KSN 2021
FULLY VIRTUAL MEETING
September 02 (Thu) - 05 (Sun)

Abstract Submission No. : 9158

What is going on under the surface? Donor-derived cell-free DNA and ongoing rejection process

Philip Halloran
University of Alberta, Canada

The clinician who follows kidney transplant patients has always wanted a non-invasive measurement in blood or urine that would indicate rejection in the transplant, beyond measurements such as proteinuria and creatinine. Measurement of plasma donor-derived cell-free DNA (dd-cfDNA) is an important step toward this objective (1).

The definitive assessment of rejection and other disease states and kidney transplants is the biopsy, interpreted by histology (using the Banff guidelines) and more recently by molecular assessments. Our laboratory developed the Molecular Microscope® Diagnostic System (MMDx), which uses genome-wide microarrays with 50,000 probe sets to measure the gene expression in the biopsy (mRNA), interpreted using ensembles of machine learning algorithms in 24 hours (2). MMDx measures T cell-mediated rejection (TCMR) (3), antibody-mediated rejection (ABMR) (4), acute kidney injury (AKI) (5), and atrophy fibrosis (CKD) (6) as recently reviewed (7-11). MMDx archetypal analysis divides TCMR into a more severe TCMR1, often associated with ABMR; and a less severe TCMR2, accompanied by fibrosis; and divides ABMR into early-stage (EABMR), fully-developed (FABMR), and late-stage (LABMR).

The comparison of MMDx with histology and donor-specific antibody (DSA) was established in INTERCOMEX (ClinicalTrials.gov NCT01299168), a prospective observational study involving 38 investigators in 25 international centers. MMDx measurements provided a superior prediction of the disease and injury states and prognosis compared to conventional assessments. MMDx has a high agreement with histology but has discrepancies (12), many of which are due to the inherent interobserver variation in visual assessment systems like histology. MMDx has the advantage of precision measurement and automated readouts, but like all tissue, assessment can be influenced by sampling error and boundary cases i.e. weak phenotypes. MMDx is also working to help evolve the guidelines for histology assessment (13) and to identify ABMR in biopsies currently called no rejection (2). DSA was present in FABMR (70%) more than EABMR (58%) or LABMR (56%) but was often negative in molecular ABMR. Thus DSA negative ABMR is often diagnosed by MMDx and is increasingly recognized by histology.

The findings in a biopsy can potentially be anticipated by dd-cfDNA, indicating when biopsies should be done and avoiding some unnecessary biopsies. All tissues release small amounts of short-lived circulating cfDNA into the blood, which is increased by injury and disease. Under some circumstances, the host cfDNA is accompanied by a small amount of cfDNA with non-host sequences:

pregnancy, cancer, and organ transplants. The distinction between host and non-host DNA is made by methods based on sequencing or PCR. This has led to a number of commercial essays applied to organ transplants that measure the dd-cfDNA, usually expressed as a fraction of the total cfDNA, which is usually >99% host-derived (1). One such assay has been developed by Natera, the Prospera assay, which uses massively multiplexed PCR to detect dd-cfDNA, currently expressed as a percentage of the total circulating cfDNA in plasma samples. (Since host cfDNA release can also vary, the long-term goal is absolute measurement of the dd-cfDNA rather than a percent.) Prospera %dd-cfDNA measurements have been shown to be elevated in the presence of rejection (14).

Our laboratory has been engaged in the Trifecta study (ClinicalTrials.gov # NCT04239703) to compare %dd-cfDNA to MMDx measurements. Trifecta is a collaboration with Natera and 28 investigators in 14 international centers as well as One Lambda/Thermo Fisher (OLI). Trifecta compared molecular and histologic findings in indication kidney transplant biopsies to the %dd-cfDNA (Prospera) and to DSA. Initial results on 300 biopsies have recently been presented (15, 16) (manuscripts in preparation).

Trifecta compared the histologic and molecular findings in 300 biopsies to %dd-cfDNA in blood drawn at the time of prospectively collected biopsies (94% for indications), from 19 centers in Europe and North America. Molecular rejection was assessed using the MMDx and by histology (by local standard-of-care). The case-mix was similar to previous INTERCOMEX studies: 175 no rejection (NR); 67 ABMR; 12 possible ABMR; 21 TCMR, 6 possible TCMR, and 19 mixed rejection. The median time of biopsy post-transplant was 455 days (range 5 days to 32 years). MMDx ABMR and Mixed rejection were strongly associated with %dd-cfDNA, while most NR biopsies had low %dd-cfDNA. All top 20 genes associated with %dd-cfDNA were previously annotated as ABMR-associated and were primarily NK cell-expressed (e.g. GNLY, CCL4, TRDC, and S1PR5) and IFNG-inducible (e.g. PLA1A, IDO1, CXCL11, and WARS). Every one of the top 20/50000 probesets (transcripts) highly correlated with %dd-cfDNA had previously been annotated as a rejection associated transcript (RAT) highly correlated with ABMR (ABMR-RAT) and all rejection (Rej-RAT).

