BENDING THE VALUE CURVE

Cell-Free DNA Sequencing, Pathogen Detection, and the Journey to Value

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A previously healthy 9-month-old girl presented to the emergency department for fever, rash, and decreased urine output for 3 days. She was in compensated shock: temperature of 39.6°C, heart rate of 168 beats per minute, and blood pressure of 130/63. Her examination was notable for a diffuse blanching rash. Laboratory studies showed an elevated procalcitonin of 21.74 ng/mL, normal white blood cell count of 7.90 K/μL, and elevated creatinine of 0.36 mg/dL. Urinalysis demonstrated large blood, negative nitrites, trace leukocyte esterase, and 30 to 49 white blood cells per high-power field. Renal ultrasound was consistent with pyelonephritis. Because of concern for septic shock, she was given 60 mL/kg of normal saline and ceftriaxone and transferred to the PICU.

In the PICU, vancomycin was added on the first day of admission because of worsening clinical status. She required vasopressor support with epinephrine for 2 days. Her initial microbiologic evaluation was unrevealing: urine culture grew 1000 to 9000 colony-forming units of normal flora, blood culture was negative, and respiratory pathogen panel result was positive for rhinovirus. Abdominal ultrasound on hospital day 1 showed developing left perinephric abscesses. Her rash worsened, and she developed lip peeling and conjunctivitis on day 2, concerning for Kawasaki disease or a toxin-mediated process, typically caused by *Staphylococcus aureus* or *Streptococcus pyogenes*. Because it is unusual for either pathogen to cause pyelonephritis in infants, and because the patient had persistent fevers, a blood sample was sent for sequencing of microbial cell-free DNA (cfDNA) on day 3. On day 7, testing resulted with evidence of *S aureus* in the blood. She was switched from vancomycin and ceftriaxone to ceftaroline for methicillin-sensitive and -resistant *S aureus* and Gram-negative bacterial coverage. She defervesced on hospital day 13 and was discharged on hospital day 15 to complete an additional 14 days of linezolid.

INTRODUCTION TO MICROBIAL CFDNA SEQUENCING

Advances in genomic technology have allowed for the detection and sequencing of cfDNA in human plasma, increasing the potential for blood-based diagnoses while avoiding highly invasive procedures.[1] Sequencing of cfDNA has most notably played a role in prenatal diagnosis, with millions of pregnant women already screened for fetal chromosomal disorders.[2] More recently, there has been interest in applying cfDNA sequencing to identify pathogens not detected using routine diagnostic methods. Although blood cultures remain the gold standard for pathogen identification, there are limitations to their use. In children, the sensitivity of a blood culture is limited by smaller blood volumes used for cultures when compared with adults and depends on the blood culture laboratory system, the type of nutrient media, and the causative organism.[3] Time-to-positivity can be long, and the yield can be low if insufficient blood volumes are obtained, especially for fastidious organisms. In addition, blood cultures are

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primarily used for bacterial identification. Although most fungi grow well in conventional blood culture systems, some fungi, such as *Candida glabrata*, *Cryptococcus neformans*, *Histoplasma*, and *Blastomyces*, may take days to weeks to grow.[4] Polymerase chain reaction–based assays remain the gold standard for virus detection[5] because blood cultures are typically not used for viral identification. The limited ability of the conventional blood culture to detect pathogens becomes most apparent in cases of culture-negative severe sepsis, in which patients are critically ill but lack a microbiologically documented infection, a phenomenon accounting for ≤2.5% of all hospitalizations in young children.[6] Without a known etiology, inappropriate or inadequate antibiotic therapy may be used, which is associated with higher morbidity and mortality in children with severe sepsis.[7] Thus, efforts have been undertaken to validate the use of cfDNA sequencing for infectious testing. Analytical and clinical validation of a commercially available cfDNA test was shown to identify potentially pathogenic organisms in >33% of patients whose sepsis etiology was not identified with conventional methods.[8] The sequencing test can identify and quantify cfDNA from >1000 clinically relevant bacteria, DNA viruses, fungi, and parasites, overcoming limitations of the conventional blood culture system.

PEDIATRIC STUDIES

Microbial cfDNA sequencing in pediatrics was initially studied in immunocompromised patients at high risk for serious infections. Researchers in one study suggested that use of cfDNA sequencing would have led to earlier initiation of appropriate treatment in the majority of immunocompromised patients.[9] cfDNA sequencing has also been used to detect fungal pathogens in immunocompromised patients, showing promise in its use to limit prolonged empirical antifungal treatment.[10] In this same study, cfDNA was used to identify viral pathogens in patients with prolonged febrile neutropenia and may have been the primary infectious source. More recently, cfDNA sequencing has been used in immunocompetent patients with prolonged or complicated infections. Researchers in one study examined the utility of cfDNA sequencing in 15 children hospitalized for complicated pneumonia. In this cohort, sequencing identified a pathogen in 13 patients and led to a change in management, specifically narrowing of antibiotics, for 7 patients.[11]

