Donor- derived cell- free DNA for the detection of kidney allograft injury

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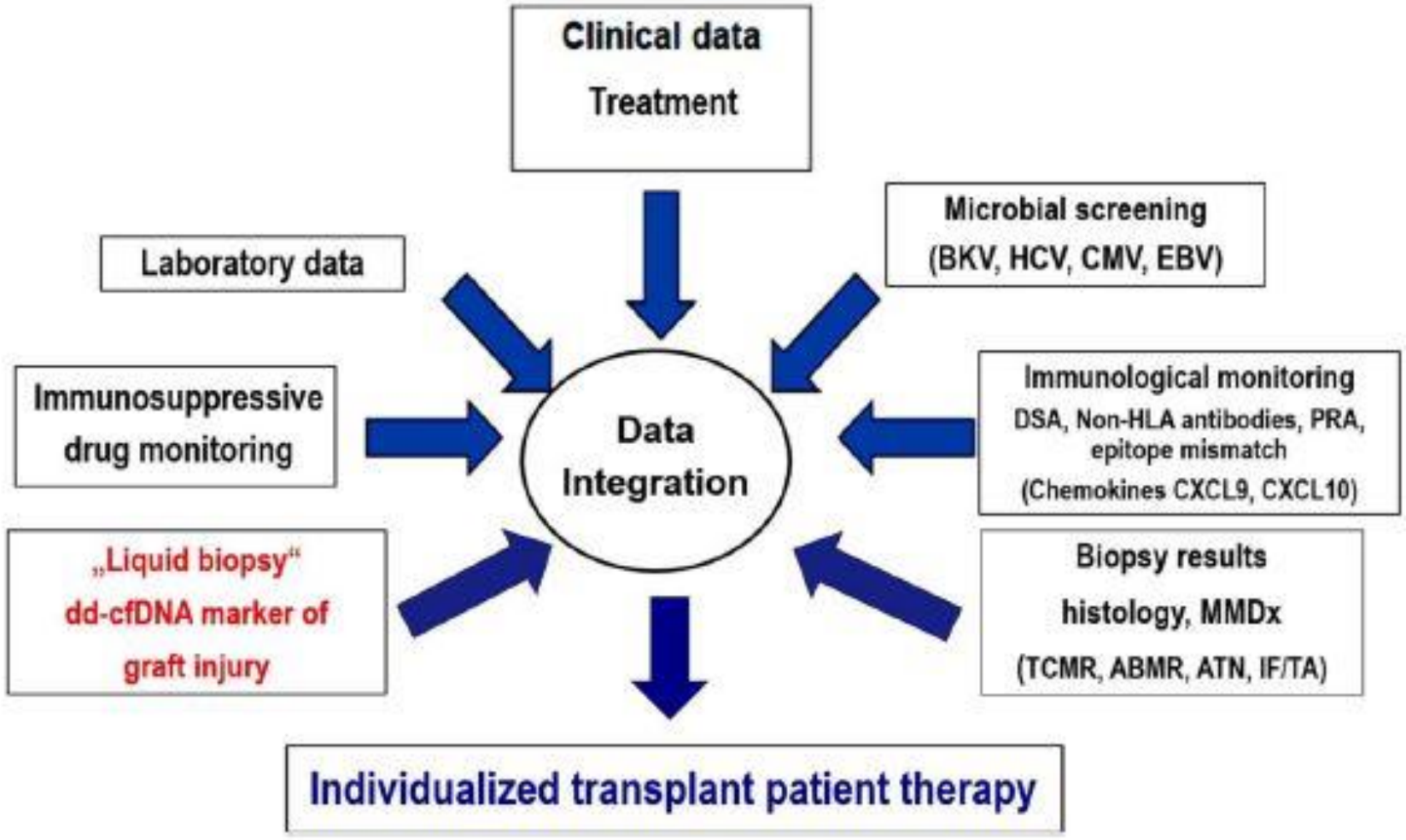
Kidney transplant

- The rate of **acute rejection** in the first year after kidney transplantation is ~12% in the USA
- The rate of **kidney graft failure at 5 years** is ~20% for grafts from deceased donors and ~10% for grafts from living donors
- The **10- year kidney allograft survival** rate is 51% for grafts from deceased donors and 69% for grafts from living donors

Pathophysiological mechanisms of rejection

- **Early clinical acute rejection** (due to T cell- mediated rejection (TCMR) and/or ABMR) is observed within 3 months of kidney transplantation in ~10–15% of recipients
- **Subclinical acute rejection** due to TCMR or ABMR might also occur in 20–25% of kidney transplant recipients in the first 12–24 months
- **Chronic rejection** is mainly due to under- immunosuppression and the development of DSAs, is usually observed after 12 months
- **ABMR** is thought to be more severe because it is associated with 20–30% risk of graft loss

kidney recipient surveillance



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kidney recipient surveillance

- **Plasma creatinine :** an **insensitive biomarker** because increase after 24–48 hours of injury
- A detectable increase depends on: the **baseline creatinine** level, the **muscle mass** of the patient and the **volume of distribution**.
- Increases in plasma creatinine levels **unrelated to rejection** might be observed owing to volume depletion or changes in medication, including renin–angiotensin system inhibitors, diuretics, NSAIDs or other nephrotoxic medications

kidney recipient surveillance

- **Routine immunosuppressive drug (ISD) monitoring** can indicate the risk of acute nephrotoxicity due to excessive drug concentrations but is not a biomarker of graft damage
- Nephrotoxicity, infections, cardiovascular events, and acute or chronic rejection are still observed in patients in whom therapeutic ISD concentrations are maintained.
- Recommended therapeutic ranges for blood ISD concentrations are suitable for most patients but might not be appropriate for individual patients

kidney recipient surveillance

- Alternative strategies such as **monitoring immune system** activity or measuring the expression of rejection- related genes are not currently used widely owing to the lack of large prospective validation studies.
- post- transplantation **HLA alloantibody screening** has also become a useful biomarker to estimate the risk of chronic antibody-mediated rejection (ABMR) and guide the decision to perform a kidney biopsy

