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# Clinical utility of plasma percent donor-derived cell-free DNA for lung allograft surveillance: A real-world single-center experience

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**KEYWORDS:**
lung transplant;
plasma donor-derived cell-free DNA;
cfDNA;
acute cellular rejection;
infection;
allograft dysfunction

**BACKGROUND:** Plasma percent donor-derived cell-free DNA (%dd-cfDNA) has been investigated as a biomarker of allograft injury after lung transplantation. We sought to determine the clinical utility of %dd-cfDNA as a screen for acute cellular rejection (ACR) and respiratory infections (RIs) among lung transplant recipients (LTRs).

**METHODS:** We retrospectively analyzed %dd-cfDNA results from 95 plasma samples collected from 81 bilateral LTRs >45 days after transplant with a paired transbronchial biopsy performed within 24 hours after sample collection. We calculated sensitivity, specificity, negative predictive value (NPV), and positive predictive value of %dd-cfDNA to detect ACR and RIs and used a generalized estimating equation model to compare %dd-cfDNA between groups.

**RESULTS:** A dd-cfDNA threshold of 0.5% had low sensitivity to detect ACR among LTRs (41.67%), as did a 70% increase in %dd-cfDNA (50.00%). The NPV was high (88.89% and 87.50%, respectively) but driven by the low prevalence of ACR (12/95 [12.6%]). The area under the receiver operating characteristic curve (AUC) was 0.499 (95% confidence interval [CI] [0.326-0.672]) and 0.360 (95%CI [0.132-0.588]) for the detection of ACR and ACR grade ≥A2, respectively. The adjusted mean %dd-cfDNA trended higher in LTRs with a definite or possible RI (1.218, 95%CI [0.671-2.212]) than in LTRs without microbial isolation (0.731, 95%CI [0.525-1.017], $p = 0.059$), but was not significantly different from those with microbial colonization (0.873, 95%CI [0.538-1.415], $p = 0.390$). The AUC for the detection of allograft dysfunction due to ACR and/or RI was 0.573 (95%CI [0.431-0.716]).

**CONCLUSIONS:** %dd-cfDNA may have limited utility as a screening tool to detect ACR and/or RI among LTRs.

JHLT Open 2024;6:100141



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https://doi.org/10.1016/j.jhlto.2024.100141

## Background

Lung transplantation (LT) is life-saving for patients with end-stage lung disease; however, the post-LT course is often fraught with complications, and long-term survival is shorter among lung transplant recipients (LTRs) than other solid organ transplant recipients.[1] Acute cellular rejection (ACR) and respiratory infections are common complications that require bronchoscopy for a definitive diagnosis.[2-4] Cell-free DNA is fragmented DNA that is continuously released from cells into the bloodstream. AlloSure (CareDx Inc., Brisbane, CA) is a next-generation sequencing assay that measures single nucleotide polymorphisms to distinguish between donor-derived and recipient-derived cell-free DNA,[5] and plasma percent donor-derived cell-free DNA (%dd-cfDNA) has been validated for differentiation of ACR, antibody-mediated rejection, and quiescence after renal and cardiac transplantation.[6-8] AlloSure Lung also quantifies plasma %dd-cfDNA, and this assay has been investigated as a noninvasive biomarker of lung allograft injury.[9,10] The test gained particular prominence during the COVID-19 pandemic due to the need for close monitoring of LTRs despite pandemic-related challenges to health care access and delivery.[11,12] A threshold dd-cfDNA value of 0.9% was associated with a positive predictive value (PPV) of 44% and a negative predictive value (NPV) of 84% based on a 25% prevalence of allograft rejection.[13] Furthermore, a cut-off of < 0.5% was associated with a lower risk of ACR and respiratory infection.[12] Last, a > 70% increase in %dd-cfDNA from prior results was found to exceed the biological variability observed in a reference population.[14-16] Despite these encouraging reports, we noticed a frequent disparity between %dd-cfDNA and ACR at our large lung transplant center, with low %dd-cfDNA observed in patients with ACR. Thus, we sought to determine the accuracy of AlloSure Lung as a screening tool to noninvasively identify ACR, respiratory infections, and lung allograft dysfunction in our cohort of LTRs.

## Materials and methods

### Study design and setting

This single-center, retrospective, noninterventional cohort study of LTRs was approved by our Institutional Review Board with a waiver of patient consent (PHX-21-500-198-73-18 dated September 5, 2023). All patient care was carried out under strict compliance with the International Society of Heart and Lung Transplantation ethics statement. Data were collected by detailed chart review. The study was not industry-sponsored, and CareDx was not involved in data collection or data analysis.

