Clinical Utility and Impact of the Metagenomic Microbial Plasma Cell-Free DNA Next-Generation Sequencing Assay on Treatment Decision: A Single-Center Retrospective Study

Akira Shishido (ashishido@som.umaryland.edu)
University of Maryland Medical Center

Myint Noe
Lee Health

Kapil Saharia
University of Maryland School of Medicine

Paul Luethy
University of Maryland School of Medicine

Research Article

Keywords: cell-free DNA, transplant infectious disease, next-generation sequencing, Karius ©, sepsis

Posted Date: January 31st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1160543/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Metagenomic next-generation sequencing (mNGS) of microbial cell-free DNA (mcfDNA) allows for non-invasive pathogen detection from plasma. However, there is little data describing the optimal role for this assay in real-world clinical decision making.

Methods

We performed a single-center retrospective cohort study of adult patients for whom a mcfDNA test was sent between May 2019 and February 2021. Clinical impact was arbitrated after review and discussion of each case.

Results

A total of 80 patients were included. The most common reason for sending the assay was unknown microbiologic diagnosis (78%), followed by avoiding invasive procedures (14%). The test had a positive impact in 34 (43%), a negative impact in 2 (3%), and uncertain or no impact in 44 (55%). A positive impact was observed in solid organ transplant recipients (SOTR, 71.4%, p=0.003), sepsis (71.4%, p=0.003), and those receiving antimicrobial agents for less than 7 days prior to mcfDNA testing (i.e., 61.8%, p=0.004).

Conclusion

Clinical utility of mcfDNA testing was highest in SOTR and patients with sepsis. Prolonged antimicrobial use prior to mcfDNA testing limited the utility of the assay. Prospective studies evaluating the utility of mcfDNA assays should be performed to further clarify its role in clinical management.

Key Points

This is a retrospective study evaluating the clinical utility of mcfDNA testing at a single center. mcfDNA positively impacted clinical care in 43% of cases. Patients admitted with sepsis, patients receiving antibiotics for less than 7 days, and solid organ transplant recipients derived the most benefit from mcfDNA testing.

Introduction

Clinical metagenomic next-generation sequencing (mNGS) is an emerging diagnostic modality that comprehensively analyzes all genetic material in a given sample of fluid or tissue.¹ mNGS platforms
sequence millions of small DNA and/or RNA fragments in parallel.\textsuperscript{1} Bioinformatic analyses then match sequences to reference genomes for identification.\textsuperscript{1} While most conventional molecular diagnostic assays target a single or limited number of pathogens, mNGS of microbial cell-free DNA (mcfDNA) allows for broad-range pathogen detection.\textsuperscript{1,2} The Karius© test (Karius, Redwood City, California) emerged in 2016 making mNGS of mcfDNA widely available. The assay amplifies small fragments of mcfDNA and then matches the sequences to a bank of reference genomes that can reportedly identify 1250 bacteria, viruses, fungi and parasites.\textsuperscript{2}

Understandably, a non-invasive assay able to detect multiple pathogens is an attractive prospect for clinicians. However, in comparison to conventional diagnostics, mcfDNA and mNGS are expensive and do not provide antimicrobial susceptibility results. Current data to define the optimal clinical context for the use of the mcfDNA assay is limited. The use of mcfDNA at our institution has been restricted to tertiary testing in scenarios where conventional assays do not provide a diagnosis or situations that require morbid invasive procedures. mcfDNA sequencing has been studied as a complimentary assay in the rapid diagnosis of sepsis, culture-negative endocarditis, pneumonia, invasive fungal infections, brain abscesses and more recently as an adjunct to conventional microbiology cultures in bloodstream infections and prosthetic joint infections.\textsuperscript{2–8} Despite results of recent clinical trials and case reports demonstrating the potential value of mcfDNA as a diagnostic tool, the overall impact of mcfDNA testing on clinical care is less certain. For example, a retrospective cohort study by Hogan and colleagues evaluating the clinical impact of mcfDNA found that despite a positivity rate of 61%, mcfDNA had a positive clinical impact in only 7.3% of cases.\textsuperscript{9} This is in contrast to a study by Rossoff et al. in which 56% of samples sent for mcfDNA provided clinically relevant information.\textsuperscript{10} Given the clinical equipoise, we performed a single center, retrospective study to assess the clinical impact of mcfDNA testing stratified by patient comorbidities, clinical syndromes, days of antimicrobial therapy and indication for testing to identify a context, if any, in which the assay may have the highest clinical utility.

