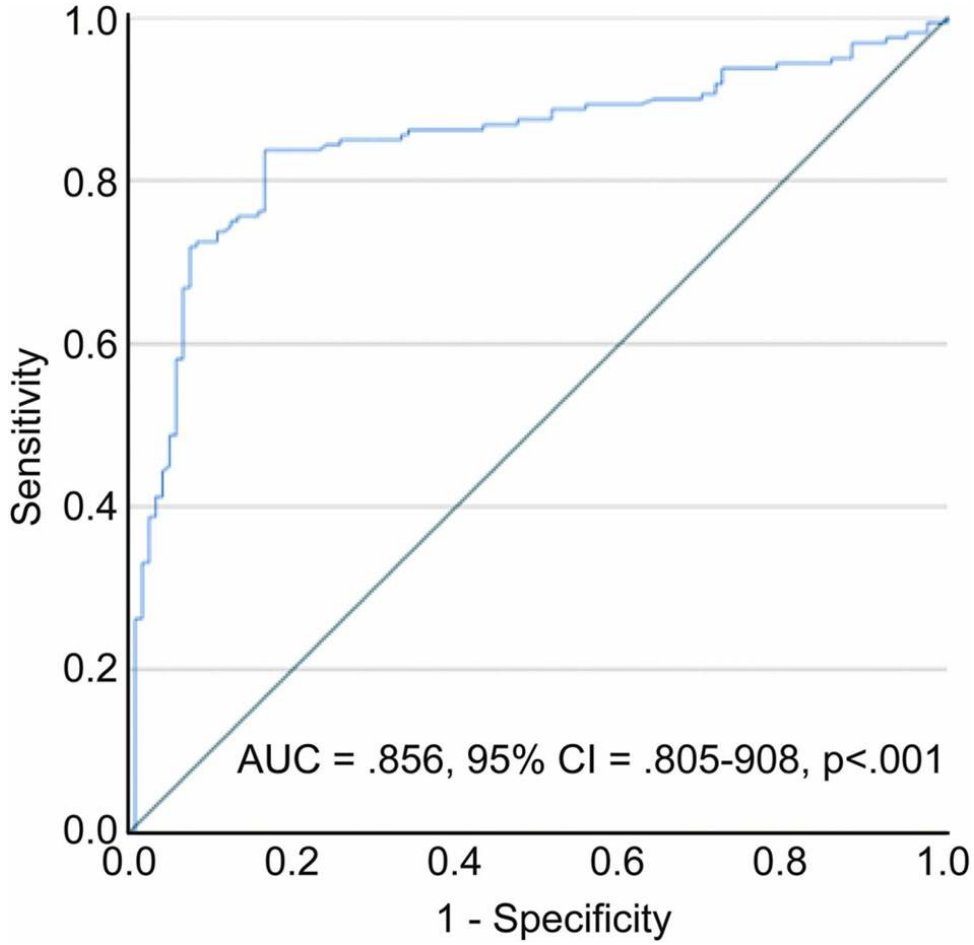
Participants that had EMI within the first 2.54 years after surgery were at greatest risk of CLAD or death with a sensitivity of 84% and specificity of 83%.



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Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

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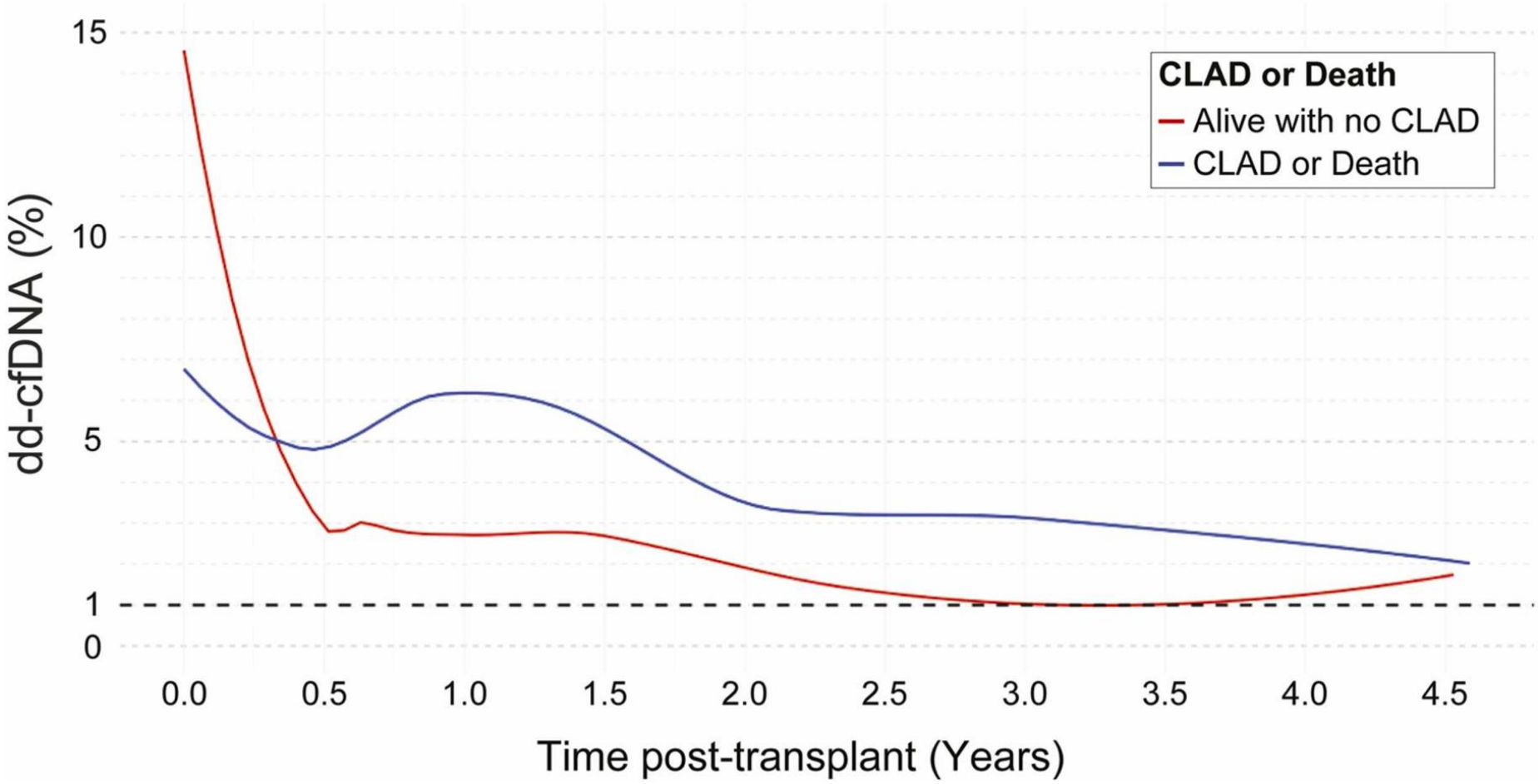
While EMI patients that developed CLAD/Death had initially lower levels of dd-cfDNA at time of EMI, they demonstrate less rapid decay in dd-cfDNA following EMI and persistently higher levels of dd-cfDNA.



