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ORIGINAL CLINICAL SCIENCE

Donor-derived cell-free DNA as a composite marker of acute lung allograft dysfunction in clinical care

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KEYWORDS:
dd-cfDNA;
lung transplant;
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BACKGROUND: As a marker of underlying lung allograft injury, donor-derived cell-free DNA (dd-cfDNA) may be used to identify episodes of acute allograft injury in lung transplant recipients. We investigated the utility of dd-cfDNA to monitor subjects at risk of acute rejection or infection in routine clinical practice.

METHODS: This multicenter, retrospective cohort study collected data from lung transplant recipients within 3 years of transplant at 4 centers between March 24, 2020 and September 1, 2020. During this period, as part of routine care during the COVID-19 pandemic, these centers implemented a home-based surveillance program using plasma dd-cfDNA in preference to surveillance bronchoscopy. Dd-cfDNA was used to detect acute lung allograft dysfunction (ALAD) − a composite endpoint of acute rejection and infection. dd-cfDNA levels in patients with ALAD were compared to stable patients. The performance characteristics of dd-cfDNA ≥ 1.0% to detect ALAD were estimated.

RESULTS: A total of 175 patients underwent 380 dd-cfDNA measurements, of which 290 were for routine surveillance purposes. dd-cfDNA was higher in patients with ALAD than stable patients (Median (IQR) 1.7% (0.63, 3.1) vs 0.35% (0.22, 0.79), $p < 0.001$). As an indication of underlying ALAD during surveillance testing, the estimated sensitivity of dd-cfDNA ≥1% was 73.9%, specificity of 87.7%, positive predictive value of 43.4% and negative predictive value of 96.5%.

CONCLUSIONS: dd-cfDNA identified acute lung allograft dysfunction in asymptomatic lung transplant patients that may not have been identified by using a clinically indicated biopsy strategy alone. dd-cfDNA <1.0% may be useful in ruling out acute rejection and infection, supporting its use as a potential noninvasive marker for surveillance monitoring.

Abbreviations: dd-cfDNA, Donor-derived cell free DNA (dd-cfDNA)

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The global pandemic of Coronavirus Disease 2019 (COVID-19) has resulted in significant disruption to health care delivery systems.[1] The need to optimize resource allocation and mitigate the spread of disease has dramatically altered the longitudinal follow up of patients with chronic medical conditions.[2-4] Emphasis has been placed on minimizing patient contact with the health care system through remote monitoring strategies and parsimonious use of diagnostic testing procedures.[5-7] These issues pose a unique challenge to providers caring for patients with complex medical conditions that require close follow up in the outpatient setting. Lung transplant patients, in particular, represent a population that requires diligent post-transplant medical follow-up, especially within the first year after transplantation.[8] Given the need for intense immunosuppressive regimens, lung transplant patients are also at increased risk of infection and severe COVID-related complications. Balancing the requirement for careful post-transplant monitoring with the risk of infection through exposure to the health care system has been especially challenging.[6,9]

A considerable aspect of post-transplant monitoring involves the performance of routine surveillance bronchoscopy with transbronchial biopsy (TBBx) and bronchoalveolar lavage (BAL) to detect asymptomatic episodes of acute allograft rejection and infection. While practice patterns vary, patients may receive routine surveillance bronchoscopy up to every 3 months during the first year after lung transplantation.[10] During the initial phase of the COVID-19 pandemic, in accordance with the International Society of Heart and Lung Transplantation (ISHLT) guidance, several lung transplant centers deferred performing routine surveillance bronchoscopies in favor of bronchoscopy solely in response to clinical signs or symptoms.[11,12] However, the absence of adequate surveillance monitoring generates concern about asymptomatic acute allograft rejection or infection, which may arise in up to 18% of lung transplant patients.[13]

While several studies have investigated other biomarkers of allograft injury,[14,15] recent observational studies in lung transplant recipients demonstrate that levels of plasma donor-derived cell free DNA (dd-cfDNA) increase during acute rejection and infection.[16-21] These studies have also defined potential dd-cfDNA thresholds with high sensitivity and negative predictive value for the detection of acute rejection. However, the utility and performance of dd-cfDNA in routine clinical care remains undefined. The ability to noninvasively screen for acute rejection and infection would prove especially valuable in the instance where performing surveillance bronchoscopy may be unnecessary or would pose undue risk. In response to the COVID-19 pandemic, to reduce the risk of exposing patients and providers to infection, 4 lung transplant centers used dd-cfDNA in place of routine surveillance bronchoscopy to monitor for acute allograft infection and rejection. This study details the first documented use of dd-cfDNA in routine clinical care of lung transplant recipients and aims to provide further assessment of the clinical utility of dd-cfDNA for the screening of acute allograft rejection and infection by estimating its performance characteristics in the clinical setting.

