AIBT Associazione Italiana di Immunogenetica e Biologia dei Trapianti
XXX Congresso Nazionale
Napoli, 10/12 ottobre 2024
"The programme of this event has been approved by the EFI Education Committee"

Il DNA libero circolante di origine del donatore: uno strumento diagnostico nel trapianto di organo solido

Silvia DEAGLIO, MD, PhD
Professor Medical Genetics
Department of Medical Sciences
University of Turin
Città della Salute e della Scienza
Turin, Italy

Il sottoscritto Silvia Deaglio
in qualità di relatore al

XXX CONGRESSO NAZIONALE AIBT
NAPOLI, 10/12 OTTOBRE 2024

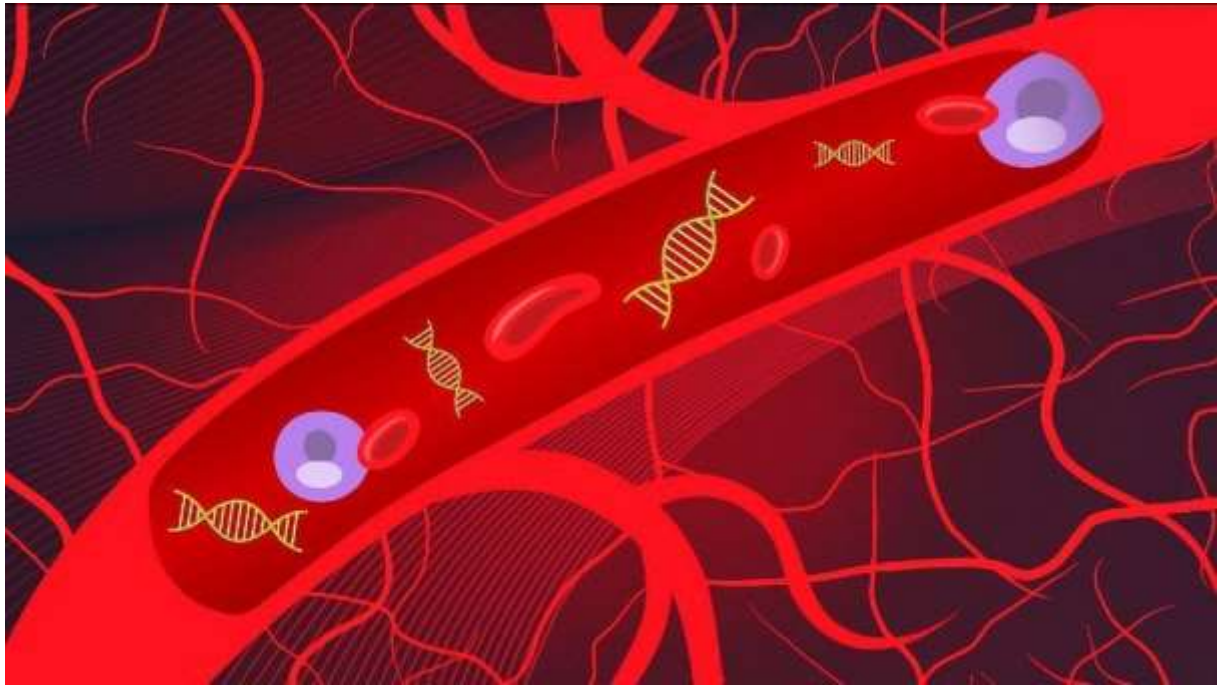
ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 18,19 dell'Accordo Stato-Regione del 19 aprile 2012, per conto di Planning Congressi srl

dichiara

che negli ultimi due anni ha avuto rapporti diretti di finanziamento con i seguenti soggetti portatori di interessi commerciali in campo sanitario:

- *Astra Zeneca (sponsored research agreement)*
- Heidelberg Pharma *(sponsored research agreement)*
- Solve Therapeutics *(sponsored research agreement)*

Cos'è il cfDNA?



- ✓ Piccoli frammenti di DNA che si trovano nel plasma di ciascuno di noi
- ✓ Tre ambiti di applicazione in medicina:
  - ambito oncologico
  - ambito prenatale
  - ambito trapiantologico

Come si usa oggi il cfDNA?

✓ In ambito oncologico il ctDNA serve per:

- diagnosi precoce
- monitoraggio effetto terapia
- monitoraggio comparsa resistenza alla malattia
- MRD

LIMITARE LE BIOPSIE TISSUTALI / ASPIRATI MIDOLLARI

✓ In ambito di diagnosi prenatale il cfDNA serve per:

- stimare il rischio di alterazioni citogenetiche con un buon livello di accuratezza
- determinare il sesso del feto

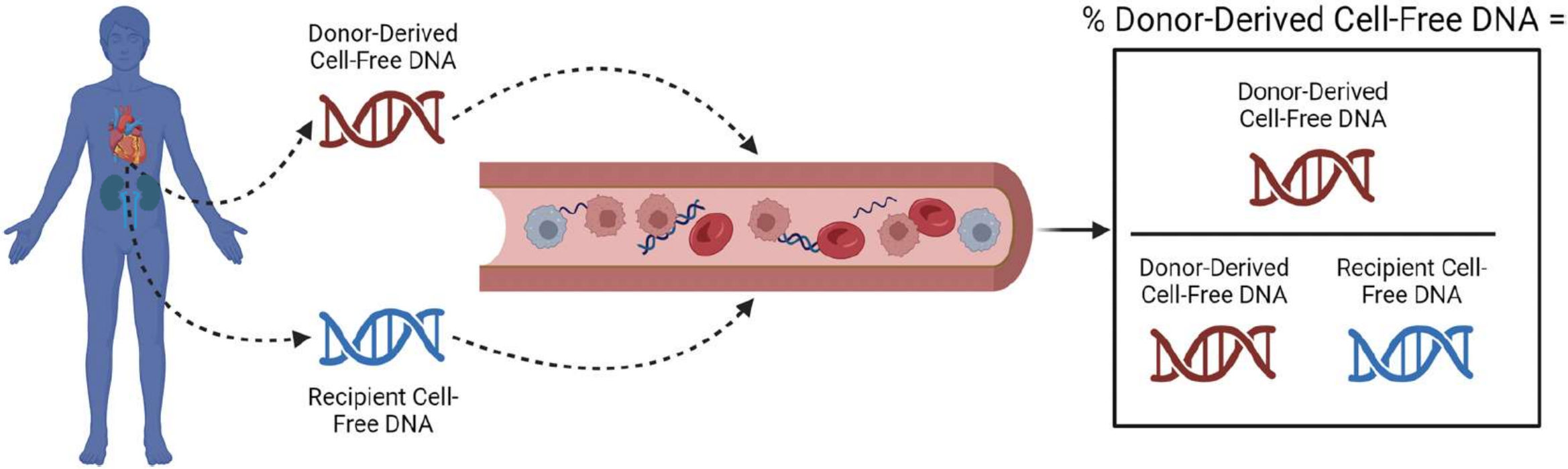
LIMITARE LA DIAGNOSI PRENATALE INVASIVA

✓ In ambito trapiantologico il dd-cfDNA serve per:

- diagnosticare il rigetto/danno d'organo
- monitorare la risposta alla terapia degli episodi di rigetto

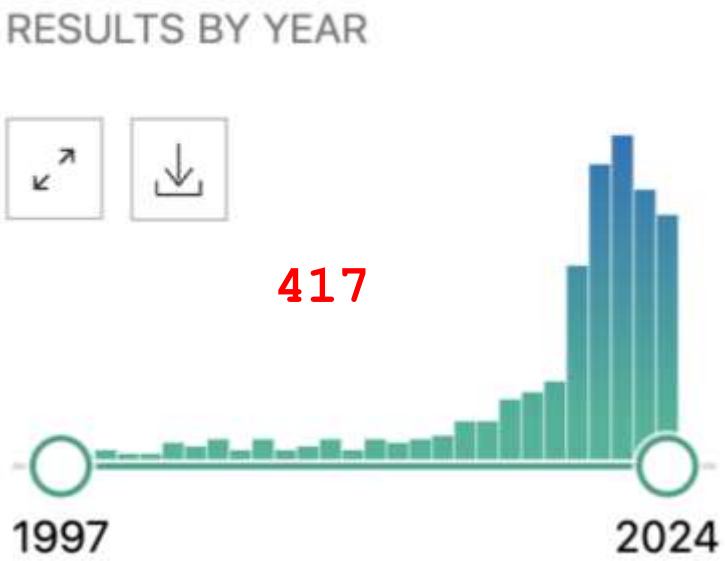
LIMITARE LE BIOPSIE DELL'ORGANO TRAPIANTATO

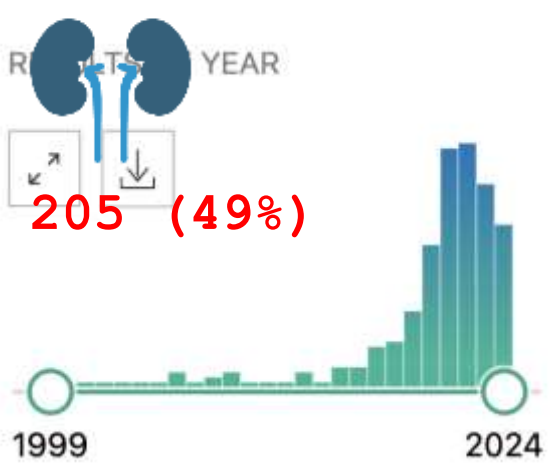
Il cfDNA nel contesto del trapianto

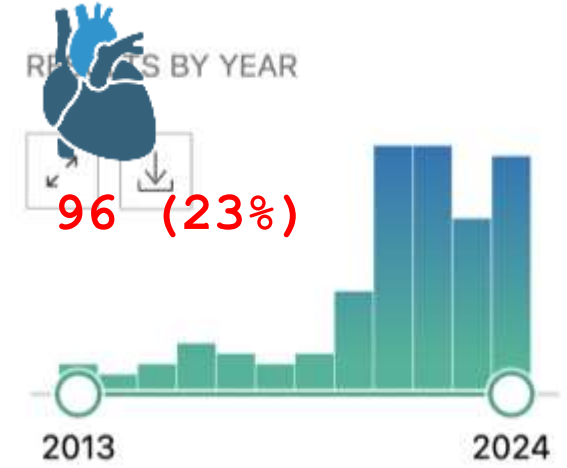


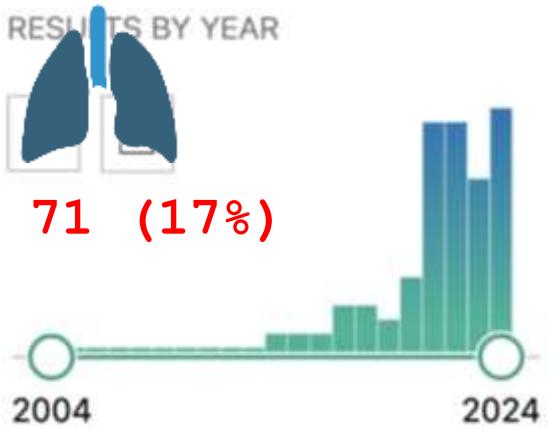
Adapted from: Current Heart failure reports, 20:493, 2023

