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Sequencing of circulating microbial cell-free DNA can identify pathogens in periprosthetic joint infections

APPENDIX

Methodology

Patient recruitment

Candidates were identified through systematic screening of electronic medical records and operating room schedules for infection-related diagnostic and procedural codes. As surgical treatment is often urgent or semi-urgent, some patients treated for prosthetic joint infection during the study period were not identified. Treating physicians were contacted to confirm observations consistent with prosthetic joint infection. Patients were excluded pre-enrollment if they were unable to provide written informed consent or carried a blood-borne pathogen requiring specialized specimen handling.

Chart review

Clinical laboratory test results, physical exam findings, microbial culture and pathology reports were gathered through in-depth review of medical records at the treatment hospital.

Pathology scoring

A positive histopathology score for infection involved pathology reports directly stating signs of infection were found that included granulation tissue and acute and chronic inflammation (STable 5).

Blood microbial cfDNA extraction

Patients' arterial or venous blood was collected into a K_2EDTA tube in the operating room prior to the index infection surgery and at the time of reimplantation for patients undergoing two-stage revisions. Subsequent blood collections for patients who underwent DAIR or one-stage revision were drawn in the office or hospital phlebotomy center during follow-up post-operative visits, usually 6 weeks after surgery. Plasma was isolated by centrifugation, frozen at -80°C and shipped in batches within weeks of collection to Karius (Redwood City, CA) for microbial cfDNA sequencing and analysis. Thawed plasma was spiked with synthetic normalization controls and centrifuged again to remove remaining cellular debris. Microbial cfDNA was isolated from 0.25 mL plasma using a modified Mag-Bind cfDNA Kit (Omega Biotek) in Hamilton STAR liquid handling workstation.

cfDNA sample processing

Plasma from hundreds of healthy subjects was previously used to validate and establish microbial cfDNA sequence abundance thresholds for ~1,300 microorganisms[1]. The presence of microbial cfDNA above a statistical threshold is reported as the number of molecules per microliter of plasma (MPM)[1].

DNA libraries were constructed using customized dual-indexed Ovation Ultralow System V2 library preparation kits (NuGEN) in Hamilton STAR liquid handling workstations. Libraries were pooled with environmental and assay controls in every batch and sequenced on Illumina NextSeq500 sequencers using a 75-cycle single-end, dual index kit[1].

The primary sequencing output was demultiplexed by bcl2fastq v2.17.1.14. The reads were quality trimmed and subsequently filtered if shorter than 20 bases by Trimmomatic v0.32[2] or aligned

against human and synthetic references using Bowtie v2.2.4[3]. The remaining reads were aligned with a highly curated microorganism reference database using BLAST v2.2.30[4]. PCR duplicates were removed based on their alignments.

Relative abundances were assigned to each taxon in a sample on the basis of the sequencing reads and their alignments. From these abundances, the number of reads arising from each taxon was aggregated up the taxonomic tree. A set of environmental control samples were processed and sequenced within each batch. Statistical significance values were computed for each estimated taxon abundance in each non-environmental control sample, and those within the CRR at high significance levels comprised our candidate calls. Final calls were made after additional filtering was applied, which accounted for read location uniformity, read percent identity and cross-reactivity originating from higher abundance calls. The microorganism calls that passed these filters were reported along with abundances in MPM (molecules per microliter).

Two significance thresholds for calls were applied to this data. The clinical threshold was set in Blauwkamp et al., 2019[1], maximizing analytical specificity and clinical sensitivity in a cohort of patients with sepsis. Sensitivity was established for bacteremia patients by comparing sequencing results to microbial culture results. Specificity was assessed using samples from 167 aseptic donors and by replicate measurements of a single well-characterized asymptomatic human plasma pool. For the latter, of the 50 replicates tested, unexpected microbial cfDNA was detected in only one replicate, yielding an overall specificity of 98% (49 of 50) and 99.998% on a per analyte basis (62,449 of 62,450). In addition, they 'spiked' well-known samples with genetically similar micro-organisms and all of the spiked-in microbial cfDNAs were identified (1,250 of 1,250), with a 99.4% positive predictive value[1]. Because microbial abundances may be low concentration in infected prosthetic joints, a research-use only (RUO) threshold was trained using the first batch of 34 samples to optimize sensitivity and specificity in prosthetic joint infections. Individuals involved in the blood cfDNA sequencing test were blinded to patient diagnosis and the pathogens identified by clinical joint cultures.

