Abstract #2084

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Performance of the Karius Plasma Next Generation Sequencing Test in Determining the Etiologic Diagnosis of Febrile Neutropenia: Results from a Pilot Study

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ABSTRACT

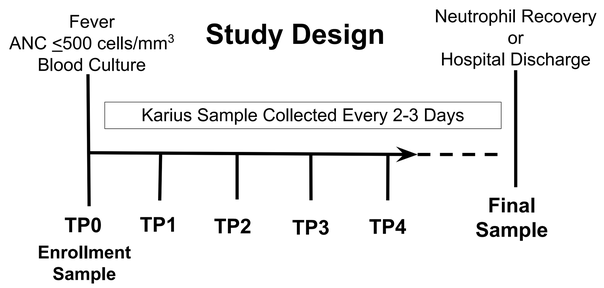
Background:
Blood cultures (BC) fail to detect a pathogen in most patients with neutropenic fever (NF). We examined the performance of the Karius plasma next generation sequencing (NGS) test compared to that of BC in chemotherapy-induced NF.

Methods:
Patients 18 years or older with absolute neutrophil count ≤500 cells/mm^3 anticipated for > 7 days were enrolled at the time of BC collection (T0) due to fever. Plasma samples were collected at T0 and twice weekly until neutrophil recovery or discharge. Samples were shipped to the Karius CLIA/CAP laboratory (Redwood City, CA) where cell-free plasma was prepared, DNA extracted, and NGS performed. After removing human reads, remaining sequences were aligned to a curated pathogen database. Organisms present at a significance level above a predefined threshold were reported. T0 BC and Karius plasma NGS results were compared, excluding organisms (e.g., viruses) not recoverable by BC. Positive agreement was defined as plasma NGS identification of at least one isolate seen on BC. Plasma NGS+/BC- results underwent adjudication by three infectious diseases specialists. Diagnosis was "Definite" if microbiology confirmed NGS result within seven days of enrollment; "Probable" if clinical, radiologic, and laboratory data were compatible with Karius plasma NGS test result; "Possible" if NGS result was compatible with a known clinical syndrome but patient had non-specific clinical findings; and "Indeterminate" where information was insufficient for classification.

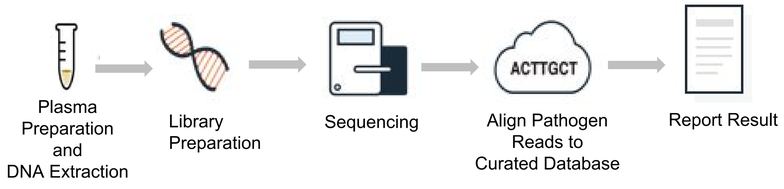
Results:
The first 32 enrolled patients were evaluated. At enrollment, 72% (23/32) of all patients were on anti-infective therapy. Of these with 56% (13/23) were on antibiotics and 22% (5/22) were on antifungals and antivirals, respectively. Five patients were BC+ (each mono-microbial) and concordant with Karius plasma NGS at T0. Plasma NGS identified additional organisms in two of the BC+ patients with surgical abdomen. Compared to BC, Karius plasma NGS positive agreement was 100% (5/5) and negative agreement was 33% (9/27). All BC-/NGS+ results were assessed for etiology of NF episode by clinical adjudication. Of these, 12 were classified as "Probable", four as "Possible", and two as "Indeterminate". These included both single and mixed organisms in patients with enterocolitis or severe mucositis. In one sample, *Aspergillus fumigatus* was detected in a patient with new lung nodules and another sample had *Rhizomucor miehei* detected in a patient previously diagnosed with *Aspergillus niger*.

Conclusions:
The etiologic diagnosis of neutropenic fever is frequently unknown, leading to broad antibiotics and sometimes delay of targeted treatment. Karius plasma NGS may provide useful data for managing NF given its ability to detect a breadth of pathogens even when patients are pretreated with antibiotics.

METHODS



Sample Processing and Workflow



RESULTS

Patient Characteristics

Demographic Characteristics	
Race – no. (%)	
Asian	4 (13)
Native America/Pacific Islander	1 (3)
White	18 (56)
Hispanic/Latino	9 (28)
Female - no. (%)	11 (34)
Median Age (range) - yrs	66 (20-82)
Clinical Characteristics	
Malignancy Type – no. (%)	
Acute Myeloid Leukemia (AML)	20 (63)
Acute Lymphocytic Leukemia	8 (25)
Acute Undifferentiated Leukemia	1 (3)
Acute Leukemia, Unspecified	3 (9)
Transplant Status – no (%)	
Stem Cell (haploidentical, MUD)	2 (6)
Antimicrobials at TP0 – no. (%)	
Antibiotics	13 (40)
Antivirals	5 (16)
Antifungals	5 (16)
Blood Culture Results at TP0 – no. (%)	
Negative	27 (84)
Positive	5 (16)