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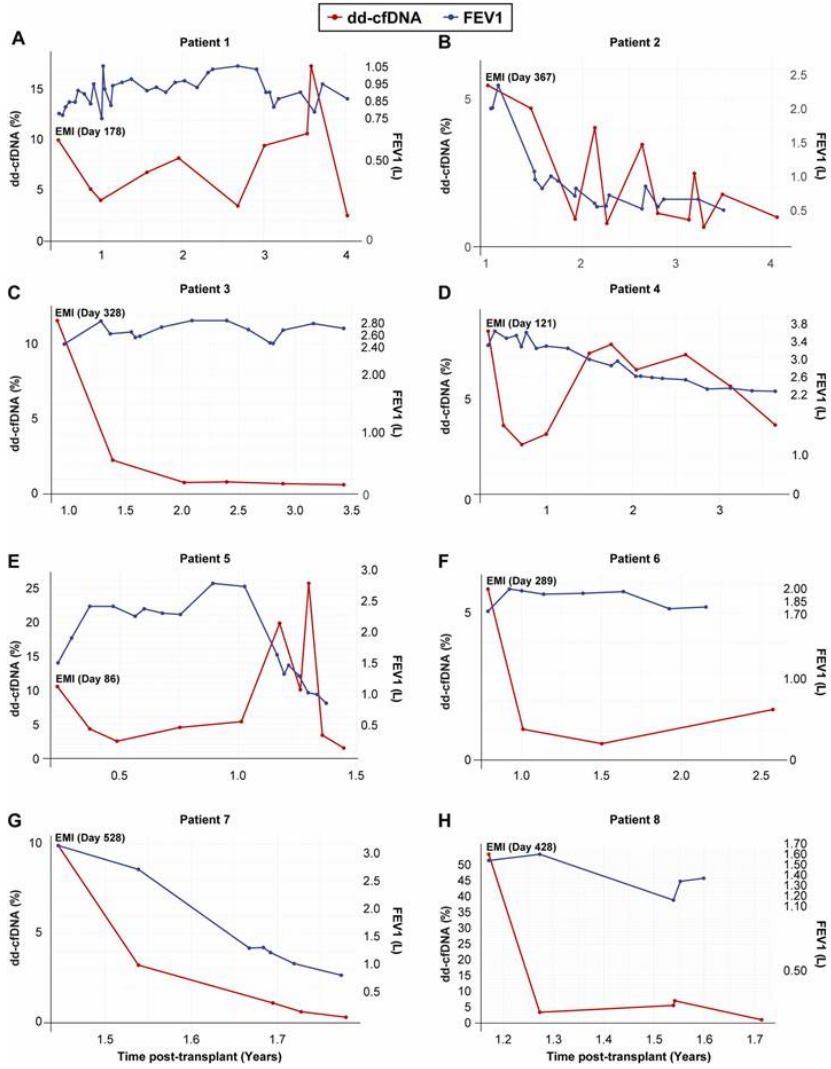
Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

Case Series of EMAD patients demonstrating longitudinal trends of dd-cfDNA & FEV1 from the time of EMI.



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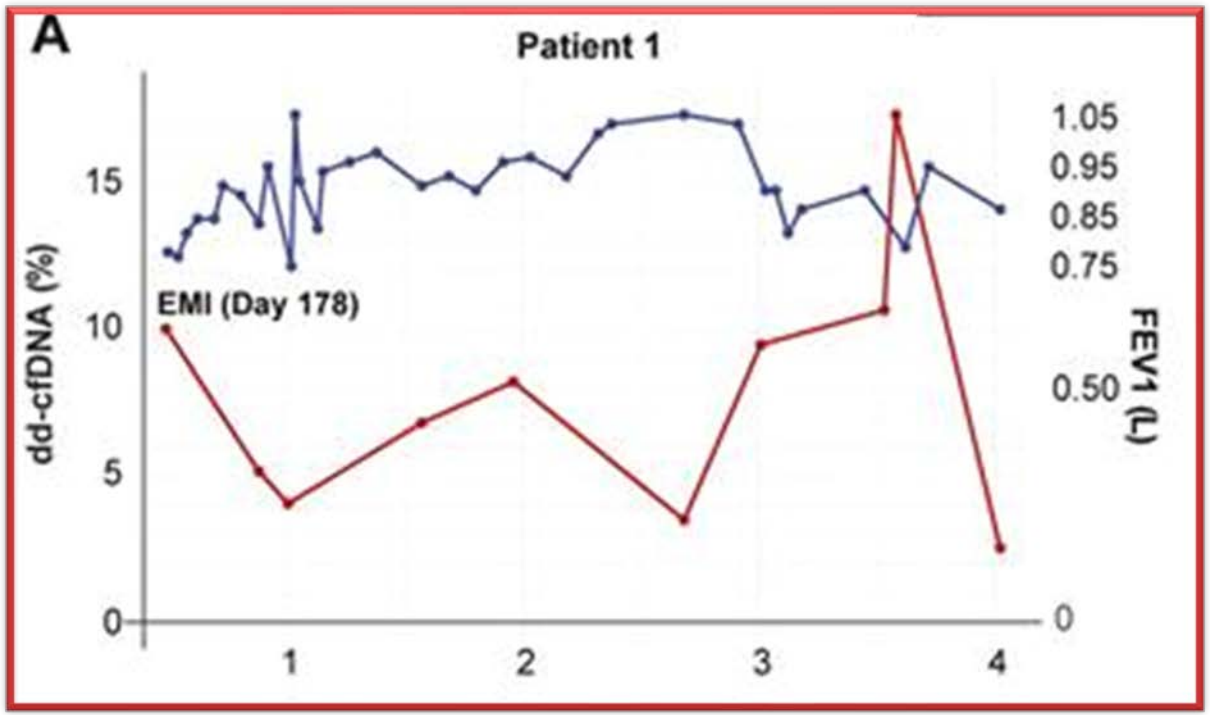

