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BRIEF REPORT

Clinical Impact of Noninvasive Plasma Microbial Cell-Free Deoxyribonucleic Acid Sequencing for the Diagnosis and Management of *Pneumocystis jirovecii* Pneumonia: A Single-Center Retrospective Study

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We present 23 cases of *Pneumocystis jirovecii* pneumonia (PCP) diagnosed with commercially available noninvasive plasma microbial cell-free deoxyribonucleic acid (mcfDNA) assay. Our findings suggest that plasma mcfDNA testing resulted in positive clinical impact for the diagnosis and treatment of PCP and coinfections in 82.6% of cases.

Keywords. PCP; Karius; mcDNA; microbial cell-free deoxyribonucleic acid; *Pneumocystis jirovecii* pneumonia.

Since the advent of highly active antiretroviral therapy (HAART) and the use of *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis, the incidence of PCP in both HIV and non-HIV immunocompromised populations (ie, hematological malignancy, transplant recipients) has declined significantly [1–4]. However, PCP remains one of the leading opportunistic infections in the HIV population especially those still undiagnosed with HIV and nonadherent to HAART [1, 2, 5–7]. A recent epidemiological study observed a significant increased incidence of PCP in non-HIV risk groups (ie, solid organ malignancy, rheumatological, and pulmonary diseases) who typically do not receive PCP prophylaxis [4].

The diagnosis of PCP is challenging in non-HIV immunocompromised hosts due to low burden of organisms [8]. Given the lack of reliable sensitivity of conventional diagnostics and potential for coinfections, there is a growing interest in metagenomic next-generation sequencing (NGS) of microbial cell-free deoxyribonucleic acid (mcfDNA) to rapidly and accurately identify *P. jirovecii* and coinfecting pathogens [9–13]. However, current data are limited to studies from China, diagnostic specimens from a respiratory source only or a mixture of blood and respiratory sources, and different NGS platforms [9–13]. Additionally, bronchoscopy is an invasive procedure, and procurement of lower respiratory specimens from bronchoscopy may not be feasible in certain patients. A recent study from the United States reported that noninvasive plasma mcfDNA sequencing has a sensitivity and specificity of 100% and 93.4%, and 48.6% and 99.1%, in proven and proven/probable PCP cases, respectively [14]. However, its clinical impact and role in coinfection detection were not evaluated in that study.

In the present study, we sought to evaluate the clinical impact of positive *P. jirovecii* plasma mcfDNA NGS in the diagnosis and management of PCP and coinfections.

METHODS

This was a retrospective study conducted at OSF St. Francis Medical Center, a 616-bed tertiary academic hospital in Peoria, Illinois, from January 1, 2019, through February 28, 2022. We reviewed the data of all hospitalized patients aged ≥18 years in whom plasma mcfDNA testing was performed during the study period.

All plasma mcfDNA testing was ordered at the request of Infectious Disease physicians at our institution. Plasma specimens were analyzed using the commercially available Karius test (KT; Karius Inc., Redwood City, CA, USA). The validation of the KT has been previously described [15]. Patients were included in this study if (1) *P. jirovecii* mcfDNA concentration met the predefined statistical thresholds; (2) compatible clinical manifestations for PCP such as fever, cough, dyspnea, or hypoxia were present; and (3) there were supportive radiographic findings.

Coinfection with PCP was defined as (1) identification of any non-*Pneumocystis* pathogen above the predefined thresholds from the same KT; and (2) receipt of intervention/management based on this result. Comprehensive record review was performed independently by 2 infectious disease–trained investigators (K.S.F. and A.A.) to determine the clinical impact of KT results according to the predefined criteria (Supplementary Table 1), as previously published [16,17]. A third investigator (M.M.) adjudicated to resolve any disagreement. We further divided patients into non-HIV and HIV groups for comparison.

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Statistical analysis was performed using SPSS, version 23.0 (IBM, Armonk, NY, USA). For descriptive statistics, we used the χ^2 or Fisher exact test for categorical variables and the Mann-Whitney *U* test for continuous variables for non–normally distributed variables. This study was approved by the Institutional Review Board of the University of Illinois.

RESULTS

During the study period, a total of 863 KTs were performed, and 28 (3.2%) of these tests were above the assay threshold for *P. jirovecii* among 23 unique inpatients, with a median (interquartile range [IQR]) of 8059 (321–36 502) molecules per microliter for *P. jirovecii*. Three patients had >1 KT performed. The median turnaround time (from test ordering to result reporting) for KTs (IQR) was 2 (2–3) days, whereas the median duration between hospital admission and Karius result reporting (IQR) was 5 (4–8) days.

