Using metagenomic sequencing in clinical practice

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Disclaimers

- These slides represent consensus views at UCSF; this is an area of evolving evidence
- No personal disclosures

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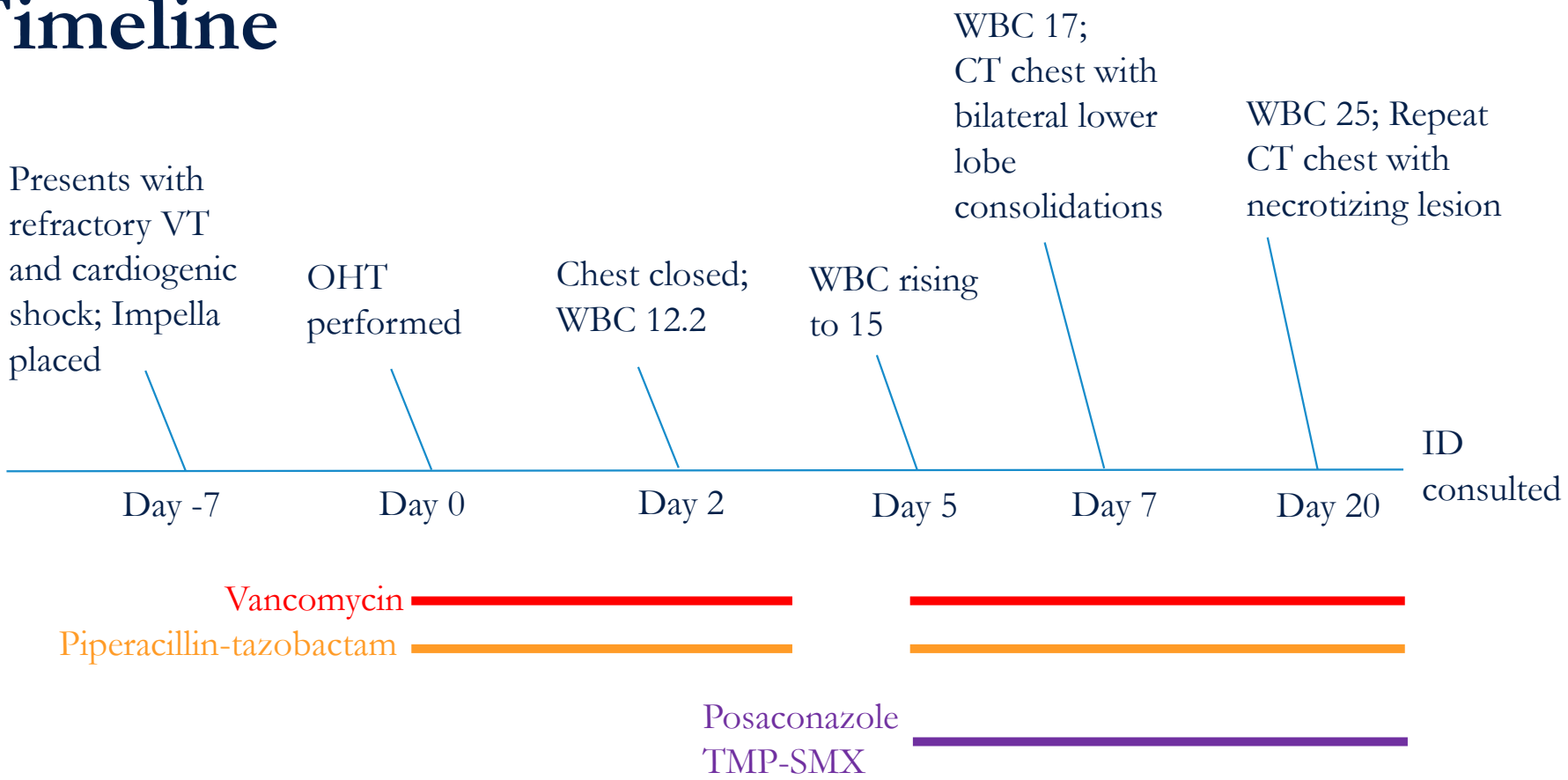
A challenging clinical case

- 56yo with orthotopic heart transplant 3 weeks prior (CMV D+/R+), thymoglobulin induced, on tacrolimus, mycophenolate mofetil and prednisone.

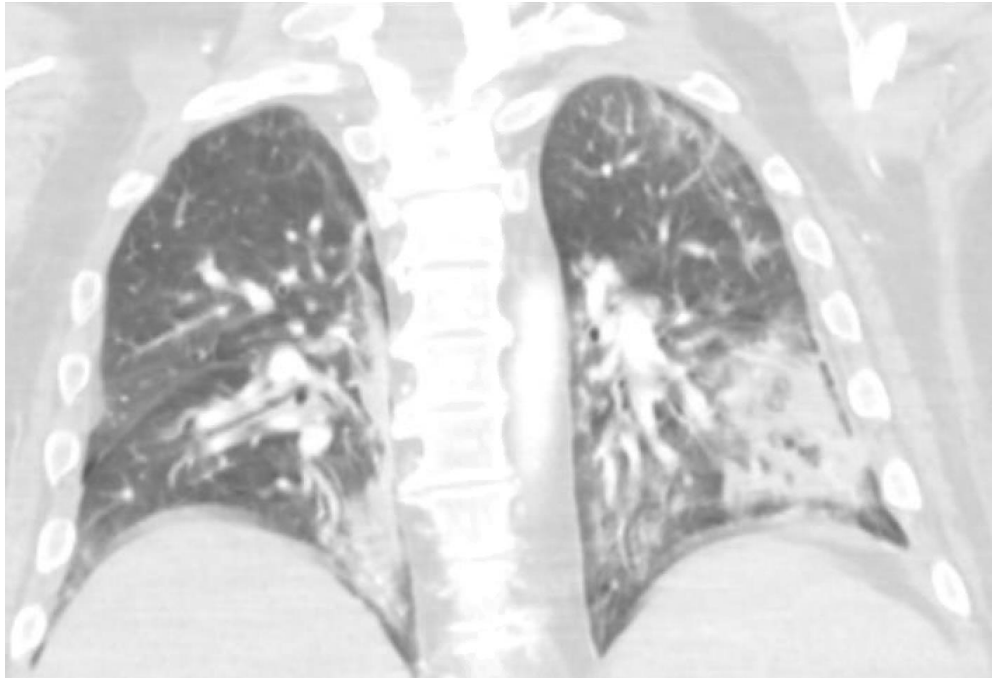
- **Consult question: diagnostics and therapeutics for cavitating pneumonia**

