LETTER TO EDITOR

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Single Center "Snapshot" Experience With Donor-Derived Cell-Free DNA After Lung Transplantation

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Lung transplantation (LT) provides a potentially curative option for many patients suffering from a spectrum of end-stage lung diseases. However, the median 5-year survival after LT remains at only 60%, notably inferior to that currently achieved with other solid organ transplants.[1] The higher mortality is primarily attributed to the prevalent complication of chronic lung allograft dysfunction (CLAD). Early detection of CLAD would allow implementation of strategies to reverse or halt further progression. Additionally, an unpredictable clinical presentation for CLAD, makes this a more challenging endeavor.

Acute cellular rejection (ACR), antibody mediated rejection (ABMR), de novo donor specific HLA antibodies (DSA), auto-antibodies and infection, have all been recognized as risk factors for CLAD.[2-7] Pulmonary function tests (PFTs), histopathology, and DSA are often required during evaluation to mitigate these risk factors. Nevertheless, conventional video-bronchoscopy with biopsies may lack sufficient sensitivity and specificity for the elucidation of ACR, ABMR, and CLAD.[8] These deficiencies underscore a need for molecular diagnostics to complement what has been provided by conventional assessments.

There is no more urgent time than the present with the CoVid-19 pandemic, to evaluate a non-invasive tool for surveillance of allograft quiescence and rejection. LT programs by necessity, have adjusted their post-transplant protocols to both conserve critical healthcare resources and to minimize the risk of exposure to these vulnerable patients. Surveillance bronchoscopy and formal pulmonary function tests, utilized at most centers to evaluate for early allograft dysfunction, have been deferred in order to mitigate patient contagion exposures. This decrease in clinical monitoring has created significant concern regarding a potential delay in diagnosis of rejection or allograft infection. Thus, this interruption in routine monitoring has challenged the transplant community to consider alternative methods to surveil LT patients.

We endeavored to assess clinical utility of a strategy implementing biomarker surveillance with plasma donor-derived cell-free DNA (dd-cfDNA) in concert with a clinical monitoring strategy. Agbor-Enoh et al, has previously described measurement of dd-cfDNA by "shotgun" methods (that require genomic sequencing of donor and recipient) with an elevation during lung acute cellular and antibody-mediated allograft rejection.[9,10] Therefore, we performed, a "Snapshot" investigation implementing a clinical-grade "next generation sequencing" (NGS) dd-cfDNA assay (AlloSure®) that interrogates a panel of single nucleotide polymorphisms (SNPs) across all 22 somatic chromosomes without requirement for prior genotyping. This dd-cfDNA assay, with a detectable range for donor (allograft) cell-free DNA fraction of 0.12% to 16.0%, has been previously validated in in large multi-center studies for kidney and heart transplantation.[11-14] This assay specifically does not require separate donor genomic material and is provided with rapid turn-around time for results (<72 hours) from a Central Clinical Laboratory Improvements Act (CLIA)-validated and College of American Pathologists (CAP)-accredited laboratory [CareDx, Inc.; Brisbane, CA, USA]. The study had been approved by our Institutional Review Board for Clinical Research (HSC20080378H) and informed patient consent was obtained, in anticipation of implementing a LT program surveillance strategy, which would include usual laboratory studies, dd-cfDNA, home self-spirometry assessment, and tele-health visits during the CoVid-19 pandemic.

We assessed 48 unique patients, between 3-months and 12-years post-LT, in cohorts with: chronic lung allograft dysfunction (CLAD, N = 10], HLA antibody-mediated rejection (ABMR, N = 9), acute cellular rejection (ACR, N = 2), stable patients without evidence for rejection or infection (STABLE, N = 11), and allograft-associated infection (INFXN, N = 16). Infectious episodes included: non-tuberculous mycobacterial (N = 2), fungal (N = 7), bacterial (N = 5) and viral (N = 2) pathogens by respiratory cultures and viral multi-plex PCR. The dd-cfDNA levels were as follows—CLAD (0.85%; IQR: 0.67%-1.5%), ABMR (1.20%; IQR: 0.82%-1.73%), ACR (0.32%; IQR: 0.29%-0.35%), and INFXN (0.52%; IQR: 0.31%-1.35%). In contrast, STABLE patients had low levels for dd-cfDNA with median of 0.150% (IQR: 0.15%-0.195%). Figure 1 depicts significant elevation in dd-cfDNA for the aggregated cohorts (CLAD+ABMR+ACR) of allograft rejection (REJXN) with median 0.95% (IQR: 0.59%-1.53%) versus STABLE patients (Mann-Whitney Rank Sum; $P < .001$). The INFXN cohort median dd-cfDNA was also significantly increased compared to STABLE patients ($P < .001$), while small