LIMITATIONS OF MICROBIAL CFDNA SEQUENCING

Although cfDNA sequencing has been demonstrated to improve the ability to initiate appropriate therapy and reduce the use of unnecessarily broad-spectrum antimicrobial agents, it is not without limitations. Bacterial identification by sequencing does not imply that the bacteria are pathogenic or causing an infection. The current paradigm of infection is one that is developed through the lens of cultivatable bacteria. The use of the standard urine culture, for example, has perpetuated the idea of urine sterility as the majority of urine cultures taken from asymptomatic people will be negative. However, by using the expanded quantitative urine culture, in which a larger volume of urine is analyzed under varying conditions, many more bacterial species can be grown from urine from asymptomatic people.[12,13] Similarly, freshly drawn blood from 62% of healthy volunteers had cultivatable bacteria.[14] With these data, we call into question notions of sterility of both blood and urine, making the differentiation between pathogenic and commensal organisms difficult. Indeed, researchers in one study looked at 16S rRNA sequencing of blood in both healthy adults and those with sepsis and found that the amount of microbial genetic material was the same between the 2 groups, although the composition and diversity of the material differed.[15]

A second limitation to sequencing tests is their reliance on DNA, which is present in both living and nonviable organisms, including bacteria, viruses, fungi, and parasites.[16] Therefore, DNA fragments may be present within the blood that may not be reflective of an active infection and, in the case of viruses, could signify reactivated viruses that may be falsely identified as the cause of symptoms or disease. The inability to determine if, where, or, more importantly, when these organisms were living is a major drawback to these techniques.

FINDING VALUE

Although cfDNA sequencing is less expensive than invasive procedures required to obtain cultures from sites of infection (ie, biopsies, bronchoalveolar lavage), commercially available cfdNA sequencing remains costly (thousands of dollars). For patients with prolonged hospital stays due to complicated illnesses of unknown etiology, pathogen identification could lead to management change and quicker illness resolution, reducing hospital length of stay. In these patients, such as the introductory case, the cost of cfDNA testing may be justified. Although *S aureus* is a common cause of perinephric abscesses,[17] it is an unusual uropathogenic organism in otherwise healthy infants. cfDNA testing provided valuable information in identifying a causative organism and led clinicians not to treat for Kawasaki disease. Without knowing the etiology of the infection, she may have completed a prolonged course of multiple intravenous antibiotics or been treated for Kawasaki disease with intravenous immunoglobulins, which has known adverse effects.[18]

The potential for cfDNA testing to be of value to pediatric hospitalists and other clinicians is promising. However, the increasing popularity of such a sensitive test carries with it the inherent risk of overdiagnosis, misdiagnosis, and questionable value. In a recent study, researchers demonstrated that cfDNA sequencing made no impact in management for nearly 87% of patients.[19] However, they also report that <4% of patients in their cohort received unnecessary treatments or diagnostic investigations or had a longer length of stay as a result of the cfDNA test. Among the patients in whom it was concluded that no impact was made, the conventional microbiologic diagnosis was confirmed by cfDNA sequencing, and antimicrobial therapy remained the same for over a quarter of the patients. Therefore, it is possible the confirmatory test prevented

broadening or prolonging of antimicrobial treatment, limited unnecessary invasive testing, and perhaps led to earlier discharge. Similar concerns regarding testing that lead to no management changes have been raised with multiplex molecular-based panels used to detect pathogens in respiratory secretions, stool, and cerebrospinal fluid; current efforts seek to either limit the use of, guide the interpretation of, or identify scenarios in which these tests add value.[20–25]

There are, however, advancements in research and implementation of cfDNA testing that could increase its value. cfDNA sequencing is not done within the majority of clinical laboratories, necessitating that these tests are sent to other facilities. Although the process of sequencing thousands of pathogens is rapid, with results generated within 24 to 48 hours of specimen receipt, in-house cfDNA sequencing test would reduce the turnaround time of test results that could more readily inform real-time clinical decision-making, thus providing future value to relevant patient populations.

A more thorough understanding of negative cfDNA testing results and the resultant implications for clinical care may expand the relevance of this technology to non-high-risk populations. The majority of studies involving cfDNA testing have investigated clinical scenarios in which clinicians already have high suspicion for bacteremia. However, in 1 study, of the 27 patients with previously confirmed presence of pathogenic microorganisms detected in the blood, 41% of patients had negative cfDNA testing,[19] raising questions about sensitivity and how antimicrobial treatment before cfDNA testing affects results. Expansion of research focusing on addressing the negative predictive value of such testing will be essential, particularly as such testing becomes more widely used.

CONCLUSIONS

As more research is being performed to validate the application of microbial cfDNA sequencing in pediatrics, pediatric hospitalists need to be aware of the potential increase in the use of sequencing tests. Although it is tempting to incorporate such a sensitive and broad-reaching test into clinical practice, there remain the possibilities for overdiagnosis and treatment of nonclinically relevant pathogens, or for testing results that require no change in management, leading to low-value care. Clinicians should practice restraint in ordering cfDNA testing while efforts to guide the appropriate implementation and interpretation of cfDNA testing are underway and until robust clinical evidence is available to guide and support the real-time use of cfDNA testing. In the meantime, there exists a growing opportunity for clinical and translational scientists to learn how cfDNA testing could best improve the outcomes for patients.

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