A total of 791 %dd-cfDNA results were obtained between November 1, 2022 and August 2, 2023 from 390 LTRs at our center. After excluding unsatisfactory samples, samples without a paired transbronchial biopsy (TBBx), or samples from LTRs with a single LT, bone marrow transplant, or multiorgan transplant, 348 samples paired with bronchoscopy with bronchoalveolar lavage (BAL) and TBBx performed within 30 days of %dd-cfDNA sample collection remained. The %dd-cfDNA results were stratified into those collected ≤45 days ($n$ = 42) and those collected > 45 days ($n$ = 306) after LT. Of the 306 %dd-cfDNA results collected > 45 days after LT, 95 were paired with BAL and TBBx performed within 24 hours after sample collection. These 95 samples were used for the primary analysis (Figure 1). An additional analysis of samples collected ≤45 days after LT is included in Supplementary Material 1, and a broader analysis of the 306 %dd-cfDNA samples collected > 45 days after LT with paired BAL and TBBx performed within 30 days of sample collection is included in Supplementary Material 2. The results of the study are reported per Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[17]

### Surveillance protocols and clinical outcomes

At our center, surveillance bronchoscopy with BAL and TBBx is performed at 1, 3, 6, 9, 12, 18, 24, 30, and 36 months after LT and "for cause" as needed to evaluate lung allograft dysfunction. The %dd-cfDNA was routinely obtained in addition to, not in lieu of, standard surveillance or for-cause testing. Primary outcomes included the sensitivity, specificity, PPV, and NPV of AlloSure Lung %dd-cfDNA to detect TBBx-confirmed ACR. ACR was graded by our highly experienced pathologists per the revised International Society of Heart and Lung Transplantation guidelines[18]: grade A (perivascular lymphocytic infiltration) subtypes A0 (absence of ACR), A1 (minimal), A2 (mild), A3 (moderate), and A4 (severe) and grade B (lymphocytic bronchiolitis) subtypes B0 to 2R.

The secondary outcome was the ability of AlloSure Lung %dd-cfDNA to detect respiratory infections. Microbial isolation from BAL fluid was defined as the culture of bacteria or fungi or the detection of viruses by real-time reverse transcription polymerase chain reaction. A *definite respiratory infection* was defined as microbial isolation from BAL fluid along with at least 2 of the following findings: bronchoscopy with purulent secretions or mucosal edema, signs and symptoms of respiratory infection on history or physical exam, and chest radiographic findings suggesting infection. *Respiratory colonization* was defined as microbial isolation from BAL fluid with only 1 or none of the 3 above-mentioned findings. When definite respiratory infection and colonization could not be differentiated, samples were classified as *possible respiratory infection*.

### %dd-cfDNA testing

To obtain specimens, venous blood was collected in Streck Cell-Free DNA Blood Collection Tubes (Streck LLC), processed, and analyzed in a Central Clinical Laboratories Improvements Act and College of American Pathologists–certified laboratory (CareDx, Inc., Brisbane,