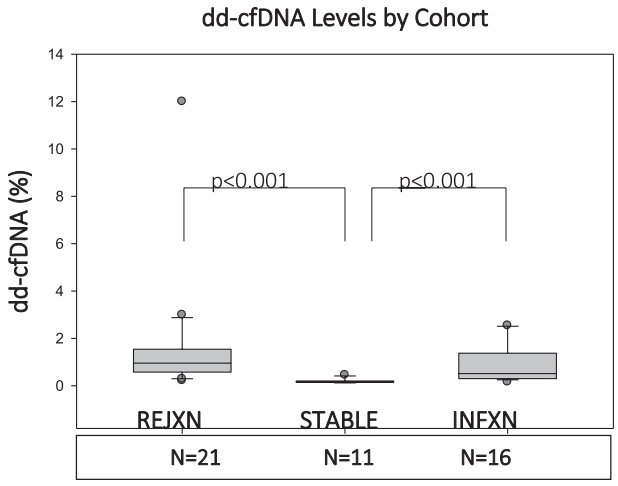

Figure 1. dd-cfDNA levels (*Y* axis) with cohorts of allograft rejection, stable, and infection. (Mann-Whitney rank sum; Box Plot with median, 25-75th percentile IQR (Box), 95% CI (Whiskers), outlier values (Dots).

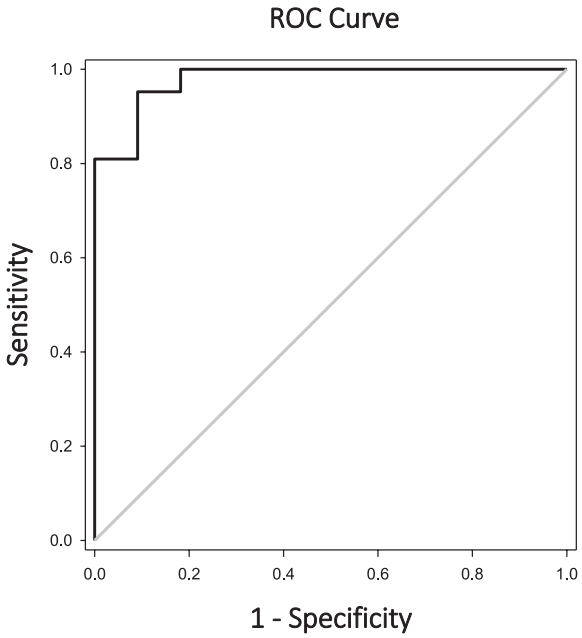

Figure 2. Receiver Operator Characteristic (ROC) curve analysis for sensitivity (true positive; *Y* axis) versus specificity (false positive; *X* axis) for aggregated diagnosis of REJECTION versus STABLE patients. Area under Curve (AUC) = 0.98 (95% CI: 0.937-1.02) and an optimal threshold determined for aggregated rejection (maximal sensitivity + specificity) for dd-cfDNA = 0.51%.

sample size for the different types of infection precluded further analysis. An AUC-ROC analysis, yielded an AUC = 0.98 (95% CI: 0.937%-1.02%) (Figure 2) and an optimal threshold for rejection was determined (maximal sensitivity + specificity) with dd-cfDNA = 0.51% whereupon sensitivity = 81% (95% CI: 58%-95%) and specificity = 100% (95% CI: 71%-100%).

We conclude that low levels of dd-cfDNA (<0.20%) typically characterize stable healthy LT allografts (Figure 3), while higher levels correlated with the spectrum of allograft rejection. The cohort with allograft infection had variability in levels of dd-cfDNA, likely due to inherent challenges in differentiation

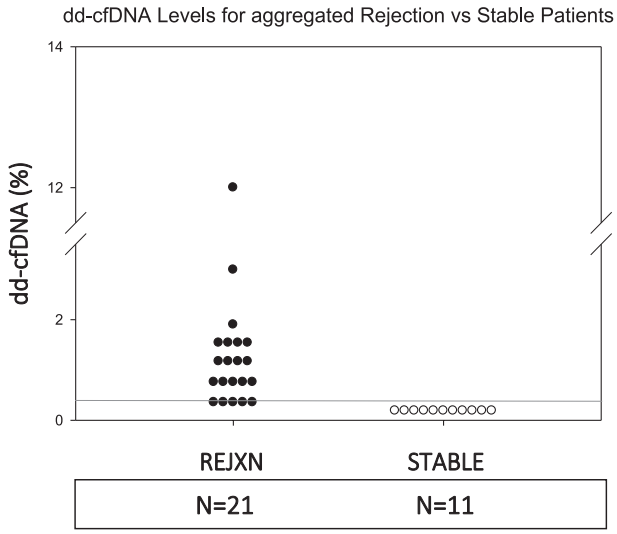

Figure 3. Dot Histogram for dd-cfDNA (*Y* axis) in aggregated Cohorts of REJECTION versus STABLE patients. An optimal threshold dd-cfDNA = 0.51% for aggregated rejection (Horizontal line) was determined by AUC-ROC analysis.

