#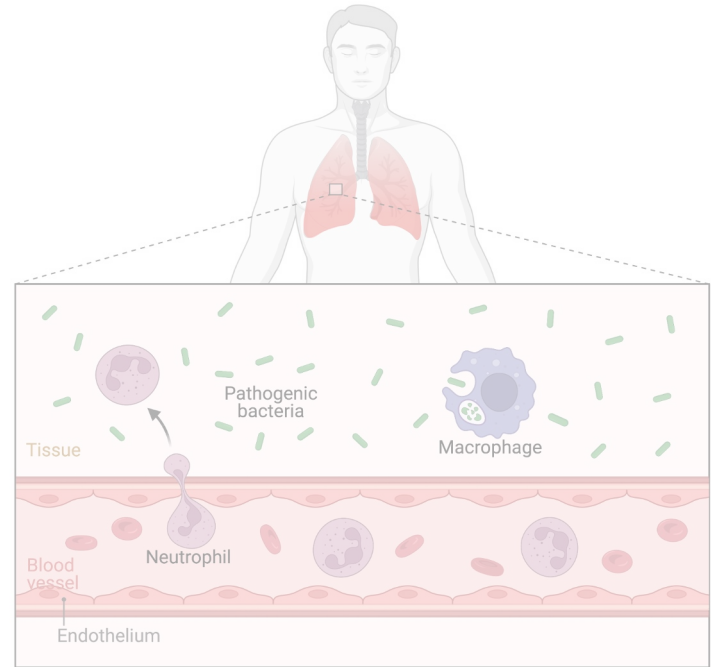 Clinical Impact of Metagenomic Next-Generation Sequencing of Plasma Cell-Free DNA for the Diagnosis of Infectious Diseases

Catherine Hogan, MD, MSc, FRCPC, DTM&H, D(ABMM)
Medical microbiologist, BCCDC
Clinical Assistant Professor, University of British Columbia
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THE UNIVERSITY OF BRITISH COLUMBIA

BC Centre for Disease Control

Disclosures

- Inflammatix: Advisor/Consulting (Ongoing)
- All relevant financial disclosures have been mitigated.

Plan

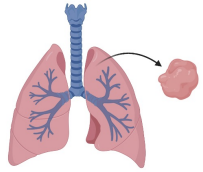Background and testing landscape – who, what?

Focused review of the literature – who, what?

Clinical impact assessment – who, what, when, why?

Opportunities and moving forward – where to?

Background

1

Infections account for significant disease burden worldwide

Clinical syndrome etiology remains unknown in up to 60%

Current state

2

Frequent need for invasive testing

Culture-dependent methods, associated with longer TAT and reduced sensitivity on antimicrobial therapy

Targeted approach requires *a priori* knowledge of what to order

Clinical metagenomics

3

Hypothesis-free, culture-independent, direct from clinical specimen

Potential for enhanced diagnostic yield and antimicrobial stewardship

Expensive, complex, challenge of standardization, challenging result interpretation

Simner PJ et al. *Clin Infect Dis* 66(1), 778–788 (2018).

Plasma testing landscape

Sample type	Company/ site	Live since	Approach	Organisms detected	Result interpretation	Turnaround time
Plasma	**Karius** (Redwood City, CA, USA)	December 2016	NGS of microbial cell-free DNA	Bacteria DNA viruses Fungi Protozoa	Organism(s) detected Quantitative result (MPM) No interpretation Reference threshold	Median 26 hours (IQR 25-28)
	UCSF - on hold (San Francisco, CA, USA)	2020 -On-hold- since Oct 2021	mNGS	Bacteria DNA viruses Fungi Parasites	Organism(s) detected Clinical interpretation as free text	1-2 weeks
	Noscendo (Reutlingen, German)	2020	NGS of microbial cell-free DNA	Bacteria DNA viruses Fungi Parasites	Organism(s) detected Clinical information as free text	<24 hours from sample receipt