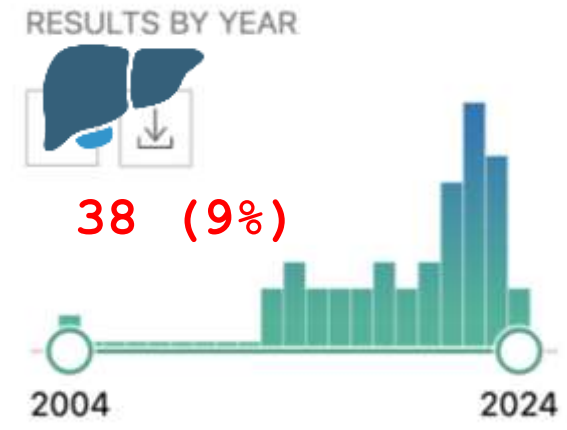
Le pubblicazioni sul tema dd-cfDNA



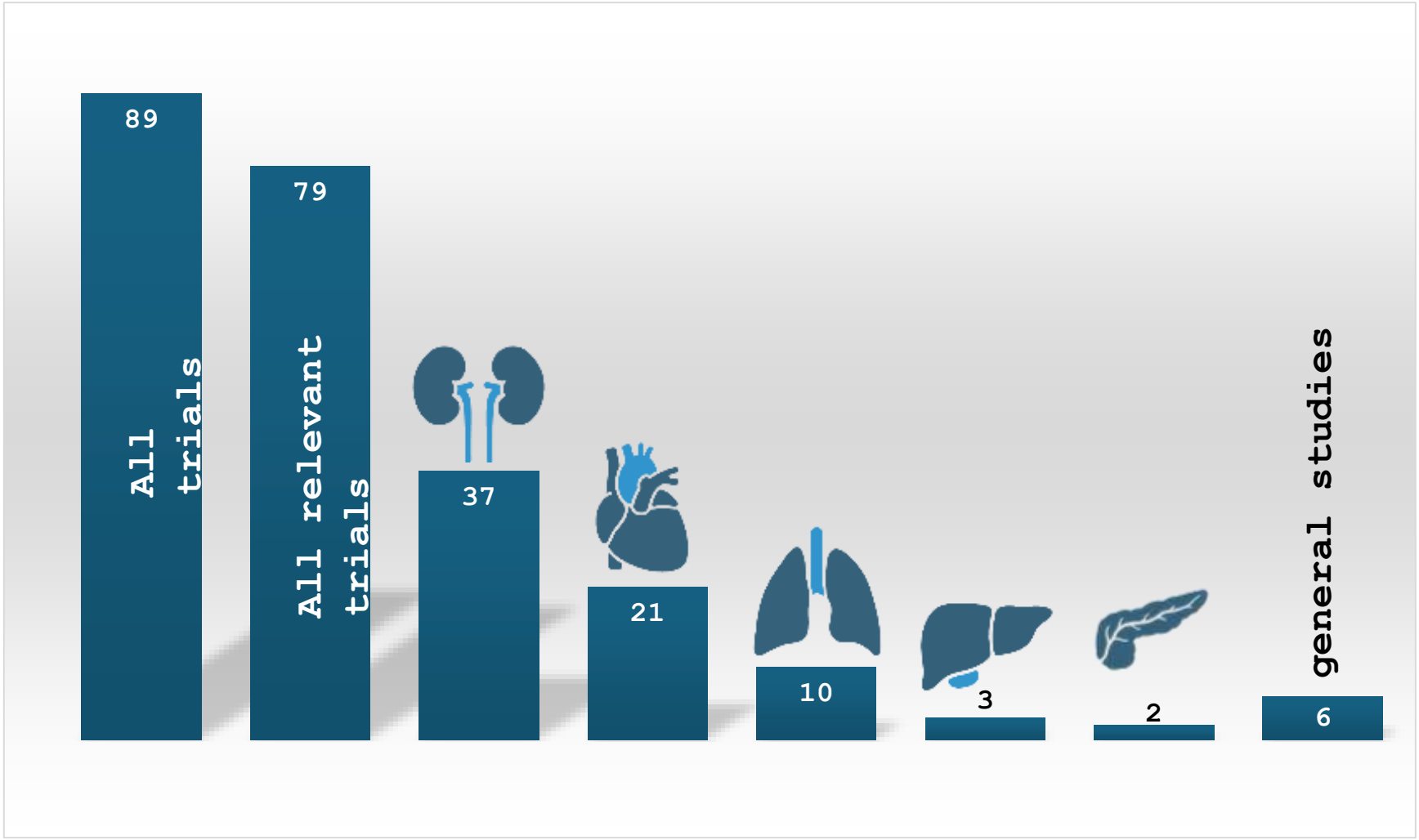








Sperimentazioni cliniche sul dd-cfDNA



33 studi stanno reclutando o cominceranno a reclutare

Il dd-cfDNA nel monitoraggio del rigetto acuto nel trapianto di rene

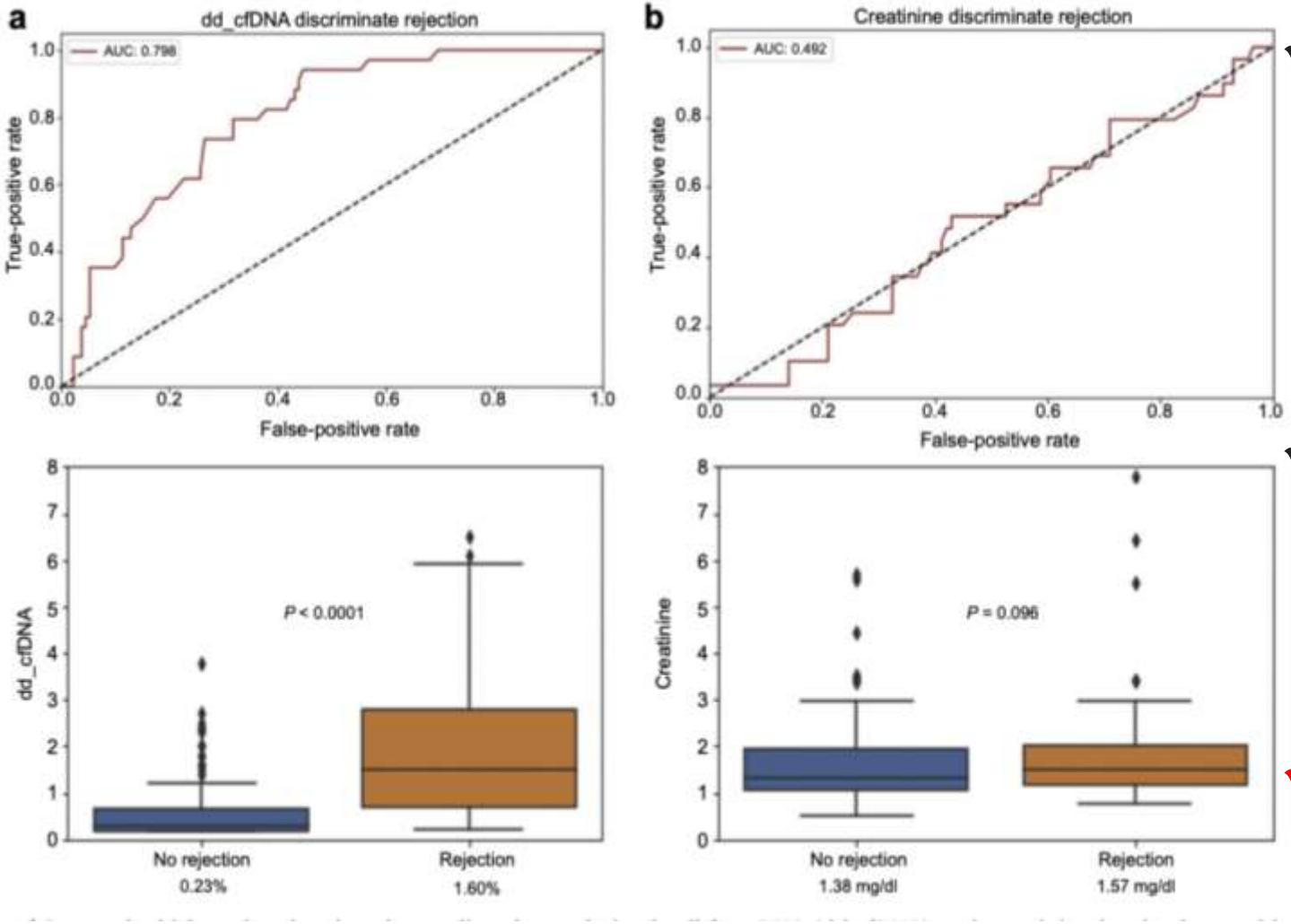
TABLE 1 | Summary of key data reviewed on donor-derived cell-free DNA for clinical acute rejection.