PCP was suspected in 16 (69.6%) patients before Karius testing. Seven (30.4%) patients underwent bronchoscopy before Karius testing, and none of the bronchoalveolar lavage (BAL) Grocott methenamine silver (GMS) stain was positive for *P. jirovecii*. Ten patients (43.5%) had HIV infection; of these patients, 6 cases were new HIV diagnosis. HIV patients with PCP were significantly younger (44 vs 61 years; $P = .002$), were predominantly male (70.0% vs 15.4%; $P = .013$), reported Black race (70% vs 0%; $P \leq .001$), had lower CD4 cell counts (17 vs 133 cells/mm^3; $P = .022$), and were more likely to receive empiric PCP treatment before Karius testing (70.0% vs 23.1%; $P = .040$). However, the HIV group with PCP was significantly less likely to have solid organ malignancy (0% vs 46.2%; $P = .019$) and receipt of immunosuppressive therapy or biologic immunomodulators in the 90 days before PCP diagnosis (0% vs 69.2%; $P = .002$). The remaining demographic and clinical characteristics are summarized in Table 1.

Overall, KT results yielded a positive clinical impact in 19 patients (10 were non-HIV, and 9 were HIV). There was no statistical difference in the positive clinical impact observed between the non-HIV and HIV groups. As depicted in Table 2, these positive clinical impacts included confirmation of clinical suspicion of PCP in 13 patients (4 cases had negative BAL GMS stain for *P. jirovecii* before Karius testing), new/earlier diagnosis of PCP in 6 cases (3 cases had negative BAL GMS stain for *P. jirovecii* before Karius testing), initiation of PCP treatment in 11 patients, and potential avoidance of invasive bronchoscopy in 14 cases. Other positive clinical impacts were new/early detection and initiation of antimicrobial treatment for coinfections in 6 and 5 patients, respectively. The coinfections identified by KT included cytomegalovirus, herpes simplex virus, *Histoplasma capsulatum*, *Aspergillus fumigatus*, *Pseudomonas aeruginosa*, *Bacteroides vulgatus,* and Kaposi sarcoma–associated herpesvirus. KT results had no clinical impact in 2 cases, in which both patients underwent bronchoscopy following KT results. The clinical impact of KT results was uncertain in 2 non-HIV patients. None of the KT results were associated with negative clinical impact.

DISCUSSION

Emerging data suggest plasma mcfDNA to be a promising noninvasive diagnostic assay for PCP with high sensitivity and specificity [14]. However, little is known about its clinical impact. In this retrospective study, we demonstrated that *P. jirovecii* plasma mcfDNA testing had a positive clinical impact on the diagnosis and management in 82.6% of patients who had no pre-established diagnosis of PCP through conventional diagnostics. We found that plasma mcfDNA testing confirmed the diagnosis for 68.4% of patients with suspected PCP and led to new/earlier diagnosis of PCP in 31.6% of cases. The PCP diagnosis may have been missed in 7 of our cohort patients when using bronchoscopy with BAL alone. Given the shorter turnaround time and earlier availability of actionable diagnostic results, more than half of our patients were initiated on PCP treatment in a timely manner. Several studies have shown that PCP diagnostic and treatment delays are associated with poorer outcomes [18–20]. Therefore, accurate and rapid diagnosis of PCP through plasma mcfDNA may have significant therapeutic implications.

As demonstrated in this study, we found that plasma mcfDNA testing may have led to avoidance of invasive respiratory sampling through bronchoscopy in 14 cases. Only 2 patients in our cohort underwent bronchoscopy following plasma mcfDNA testing. Although both cases had positive BAL GMS stain for *P. jirovecii*, the BAL result did not change the clinical management. BAL sampling from bronchoscopy remains the reference standard for the diagnosis of PCP. However, bronchoscopy may not be always feasible in certain patient populations and is often not readily available in resource-limited settings [21,22]. Recent data also suggest a heterogeneity in the practice for bronchoscopy across US hospitals [23,24]. Taken together, plasma mcfDNA NGS might prove valuable to diagnose PCP by noninvasive means with the potential to significantly improve patient outcomes. Moreno et al. proposed noninvasive plasma mcfDNA to be an initial diagnostic tool in a PCP testing algorithm [14].