2. Focused review of the literature

Plasma – test performance

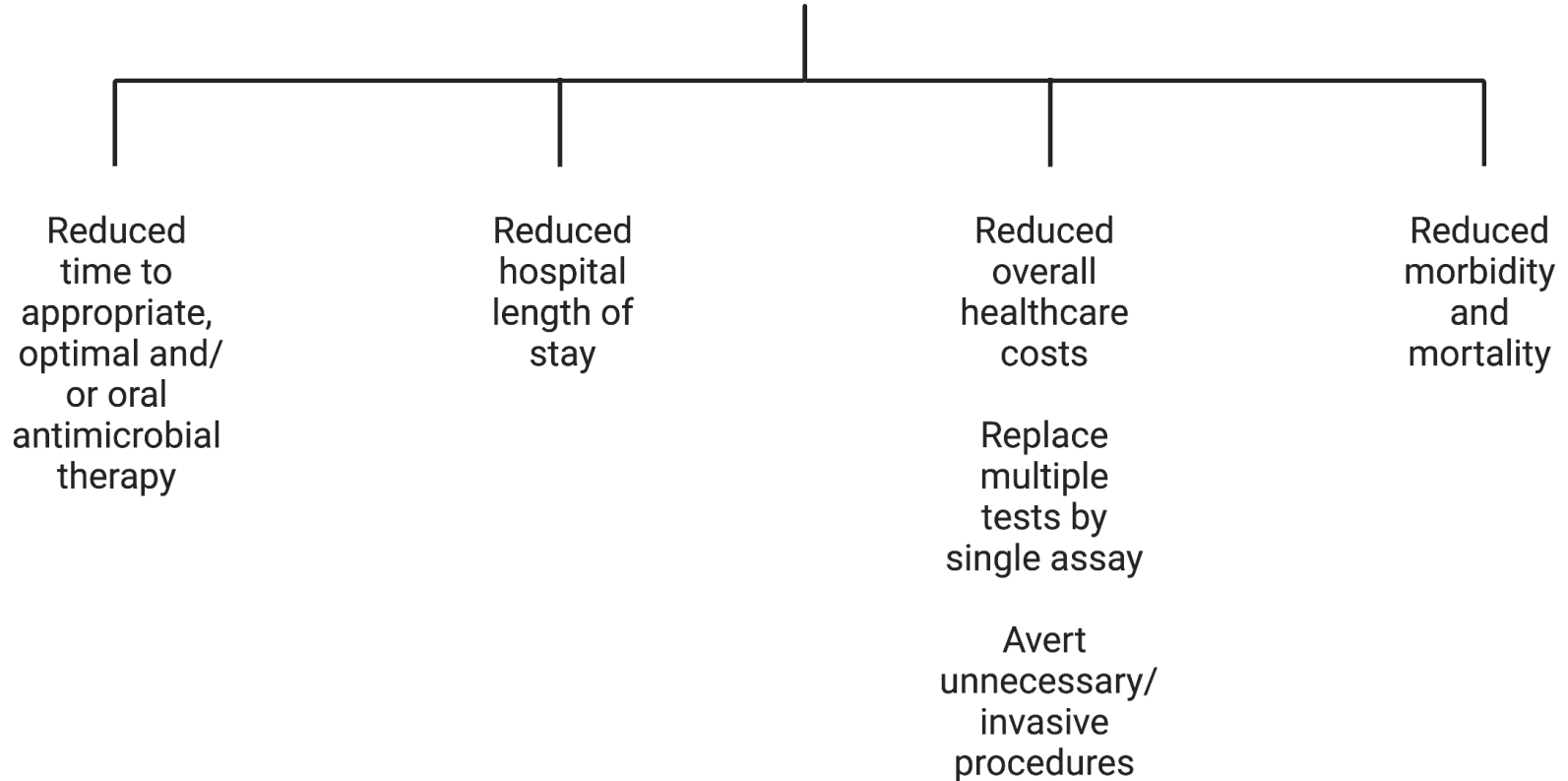| Reference	Assay	# samples	Patient population	Positivity rate	Category	Main findings
Blauwkamp et al. Nature Micro 2019	**Karius**	358 contrived 2,625 in silico 580 clinical	IC Sepsis Endocarditis Complicated pneumonia	53.7% (of which 50.4% poly)	Contrived samples with 13 organisms Individuals with sepsis alert (n=348)	LoD 33-74 molecules/uL (MPM) Except *P. aeruginosa* 415 MPM vs initial BCx: **Sen: 93.7%** (95%CI 84.5-98.2) **Spe 40.0%** (95% CI 34.3–45.9) vs all micro testing: **Sen: 84.8%** (95%CI 77.6–90.5) **Spe 48.2%** (95% CI 44.3–55.0)
Unpublished	**UCSF**	≅200	-	-	-	mix of comparator: **Sen: 77%** **Spe: 86%**
Grumaz et al. Critical Care Medicine: May 2019 - Volume 47 - Issue 5 - p e394-e402	**Noscendo**	256 samples of 48 septic patients	Septic shock	66.7%	Septic shock patients	vs initial BCx, after excluding FP: **Sen: 71.4%** **Spe: 28.3%**

3. Clinical impact assessment

'Of ultimate importance is the ability of a new technology to impact favorably on infectious disease outcomes.'


