KARIUS® | STUDY SUMMARY

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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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.

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.

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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/mm^3 and documented fever or report of fever ≥38.3C or ≥38.0 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 a curated pathogen database that includes bacteria, viruses, fungi and parasites.

Sample Processing and Workflow

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.
e.g. Karius found HSV1 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 a likely 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.

Unlikely: 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.

False Negative: Karius Test is negative but other microbiologic tests are positive and adjudicated as the cause of infection OR microbiologic test results are negative and there is a localized infection diagnosed using history, exam, and non-microbiologic data.

True Negative: Karius Test result is negative and other microbiologic tests are negative results AND the cause of fever can be attributed to a non-infectious cause.

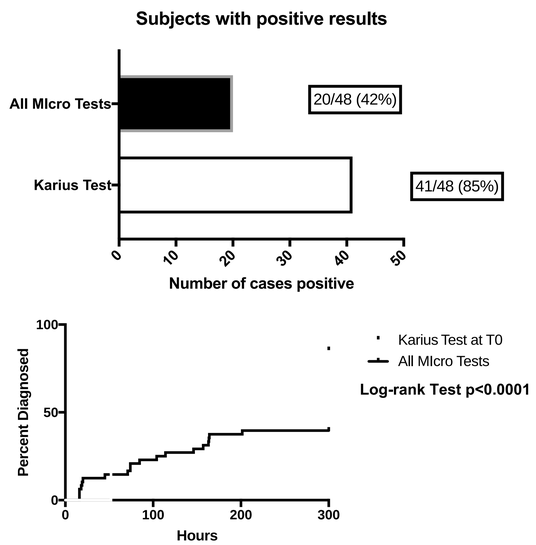
RESULTS

Demographic and Clinical Characteristics*

Demographic Characteristics	Total N=55
Race – no. (%)	
Asian	8 (14)
Native American/Pacific Islander	2 (4)
White	32 (58)
Hispanic/Latino	12 (22)
Unknown / Not Reported	1 (2)
Female – no. (%)	24 (44)
Median Age in years (range)	60 (20-82)
Clinical Characteristics – no. (%)	
Hematological Malignancy Type	
Acute Myeloid Leukemia**	43 (78)
Acute Lymphocytic Leukemia	9 (16)
Acute Undifferentiated Leukemia	2 (4)
Myelodysplastic Syndrome	1 (2)
Allogeneic Stem Cell Transplant – no (%)	3 (6)
Antimicrobials at presentation – no. (%)	
Antibiotics	19 (35)
Antivirals	11 (20)
Antifungals	9 (16)
Results of T0 sample – no. (%)	
Blood Culture Positive	10 (18)
Karius Test Positive	41 (75)
Karius Test Polymicrobial	25 (61)

*Modified intent to diagnose population
** 4/43 were Acute Myeloid Leukemia secondary to Myelodysplastic Syndrome

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



Note: Includes cases adjudicated as Definite, Probable, Possible and False Negative (48/55 mITD cases). 7/55 mITD cases were adjudicated as True Negative and were excluded from this analysis. Karius Test turn around time was estimated using current CLIA/CAP clinical laboratory performance.

Karius Test vs Blood Culture at T0

	Blood Culture Positive	Blood Culture Negative
Karius Test Positive	**9**	31
Karius Test Negative	1*	**14**

*Discordant positive. Blood culture detected *Streptococcus parasanguinis*. Karius Test detected Human herpesvirus 1 and *Prevotella oralis*

Positive Agreement = 90% (9/10)
Negative Agreement = 31% (14/45)

Karius Test vs Clinical Diagnosis at T0

	Clinical Diagnosis Positive**	Clinical Diagnosis Negative***
Karius Test Positive	**41**	0
Karius Test Negative	7	**7**

**Definite (n=12), Probable (n=19), Possible (n=10) and False Negatives (n=7) adjudicated cases. False Negatives included: 2 cases of cellulitis, 2 tooth infections, 2 cases of pulmonary nodules, 1 case of persistent neutropenic fever with ICU transfer
*** Unlikely (n=0) and True Negative (n=7) adjudicated cases

Sensitivity = 85.4% (41/48)
Specificity = 100% (7/7)

Note: Karius results that were "Possible" causes of FN episodes were adjudicated to have resulted in change of anti-microbial management in 9 of 10 cases, with the remaining case deemed to already be on appropriate therapy for the pathogens detected by the Karius Test.

Impact of Karius Test Results on Antimicrobial Management

Antimicrobial Management	Total N=55
Antimicrobial therapy changed if Karius Test results were available in real-time – no. (%)	26 (47)
Antibiotic	22
Addition	10
Broadening (non-specified)	1
MRSA coverage added	1
Anaerobic coverage added	7
Anti-mycobacterial coverage added	1
Withdrawal	13
Narrowing (non-specified)	8
MRSA coverage withdrawn	5
Antiviral	8
HSV coverage added	7
CMV coverage added	1
Anti-parasitic	1
Anti-parasitic coverage added	1
Anti-fungal	2
Anti-fungal coverage added	2

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.