Data Analysis and Statistics

Due to the observational nature of the study and the restriction of the population to those with prosthetic joint infections, power analyses and sample size calculations were not performed; measures should therefore be considered exploratory.

Post-hoc statistical analysis was performed to examine potential confounding variables and their relationship with the pathogen identification performance of cfDNA sequencing. This included surgical site, antimicrobial use, timing of infection onset, infectious pathogen and histopathology. An odds ratio was calculated to test for the association of these variables with the correct identification of the causative pathogen by microbial cfDNA sequencing. Fisher's exact test was performed to qualify the possible statistical difference in performance between groupings based on potential confounding variables. An alpha of 0.05 was used for significance.

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Patients included

Patient	Joint	Clinical diagnosis	Cultured organism	Procedure	Blood CRP (mg/dL)	Blood ESR (mm/hr)	Joint fluid WBC (cells/uL)	Neutrophils (%)	Antibiotics at sampling	Blood culture	Joint tissue culture	Joint fluid culture	Plasma mcfDNA-sequencing (molecules per microliter, mpm)
1	Knee	Infection	*Streptococcus agalactiae*	2 stage	3.5	61	17250	96	No	n/a	*Streptococcus agalactiae*	*Streptococcus agalactiae*	*Streptococcus agalactiae (250)*
4	Hip	Infection	*Staphylococcus lugdunensis*	DAIR	0.7	14	83750	99	No	n/a	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis (13)** *Streptococcus mitis (16)** *Staphylococcus hominis (14)**
5	Hip	Infection	*Streptococcus mitis group*	1 stage	7.0	68	12100	99	No	n/a	*n/a*	*Streptococcus mitis group*	*Streptococcus gordonii (15)**
7	Hip	Infection	*Staphylococcus haemolyticus*	1 stage	1.4	65	n/a	n/a	No	n/a	*Staphylococcus haemolyticus*	*Staphylococcus haemolyticus*	*Pseudomonas aeruginosa (24)* *Staphylococcus haemolyticus (34)*
8	Hip	Infection	*Staphylococcus lugdunensis*	2 stage	8.6	75	325	1	No	n/a	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis (21)*
9	Knee	Infection	*Staphylococcus lugdunensis*	1 stage	n/a	n/a	47100	96	No	n/a	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis*	No organism detected
10	Knee	Infection	*Staphylococcus epidermidis (MSSE)*	2 stage	5.5	34	30100	97	No	No growth	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis (43)* *Staphylococcus hominis (43)*
11	Hip	Infection	*Staphylococcus aureus* (MRSA)	2 stage	19.0	121	146500	98	Yes	No growth	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus (1403)* *Rothia mucilaginosa (71)*
12	Knee	Infection	*Staphylococcus aureus* (MRSA)	2 stage	0.7	5	32375	98	Yes	n/a	*MRSA*	*MRSA*	No organism detected
13	Knee	Infection	*Staphylococcus aureus* (MSSA)	1 stage	4.2	73	70000	97	Yes	n/a	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus (73)*
15	Knee	Infection	*Candida parapsilosis*	2 stage	1.2	36	4625	96	Yes	n/a	*Candida parapsilosis*	*Candida parapsilosis*	No organism detected
16	Hip	Infection	*Staphylococcus aureus* (MSSA)	Spacer exchange	0.7	19	5125	91	Yes	n/a	*Staphylococcus aureus*	*Staphylococcus aureus*	*Bacteroides uniformis (14)**
17	Knee	Infection	Enterobacter cloacae and Staphylococcus epidermidis (MRSE)	DAIR	3.2	36	52500	95	No	No growth	*Enterobacter cloacae*	*Enterobacter cloacae*	*Enterobacter cloacae* complex *(22)**
19	Knee	Infection	ESBL *Klebsiella pneumoniae*	2 stage	2.2	11	4125	98	Yes	n/a	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae (19)**
20	Hip	Infection	Staphylococcus epidermidis (MRSE)	2 stage	3.6	27	24600	98	No	n/a	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No organism detected