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Methods

Study design and population

This multicenter, retrospective, observational cohort study included patients from 4 comprehensive transplant centers in the United States during the time period between March 24, 2020 and September 1, 2020. This time period coincided with the onset of the COVID-19 pandemic in the United States. As part of routine clinical care, in an effort to mitigate the risk of COVID-19 infection among lung transplant patients and providers, these centers reduced traditional post-transplant surveillance bronchoscopy and clinic-based pulmonary function testing, and instead, consented patients for a remote, home-based monitoring program (RemoTraC) using plasma dd-cfDNA (Allosure, CareDx) in order to non-invasively monitor for evidence of acute rejection or infection, in conjunction with home based spirometry. All patients > 18 years, between 30 days and 3 years post-transplant who received routine dd-cfDNA testing were included in the study. The rationale for excluding patients > 3 years post-transplant is based on prior observations by our lab suggesting that dd-cfDNA levels tend to rise after year 3 post-transplant, potentially due to a decrease in recipient-derived cell free DNA levels. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines for reporting observational studies were followed. This study was approved by the institutional review board at each center (Johns Hopkins Hospital IRB, #IRB00138643; Inova Fairfax IRB #U20-08-4227, WIRB #20204615; University of Maryland IRB #HP-00092685; University of Texas San Antonio IRB #HSC20080378H).

Surveillance protocol

Prior to the time period of this study, these 4 lung transplant centers performed surveillance bronchoscopy at 1, 3, 6, 9, 12- and 18-months post-transplant. Throughout the follow-up period, telemedicine-based clinic visits predominated over in-person visits due to restrictions on clinic capacity. Patients enrolled in remote monitoring underwent routine surveillance blood draws into specialized tubes with preservative to prevent cell lysis (Streck tube). Blood samples were shipped overnight to CareDx for plasma dd-cfDNA measurements. All four centers developed consensus guidance regarding the frequency of asymptomatic dd-cfDNA monitoring and interpretation with monthly surveillance plasma dd-cfDNA testing for patients less than 1-year post-transplant and every 3 months for patients > 1-year post-transplant (Figure E1a in the online data supplement). Prior observational data indicated

a potential optimal threshold value of 1.0% for the diagnosis of acute rejection or infection.[17,18,21] Based on this data, patients with surveillance dd-cfDNA ≥1.0% were recommended to proceed to surveillance bronchoscopy, donor-specific antibody (DSA) testing, and in-clinic spirometry. Asymptomatic patients with a level between 0.5% and 1.0% were recommended to undergo a re-check within 1 to 2 weeks and proceed with bronchoscopy if the value was ≥1.0% on recheck (Figure E1b in the online data supplement). Patients with a dd-cfDNA level <0.5% did not receive further evaluation and continued with monthly (or Q3M) surveillance levels. Dd-cfDNA testing was also performed, per each center's testing availability, for non-surveillance purposes (for cause) in response to a decline in forced expiratory volume in 1 second (FEV1) on home or office-based spirometry, signs or symptoms suggesting a change in respiratory status (fever, dyspnea, chest pain, cough, and radiographic changes) and/or treatment follow up. In addition, bronchoscopy was performed in the setting of signs and symptoms of allograft dysfunction at the discretion of the provider (for cause bronchoscopy).

Data collection

Retrospective chart review was performed on all patients enrolled in the home monitoring program over the course of the study period. All plasma dd-cfDNA levels were identified. An indication for each dd-cfDNA level was assigned as follows: routine surveillance, change in respiratory signs or symptoms (fever, dyspnea, chest pain, hypoxia, cough and/or radiographic changes), decline in forced expiratory volume in 1 second (FEV1) ≥ 10% from established baseline value, follow up of abnormal dd-cfDNA value, follow up for treatment of a respiratory event or other. More than one indication could be assigned for each value. (ex. signs and symptoms + decline in FEV1). In addition, based on provider documentation, orders and results at the time of the dd-cfDNA draw, a diagnostic response to each dd-cfDNA value was assigned as follows: bronchoscopy, Pulmonary Function Testing (PFT), CT scan, or other. The diagnostic test must have also been performed within 1 month of the dd-cfDNA test in order to be assigned.