Authors	Study design	Number of samples	Results
[9]	Prospective observational, Multicenter	107 biopsies 27 rejections 80 no rejection	For any rejection (AUROC 0.74, PPV 0.61, NPV 0.84)
[10]	Subgroup analysis of prospective observational, Multicenter	87 patients 16 ABMR 53 no rejection	For ABMR with + DSA and dd-cfDNA >1%, (AUROC 0.86, PPV 0.81, NPV 0.83]
[2]	Retrospective analysis of biorepository samples, Single center	217 biopsies 38 rejections 179 no rejection	For any rejection for cause + SubAR (AUROC 0.87, PPV 0.52, NPV 0.95)
[11]	Prospective observational, Single center	63 biopsies 34 rejections 29 no rejection	For any rejection (AUROC 0.71, PPV 0.77, NPV 0.75)
[12]	Prospective observational, Single center	189 patients 22 rejections 395 stable samples	For any rejection (Absolute concentration of dd-cfDNA (AUROC 0.83) is better than dd-cfDNA (%) (AUROC 0.73)
[13]	Prospective cross-sectional, Multicenter (n = 2)	61 biopsies 13 ABMR 48 no rejection	For ABMR (absolute concentration AUROC 0.91 vs. dd-cfDNA (%) 0.89)
[14]	Subgroup analysis of prospective observational, Multicenter	79 patients with TCMR 1A/ borderline changes	Subjects with TCMR (1A and borderline) with high dd-cfDNA had worse clinical outcomes compared to those with low dd-cfDNA
[15]	Cross-sectional for DSA screening/Retrospective testing of dd-cfDNA on bio-banked samples, Single center	From 2 independent cohort 45/30 biopsies 25/17 ABMR 20/13 no ABMR	For ABMR with +DSA AUROC for dd-cfDNA alone 0.89/0.69 AUROC for DSA alone 0.88/0.77
[3]	Prospective observational, multicenter (ADMIRAL)	219 biopsies 113 rejections 106 no rejection	For any rejection dd-cfDNA (AUROC 0.8, PPV 0.5, NPV 0.9)
[16]	Prospective observational, Single center	208 biopsies 162 rejections by histology 46 no rejection by histology	For any rejection dd-cfDNA and MMDx (AUROC 0.80), dd-cfDNA and histology (AUROC 0.75)
[17]	Prospective observational, multicenter (TRIFECTA)	300 biopsies 120 rejections 180 no rejection	dd-cfDNA levels are strongly associated with the active molecular rejection phenotype (MMDx), particularly with AMR, mixed, and active TCMR
[18]	Prospective observational, multicenter (TRIFECTA)	367 biopsies 83 (histology test set) rejection 71 (MMDx test set) rejection	Any rejection prediction AUROC in test set by logistic regression model using both dd-cfDNA (%) and absolute concentration • 0.88 for MMDx • 0.82 for histologic rejection

AUROC, area under the receiver operating characteristic curve; DSA, donor-specific antibody; MMDx, the molecular microscope diagnostic system; NPV, negative predictive value; PPV, positive predictive value.

European Society of Organ Transplantation Consensus Statement on Testing for Non-Invasive Diagnosis of Kidney Allograft Rejection,

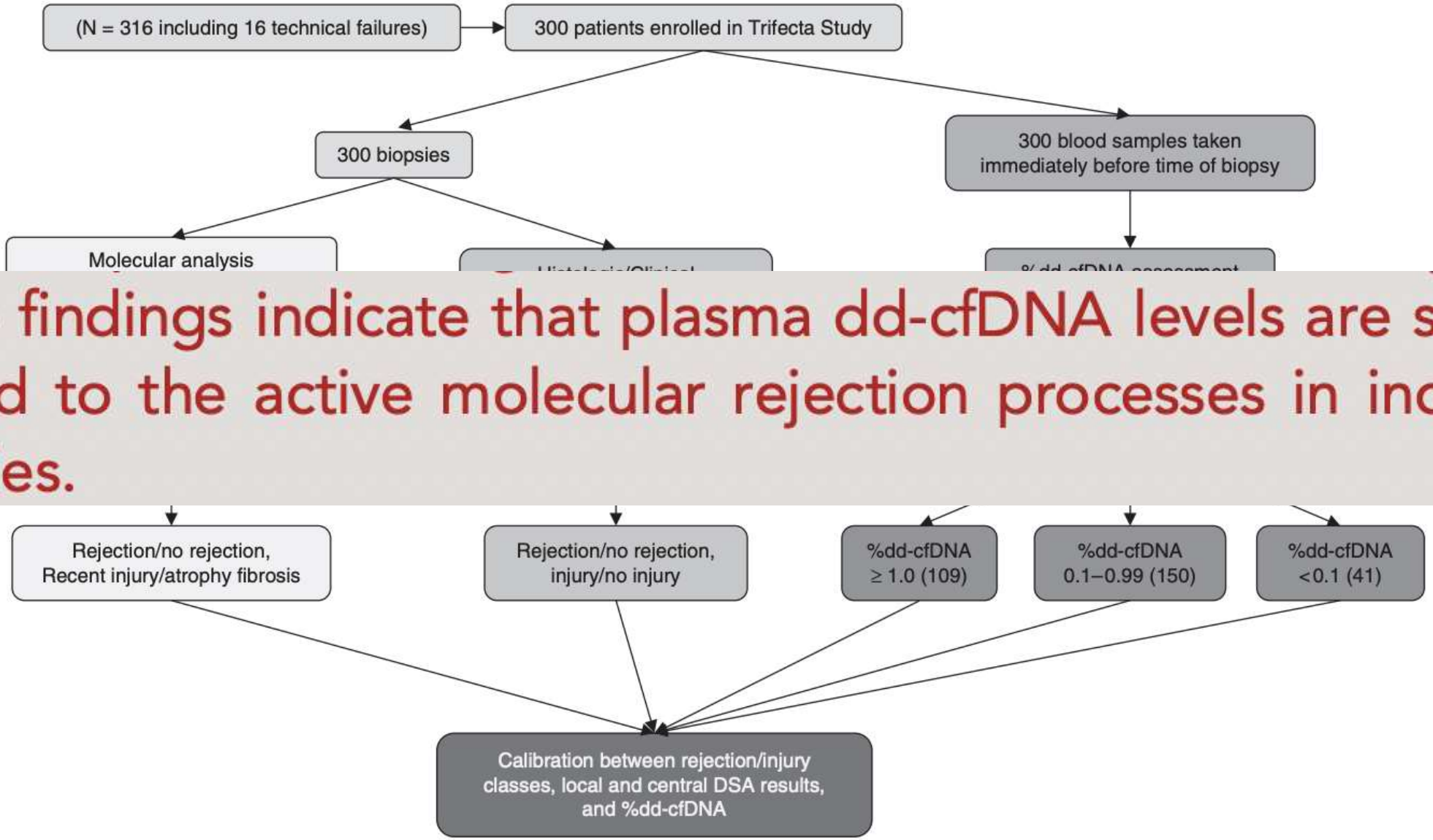
Il ruolo del dd-cfDNA nel trapianto di rene: lo studio ADMIRAL



- ✓ 1092 kidney transplant recipients monitored for dd-cfDNA over a three-year period
- ✓ association of dd-cfDNA with histologic evidence of allograft rejection
- ✓ **persistently low dd-cfDNA levels may accurately identify allograft quiescence or absence of injury**

Bu et al, Kidney Int., 2022 (US)

Il ruolo del dd-cfDNA nel trapianto di rene: lo studio TRIFECTA



These findings indicate that plasma dd-cfDNA levels are strongly related to the active molecular rejection processes in indication biopsies.

Halloran et al, JASN, 2022 (Canada)

Il ruolo del dd-cfDNA nel trapianto di rene

nature medicine

Article

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

Cell-free DNA for the detection of kidney allograft rejection

- ✓To assess the association of dd-cfDNA with rejection, disease activity and severity
- ✓To assess its independent and added value to detect rejection beyond standard of care monitoring

Aubert et al, Nature Medicine. 2024

Il disegno dello studio

Cohort: **3,733 kidney transplant evaluations** between 2013 and 2022

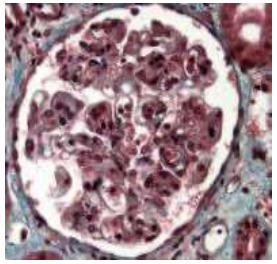
French development cohort : 1,415 evaluations

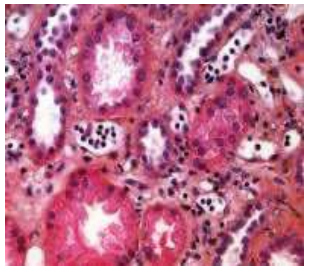
Validation cohort : 2,318 evaluations in the US (11 centers) and Belgium (1center)

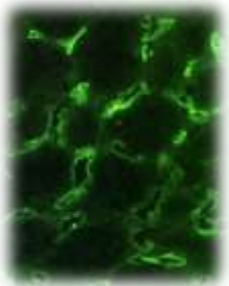
Evaluations (n=3,733)

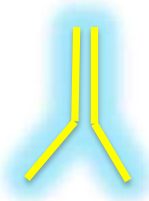
- Baseline characteristics
- Renal function + Proteinuria (P/C)
- HLA DSA at the time of TX
- HLA DSA at the time of biopsy
- Histology :
  - Banff 2019 scores
  - Diagnoses
- **dd-cfDNA (AlloSeq and AlloSure)**

Rejection Banff scores









Aubert et al, Nature Medicine. 2024

Il dd-cfDNA mostra significativa associazione con il rigetto

Derivation cohort

		Number of biopsies	Number of events	OR	95% CI	p	OR 95% OR bootstrap BCA
eGFR (mL/min/1.73 m²)		1,395	226	0.989	(0.979-0.999)	**0.033**	(0.979-1.000)
Proteinuria (g/g) (log transformation)		1,395	226	1.180	(1.031-1.350)	**0.016**	(1.029-1.350)
Kidney graft instability*	**No** **Yes**	1,170 225	159 67	1 1.768	- (1.144-2.711)	**0.010**	- (1.102-2.894)
Previous episode of Rejection	**No** **Yes**	1,300 95	178 48	1 4.756	- (2.852-7.920)	**4.754e-09**	- (2.781-7.827)
Anti-HLA DSA MFI	**<500** **≥500 – 3,000** **≥3000 – 6,000** **≥6,000**	859 424 54 58	76 93 20 37	1 3.081 3.310 6.338	- (2.152-4.429) (1.618-6.597) (3.186-12.791)	**2.125e-12**	- (2.062-4.496) (1.726-6.607) (3.410-12.258)
dd-cfDNA (log transformation)		1,395	226	2.275	(1.902-2.739)	**<2.2e-16**	(1.805-2.801)