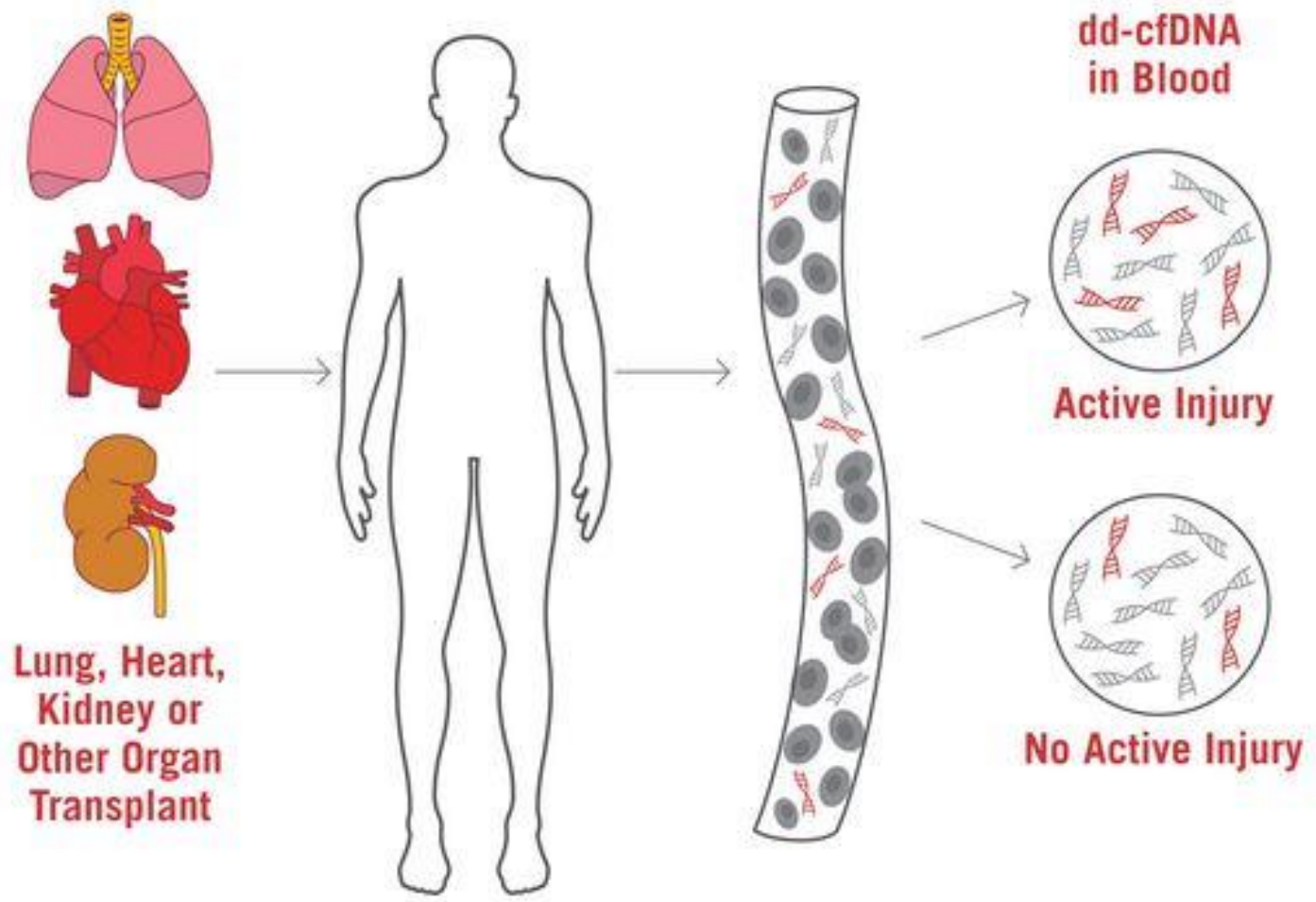
kidney recipient surveillance

- Biopsy of the kidney graft is still the **gold standard** for confirming graft damage, although histopathological assessment of kidney transplant biopsy samples has its limitations.
- The use of **serial biopsies** to assess graft integrity is clinically impractical, burdensome and expensive, and is associated with a 1% **major complication** rate. Additionally, up to 25% of biopsies yield an **inadequate specimen**, and are subject to sampling and interpretation errors

Essential features of a minimally invasive biomarker in kidney transplantation

- Enables early **detection** or exclusion of acute or chronic rejection
- Detects asymptomatic graft injury (subclinical rejection)
- Enables assessment of **minimum immunosuppressive drug dosage** required to prevent immune activation
- Enables **personalized immunosuppression** to improve long- term outcomes
- Can be implemented practically, with a reasonable turnaround time for test results and at an **affordable cost**