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21	Knee	Infection	*Staphylococcus aureus* (MSSA)	2 stage	17.3	92	53570	93	Yes	n/a	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus (313)*
22	Knee	Infection†	*Streptococcus pneumoniae†*	1 stage	n/a	n/a	n/a	n/a	Yes	n/a	No growth†	No growth†	*Streptococcus pneumoniae (13)†*
24	Knee	Infection	*Streptococcus mitis*	DAIR	22.9	132	119500	93	No	No growth	*Streptococcus mitis§*	*Streptococcus mitis§*	*Streptococcus tigurinus (2226)§*
25	Knee	Infection	*Streptococcus mitis*	DAIR	23.5	41	21625	89	No	No growth	*Streptococcus mitis§*	*Streptococcus mitis§*	*Streptococcus oralis (68)§ Herpes Simplex Virus 1 (HSV-1) (22)*
26	Knee	Infection	*Bacteroides thetaiotamicron*	DAIR	25.0	112	18963	96	No	No growth	*Bacteroides thetaiotaomicron*	n/a	*Bacteroides thetaiotaomicron (20)*
27	Knee	Infection	*Staphylococcus aureus* (MRSA)	2 stage	1.0	30	8625	96	No	n/a	*Staphylococcus aureus*	*Staphylococcus aureus*	No organism detected
28	Knee	Infection	*Enterococcus faecalis* and MRSE	2 stage	5.9	14	22000	78	Yes	No growth	*Enterococcus faecalis*	*Enterococcus faecalis*	*Staphylococcus hominis (43) Saccharomyces cerivisiae (33) Enterococcus faecalis (20)*
29	Knee	Infection†	VRE infection history in same joint†	2 stage	2.7	66	31750	98	Yes	n/a	*Serratia liquifaciens* (1 of 8 cultures)†‖	No growth†	No organism detected†
30	Hip	Infection	*Staphylococcus capitis*	2 stage	4.4	42	6000	95	No	n/a	*Staphylococcus capitis*	*Staphylococcus capitis*	No organism detected
32	Knee	Infection	*Propionibacterium propionicum*	DAIR	7.2	83	25250	93	Yes	n/a	*Propionibacterium propionicum* (16S PCR) ◊	*Propionibacterium propionicum* (16S PCR) ◊	*Actinomyces massilensis (106)* ◊
35	Hip	Infection	*Staphylococcus lugdunensis*	2 stage	5.5	66	20375	89	Yes	No growth	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis*	*Streptococcus gordonii (109) Streptococcus oralis (192)*
36	Hip	Infection	*Enterococcus faecium*	DAIR	12.6	34	4575	98	No	*Staphylo-coccus epidermidis* (1 of 7 cultures)§	*Enterococcus faecium*	*Enterococcus faecium*	*Enterococcus faecium (88) Staphylococcus hominis**
37	Knee	Infection†	Broth only strep mitis group (strep gordonii)	2 stage	8.4	54	n/a	n/a	Yes	n/a	Streptococcus mitis group, Staph epidermidis. Not simultaneous	n/a	No organism detected
39	Hip	Infection	*Staphylococcus aureus* (MSSA)	2 stage	6.7	121	n/a	n/a	No	n/a	*Staphylococcus aureus*	*Staphylococcus aureus*	No organism detected
42	Knee	Infection	Staphylococcus epidermidis (MRSE)	2 stage	5.8	44	13105	91	Yes	n/a	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis (188)*
43	Knee	Infection	Staphylococcus epidermidis (MRSE)	2 stage	3.3	39	35087	98	No	n/a	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No organism detected