**Methods**

**Ethical Considerations**

The study protocol was approved by the institutional review board (IRB) at University of Maryland, Baltimore (UMB).

**Study Design**

We performed a retrospective cohort study of adult patients for whom a mcfDNA test was sent from our institution between May 2019 and February 2021. We predefined clinical impact categories (Table 1) based on criteria used by Hogan et al. and performed comprehensive record reviews for each case. We included and stratified data by patient co-morbidities, infectious syndromes, duration of antimicrobial therapy prior to mcfDNA testing, reasons for sending the test, and final clinical diagnosis. Cases that fit within multiple predefined categories were included and analyzed in all categories to which they applied.
Clinical impact was arbitrated by all authors based on the actions of the treatment team and patient outcome after review and discussion of each case. The mcfDNA assay was performed by Karius© as described by Blauwkamp et al. 2019.²

Table 1
Clinical Impact Categories and their Predefined Criteria

| Category                  | Definition                                                                 |
|---------------------------|-----------------------------------------------------------------------------|
| Positive                  | Test result led to a new diagnosis when conventional tests were negative.   |
|                           | Test result confirmed clinical diagnosis.                                   |
|                           | Test result led to an earlier diagnosis.                                    |
|                           | Test result negated invasive or costly procedures or tests.                 |
|                           | Test result helped reduce length of hospital stay.                          |
|                           | Test result led to the initiation of appropriate antimicrobial therapy.      |
|                           | Test result led to de-escalation or discontinuation of antimicrobial therapies. |
| Negative                  | Test result led to unnecessary antimicrobial treatment.                     |
|                           | Test result led to unnecessary diagnostic investigation or procedures.      |
|                           | Test result led to an unnecessarily prolonged hospital stay.                |
| Uncertain or No impact    | Test result did not change any clinical management or unable to determine the clinical impact. |

Legend: *Test Result* - result of microbial cell-free DNA assay; *conventional tests* - standard serological, microbiological, and molecular, histopathological and biochemical results.

Statistical Analysis

Categorical variables were summarized using frequency and percentages and continuous variables were summarized using median and quartiles as the variables were non-normal. Comparative analysis of categorical variables was conducted by the Fisher’s exact test or chi-square test as appropriate and continuous data were compared using Mann-Whitney U test. Statistical tests were performed using SPSS (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) with p-values ≤ 0.05 as the significance threshold.

Results

A total of 80 patients had mcfDNA testing over the course of the study period (Table 2). The median age was 54.5 and 60% of the patients were male. The most common reason for sending the assay was unknown microbiologic diagnosis (78%), followed by avoiding invasive procedures (14%), confirmatory
testing (5%) and early diagnosis (3%). Forty-five patients (56%) were immunocompromised (Table 3). The most common immunocompromising condition was hematologic malignancy (27%), followed by solid organ transplantation (26%). Fourteen patients (18%) had prosthetic hardware or grafts, the majority of which were prosthetic heart valves (10%) followed by vascular grafts (7%). The most common clinical syndrome was respiratory failure/pneumonia (31%) followed by sepsis/septic shock (15%). Thirty-seven patients (51%) received more than 7 days of antimicrobial therapy prior to mcfDNA testing.

Table 2
Patient Demographic and Baseline Characteristics

| Total patients | N=80 |
|----------------|------|
| Age (years), median (+/-SD) | 54.5 (15.59) |
| Gender, n (%) |       |
| Female | 32 (40) |
| Male | 48 (60) |
| Reason for mcfDNA Assay, n (%) |       |
| Microbiologic diagnosis unknown | 74 (78) |
| Avoid invasive diagnostic procedure | 13 (14) |
| Confirmatory test | 5 (5) |
| Early diagnosis | 3 (3) |
| Types of Clinical Impact, n (%) |       |
| Negative | 2 (3) |
| Positive | 34 (43) |
| Uncertain or No impact | 44 (55) |
| Consistency with Final Clinical Diagnosis, n (%) |       |
| Yes | 52 (65) |
| No | 25 (31) |
| NA | 3 (4) |