Aubert et al, Nature Medicine. 2024

Il dd-cfDNA mostra significativa associazione con il rigetto

Validation cohort

		Number of biopsies	OR	95% CI	p
eGFR (mL/min/1.73 m²)		1,911	0.990	(0.984-0.996)	**0.001**
Proteinuria	**Absence** **Presence**	1,341 570	1 1.468	- (1.134-1.900)	**0.016**
Kidney graft instability	**No** **Yes**	1,505 406	1 1.397	- (1.028 – 1.896)	**0.004**
Previous episode of rejection	**No** **Yes**	1,538 373	1 2.661	- (2.016-3.516)	**5.17e-12**
Anti-HLA DSA	**Absence** **Presence**	1,498 413	1 3.134	- (2.402-4.096)	**< 2e-16**
dd-cfDNA (log transformation)		1,911	2.317	(2.083-2.588)	**< 2e-16**

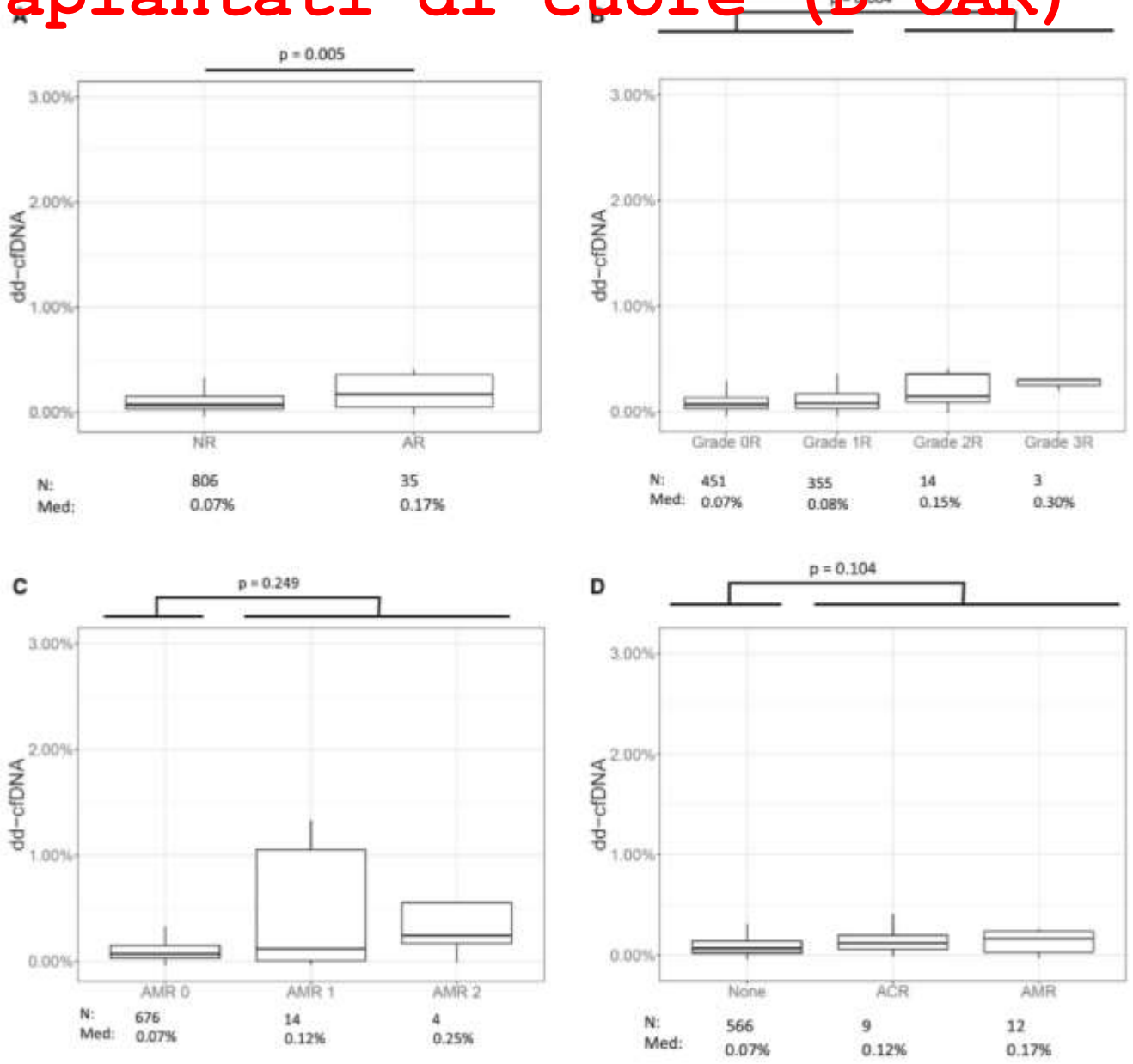
Aubert et al, Nature Medicine. 2024

Il ruolo del dd-cfDNA nel trapianto di cuore

Study Name, Year of Publication	Study Design	Key Study Findings
dd-cfDNA studies		
Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection, 2014[13]	• Prospective study on Genome Transplant Dynamics (GTD) using SNV genotyping • 65 adult and pediatric HT recipients with 565 longitudinally obtained plasma samples compared with EMB	• Established rapid clearance of dd-cfDNA after HT and a stable baseline allowing for implementation of a time-independent threshold for the diagnosis of AR when collected more than 2 wks after HT • dd-cfDNA detected both ACR and AMR, and levels correlated with severity • ROC analysis for detection of ACR (≥2R/3A or AMR) by dd-cfDNA revealed AUC of 0.83 (sens = 0.58, spec= 0.93 at a dd-cfDNA threshold level of 0.25%) • Findings indicate that dd-cfDNA could replace EMB
Donor-Derived Cell-Free DNA-Outcomes AlloMap Registry (D-OAR), 2019[6]	• Prospective observational cohort of 740 HT recipients from 26 U.S. centers aged ≥15 y and >55 d post-HT who were undergoing AlloMap GEP and AlloSure dd-cfDNA testing for surveillance, plus a single-center cohort of 33 patients at high risk for AMR • Study period: 2014-2018	• Median dd-cfDNA was 0.07% in reference HT recipients (2,164 samples) and 0.17% with acute rejection (35 samples; $P = 0.005$) • At a 0.2% threshold, dd-cfDNA had a 44% sensitivity to detect rejection and a 97% NPV • In the cohort at risk for AMR (110 samples from 33 patients), dd-cfDNA levels were elevated 3-fold in AMR compared with patients without AMR (99 samples; $P = 0.004$)
Genomic Research Alliance for Transplantation (GRAfT), 2021[7]	• Prospective observational cohort study of HT recipients aged ≥18 y from 5 U.S. centers • Aimed to validate the test characteristics of dd-cfDNA for acute rejection and to determine the ability to predict long-term outcomes including CAV, graft failure, and mortality • dd-cfDNA measured by shotgun sequencing and included donor-recipient-paired genotyping to identify SNVs (research grade assay-not available clinically) • Study period: 2015-ongoing	• Median dd-cfDNA levels were 0.34% vs 0.04%; $P < 0.006$ for ACR grade ≥2 vs ACR grade 1 • Median levels were 0.63% vs 0.02%; $P < 0.001$ for AMR 1 versus grade 0 rejection and 1.68% vs 0.63%; $P = 0.039$ for AMR ≥2 vs AMR 1 • dd-cfDNA levels rose as early as 120 d before acute rejection, and fragment length >100 bp was associated with AMR diagnosis
A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation (DEDUCE), 2022[14]	• Observational 2 center study with retrospective and prospective component • Using the clinically available Prospera dd-cfDNA test (Natera) • Study period: 2017-2022	• 811 samples from 223 patients with dd-cfDNA testing and contemporaneous EMB with 49 EMBs showing AR in 35 patients • dd-cfDNA was significantly higher in AR (median 0.58%; IQR: 0.13%-1.68%) compared with non-AR (median 0.04%, IQR: 0.01%-0.11%, P corrected for multiple comparisons < 0.001) • AUC-ROC of 0.86 (95% CI: 0.77-0.96) • For dd-cfDNA ≥0.15% 78.5% sens (95% CI: 60.7%-96.3%) and 76.9% spec (95% CI: 71.1%-82.7%) for AR • PPV 25.1% (95% CI: 18.8%-31.5%) and NPV 97.3% (95% CI: 95.1%-99.5%)
Surveillance HeartCare Outcomes Registry (SHORE) [NCT03695601]	• Prospective observational registry study, HT patients with HeartCare (AlloMap and AlloSure) monitoring initiated within 3 mo of HT compared with historical control group not monitored with HeartCare • Planned enrollment of 2,300 patients completed (plan to match with 1,150 historical controls) • Primary outcome: Percentage of patients alive at 1, 2, and 3 y post-HT • Study period: 2018-2024	• NA