Clinical outcomes

Acute Cellular Rejection (ACR) was defined as histopathologic evidence of ACR on transbronchial biopsy or clinically diagnosed ACR for subjects with clinical signs of allograft dysfunction and negative biopsy prompting empiric treatment for ACR, as has been previously defined.[22-24] Histopathology of ACR was graded by pathologists at each center in accordance with revised International Society of Heart and Lung Transplant (ISHLT) guidelines.[25] The outcome of Antibody Mediated Rejection (AMR) was assigned to a patient according to ISHLT consensus guidelines for the diagnosis of "probable" AMR.[26] Diagnosis of infection was defined as the presence of a lower respiratory tract infection in accordance with ISHLT consensus guidelines for the diagnosis of "proven" bacterial, viral, or fungal pneumonia which includes positive microbiology on bronchoalveolar lavage (BAL) specimens, sputum samples or real time reverse transcription polymerase chain reaction (RT-PCR) nasopharyngeal swab along with documented initiation of treatment and radiographic evidence of new infiltrates on chest imaging.[27] The composite outcome of ACR, AMR, or infection with or without a decline in FEV1 was termed Acute Lung Allograft Dysfunction (ALAD).[28] Individual dd-cfDNA levels were assigned an outcome of "stable" if the patient did not experience any episode of infection, ACR, AMR, new respiratory signs/symptoms or decline in >10% FEV1 for the duration of the study period with a requirement of at least 30 days of follow-up from the date of the dd-cfDNA draw. A review of the definitions of clinical outcomes can be found in Table E1 of the online data supplement.

Statistical analysis

Continuous variables were summarized using mean (SD) or median (IQR), and categorical variables were summarized using counts (%). Nonparametric tests were used when indicated. Levels of dd-cfDNA were log10-transformed and compared using linear mixed models to account for repeated measures within each patient. Logistic regression was used to estimate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Generalized estimating equation (with exchangeable working correlation matrix) was used to account for repeated measures. ROC curves were plotted using *R* (version 4.1.2) package *pROC* (version 1.18.0).[29] Two-sided *p*-values were reported. SAS version 9.4 was used for all other analysis.

Results

Cohort description and study endpoints

198 patients were enrolled in the remote dd-cfDNA monitoring program between March 24, 2020 and September 1, 2020. 23 patients were > 3 years post-transplant and excluded, leaving 175 patients included in the final analysis (Figure 1). The mean (SD) age of transplantation was 58.5 (12.7) years and the mean (SD) time post-transplant was 13.1 (8.7) months at enrollment. A total of 99 (57%) patients were <1-year post-transplant at enrollment. A total of 46% were female, 64% were white, 18% black, 3% Asian. 82% underwent bilateral lung transplant (Table 1). Overall, 37 episodes of ALAD were detected: 12 episodes of ACR (8 biopsy confirmed, 4 treated without biopsy confirmation – Table E2 in the online data supplement), 8 episodes of AMR, 2 episodes of combined ACR + AMR and 15 episodes of infection (10 Bacterial, 3 fungal, 2 viral - Table E3 in the online data supplement).

dd-cfDNA testing

Over the course of the study period, a total of 380 dd-cfDNA levels were performed in 175 patients. A total of 157/175 (89.7%) patients received dd-cfDNA testing for routine surveillance purposes. The remaining 18/175 (10.3%) presented initially with clinical signs or symptoms of infection, PFT decline or were receiving prior treatment for established allograft injury and were not assigned any "routine surveillance" draws - only "follow up for treatment" draws. Of the 380 dd-cfDNA measurements, 290 were for routine surveillance, 24 in response to clinical signs and symptoms, 17 for a decline in