Timeline

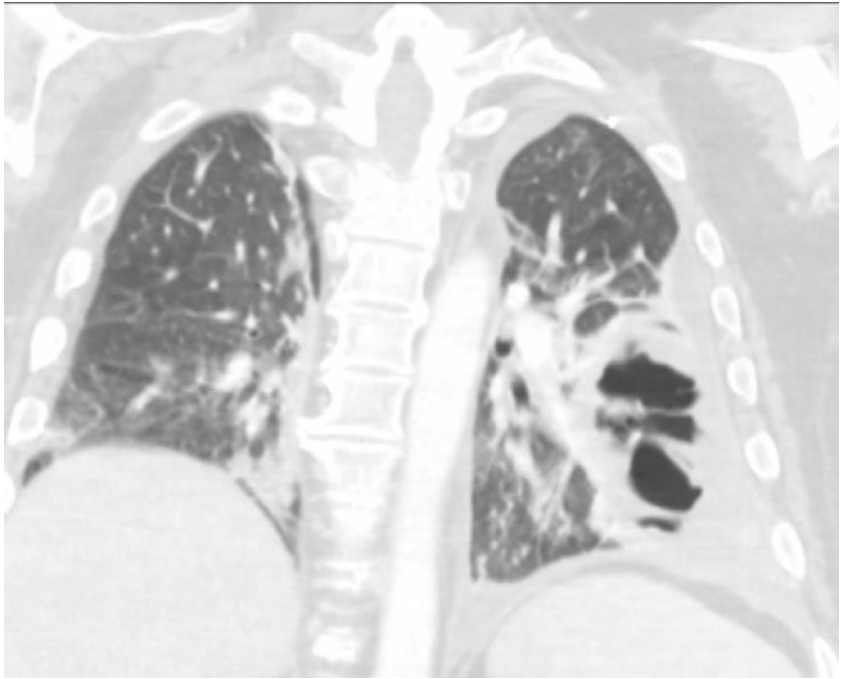

Presents with refractory VT and cardiogenic shock; Impella placed
OHT performed
Chest closed; WBC 12.2
WBC rising to 15
WBC 17; CT chest with bilateral lower lobe consolidations
WBC 25; Repeat CT chest with necrotizing lesion
ID consulted
Day -7
Day 0
Day 2
Day 5
Day 7
Day 20
Vancomycin
Piperacillin-tazobactam
Posaconazole
TMP-SMX

The imaging

Day 7 post-transplant



Day 20 post-transplant



Workup to date

Cultures:
Blood cultures x2 negative
Sputum cultures:
- Bacterial with oral flora
- Mycobacterial negative
- Fungal negative

Antibodies:
Coccidioides antibodies: negative
Histoplasma antibodies: negative
HIV antibody: negative
Quantiferon gold: negative

Antigens:
Cryptococcus Ag negative
Coccidioides Ag: negative
Histoplasma Ag: negative

PCR tests:
COVID negative
Respiratory viral panel negative
Nasal legionella: negative
CMV plasma: negative

Fungal markers:
B-d-glucan: 31 (normal)
Galactomannan: 0.2 (normal)

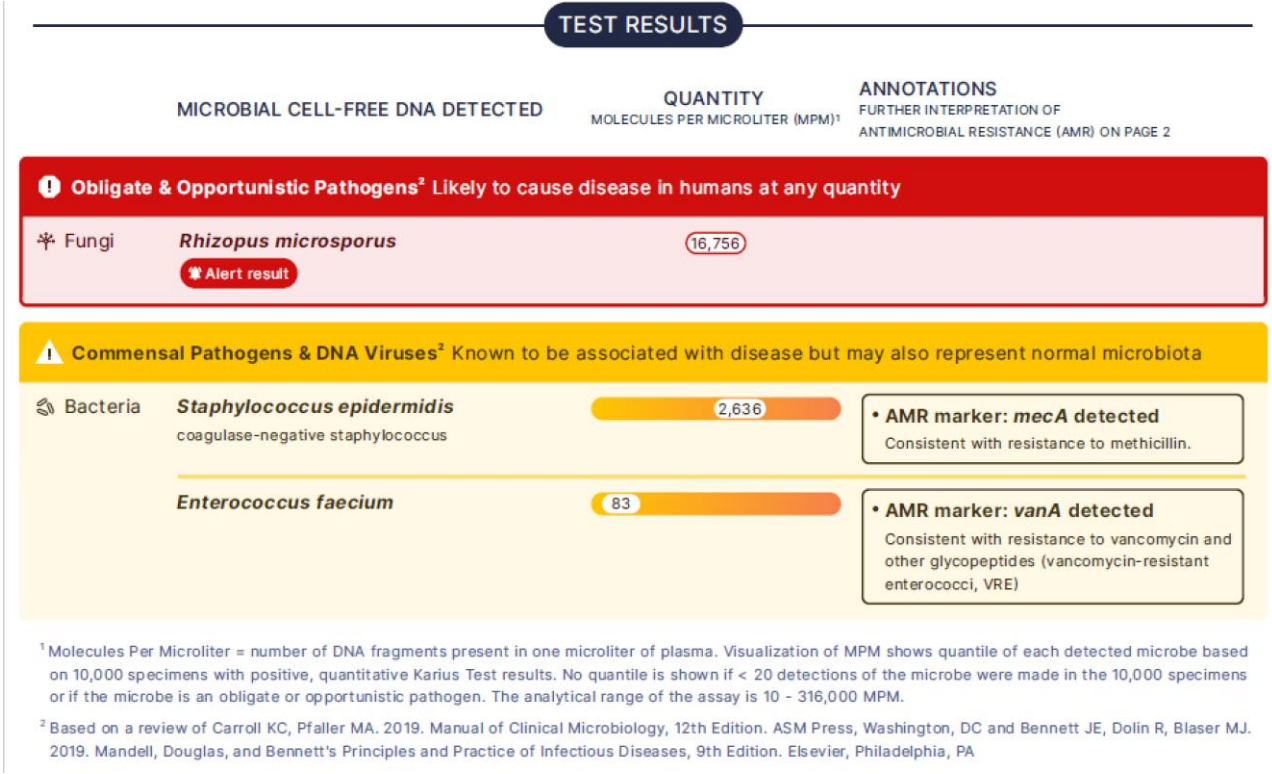
Pleural fluid from thoracentesis
- Bacterial cultures: negative
- Mycobacterial cultures: negative to date
- Fungal cultures: negative to date

Pulmonology is consulted for bronchoscopy; defers pending pleural results

Question 1: What empiric therapies would you recommend now?