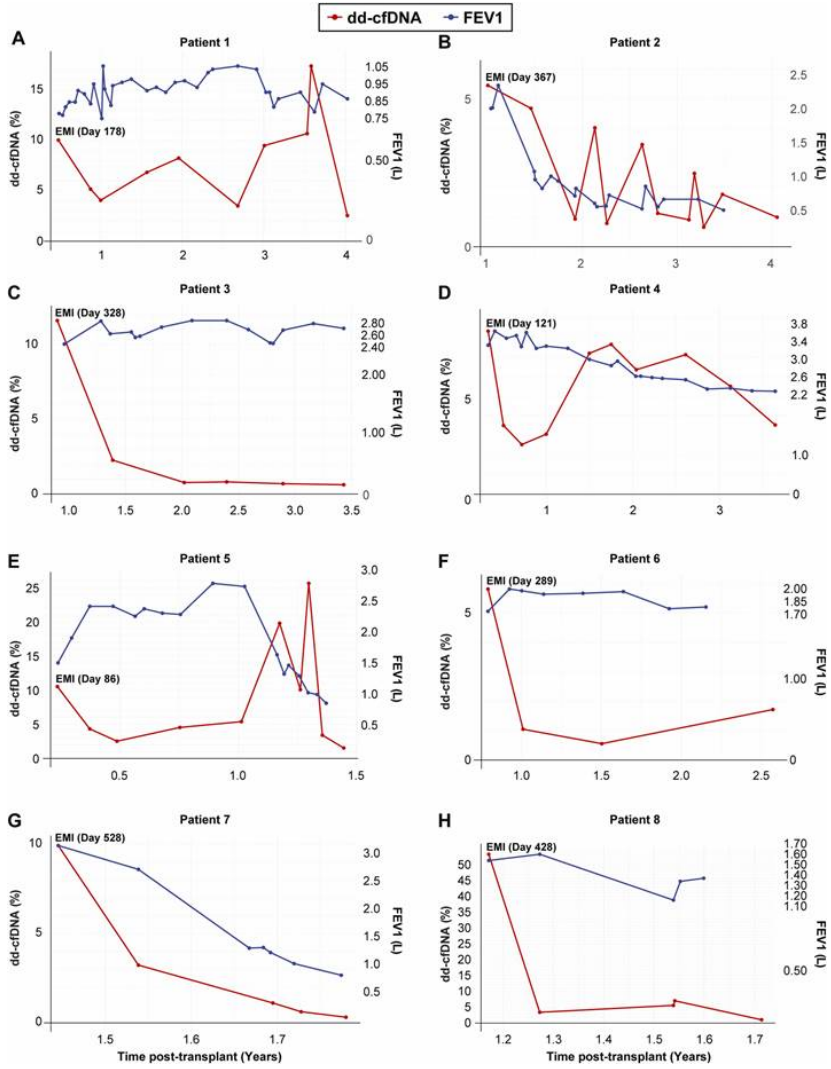

- **Primary EMI at post-transplant day 178**
- **AMR developed on day 710**
- **Death on day 1561**

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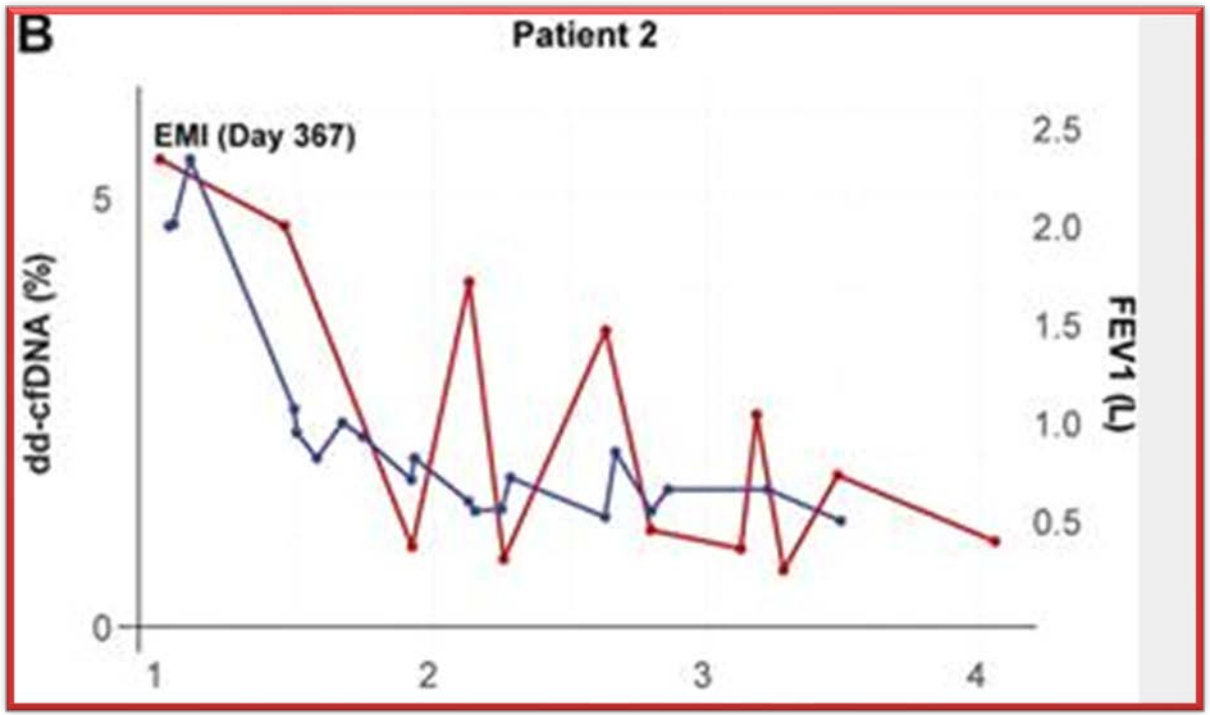

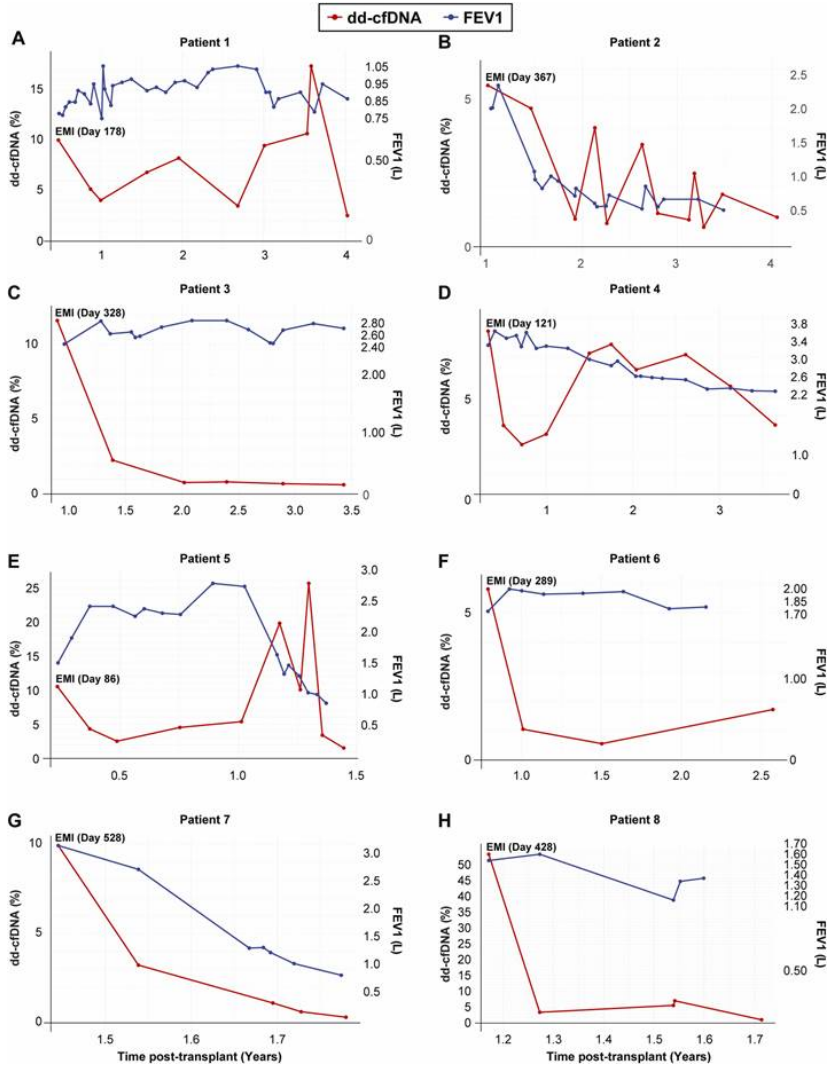

- **Secondary EMI (RSV) on post-transplant day 367**
- **Development of CLAD on day 379**
- **Death on day 1529**

