Karius Test for the Diagnosis and Management of Febrile Neutropenia

Evaluation of the Karius Plasma Next Generation Sequencing Cell-free Pathogen DNA Test to Determine the Etiology of Infection and Impact on Anti-Microbial Management in Patients with Severe Neutropenia and Fever

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PATIENT POPULATION
This prospective, observational study enrolled 57 high-risk leukemia patients receiving chemotherapy who presented with severe febrile neutropenia.

STUDY DESIGN
The Karius Test was performed on blood samples collected within 24 hours of fever onset.

The Karius results were compared to blood cultures and clinical diagnosis as adjudicated by an independent panel of infectious disease physicians.

RESULTS
Compared to blood culture, the Karius Test showed a positive agreement of 90% (9/10) and negative agreement of 31% (14/45).

The Karius Test had 83.8% sensitivity (41/49) and 100% specificity (6/6) compared to clinical diagnosis - a composite of laboratory, radiology, and clinical data - as adjudicated by the panel.

The adjudication panel also determined that antimicrobial therapy could potentially have been changed in 26 out of 55 patients (47% of the time) if Karius Test results had been available in real-time. This includes either broadening or narrowing of antibiotics or the addition of antivirals, antifungals, or other antimicrobial agents.

The Karius Test can provide fast and accurate detection of pathogens and clinically relevant polymicrobial infections in febrile neutropenic patients which can inform treatment decisions.
**BACKGROUND**

Standard microbiological testing fails to identify a pathogen in most chemotherapy recipients with febrile neutropenia, who then receive prolonged empiric courses of broad spectrum antimicrobials. We evaluated the use of the Karius next-generation sequencing plasma test (Karius® Test) to identify infectious etiologies of neutropenic fever (NF) and its impact on antimicrobial management.

**METHODS**

This prospective, observational study enrolled 57 patients with ≤500 neutrophils/mm³ and documented fever or report of fever ≥38.3°C or ≥38.0°C on two episodes separated by at least one hour. Samples were collected within 24h of fever onset (T0) and every 2-3 days up to 5 time points. Cell-free plasma DNA was prepared and sequenced in a CLIA/CAP laboratory, human reads were excluded, and remaining sequences aligned to discordant positives).

**Sample Processing and Workflow**

**RESULTS**

**Determination of Agreement of Karius Test vs Blood Culture**

Concordant results between Karius Test and blood culture included:

- **True Positive**: Karius Test is concordant with positive blood culture (excluding contaminants) for at least one pathogen.
- **True Negative**: Karius Test and blood culture are both negative.

Discordant results between Karius Test and blood culture included:

- **False Positive**: Karius Test has any result and blood culture is negative.
- **False Negative**: Karius Test is negative and blood culture is positive (excluding contaminants) OR Karius Test is positive for a different microorganism than what is grown by blood culture (i.e. discordant positives).

**Clinical Diagnosis Definitions**

Karius® Test results were adjudicated by 3 independent infectious disease specialists using (i) laboratory data; (ii) radiology results; and (iii) discharge summaries:

- **Definite**: Karius Test result is concordant with positive blood culture (excluding contaminants) for at least one pathogen OR a microbiologic test performed within 7 days of Karius sample collection that is likely a cause of febrile neutropenia.
- **Probable**: Karius Test result is likely cause of neutropenic fever based on clinical, radiologic or laboratory findings.
- **Possible**: Karius Test result is consistent with an infection but not a common cause of febrile neutropenia AND diagnosis must be made using history, exam, and non-microbiologic lab testing (e.g. elevated WBC with left shift, elevated CRP) AND no other focal point has been reported.
- **Utility**: Karius Test result is positive but discordant with other microbiologic test results and Karius Test is either not a plausible cause of infection OR there is a more likely explanation for the febrile event that does not meet the “Possible” or “Probable” case classification criteria.

**Karius Test identified a plausible cause of neutropenic fever 2x more often and faster than all other microbiology tests**

Subjects with positive results

- **Antimicrobial Therapy**
  - **Karius Test vs Blood Culture at T0**
  - **Karius Test vs Clinical Diagnosis at T0**

- **Per adjudication, antimicrobial therapy could have been changed in 47% of patients (26/55) had the Karius Test results been available in real-time within 52-100 hours.**

Using current CLIA/CAP clinical laboratory turn around time, study result time was estimated as 52 hours from time of draw and 100 hours from time of draw for samples that did not meet quality control metrics and required repeat testing (< 5% of samples).

**CONCLUSIONS**

The Karius Plasma NGS Test:

- Detected nearly all organisms detected by blood culture (90%).
- Identified clinically significant polymicrobial infections not detected by standard microbiologic testing.
- Identified a plausible cause of neutropenic fever 2x more often than all other standard microbiology tests.
- Identified a plausible cause of neutropenic fever faster than all other standard microbiology tests.

Would have changed antimicrobial management in 47% of the cases if the results had been available in real-time.