1) Continue vancomycin and piperacillin-tazobactam only
2) Add azithromycin for atypical organism coverage
3) Add liposomal amphotericin for additional fungal coverage
4) Call back Pulmonology
5) Consult a surgical team

A diagnostic test returns…



TEST RESULTS

MICROBIAL CELL-FREE DNA DETECTED	QUANTITY (MOLECULES PER MICROLITER (MPM)[1])	ANNOTATIONS (FURTHER INTERPRETATION OF ANTIMICROBIAL RESISTANCE (AMR) ON PAGE 2)
Obligate & Opportunistic Pathogens[2] Likely to cause disease in humans at any quantity		
Fungi — ***Rhizopus microsporus*** Alert result	16,756	
Commensal Pathogens & DNA Viruses[2] Known to be associated with disease but may also represent normal microbiota		
Bacteria — ***Staphylococcus epidermidis*** coagulase-negative staphylococcus	2,636	• **AMR marker: *mecA* detected** Consistent with resistance to methicillin.
Enterococcus faecium	83	• **AMR marker: *vanA* detected** Consistent with resistance to vancomycin and other glycopeptides (vancomycin-resistant enterococci, VRE)

[1] Molecules Per Microliter = number of DNA fragments present in one microliter of plasma. Visualization of MPM shows quantile of each detected microbe based on 10,000 specimens with positive, quantitative Karius Test results. No quantile is shown if < 20 detections of the microbe were made in the 10,000 specimens or if the microbe is an obligate or opportunistic pathogen. The analytical range of the assay is 10 - 316,000 MPM.

[2] Based on a review of Carroll KC, Pfaller MA. 2019. Manual of Clinical Microbiology, 12th Edition. ASM Press, Washington, DC and Bennett JE, Dolin R, Blaser MJ. 2019. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 9th Edition. Elsevier, Philadelphia, PA

- Plasma mNGS returns
- Urgently taken to OR by thoracic surgery for LLL lobectomy
- Liposomal amphotericin started in addition to posaconazole

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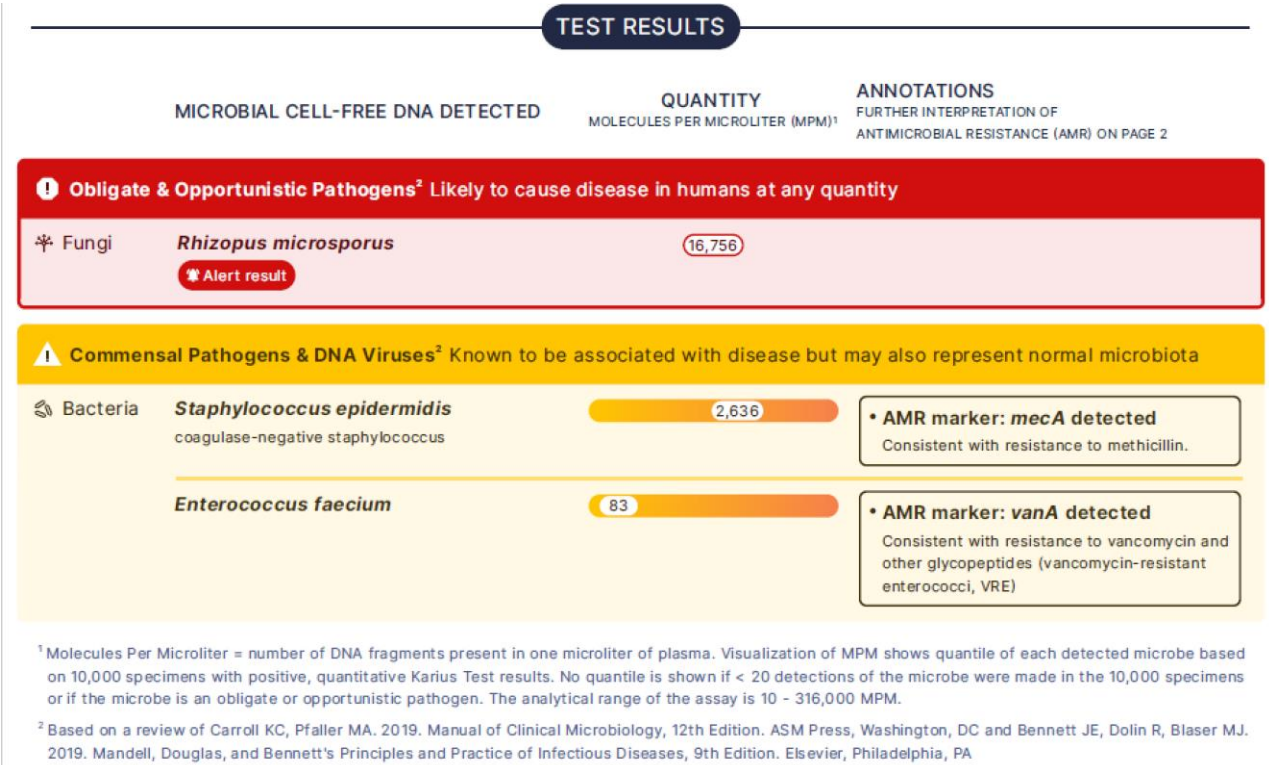

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Question 2: With this test result, would you treat the bacterial organisms?

1) Yes
2) No
3) Maybe – it depends

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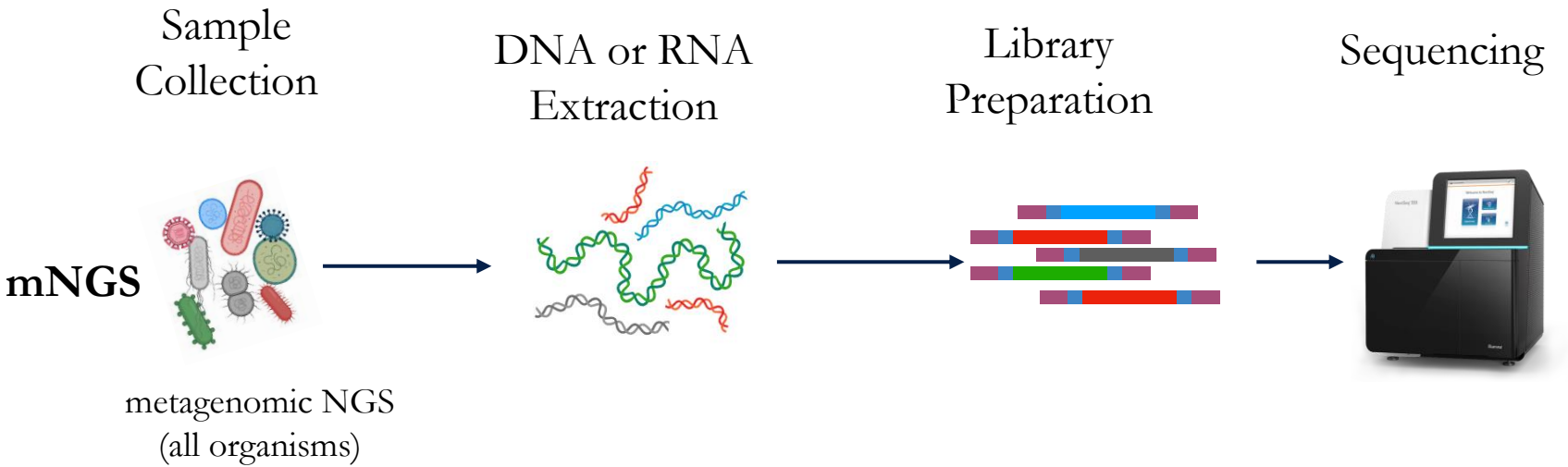
Talk goals