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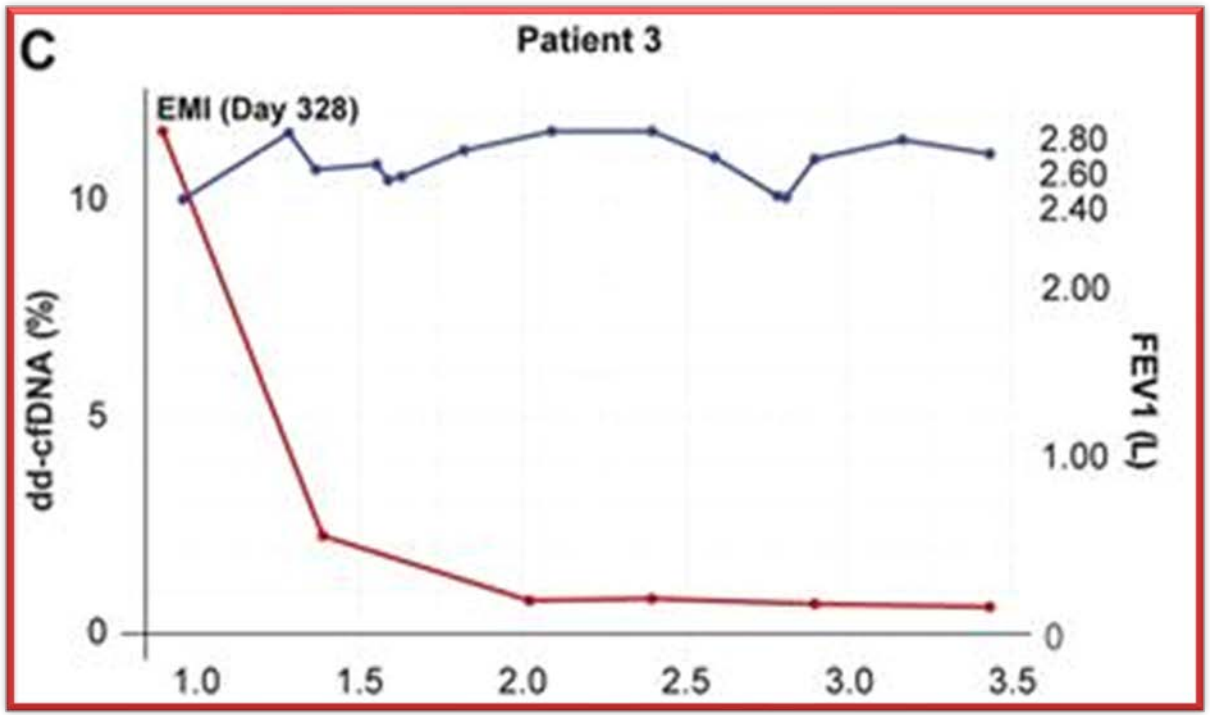
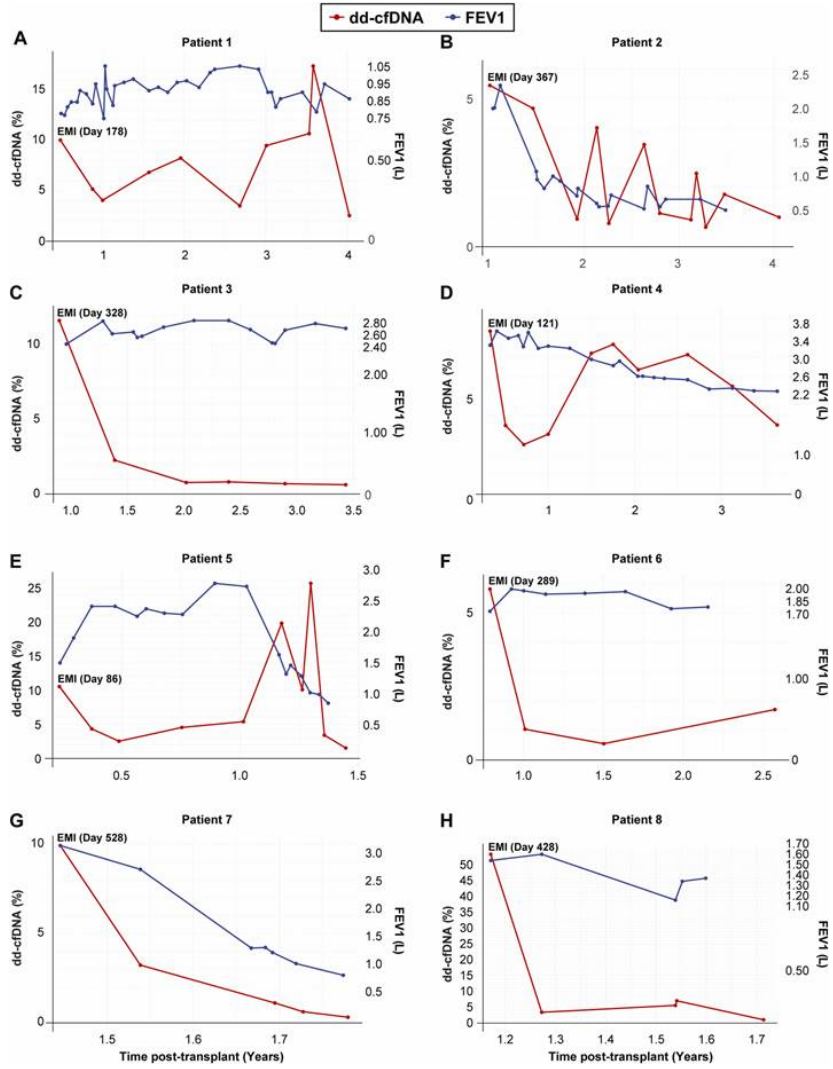
Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

Case Series of EMAD patients demonstrating longitudinal trends of dd-cfDNA & FEV1 from the time of EMI.





- **Secondary EMI (Grade 4 graft dysfunction) on day 328**
- **Lung function recovered to baseline on day 351**
- **dd-cfDNA levels rapidly decreased | Alive, CLAD-free**

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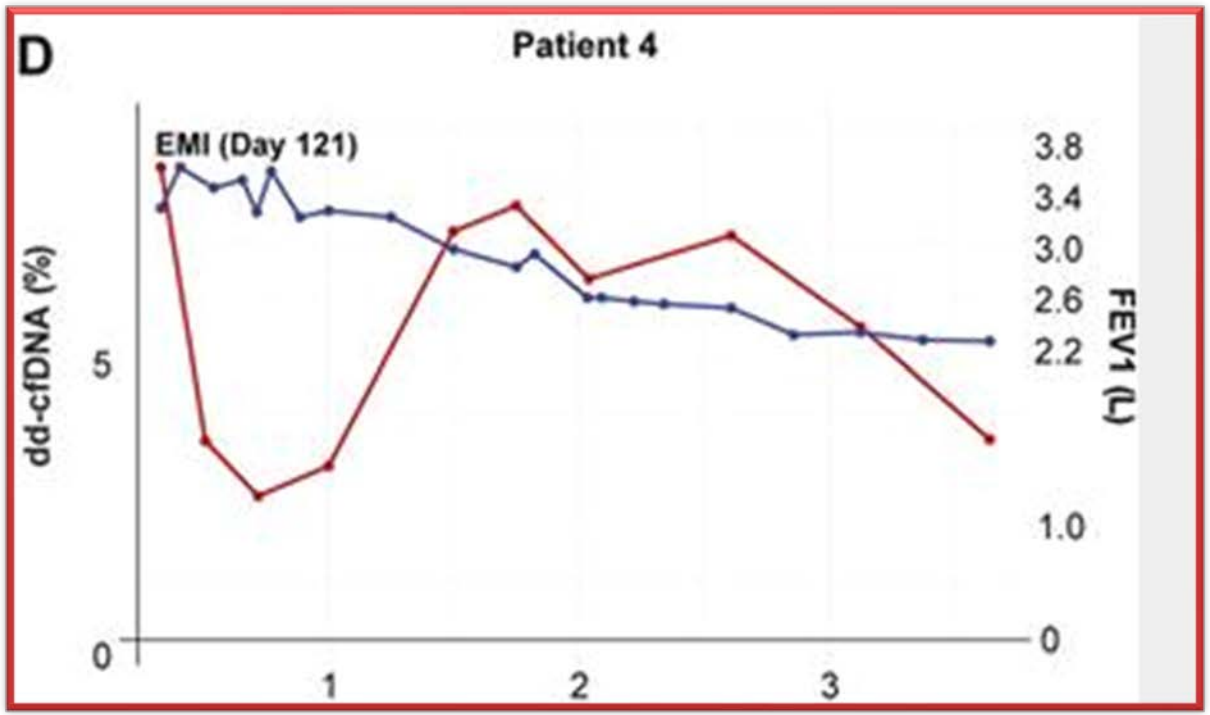