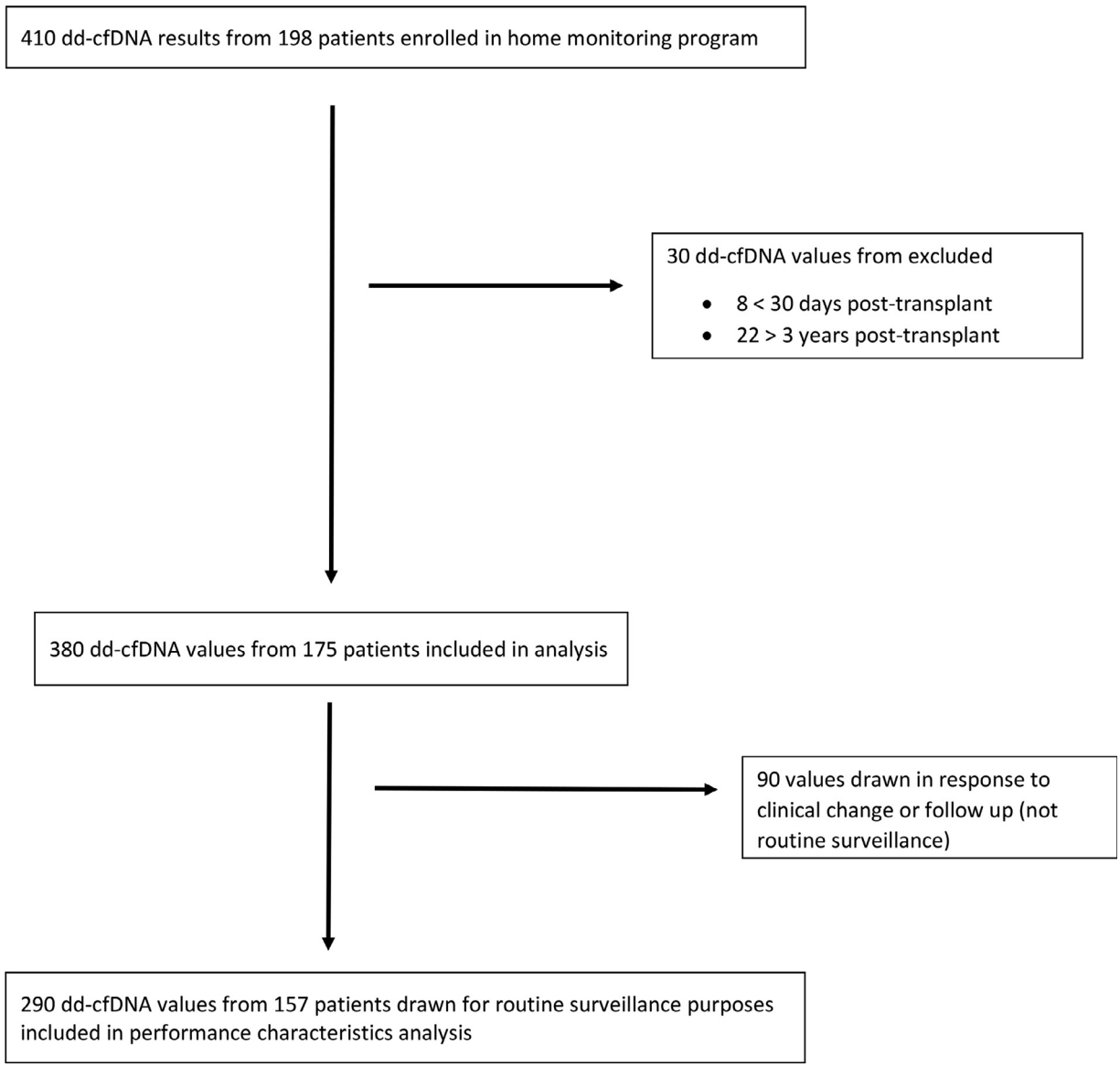

Figure 1 Study enrollment and inclusion criteria for overall analysis and performance characteristics analysis in the surveillance bronchoscopy group.

Table 1 Patient Demographics

Recipient Age (years)	58.5 (12.7)
Female Recipient (%)	46%
Bilateral Transplant (%)	82%
Mean Time Post-Transplant (months)	13.1 (8.7)
Patients < 1 year Post transplant (%)	57%
Race	
White	64%
Black	18%
Asian	3%
Other	15%
Diagnosis	
COPD	15.7%
Cystic Fibrosis	7%
Interstitial Lung Disease	62.6%
Pulmonary Arterial Hypertension	3%
Sarcoidosis	5.6%
Retransplantation	1.5%
Other	4.5%

FEV1 >10%, 66 for follow up of a prior abnormal value and 28 for follow up of treatment (one value may have been assigned multiple designations). The distribution of dd-cfDNA values is provided in Figure E2 of the online data supplement.

