Table 1: Clinical Impact Categories and their Predefined Criteria

Category	Definition
Positive	Karius test led to a new diagnosis when conventional tests were negative. Karius test led to the confirmation of the diagnosis. Karius test led to an earlier diagnosis. Karius test helped to avoid invasive or costly procedures or tests. Karius test helped to avoid a prolonged hospital stay. Karius test result led to the initiation of appropriate antimicrobial therapy. Karius test result led to de-escalate or stop antimicrobial therapies.
Negative	Karius test led to unnecessary antimicrobial treatment. Karius test led to unnecessary diagnostic investigation or procedures. Karius test led to an unnecessarily prolonged hospital stay.
Uncertain or No impact	Karius test result did not change any clinical management or unable to determine the clinical impact.

Results. A total of 80 patients were included. 45 patients (56%) were immunocompromised, and 14 (18%) had prosthetic hardware or grafts. The most common clinical syndrome was pneumonia/respiratory failure (31%), followed by sepsis/septic shock (15%) and endocarditis (13%). 72 patients (90%) received antibiotics prior to sending the Karius assay. The most common reason for sending the assay was unknown microbiologic diagnosis (78%), followed by avoiding invasive procedures (14%). The test was consistent with the final diagnosis in 65% of cases and had a positive impact in 34 (43%), a negative impact in 2 (3%), and uncertain or no impact in 44 (55%) (Table 2). A positive impact was observed in solid organ transplant recipients (SOTR, 71.4%, p=0.003), sepsis (71.4%, p=0.003), and those receiving antimicrobial agents for less than 7 days prior to Karius testing (i.e., 61.8%, p=0.004) (Table 3).

Table 2: Patient Demographics and Characteristics of the Karius® Assay

Total patients	N=80
Age (years), median (+/-SD)	54.5 (15.59)
Gender, n (%)	
Female	32 (40)
Male	48 (60)
Reason for Karius Assay, n (%)	
Microbiologic diagnosis unknown	74 (78)
Avoid invasive diagnostic procedure	13 (14)
Confirmatory test	5 (5)
Early diagnosis	3 (3)
Types of Clinical Impact, n (%)	
Negative	2 (3)
Positive	34 (43)
Uncertain or No impact	44 (55)
Consistency with Final Clinical Diagnosis, n (%)	
Yes	52 (65)
No	25 (31)
NA	3 (4)

Abbreviations: SD, standard deviation; NA, not applicable

Table 3: Patient Characteristics and Relationship to Clinical Impact †

Comorbidities	n (%)	Uncertain or No impact, n (%)	Positive Impact, n (%)	P-value
Immunocompromised	45 (56)	24 (53.3)	21 (46.7)	0.522
Organ transplant	21 (26)	6 (28.6)	15 (71.4)	0.003
Stem cell transplant	3 (4)	3 (100)	0	
Solid tumor	2 (2)	2 (100)	0	
Hematologic malignancy	22 (27)	15 (68.2)	7 (31.8)	0.189
HIV/AIDS	1 (1)	1 (100)	0	
Autoimmune disease	3 (4)	1 (33.3)	2 (66.7)	
Hardware or prosthesis	14 (18)	7 (50)	7 (50)	0.593
Vascular graft	6 (7)	4 (66.7)	2 (33.3)	0.691
Prosthetic joint or orthopedic hardware	1 (1)	0	1 (100)	
Mechanical Cardiac Device	1 (1)	0	1 (100)	
Prosthetic valve	8 (10)	3 (37.5)	5 (62.5)	0.285
Diabetes	13 (16)	6 (50)	6 (50)	0.626
Infectious Syndrome/Clinical Diagnosis				
Sepsis/Septic shock	15 (15)	4 (28.6)	10 (71.4)	0.02
Bacteremia	3 (3)	1 (33.3)	2 (66.7)	
Vascular graft infection	7 (7)	4 (57.1)	3 (42.9)	1
Endocarditis	13 (13)	6 (46.2)	7 (53.8)	0.414
Respiratory failure/pneumonia	30 (31)	14 (48.3)	15 (51.7)	0.265
Bone/joint infection	4 (4)	3 (75)	1 (25)	
CNS infection (meningoencephalitis)	10 (10)	7 (77.8)	2 (22.2)	0.285
Fever unknown origin	10 (10)	5 (50)	5 (50)	0.74
Unexplained leukocytosis	2 (2)	1 (50)	1 (50)	
Sinusitis	1 (1)	1 (100)	0	
Skin and soft tissue infection	1 (1)	0	1 (100)	
Others	1 (1)	1 (100)	0	
Antimicrobial agents administered prior to Karius test	72 (90)	39 (55.7)	31 (44.3)	1
Less than 7 days	35 (49)	13 (38.2)	21 (61.8)	0.004
More than 7 days	37 (51)	26 (72.2)	10 (27.8)	
No antimicrobial agents prior to Karius test	8 (10)	5 (62.5)	3 (37.5)	1
Final Diagnosis				
Bacterial	26 (31)	15 (60)	10 (40)	0.661
Fungal	21 (25)	12 (57.1)	9 (42.9)	0.937
Viral	10 (12)	6 (60)	4 (40)	1
Non-infectious	28 (33)	14 (51.9)	13 (48.1)	0.555
Days of Hospitalization before sending Karius test, Median (Q1, Q3)		11.00 (5.00,21.50)	9.50 (3.00,18.25)	0.361