JACC: HEART FAILURE, 11:263-276, 2023

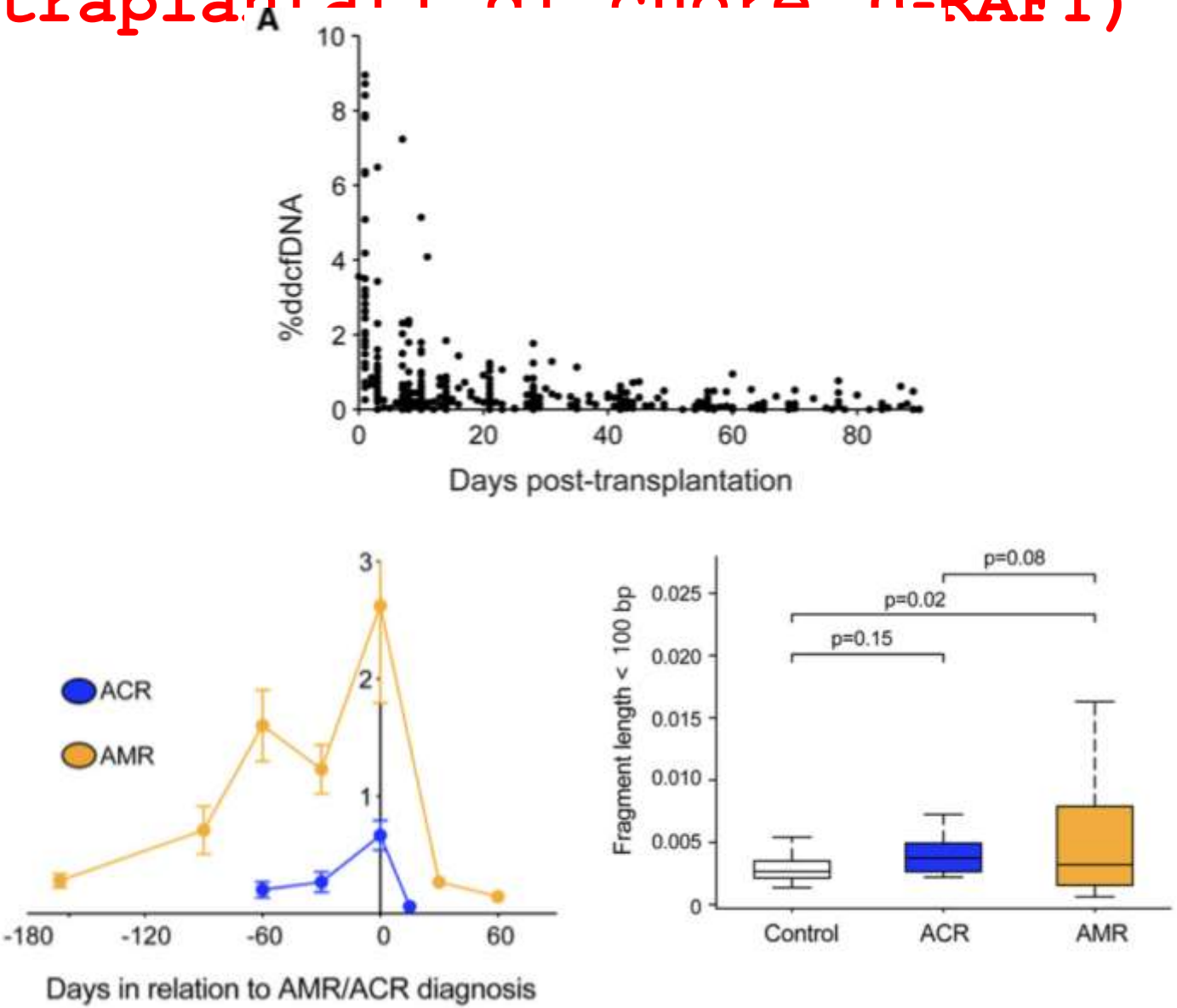
Il dd-cfDNA come marcatore di rigetto nei trapiantati di cuore (D-QAR)



- ✓ 740 pazienti da 26 centri con biopsie seriali
- ✓ La misurazione standardizzata di dd-cfDNA discrimina rigetto acuto da non-rigetto

Kuush et al, Am J Transplant. 2019

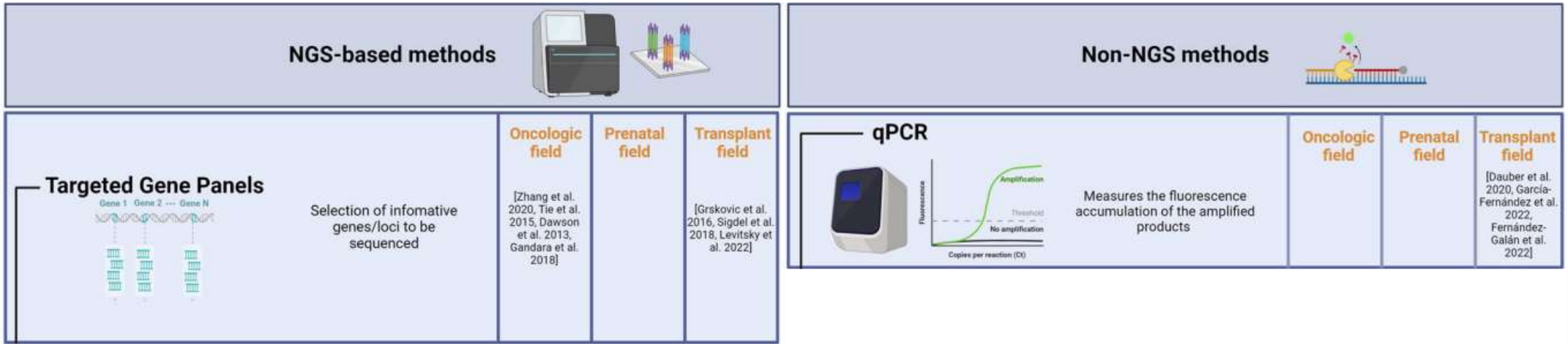
Il dd-cfDNA come marcatore di rigetto nei trapiantati di cuore (CRAFT)



- ✓ 171 pazienti
- ✓ Il test è attendibile 4 settimane dopo il trapianto
- ✓ Il dd-cfDNA si alza circa 4 mesi prima dell'evidenza istologica di rigetto
- ✓ La lunghezza dei frammenti di dd-cfDNA è suggestiva del tipo di rigetto
- ✓ Il test può evitare un numero significativo di biopsie protocollari

Agbor-Enoh et al, Circulation, 2021

Come monitoriamo il dd-cfDNA?



NGS sequencing of >200 SNPs vs 50 in/del markers

Highly quantitative PCR conditions, may be multiplexed

Sorbini et al, Biomolecules, 14:498, 2024

Cosa è disponibile in Europa?

TABLE 2 Commercially-Available GEP and dd-cfDNA Assays

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

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

JACC: HEART FAILURE, 11:263-276, 2023

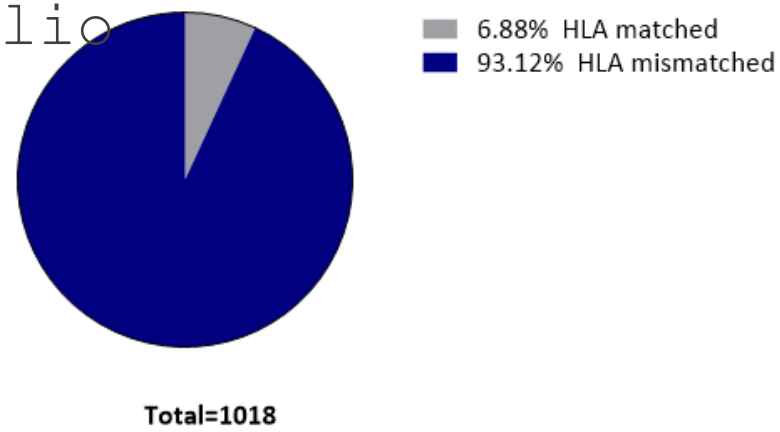
La nostra esperienza sfruttando gli *HLA DRB1* in ddPCR

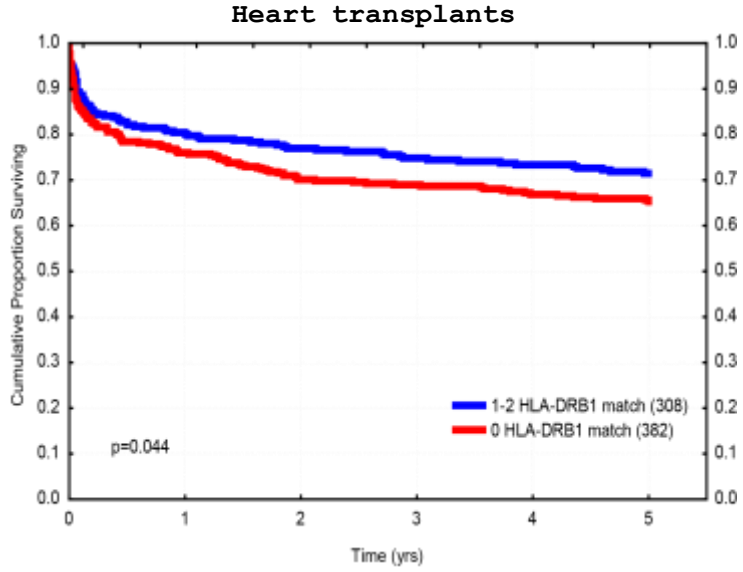
Perché il gene *HLA-DRB1*?