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45	Hip	Infection	*Staphylococcus aureus* (MRSA)	DAIR	1.3	29	189500	95	No	n/a	*Staphylococcus aureus* (MRSA)	Staphylococcus aureus (MRSA)	No organism detected
46	Knee	Infection	*Proteus mirabilis*	DAIR	4.3	42	5075	68	No	No growth	*Proteus mirabilis*	n/a	*Proteus mirabilis* (56)
47	Knee	Infection	*Streptococcus agalactiae*	DAIR	8.8	66	63375	96	Yes	No growth	*Streptococcus agalacticae*	*Streptococcus agalacticae*	*Streptococcus agalacticae (959)*
50	Knee	Infection†	*Escherichia coli†*	DAIR	4.4	>140	n/a	n/a	Yes	No growth	No growth†	No growth†	*Escherichia coli (944)†*
52	Hip	Infection	*Staphylococcus aureus* (MRSA) and *Staphylococcus Intermedius*	2 stage	22.0	47	20274	97	No	n/a	*Staphylococcus intermedius*	*Staphylococcus aureus* (MRSA)	*Staphylococcus aureus (86)*
53	Knee	Infection	*Staphylococcus epidermidis*	DAIR	2.9	36	13600	92	No	n/a	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis (40)*
54	Knee	Infection	*Staphylococcus lugdunensis*	DAIR	15.2	83	61125	92	No	n/a	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis*	*Staphylococcus lugdunensis (259)*
55	Knee	Polymicrobial PJI	*Staphylococcus hominis subspecies hominis. Staphylococcus epidermidis*	2 stage	2.4	30	15250	96	No	n/a	*Staphylococcus hominis subspecies hominis. Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No organism detected
56	Knee	Infection	*Streptococcus sanguinis*	DAIR	5.6	83	15125	93	No	n/a	Superfacial culture - *streptococcus sanguinis*	*Streptococcus sanguinis*	*Streptococcus sanguinis (66)*
57	Knee	Infection	*Streptococcus agalacticae*	1 stage	5.8	5	103000	84	Yes	No growth	*Streptococcus agalacticae*	No growth	*Streptococcus agalactiae (2794)*
60	Knee	Infection	*Candida orthopsilosis*	2 stage	1.9	30	2625	90	No	n/a	*Candida orthopsilosis*	*Candida orthopsilosis*	*Klebsiella pneumoniae (13)*
61	Knee	Infection	Negative	2 stage	5.7	78	n/a	n/a	Yes	n/a	No growth	No growth	*Streptococcus agalactiae (18)*
62	Knee	Infection	*Staphylococcus epidermidis*	2 stage	31.1	110	28489	98	No	No growth	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis (41)*
63	Knee	Infection	*Pseudomonas aeruginosa*	2 stage	3.5	75	n/a	n/a	No	n/a	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Staphylococcus warneri (669) Streptococcus mitis (189) Corynebacterium afermentans (72)*
64	Knee	Infection	Beta hemolytic group g *streptococcus*	DAIR	41.4	102	n/a	n/a	Yes	No growth	No growth	(Beta hemolytic group g *streptococcus)*	*Escherichia coli (193) Herpes Simplex Virus type 1 (HSV-1) (100) Streptococcus dysgalactiae (768)*
66	Hip	Infection	*Staphylococcus aureus*	DAIR	24.3	109	45750	90	Yes	n/a	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus (121)*