Abbreviations: HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency syndrome; CNS, central nervous system
† Some patients had more than one comorbidity and clinical syndrome. 2 patients with negative impact were not included in the analysis.

Conclusion. In our cohort, clinical utility of Karius testing was highest in SOTR and in patients with sepsis. Prolonged antimicrobial use (> 7 days) prior to Karius testing limited the utility of the assay. Prospective studies evaluating the utility of mNGS mcfDNA assays should be performed to further clarify its role in clinical management.

Disclosures. **All Authors**: No reported disclosures

662. Using Machine Learning to Aid in the Diagnosis of Multisystem Inflammatory Syndrome in Children

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Session: P-30. Diagnostics: Typing/sequencing

Background. Multisystem inflammatory syndrome in children (MIS-C) is a newly recognized inflammatory syndrome that occurs post Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) infection. It affects multiple organ systems - particularly cardiac, gastrointestinal, dermatologic and neurologic. Clinicians may have difficulty diagnosing MIS-C due to its novelty and similarity to Kawasaki disease. Our goal was to use machine learning to predict whether children would have MIS-C based on symptoms and laboratory values.

Methods. A retrospective review was conducted of patients admitted to Loma Linda University Children's Hospital who were suspected of having MIS-C. Demographic, symptom (such as fever, abdominal pain, diarrhea, shock, etc), and laboratory data were collected from the electronic medical record. For the 115 patients and 20 laboratory values, there was a total of 130 missing values (5.7%). Missing laboratory values were imputed using the median value based on the presence or absence of MIS-C. The data were split into a training (93 patients, 80%) and testing (22 patients, 20%) set. The training set was used to train a random forest model and the testing set was used to evaluate model performance. R 4.0.2 was used for modeling with the following packages: tidymodels and randomForest.

Results. There were 115 patients of which 49 were females, and 77 were diagnosed with MIS-C. The median age of the patients with MIS-C was 115 months and 79 months for those without MIS-C. In the testing set, all 15 patients with MIS-C were classified correctly but of the 7 without MIS-C, the model predicted 4 of the patients correctly. This gives a sensitivity of 100% and specificity of 57%. When changing the seed and testing set, the sensitivity remained 100% but the specificity improved to 86%. The random forest algorithm showed that the most important features were pro-calcitonin, ferritin, pro-BNP, and CRP.

Conclusion. During the height of the SARS-CoV-2 pandemic, many children were being admitted with suspected MIS-C, but clinicians struggled to confirm the diagnosis. We have found a model predicting which of these patients had MIS-C with high sensitivity. This model is a first step of many toward creating the foundation of personalized medicine for children.