- ✓ Non è troppo polimorfico nella popolazione
- ✓ Sonde e primers erano commercialmente disponibili
- ✓ I trapianti "matched" per HLA-DR vanno in generale meglio

Probe ID	Target allele
dHsaEXD29156242	*HLA-DRB1*01*
dHsaEXD93426015	*HLA-DRB1*03*
dHsaEXD67695788	*HLA-DRB1*04*
dHsaEXD41965561	*HLA-DRB1*07*
dHsaEXD16235334	*HLA-DRB1*08*
dHsaEXD80505107	*HLA-DRB1*11*
dHsaEXD54774880	*HLA-DRB1*13*
dHsaEXD29044653	*HLA-DRB1*15/16*

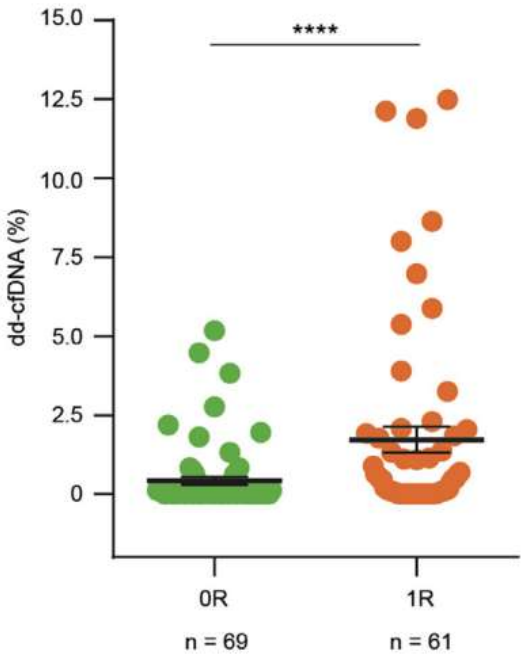
Zou et al, Hum Immunol 2017

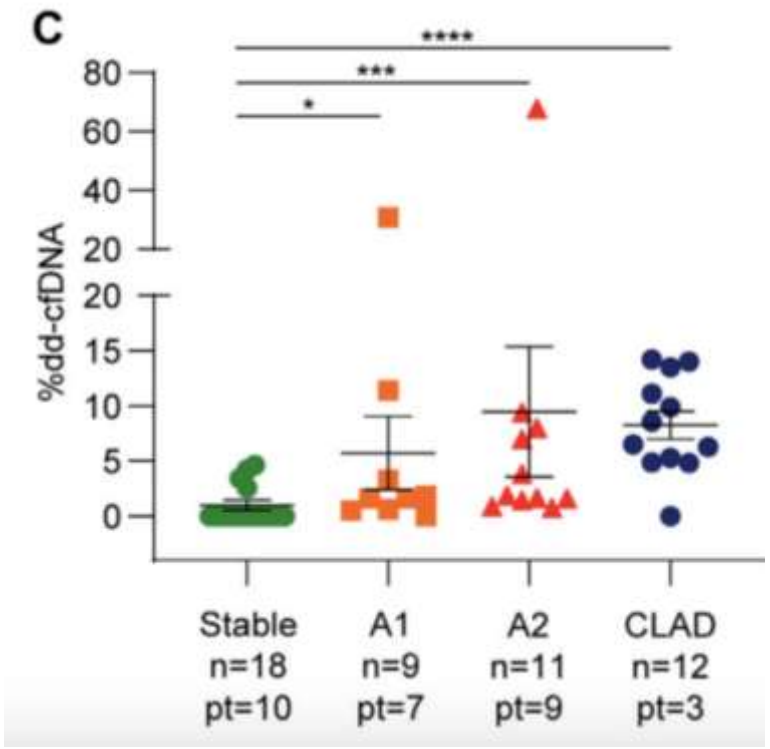


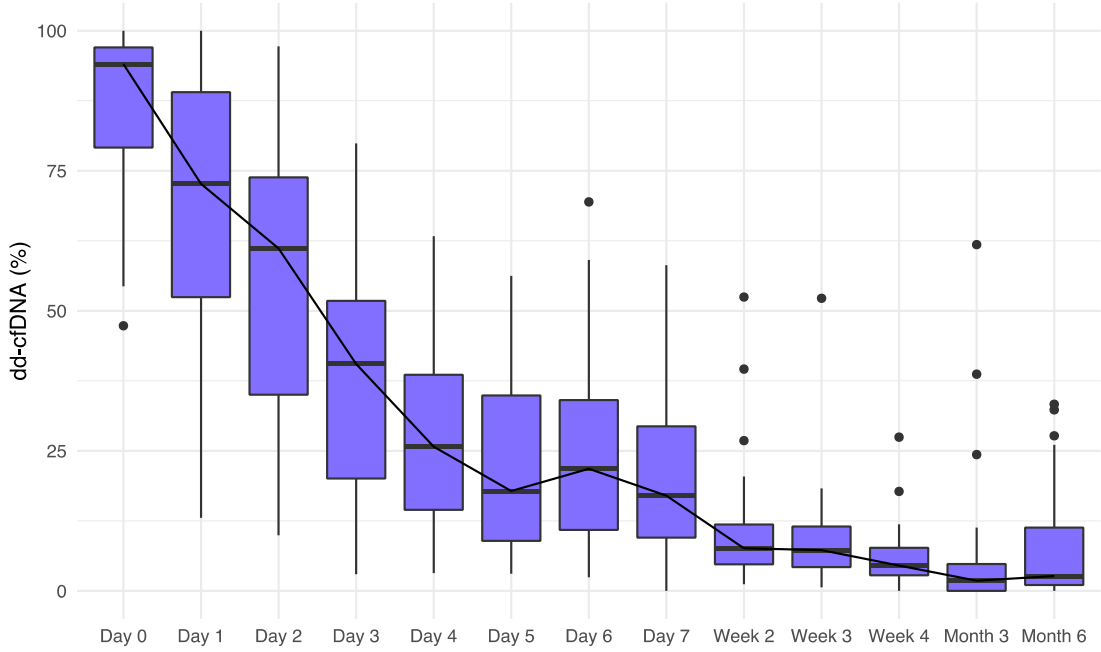


Patent P2021/10

Validazione di un test "home-made"







Sorbini et al., J Heart Lung

Sorbini et al., Transplant

Sorbini M, Carradori T, et al.,

Quando e come usare questo test?

- ✓ Poiché ha un valore predittivo negative lo potremmo usare per limitare le biopsie di sorveglianza

- ✓ Abbiamo offerto a una popolazione di pazienti pediatrici trapiantati di cuore e in follow-up presso il Centro di Torino il monitoraggio del dd-cfDNA come parte dei loro esami di controllo a ogni appuntamento per 12 mesi consecutive

- ✓ Al termine dello studio abbiamo correlato i dati di dd-cfDNA con le informazioni cliniche, di laboratorio, di imaging e di biopsia per capire che cosa aggiunge questo monitoraggio

La coorte pediatrica di trapiantati di cuore

TABLE 1.