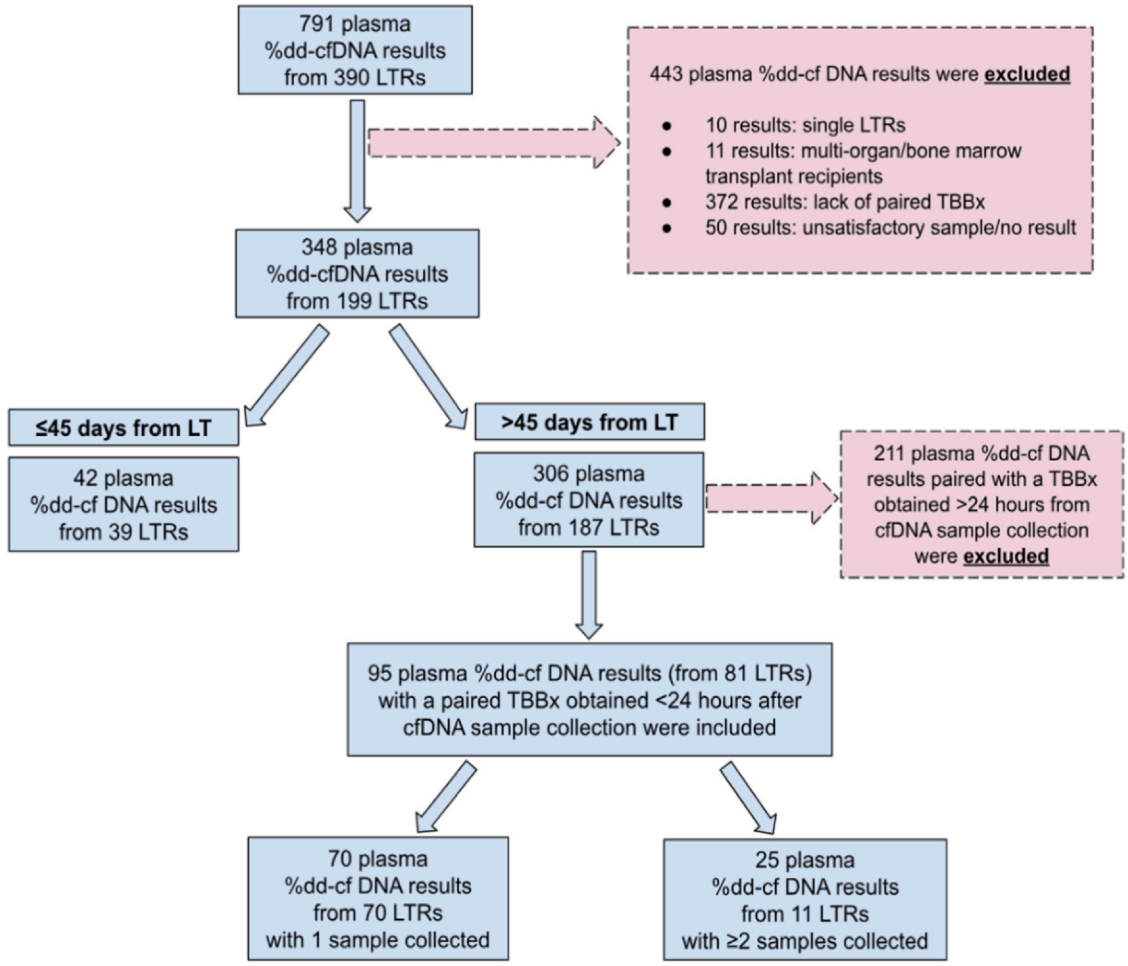


**Figure 1** Flowchart showing study enrollment and exclusion criteria. %dd-cfDNA, percent donor-derived cell-free DNA; cfDNA, cell-free DNA; LT, lung transplantation; LTR, lung transplant recipient; TBBx, transbronchial biopsy.

CA). Standardized specimen processing protocols and analytical methodology for targeted next-generation sequencing dd-cfDNA have been previously published.[13]

## Statistical analysis

Summary statistics for categorical variables were expressed as count and percentage, and continuous variables as median and interquartile range (IQR). Receiver operating characteristic (ROC)–area under the curve (AUC) analysis was performed for ACR (any grade and grade ≥A2) and percent change in %dd-cfDNA for detection of ACR. Sensitivity, specificity, PPV, and NPV were calculated. We used the generalized estimating equation (GEE) model with exchangeable working correlation matrix, gamma–distributed dependent variable, and log link function to account for repeated measures within subjects, compute the effect of predictors on %dd-cfDNA levels, and calculate the estimated marginal means to report the adjusted %dd-cfDNA levels. All reported $p$-values were 2-sided, and $p < 0.05$ was considered significant. Statistical analysis was carried out using IBM SPSS Statistics v.29 (IBM Corp., Armonk, NY).

## Results

We identified 306 %dd-cfDNA results that were collected >45 days after LT from 187 LTRs and paired with a bronchoscopy performed within 30 days of sample collection. Of these, 95 %dd-cfDNA results from 81 LTRs had a paired bronchoscopy with TBBx performed within 24 hours after sample collection. These 95 samples were used for the primary analysis. Demographic and clinical data are presented in Table 1. An additional analysis of samples obtained ≤45 days after LT is detailed in Supplementary Material 1, and a broader analysis of the 306 plasma samples collected >45 days with a paired bronchoscopy with TBBx performed within 30 days after LT is detailed in Supplementary Material 2.