Disclosures. **All Authors**: No reported disclosures

663. Two (Plus) Birds, One Stone: The Rapid, Comprehensive, Non-invasive Detection of Co-Pathogens of Critical Importance Using A Plasma-based Microbial Cell-free DNA Next-generation Sequencing Test

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Session: P-30. Diagnostics: Typing/sequencing

Background. Immunocompromised (IC) patients are at risk for infections by a spectrum of invasive pathogens. The overlap in presentation makes it challenging to differentiate among infectious etiologies and critical co-infections (CI) may remain undiagnosed. Open-ended, comprehensive assessment of infection through microbial cell-free DNA (mcfDNA) next-generation sequencing (NGS) of plasma offers the potential for the rapid identification of multiple co-infecting pathogens of critical importance (CI-POCI) with one test.

Methods. Karius Test™ (KT) results from patients who underwent clinical testing from December 2016 to April 2021 were reviewed for detections of two or more CI-POCI including parasites, fungi (*Pneumocystis jirovecii, Trichosporon sp*, endemic mycoses, *Aspergillus sp*., *Mucorales*, Non-*Aspergillus*/Non-*Mucorales* molds), mycobacteria, *Legionella sp*., *Nocardia sp*. and *Listeria*. KT, developed and validated in Karius' CLIA certified/CAP accredited lab, detects mcfDNA from plasma. McfDNA is extracted, NGS performed, human sequences removed and remaining sequences aligned to a curated pathogen database of > 1500 organisms. Organisms present above a statistical threshold are reported and quantified. For > 85% of tests the time to result reporting is the next day from sample receipt.

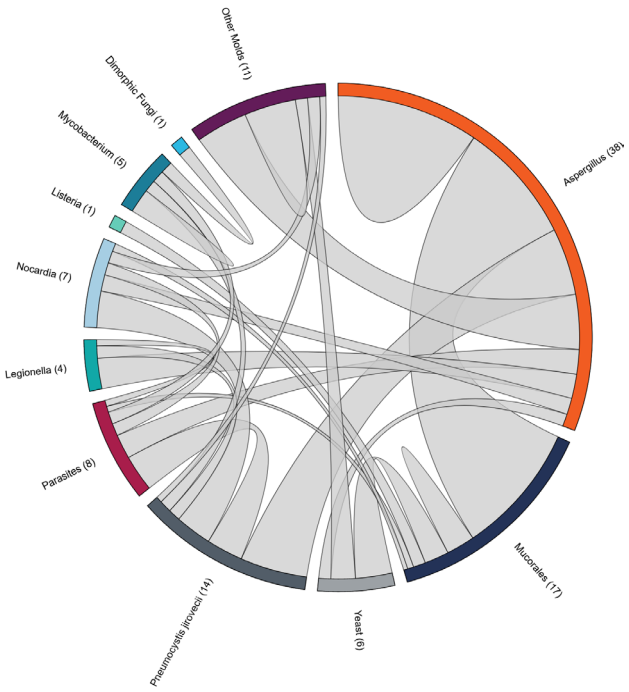
Results. KT detected CI of two or more POCI in 59 samples (75% adults, 25% children). The most common partnering co-pathogens of critical importance were *Aspergillus sp* (38), *Mucorales* (17) and PJP (14); the most common combinations were two or more distinct *Aspergillus sp* (14) followed by an *Aspergillus sp* and a *Mucorales* (12). There were 17 samples with the detection of three or more CI-POCI (29%).