Molecular classifiers and gene sets correlating most significantly with %dd-cfDNA were also ABMR- or all rejection-related, and IFNG-inducible. Acute kidney injury and atrophy-fibrosis correlated weakly with %dd-cfDNA, indicating that other forms of renal injury can also release dd-cfDNA. Nevertheless, in principal component analysis, %dd-cfDNA behaved like a molecular rejection feature; particularly related to active ABMR. The AUC of %dd-cfDNA for all molecular rejection was 0.82, higher than for histologic rejection (0.72).

The %dd-cfDNA was highly related to active antibody-mediated rejection (EABMR and FABMR). Late-stage ABMR (LABMR) gave less consistent %dd-cfDNA elevation. The association of %dd-cfDNA with pure T cell-mediated rejection (TCMR) was weaker, particularly for weak TCMR2: some TCMR2 does not release dd-cfDNA. Thus plasma %dd-cfDNA at biopsy strongly correlates with active molecular rejection activity and is a robust screen for active molecular rejection.

Trifecta also provided a comparison between MMDx, %dd-cfDNA, and DSA. Central HLA antibody assessment was performed at OneLambda and interpreted by Dr. Luis Hidalgo using local genotyping. Each center also provided local DSA assessment. Molecular ABMR was frequently DSA

negative, both centrally and locally, consistent with the increased recognition of DSA-negative ABMR. DSA associated with MMDx ABMR was usually class II. Because histologic ABMR uses DSA positivity for diagnosis, histology sometimes missed DSA-negative ABMR, but in DSA positive ABMR MMDx and histology usually agreed. Molecular ABMR classifiers predicted central DSA with AUC 0.79 (class II). The %dd-cfDNA predicted central DSA with AUCs of 0.73 (class II). We conclude that there is a strong relationship between %dd-cfDNA, DSA (particularly class II), and rejection, particularly active ABMR. These relationships are stronger for MMDx than histology diagnoses.

Many ABMR cases are DSA-negative but still have high %dd-cfDNA (Figure 1), indicating that %dd-cfDNA may be a more robust feature of ABMR than the current definitions of DSA, and probably both %dd-cfDNA and DSA should be recognized in the diagnostic criteria.

Trifecta and other ongoing studies are designed to define the clinical utility of following dd-cfDNA in organ transplant patients, either at times of organ dysfunction or in routine surveillance, but cost-effectiveness will need to be established to guide the use of %dd-cfDNA in clinical practice.

Reference LIst

1. Kataria A, Kumar D, Gupta G. Donor-derived Cell-free DNA in Solid-organ Transplant Diagnostics: Indications, Limitations, and Future Directions. Transplantation. 2021;105(6):1203-11.
2. Madill-Thomsen KSB, G.A., Bromberg J, Einecke G, Eskandary F, Gupta G, Hidalgo LG, et al. Donor-specific antibody is associated with increased expression of rejection transcripts in renal transplant biopsies classified as no rejection. J Am Soc Nephrol. 2021;32(7):DOI: https://doi.org/10.1681/ASN.2021040433.
3. Venner JM, Famulski KS, Badr D, Hidalgo LG, Chang J, Halloran PF. Molecular landscape of T cell-mediated rejection in human kidney transplants: prominence of CTLA4 and PD ligands. Am J Transplant. 2014;14(11):2565-76.
4. Venner JM, Hidalgo LG, Famulski KS, Chang J, Halloran PF. The molecular landscape of antibody-mediated kidney transplant rejection: evidence for NK involvement through CD16a Fc receptors. Am J Transplant. 2015;15(5):1336-48.
5. Famulski KS, de Freitas DG, Kreepala C, Chang J, Sellares J, Sis B, et al. Molecular phenotypes of acute kidney injury in kidney transplants. J Am Soc Nephrol. 2012;23(5):948-58.
6. Venner JM, Famulski KS, Reeve J, Chang J, Halloran PF. Relationships among injury, fibrosis, and time in human kidney transplants. JCI Insight. 2016;1(1):e85323.
7. Halloran PF, Famulski K, Reeve J. The molecular phenotypes of rejection in kidney transplant biopsies. Curr Opin Organ Transplant. 2015;20(3):359-67.
8. Halloran PF, Reeve JP, Pereira AB, Hidalgo LG, Famulski KS. Antibody-mediated rejection, T cell-mediated rejection, and the injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies. Kidney Int. 2014;85(2):258-64.
9. Reeve J, Halloran PF, Kaplan B. Common errors in the implementation and interpretation of microarray studies. Transplantation. 2015;99(3):470-5.
10. Halloran PF, Famulski KS, Reeve J. Molecular assessment of disease states in kidney transplant biopsy samples. Nat Rev Nephrol. 2016;12(9):534-48.
11. Halloran PF, Venner JM, Madill-Thomsen KS, Einecke G, Parkes MD, Hidalgo LG, et al. Review: The transcripts associated with organ allograft rejection. Am J Transplant. 2018;18(4):785-95.