- Explain how mNGS fits into the infectious disease diagnostic armamentarium
- Review evidence for plasma mNGS for diagnosis of immunocompromised patients with pulmonary lesions
- Review evidence for plasma mNGS for diagnosis in neutropenic fever
- Discuss the current UCSF guidelines for plasma mNGS use

Metagenomic sequencing



Go Fish and diagnostic bias

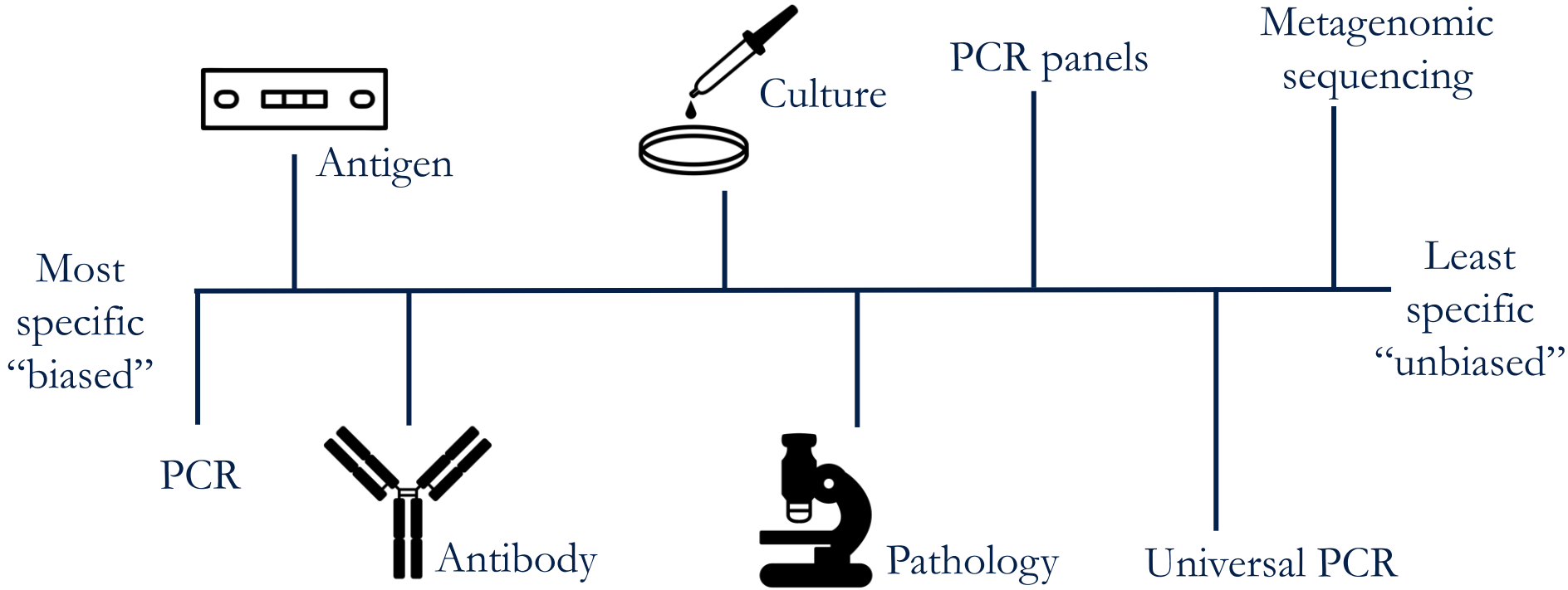
A biased test answers the question: "Do you have a *yellow* fish?"

An unbiased test answers the question: "What fish do you have?"