Legend: SD - Standard deviation; NA - Not applicable; mcfDNA - microbial cell-free DNA; Clinical Impact - defined in Table 1.
| Comorbidities                        | n (%) | Uncertain or No impact, n (%) | Positive Impact, n (%) | P-value |
|-------------------------------------|-------|-----------------------------|------------------------|---------|
| Immunocompromised                   | 45 (56) | 24 (53.3) | 21 (46.7) | 0.522 |
| Organ transplant                    | 21 (26) | 6 (28.6) | 15 (71.4) | 0.003 |
| Stem cell transplant                | 3 (4) | 3 (100) | 0 | |
| Solid tumor                         | 2 (2) | 2 (100) | 0 | |
| Hematologic malignancy              | 22 (27) | 15 (68.2) | 7 (31.8) | 0.189 |
| HIV/AIDS                            | 1 (1) | 1 (100) | 0 | |
| Autoimmune disease                  | 3 (4) | 1 (33.3) | 2 (66.7) | |
| Hardware or prosthesis              | 14 (18) | 7 (50) | 7 (50) | 0.593 |
| Vascular graft                      | 6 (7) | 4 (66.7) | 2 (33.3) | 0.691 |
| Prosthetic joint or orthopedic hardware | 1 (1) | 0 | 1 (100) | |
| Mechanical Cardiac Device           | 1 (1) | 0 | 1 (100) | |
| Prosthetic valve                    | 8 (10) | 3 (37.5) | 5 (62.5) | 0.285 |
| Diabetes                            | 13 (16) | 6 (50) | 6 (50) | 0.626 |
| Infectious Syndrome/Clinical Diagnosis | | | | |
| Sepsis/Septic shock                 | 14 (15) | 4 (28.6) | 10 (71.4) | 0.02 |
| Bacteremia                          | 3 (3) | 1 (33.3) | 2 (66.7) | |
| Vascular graft infection            | 7 (7) | 4 (57.1) | 3 (42.9) | 1 |
| Endocarditis                        | 13 (13) | 6 (46.2) | 7 (53.8) | 0.414 |
| Condition                                    | Count (Percentage) | Count (Percentage) | Count (Percentage) | p-value |
|----------------------------------------------|--------------------|--------------------|--------------------|---------|
| Respiratory failure/pneumonia                | 30 (31)            | 14 (48.3)          | 15 (51.7)          | 0.265   |
| Bone/Joint infection                         | 4 (4)              | 3 (75)             | 1 (25)             |         |
| CNS infection (meningoencephalitis)          | 10 (10)            | 7 (77.8)           | 2 (22.2)           | 0.285   |
| Fever unknown origin                         | 10 (10)            | 5 (50)             | 5 (50)             | 0.74    |
| Unexplained leukocytosis                     | 2 (2)              | 1 (50)             | 1 (50)             |         |
| Sinusitis                                     | 1 (1)              | 1 (100)            | 0                  |         |
| Skin and soft tissue infection               | 1 (1)              | 0                  | 1 (100)            |         |
| Others                                        | 1 (1)              | 1 (100)            | 0                  |         |
| Antimicrobial agents administered prior to mcfDNA test | 72 (90)          | 39 (55.7)          | 31 (44.3)          | 1       |
| Less than 7 days                             | 35 (49)            | 13 (38.2)          | 21 (61.8)          | 0.004   |
| More than 7 days                             | 37 (51)            | 26 (72.2)          | 10 (27.8)          | 0.004   |
| No antimicrobial agents prior to mcfDNA test | 8 (10)             | 5 (62.5)           | 3 (37.5)           | 1       |
| Final Diagnosis                              |                    |                    |                    |         |
| Bacterial                                    | 26 (31)            | 15 (60)            | 10 (40)            | 0.661   |
| Fungal                                       | 21 (25)            | 12 (57.1)          | 9 (42.9)           | 0.937   |
| Viral                                        | 10 (12)            | 6 (60)             | 4 (40)             | 1       |
| Non-infectious                               | 28 (33)            | 14 (51.9)          | 13 (48.1)          | 0.555   |
| Days of Hospitalization before sending mcfDNA test, Median (Q1, Q3) | 11.00 (5.00,21.50) | 9.50 (3.00,18.25)  | 0.361   |

Legend: HIV/AIDS: Human immunodeficiency virus/Acquired immunodeficiency syndrome; CNS: Central Nervous System.