### %dd-cfDNA as a screening tool for ACR > 45 days after LT

The median time from LT to plasma sample collection was 18.15 months [IQR, 9.17-24.00 months]. Of the 95 samples obtained >45 days after LT, 12 (12.6%) had ACR (any grade) confirmed by TBBx, and 5 (5.3%) had ACR grade ≥A2. Of those with ACR, 5 of 12 (41.7%) samples had

**Table 1** Demographics and Clinical Data

| Variable | Primary Cohort ($n = 95$ samples)* |
|---|---|
| *Demographics* | |
| Age, median [IQR], years | 67 [60.00, 71.00] |
| Female sex | 30 (31.6) |
| Time from transplant to sample collection, median [IQR], months | 18.15 [9.17-24.00] |
| 0-1.5 months after LT | - |
| 1.5-12 months after LT | 34 (35.8) |
| 12-36 months after LT | 47 (49.5) |
| > 36 months after LT | 14 (14.7) |
| *Clinical data* | |
| ACR (any grade)[a] | 12 (12.6) |
| ACR grade ≥A2[a] | 5 (5.3) |
| Microbial isolation | 33 (34.7) |
| Only viral | 10 |
| Only fungal | 6 |
| Only bacterial | 10 |
| Only mycobacterial | 1 |
| Polymicrobial | 6 |

Abbreviations: ACR, acute cellular rejection; IQR, interquartile range; LT, lung transplant.

*Data presented as no. (%) unless otherwise indicated.

[a]As confirmed by bronchoscopy with transbronchial biopsy and histopathology within 24 hours after sample collection for %dd-cfDNA.

dd-cfDNA ≥0.5% and 2 of 12 (16.7%) had dd-cfDNA ≥0.9%. Of those with ACR grade ≥A2, 1 of 5 (20.0%) samples had dd-cfDNA ≥0.5%, and 0 of 5 (0.0%) had dd-cfDNA ≥0.9%.

The median %dd-cfDNA of the samples was 0.36 [IQR 0.16-0.65]. Furthermore, the median %dd-cfDNA was low irrespective of the presence or absence of ACR (ACR, 0.28 [IQR 0.15-0.74]; no ACR, 0.36 [IQR 0.17-0.65]). There was no statistically significant difference in the estimated marginal means between samples with ACR and those without ACR (0.515, 95% confidence interval [CI] [0.299-0.886] vs 0.709, 95%CI [0.511-0.983], respectively, $p = 0.195$). However, LTRs with ACR grade ≥2 had a significantly lower estimated marginal mean %dd-cfDNA than those with ACR grade 1 or no ACR (0.262, 95%CI [0.164-0.419] vs 0.719, 95%CI [0.521-0.992], respectively, $p < 0.001$). The sensitivity and PPV of %dd-cfDNA to detect ACR remained low at both the 0.9% and 0.5% cut-offs (Table 2) with ROC curves showing an AUC of 0.499 (95%CI [0.326-0.672]) for the detection of ACR and an AUC of 0.360 (95%CI [0.132-0.588]) for the detection of ACR grade ≥A2 (Figure 2A and B). Notably, the NPV was high at both the 0.9% and 0.5% thresholds (87.50% and 88.89%, respectively); however, this was due to the low prevalence of ACR in the cohort.

## Impact of advanced age on %dd-cfDNA levels in LTRs

A GEE model using age ≥70 years and ACR any grade as predictors showed that LTRs aged ≥70 years had a lower adjusted mean %dd-cfDNA than LTRs aged < 70 years (0.587, 95%CI [0.401-0.860] vs 0.679, 95%CI [0.464-0.993]), $p < 0.001$). LTRs with ACR had a lower adjusted mean %dd-cfDNA than LTRs without ACR (0.542, 95%CI [0.317-0.928] vs 0.735, 95%CI [0.526-1.03]), but the difference was not statistically significant ($p = 0.205$). Similarly, a GEE model using age ≥70 years and ACR grade ≥2 as predictors showed that LTRs with age ≥70 years had a lower adjusted mean %dd-cfDNA than LTRs aged < 70 years (0.348, 95%CI [0.248-0.489] vs 0.401, 95%CI [0.285-0.563]), $p = 0.009$), and LTRs with ACR grade ≥2 had a significantly lower adjusted mean %dd-cfDNA than those with ACR grade 1 or no ACR (0.198, 95%CI [0.131-0.299] vs 0.705, 95%CI [0.516-0.963]), $p < 0.001$).

