Rapid, Non-invasive Detection of Invasive *Mycoplasma hominis* Infection using the Karius Test, A Next-Generation Sequencing Test for Microbial Cell-free DNA in Plasma

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Background

Mycoplasma hominis is a rare cause of invasive infections, often in immunocompromised hosts. Diagnosis is difficult due to challenges in culturing these organisms. Current molecular diagnostics are targeted tests that require an index of suspicion which may not be present at the time of tissue sampling. Accurate, rapid diagnosis of invasive *Mycoplasma hominis* infections are important for antibiotic management.

Cases

Case 1

A young woman with lupus nephritis status post renal transplant developed persistent fever with progressive multifocal culture-negative osteoarticular infection despite empiric ceftriaxone.

Case 2

An adolescent female presented with an ascending pelvic infection progressing to purulent polymicrobial intra-abdominal abscesses requiring surgical debridement and cefepime, metronidazole and micafungin therapy. Her course was complicated by progressive peritonitis/abscesses.

Parameter	Case 1	Case 2
Age	26	15
Gender	female	female
Pre/comorbid underlying condition(s)	Renal transplant, SLE	None
Immunosuppressive medications	Mycophenolate, tacrolimus, prednisone (5mg/day), hydroxychloroquine. Recent alemtuzumab	None
Presenting symptoms and duration of infection	Right hip pain for 13 days	PID, septic shock
Antecedent symptoms (URI, genital infection , etc.)	None	PID
Tmax/Fever at presentation	Afebrile (later had self resolving fever)	Persistently febrile, Tmax 39.9 until post drainage
WBC with %N	13.5k with 98% neutrophils (admission)	14.6k with 74% Neutrophils (admission)
ESR mm per hr/CRP mg per dL	Max ESR 108 (day 20 post adm) Max CRP 14.7(day 20 post adm)	Max ESR 58 Max CRP 22.6 (day prior to abscess drainage)
Blood culture result	negative	negative
Other culture or infectious diseases test	See below *	*S agalactiae, S dysgalatciae, Fusobacterium necrophorum*, MSSA, *Prevotella spp*, and *C glabrata*
Location and type of infection	Septic arthritis of right hip, right wrist and bilateral shoulder joints	PID with necrotizing uterus/ovaries, s/p multiple debridement with multiple subsequent abdominal & pelvic abscesses
Biopsy fluid parameters results (WBC with %N)	Right wrist (56k wbc with 93% segs)	Not done
Biopsy culture or molecular (16S) results	Not done	16S from abscess fluid with *Ureaplasma parvum*
Imaging modality	MRI pelvis showed R sacroiliitis and sacral osteomyelitis X ray of right wrist and shoulder- unremarkable	Multiple large abdominal/ pelvic abscesses noted on abdominal CTs, MRIs and USG
Empiric antibiotics	vancomycin + ceftriaxone	cefepime+ metronidazole + micafungin
Antibiotic pretreatment duration prior to Karius Test	18 days	4 weeks
Choice of antibiotics after Karius Test and clinical impact	Doxycycline+ levofloxacin (clinical improvement)	Doxycycline+ piperacillin /tazobactam + micafungin (clinical improvement)
Duration of antibiotics (IV/PO)	6 weeks after diagnosis	7 weeks total (10days of doxycycline)
Duration of hospitalization	35 days (excluding rehab)	> 8 weeks
Outcome	Resolution of symptoms	Resolution of abscesses
Time to result from Karius Test collection	2 days	4 days
Time to result from Karius Test sample receipt	1 day	2 days
Karius Test result molecule/ microliter (MPM)	*Mycoplasma hominis* 3251 MPM	*Mycoplasma hominis* 3914 MPM, S *dysgalactiae* 393 MPM, *Fusobacterium necrophorum* 583 MPM, CMV 3997 MPM, EBV 812 MPM **

*Imaging: CXR, CT Chest; Culture: routine blood, AFB blood, fungal blood, urine; Serology: Lyme, Brucella, Bartonella; Antigen: serum Cryptococcal, urine Coccidioides, serum and urine Histoplasma; PCR: Parvovirus, Bartonella; serum BDG and galactomannan; RPR; all above tests negative

****Ureaplasma parvum*** reads were present in the raw data

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Methods

Two cases of invasive *Mycoplasma hominis* infections are presented in which the Karius test was used to make the diagnosis. The Karius test was developed and validated in Karius' CLIA certified/CAP accredited lab in Redwood City, CA. It is a next-generation sequencing (NGS) blood test that detects circulating microbial cell-free DNA (mcfDNA) in plasma. After mcfDNA is extracted and NGS performed, human reads are removed and remaining sequences are aligned to a curated database of > 1400 organisms. McfDNA from organisms present above a statistical threshold are reported and quantified in molecules/microliter (MPM). Case review was performed by infectious disease consultants and included for clinical correlation.

Results

Karius testing detected high-levels of *Mycoplasma hominis* mcfDNA in both cases – at 3251 molecules/ microliter (MPM) in the first case and 3914 MPM in the second case. The normal range of *Mycoplasma hominis* mcfDNA in a cohort of 684 normal adults is 0 MPM. The patients rapidly improved with atypical coverage with doxycycline and levofloxacin.

Conclusion

Open-ended, plasma-based NGS for mcfDNA provides a rapid, non-invasive method to diagnose invasive *Mycoplasma hominis* infection. Furthermore, these cases highlight the potential of this technique to diagnose infections caused by fastidious/unculturable pathogens.