Rapid, Non-invasive Detection and Monitoring of *Bartonella quintana* Endocarditis by Plasma-based Next-generation Sequencing of Microbial Cell-free DNA

Dipesh Solanky, MD[1]; Asim Ahmed, MD[2]; Joshua Fierer, MD[3,4]; Sanjay Mehta, MD, D(ABMM), DTM&H[3,4]

[1]University of California, San Diego, Dept of Internal Medicine, San Diego, United States
[2]Karius, Redwood City, United States
[3]University of California, San Diego, Dept of Infectious Diseases, San Diego, United States
[4]VA San Diego Healthcare System, Infectious Diseases Section, La Jolla, United States

Background

- Infective endocarditis (IE) mortality remains high, at >30% within the first 1 year of diagnosis[1,2]
- Culture negative endocarditis (CNE) comprises ~20% of annual cases of IE in the US[3]
- Treatment duration and timing of surgical intervention remain significant challenges for providers
- Plasma next-generation sequencing (NGS) for circulating microbial cell-free DNA (mcfDNA) has shown utility in diagnosing and monitoring the response to treatment in IE[4-6]

Study Aims

- Evaluate the efficacy of NGS of plasma mcfDNA in diagnosis and monitoring treatment response in a patient with *Bartonella quintana* endocarditis treated with antibiotics and aortic valve replacement
- Characterize mcfDNA signal decay kinetics and half-life after debridement of infectious focus

Methods

- Serial blood samples obtained prior to and after aortic valve replacement
- Microbial cfDNA extracted from plasma and NGS performed by Karius (CLIA-certified/CAP accredited laboratory in Redwood City, CA)
- Human sequences removed and remaining sequences aligned to a curated database of over 1,400 pathogens
- Organisms above a predefined statistical significance threshold were reported and quantified in DNA molecules per microliter (MPM)
- Chart review performed for clinical correlation

References

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Contact: sdipesh@health.ucsd.edu
IDWeek Poster 711

Results

Case Presentation

- An adult male with a history of homelessness, well-controlled HIV infection and a bioprosthetic aortic valve presented with symptomatic severe aortic stenosis and elevated inflammatory markers three years following valve surgery.
- Transesophageal echocardiography showed a focal soft tissue density on the aortic valve and paravalvular leak (Fig. 1a-b).
- *Bartonella quintana* was detected by Karius mcfDNA NGS (in parallel *Bartonella henselae* serologies were positive).
- Parenteral antibiotics were initiated and he ultimately underwent surgical aortic valve replacement.
- Serial Karius testing was performed after four weeks of antibiotic therapy, as well as immediately prior to and following valve replacement.
- After 4 weeks of parenteral antibiotics, repeat Karius testing demonstrated a 78% (4.5-fold) decrease in the *Bartonella quintana* mcfDNA signal to 8813 MPM (Table 1).
- *Bartonella quintana* mcfDNA rises dramatically after the operative manipulation of the infected valve; the signal drops 81-fold in the first 4 hours following valve extraction (intracardiac sampling). Half-life calculations of mcfDNA over this time range from 35 (TP 3–7) to 115 minutes (TP 4–8) (Fig. 2).
- 24 hours post-valve extraction, Karius testing shows rapid decay of the *Bartonella quintana* mcfDNA signal to 103 MPM (Fig. 2).
- The patient completed three months of oral antibiotics post-operatively, ultimately returning to his clinical baseline.

Bioprosthetic Aortic Valve Abnormalities in a Patient with *Bartonella quintana* Endocarditis

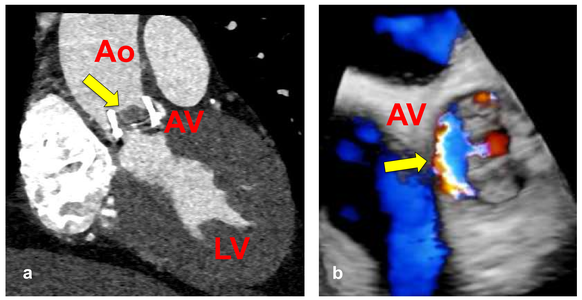

Figure 1a: Cardiac CT showing soft tissue density on the bioprosthetic valve measuring 11 x 8mm (arrow). **1b:** Three-dimensional rendition of bioprosthetic aortic valve visualized on transesophageal echocardiogram showing regurgitation and paravalvular leak (arrows) at the time of diagnosis of *B. quintana* endocarditis. Ao = aorta; AV = aortic valve; LV = left ventricle.