Most tests are *biased*

Wikipedia image

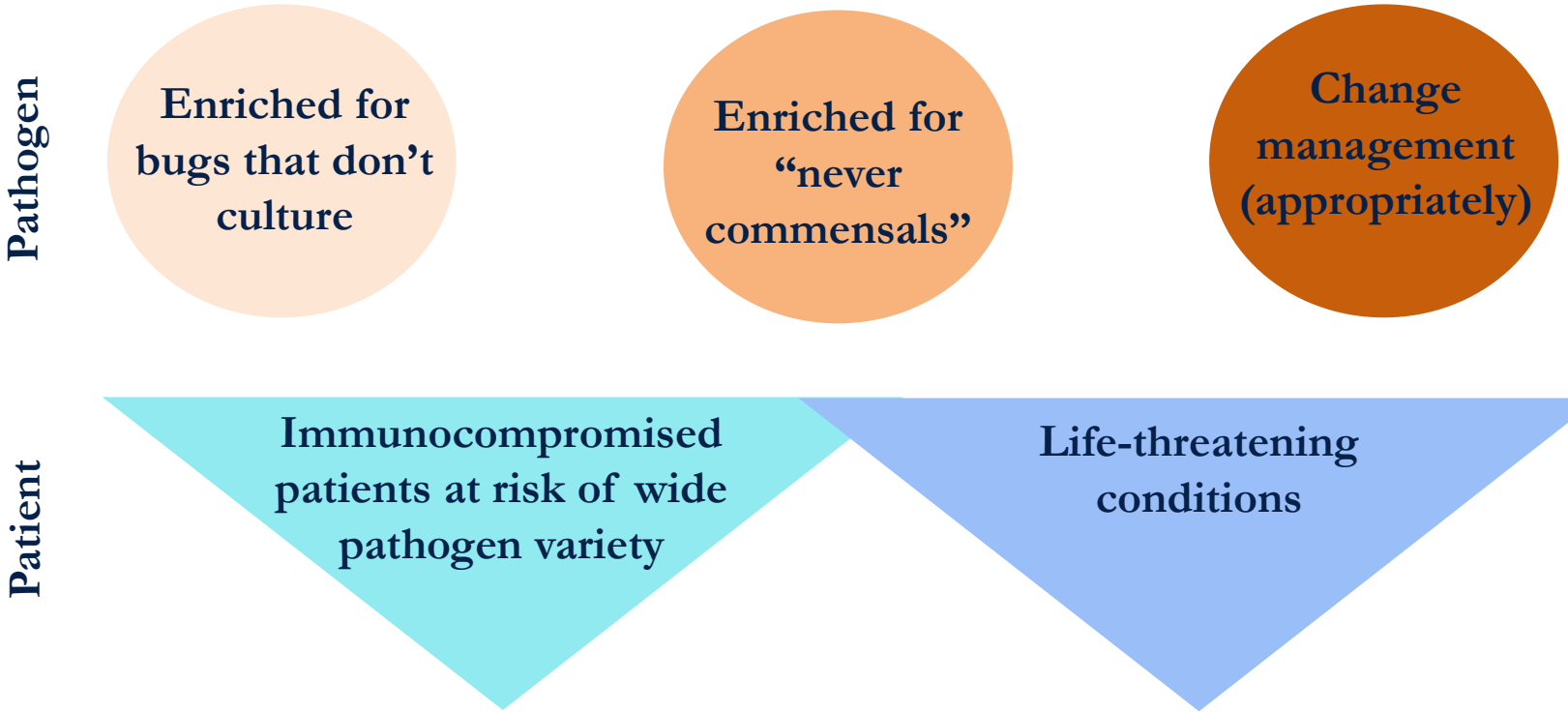
Clinical diagnostics summary



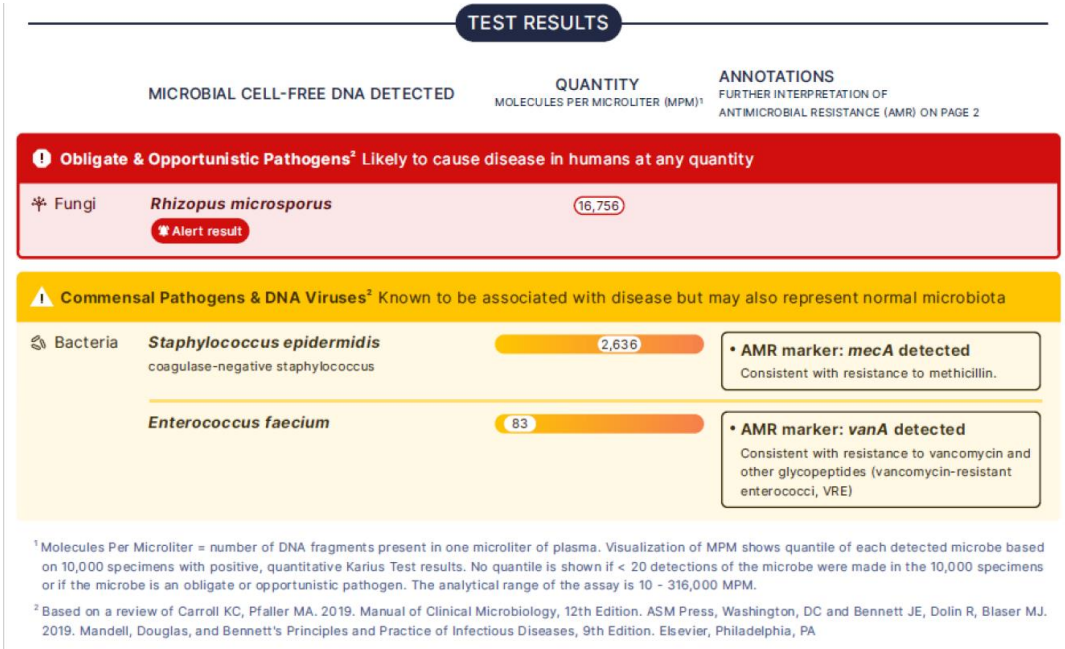
A clinician's view of mNGS challenges

Specificity	Technical contaminants	*Delftia,* other water-dwelling organisms
	Biologically present but of unclear relevance	Frequent detection of GI/oral flora in critically ill patients. mNGS plasma detection ≠ blood culture. DNA viruses of unclear significance Risk of **inappropriate antimicrobial escalation**
Sensitivity	Negative predictive value not known	Most likely not as sensitive as PCR given no amplification steps Unclear how to interpret a negative Risk of **inappropriate antimicrobial de-escalation**

What makes a mNGS diagnosis high impact?



Interpreting plasma mNGS: the bugs



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Obligate/Opportunistic pathogens

- Always assume true pathogen = most fungi, highly pathogenic bacteria (*Legionella*)

Commensals/DNA Viruses

- Do not assume that mNGS detection = bacteremia
- May indicate gut or mouth "leak"
- DNA viruses may be present but not causing pathology
- We send orthogonal test if unclear (CMV PCR, bacterial blood culture)

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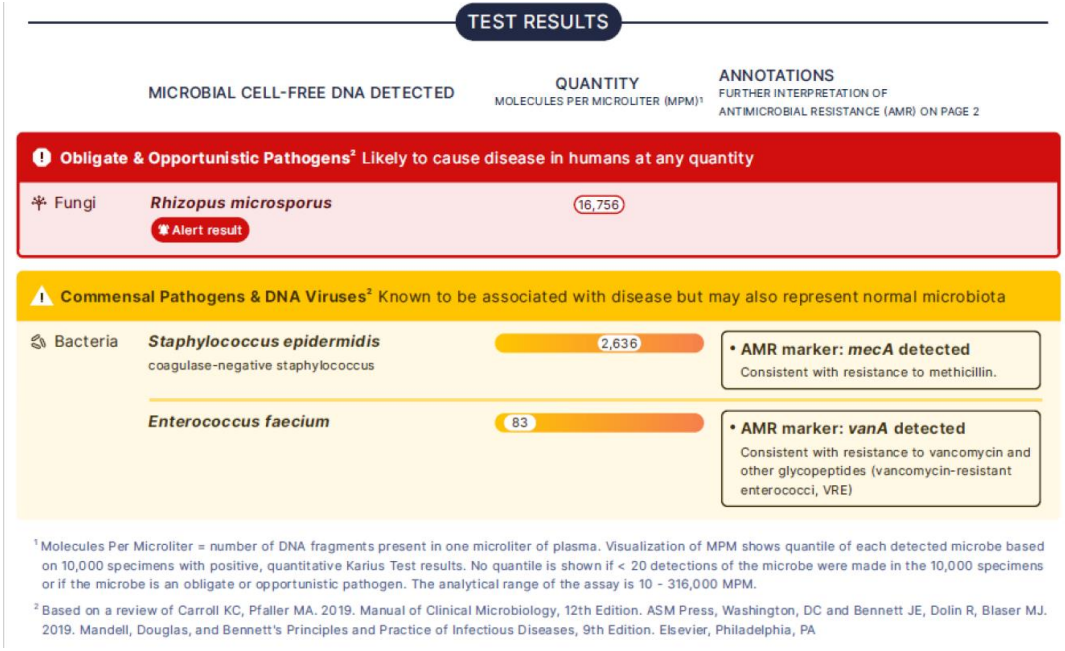
Interpreting plasma mNGS: MPM and AMR