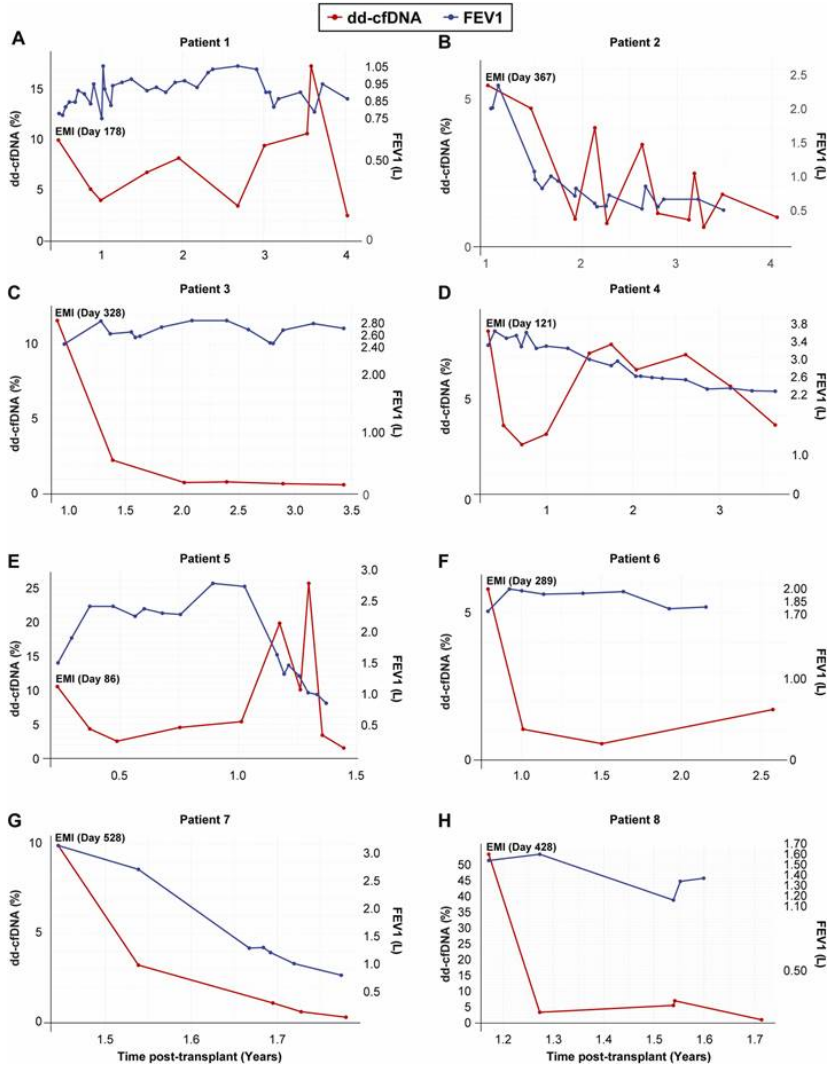

- **Primary EMI on post-transplant day 121**
- **Sustained elevations in dd-cfDNA with AMR on day 548**
- **CLAD on day 744.**

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Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

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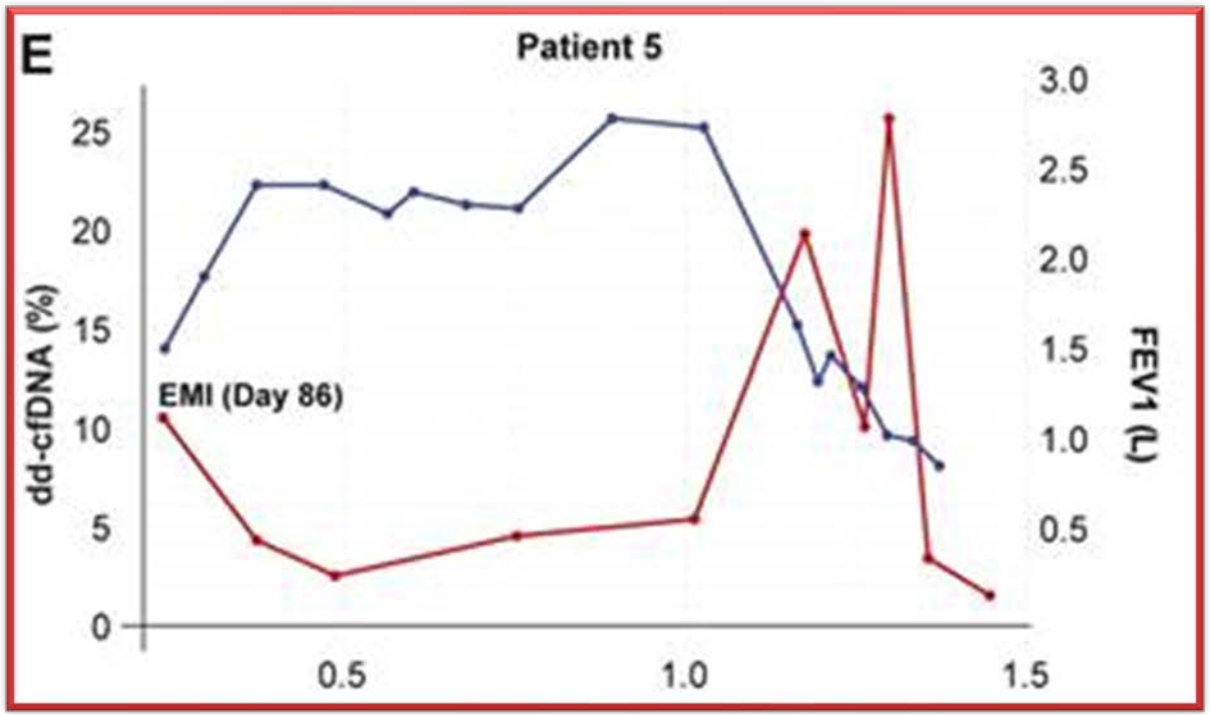