## Increase in %dd-cfDNA as a screening tool for ACR > 45 days after LT

We identified 11 LTRs with ≥2 available samples (25 samples in total) > 45 days after LT. Of the 14 samples

**Table 2** Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of Plasma Percent Donor-Derived Cell-Free DNA (%dd-cfDNA) for the Detection of Acute Cellular Rejection (ACR) After Lung Transplantation (LT)

| %dd-cfDNA | ACR ≥A1 | No ACR | | ACR ≥A2 | ACR < A2 | |
|---|---|---|---|---|---|---|
| ≥0.9 | 2 | 13 | PPV **13.33%** | 0 | 15 | PPV **0.00%** |
| < 0.9 | 10 | 70 | NPV **87.50%** | 5 | 75 | NPV **93.75%** |
| | Sensitivity **16.67%** | Specificity **84.34%** | Total 95 | Sensitivity **0.00%** | Specificity **83.33%** | Total 95 |
| > 0.5 | 5 | 27 | PPV **15.63%** | 1 | 31 | PPV **3.13%** |
| ≤0.5 | 7 | 56 | NPV **88.89%** | 4 | 59 | NPV **93.65%** |
| | Sensitivity **41.67%** | Specificity **67.47%** | Total 95 | Sensitivity **20.00%** | Specificity **65.56%** | Total 95 |

Bold font indicates sensitivity, specificity, PPV, and NPV (plain font indicates the number of samples).

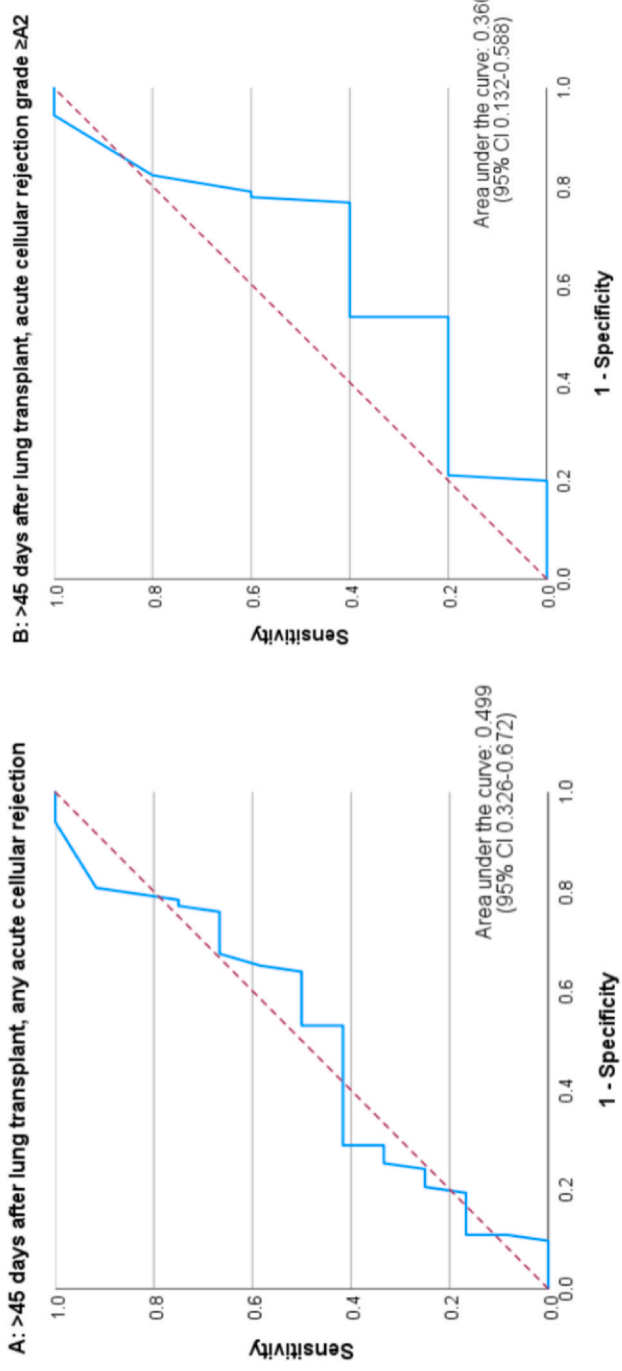


**Figure 2** Receiver operating characteristic curve of a ≥0.5% threshold plasma percent donor-derived cell-free DNA threshold to detect acute cellular rejection after lung transplantation. (A) > 45 days after lung transplant, any acute cellular rejection. (B) > 45 days after lung transplant, acute cellular rejection grade ≥A2. CI, confidence interval.

**Table 3** Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of > 70% Change in Plasma Percent Donor-Derived Cell-Free DNA (%dd-cfDNA) for the Detection of Acute Cellular Rejection (ACR) After Lung Transplantation (LT)

| > 70% change in %dd-cfDNA[a] | ACR ≥ A1 | No ACR | |
|---|---|---|---|
| Yes | 1 | 5 | PPV **16.67%** |
| No | 1 | 7 | NPV **87.50%** |
| | Sensitivity **50.0%** | Specificity **58.33%** | Total 14 |

Bold font indicates sensitivity, specificity, PPV, and NPV (plain font indicates the number of samples).