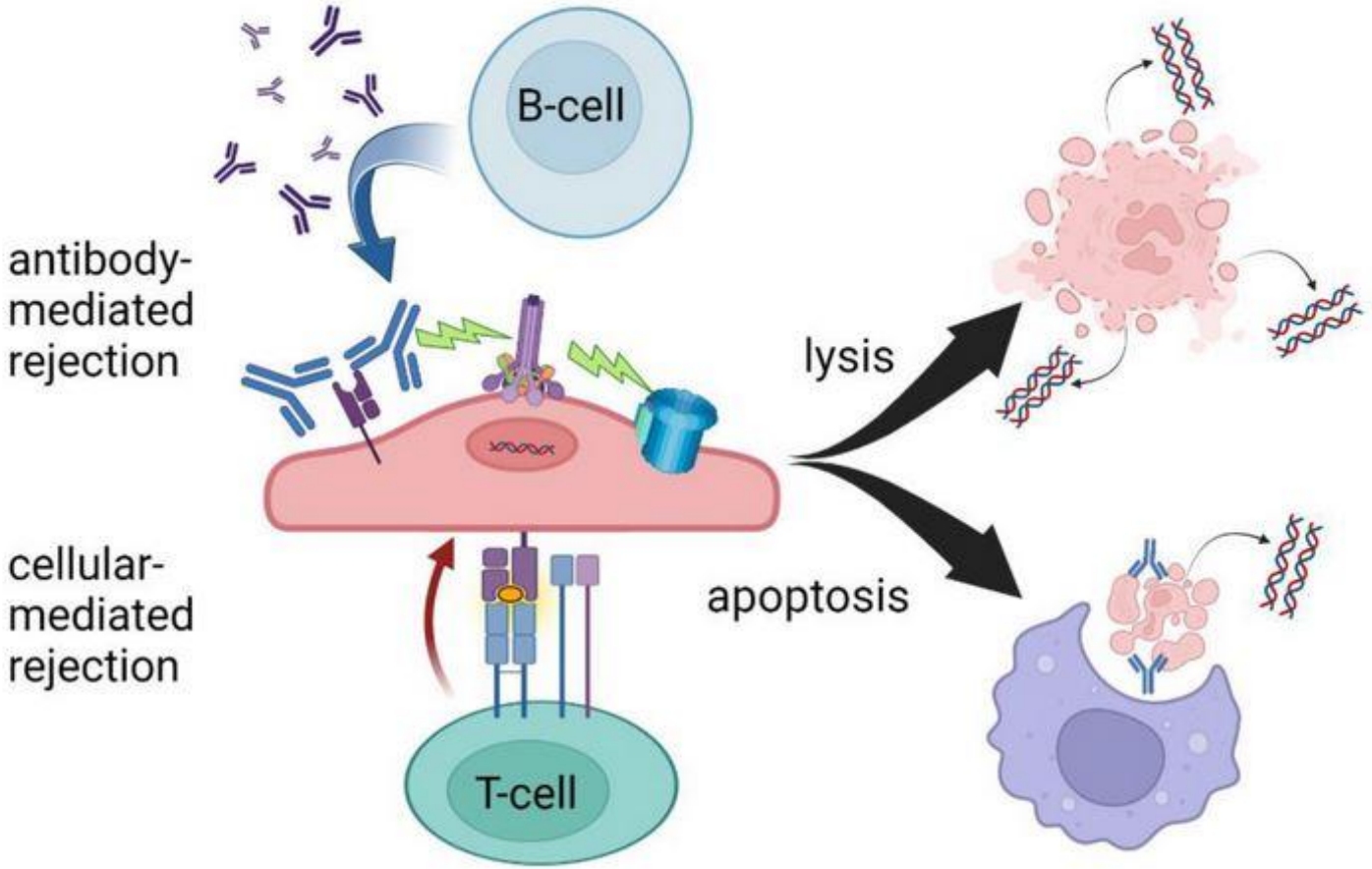
Cell-free DNA: A Clear Biomarker for Organ Injury



dd- cfDNA as a graft rejection biomarker

- Organ transplants are also **genome transplants**
- The presence of donor- specific DNA in plasma of kidney transplant recipients was first described in 1998, using a Y- chromosome-specific PCR in female recipients of organs from male donors
- As cells undergo **apoptosis** or **necrosis**, nucleosomes are released into the bloodstream and circulate in the plasma
- Necrosis yields larger cfDNA fragments (~10,000 bp), whereas apoptosis typically results in the release of smaller nucleosomal units of ~170 bp

