Noninvasive Diagnosis of Pulmonary Infection in Pediatric Bone Marrow Transplant Patients Using Microbial Cell-Free DNA

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Abstract

Pediatric hematopoietic stem cell transplant patients are highly immune compromised with a significantly increased risk for severe pulmonary infections. In these two case reports, we review the use of plasma microbial cell-free DNA next generation sequencing, also known as the Karius Test (KT), as a non-invasive diagnostic tool. These cases illustrate how the KT can provide early species-level identification of pathogens for patients that are either unable to tolerate sedated bronchoscopy, or those with negative bronchoalveolar lavage due to heavy antibiotic pre-treatment. These two clinical cases illustrate how use of KT can reduce infection-related morbidity and mortality.

INTRODUCTION

Pediatric hematopoietic stem cell transplant (HSCT) is associated with increased risk for severe pulmonary infections. Advances in non-invasive diagnostics can potentially reduce infection-related morbidity and mortality by providing an earlier and more accurate diagnosis. Plasma microbial cell-free DNA (mcfDNA) next generation sequencing (NGS), also known as the Karius Test (KT), is an open-ended assay that can detect >1400 DNA pathogens. This test has shown feasibility and utility in diagnosing infections in adult and pediatric immunocompromised hosts[1, 2] and can assist in the detection – and even the prediction – of sepsis in immunocompromised pediatric patients[3].

In this report, we describe two patients with suspected pneumonia where conventional testing was not possible or failed to identify the offending organism. The use of KT provided informative data that tailored treatment, which ultimately led to resolution of the pulmonary infection.

PATIENT 1.

A 9 yo male with multiply relapsed acute lymphoblastic leukemia, who had previously undergone matched related HSCT and chimeric antigen receptor (CAR) T cell therapy, presented for evaluation of fever, cough and anorexia. He last received inhaled pentamidine for *Pneumocystis jirovecii* pneumonia (PJP) prophylaxis 5 months earlier.

Vital signs on admission were notable for fever and tachycardia, but no hypoxia. He was initially non-toxic appearing with a normal cardiopulmonary exam. Labs were obtained (see Table 1) and he was treated with empiric piperacillin-tazobactam. He developed increasing cough and oxygen requirement within 24 hours. Chest computed tomography (CT) scan revealed bilateral diffuse ground-glass opacities (Figure 1A), prompting addition of vancomycin and liposomal amphotericin B. Flexible bronchoscopy with bronchoalveolar lavage (BAL) failed to reveal an infectious etiology. Of note, KT collected shortly after bronchoscopy showed a strong *Pneumocystis jirovecii* signal (5049 MPM). Intravenous trimethoprim-sulfamethoxazole (TMP/SMX)

was initiated at treatment dosing (15 mg/kg/day) that was converted to oral dosing after 3 days. He weaned off supplemental oxygen, and successfully completed a three week course of TMP/SMX.

PATIENT 2.

A hydropic neonate of 32 weeks gestational age was found to have severe anemia (Hgb 2 g/dl) and pancytopenia at birth (see Table 1). A bone marrow aspirate at seven days of life revealed severe hypoplasia. She was ventilator-dependent for the first two weeks of life. Due to her severely immune compromised state, she received prophylactic antimicrobials including IV micafungin, piperacillin-tazobactam, Pentamidine and intravenous immunoglobulin. She also required regular transfusion support with platelets and packed red blood cells. Over the ensuing three months she developed bacteremia with *S. haemolyticus* on multiple occasions (detected both by KT and blood culture) that responded to vancomycin. Surveillance of infection with KT to follow *S. haemolyticus* on day +74 of life coincidentally revealed a positive signal for *Aspergillus calidoustus* (65 MPM). To further investigate, a chest CT scan revealed a right middle lobe consolidation with a ring enhancing lesion measuring 0.6 x 0.7 cm (Figure 1B). This prompted discontinuation of micafungin and initiation of liposomal amphotericin B (5 mg/kg/day). Repeat KT two weeks later revealed a rising signal for *Aspergillus calidoustus* , and granulocyte transfusions were thus initiated. After six weeks of liposomal amphotericin B and three weeks of daily granulocyte transfusions, imaging studies demonstrated shrinkage of the right lung mass with negative KT testing. Shortly thereafter, the patient underwent reduced intensity immunoablative conditioning followed by a matched unrelated HSCT. She engrafted with an absolute neutrophil count > 500/ul at 10 days post HSCT, and liposomal amphotericin B was discontinued three weeks later. She remains healthy two years post HSCT.

DISCUSSION

Pulmonary infections are a significant cause of morbidity in immunocompromised pediatric patients. Clinical symptoms are often non-specific, presenting a diagnostic challenge that complicates therapeutic intervention. Host immune responses to common childhood respiratory viruses and opportunistic pathogens [4] [5, 6] are often attenuated or absent. As such, a need exists for diagnostic tools that can rapidly identify the etiology of invasive infections in this vulnerable population.