† Some patients had more than one comorbidity and clinical syndrome. 2 patients with negative impact were not included in the analysis.
The mcfDNA test result was consistent with the final diagnosis in 65% of cases and had a positive impact in 34 cases (43%), a negative impact in 2 cases (3%), and uncertain or no impact in 44 cases (55%) (Table 2). The only patient characteristic associated with a positive impact from mcfDNA testing was solid organ transplantation (71.4%, p=0.003). Other variables associated with a positive impact from mcfDNA testing in univariate analysis were presence of sepsis (71.4%, p=0.02) and antibiotic duration less than 7 days prior to mcfDNA testing (61.8%, p=0.004). No other patient comorbidities, clinical syndromes or variables analyzed yielded a statistically significant association with positive impact from mcfDNA testing.

Pathogens were identified via mcfDNA test in 49/80 cases. Of the pathogens identified 31% were bacteria, 10% were viruses, and 21% were mold/fungi (Table 3). In cases in which pathogens were identified, 55.1% yielded a positive impact (Table 4). In 31/80 cases, mcfDNA testing yielded a negative result. In 7 cases, the negative test supported a non-infectious etiology of the patient's syndrome and antibiotics were either de-escalated or stopped. Positive impact was driven primarily by mcfDNA test results leading to de-escalation (47%) of antimicrobial therapy (Table 5).

| Result Type                                | Uncertain, No Impact or Negative Impact, n (%) | Positive Impact, n (%) |
|-------------------------------------------|-----------------------------------------------|------------------------|
| Positive with quantitative result         | 19 (45.2%)                                    | 23 (54.8%)             |
| Positive with qualitative result          | 3 (42.9%)                                     | 4 (57.1%)              |
| Negative                                  | 24 (77.4%)                                    | 7 (22.6%)              |
| Did not Meet QC and was not run           | 3                                              | 0                      |

Legend: QC - Quality Control, as specified by Karius©
Table 5

| Reason for positive Impact | N (%) |
|----------------------------|-------|
| Led to new diagnosis       | 6 (17.6) |
| Led to confirmation of diagnosis | 11 (32.4) |
| Led to earlier diagnosis   | 2 (5.9) |
| Avoided invasive procedure | 3 (8.8) |
| Led to appropriate antimicrobials | 6 (17.6) |
| Led to de-escalation of antimicrobials | 16 (47.1) |

Legend: N/A

Discussion

While mNGS is an attractive diagnostic modality for its non-invasive broad-range pathogen detection, its clinical utility and impact on clinical decision-making remains poorly defined. In this single-center retrospective cohort study, we show that the overall clinical utility of mNGS for pathogen identification remains low (43%). Additionally, we identified three contextual factors within our cohort wherein a mNGS testing had a positive impact on clinical decision-making: SOTR (71.4%, p=0.003), sepsis (71.4%, p=0.02) and antimicrobial therapy for fewer than 7 days prior to assay collection (61.8%, p=0.004). The clinical impact was driven primarily by mcfDNA testing leading to de-escalation of antimicrobials and confirming clinical diagnosis (Table 5).

Our finding of 43% overall clinical benefit is higher than prior studies evaluating clinical impact of mNGS for pathogen detection in CSF (3.4%) and plasma (7.3%). This may be due in part because mcfDNA testing is strictly regulated within our institution. All mcfDNA testing is reviewed by an Infectious Disease specialist in consultation with the clinical microbiology director. Therefore, all mcfDNA testing is performed on a narrow, vetted patient population. Additionally, 33% of patients in the study by Hogan et al. had a preestablished microbiological diagnosis through conventional testing, whereas in our study mcfDNA testing was often performed when diagnosis was in question. Lack of clinical impact was most commonly due to identification of a new organism that was not acted upon or confirmation of a conventional result that was not acted upon. In our analysis, we designated mcfDNA confirmation of diagnosis that led to de-escalation of antimicrobials as a positive impact and found this to be the primary driver of clinical benefit. Additionally, our results appear consistent with a similarly executed study by Rossoff et. al that explored the diagnostic capabilities of mcfDNA testing for pediatric infections. While Rossoff et. al did not formally assess clinical outcomes on mcfDNA-based decision-making, they found
that mcfDNA testing netted a higher yield of clinically relevant pathogens in immunocompromised patients (61%) than in immunocompetent patients (35%).