Table 1. Categorization of Co-infections with Pathogens of Critical Importance*

Pathogens	# of co-infections	Pathogens	# of co-infections	Pathogens	# of co-infections
***Aspergillus* sp.** (Asp)	**38**	***Mucorales*** (Muc)	**17**	**Parasites**	**8**
Asp + Asp (9) -*Aspergillus flavus*/*oryzae* + *Aspergillus fumigatus* -*Aspergillus niger* + *Aspergillus fumigatus* -*Aspergillus flavus*/*oryzae* + *Aspergillus nivoflumigatus* -*Aspergillus flavus*/*oryzae* + *Aspergillus tubingensis*	5 2 1 1	Asp + Muc (8)* Muc + Muc + Trich (2) -*Rhizopus microsporus* + *Rhizopus oryzae* Muc + Muc (1) -*Rhizopus microsporus* + *Rhizopus microsporus* Muc + Trich (1) -*Rhizomucor pusillus* + *Trichosporon asahii* Muc + List (1) -*Rhizomucor pusillus* + *Listeria monocytogenes* Asp + Muc + Parasite (1)* Asp + Asp + Muc (1)* Asp + Asp + Asp + Muc (1)* Asp + Muc + Muc + Noc (1)*	8 2 1 1 1 1 1	PJP + Parasite (3)* Parasite + Noc (1) -*Trichomonas vaginalis* + *Nocardia elegans* Parasite + Myco (1) -*Mycobacterium haemophilum* + *Amoeba* Asp + Muc + Parasite (1)* Asp + Leg + Parasite (1)* Asp + Muc + Parasite (1)*	3 1 1 1 1 1
Asp + Muc (8) -*Aspergillus fumigatus* + *Rhizopus microsporus* -*Aspergillus fumigatus* + *Rhizopus oryzae* -*Aspergillus fumigatus* + *Rhizomucor miehei* -*Aspergillus flavus*/*oryzae* + *Rhizopus oryzae* -*Aspergillus niger* + *Rhizopus delemar* -*Aspergillus flavus*/*oryzae* + *Rhizomucor pusillus* -*Aspergillus fumigatus* + *Rhizomucor pusillus* Asp + PJP (5) -*Aspergillus fumigatus* + *Pneumocystis jirovecii* -*Aspergillus oystoni* + *Pneumocystis jirovecii* Asp + NA/NM + NA/NM (2) -*Aspergillus chevalieri* + *Trichoderma longibrachiatum*/*atroviride* + -*Aspergillus calidoustus* + *Trichoderma longibrachiatum* -*Aspergillus calidoustus* + *Trichoderma longibrachiatum* + *Fusarium proliferatum* Asp + Leg (1) -*Aspergillus flavus*/*oryzae* + *Legionella pneumophila* Asp + Noc (1) -*Aspergillus fumigatus* + *Nocardia vinacea*/*fusca* Asp + Parasite (1) -*Aspergillus fumigatus* + *Acanthamoeba* Asp + Trich (1) -*Aspergillus fumigatus* + *Trichosporon asahii* Asp + NA/NM (1) -*Aspergillus flavus*/*oryzae* + *Alternaria alternata* Asp + Asp + NA/NM (1) -*Aspergillus subglaucus* + *Aspergillus fumigatus* + *Fusarium proliferatum* Asp + NA/NM + PJP (1) -*Aspergillus fumigatus* + *Fusarium fujikuroi* + *Pneumocystis jirovecii* Asp + Muc + Parasite (1) -*Aspergillus fumigatus* + *Lichtheimia ramosa* + *Strongyloides stercoralis* Asp + Asp + Muc (1) -*Aspergillus flavus*/*oryzae* + *Aspergillus terreus* + *Lichtheimia corymbifera* Asp + Leg + Parasite (1) -*Aspergillus fumigatus* + *Legionella feeleii*/*tunisiensis* + *Exophiala*/*salmonis* Asp + Asp + Leg (1) -*Aspergillus calidoustus* + *Aspergillus fumigatus* + *Legionella feeleii* Asp + Asp + Asp + Muc (1) -*Aspergillus fumigatus* + *Aspergillus flavus*/*oryzae* + *Aspergillus tubingensis* + *Rhizomucor pusillus* Asp + Asp + Asp + NA/NM (1) -*Aspergillus fumigatus* + *Aspergillus flavus* + *Aspergillus terreus* + *Nechia haematococca*/*cuenoi solani* Asp + Muc + Muc + Noc (1) -*Aspergillus fumigatus* + *Rhizomucor pusillus* + *Rhizopus microsporus* + *Nocardia farcinica*	38 5 2 1 1 2 1 1 1 1 1 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	***Pneumocystis jirovecii*** (PJP) Asp + PJP (5)* PJP + Parasite (3) -*Pneumocystis jirovecii* + *Toxoplasma gondii* -*Pneumocystis jirovecii* + *Enterocytozoon bieneusi* PJP + Noc (2) -*Pneumocystis jirovecii* + *Nocardia cyriacigeorgica* -*Pneumocystis jirovecii* + *Nocardia asiatica* PJP + Leg (1) -*Pneumocystis jirovecii* + *Legionella pneumophila* PJP + Myco (1) *Pneumocystis jirovecii* + *Mycobacterium magenticense* Asp + NA/NM + PJP (1)*	**14** 5 2 1 2 1 1 1 1	***Mycobacteria sp.*** (Myco) Myco + EM (1) -*Mycobacterium avium complex* + *Histoplasma capsulatum* Myco + Myco + Myco (1) -*Mycobacterium abscessus*/*chelonae*/*Mycobacterium mucogenicum* + *Mycobacterium bolletii*/*arenes* PJP + Myco (1)* Parasite + Myco (1)*	**4** 1 1 1 1
		***Non-Aspergillus* Non-*Mucorales* Molds** (NA/NM)	**10**	***Legionella sp.*** (Leg) Asp + Leg (1)* Asp + Leg + Parasite (1)* Asp + Asp + Leg (1)* PJP + Leg (1)*	**4** 1 1 1 1
		NA/NM + NA/NM + NA/NM (3) -*Aureobasidium pullulans* + *Alternaria alternata* + *Exophiala xenobiotica* -*Aureobasidium pullulans* + *Aureobasidium subglaciale* + *Filobasidium wieringae* -*Trichoderma harzianum* + *Ophiostoma piceae* + *Graphilbum fragrans* Asp + NA/NM (2)* Asp + Asp + NA/NM (1)* NA/NM + Noc (1) *Lomentospora prolificans* + *Nocardia novacoligeans* Asp + NA/NM + NA/NM (1)* Asp + NA/NM + PJP (1)* Asp + Asp + Asp + NA/NM (1)*	1 1 1 2 1 1 1 1 1 1	***Trichosporon asahii*** (Trich) Muc + Muc + Trich (2)* Asp + Trich (1)* Muc + Trich (1)*	**4** 2 1 1
				Endemic mycoses (EM) Myco + EM (1)*	**1** 1
				Listeria monocytogenes (List) Muc + List (1)*	**1** 1