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- MPM = molecules per microliter, rough estimate of how much pathogen is present
- May be correlated with PCR measures within a particular organism (e.g. CMV)
- Cannot really compare across different organisms
- Antimicrobial resistance (AMR) is in beta-testing. Our practice:
  - Gram-positives: better data, may broaden abx based on this result
  - Gram negatives: really insufficient data, would not change therapy

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Plasma mNGS: the logistics

- Goes to commercial company (Karius)
- Turn around time variable, but usually 3-4 weekdays
- Tests are often rejected due to incorrect collection
- Cost: ~$1500/sample

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Severely immunocompromised patients with pneumonia

- Key study: PICKUP
  - Multicenter, prospective, observational study comparing Karius mNGS to standard of care in immunocompromised adults*
  - All patients had pneumonia of unclear etiology

Bergin et al 2024 CID

*Immunocompromised = hematopoetic stem cell transplant or hematologic malignancy

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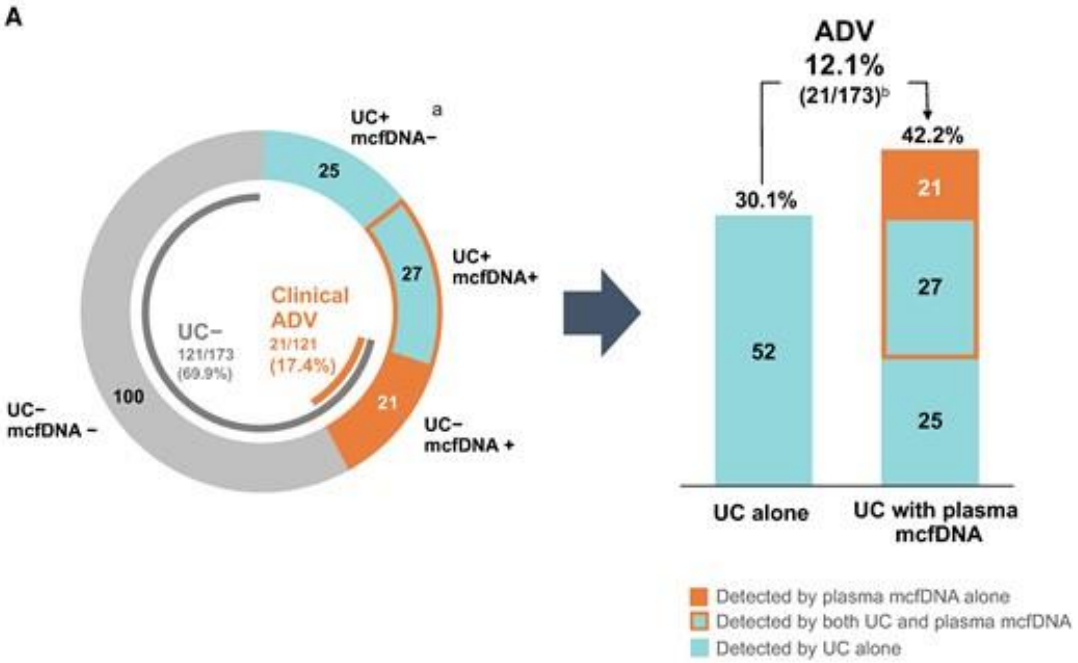
How Karius performed in PICKUP



- 30.1% of patients received a diagnosis through usual care (UC)
- 42.2% of patients received a diagnosis through a composite of UC and Karius testing
- 21/173 (12.1%) patients exclusively diagnosed by Karius

Bergin et al 2024 CID

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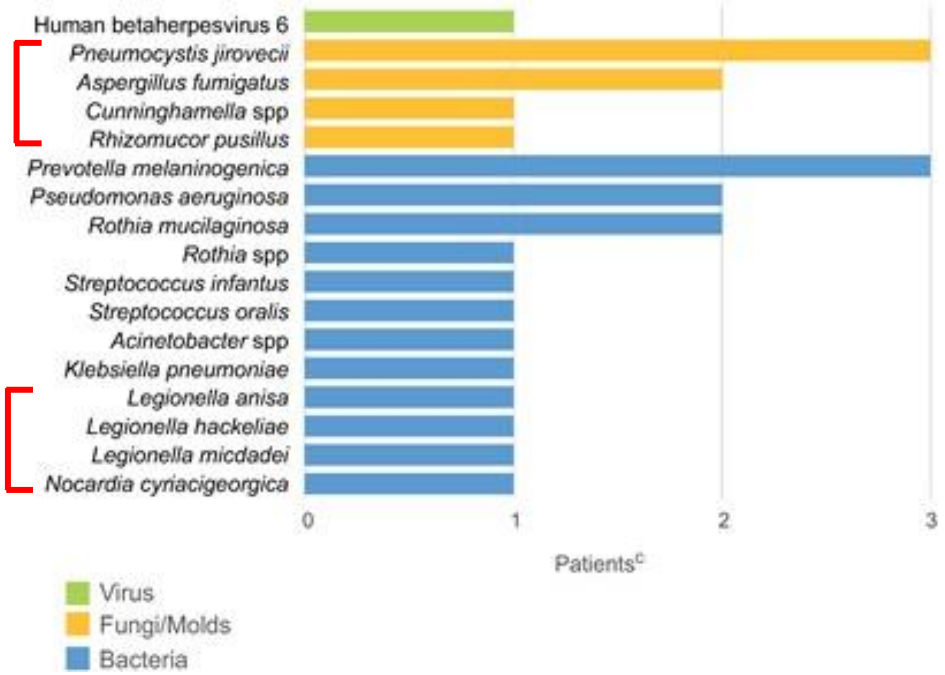
Patients diagnosed only by mNGS

- Of the 21 patients exclusively diagnosed by Karius, pathogens included fungi, bacteria, and viruses
- Retrospective review indicated that 17 diagnoses might have changed management (9.8% total patients)
- Notably, not all patients can get bronchoscopies, so this may *underestimate* benefit



Bergin et al 2024 CID

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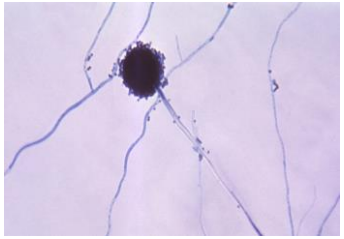
The diagnoses missed by mNGS

- 25/173 patients (14.5%) had positive diagnoses by usual care but negative Karius tests
- Inherently misses RNA viral pathogens
- Intriguing finding that mNGS may be worse at detection of *Aspergillus* than other fungi
- Another study (Hill et al 2021) have also shown what appears to be worse detection of *Aspergillus*



Bacteria **N = 7**	• All common respiratory bacterial pathogens
Viruses **N =4**	• All RNA viruses • Rhinovirus, SARS-CoV-2, parainfluenza
Fungi **N = 17**	• 1 Cryptococcus • 16 *Aspergillus* species

Bergin et al 2024 CID; Hill et al 2021 CID; Wiki

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Defining the problem of FN

Concern for infection

Core temperature

≥38.3 C once or ≥ 38.0 C for ≥ 1 hour

+

Profound immunocompromise

UCSF definition:
Absolute neutrophil count <500 *or* <1000 and falling

Usually from:
Cancer
Chemotherapy
Bone marrow transplant

- Most patients are on prophylactic antibiotics if neutropenic, before developing fever
- Only 20-30% of patients usually receive a microbiologic diagnosis

Friefield et al. CID. 2011

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A key febrile neutropenia study

Benamu et al. CID. 2022.