Based on ilustration by E.H. Shepard

Doern GV. *J Clin Microbiol*. 52(5):1314-6 (2014).

Potential benefits of plasma metagenomics

Reduced time to appropriate, optimal and/ or oral antimicrobial therapy

Reduced hospital length of stay

Reduced overall healthcare costs

Replace multiple tests by single assay

Avert unnecessary/ invasive procedures

Reduced morbidity and mortality

Ref.	# patients	Study population	Study design	Approval required	Indication	Definition of impact	Positivity rate	Main findings
Rossoff et al. OFID 2019	79 (100 tests)	Pediatric (100%) IC (76%)	Retrospective single site study, Chicago (timing not specified)	No	Suspected IFI Sepsis Fever LN	Management decision based on result	70 (70.0%) 33 poly	56 (80%) clinically relevant 14 mNGS only ↑ utility IC
Niles DT et al. JCM 2020	60	Pediatric (100%) IC (62%)	Retrospective single site study, Houston Concurrent testing (± 1wk)	No	Lung lesion Unclear FN Sepsis	Addn/Δ ATBx	38 (63.3%) 16 poly	PPA 61% NPA 58% CT 3.5d earlier Addn: 74%: no Δ
Lee et al. JCM 2020	54 (59 tests)	Pediatric (100%) IC (56%)	Retrospective single site study, Boston (testing median 8 days into workup)	Yes	Resp FUO Multisite	Standardized criteria/ research team assessment	29 (49%) 10 poly	Impact 14% PPA 53% NPA 79% ↑utility IC
Hogan CA et al. CID 2021	82 (98 tests)	Adults (47.6%) Children (52.4%) IC (65%)	Retrospective multicenter study, 5 U.S. sites (± 1wk)	No->yes	FUO Resp IE	Standardized criteria/MD assessment	50 (61.0%) 25 poly	No impact 86.6% Pos impact 7.3% Neg impact 3.7%
Duan H et al. BMC Inf Dis 2021	109 total 37 blood	Adult (100%) IC (NA)	Retrospective single site study, Shanghai (timing not specified)	NA	Resp BSI	Multivariable analysis of Px	79 (72.5%) Overall NA	mNGS-pos = poor Px
Shishido AA et al. BMC Inf Dis 2022	80	Adult (100%) IC (56%)	Retrospective single site study, Baltimore (up to weeks in course)	Yes	Resp Sepsis IE	Standardized criteria/MD assessment	49 (61.3%) NA	No impact 55% Pos impact 43% Neg impact 3%