The positive clinical impact observed in our study was largely driven by mcfDNA leading to de-escalation of antimicrobials. This benefit was often derived from situations in which the assay either identified pathogens that were felt to not be clinically relevant, or no pathogen at all (Table 5). While different from our study in its scope, a study by Eichenberger, et al. demonstrated that mcfDNA persisted in plasma well beyond conventional blood cultures in blood stream infections and that persistence was associated with an increased risk of metastatic infection. Taken together, the safe de-escalation or discontinuation of antimicrobials in the context of negative or decreasing levels of mcfDNA may highlight a role for mcfDNA in the realm of stewardship or determining antimicrobial course duration.

The fact that the clinical factors associated with positive clinical impact by mcfDNA testing were SOTR, sepsis and short antimicrobial courses may be due to both host and environmental factors. Immunocompromised patients who lack adequate T-cell responses may present with atypical presentations of infections that are less amenable to detection by conventional cultures. Additionally, these patients often receive broad empiric antimicrobial therapy thus reducing the yield of conventional cultures. Patients with sepsis may also have altered immune system function and receive broad therapy affecting conventional techniques.

That other immunosuppressed populations in our cohort (i.e., stem cell transplant patients) did not display the same level of benefit from mcfDNA testing may be explained by their underrepresentation and small contributing numbers to the overall data as well as standardized algorithmic approach to management. A recent study by Benamu et al. assessed the utility of early mcfDNA testing in patients with neutropenic fever by prospectively obtaining mcfDNA within 24 hours of fever onset. The authors concluded that mcfDNA testing could have allowed earlier optimization of antimicrobials in 47% of patients. While the utility of mcfDNA testing in this study was similar to our overall results, the lack of effect we observed specifically in hematologic malignancy and stem cell transplant patients compared to Benamu et al. may be due to several factors. First, real-world management of febrile neutropenia remains institution-specific and protocol-driven. Antimicrobial de-escalation may not occur even in the presence of identified pathogens. Therefore, the lack of observed utility in our study may be because the primary team did not change management based on the mcfDNA result even if it identified a true pathogen. Second, the prior study did not base utility on clinical outcomes, but instead on an arbitration of whether the mcfDNA result could have made an impact – thereby attenuating any potential algorithmic impact on clinical management results. Third, Benamu et al. collected mcfDNA within 24 hours of fever onset whereas patients in our study often had mcfDNA evaluated weeks into their course. Lastly, stem cell patients were underrepresented in our cohort.

The retrospective nature of the study comes with inherent limitations. Despite the basis of clinical impact of mNGS on the clinical team's management and patient outcome, the retrospective arbitration process to assign utility comes with inherent subjectivity. Additionally, the retrospective nature of the study does not
allow for control of timing of testing and patient and disease characteristics, therefore there was considerable variability among factors and many underrepresented patient populations and diseases. Lastly, the small sample size and retrospective nature allow for only hypothesis-generating conclusions to be made.

In conclusion, while mNGS of mcfDNA is a promising technology for rapid microbial diagnosis, the exact clinical context and utility of the test remains undefined. Our study identifies several factors for which the assay may have a higher likelihood of providing clinical benefit: SOTR, sepsis and patients who have received fewer than 7 days of antimicrobial therapy. Positive clinical impact was driven primarily by de-escalation of antimicrobial therapy suggesting a potential role for mcfDNA in the realm of stewardship. Further studies should explore this relationship and the utility of mcfDNA testing specifically in this context.

Declarations

Ethics approval and consent to participate:

This study protocol was approved by the Human Research Protections Office (HRPO) of the University of Maryland, Baltimore. This study was retrospective in nature and deemed exempt from informed consent by the HRPO. All research performed in this study was done in accordance with the standards set by the HRPO.

Consent for publication:

N/A

Availability of data and materials:

De-identified data are available as supplementary material (Supplementary Table 1) or by request to the corresponding author.

Conflicts of Interest:

The authors have no conflicts of interest to disclose.