of invasive infection versus airway colonization. These results support the concept that further investigation and serial trend monitoring of dd-cfDNA, may serve valuable for assessment of LT allograft dysfunction and to preserve allograft health.

Authors' Contributions

DJL and DJR contributed to concept, design, and initial draft of study. All authors participated equally in implementation of study, data analysis, manuscript development, and final manuscript approval.

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REFERENCES

1. Chambers DC, Cherikh WS, Harhay MO, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-sixth adult lung and heart-lung transplantation report-2019; focus theme: donor and recipient size match. *J Heart Lung Transplant*. 2019;38:1042-1055. doi:10.1016/j.healun.2019.08.001.
2. DerHovanessian A, Wallace WD, Lynch JP III, Belperio JA, Weigt SS. Chronic lung allograft dysfunction: evolving concepts and therapies. *Semin Respir Crit Care Med*. 2018;39:155-171. doi:10.1055/s-0037-1618567.
3. Van Herck A, Verleden SE, Sacreas A, et al. Validation of a post-transplant chronic lung allograft dysfunction classification system. *J Heart Lung Transplant*. 2019;38:166-173. doi:10.1016/j.healun.2018.09.020.
4. Verleden GM, Raghu G, Meyer KC, Glanville AR, Corris P. A new classification system for chronic lung allograft dysfunction. *J Heart Lung Transplant*. 2014;33:127-133. doi:10.1016/j.healun.2013.10.022.
5. Shino MY, DerHovanessian A, Sayah DM, et al. The impact of allograft CXCL9 during respiratory infection on the risk of chronic lung allograft dysfunction. *OBM Transplant*. 2018;2:029. doi:10.21926/obm.transplant.1804029.
6. Levine DJ, Glanville AR, Aboyoun C, et al. Antibody-mediated rejection of the lung: a consensus report of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2016;35:397-406. doi:10.1016/j.healun.2016.01.1223.
7. Rao U, Sharma M, Mohanakumar T, Ahn C, Gao A, Kaza V. Prevalence of antibodies to lung self-antigens (Kalpha1 tubulin and collagen V) and donor specific antibodies to HLA in lung transplant recipients and implications for lung transplant outcomes: Single center experience. *Transpl Immunol*. 2019;54:65-72. doi:10.1016/j.trim.2019.02.006.

8. Roden AC, Aisner DL, Allen TC, et al. Diagnosis of acute cellular rejection and antibody-mediated rejection on lung transplant biopsies: a perspective from members of the pulmonary pathology society. *Arch Pathol Lab Med*. 2017;141:437-444. doi:10.5858/arpa.2016-0459-SA.
9. Agbor-Enoh S, Jackson AM, Tunc I, et al. Late manifestation of alloantibody-associated injury and clinical pulmonary antibody-mediated rejection: Evidence from cell-free DNA analysis. *J Heart Lung Transplant*. 2018;37:925-932. doi:10.1016/j.healun.2018.01.1305.
10. Agbor-Enoh S, Wang Y, Tunc I, et al. Donor-derived cell-free DNA predicts allograft failure and mortality after lung transplantation. *EBioMedicine*. 2019;40:541-553. doi:10.1016/j.ebiom.2018.12.029.
11. Grskovic M, Hiller DJ, Eubank LA, et al. Validation of a clinical-grade assay to measure donor-derived cell-free DNA in solid organ transplant recipients. *J Mol Diagn*. 2016;18:890-902. doi:10.1016/j.jmoldx.2016.07.003.
12. Bloom RD, Bromberg JS, Poggio ED, et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol*. 2017;28:2221-2232. doi:10.1681/ASN.2016091034.
13. Bromberg JS, Brennan DC, Poggio E, et al. Biological variation of donor-derived cell-free DNA in renal transplant recipients. *J Appl Lab Med*. 2017;2:309-321.
14. Khush KK, Patel J, Pinney S, et al. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: a prospective multicenter study. *Am J Transplant*. 2019;19:2889-2899. doi:10.1111/ajt.15339.

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