* Previously Listed

Figure 1. Chord Plot of Co-infections with Pathogens of Critical Importance



The outer circle sections represent Karius Test detections belonging to different taxonomic groups. The length of each circle section is proportional to the total number of detections of a taxon belonging to that group. The chords connecting a pair of circle sections are proportional to the number of times two taxa from those groups were observed together, weighted by the total number of taxa detected.

Conclusion. Plasma mcfDNA NGS offers a rapid, comprehensive non-invasive means of detecting CI-POCI in IC patients with one test. Although rare, co-infections with POCI can greatly increase mortality. The KT can provide important insights into pathogen-pathogen interactions in complex hosts and help optimize therapy.

Disclosures. **Matthew Smollin, PharmD**, **Karius, Inc.** (Employee) **Martin S. Lindner, PhD**, **Karius, Inc.** (Consultant) **Nicholas R. Degner, MD, MPH, MS**, **Karius Inc.** (Employee, Shareholder) **Ricardo Castillo-Galvan, MD MPH**, **Karius Inc.** (Consultant) **Jose Alexander, MD, D(ABMM), FCCM, CIC, SM, MB(ASCP), BCMAS**, **Karius** (Employee) **Ann Macintyre, DO**, **Karius, Inc.** (Employee) **Bradley Perkins, MD**, **Karius, Inc.** (Employee) **Asim A. Ahmed, MD**, **Karius, Inc.** (Employee) **Aparna Arun, MD**, **Karius** (Employee)

664. Clinical Impact of Cell-Free DNA Metagenomics in Diagnosing Infectious Diseases in Pediatrics: A Single-Center Experience

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Session: P-30. Diagnostics: Typing/sequencing

Background. Metagenomic next-generation sequencing (mNGS) of plasma cell-free DNA has significant potential to improve infectious diseases diagnostics through unbiased detection of pathogens. However, the optimal patient population or clinical condition for this testing has not been determined.

Methods. We performed a retrospective review of all orders for plasma cell-free DNA mNGS using the Karius test (Karius, Redwood City, CA) from The Children's Hospital of Philadelphia from 7/1/19-4/30/21. Chart review then determined if the test had a positive, negative, or no clinical impact.