[a]Lung transplant recipients with ≥2 available samples of %dd-cfDNA were included in the analysis. A > 70% change was defined as a > 70% increase from %dd-cfDNA nadir.

included in the analysis, 6 samples had a > 70% increase in %dd-cfDNA from the nadir result (Table 3). Of these 6 samples with a > 70% within-subject increase in %dd-cfDNA, only 1 (16.7%) had ACR. Similarly, of the 8 samples that did not show a > 70% within-subject increase in %dd-cfDNA, 1 (12.5%) had ACR. Among the 2 samples with ACR, only 1 showed a > 70% increase in %dd-cfDNA from the nadir, thereby demonstrating a low sensitivity and PPV (50.0% and 16.7%) for detection of ACR based on a within-subject %dd-cfDNA increase from the nadir result (AUC of 0.458, 95%CI [0.005-0.912]).

## %dd-cfDNA as a screening tool for respiratory infection > 45 days after LT

Of the 12 bronchoscopies with ACR, 5 had a concurrent fungal or bacterial isolate, and only 1 was associated with an elevated %dd-cfDNA (1.8%). In addition, 1 bronchoscopy had ACR and a concurrent viral isolate (severe acute respiratory syndrome coronavirus 2) and was not associated with an elevated %dd-cfDNA (0.23%). The remaining 6 bronchoscopies with ACR had no concurrent microbial isolation.

Of the 83 bronchoscopies without ACR, solely viruses were identified in 9 (10.8%) BAL samples, fungi in 5 (6.0%), bacteria in 7 (8.4%), mycobacteria in 1 (1.2%), and multiple organisms were identified in 5 (6.0%) BAL samples (Table 4). The median %dd-cfDNA remained low in plasma samples paired with viral (0.52 [IQR 0.28-1.44]), fungal (0.17 [IQR 0.12-0.67]), bacterial (0.15 [IQR 0.12-1.30]), and polymicrobial (0.28 [IQR 0.12-2.22]) BAL isolations.

Notably, %dd-cfDNA was comparable between patients with a clinically significant (definite or possible) respiratory infection, those with microbial colonization, and those without microbial isolation on unadjusted analysis (Figure 3). The adjusted mean %dd-cfDNA in patients with definite or possible infection (1.218, 95%CI [0.671-2.212]) trended higher than that of patients with no microbial isolation (0.731, 95%CI [0.525-1.017], $p = 0.059$) but was not significantly different from patients with microbial colonization (0.873, 95%CI [0.538-1.415], $p = 0.390$). There was no significant difference in the adjusted mean %dd-cfDNA between patients with microbial colonization and no microbial isolation ($p = 0.464$).

## %dd-cfDNA as a screening tool for lung allograft dysfunction due to ACR, respiratory infection, or both

We assessed the utility of %dd-cfDNA as a screening tool for lung allograft dysfunction by creating a composite outcome variable comprising ACR (any grade) and definite or possible respiratory infection. The results were similar to

**Table 4** Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of Plasma Percent Donor-Derived Cell-Free DNA (%dd-cfDNA) for Detection of Microbial Isolation After Lung Transplantation (LT) Among Patients Without Acute Cellular Rejection (ACR)

| %dd-cfDNA | No microbial isolation | Only bacterial isolation | | Only fungal isolation | | Only viral isolation | | Polymicrobial isolation | |
|---|---|---|---|---|---|---|---|---|---|
| ≥0.9 | 7 | 2 | PPV **22.22%** | 1 | PPV **12.50%** | 2 | PPV **22.22%** | 1 | PPV **12.50%** |
| < 0.9 | 49 | 5 | NPV **90.74%** | 4 | NPV **92.45%** | 7 | NPV **87.50%** | 4 | NPV **92.45%** |
| | Specificity **87.50%** | Sensitivity **28.57%** | Total 63 | Sensitivity **20.00%** | Total 61 | Sensitivity **22.22%** | Total 65 | Sensitivity **20.00%** | Total 61 |
| > 0.5 | 15 | 3 | PPV **16.67%** | 1 | PPV **6.25%** | 5 | PPV **25.00%** | 2 | PPV **11.76%** |
| ≤0.5 | 41 | 4 | NPV **91.11%** | 4 | NPV **91.11%** | 4 | NPV **91.11%** | 3 | NPV **93.18%** |
| | Specificity **73.21%** | Sensitivity **42.86%** | Total 63 | Sensitivity **20.00%** | Total 61 | Sensitivity **55.56%** | Total 65 | Sensitivity **40.00%** | Total 61 |

Bold font indicates sensitivity, specificity, PPV, and NPV (plain font indicates the number of samples).