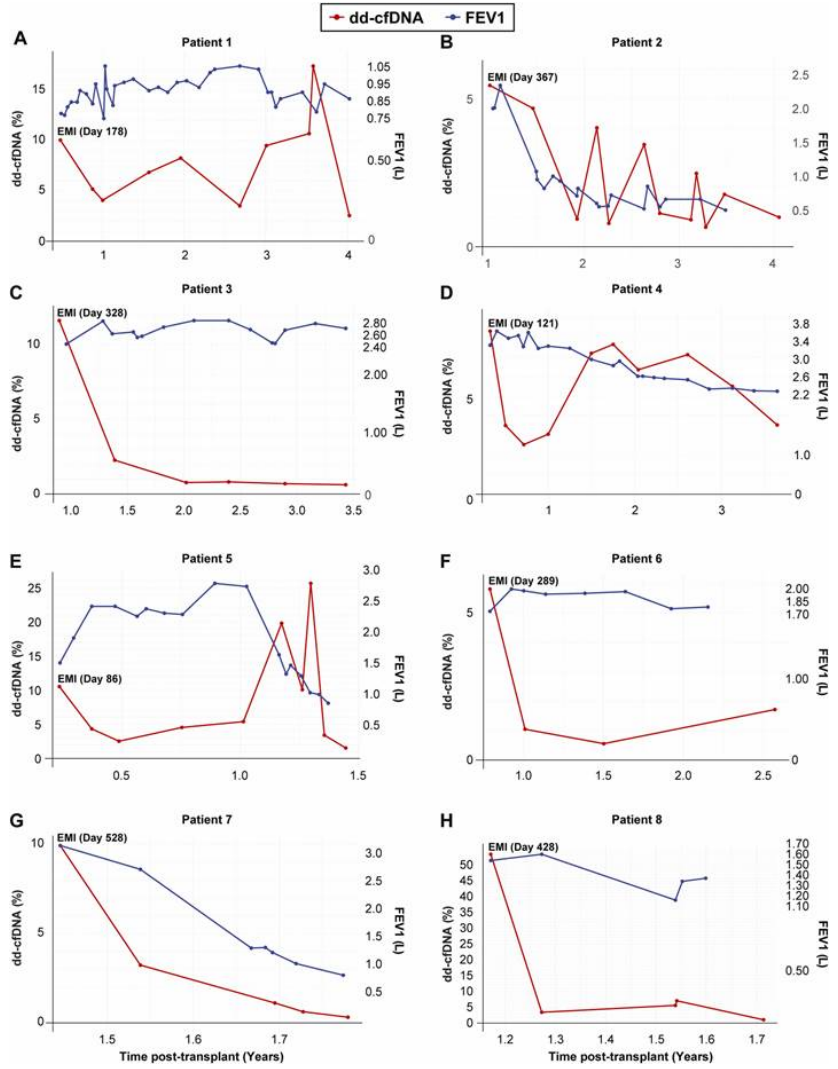

- **Secondary EMI (AMR) on post-transplant day 86**
- **FEV1 improves; continued lung injury/CLAD on day 425**
- **Death on day 573**

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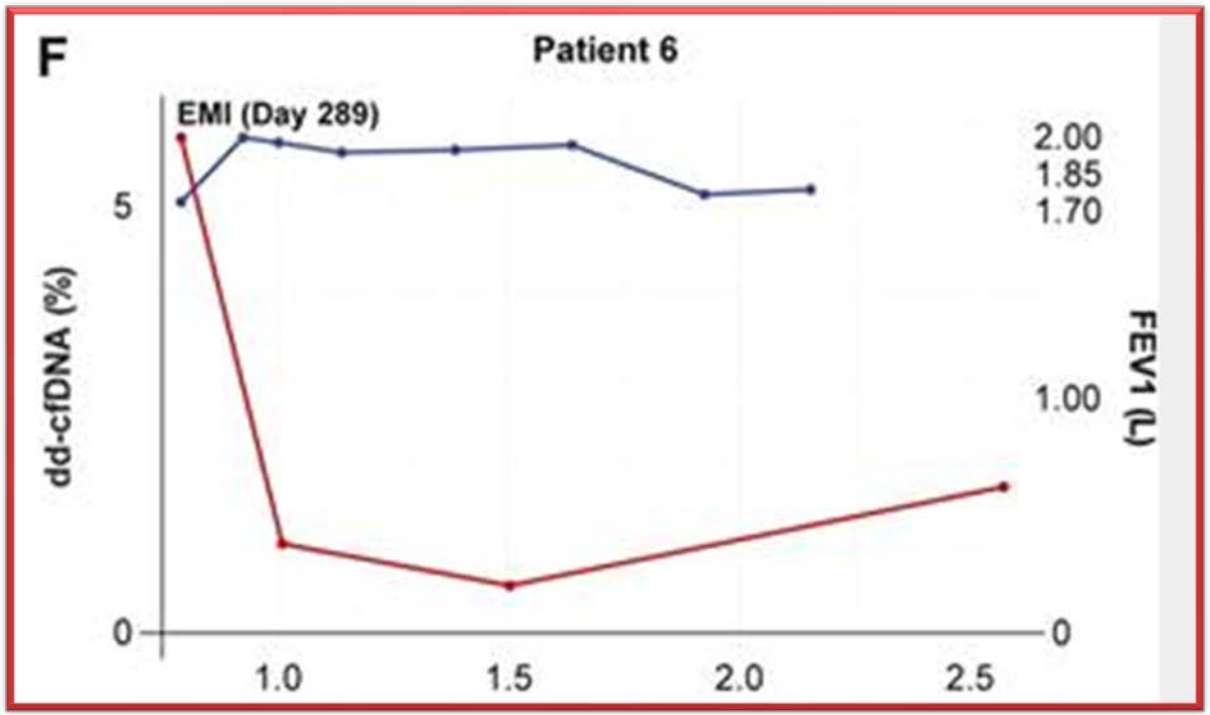
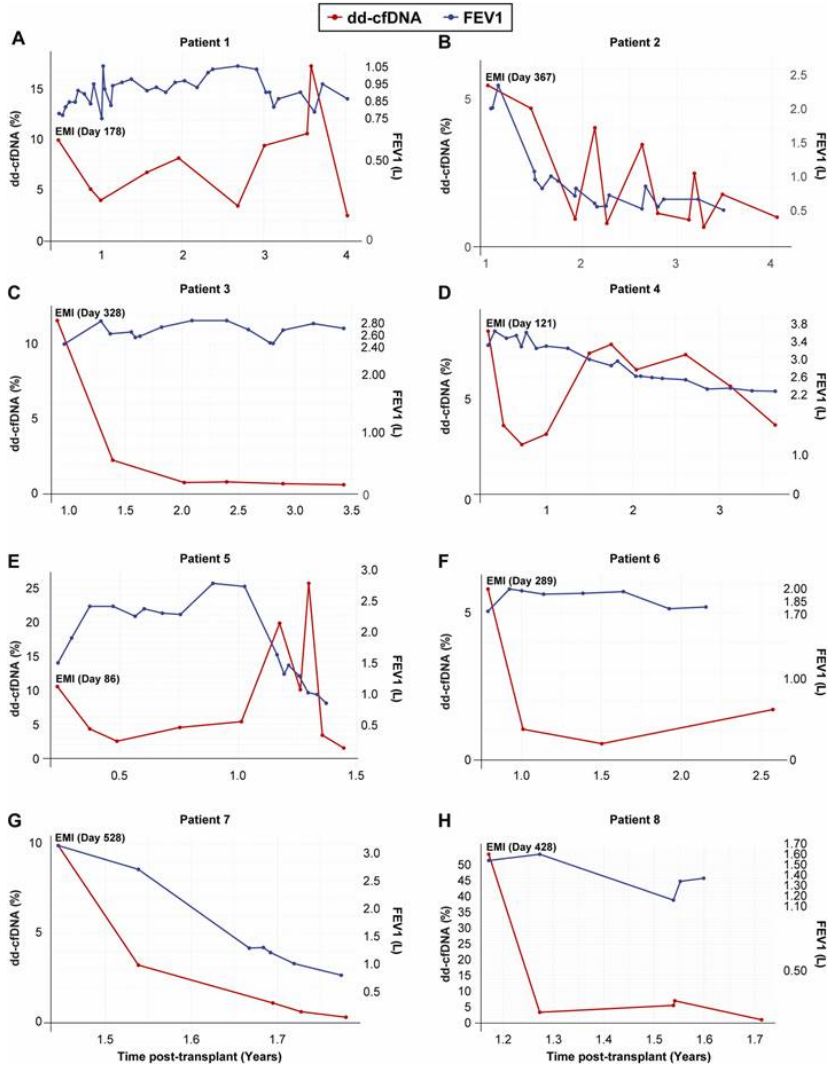
Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

Case Series of EMAD patients demonstrating longitudinal trends of dd-cfDNA & FEV1 from the time of EMI.