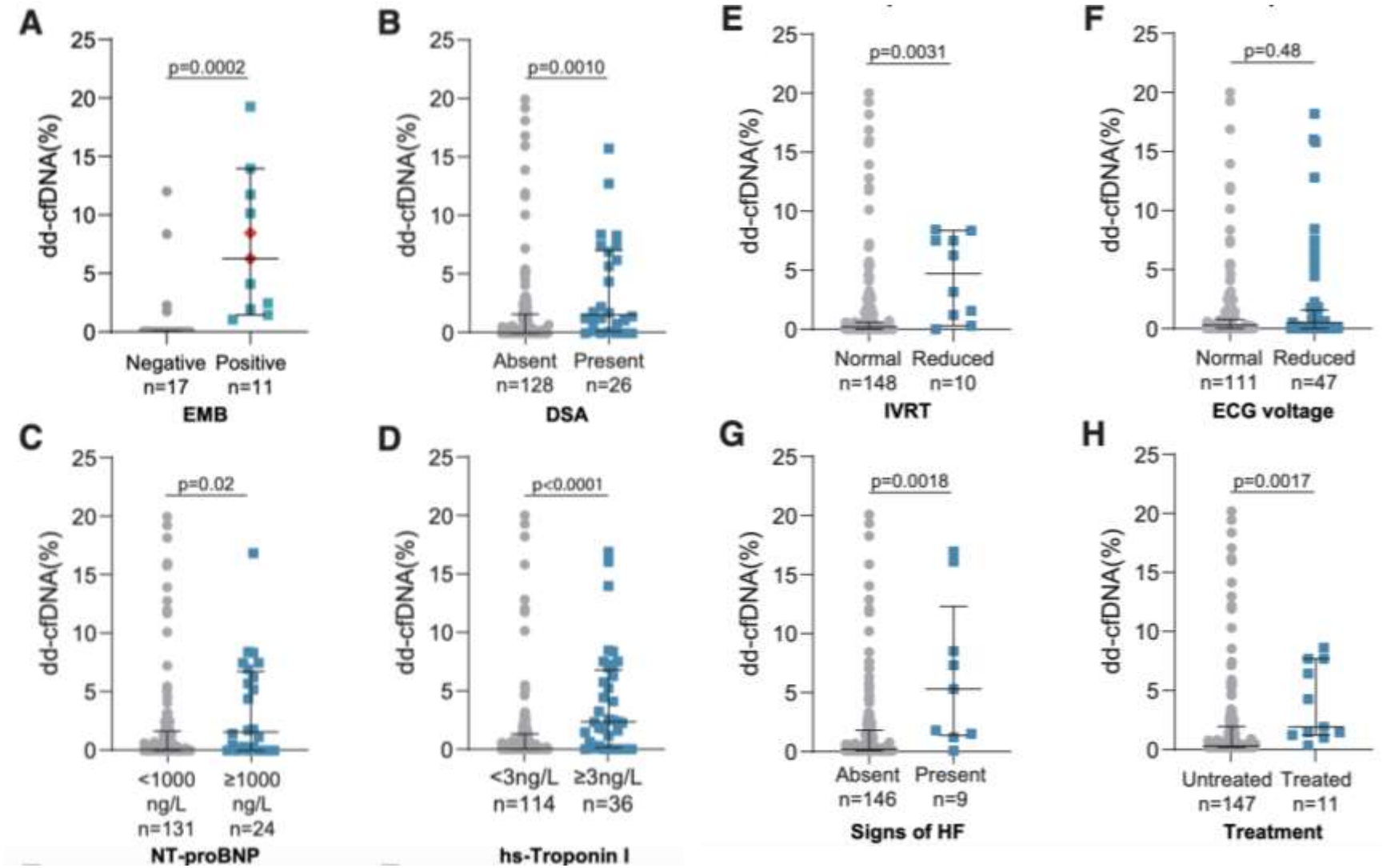
Characteristics of the enrolled cohort

ID	Sex	Age, y	Diagnosis	Transplant date	Enrollment date	Time since transplant, y	No. of EMB	dd-cfDNA determinations
1	F	18.6	CHD	01/10/06	02/22/22	16.13	1	4
2	F	15.3	DCM	07/03/08	04/26/22	13.82	0	6
3	F	17.9	DCM	02/09/10	03/08/22	12.08	1	4
4	F	15.1	CHD	08/27/10	03/29/22	11.59	1	1
5	M	14.1	DCM	12/31/10	10/04/22	11.77	1	1
6	M	16.6	CHD	04/25/11	04/08/22	10.96	1	8
7	F	12.6	HCM	07/02/11	03/21/22	10.73	1	4
8	F	13.9	DCM	03/07/13	04/05/22	9.08	1	6
9	M	11.4	DCM	04/17/13	02/24/22	8.86	1	9
10	F	10.3	DCM	01/09/14	03/01/22	8.15	1	6
11	M	10.2	CHD	09/18/14	03/14/22	7.49	0	3
12	F	8.4	DCM	06/20/15	02/24/22	6.69	1	5
13	F	18.5	CHD	01/08/16	03/09/22	6.17	1	8
14	F	12.7	CHD	07/16/16	05/09/22	5.82	1	5
15	F	7.0	DCM	10/21/16	04/19/22	5.50	1	5
16	F	8.3	DCM	04/26/17	05/23/22	5.08	1	4
17	F	7.2	DCM	08/01/17	03/03/22	4.59	1	3
18	F	12.0	DCM	12/05/17	02/28/22	4.24	1	5
19	M	5.6	DCM	11/04/18	02/23/22	3.31	4	11
20	M	7.0	CHD	06/16/19	05/02/22	2.88	1	5
21	F	3.5	DCM	11/12/20	04/11/22	1.41	1	4
22	F	9.9	CHD	12/22/20	03/15/22	1.23	1	7
23	M	14.6	RCM	07/01/21	02/25/22	0.65	2	13
24	M	16.5	CHD	07/17/21	03/10/22	0.65	1	5
25	F	2.4	DCM	08/25/21	03/14/22	0.55	1	7
26	M	14.7	CHD	03/17/22	03/24/22	0.02	1	11
27	M	0.8	CHD	07/30/22	10/20/22	0.22	0	2
28	F	7.1	DCM	01/08/23	01/20/23	0.03	0	3
29	M	11.3	CHD	01/11/23	02/13/23	0.09	0	3

For each patient, sex, age, diagnosis, date of transplant and enrollment, and number of EMBs, and dd-cfDNA samples are listed.
CHD, congenital heart defect; DCM, dilated cardiomyopathy; dd-cfDNA, donor-derived cell-free DNA; EMB, endomyocardial biopsy; F, female; HCM, hypertrophic cardiomyopathy; M, male; RCM, restrictive cardiomyopathy.