12. Madill-Thomsen K, Perkowska-Ptasinska A, Bohmig GA, Eskandary F, Einecke G, Gupta G, et al. Discrepancy analysis comparing molecular and histology diagnoses in kidney transplant biopsies. Am J Transplant. 2020;20(5):1341-50.
13. Colombat M, Holifanjaniaina S, Hirschi S, Mal H, Stern M. Histologic reconstruction of bronchiolar lesions in lung transplant patients with bronchiolitis obliterans syndrome. Am J Surg Pathol. 2014;38(8):1157-8.
14. Callemeyn J, Lerut E, de Loor H, Arijs I, Thaunat O, Koenig A, et al. Transcriptional Changes in Kidney Allografts with Histology of Antibody-Mediated Rejection without Anti-HLA Donor-Specific Antibodies. J Am Soc Nephrol. 2020;31(9):2168-83.
15. Halloran PF, Demko Z, Prewett A, Reeve J, Billings P. The Trifecta Study: Calibrating Circulating Donor-Derived Cell-Free DNA at the Time of Indication Biopsies Against the Molecular Phenotype of the Biopsy Reveals a Prominent Association With NK Cell Genes. Am J Transplant. 2021;21(S3).
16. Halloran PF, Hidalgo LG, Reeve J, Demko Z, Prewett A, Billings P, et al. Relationship Between %dd-cfDNA, MMDx Molecular Diagnoses, and Donor-specific Antibody in the Trifecta Study. Am J Transplant. 2021;21(S3).