Levels of dd-cfDNA in patients diagnosed with acute rejection or infection

Levels of dd-cfDNA in patients with ALAD at the time of diagnosis were higher than in stable patients (Median (IQR) 1.7% (0.63, 3.1) vs 0.35% (0.22, 0.79), $p < 0.001$ using linear mixed model) (Figure 2). Levels of dd-cfDNA did not significantly differ between biopsy confirmed ACR vs ACR treated without biopsy confirmation ($p = 0.21$) or between ACR vs AMR ($p = 0.26$). Levels of dd-cfDNA did not significantly differ between acute rejection (AMR + ACR) vs infection (1.6% (0.38, 3.4) vs 1.8% (0.84, 2.7), $p = 0.82$)

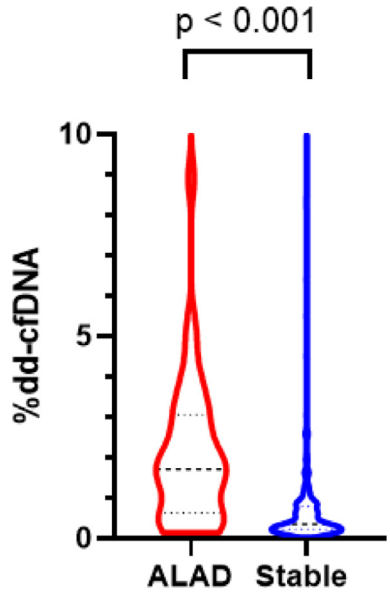

Figure 2 Comparison of levels of dd-cfDNA between patients with ALAD (n = 37) at the time of diagnosis vs stable patients (n = 258) displayed with violin plots including median and interquartile range. Levels of dd-cfDNA are higher in patients at the time of diagnosis of ALAD vs stable patients. ALAD: Acute Lung Allograft Dysfunction.

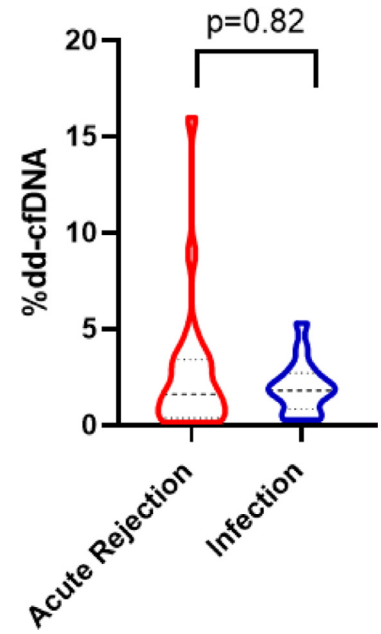

Figure 3 Comparison of levels of dd-cfDNA between patients with acute rejection (AMR and ACR, n = 22) vs infection (n = 15) displayed with violin plots including median and interquartile range. There is no difference in dd-cfDNA levels between patients at the time of diagnosis of acute rejection vs infection. AMR: Antibody Mediated Rejection; ACR: Acute Cellular Rejection.

(Figure 3). There was no difference in levels of dd-cfDNA between bacterial infections vs viral or fungal infections (p = 0.53). In patients with ALAD, there was no difference in dd-cfDNA in single vs double lung transplant (1.7% (0.38, 3.45) vs 1.7% (0.70, 3.40), p = 0.80), although the number of single lung transplant patients with ALAD is too small to make conclusions (n = 5). In patients classified as stable, the median dd-cfDNA in single lung transplant patients was lower than in double lung transplant patients (0.24% (0.12, 0.39) vs 0.37% (0.23,0.79), $p < 0.01$). Levels of dd-cfDNA were higher in patients who experienced spirometric decline of ≥ 10% vs stable patients (0.70% vs 0.35%, p = 0.02). Further description of patients presenting with signs and symptoms of allograft dysfunction is included in page 2 of the online data supplement.

Performance characteristics of dd-cfDNA in screening for acute rejection or infection

We next analyzed the performance characteristics of a surveillance dd-cfDNA level of ≥1% to detect ALAD in the 157 patients that received dd-cfDNA draws for routine surveillance. We excluded 14 routine surveillance levels classified as stable for which there was not at least 1 month of follow up (i.e., those performed between August 1 and September 1). Out of 290 measured dd-cfDNA levels, 53 (18%) were ≥ 1%. 23/53 (43.4%) of these levels were associated with ALAD. Only 30/53 (56.6%) patients with dd-cfDNA levels ≥ 1% were designated as stable. A total of 237/290 (81.7%) of the dd-cfDNA levels were < 1%. Only 9/237 (3.8%) of these levels were associated with evidence of ALAD. The accuracy of surveillance dd-cfDNA for detecting ALAD as assessed by the area under the receiver-operator characteristic curve (AUC) was 0.82 (95% CI, 0.73, 0.91) with an optimal threshold value of 0.91% (Figure 4). As an indication of underlying ALAD during surveillance testing, the sensitivity (95% CI) of a dd-cfDNA level ≥1% was 73.9% (54.3%, 87.1%) with a specificity of 87.7% (81.9%,91.9%), positive predictive value (PPV) of 43.4% (31.0%, 56.7%) and negative predictive value of