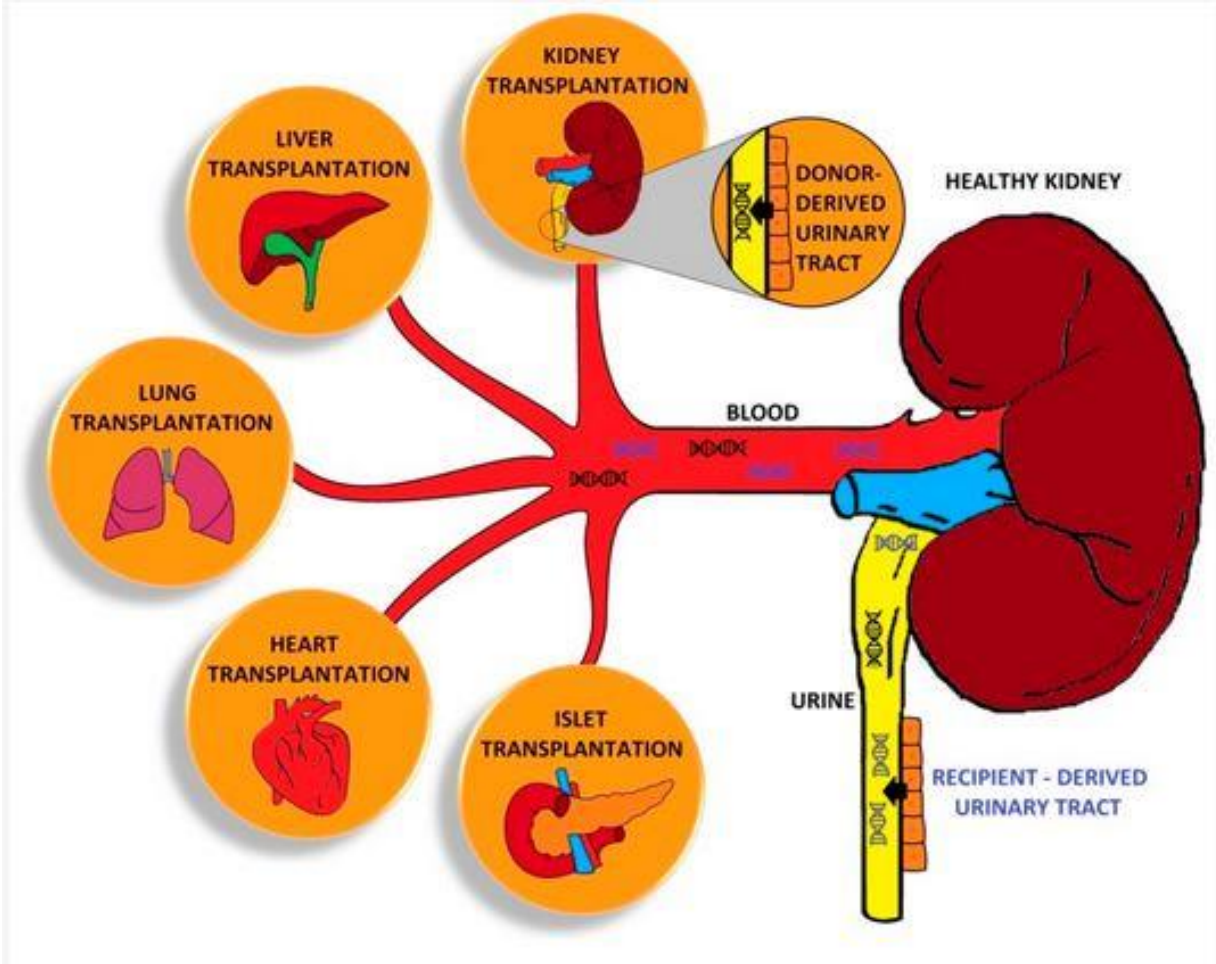
T-cell- and antibody-mediated rejection leading to cell lysis and/or apoptosis



dd- cfDNA as a graft rejection biomarker

- Most cfDNA (~90%) present in a plasma sample originates from apoptotic circulating recipient white blood cells
- **dd- cfDNA** accounts for only a small fraction of total cfDNA in the recipient's blood
- Once released, cfDNA is rapidly cleared and has a median **half- life** in the circulation of ~30 minutes to 2 hours
- Clearance of cfDNA involve a combination of nuclease cleavage, renal clearance and uptake by the liver and spleen
- Available technique are **next-generation sequencing** or digital polymerase chain reaction (PCR)

dd-cell-free DNA in a kidney transplant is secreted by donor-derived urinary tract and secreted into the blood.



Time dependence of dd- cfDNA

- Over the first 2 weeks after transplantation, dd- cfDNA typically declines exponentially to baseline levels
- An abnormal decline was associated with the presence of a **urinary tract infection**, pre- renal- mediated **acute kidney injury**, **surgical complications** or hydronephrosis
- In kidney transplant recipients of grafts from **living donors**, dd-cfDNA(%) and dd- cfDNA(cp/ml) were lower in the first 5 days after transplantation compared with dd- cfDNA in recipients of grafts from **deceased donors**.

Diagnostic performance of plasma dd- cfDNA

- High dd- cfDNA levels were found in kidney transplant recipients as early as **several days or even weeks** before the clinical manifestations of acute rejection
- Diagnostic accuracy in identifying kidney transplant recipients with rejection might improve even further by combining **DSA and dd-cfDNA** measurements
- Elevated dd- cfDNA was also a risk factor for persistent rejection and deterioration of estimated glomerular filtration rate (eGFR).
- As observed in liver or heart transplant recipients, dd- cfDNA **declined rapidly to baseline** levels after successful rejection treatment

Donor-derived Cell-free DNA in Solid-organ Transplant Diagnostics: Indications, Limitations, and Future Directions

Ashish Kataria, MD,[1] Dhiren Kumar, MD,[2] and Gaurav Gupta, MD[2,3]

- Although sensitivity for the diagnosis of **antibody-mediated rejection** is excellent, it is less so for T-cell–mediated rejection.
- It is possible that combining dd-cfDNA with other novel urine- or blood-based biomarkers may increase the sensitivity for the diagnosis of rejection

dd-cfDNA and Kidney Transplantation

- Two meta-analysis by Wijtvliet et al included 7 studies, the one by Xiao et al included 9 studies revealed **significantly higher median dd-cfDNA fractions** in patients with antibody mediated rejection (**AMR**) than patients without rejection
- The diagnostic accuracy was much poorer for patients with early T-cell mediated rejection (TCMR).
- Nevertheless, a large majority of “stable” patients had a low dd-cfDNA measurements (median = 0.21%) with a **negative predictive value** of 95% to rule out active rejection.