Figure 1. Dd-cfDNA release in DSA-negative Antibody-mediated rejection

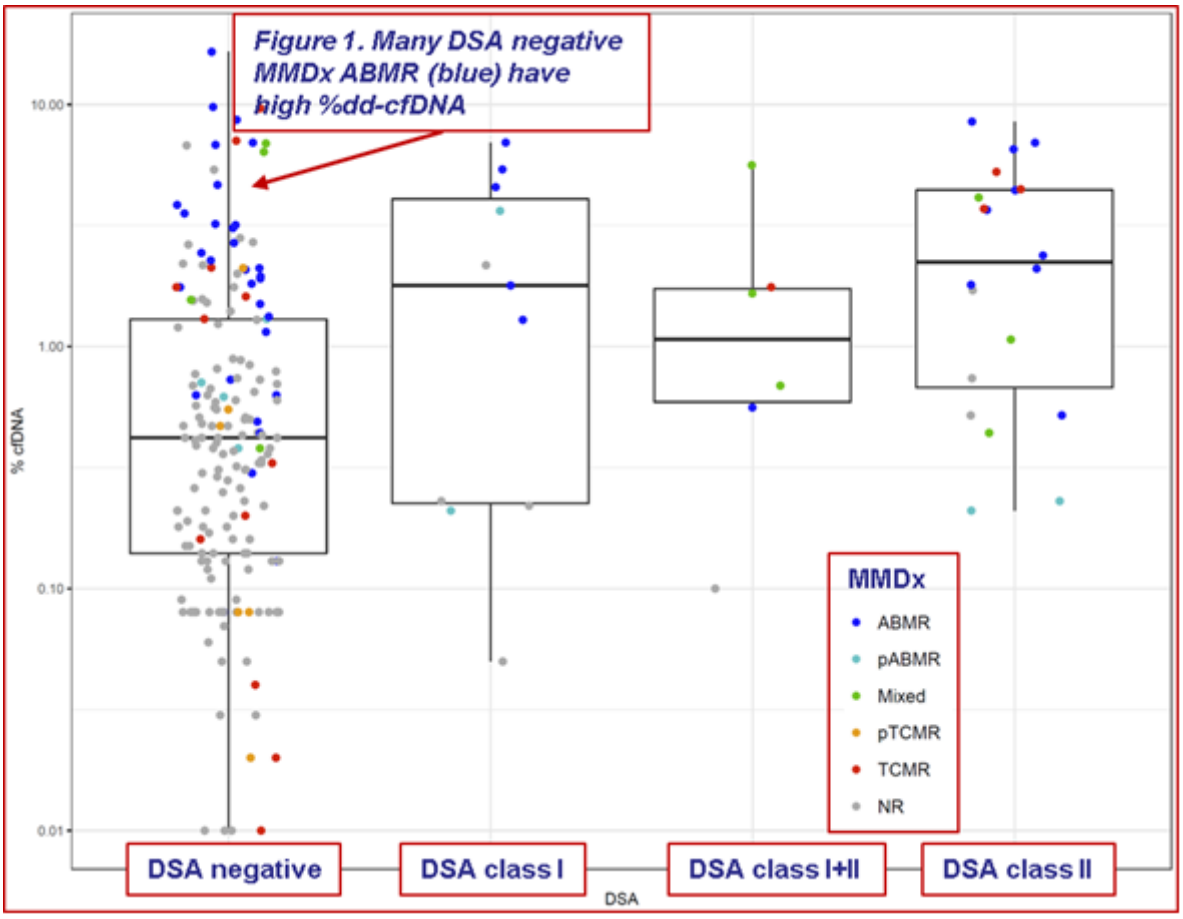

Table 1. The transcripts associated with dd-cfDNA release are all previously annotated as associated with antibody-mediated rejection and all rejection.

Table 1. Top 20 probesets positively correlated with %dd-cfDNA in the Trifecta Study (N=300)

Affy	SYMB	Name	Annotation as rejection-associated transcripts (RAT)	Spearman correlation	p-value
11751857_a_at	GNLY	granulysin	ABMR-RAT, Rej-RAT	0.56	3.8E-26
11763715_a_at	GNLY	granulysin	ABMR-RAT, Rej-RAT	0.55	3E-25
11746954_s_at	CCL4	chemokine (C-C motif) ligand 4	ABMR-RAT,Rej-RAT	0.52	4.3E-22
11761790_x_at	TRDC	T cell receptor delta constant	ABMR-RAT,Rej-RAT	0.51	1.5E-21
11718982_s_at	CCL4	chemokine (C-C motif) ligand 4	ABMR-RAT,Rej-RAT	0.51	1.7E-21
11718983_x_at	CCL4	chemokine (C-C motif) ligand 4	ABMR-RAT,Rej-RAT	0.51	1.9E-21
11743168_at	IDO1	indoleamine 2,3-dioxygenase 1	ABMR-RAT, Rej-RAT	0.51	7.4E-21
11749245_a_at	CXCL11	chemokine (C-X-C motif) ligand 11	ABMR-RAT,Rej-RAT	0.50	3.4E-20
11753484_x_at	KLRD1	killer cell lectin-like receptor subfamily D, member 1	ABMR-RAT,Rej-RAT	0.50	4E-20
11727116_a_at	PLA1A	phospholipase A1 member A	ABMR-RAT, Rej-RAT	0.50	4.9E-20
11729649_at	PRF1	perforin 1 (pore forming protein)	ABMR-RAT, Rej-RAT	0.50	4.5E-20
11740452_x_at	KLRD1	killer cell lectin-like receptor subfamily D, member 1	ABMR-RAT, Rej-RAT	0.50	5.6E-20
11724900_a_at	GZMB	granzyme B	ABMR-RAT, Rej-RAT	0.49	6.9E-20
11726287_a_at	WARS	tryptophanyl-tRNA synthetase	ABMR-RAT, Rej-RAT	0.49	8.9E-20
11733353_at	CRTAM	cytotoxic and regulatory T-cell molecule	ABMR-RAT, Rej-RAT	0.49	9E-20
11756632_a_at	GNLY	granulysin	ABMR-RAT, Rej-RAT	0.49	1.2E-19
11744660_s_at	CCL4L1	chemokine (C-C motif) ligand 4-like 1	ABMR-RAT, Rej-RAT	0.49	1.5E-19
11732466_a_at	CXCL11	chemokine (C-X-C motif) ligand 11	ABMR-RAT, Rej-RAT	0.49	1.9E-19
11752664_a_at	S1PR5	sphingosine-1-phosphate receptor 5	ABMR-RAT, Rej-RAT	0.49	2.7E-19
11763756_x_at	GNLY	granulysin	ABMR-RAT, Rej-RAT	0.49	2.7E-19