Non-invasive radiological testing (CXR or CT scans) can help define general patterns of pulmonary infiltrates, but are non-specific and may underestimate the extent of disease [7, 8] . Blood cultures from patients with suspected pneumonia identify causative pathogens in only 0.5%-14% of cases [9, 10] . In cases where fungal infections are suspected, serum markers such as (1-3) B-D glucan (BG) and galactomannan (GM) are often examined. Galactomannan is a major cell wall component of Aspergillus with variable sensitivity as well as cross-reactivity with Fusarium, Blastomyces, Histoplasma and Cryptococcus [11]. A recent study examined the performance of the Fungitell BG and GM assays on cases of confirmed invasive aspergillosis. While the BG assay was more sensitive in detecting Aspergillus (81% vs. 49%), the GM assay was found to be more specific (97% vs 82%)[12]. BG is also known to produce false positive results, particularly in patients on antibiotics (cefepime, trimethoprim-sulfamethoxazole and ampicillin-sulbactam) or in those on hemodialysis or receiving blood products filtered through cellulose filters [13, 14].

Invasive diagnostic studies include flexible bronchoscopy with BAL or lung biopsy. Bronchoscopy is generally a well-tolerated procedure with <0.1% mortality, but carries risk for airway trauma, bronchospasm, pneumothorax or even respiratory failure [15]. The yield of clinically useful information from this procedure varies widely in HSCT patients who often have been pre-treated with broad spectrum antimicrobials, increasing the chances of false negative results [8]. Multiple retrospective studies examining the diagnostic utility of BAL in HSCT patients showed the causative organism identified in 25-67%% of cases [16-19] . Lung biopsy may be considered the gold standard for pathogen identification in adults, but is more problematic in immunocompromised children and associated with significant morbidity [20, 21]. PJP is a major cause of preventable morbidity and mortality in immune compromised patients. The individual described in case #1 likely developed PJP secondary to a lapse in chemoprophylaxis. Without appropriate prophylaxis, up to 25% of pediatric oncology patients develop PJP [22] . Because *P. jirovecii* is difficult to culture *in vitro*

, diagnosis historically relies on microscopic examination of BAL fluid. However, staining for *P. jirovecii* may be falsely negative in patients with low disease burden, with some advocating the use of PCR to aid in diagnosis [23].

In both of the clinical cases outlined above, KT identified pathogens that permitted targeted treatment. This non-invasive diagnostic test has demonstrated sensitivity of 98% and specificity of 100% in cases of febrile neutropenia with positive blood cultures [24]. There is also evidence to suggest that KT can identify pathogens in immunocompromised patients where suspicion for infection is generally higher. Looking at a pediatric hematology oncology patient population, NGS testing had a higher yield, with 61% testing positive for clinically relevant pathogens vs. 35% in non-immunocompromised patients. In a study of 33 pediatric febrile neutropenia patients, plasma NGS testing identified a pathogen in 4 out of 6 patients with proven invasive fungal infection, allowed identification of PJP in a patient with presumed invasive fungal infection based on positive B-D glucan, Candida glabrata in one patient and alternative fever sources in another 16 patients [25]. The coincidental detection of emerging invasive fungal infection before it was clinically evident in case #2 is compelling. It suggests that mcfDNA NGS surveillance may hold promise in predicting severe life-threatening infections in an at-risk population, similar to the prediction of sepsis illustrated by Goggin, Wolf and colleagues[26] In instances where cultures and serologic markers may be unreliable, KT can provide species level identification of pathogens missed by conventional testing methods. However, results must be interpreted with caution as such assays have potential to detect translocating enteric organisms and cannot identify precisely which organ(s) is/are infected. In addition, only DNA-containing microbes will be identified, thus limiting its effectiveness in detecting RNA viral infections.

In conclusion, pediatric HSCT patients have an elevated risk for pulmonary infection and continue to present challenges regarding precise and timely diagnosis. Such patients undergo extensive testing, and are often treated empirically for a wide array of potential pathogens. Our cases highlight the value of non-invasive plasma NGS to enhance real-time clinical decision making.

Conflict of Interest

FG is on the Advisory Board for Karius. LS and SM have no conflict of interest

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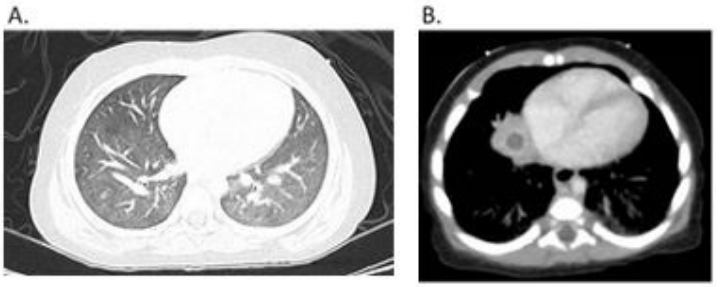
Figure 1.



A. CT chest from patient 1 exhibiting bilateral centrally distributed ground glass opacities. B. CT chest of patient 2 showing right middle lobe consolidation with ring enhancing lesion.

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