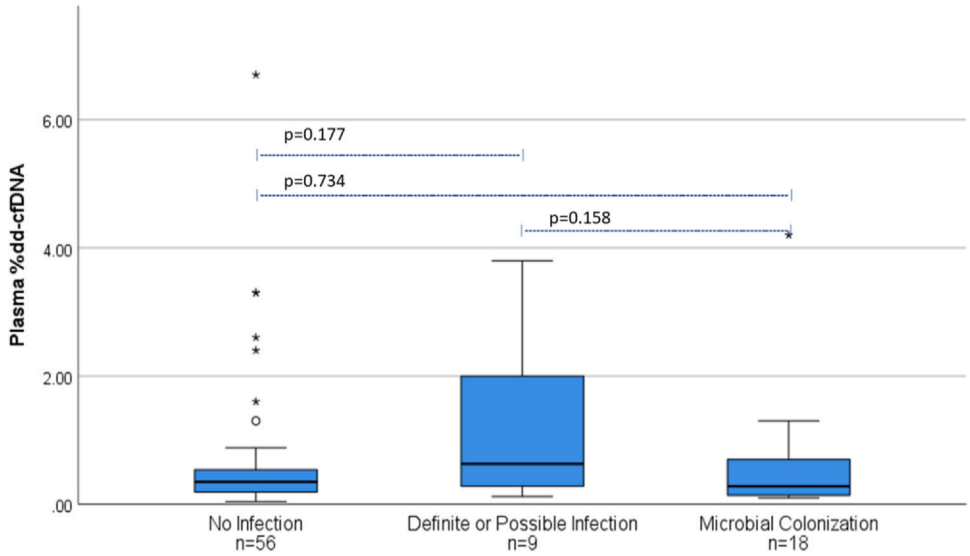


**Figure 3** Unadjusted analysis of plasma percent donor-derived cell-free DNA (%dd-cfDNA) stratified by the presence or absence of respiratory microbial colonization or infection, but no acute cellular rejection.

our previous findings, with the ROC curve showing an AUC of 0.573 (95%CI [0.431-0.716]) for the detection of allograft dysfunction due to ACR or respiratory infection.

## Discussion

In this study, we describe the real-world clinical utility of plasma %dd-cfDNA for lung allograft surveillance. We found that LTRs with ACR grade ≥2 had a lower adjusted mean %dd-cfDNA than those with ACR grade 1 or no ACR. In addition, the sensitivity of %dd-cfDNA for ACR detection was low, whereas the NPV was high, which was likely driven by the low prevalence of ACR in our cohort. Furthermore, a 70% increase in %dd-cfDNA also had low sensitivity and a low PPV for ACR detection, thus serial measurements of %dd-cfDNA may also have limited clinical utility. Although the %dd-cfDNA trended higher in patients with a definite or possible respiratory infection than in those with no microbial isolation, the assay may not be able to differentiate between patients with respiratory infections and those with airway colonization, thereby limiting its diagnostic use.

The clinical utility of %dd-cfDNA to detect lung allograft rejection at a threshold ranging from 0.85% to 1% has been supported by several publications, with a reported sensitivity of 55.6% to 73.9%, specificity of 75.8% to 87.7%, PPV of 43.3% to 43.4%, and NPV of 83.6% to 96.5%.[9,12,13,19-23] The results of our study differ from these publications, as a dd-cfDNA threshold of 0.9% was insensitive (sensitivity for ACR of any grade and ACR grade ≥A2: 16.67% and 0.00%, respectively) and had a low PPV (13.33% and 0.00%, respectively; Table 2). Furthermore, neither the sensitivity nor the PPV significantly improved when the threshold was reduced to 0.5%. The ROC curves of %dd-cfDNA for the detection of ACR showed an AUC below 0.5 (Figure 2A and B), thereby highlighting the absence of a %dd-cfDNA threshold associated with an acceptable sensitivity or specificity for ACR detection.