Diagnostic Potential of Minimally Invasive Biomarkers: A Biopsy-centered Viewpoint From the Banff Minimally Invasive Diagnostics Working Group

Edmund Huang, MD,[1] Michael Mengel, MD,[2] Marian C. Clahsen-van Groningen, MD, PhD,[3,4,5] and Annette M. Jackson, PhD[6]

- In the **Banff criteria** for kidney allograft pathology, donor-specific antibody (**DSA**) is utilized for a diagnosis of AMR
- With the introduction of newer minimally invasive biomarkers to the market, there is now an opportunity to consider biomarkers other than DSA for inclusion in the Banff classification

A diagnostic biomarker should:

(1) differentiate rejection from the absence of rejection;

(2) be specific for rejection;

(3) hold the potential to replace biopsies or,at minimum, have additive value over histology alone;

(4) have prognostic value

And if possible distinguish between rejection types, that is,

AMR and T cell-mediated rejection (TCMR)

Candidate criteria

- Applying these criteria, we chose 3 broad categories for consideration:

(1) donor-derived cell-free DNA(dd-cfDNA)

(2) blood or urine transcriptomics

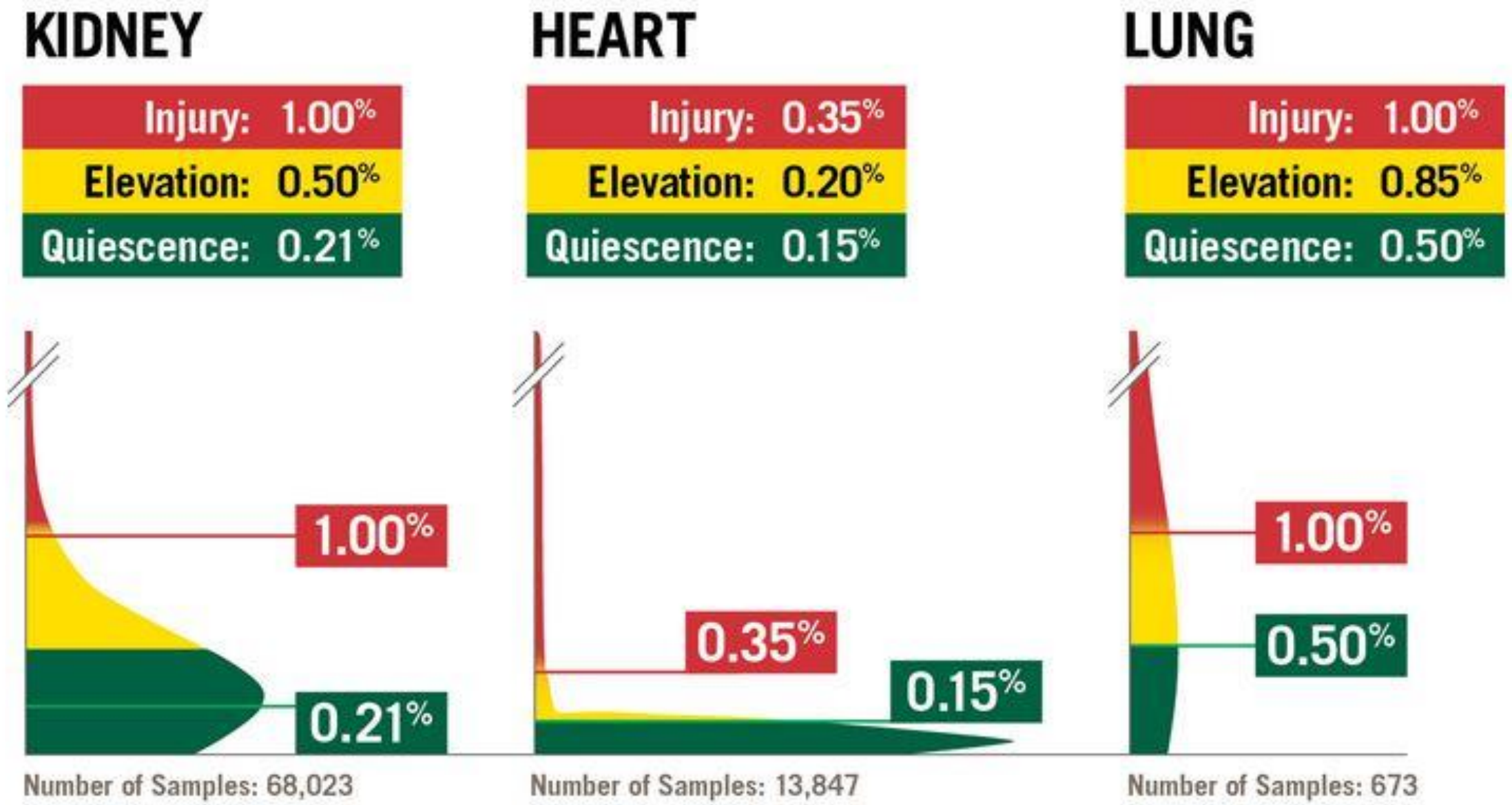
(3) urinary protein chemokines

dd-cfDNA

- cfDNA are fragments of DNA released from cells and, due to rapid turnover, can detect allograft injury in real time
- AMR was more readily detected using a higher dd-cfDNA positive **threshold (1%),** whereas lower thresholds were needed to correlate with TCMR, indicating that dd-cfDNA has a lower sensitivity for TCMR

dd-cfDNA as an Additive Diagnostic Marker in the Banff Classification

- The ability of dd-cfDNA tests to accurately diagnose rejection is highly dependent upon the positive **threshold** used, the type of rejection, and the composition of the study cohort
- The sensitivity of dd-cfDNA is too low to be considered as a **stand-alone diagnostic test**
- Recent studies assessing the correlation between dd-cfDNA, biopsy transcriptomics (MMDx), and histology suggest that dd-cfDNA is more strongly correlated with AMR than TCMR