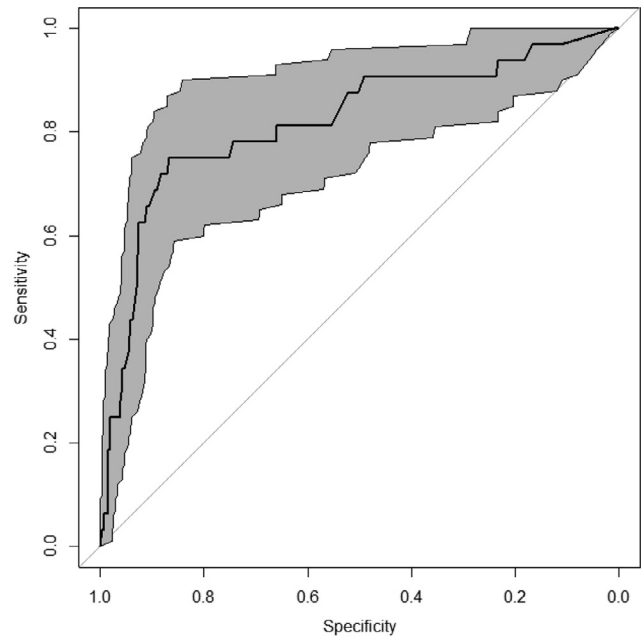

Figure 4 Receiver-operator characteristic curve demonstrating the accuracy of surveillance %ddcfDNA ≥ 1% to detect underlying ALAD with an AUC (95% CI) of 0.82 (0.73, 0.91).

Table 2 Performance Characteristics of Routine Surveillance ddcfDNA ≥1%

	ALAD	Stable	
≥1%	23	30	Total ≥ 1% 53
<1%	9	228	Total < 1% 237
	Total ALAD 32	Total Stable 258	Total Samples 290

96.5% (92.5%, 98.4%) (Table 2). A sensitivity analysis including patients > 3 years post-transplant demonstrated similar results, with an AUC of 0.81 (0.71, 0.90).

As an additional sensitivity analysis, we analyzed performance characteristics using the designation of stable for patients with at least 3 months follow-up without ALAD, decline in PFTs and signs/symptoms. By extending the duration of time without ALAD after a given test, this increases the confidence that a given value was not associated with underlying, asymptomatic pathology at the time of the draw. Using this definition, the AUC was 0.79 (0.71, 0.88) (Figure E3 in the online data supplement) with a sensitivity of a dd-cfDNA level ≥1% of 66.8% (48.7%, 81.0%), with a specificity of 87.8% (81.5%, 92.2%), PPV of 65.0% (53.3%, 75.2%) and NPV of 91.1% (84.1%, 95.2%). Further extending this sensitivity analysis, a total of 52 patients received surveillance bronchoscopy independent of dd-cfDNA level, based on clinician preference − generating a pool of patients who each received a "gold standard" work up along with a corresponding dd-cfDNA level. In this group of patients, a dd-cfDNA level of ≥1% demonstrated a sensitivity of 76.2% (54.0%, 89.7%), specificity of 70% (50.7%, 84.6%) PPV of 66.7% (48%, 81.3%) and NPV of 79.2% (59.4%, 90.8%) for the diagnosis of ALAD.

Reduction in surveillance bronchoscopies

Seventy-seven total bronchoscopies were performed over the study period. Fifty-two were performed for routine surveillance purposes. Accounting for time post-transplant, the expected number of surveillance bronchoscopies performed using the traditional surveillance bronchoscopy schedule would have been approximately 277. Accordingly, an estimated total of 225 less surveillance bronchoscopies were performed than typically would have over this time frame (82.1% reduction).