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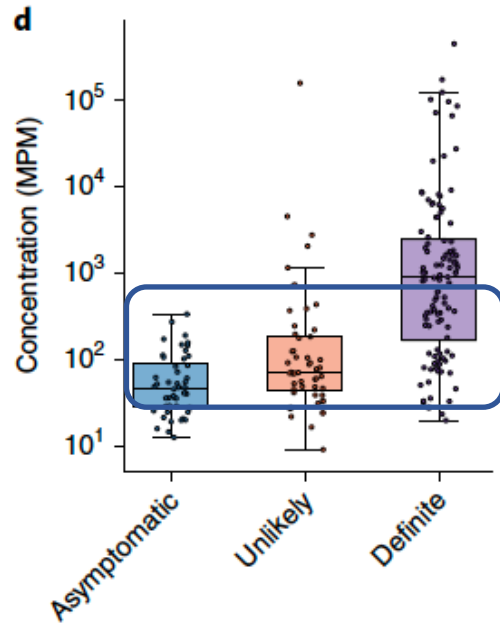# Quantitative result interpretation
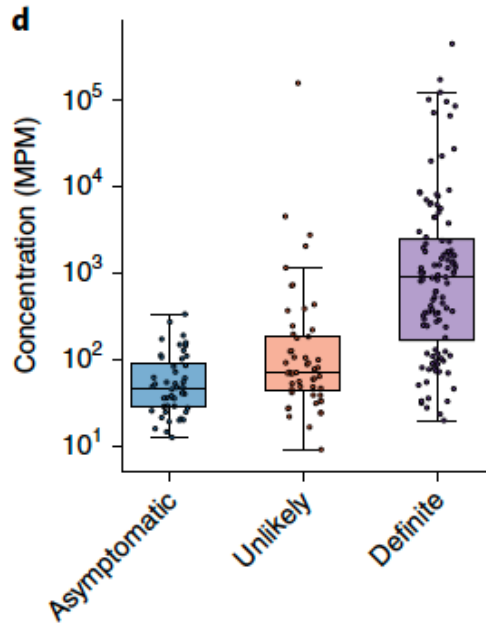
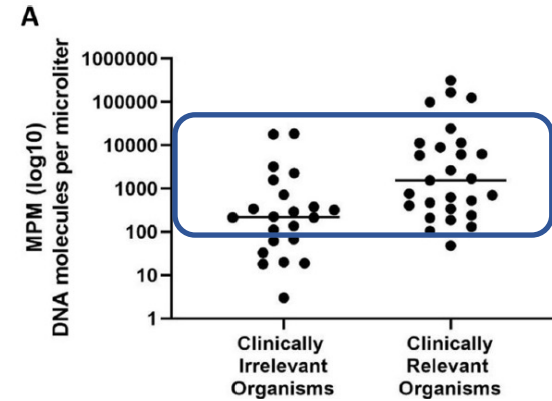Blauwkamp, T.A. *et al. Nat Microbiol* 4, 663–674 (2019).
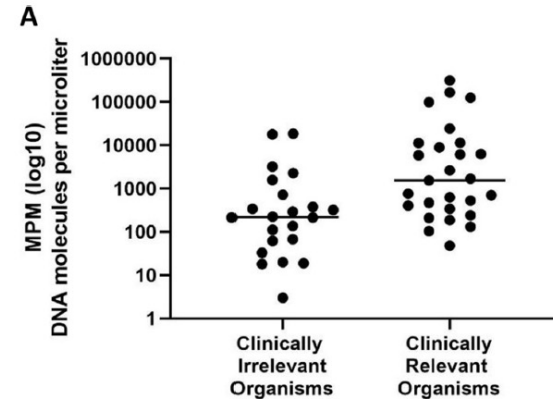
Lee, R.A. *et al. J Clin Microbiol*. 58(7):e00419-20 (2020).

Quantitative result interpretation

Blauwkamp, T.A. *et al. Nat Microbiol* 4, 663–674 (2019).

Lee, R.A. *et al. J Clin Microbiol*. 58(7):e00419-20 (2020).

Febrile neutropenia and invasive fungal infection

Ref	# patients	Study population	Study design	Definition of impact	Positivity rate	Main findings
Benamu E et al. CID 2021	55	Adults with acute leukemia and febrile neutropenia (plasma within 24 hours of fever)	Prospective cohort study, single site, Stanford	Hypothetical assessment by clinical adjudication	85% of all samples 61% poly	47% of patients may have benefitted from earlier antimicrobial optimization
Hill et al. CID 2020.	114	Adults with pulmonary IFI post-HSCT (plasma within 14 days of diagnostic testing)	Retrospective, single site, Seattle	Incremental diagnostic test performance	38 (51%) of proven/probable IFI detected mold	Moderate sensitivity Additional yield to GM (84% Sen combined)

Understanding drivers of clinical impact

Different anti-microbial treatment proposed had KT result been available in real time	No	29 / 55 (52.7)
	Yes	26 / 55 (47.3)
Antibiotic	Addition**	11 / 55 (20)
	No Change	33 / 55 (60)
	Withdrawal***	14 / 55 (25.5)
Antiviral	Addition	8 / 55 (14.5)
	No Change	47 / 55 (85.5)
Antifungal	Addition	2 / 55 (3.6)
	No Change	51 / 55 (92.7)
	Withdrawal	1 / 55 (1.8)
	Not applicable	1 / 55 (1.8)
MRSA coverage	Addition	1 / 55 (1.8)
	Withdrawal	5 / 55 (9.1)
	Not applicable	49 / 55 (89.1)
Anaerobes coverage	Addition	7 / 55 (12.7)
	Not applicable	48 / 55 (87.3)
Narrowing of antibiotic spectrum	Yes	8 / 55 (14.5)
	Not applicable	47 / 55 (85.5)
Broadening of antibiotic spectrum	Yes	1 / 55 (1.8)
	Not applicable	54 / 55 (98.2)
Anti-viral coverage CMV	Addition	1 / 55 (1.8)
	Not applicable	54 / 55 (98.2)
Anti-viral coverage HSV	Addition	7 / 55 (12.7)
	Not applicable	48 / 55 (87.3)

Benamu E *et al. Clin Infect Dis* 74(9):1659-1668 (2021).