- **Secondary EMI (Influenza A) on post-transplant day 289**
- **Rapid decrease in dd-cfDNA levels**
- **No further infection, rejection or CLAD | Alive**

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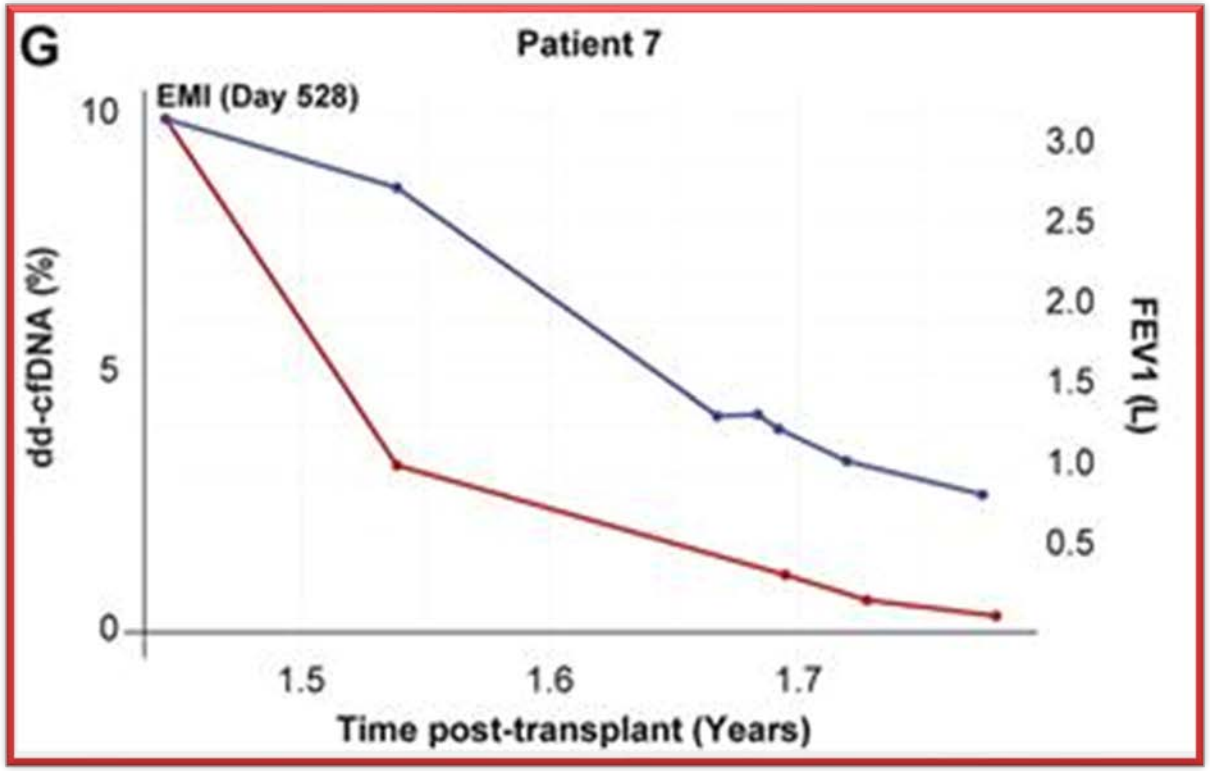
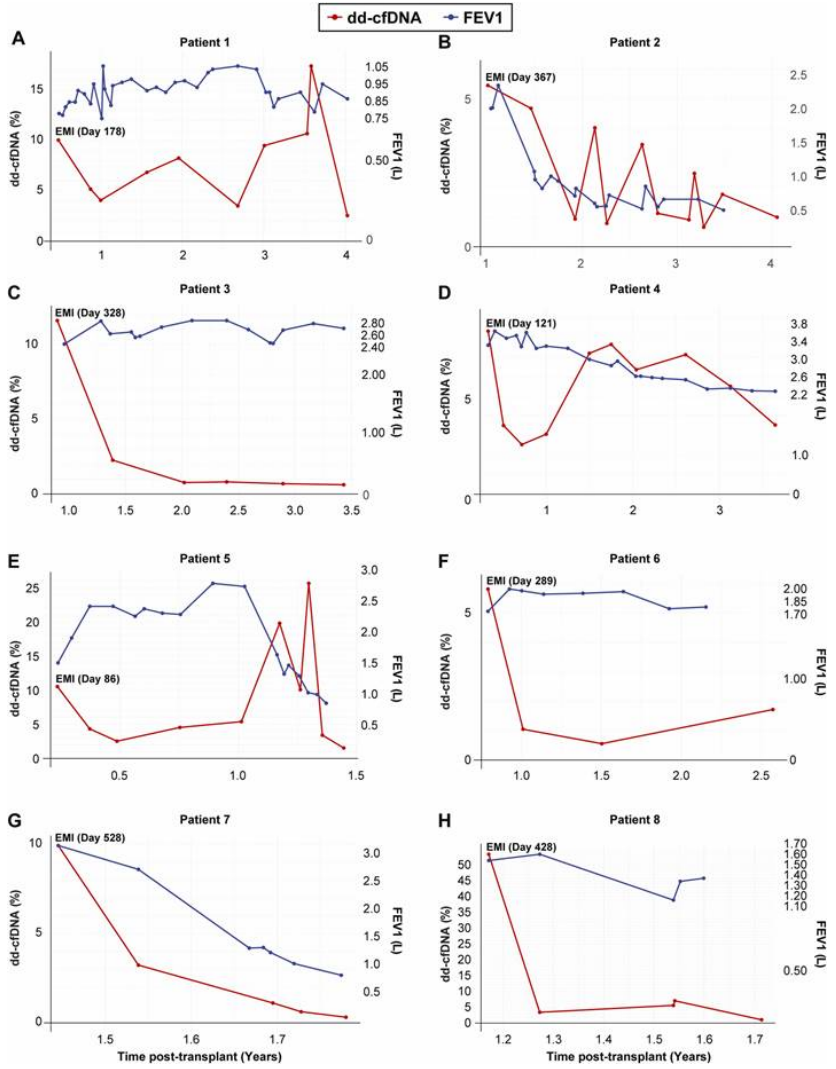
Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

Case Series of EMAD patients demonstrating longitudinal trends of dd-cfDNA & FEV1 from the time of EMI.