KIDNEY
Injury: 1.00%
Elevation: 0.50%
Quiescence: 0.21%
1.00%
0.21%
Number of Samples: 68,023
HEART
Injury: 0.35%
Elevation: 0.20%
Quiescence: 0.15%
0.35%
0.15%
Number of Samples: 13,847
LUNG
Injury: 1.00%
Elevation: 0.85%
Quiescence: 0.50%
1.00%
0.50%
Number of Samples: 673

dd-cfDNA as an Additive Diagnostic Marker in the Banff Classification

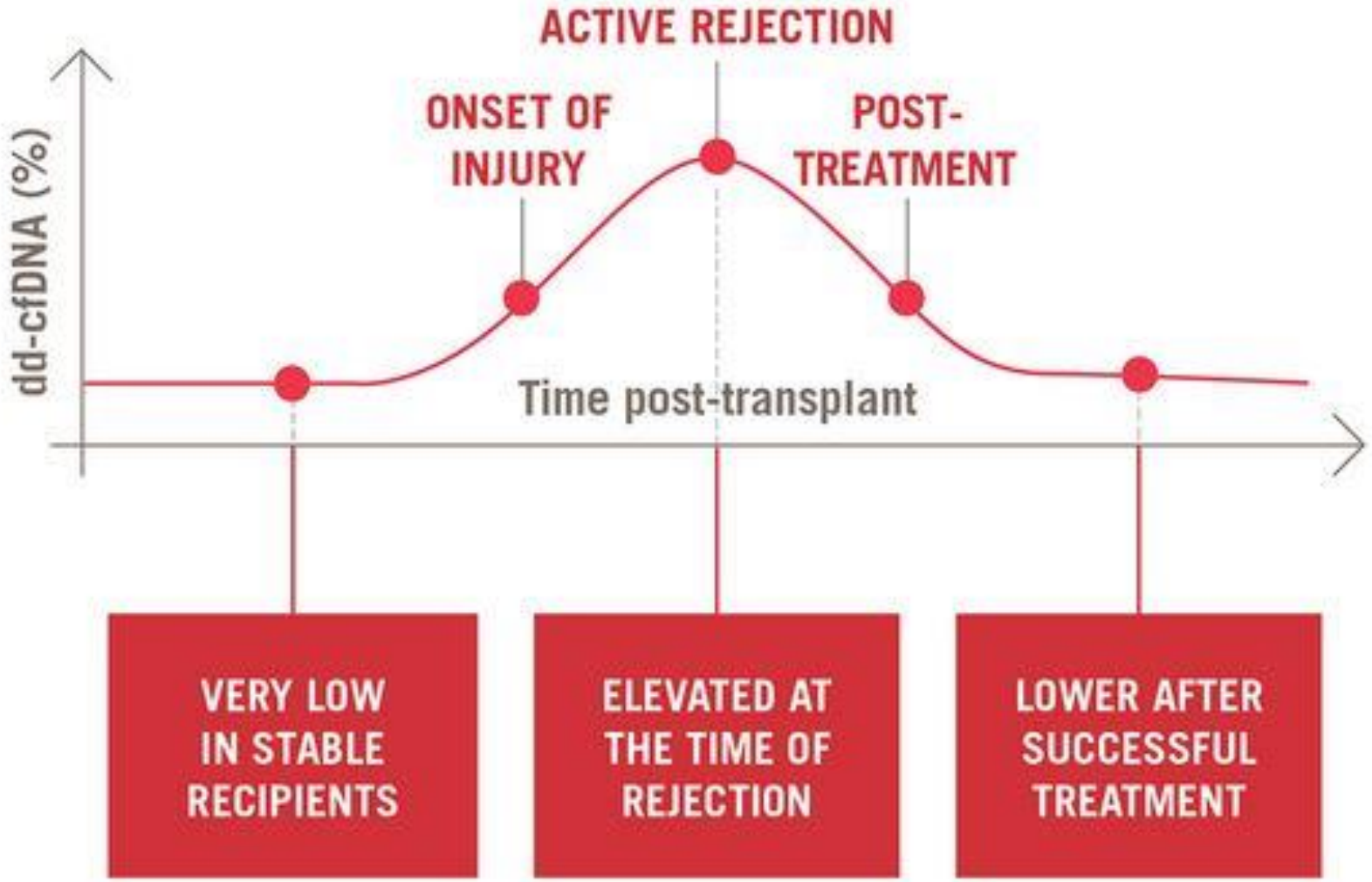
- Gupta et al reported that among **DSA-negative** patients with dd-**cfDNA ≥1%,** several cases of no rejection or TCMR by histology were reclassified as AMR or mixed rejection by MMDx
- So high levels of dd-cfDNA might indicate AMR even if histology suggests otherwise
- A recent study of dd-cfDNA in heart transplantation suggested that nearly two-thirds of "false positive" dd-cfDNA test results, with normal surveillance **endomyocardial biopsies**, were associated with concurrent allograft dysfunction, later dysfunction, or **future rejection**

dd-cfDNA as an Additive Diagnostic Marker in the Banff Classification

- Reports in kidney and lung have shown elevations in dd-cfDNA can **precede de novo DSA detection**

- There are also reports that decreased dd-cfDNA levels correlate with improved function following **rejection treatment**, suggesting some prognostic potential

Allograft Surveillance with dd-cfDNA Testing: Assessment of Injury Risk



dd-cfDNA as an Additive Diagnostic Marker in the Banff Classification

- Huang et al found that dd-cfDNA measurements combined with Banff chronicity scores were predictive of estimated glomerular filtration rate (eGFR) over time
- Stites et al found that among patients with TCMR 1A/borderline rejections, an elevated dd-cfDNA (≥0.5%) was associated with greater eGFR decline, an increased risk of de novo DSA, and future or persistent rejection

