**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/mm³ 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 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-inflammatory therapy. Of these with 56% (13/24) 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% (9/9) and negative agreement was 93% (9/22). 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.

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**METHODS**

**Sample Processing and Workflow**

**Study Design**

- **Fever ANC <500 cells/mm³**
- **Study Sample**
  - **Karius Sample Collection**
  - **Every 2-3 Days**
  - **Hospital Discharge**

**RESULTS**

**Matched Karius Test and Blood Culture Results**

<table>
<thead>
<tr>
<th>Positive Blood Culture</th>
<th>Karius Positive</th>
<th>Karius Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Agreement 100%</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Negative Agreement 33%</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

**Cases 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 hypalform forms were identified on histopathology. Rhizomucor miehei 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.

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**CONCLUSIONS**

- **Karius plasma NGS** detected all organisms found by blood culture.
- **Identified polymicrobial infections not detected by standard microbiologic testing.**
- **Detected 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.

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**Karius Plasma NGS Results**

- **HD1: Negative**
- **HD4: Rhizomucor miehei**
- **HD8: Rhizomucor miehei, Veillonella sp.**

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Clinical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Pneumatosis intestinale on CT</td>
</tr>
<tr>
<td>Staphylococcus aureus, HSV1</td>
<td>Facial cellulitis, HSV PCR from lip vesicle positive</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Admitted with nausea, vomiting, diarrhea</td>
</tr>
<tr>
<td>Rhizomucor miehei, Veillonella sp.</td>
<td>Infectious enterocolitis</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>Abdominal pain, enteritis</td>
</tr>
<tr>
<td>Fusobacterium nucleatum, Bacteroides thetaiotaomicron</td>
<td>Scattered pulmonary nodules</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Dental caries tooth #2, chest CT with ground glass opacities and indeterminate small nodules</td>
</tr>
<tr>
<td>Streptococcus agalactiae, Streptococcus mitis, Veillonella dispar</td>
<td>Severe sepsis, bilateral upper lobe consolidative opacities, pulmonary nodules</td>
</tr>
<tr>
<td>Enterococcus faecium, Staphylococcus epidermidis, Streptococcus mitis</td>
<td>Abdominal pain, enteritis</td>
</tr>
<tr>
<td>Mycobacterium abscessus, Helicobacter pylori</td>
<td>Antibody positive (H. pylori)</td>
</tr>
</tbody>
</table>

**Possible (N=4)**

- **Streptococcus mitis**

**Non-specific findings**

- **Rhizomucor miehei, Staphylococcus epidermidis**
- **Mycobacterium abscessus, Helicobacter pylori**
- **Enterococcus faecalis**
- **Bacteroides fragilis, Clostridium perfringens**

**Non-specific findings**

- **HSV2 PCR positive at later time point**
- **HSV2 PCR positive at later time point**

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**Case Study**

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

- **Right lower lobe lesion**

- **Occlpital lobe enhancement**

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**Conventional Test Results:**

- **Positive BAL for Aspergillus niger**

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**RESULTS**

**Patient Characteristics**

**Demographic Characteristics**

- **Race – % (No.)**
  - Asian: 4 (13)
  - Native American/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)
- **Antimicrobial at T0 – % (No.)**
  - Sterilization: 2 (6)
  - Antibiotics: 13 (40)
- **Blood Culture Results at T0 – % (No.)**
  - Negative: 27 (84)
  - Positive: 5 (16)

**Karius Plasma NGS vs Blood Culture at Time 0**

<table>
<thead>
<tr>
<th>Blood Culture Positive</th>
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<th>Positive Agreement 100%</th>
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