How do you measure clinical impact?

- **Stewardship metrics**
  - Time to first antibiotic change
  - Time to appropriate antibiotic escalation or de-escalation
  - Days of therapy (DOT) of antibiotics

- **Infection control endpoints**
  - Acquisition of new hospital-acquired infections

- **Clinical outcomes**
  - All-cause mortality, cause-specific mortality
  - Hospital/ED length-of-stay (LOS)
  - ICU admission rates
  - Adverse events rates:
    - Acute kidney injury (AKI)
    - *C. difficile* infection (CDI)

- **Cost**

Munson EL *et al*. *JCM* 41:495-497 (2003)
Beekman SE *et al*. *JCM* 41: 3119-3125 (2003)
Banerjee R *et al*. *CID* 61(7):1071-1080 (2015)

Key clinical variables for plasma mNGS impact

Variable	Target
Patient population	Define high-yield patient populations most likely to maximize impact
Testing indication	Identify clinical syndromes most likely to maximize impact
Testing timeline	Define optimal timing of mNGS relative to conventional testing
Breadth of testing	Define degree of unbiased testing required
Evidence base	Assess impact through prospective, population-level data, not top hits only
Clinical impact	Standardize definitions for research, differentiate hypothetical vs real world
Provider and patient behavior	Build-in qualitative research to understand barriers and optimize impact
Multidisciplinary team approach	Partner with key stakeholders to improve interpretation and increase impact

Welcome to the real world!

'Anyone who has worked on ward XYZ knows that it doesn't matter what result you bring to the treating team, they will only de-escalate once patient has clinically improved and they feel comfortable doing so.'

- Anonymized colleague

4.

Opportunities and moving forward

Prospective studies

Head-to-head data comparing different approaches

Standardization of testing chronology and comparator conventional diagnostic tests

Defined infectious clinical syndromes

Incremental value and cost-effectiveness analyses for plasma metagenomics

Improved testing strategies

Optimized classification of pathogen vs colonizer

Integration of genotypic predictions of antimicrobial resistance

Integration of host-response results

Multidisciplinary partnerships

Optimized criteria of who and when to test

Tight collaboration between:
Clinical teams
ASP
Laboratory medicine

Collaboration with social sciences colleagues for identification and engagement on factors influencing provider willingness to act on results

Take-home points

- **Who:** varies (need more data!)
- **What:** single plasma metagenomics assay currently in North America
- **When:** depends (need more data!)
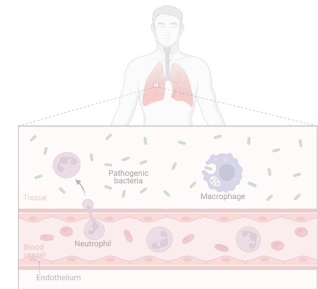
Take-home points

- Who: varies (need more data!)
- What: single plasma metagenomics assay currently in North America
- When: depends (need more data!)

- Test performance varies across indications and organisms
- Plasma metagenomics holds the potential to improve diagnosis of infectious diseases and clinical patient outcomes
  - Several important challenges remain to leverage this possible impact
  - Need standardized approaches
- Best integrated within multidisciplinary effort with stewardship
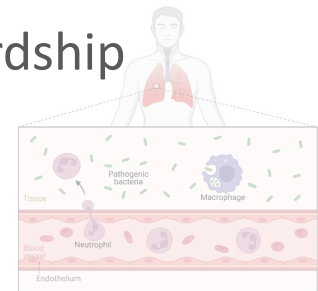
Thank you!

- Acknowledgments:

    - BCCDC (John Tyson) for methodological input

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    - Representatives from Karius (Matt Smollin), UCSF (Steve Miller) and Noscendo for input on test information

    - BioRender for slide design