	Advantages	Challenges	Potential solutions
dd-cfDNA			
Provides information specific to donor allograft	Donor specific	% measurements can be influenced by elevated recipient cfDNA Potential interference/false positives from blood products or multiorgan transplants	Absolute quantity measurements with corrections for allograft size/quality. Mitigate with algorithms using donor-specific genotypes
Provides information specific to rejection	High NPV	Low PPV, elevations due to nonrejection injury/dd-cfDNA release	Combine with companion diagnostics to rule out infection, recurrent disease, drug toxicity, ischemic injury Interrogation of cfDNA epigenetics to identify which cells within the allograft are injured
Defines specific types of injury on histopathology	High sensitivity for cell lysis/DNA release	Low sensitivity for injuries not associated with cell lysis/DNA release	Correlate with well-annotated rejection and nonrejection lesions Correlation with sequential biopsies of rejection/ nonrejection lesions with and without interventions
Specificity: AMR/TCMR	Sensitive for injury	Not specific for AMR/TCMR	Improved PPV when combined with DSA/infection/transcriptomics/epigenetics
Severity, prognostic value AMR/TCMR	Sensitive for injury Potential association with eGFR decline	Potential low sensitivity for DSA-mediated lesions not associated with cell lysis/DNA release (vasculopathy) Low sensitivity for cellular infiltration/ inflammation without cell lysis/DNA release	Combine with companion diagnostics such as detailed DSA characteristics/ transcriptomics Correlate with granzyme+/perforin+ infiltrates

Correlation of Donor-derived Cell-free DNA With Histology and Molecular Diagnoses of Kidney Transplant Biopsies

Gaurav Gupta, MD,[1] Irfan Moinuddin, MD,[1] Layla Kamal, MD,[1] Anne L. King, MD,[1] Ryan Winstead, PharmD,[2] Moses Demehin, PharmD,[2] Le Kang, PhD,[3] Pamela Kimball, PhD,[2] Marlon Levy, MD,[2] Chandra Bhati, MD,[2] H. Davis Massey, MD, PhD,[4] Dhiren Kumar, MD,[1] and Philip F. Halloran, MD, PhD[5]

- Methods. In this single-center prospective study of 208 biopsies (median = 5.8 mo) posttransplant, we report cfDNA with simultaneous biopsy assessments using **MMDx** and **histology** by area under the curve (AUC) analyses for optimal criterion, as well as for, previously published cfDNA cutoffs ≤ 0.21% to “rule-out” rejection and ≥1% to “rule-in” rejection

Distribution of important clinical diagnoses by traditional histology and molecular microscope

	Histologic diagnosis	Median (range) cfDNA (%)	MMDx diagnosis	Median (range) cfDNA (%)	*P*
N	208		208		
AKI/ATN	33 (16%)	0.48 (0.15–1.9)	55 (26%)	0.3 (0.15-2.4)	0.11
BKV/other nephritis	13 (6%)	0.3	–	–	
Any rejection	79 (38%)	1.0 (0.15–16)	92 (44%)	1.1 (0.18–16)	0.85
Mixed rejection	8 (4%)	0.95 (0.19–2.2)	14 (7%)	1.3 (0.19–16)	0.92
T cell–mediated rejection	27 (13%)	0.67 (0.15–16)	13 (6%)	1.2 (0.19–13)	0.44
Borderline	8 (4%)	0.34 (0.15–4.9)	–	–	
1A	13 (6%)	0.92 (0.19–4.1)	–	–	
1B	6 (3%)	1.2 (0.19–16)	–	–	
Antibody-mediated rejection	43 (21%)	1.2 (0.19–7.9)	65 (31%)	1.1 (0.18–7.9)	0.70
Acute	22 (11%)	0.95 (0.19–7.9)	–	–	
Chronic active	21 (10%)	1.55 (0.21–6.9)	–	–	

Donor-Derived Cell-free DNA for Personalized Immunosuppression in Renal Transplantation

Michael Oellerich, MD, Klemens Budde, MD,† Bilgin Osmanodja, MD,† Kirsten Bornemann-Kolatzki, PhD,‡ Julia Beck, PhD,‡ Ekkehard Schütz, MD,‡ and Philip D. Walson, MD**

- In a multicenter diagnosing active rejection in kidney transplant recipients (**DART**) study using targeted **next-generation sequencing** (NGS) with 266 SNPs, acute and chronic ABMRs were accurately detected using the dd-cfDNA fraction. However, the TCMR detection was insufficient for this assay

Results

- It detects rejection episodes early at an actionable stage and reflects the severity of graft injury without being rejection specific.
- Owing to its high **negative predictive value**, dd-cfDNA **is very useful for ruling out graft injury.**
- Dd-cfDNA complements histological findings and **can help in avoiding unnecessary biopsies.**
- It indicates a response to rejection treatment and **detects under-immunosuppression**

Cell-Free DNA and Active Rejection in Kidney Allografts

Roy D. Bloom,* Jonathan S. Bromberg,† Emilio D. Poggio,‡ Suphamai Bunnapradist,§ Anthony J. Langone,‖ Puneet Sood,¶ Arthur J. Matas,** Shikha Mehta,†† Roslyn B. Mannon,††‡‡ Asif Sharfuddin,§§ Bernard Fischbach,‖‖ Mohanram Narayanan,¶¶ Stanley C. Jordan,§*** David Cohen,††† Matthew R. Weir,‡‡‡ David Hiller,§§§ Preethi Prasad,‖‖‖ Robert N. Woodward,¶¶¶ Marica Grskovic,¶¶¶ John J. Sninsky,¶¶¶ James P. Yee,‖‖‖ and Daniel C. Brennan,**** for the Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Active Rejection in Kidney Transplant Recipients (DART) Study Investigators

- In 102 kidney recipients, we measured plasma levels of **dd-cfDNA** and correlated the levels with allograft rejection status ascertained by **histology** in 107 biopsy specimens.
- The dd-cfDNA level discriminated between biopsy specimens showing any rejection (TCR or ABMR]) and controls (no rejection histologically), P,0.001
- **Positive and negative predictive values** for active rejection at a cutoff of 1.0% dd-cfDNA were 61% and 84%, respectively.