- **Primary EMI on post-transplant day 528**
- **Slow decay in dd-cfDNA levels, CLAD on day 609**
- **Death on day 573**

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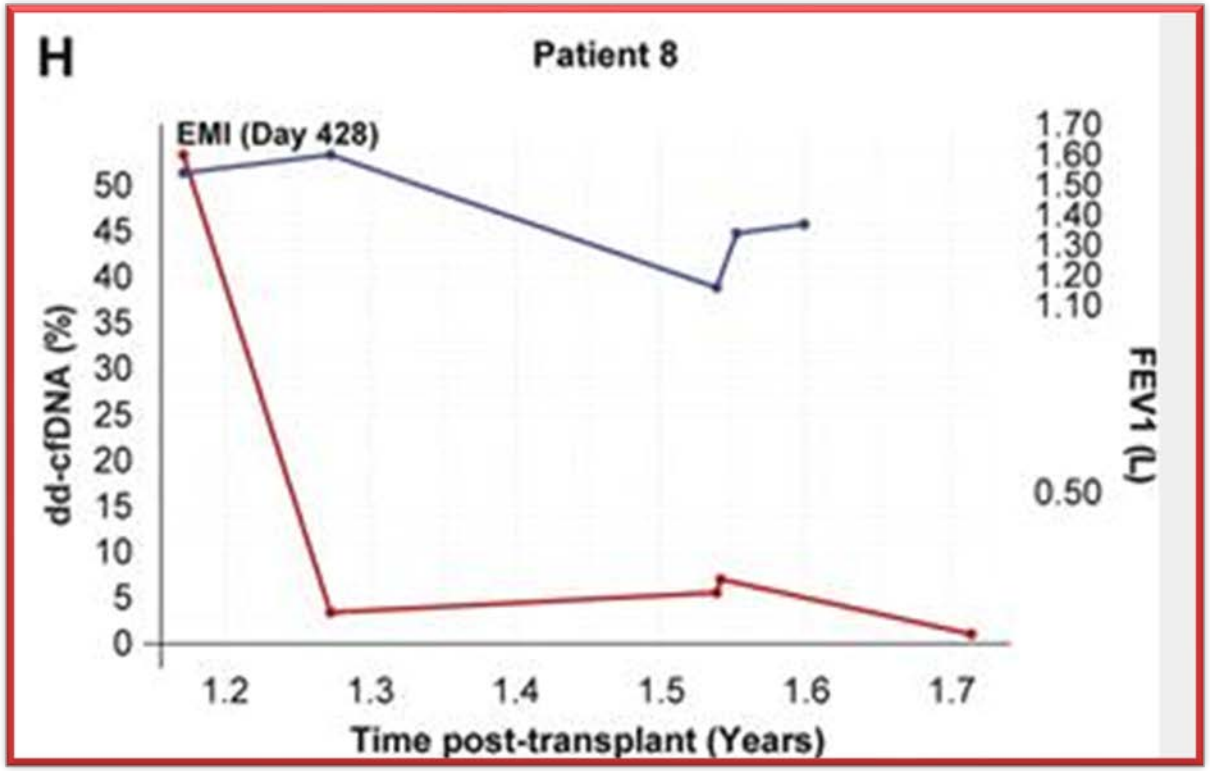
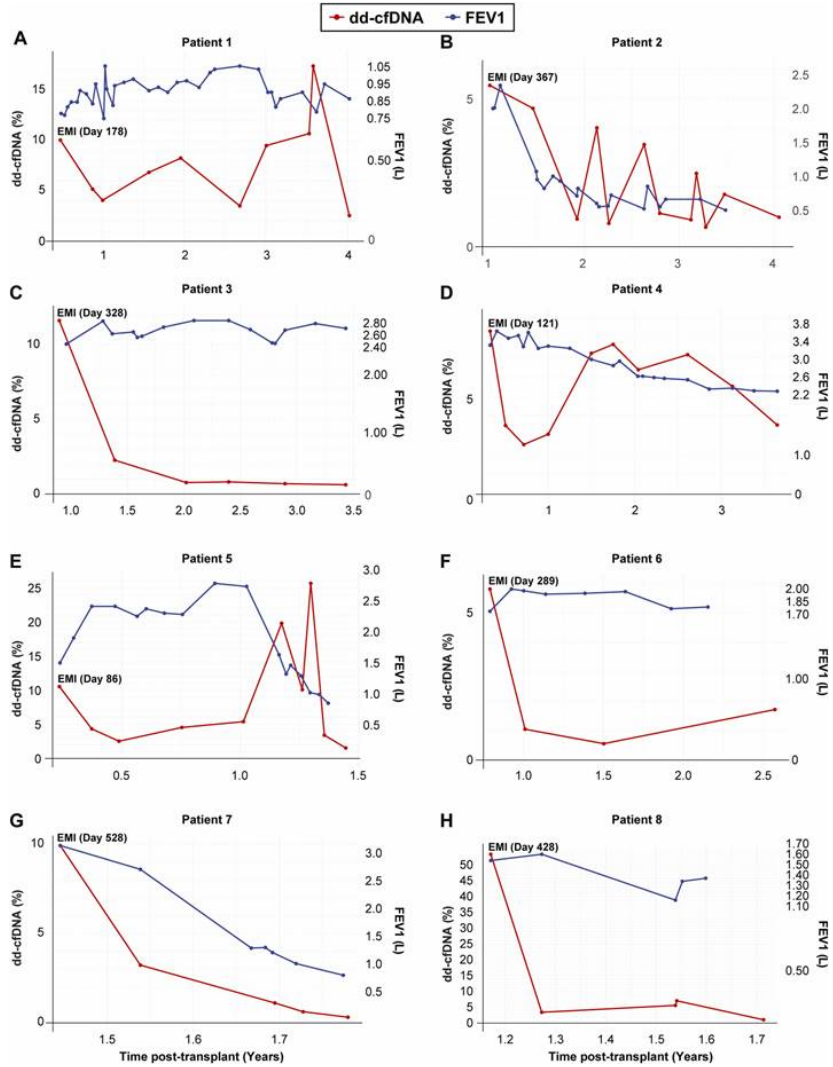
Extreme elevations of dd-cfDNA increases the risk of CLAD and death (2024)

Keller MB, et al.

Case Series of EMAD patients demonstrating longitudinal trends of dd-cfDNA & FEV1 from the time of EMI.





- **Primary EMI on post-transplant day 428**
- **dd-cfDNA levels decrease rapidly to baseline**
- **Alive and CLAD-free on follow up**

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RESULTS | Table 2. Association of Extreme Molecular Injury with CLAD or death

Models	Severe CLAD		Death		Composite: CLAD/Death	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Unadjusted Model						
EMI	2.93 (1.65 – 5.06)	<0.001	1.87 (1.13 – 3.11)	0.015	2.52 (1.10 – 3.82)	0.024
Adjusted multivariable model*						
EMI	3.90 (1.42 – 10.73)	0.008	3.88 (1.96 – 7.70)	<0.001	2.78 (1.26 – 6.22)	0.012

*Multivariable analysis was adjusted for recipient age, race, sex, native lung disease, PGD 3, bilateral vs single transplant, center, and prior episodes of ACR

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LIMITATIONS

- Observational study
- Small number of EMI events
  - Residual confounding may exist
    - Center-level differences in practice patterns may contribute to this
- Precise incidence of EMI is difficult to identify

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CONCLUSIONS

- Episodes of extreme molecular injury in lung transplant recipients are associated with an increased risk of subsequent severe CLAD or death, independent of concomitant rejection, infection or PFT decline.

- These findings offer the potential for a novel method of assessing allograft health and risk stratification in solid organ transplantation to improve long term outcomes.

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