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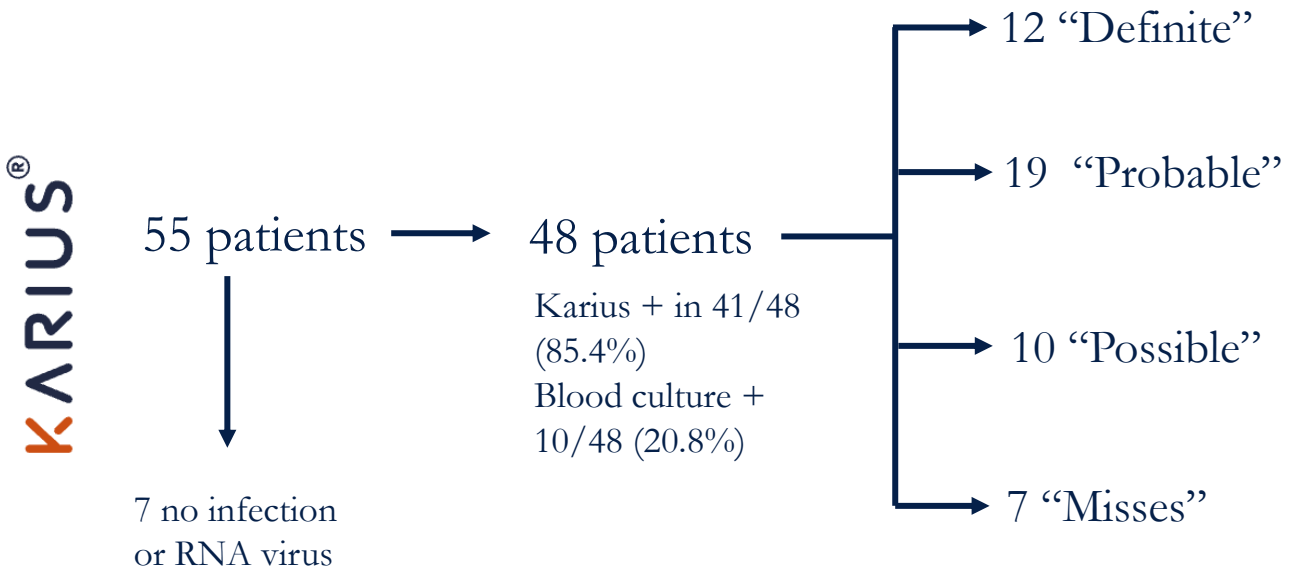
mNGS and neutropenic fever: the results



mNGS misses

- 2 with dental infections
- 2 with skin infections
- 2 with pulmonary *Aspergillus* infections
- 1 unclear

Authors estimate antimicrobial management would have changed in 26 patients (47.3%), but this assumes de-escalation on negative results…

Benamu et al. CID. 2022.

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A second FN study highlights hurdles

DISQVER®

98 FN episodes
61 patients

DISQVER + in
42/97 (43.3%)
Blood culture +
14 (14.3%)

Bacteria (n = 24)
6/24 probable skin flora or
mNGS contaminants

Viruses (n = 15)
7/15 HHV6

Fungi (n = 5)
3 Candida, 2 Aspergillus

Clinical Utility

In patients with +mNGS, ID doctors tried to answer: Would this result have changed management?

In 60% of cases, doctors could not agree

Schulz et al. Open Forum Infectious Disease. 2022.

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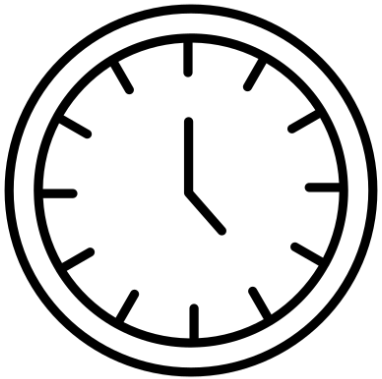
Question 3: How often do you use metagenomic next-generation sequencing?

1) Never
2) 1-2x a year
3) Monthly
4) Weekly
5) Daily

Defining timing

- **First line** = Sent concurrently with other immediate diagnostics (blood cultures, imaging, etc).
  - For inpatients, implies from the ED or immediately after admission.



- **Second line** = Sent following other syndrome-appropriate first-line diagnostics, including blood cultures, cryptococcal antigen, viral PCRs, and/or imaging.
  - For inpatients, implies ~2-3 days after admission.

UCSF Plasma mNGS: First Line Indications

1. Severely immunocompromised* patient with pneumonia, especially if:

- Concern for high concern for atypical infection such as IFI, Nocardia, Legionella
- Not responding to standard care

2. Fulminant CNS infection *and* sampling of CSF/CNS is not feasible or delayed

- High concern for CNS infection that reflects disseminated illness
- CNS/CSF sampling not technically possible

3. Fulminant infection *and* strong epidemiologic concern for atypical infection:

- High specific concern for disseminated *M. tuberculosis*, Nocardia, Coxiella/Q fever, Brucella, tularemia; invasive fungal infection

***Severely immunocompromised = bone marrow transplant within 1 year, solid organ transplant within 1 year, primary severe immunodeficiency**

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UCSF Plasma mNGS: Second Line Indications

1. Culture negative endocarditis *and*

- Negative first line workup (blood cultures, serological tests/antibodies as indicated)

2. Fever of unknown origin *and*

- Negative first line workup (blood cultures, imaging, malignancy/autoimmune workup)
- If patient is on empiric antibiotics, a trial of stopping them should be considered