Serial Timepoints of *Bartonella quintana* mcfDNA Pre- and Post-Valve Extraction

Sample	Time Off Pump (min)	Run 1 (MPM)	Run 2 (MPM)	Average (MPM)	SD
Pre-Op	-279	9042	8583	8812.5	324.6
Pre-Valve Ex/On pump	-189	89525	35120	62322.5	38470.1
Post Valve Ex/On pump	-114	675272	323442	499357	248781.4
Off pump + 15 min	15	13504	16428	14966	2067.6
Off pump + 30 min	30	24210	20321	22265.5	2749.9
Off pump + 60 min	60	6125	6164	6144.5	27.6
Off pump + 120 min	120	5805	6471	6138	470.9
Off pump + 300 min	455	1062	777	919.5	201.5
Off pump + 1 day	1451	109	97	103	8.5
Off pump + 2 day	2931	148	99	123.5	34.6

Table 1: *B. quintana* mcfDNA signal over serial timepoints. "Pre-Op" refers to time prior to transport to the operating room, after 4 weeks of parenteral antibiotics. "On pump" refers to timepoint while the patient was on cardiopulmonary bypass used for surgical valve replacement. "Off pump" refers to timepoint off cardiopulmonary bypass. Operative samples were obtained via intracardiac catheter sampling. Ex = extraction; mcfDNA = microbial cell-free DNA; MPM = molecules per microliter; min = minutes; SD = standard deviation.

Bartonella quintana mcfDNA Decay Following Valve Extraction

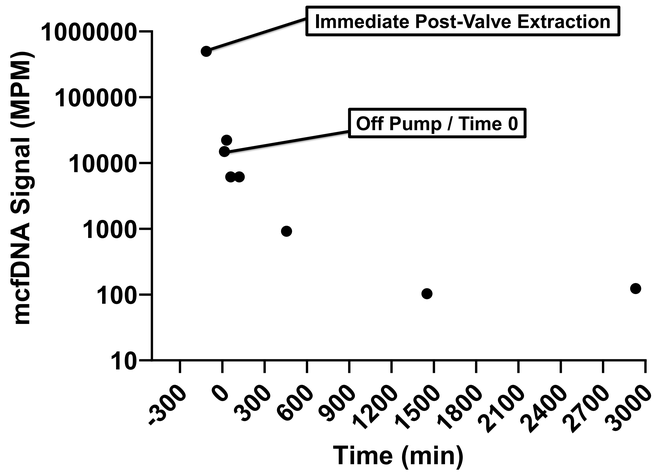

Figure 2: Decay of *B. quintana* mcfDNA following removal of the infected valve. Time 0 min represents time after patient was removed from cardiopulmonary bypass used for valve replacement surgery. The first timepoint (-114 min) represents time of valve extraction. mcfDNA = microbial cell-free DNA; MPM = molecules per microliter; min = minutes.

Discussion

- NGS of mcfDNA in plasma enabled pathogen identification in this case of culture negative prosthetic valve endocarditis, allowing for targeted therapy.
- Monitoring mcfDNA kinetics after definitive debridement of a focal site of infection offers a unique opportunity to estimate the half-life of mcfDNA.
- Estimates of mcfDNA half-life of mcfDNA in this case range from 35 – 115 minutes which is consistent with estimates of the half-life of human cell-free DNA
- Plasma-based NGS assays for mcfDNA offer a unique means of pathogen detection, assessment of infection burden and monitoring of response to both medical treatment and definitive source control as demonstrated in this case of *Bartonella quintana* endocarditis.