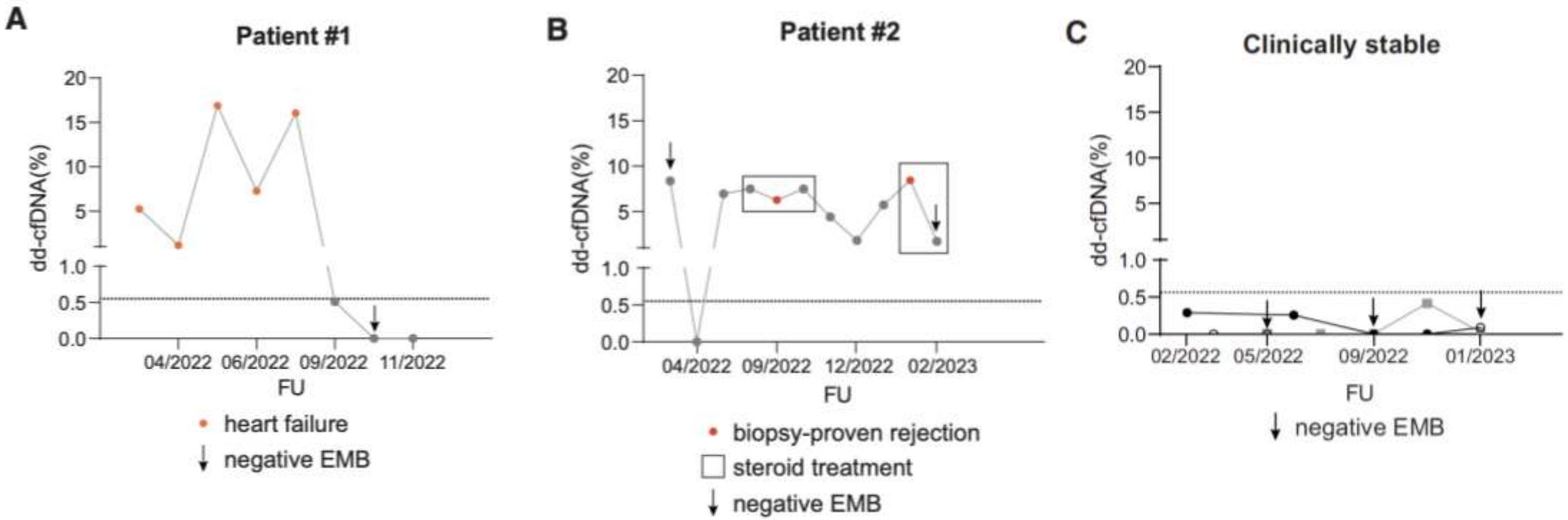
Sorbini et al., Transpl. Direct,

La coorte pediatrica di trapiantati di cuore



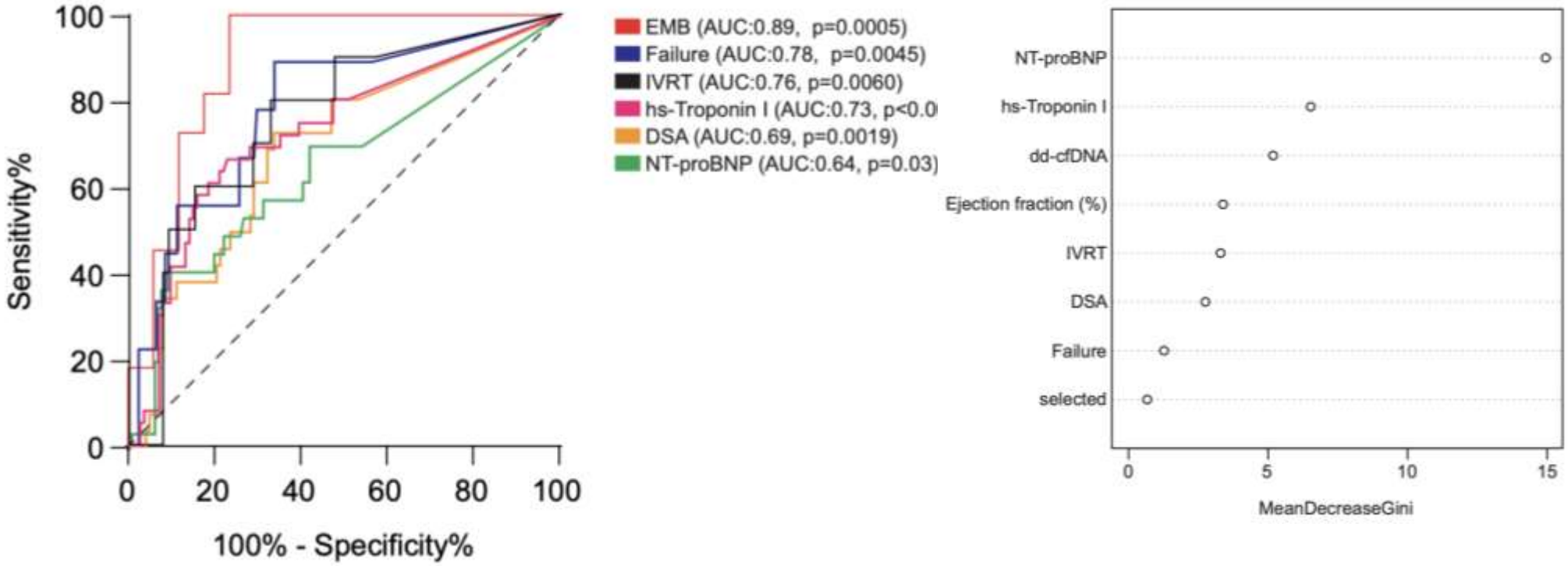
Sorbini et al., Transpl. Direct,

The pediatric heart transplant cohort



Sorbini et al., Transpl. Direct,

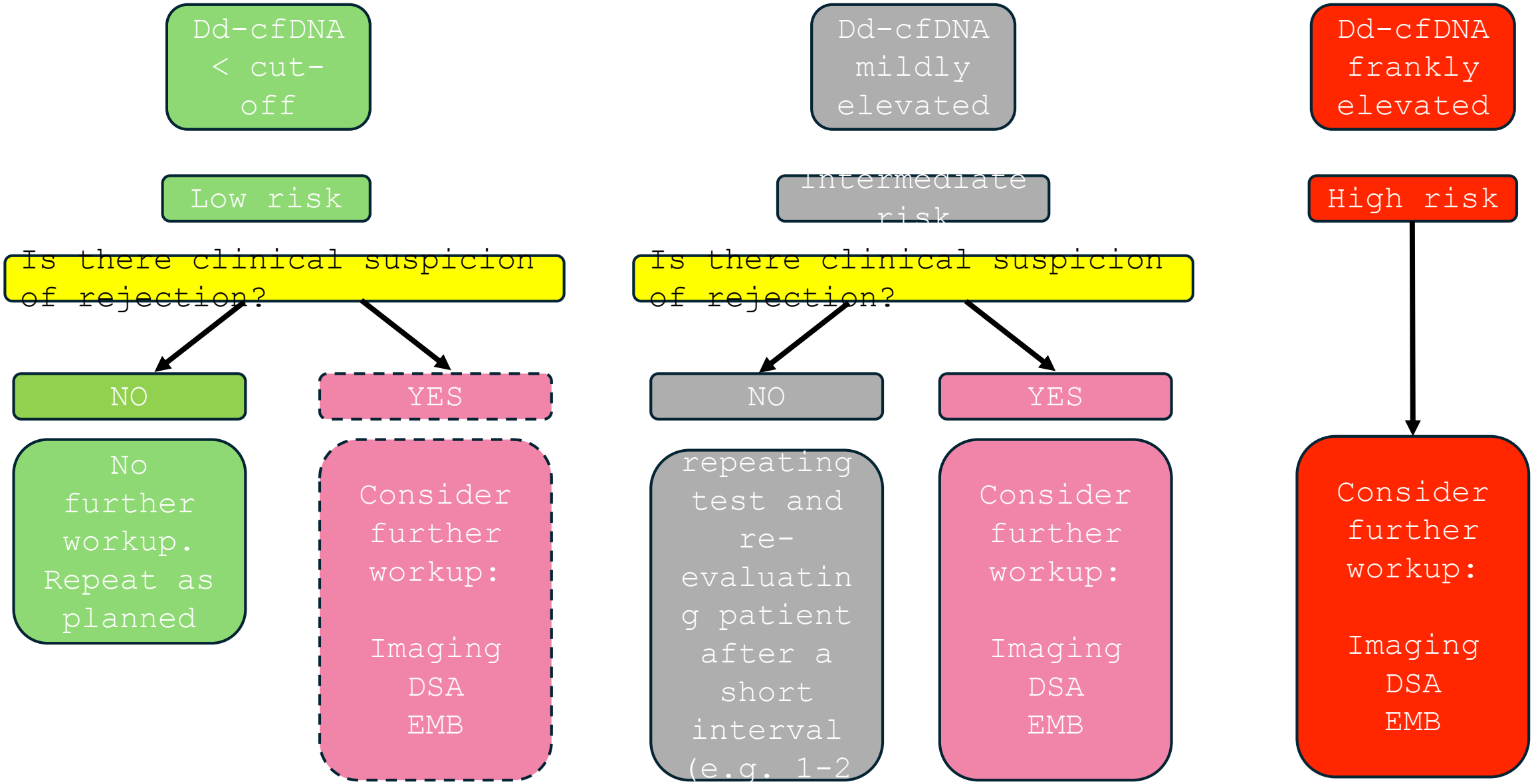
The pediatric heart transplant cohort



- ✓ Con il cut-off di 0.55%, il test aveva il 100% di sensibilità
- ✓ Pazienti con livelli di dd-cfDNA levels sotto il cut-off potrebbero posporre la loro biopsia di sorveglianza
- ✓ Con questi criteri avremmo evitato una biopsia a 7 pazienti, tutte con esito 0R

Sorbini et al., Transpl. Direct,

Un modello operativo per usare il dd-cfDNA nei trapiantati di cuore



E nel trapianto di rene come lo usiamo? Le raccomandazioni ESOT

Question 1. In kidney transplant patients with stable graft function, is plasma dd-cfDNA measurement a reliable diagnostic tool for subclinical acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or surveillance biopsy)?

Recommendation 1.1 - We suggest that clinicians consider measuring serial plasma dd-cfDNA in patients with stable graft function to exclude the presence of subclinical antibody-mediated rejection. Quality of Evidence - Moderate Strength of Recommendation - Weak in Favor

QUANDO? Insieme ai DSA (4/5 volte nei primi 24 mesi) e/o in concomitanza di una biopsia nei centri che eseguono biopsie protocollari

Question 2. In kidney transplant patients with acute allograft dysfunction, is plasma dd-cfDNA measurement a reliable diagnostic tool for acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or for cause biopsy)?

Recommendation 2.1 - We recommend that clinicians measure plasma dd-cfDNA in patients with acute graft dysfunction to exclude the presence of rejection, particularly antibody-mediated rejection. Quality of Evidence - Moderate. Strength of Recommendation - Moderate in Favor

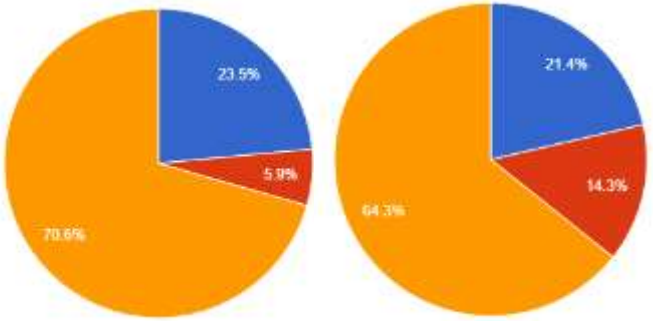
QUANDO? In concomitanza della biopsia. Ma cosa aggiunge?

SURVEY cfDNA: domande

- Quanti analisi eseguite in un anno (se recentemente introdotto quante in un mese)?
- Per quali pazienti eseguite il test?
- Per quali indicazioni cliniche eseguite il test?
- Quanti e quali plasmi sono analizzati per ciascun paziente?
- Quali provette sono utilizzate per la raccolta del plasma
- Come viene estratto il DNA?
- Tipo di kit estrazione automatica
- Viene valutata la quantità del DNA estratto prima del test
- Se sì, con quale metodica?
- Quale metodica è utilizzata per il test
- Con quale software analizzate i risultati?
- Quale cut off (%) viene utilizzato come soglia?

SURVEY cfDNA: risposte (17 centri)

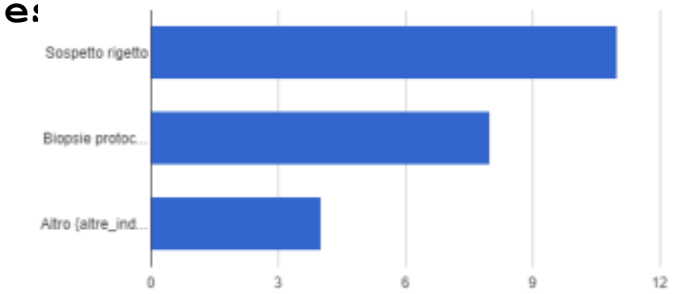
Quanti test eseguiti in un anno ?



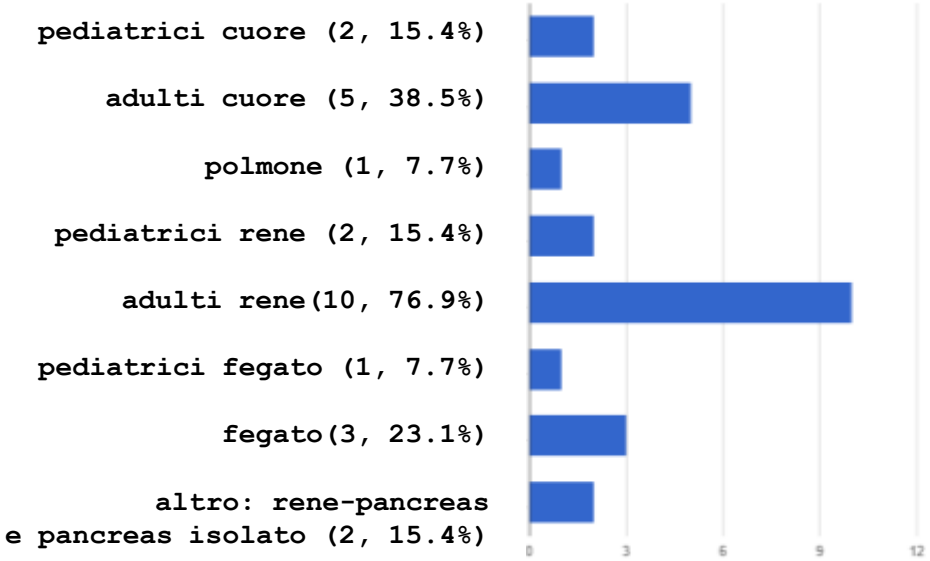
Quanti test eseguiti in un anno ?

< 10 (3, 21.4%)
> 10 (2, 14.3%)
non ancora introdotto (9, 64.3%)

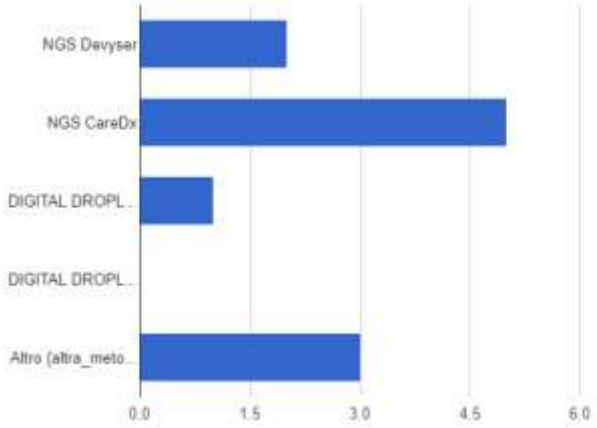
Per quali indicazioni cliniche e:



Per quali pazienti eseguite il test?



Quale metodica è utilizzata per



SURVEY cfDNA: next

- Cut-off definito per ciascun organo/kit utilizzato
- Controlli di qualità
- Identificare una prestazione con dicitura/costo compatibile con il test

Per concludere

- ✓ Il monitoraggio del dd-cfDNA nel trapianto di rene e di cuore sta acquistando importanza
- ✓ I sistemi più consolidati per il suo monitoraggio sono basati su NGS (in Europa) e su NGS e dd-PCR (negli Stati Uniti)
- ✓ Il test ha un valore predittivo negativo elevato
- ✓ Occorre disegnare delle sperimentazioni cliniche multicentriche e coordinate per potere analizzare numeri alti di pazienti

Lavoro di squadra!

SCDU Immunogenetica e Biologia dei Trapianti

Claudia Rosso
Cristiana Caorsi
Gabriele Togliatto
Rossella Chidichimo
Jessica Patuto
Elena Navaretti
Tullia Carradori
Tiziana Vaisitti
Silvia Deaglio

TESE

Biobanca

Monica Sorbini

Centro Trapianto Cuore e Polmone

Mauro Rinaldi
Massimo Boffini
Erika Simonato
Matteo Marro

SC. Cardiochirurgia pediatrica

Carlo Pace Napoleone
Enrico Aidala

Centro Trapianti Fegato

Renato Romagnoli
Damiano Patrono

Coordinamento RegionaleTrapianti

Federico Genzano Besso
Morteza Mansouri

SCDU Anatomia Patologica 1U

Mauro Papotti
Paola Cassoni

DIPARTIMENTO di SCIENZE MEDICHE unito.it

AZIENDA OSPEDALIERO-UNIVERSITARIA
Città della Salute e della Scienza di Torino