Multiple concurrent infections can occur in immunocompromised populations, making accurate diagnosis of these infections challenging. In our cohort, all patients had immunocompromising conditions attributable to uncontrolled HIV infection, receipt of immunosuppressive therapy (including biologic immunomodulators), or solid organ malignancy. The positive clinical impact of plasma mcfDNA observed in our study was also driven by new/early detection of coinfections in 31.6% of patients and subsequent initiation of antimicrobial therapy in 26.3% of these cases. In addition to the ability to detect viral and bacterial coinfection in our cohort, the rapid

Table 1. Demographic and Clinical Characteristics of Hospitalized Patients With Positive Karius Test Results for *Pneumocystis jirovecii* (n = 23)

Variable	All Patients (n = 23)	Non-HIV (n = 13)	HIV (n = 10)	*P* Value
Demographic				
Age, median (IQR), y	55 (45–62)	61 (55–74)	44 (38–54)	.002
Gender				.013
Female, No. (%)	14 (60.9)	11 (84.6)	3 (30.0)	
Male, No. (%)	9 (39.1)	2 (15.4)	7 (70.0)	
Race				<.001
White, No. (%)	16 (69.6)	13 (100)	3 (30.0)	
African American, No. (%)	7 (30.4)	0	7 (70.0)	
Comorbidities				
Chronic lung disease, No. (%)	10 (43.5)	6 (46.2)	4 (40.0)	.999
Congestive heart failure, No. (%)	7 (30.4)	6 (46.2)	1 (10.0)	.089
Chronic kidney disease, No. (%)	7 (30.4)	6 (46.2)	1 (10.0)	.089
Chronic liver disease, No. (%)	1 (4.3)	1 (7.7)	0	.999
Diabetes mellitus, No. (%)	10 (43.5)	9 (69.2)	1 (10.0)	.010
Potential predisposing factors				
Exposure to systemic corticosteroid 90 d prior admission, No. (%)	10 (43.5)	7 (53.8)	3 (30.0)	.402
Exposure to immunosuppressive therapy or biologic immunomodulators, No. (%)	9 (39.1)	9 (69.2)	0	.002
Hematologic malignancy, No. (%)	2 (8.7)	2 (15.4)	0	.486
Solid organ malignancy, No. (%)	6 (26.1)	6 (46.2)	0	.019
Hematopoietic stem cell transplant, No. (%)	1 (4.3)	1 (7.7)	0	.999
Receipts of PCP prophylaxis before admission	2 (8.7)	0	2 (20.0)	.178
Clinical presentation				
Fever, No. (%)	10 (43.5)	3 (23.1)	7 (70.0)	.040
Cough, No. (%)	16 (69.6)	7 (53.8)	9 (90.0)	.089
Shortness of breath, No. (%)	17 (73.9)	8 (61.5)	9 (90.0)	.179
Duration of symptoms before admission, median (IQR), d	7 (3–21)	14 (6–26)	3 (3–21)	.285
Extreme vital signs				
Temperature, median (IQR), °F	101.1 (98.3–102.2)	99.2 (98.3–101.7)	100.9 (98.0–102.4)	.308
Respiratory rate, median (IQR), breaths/min	27 (23–32)	24 (21–31)	28 (25–32)	.184
Peripheral oxygen saturation, median (IQR), %	90 (84–94)	88 (81–92)	94 (85–95)	.162
Heart rate, median (IQR), beats/min	118 (108–135)	118 (103–131)	117 (108–135)	.952
Oxygen supplementation				.139
None, No. (%)	3 (13.1)	1 (7.7)	2 (20.0)	
Nasal cannula, No. (%)	5 (21.7)	1 (7.7)	4 (40.0)	
Noninvasive mechanical ventilation, No. (%)	9 (39.1)	6 (46.2)	3 (30.0)	
Invasive mechanical ventilation, No. (%)	6 (26.1)	5 (38.4)	1 (10.0)	
Septic shock, No. (%)	5 (21.7)	4 (30.8)	1 (10.0)	.339
ICU admission, No. (%)	13 (56.5)	8 (61.5)	5 (50.0)	.685
Laboratory value				
White blood cells, median (IQR), cells/mm^3	7.72 (4.62–12.81)	7.72 (5.00–13.27)	7.89 (4.42–12.38)	.779
Absolute lymphocytes, median (IQR), cells/mm^3	0.61 (0.28–0.82)	0.56 (0.16–1.28)	0.64 (0.39–0.82)	.596
CD4 cell count, median (IQR), cells/mm^3	25 (5–96)	133 (25–238)	17 (4–25)	.022
LDH, median (IQR), U/L	528 (387–722)	521 (395–644)	548 (379–917)	.728
Positive beta-D-glucan (n = 18)				.600
60–500 pg/mL	6 (33.3)	5 (41.7)	1 (16.7)	
> 500 pg/mL	12 (66.7)	7 (58.3)	5 (83.3)	
Radiographic findings				.079
Ground-glass opacities, No. (%)	20 (87.0)	12 (92.3)	8 (80.0)	
Consolidations or nodules, No. (%)	15 (65.2)	9 (69.2)	6 (60.0)	
Cavitation, No. (%)	2 (8.7)	1 (7.7)	1 (10.0)	
Clinical suspicion for PCP before Karius testing, No. (%)	16 (69.6)	8 (61.5)	8 (80.0)	.405
Bronchoscopy with BAL before Karius testing, No. (%)	7 (30.4)	4 (30.8)	3 (30.0)	.999
Positive BAL GMS stain for *P. jirovecii*, No. (%)	0	0	0	
Empiric treatment for PCP before Karius testing, No. (%)	10 (43.5)	3 (23.1)	7 (70.0)	.040
Length of stay, median (IQR), d	11 (7–24)	9 (6–12)	12 (7–13)	.904
Death, No. (%)	8 (34.8)	7 (53.8)	1 (10.0)	.074