Discussion

This multicenter, observational cohort study describes the first reported use of dd-cfDNA as part of routine clinical care of lung transplant patients. This study demonstrates that levels of dd-cfDNA increase in the setting of acute rejection and infection. The use of dd-cfDNA for surveillance screening purposes identified early episodes of acute rejection and infection that would not have been identified using a clinically indicated biopsy strategy alone. Furthermore, the use dd-cfDNA for surveillance screening may demonstrate good performance characteristics for the detection of acute rejection or infection.

These findings lend further credence to accumulating evidence supporting dd-cfDNA as a predictor of allograft injury in solid organ transplantation.[17,18,30-32] Previous studies have established that dd-cfDNA increases in lung transplant recipients with ACR, AMR, primary graft dysfunction and chronic lung allograft dysfunction (CLAD).[16,18,30] Notably, in contrast to the findings of these studies, our data indicate that dd-cfDNA also increases in the setting of acute lower respiratory tract infection. This likely reflects the refined classification of infection in our analysis.[27] While several prior studies classified infection solely by microbiological isolation of a pathogen, this study required additional radiographic changes and the initiation of treatment − allowing for better discrimination between colonization and pneumonia. Increases of dd-cfDNA in the setting of both acute rejection and respiratory infection is consistent with the notion that dd-cfDNA serves as a sensitive marker of allograft injury, regardless of the cause of injury, and not specific to the underlying pathology stimulating allograft cellular destruction. Furthermore, this evidence supports our use of a composite outcome of acute rejection and infection (ALAD).

It is notable that increases in dd-cfDNA often occurred in asymptomatic patients experiencing underlying ALAD − cases that would not have been identified at the time of diagnosis using clinically indicated biopsy alone. This was found to be highly beneficial during the COVID-19 pandemic (when the performance of routine surveillance bronchoscopy was deferred), however, these findings also raise the potential for dd-cfDNA to serve as a noninvasive method of surveillance in place of surveillance bronchoscopy under traditional circumstances. Our data reveal that the use of dd-cfDNA for surveillance purposes may possess good discriminative capability for the diagnosis of clinically silent ALAD with an estimated AUC of 0.82. Of particular importance, the high negative predictive value of dd-cfDNA ≥ 1% may reliably exclude patients without underlying rejection or infection, obviating the need for many patients to undergo bronchoscopy with transbronchial biopsy for surveillance purposes.. While the PPV at a dd-cfDNA threshold of ≥1% may appear modest, a high NPV may be more desirable for surveillance screening purposes, especially considering that the underlying conditions may have serious clinical consequences, are largely asymptomatic and amenable to early treatment.[33,34] While our sensitivity analysis using a required follow-up time from dd-cfDNA testing of at least 3 months to ensure clinical stability remained consistent with our primary analysis, it is notable that the performance characteristics differed in the subset of patients who all received dd-cfDNA and "gold standard" bronchoscopy in parallel. While the PPV increased in this group, the NPV value decreased to 79.2%. While the sample size of this subset of patients is small, it raises the likelihood of an overestimation of the NPV from our primary analysis. Despite our clinical definition of stable (absence of the

development of ACR, AMR, Infection, PFT decline or symptoms throughout the entire study period with at least 1-month follow-up from the dd-cfDNA draw) and corresponding sensitivity analysis using at least 3-month follow-up from the dd-cfDNA, we can cannot exclude the possibility of subclinical, asymptomatic ALAD with absolute certainty in patients classified as stable. We did find that levels of dd-cfDNA for stable patients in this study are consistent with levels from two other prospective cohort studies that defined stability based on concomitant gold standard monitoring tools.[17,21] Further, while these results remain consistent with a recent prospective cohort study in lung transplant patients demonstrating an NPV of 90% for acute rejection at a 1% threshold,[21] they remain exploratory in nature and support the conduct of future studies evaluating the performance characteristics and optimal threshold values of dd-cfDNA in the clinical setting.

The use of surveillance bronchoscopy is a widely adopted practice utilized by up to 70% of lung transplant centers.[10,35] Despite its widespread use, no randomized controlled trials have assessed the effectiveness of surveillance bronchoscopy to prevent relevant clinical outcomes and controversy remains regarding the clinical utility of this surveillance strategy.[35-37] Advocates for its use postulate that there is an increased rate of acute rejection or infection diagnosed by surveillance biopsy, particularly in the first year after transplant.[13] Proponents further argue that the early detection and treatment of allograft injury may improve outcomes, namely a reduction in the development of CLAD − which has the greatest impact on long term survival in lung transplant recipients. In contrast, opponents argue that routine surveillance bronchoscopy is costly, time consuming, places the patient at risk of complications and has not been demonstrated to improve outcomes.[38,39] In addition, the utility of performing surveillance bronchoscopy may decrease considerably after 3 months as the presence of asymptomatic rejection or infection decreases.[40,41] As a convenient, accurate and noninvasive marker of allograft injury, dd-cfDNA may serve to bridge the controversial divide surrounding the use of surveillance bronchoscopy and set the stage for a randomized control trial comparing these two surveillance strategies.