Results. 25 mNGS tests were ordered on 24 unique patients. The majority of tests were ordered on immunocompromised patients (Table 1). Most mNGS tests were ordered after completion of routine microbiological testing (17/25, 71%). Three tests were not completed as ordered. Most completed tests (18/22, 82%) had no impact on clinical care as they confirmed the known diagnosis or were not acted upon (Figure 1). mNGS testing had a positive impact in 2 cases. For one patient with congenital heart disease presented with persistent fever and concern for endocarditis despite negative infectious workup, a negative mNGS result allowed for continued monitoring without therapy. Another patient with a lymphatics disorder had mNGS performed due to persistent clinical instability; testing was positive for *Candida parapsilosis*, allowing for early initiation of antifungal therapy. However, test results had a negative clinical impact in 2 other patients. In a patient with congenital heart disease and fever, identification of two organisms led to prolonged antibiotic therapy for endocarditis without resolution of symptoms. In a patient with leukemia, report of a dematiaceous mold led to further diagnostic testing, including a lumbar puncture, as well as treatment with antifungal therapy despite no clear diagnosis.

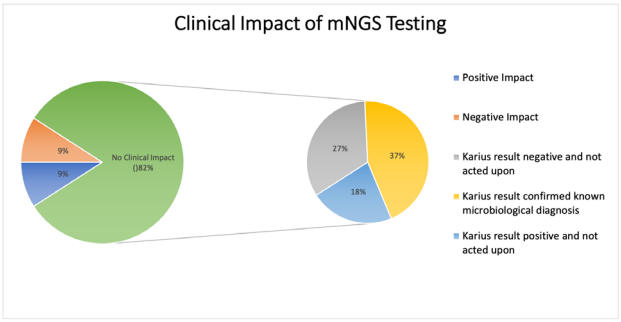

Table 1

Table 1: Clinical characteristics of patients who underwent mNGS testing and test results

Characteristic	Total (n=24)*
Age, years (median and range)	11.0 (0.2 – 28.0)
Male sex, n (%)	14 (58%)
Underlying condition	
Hematopoietic cell transplant, n (%)	5 (21%)
Active malignancy, n (%)	6 (25%)**
Primary Immunodeficiency, n (%)	3 (13%)
Rheumatologic disease, n (%)	2 (8%)
Other immunocompromised state, n (%)	3 (13%)
Congenital heart disease, n (%)	4 (17%)
None	1 (4%)
Admitted to ICU at time of testing, n (%)	12 (50%)
mNGS testing ordered after completion of routine microbiological testing, n (%)	17 (71%)
Completed Test	22 (88%)
Positive Test Results	15 (60%)
Patient ultimately died, n (%)	11 (46%)

Abbreviations: ICU, intensive care unit; mNGS, metagenomic next generation sequencing

*Twenty-four patients contributed 25 mNGS tests during the study period

**Including 2 patients who received chimeric antigen receptor T cell therapy

Conclusion. In this study, the majority of plasma cell-free mNGS tests had no impact on clinical care. mNGS testing did positively impact care in 2 patients, but did had a negative impact on care in 2 instances, leading to further testing and unnecessary treatment. Further investigation is needed to determine the ideal population or clinical condition for testing and the ideal time of sending plasma cell-free mNGS tests.

Disclosures. **All Authors**: No reported disclosures

665. Clinical and Financial Impact of Next Generation Sequencing (NGS) in addition to Conventional Microbiology Testing in our Urban Referral Health Center

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Session: P-30. Diagnostics: Typing/sequencing

Background. Clinical microbiology traditionally relies on culture methodology and serological testing, that have inherent limitations. Newer diagnostic techniques such as Next Generation Sequencing (NGS) have shown promise to improve microbial identification. In select scenarios, we send clinical specimens to reference laboratories for NGS testing in addition to current standard of care (SOC) diagnostics. We wanted to determine how this diagnostic approach has impacted patient care. We also wanted to review the financial burden through cost-benefit analysis for these 'send-out' tests.

Methods. We performed a retrospective chart review of all cases over a 3-year period in which NGS was submitted. Data, including demographics, comorbidities, antimicrobial use, and diagnosis (by SOC and NGS) were gathered. We delineated how often there was concordance or discordance between SOC and NGS. We also obtained