Karius Plasma NGS vs Blood Culture at Time 0

	Blood Culture Positive	Blood Culture Negative
Karius Positive	5	18[1]
Karius Negative	0	9

Positive Agreement 100%
Negative Agreement 33%

[1]Plasma NGS identified organisms that cannot be isolated from blood culture in 4 subjects

Matched Karius Test and Blood Culture Results

Positive Blood Culture (Time Point 0)	Plasma NGS Results
Serratia marcescens	***Serratia marcescens***, HHV6B
Escherichia coli	***Escherichia coli***
Morganella morganii	***Morganella morganii*** *Escherichia coli* *Granulicatella adiacens* *H. parainfluenzae* *Streptococcus mitis*
Pseudomonas aeruginosa	***Pseudomonas aeruginosa,*** *Bacteroides caccae* *Citrobacter koseri* *Staphylococcus aureus*
Escherichia coli	***Escherichia coli***

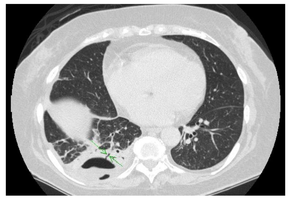
Clinical Adjudication for Patients with Positive Karius Test and Negative Blood Culture (N=18)[1]

Plasma NGS Results	Clinical Findings
Probable[2] (N=12)	
Escherichia coli	Pneumatosis intestinalis on CT
Staphylococcus aureus, HSV1	Facial cellulitis, HSV PCR from lip vesicle positive
Prevotella intermedia, Prevotella melaninogenica, Rothia aeria, Rothia mucilaginosa. Streptococcus parasanguinis, Rothia dentocariosa Streptococcus mitis, Streptococcus pseudopneumoniae Porphyromonas gingivalis	Admitted with nausea, vomiting, diarrhea
Morganella morganii	Severe mucositis
Cronobacter sakazakii	Colitis
Aspergillus fumigatus	Chest CT with pulmonary lesions
Haemophilus parainfluenzae, Rothia mucilaginosa, Streptococcus agalactiae, Streptococcus mitis, Veillonella dispar Prevotella melaninogenica	Infectious enterocolitis
Enterococcus faecalis	Scattered pulmonary nodules
Fusobacterium nucleatum, Bacteroides thetaiotamicron	Dental caries tooth #32, chest CT with ground glass opacities and indeterminate small nodules
Escherichia coli, Pseudomonas aeruginosa	Severe esophagitis, bilateral upper lobe consolidative opacities, pulmonary nodules
Enterococcus faecium, Staphylococcus epidermidis, Streptococcus sanguinis	Abdominal pain, enteritis
Mycobacterium abscessus, Helicobacter pylori	Antibody positive *(H. pylori)*
Possible[3] (N=4)	
Streptococcus mitis	Non-specific findings
Rothia mucilaginosa, Staphylococcus epidermidis Streptococcus mitis	Non-specific findings
Bacteroides fragilis, Clostridium perfringens Escherichia coli, HSV2	HSV2 PCR positive at later time point
Haemophilus influenzae	Non-specific findings

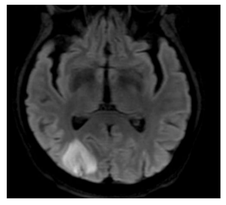
[1] Result was adjudicated as indeterminate for 2 cases where there was insufficient information available
[2] Result is plausible organism(s) known to cause clinical syndrome in susceptible host
[3] Result is plausible organism(s) that meets criteria for Probable with non-specific clinical findings

Case Study

66 year old female with AML presented with febrile neutropenia and pulmonary nodules on computed tomography (CT) of chest. Empiric voriconazole was started. Ten days later, CT showed nodule progression with new right parapneumonic effusion. Amphotericin was added. Culture from bronchoscopy grew six colonies of *Aspergillus niger*. Two weeks later, chest CT showed new areas of necrosis, cavitation and a bronchopulmonary fistula. MRI of the brain weeks later showed a ring enhancing lesion. Biopsy was performed. Fungal and bacterial sequencing were negative but hyphal forms were identified on histopathology. *Rhizomucor meihei* was identified with NGS on HD4 and HD8. **The Karius plasma NGS test detected a clinically compatible fungal pathogen that would have led to earlier optimization of antifungal therapy**.



Right lower-lobe lesion



Occipital lobe enhancement

Conventional Test Results:
Positive BAL for *Aspergillus niger*

Karius Plasma NGS Results:
HD1: Negative
HD4: *Rhizomucor miehei*
HD8: *Rhizomucor miehei, Veillonella sp.*

CONCLUSIONS

Karius plasma NGS:
- detected all organisms found by blood culture;
- identified polymicrobial infections not detected by standard microbiologic testing;
- identified a plausible cause of neutropenic fever more often than blood culture alone;
- detected an alternative fungal pathogen compatible with the clinical scenario. These results would have resulted in earlier optimization of antifungal therapy.

The Karius plasma NGS test has great potential for a diagnostic tool in fever and neutropenia.