J Am Soc Nephrol 28: 2221–2232, 2017

The Trifecta Study: Comparing Plasma Levels of Donor-derived Cell-Free DNA with the Molecular Phenotype of Kidney Transplant Biopsies

Philip F. Halloran,[1,2,3] Jeff Reeve,[1] Katelynn S. Madill-Thomsen,[1,3] Zachary Demko,[4] Adam Prewett,[4] Paul Billings,[4] and the Trifecta Investigators*

[1] Alberta Transplant Applied Genomics Center, Edmonton, Canada
[2] Department of Medicine, University of Alberta, Edmonton, Canada
[3] Transcriptome Sciences Inc., Edmonton, Canada
[4] Natera, San Carlos, California

- Trifecta is a prospective trial defining relationships between donor-derived cell-free DNA (ddcfDNA),donor-specific antibody (DSA), and molecular findings [Molecular Microscope Diagnostic System (MMDx)] in kidney transplant biopsies

J Am Soc Nephrol. 2022 Feb; 33(2): 387–400.

Study design

- The prospective Trifecta study examined **relationships between dd-cfDNA(%) measured at the time of indication biopsy and the genome-wide molecular findings** in 300 biopsies from kidney transplant recipients assessed by microarrays.
- The dd-cfDNA(%) correlated with active rejection in the biopsy, and **molecular scores** predicted dd-cfDNA(%) > 1.0% better than **histologic** scores.

J Am Soc Nephrol. 2022 Feb; 33(2): 387–400.

Histologic diagnoses and DSA status in the Kidney 300 cohort (*n*, % of total)

	Molecular Diagnoses		Histology Diagnoses	
	***n* (% of 300)**	**% DSA Positive of Those Tested**	***n* (% of 300)**	**% DSA Positive of Those Tested**
No rejection	175 (58)[a]	6	128 (43)[a]	9
Possible TCMR (pTCMR)	6 (2)	0	31 (10)	10
TCMR	21 (7)[a]	29	31 (10)[a]	19
Possible ABMR (pABMR)	12 (4)	50	35 (12)	26
ABMR	67 (22)[a]	33	45 (15)[a]	44
Mixed (ABMR+TCMR)	19 (6)[a]	50	15 (5)[a]	40
All ABMR (including Mixed)	86 (29)	36	60 (20)	37
Inadequate	—	—	6 (2)	—
Missing	—	—	9 (3)	—

Results

- 60% no rejection, 30% AMR, and 10% T cell–mediated rejection (TCMR) or mixed rejection
- Dd-cfDNA was strongly related to active molecular rejection in the biopsies.
- The top genes in the biopsy correlating with %dd-cfDNA had all been previously correlated with AMR activity, including **natural killer** and ***IFNG*-inducible** genes

J Am Soc Nephrol. 2022 Feb; 33(2): 387–400.

Results

- Trifecta finds that dd-cfDNA(%) can be useful at the time of an indication biopsy—if **dd-cfDNA(%) is very low, it means the probability of active rejection is low**.
- dd-cfDNA(%) could potentially be used to follow response-to-treatment, and avoid follow-up biopsies

J Am Soc Nephrol. 2022 Feb; 33(2): 387–400.

Antibody-mediated Rejection Without Detectable Donor-specific Antibody Releases Donor-derived Cell-free DNA: Results From the Trifecta Study

Philip F. Halloran, MD, PhD,[1,2,3] Jeff Reeve, PhD,[1] Katelynn S. Madill-Thomsen, PhD,[3] Zachary Demko, PhD,[4] Adam Prewett, MBA,[4] Philippe Gauthier, MD,[4] Paul Billings, MD, PhD,[4] Christopher Lawrence, MD,[5] Dave Lowe, PhD,[5] Luis G. Hidalgo, PhD,[6] and the Trifecta Investigators*

- The present study analyzed the triple results in 280 biopsies, focusing on the question of dd-cfDNA levels in DSA-negative antibody-mediated rejection (AMR).
- DSA was frequently negative in both molecular (56%) and histologic (51%) AMR.
- All AMRs—DSA-positive or -negative—showed elevated %dd-cfDNA
- In AMR, %dd-cfDNA ≥1.0 was more frequent (75%) than DSA positivity (44%).

Conclusion

- In predicting AMR at the time of indication biopsies, **dd-cfDNA is superior to DSA**, reflecting the prevalence of DSA-negative AMR, but the optimal predictions incorporated both dd-cfDNA and DSA.

Take home message

- cfDNA measured as a percent of total cfDNA has been proposed as a **screening test** for kidney transplant rejection.
- All causes of graft injury that lead to cell death, including rejection, acute tubular necrosis, trauma, and infections, cause elevated levels of dd-cfDNA
- Good NPV for R/O of rejection
- Sensitive for Injury not specific for rejection
- Is not a single appropriate test for rejection

Take home message

- High dd- cfDNA levels were found as early as **several days or even weeks** before the clinical manifestations of acute rejection
- Dd-cfDNA complements histological findings and can help in avoiding unnecessary biopsies.
- It indicates a response to rejection treatment and detects under-immunosuppression.
- It is useful marker for monitoring after treatment of rejection

Thanks for your attention