Abbreviations: BAL, bronchoalveolar lavage; GMS, Grocott methenamine silver; ICU, intensive care unit; IQR, interquartile range; LDH, lactate dehydrogenase; PCP, *Pneumocystis jirovecii* pneumonia.

Table 2. Distribution of Reasons for Positive Clinical Impact From Karius Test Results Among 19 Patients

Reason for Positive Clinical Impact	All Patients (n = 19)	Non-HIV (n = 10)	HIV (n = 9)	*P* Value
Confirmed suspected PCP, No. (%)	13 (68.4)	6 (60.0)	7 (77.8)	.629
Led to a new/earlier diagnosis of				
PCP, No. (%)	6 (31.6)	4 (40.0)	2 (22.2)	.629
Coinfection, No. (%)	6 (31.6)	2 (20.0)	4 (44.4)	.350
Avoided invasive bronchoscopy, No. (%)	14 (73.7)	8 (80.0)	6 (66.7)	.629
Led to the timely initiation of antimicrobial therapy for				
PCP, No. (%)	11 (57.9)	8 (80.0)	3 (33.3)	.070
Coinfection, No. (%)	5 (26.3)	2 (20.0)	3 (33.3)	.629
Led to appropriate de-escalation of antimicrobial therapy, No. (%)	1 (5.3)	0	1 (11.1)	1
Led to appropriate discontinuation of antimicrobial therapy, No. (%)	7 (36.8)	3 (30.0)	4 (44.4)	.650

Abbreviation: PCP, *Pneumocystis jirovecii* pneumonia.

detection of unusual and unexpected fungal pathogens such as *Histoplasma capsulatum* and *Aspergillus fumigatus* by plasma mcfDNA without resorting to conventional diagnostic methods with high turnaround times or invasive procedures was worth noting. Previous studies also observed similar diagnostic benefits of plasma mcfDNA over conventional microbiology diagnostics in detecting clinically significant polymicrobial infections, especially among immunocompromised patients [25,26].

The high clinical impact of plasma mcfDNA observed in this study is likely due to restriction of test ordering to infectious disease physicians and resultant patient selection with a high pretest probability of infections. This further highlights the important role of infectious disease expertise in diagnostic stewardship through judicious test ordering, careful interpretation of the results of plasma mcfDNA, and guidance on subsequent management decisions. Although the findings in this study are promising, there are several limitations. First, this is a retrospective case series study conducted in a single health system. Our findings are limited by a small sample size of 23 patients. We were unable to conclude that difference in clinical impact of plasma mcfDNA did not exist between the HIV and non-HIV groups. Second, we did not include patients with suspected PCP with a negative *P. jirovecii* plasma mcfDNA or patients in whom PCP was confirmed through conventional diagnostics who had a negative *P. jirovecii* plasma mcfDNA. As a result, this could contribute to the overall high positive clinical impact of KT observed in our study. Lastly, interpretation of clinical impact is subjected to ascertainment bias, which may overestimate the positive impact in our study.

In conclusion, unbiased plasma mcfDNA assay could potentially offer a noninvasive and rapid diagnosis of PCP and coinfections with actionable and clinically impactful results. However, KT is often performed in situations with clinical equipoise, and, therefore, the overall positive clinical impact of KT would likely be much lower if conducted for all patients with suspected PCP. Future multicenter prospective studies with a larger number of patients are needed to validate our findings, to further explore the clinical impact of positive *P. jirovecii* plasma mcfDNA between HIV vs non-HIV patients, and to determine the effect of clinical outcomes on cost, length of hospital stay, and mortality.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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