The difference between previously reported findings and those in our study may be related to a divergence in methodology. In the study by Keller et al,[12] surveillance bronchoscopy was recommended only to patients with surveillance dd-cfDNA ≥1.0%. Patients with dd-cfDNA between 0.5% and 1.0% were rechecked within 1 to 2 weeks, and patients with dd-cfDNA < 0.5% continued with monthly (or every 3-month) surveillance. Whereas in our study, %dd-cfDNA testing was obtained in addition to rather than in lieu of surveillance bronchoscopies, thus, removing the verification bias that may have influenced the findings of Keller et al.[12]

In a later study, Keller et al[14] explored the biological variability of the Allosure Lung assay in LTRs with stable allograft function. This time, they studied 109 %dd-cfDNA results from 30 LTRs and found that fluctuations in %dd-cfDNA levels of up to 70% from baseline (measures based on an average of 3.6 samples per patient) or levels less than 1% were within normal biological variation.[14-16] Based on their findings, we analyzed the utility of a > 70% within-subject increase from a nadir %dd-cfDNA value for the detection of ACR. However, the test continued to exhibit low sensitivity and PPV, and an AUC of 0.458 (95%CI [0.005-0.912]), suggesting that serial measurements of %dd-cfDNA cannot identify LTRs with ACR.

Respiratory infections also put LTRs at risk for allograft injury. However, microbial isolation from BAL fluid is common among LTRs and does not always indicate a clinically significant infection; thus, the decision to treat patients with antimicrobials often relies on a combination of microbial isolation, broncohoscopic findings, infectious symptoms, and radiographic abnormalities.[24] However, these symptoms and abnormalities are often subtle and difficult to tease out clinically as LTRs may lack common manifestations of respiratory infection or may develop allograft dysfunction despite adequate resolution of an acute infection.[25-27] Hence, studies have attempted to understand the association of %dd-cfDNA with

microbial isolation. Bazemore et al[28] found that the presence of microbial isolates in BAL samples from 51 LTRs was not associated with elevated %dd-cfDNA, but %dd-cfDNA was elevated in a subset of patients with microbial isolates that pose a higher risk of allograft injury, such as influenza, *Pseudomonas aeruginosa*, and *Aspergillus fumigatus*. However, their study focused only on microbial isolates rather than specific symptoms of infection. Our study mirrored their initial finding of low %dd-cfDNA associated with microbial isolation and additionally found that adjusted mean %dd-cfDNA trended higher in patients with a definite or possible respiratory infection than in those with no microbial isolation; however, the assay may not be able to differentiate between patients with respiratory infections and those with airway colonization, thereby limiting the predictive value of this biomarker.

Our study is limited by its single-center, retrospective design and a low number of samples associated with ACR or with a definite or possible infection. Furthermore, our study is enriched for stable patients undergoing surveillance bronchoscopies, which may limit its generalizability in regards to measuring plasma %dd-cfDNA to differentiate pulmonary and extrapulmonary causes of spirometric decline. Despite these limitations, our study is strengthened by the correlation of %dd-cfDNA results with a paired TBBx < 24 hours after %dd-cfDNA sample collection and the availability of clinical data to distinguish between definite infection and microbial colonization. Given the divergence of our results from previously published data, this study should be repeated by other transplant centers.

In conclusion, our study highlights the limited clinical utility of %dd-cfDNA as a screening tool to detect lung allograft injury due to ACR or respiratory infection in LTRs in a real-world setting. Although AlloSure assays have been useful for allograft surveillance of other solid organ transplants (e.g., kidney), their use after LT may be precluded by its poor sensitivity and PPV based on our experience. Of note, our center commonly transplants older patients, and older LTRs are predisposed to multiple comorbidities that may drive a higher cell turnover. Perhaps this turnover increases the amount of circulating recipient-derived cell-free DNA, thereby reducing the fraction of dd-cfDNA. Accordingly, our study found that LTRs ≥70 years of age had a lower adjusted mean %dd-cfDNA than those aged < 70 years. However, irrespective of the effect of age on %dd-cfDNA levels, the adjusted mean %dd-cfDNA was significantly lower among LTRs with ACR grade ≥2 than those with ACR grade 1 or no ACR, thereby limiting its clinical utility as a biomarker of ACR. The need for a noninvasive, reliable, and accurate biomarker to predict lung allograft injury to improve post-LT outcomes in this vulnerable patient population remains.

## Author contributions

**Devika Sindu**: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, and visualization. **Curt Bay**: methodology, validation, formal analysis, and visualization. **Katherine Grief**: conceptualization, data curation, writing – original draft, writing – review and editing. **Rajat Walia**: conceptualization, methodology, writing – original draft, writing – review and editing. **Sofya Tokman**: conceptualization, methodology, validation, formal analysis, investigation, writing – original draft, writing – review and editing, and supervision.

## Disclosure statement

R.W. served on the board of advisors for CareDx (consulting/advisory fees). D.S., C.B., K.G., and S.T. have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors thank Kristine Nally for editorial assistance and Marco Marchionni for graphic design.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2024.100141.

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