Evaluation of Plasma Microbial Cell-Free DNA Sequencing to Predict Bloodstream Infection in Pediatric Patients With Relapsed or Refractory Cancer

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**IMPORTANCE** Bloodstream infection (BSI) is a common, life-threatening complication of treatment for cancer. Predicting BSI before onset of clinical symptoms would enable preemptive therapy, but there is no reliable screening test.

**OBJECTIVE** To estimate sensitivity and specificity of plasma microbial cell-free DNA sequencing (mcfDNA-seq) for predicting BSI in patients at high risk of life-threatening infection.

**DESIGN, SETTING, AND PARTICIPANTS** A prospective pilot cohort study of mcfDNA-seq for predicting BSI in pediatric patients (<25 years of age) with relapsed or refractory cancers at St Jude Children's Research Hospital, a specialist quaternary pediatric hematology-oncology referral center. Remnant clinical blood samples were collected during chemotherapy and hematopoietic cell transplantation. Samples collected during the 7 days before and at onset of BSI episodes, along with negative control samples from study participants, underwent blinded testing using a mcfDNA-seq test in a Clinical Laboratory Improvement Amendments/College of American Pathologists–approved laboratory.

**MAIN OUTCOMES AND MEASURES** The primary outcomes were sensitivity of mcfDNA-seq for detecting a BSI pathogen during the 3 days before BSI onset and specificity of mcfDNA-seq in the absence of fever or infection in the preceding or subsequent 7 days.

**RESULTS** Between August 9, 2017, and June 4, 2018, 47 participants (27 [57%] male; median age [IQR], 10 [5-14] years) were enrolled; 19 BSI episodes occurred in 12 participants, and predictive samples were available for 16 episodes, including 15 bacterial BSI episodes. In the 3 days before the onset of infection, predictive sensitivity of mcfDNA-seq was 75% for all BSIs (12 of 16; 95% CI, 51%-90%) and 80% (12 of 15; 95% CI, 55%-93%) for bacterial BSIs. The specificity of mcfDNA-seq, evaluated on 33 negative control samples from enrolled participants, was 82% (27 of 33; 95% CI, 66%-91%) for any bacterial or fungal organism and 91% (30 of 33; 95% CI, 76%-97%) for any common BSI pathogen, and the concentration of pathogen DNA was lower in control than predictive samples.

**CONCLUSIONS AND RELEVANCE** A clinically relevant pathogen can be identified by mcfDNA-seq days before the onset of BSI in a majority of episodes, potentially enabling preemptive treatment. Clinical application appears feasible pending further study.

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Serious infections, especially bloodstream infections (BSIs), are among the most important complications affecting patients receiving treatment for cancer. An incident of BSI-related sepsis can cause death,1,3 multiorgan failure, and neurocognitive damage.4,6 Although a predictive test that enables preemptive, pathogen-directed therapy could reduce BSI-related morbidity and mortality, no validated test is available. Novel metagenomic microbiologic diagnostics, including plasma microbial cell-free DNA sequencing (mcfDNA-seq), show promise as diagnostic tests,7–9 but none has yet been systematically evaluated for BSI prediction.3 This prospective pilot study tested the novel hypothesis that mcfDNA-seq can identify a causative pathogen in the days before BSI develops.

### Methods

#### Study Design and Ethics

This study was approved by the St Jude Children’s Research Hospital Institutional Review Board and followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

#### Participants

Participants were pediatric patients receiving treatment for relapsed or refractory cancer at St Jude Children’s Research Hospital. Informed consent and assent for participation were obtained. Participation continued until death, loss to follow-up, transfer of care, resolution of gastrointestinal graft-vs-host disease, 30 days after hematopoietic cell transplantation, or participant request.

#### Clinical Data and Definitions

The Centers for Disease Control and Prevention’s National Healthcare Safety Network definitions were used for BSI, with onset defined at the time of collection of the first positive blood culture.10 Institutional practice is to collect blood cultures in all episodes of fever or suspected infection. The a priori predictive period comprised the 3 days before BSI onset (eFigure 1 in Supplement 1). Negative control samples were obtained from participants on a day for which no fever or infection was documented within the prior or subsequent 7 days. Participants could contribute multiple BSI and negative control episodes.

#### Laboratory Procedures

Leftover blood was available from most samples collected for clinical hematology studies and was stored at 4°C until processed to plasma and frozen. Plasma mcfDNA-seq was performed in a Clinical Laboratory Improvement Amendments/College of American Pathologists-accredited laboratory (Karius Inc, Redwood City, California) as previously described.11 Briefly, cell-free DNA was extracted, DNA libraries were prepared, and sequencing was performed. Nonhuman sequencing reads were aligned to a curated pathogen database, and the concentration of pathogen-specific DNA fragments for each organism was reported in molecules per microliter (MPM). Available samples collected up to 7 days before each BSI episode were tested alongside 2 negative control samples per episode. The laboratory was blinded until results were finalized.

#### Power and Statistical Considerations

The Simon 2-stage design was used, with minimal acceptable sensitivity of 30% and favorable sensitivity of 50%.12 This pilot reports the preplanned first stage analysis.

Predictive sensitivity was estimated for the 3-day predictive period and for each of the 7 days before BSI onset. Diagnostic sensitivity was estimated from samples collected on day of BSI onset. Predictive sensitivity was defined as the proportion of episodes for which mcfDNA-seq identified the same organism subsequently identified in blood culture. Logical derivation was used to impute missing values for sensitivity (eFigure 2 in Supplement 1). Overall sensitivity was estimated for different models of testing frequency.