There are inherent limitations to this study. Although the centers developed a consensus algorithm for dd-cfDNA surveillance monitoring based on prior published data, there may have been center-level and provider-level differences in use of the algorithm. However, while there were between-center differences in the number of dd-cfDNA values drawn, the total amount of dd-cfDNA levels actually drawn was 79% of expected based on the protocol (Table E4 in the online data supplement). The interpretation of our results may also be influenced by the presence of verification bias − whereby by the results of the diagnostic test (dd-cfDNA) may affect the decision to perform the "gold standard" procedure (bronchoscopy) in order to verify the test result. The presence of this bias is further supported by the fact that patients with dd-cfDNA levels <1% underwent far less diagnostic testing than those with levels ≥ 1%, as would be expected by the protocol (42 vs 83 total procedures, Table E5 in the online data supplement). This may have resulted in missed episodes of clinically silent ALAD, overestimating the sensitivity and underestimating the specificity of dd-cfDNA for the diagnosis of ALAD.[42] However, as previously stated, dd-cfDNA levels were only designated as stable if the patient did not develop any decline in FEV1, signs/symptoms or a diagnosis of infection or acute rejection over the entire course of the study period with at least 1-month of follow-up (3-month follow up in the sensitivity analysis). In addition, the interpretation of our results may have been influenced by diagnostic review bias − whereby knowledge of the results of the diagnostic test (dd-cfDNA) may influence the interpretation of the "gold standard." This would primarily affect the diagnosis of clinically diagnosed ACR and AMR. However, we did not find any significant difference in dd-cfDNA levels between episodes of rejection diagnosed with or without biopsy confirmation. The time frame between the dd-cfDNA test and further diagnostic testing may have also resulted in disease progression bias, however, most of the diagnoses were made either concurrently or shortly after dd-cfDNA testing (mean 2.86 days). It is notable that only 1 patient from our cohort was assigned infection with COVID-19 pneumonia, perhaps lower than one would expect. While this may be attributable to cases missed by chart review, some of these patients were excluded from phlebotomy during the period of home quarantine and not captured by our analysis. This may warrant future studies assessing the characteristics of dd-cfDNA levels in patients with COVID-19 pneumonia. The time period for follow-up in this study was relatively short. However, we plan to follow this cohort of patients in order to determine the impact of this time period without routine surveillance bronchoscopy on long term outcomes, including CLAD. Given the modest sample size of our cohort, particularly in the sensitivity analysis using all patients who underwent bronchoscopy, our findings of the performance characteristics of dd-cfDNA should be considered exploratory and warrant further evaluation with appropriately designed studies. Furthermore, additional studies thoroughly assessing the differences in dd-cfDNA values and performance characteristics between single vs double lung transplant patients are needed. Lastly, while the number of surveillance bronchoscopies performed in this cohort was substantially lower than would be expected under routine clinical care, future studies should evaluate the cost effectiveness of this approach by weighing the costs of testing vs the costs of performing surveillance bronchoscopy and its associated procedural complications.

In conclusion, we report the findings of the first documented use of dd-cfDNA in the routine clinical monitoring of lung transplant recipients. Dd-cfDNA increased in patients who developed acute rejection and infection and identified episodes of acute rejection and infection that may not have been identified by clinically indicated biopsy alone. This study supports the feasibility of using dd-cfDNA for routine surveillance purposes in a real world

setting and the conduct of further investigation into the impact of dd-cfDNA guided surveillance monitoring on post-transplant outcomes.

Author contributions

MK, and SA-E conceived the study and designed the analysis. JM, IT, PS, DL, SA, and AI recruited and monitored patients. PS, DL, SA and IT adjudicated outcomes. JM, CG, CM, and AV provided substantial contributions to data acquisition. MK, SA-E, and JS performed statistical analysis and compiled results. MK wrote the initial manuscript draft and all authors reviewed the manuscript and revisions.

Disclosure statement

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.healun.2021.12.009.

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