Funding:

None

Author Contribution:

Original research performed by all authors. AS drafted original manuscript. MN, PL and KS contributed additional sections and references. All authors reviewed and approved the final manuscript.

Acknowledgements:
Nivya George (Institute of Human Virology, Baltimore, Maryland, USA) aided with statistical analysis. Preliminary analysis of this data was previously presented at IDWeek2021© with abstract published in Open Forum Infectious Diseases.\(^\text{12}\)

**References**

1. Chiu CY, Miller SA. Clinical metagenomics. *Nature Reviews Genetics*. 2019;20(6):341-355. doi:10.1038/s41576-019-0113-7

2. Blauwkamp TA, Thair S, Rosen MJ, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nature Microbiology*. 2019;4(4):663-674. doi:10.1038/s41564-018-0349-6

3. Hong DK, Blauwkamp TA, Kertesz M, Bercovici S, Truong C, Banaei N. Liquid biopsy for infectious diseases: sequencing of cell-free plasma to detect pathogen DNA in patients with invasive fungal disease. *Diagnostic Microbiology and Infectious Disease*. 2018;92(3):210-213. doi:10.1016/j.diagmicrobio.2018.06.009

4. Downey RD, Russo SM, Hauger SB et al. Identification of an Emergent Pathogen, Bartonella vinsonii, Using Next-Generation Sequencing in a Patient With Culture-Negative Endocarditis [published online ahead of print, 2020 Feb 24]. *J Pediatric Infect Dis Soc*. 2020;piaa014. doi:10.1093/jpids/piaa014

5. Marks KM, Kondo M, Dalai SC, et al. Diagnosis and Genotyping of Coxiella burnetii Endocarditis in a Patient with Prosthetic Pulmonary Valve Replacement Using Next-Generation Sequencing of Plasma Microbial Cell-Free DNA. *Open Forum Infectious Diseases*. 2019;6(6):1-4. doi:10.1093/ofid/ofz242

6. Shishido A, Vostal A, Mayer R, Ho Y, Baddley JW. Diagnosis of central nervous system invasive aspergillosis in a liver transplant recipient using microbial cell-free next generation DNA sequencing. 2021;(February):1-6. doi:10.1111/tid.13592

7. Eichenberger EM, de Vries CR, Ruffin F, et al. Microbial Cell-Free DNA Identifies Etiology of Bloodstream Infections, Persists Longer Than Conventional Blood Cultures, and Its Duration of Detection Is Associated With Metastatic Infection in Patients With Staphylococcus aureus and Gram-Negative Bacteremia. *Clin Infect Dis*. 2021;(Online ahead of print). doi:10.1093/cid/ciab742

8. Echeverria AP, Cohn IS, Danko DC, et al. Sequencing of Circulating Microbial Cell-Free DNA Can Identify Pathogens in Periprosthetic Joint Infections. *Journal of Bone and Joint Surgery*. 2021;103(18):1705-1712. doi:10.2106/jbjs.20.02229

9. Hogan CA, Yang S, Garner OB, et al. Clinical Impact of Metagenomic Next-Generation Sequencing of Plasma Cell-Free DNA for the Diagnosis of Infectious Diseases: A Multicenter Retrospective Cohort Study. *Clinical Infectious Diseases*. 2021;72(2):239-245. doi:10.1093/cid/ciaa035

10. Rossoff J, Chaudhury S, Soneji M, et al. Noninvasive diagnosis of infection using plasma next-generation sequencing: A single-center experience. *Open Forum Infectious Diseases*. 2019;6(8). doi:10.1093/ofid/ofz327
11. Benamu E, Gajurel K, Anderson JN, et al. Plasma Microbial Cell-free DNA Next Generation Sequencing in the Diagnosis and Management of Febrile Neutropenia. doi:10.1093/cid/ciab324/6236109

12. Noe M, Shishido AA, Kapil Saharia ;, Luethy P. Clinical Utility and Impact of the Metagenomic Microbial Plasma Cell-Free DNA Next-Generation Sequencing Assay on Treatment Decision: A Single-Center Retrospective Study. *Open Forum Infectious Diseases*. 2021;8(Supplement 1):S432-S433. https://academic.oup.com/ofid/article/8/Supplement_1/S432/6450531

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- ClinicalUtilitymcfDNArawdataV2.xlsx