Specificity was defined as the proportion of negative control samples for which no bacterial or fungal organisms were identified by mcfDNA-seq. Specificity for common BSI pathogens, genera comprising 1% or more of central line–associated BSI in the Children’s Hospital Association Childhood Cancer & Blood Disorders Network BSI database,13 was an additional ad hoc measure (eTable 1 in Supplement 1). A third-degree penalized B-spline curve was used to analyze temporal trends in pathogen-specific DNA concentration. Data analysis was performed using SAS, version 9.4 (SAS Institute Inc).

#### Results

#### Population and BSI Episodes

Between August 9, 2017, and June 4, 2018, 47 participants were enrolled (Table). Nineteen BSI episodes occurred in 12 participants (3.3 per 1000 patient-days; 95% CI, 2.5–5.2 per 1000 patient days) (eTable 2 in Supplement 1). Eight episodes (42%) were associated with signs of sepsis, including hypotension (n = 3), requirement for urgent intervention (n = 5), or intensive care unit admission (n = 2). A predictive period sample was available in 16 episodes. Broad-spectrum antibacterial therapy was administered during the prior week in 17 of 19 (89%) episodes, so a comparative subgroup analysis of the effect of pretreatment was not feasible.

#### Sensitivity of mcfDNA-seq

The BSI pathogen was identified by mcfDNA-seq during the predictive period in 12 of 16 BSI episodes (predictive sensitivity,
75%; 95% CI, 51%-90%) and in 12 of 15 bacterial BSI episodes (predictive sensitivity, 80%; 95% CI, 55%-93%). Diagnostic sensitivity was 83% (15 of 18; 95% CI, 61%-94%); diagnostic sensitivity for bacterial BSI was 88% (15 of 17; 95% CI, 66%-97%). Blood cultures were collected during the week before BSI in 10 episodes (eTable 4 in Supplement 1).

Daily predictive sensitivity of mcfDNA-seq for BSI is shown in Figure 1 and eFigure 4 in Supplement 1. Pathogen-specific DNA concentrations typically increased in the days approaching BSI onset (Figure 2 and eTable 3 and eFigure 3 in Supplement 1). Assuming same-day results, projected median predictive sensitivity for bacterial BSI was 71% for twice-weekly testing (eTable 5 and eFigures 5 and 6 in Supplement 1).

Specificity of mcfDNA-seq
Thirty-three negative control samples obtained from study participants underwent mcfDNA-seq testing. (eTable 6 in Supplement 1); 27 of 33 had no bacterial or fungal organism identified (specificity, 82%; 95% CI, 66%-91%) and 30 of 33 had no common BSI pathogen identified (specificity, 91%; 95% CI, 76%-97%). The concentration of bacterial DNA in negative control samples was typically lower than in predictive samples, with a maximum of 609 MPM for any bacteria and a maximum of 112 MPM for common BSI pathogens compared with higher than 609 MPM in 11 of 16 predictive episodes (69%; 95% CI, 44%-86%) and higher than 112 MPM in 12 of 16 episodes (75%; 95% CI, 51%-90%).

Additional bacteria, including common pathogens, were identified by mcfDNA-seq in many samples collected before BSI episodes (eTable 7 in Supplement 1). We attempted to assess the clinical significance of these, but all identified bacteria were potentially susceptible to empirical antimicrobial therapy, so treatment failure associated with untreated organisms was not evaluable. Fungal DNA was also identified by mcfDNA-seq in 2 participants with and 1 participant without evidence of invasive fungal infection.

Discussion
This prospective pilot study shows that mcfDNA-seq has the potential to predict most episodes of BSI before onset in high-risk pediatric cancer patients. The estimated predictive sensitivity of 75% (95% CI, 51%-90%) exceeds the predefined favorable value of 50%, which was chosen because it represents the approximate efficacy of antibacterial prophylaxis.14,15 In addition to BSI organisms, viruses and invasive fungi that infect immunocompromised patients were also detected. Further studies are needed to determine whether mcfDNA-seq can
reliably predict infection with nonbacterial pathogens in this patient population.

Limitations
This study does have important limitations. A specificity of 82% would make mcfDNA-seq screening impractical because of the high false-positive rate; specificity might be improved by excluding uncommon BSI pathogens, applying quantitative break points, or performing short interval repeat testing.7,8 Return of mcfDNA-seq results in a time frame that allows implementation of screening may not yet be feasible in most centers. Technological and practical advances will be required to reduce turnaround time considerably and might also improve sensitivity to predict BSI even earlier. Application of mcfDNA-seq screening to febrile neutropenia and clinically documented and other microbiologically documented infections was not evaluated in this study and will be important for future studies. Once these challenges are overcome, implementation of predictive mcfDNA-seq has the potential to significantly reduce treatment-related morbidity and mortality of pediatric cancer patients and could potentially be applied to other immunocompromised patient populations.

Conclusions
We provide to our knowledge the first evidence that mcfDNA-seq can predict infections in approximately 75% of relapsed pediatric cancer patients with impending BSI with a specificity of more than 80%. Strategic implementation and continued technological advancements may enable the use of mcfDNA-seq to guide preemptive therapy and reduce infection-related morbidity and mortality in high-risk immunocompromised patients.

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