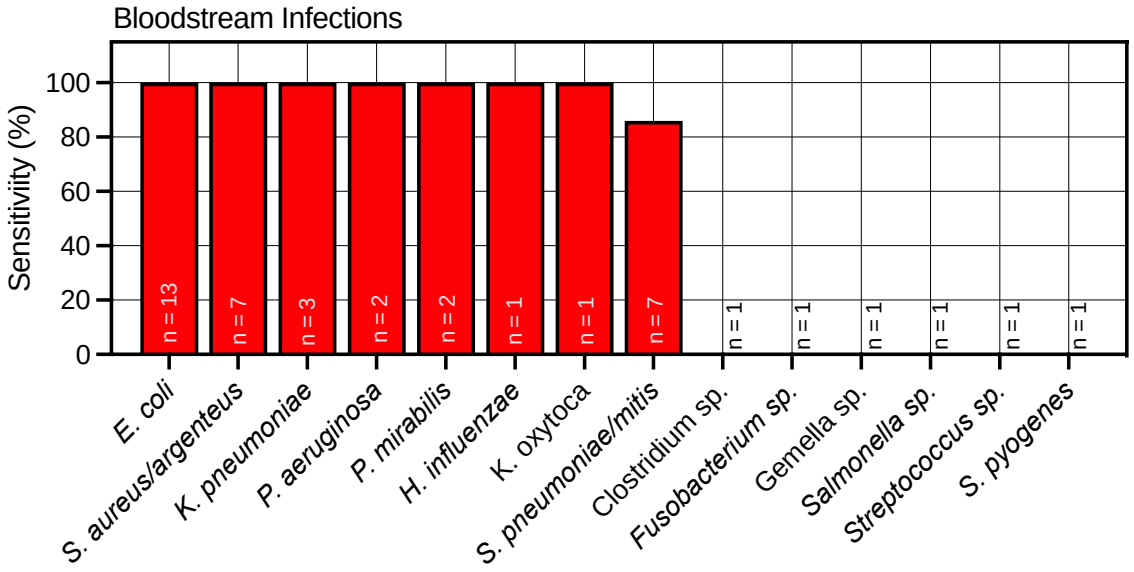
3. Deep seated lesions/abscesses (epidural, hepatic, splenic, peri-renal, pleural effusion) *and*

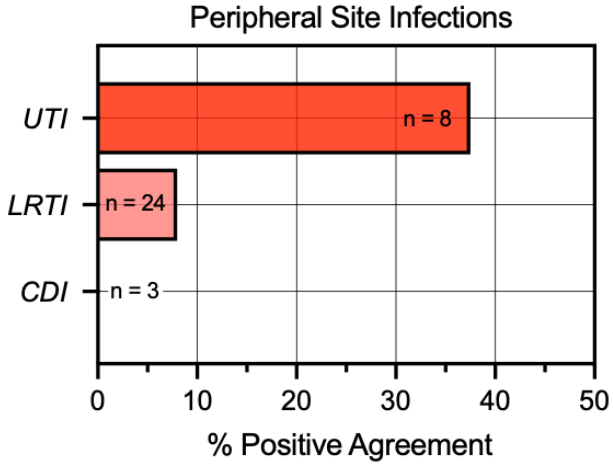
- Blood cultures negative
- Sampling of site unfeasible and/or unrevealing

4. Persistent febrile neutropenia *and*

- Negative first line workup (blood cultures, cross-sectional imaging)

Emerging indications: could mNGS be useful in sepsis?



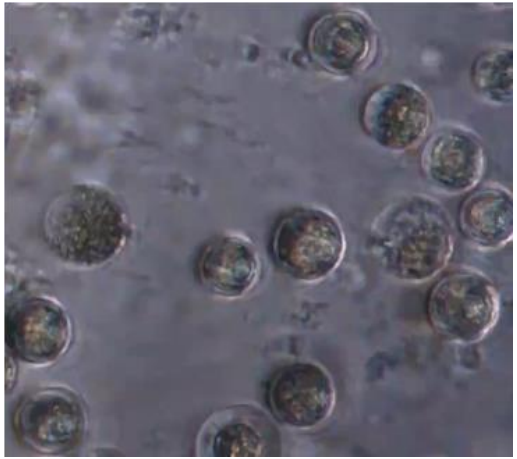


- Specificity 78%
- Possible pathogens identified in 42% of patients with culture negative sepsis

Kalantar and Neyton et al. Nature Microbiology. 2022

Plasma mNGS can sometimes diagnose CNS infection

- Patient with no past medical history developed neurological symptoms and fever.
- Lumbar puncture consistent with infection, but CSF cultures negative.
- Imaging ambiguous: infection or non-infectious autoimmune process
- Plasma mNGS sent (Karius). Patient discharged



- **Diagnosis: *Balamuthia mandrillaris*.**
- Multidisciplinary ameba team promptly involved
- Therapy started within 1-2 days

Image from DeRisi lab.

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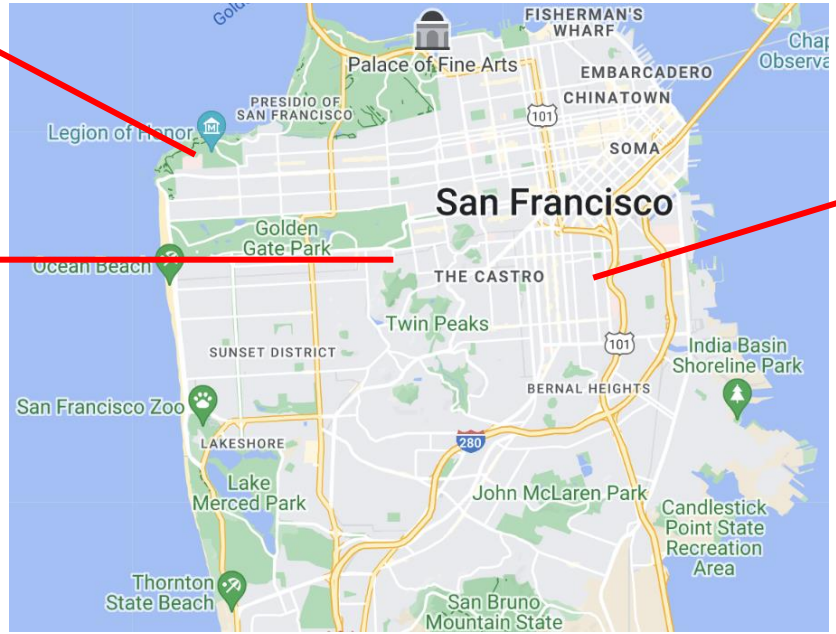
A word on equity: a tale of three hospitals

San Francisco Veterans Association

UCSF Parnassus



Zuckerberg San Francisco General Hospital

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Conclusions

- Metagenomic sequencing is an exciting new tool to understand what is making patients sick
- Uniquely can detect pathogens that the physician may not be thinking about (unbiased diagnostic).
- Benefit of plasma mNGS is dependent on syndrome being tested
- Perhaps the strongest evidence is for highly immunocompromised patients with pneumonia
- More work is needed to determine the best use case of this test!

Thank you!
Questions?

References

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3. J. A. Hill *et al.*, Liquid Biopsy for Invasive Mold Infections in Hematopoietic Cell Transplant Recipients With Pneumonia Through Next-Generation Sequencing of Microbial Cell-Free DNA in Plasma. *Clin Infect Dis* 73, e3876-e3883 (2021).
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