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67	hip	Infection	*Staphylococcus aureus*	DAIR	5.7	118	77792	94	No	n/a	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus (22)**
68	Knee	Infection	No growth	2 stage	5.5	49	13783	93	Yes	No growth	No growth	No growth	No organism detected
69	hip	Infection	*Pseudomonas aeruginosa & MRSA*	DAIR	2.3	36	n/a	n/a	No	No growth	*Pseudomonas aeruginosa & MRSA*	*Pseudomonas aeruginosa & MRSA*	*Enterococcus faecalis (80) Pseudomonas aeruginosa (128)*
70	Knee	Infection	*Pseudomonas aeruginosa*	DAIR	8.4	61	6696	92	Yes	No growth	No growth	*Pseudomonas aeruginosa*	*Rothia dentocariosa (19)*
71	Hip	Infection	*Staphylococcus aureus*	DAIR	19.6	94	44343	63	No	No growth	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus aureus (41)*

Patients excluded

Patient	Joint	Clinical diagnosis	Cultured organism	Procedure	Blood CRP (mg/dL)	Blood ESR (mm/hr)	Joint fluid WBC (cells/uL)	Neutrophils (%)	Antibiotics at sampling	Joint tissue culture	Joint fluid culture	Plasma mcfDNA-sequencing (molecules per microliter, mpm)	Reason for exclusion
3	Knee	Not infected	Negative	2 stage	3.9	n/a	28600	87	Yes	n/a	No growth	*Herpes Simplex Virus 1 (HSV-1) (129)*	Not infected
6	Hip	Infection	Negative	DAIR	1.1	32	n/a	n/a	No	No growth	No growth	*Helicobacter pylori (70) Pseudomonas aeruginosa (355)*	Diagnosis inconclusive
14	Hip	Not infected	Negative	DAIR	3.4	30	16200	80	No	No growth	No growth	No organism detected	Not infected
18	Knee	Not infected	Negative	DAIR	n/a	n/a	n/a	n/a	No	n/a	No growth	No organism detected	Not infected
23	Hip	Infection†	Coagulase negative *staphylococcus*†	2 stage	2.9	37	28975	96	No	No growth†	No growth†	No organism detected†	Diagnosis inconclusive
31	Knee	Not infected	Negative	DAIR	5.5	27	4100	68	Yes	n/a	Staphylococcus epidermidis (broth only)‖	No organism detected	Not infected
33	Knee	Infection†	MSSA†	DAIR	6.8	75	6750	98	Yes	No growth†	n/a†	No organism detected†	Infection determined not to be in joint

34	Hip	Not infected	Negative	DAIR	3.9	41	500	90	Yes	n/a	*Staphylococcus aureus* (1 of 6 cultures)‖	No organism detected	Not infected
38	Hip	Infection	Staphylococcus epidermidis (MRSE)	2 stage	4.5	53	3625	83	No	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	No organism detected	Infection determined not to be in joint
40	Knee	Not infected	Negative	2 stage	<0.7	24	n/a	n/a	Yes	n/a	No growth	No organism detected	Not infected
41	Knee	Not infected	Negative	2 stage	1.5	27	1432	85	No	n/a	No growth	No organism detected	Not infected
44	Hip	Infection	Culture negative PJI	DAIR	5.0	71	n/a	n/a	Yes	*Enterococcus faecium* (1 of 7 cultures)‖	n/a	No organism detected	Diagnosis inconclusive
48	Hip	Infection	Culture negative PJI	2 stage	3.8	36	58800	80	Yes	No growth	No growth	No organism detected	Diagnosis inconclusive
49	Knee	Infection	Culture negative PJI	2 stage	1.4	24	714	70	Yes	No growth	No growth	*Staphylococcus epidermidis (47)*	Partial knee replacement
51	Knee	Not infected	Negative	2 stage	<0.7	16	325	2	No	n/a	No growth	No organism detected	Not infected
58	Knee	Infection	Culture Negative Infection	2 stage	<0.7	22	207	0	No	No growth	No growth	No organism detected	Diagnosis inconclusive
59	Hip	Not infected	*Finegoldia magna*	DAIR	3.5	75	6875	70	No	No growth deep. *Escherichia coli, Enterococcus faecalis* surface	*Finegoldia magna*	*Kytococcus sedentarius (110)* *Micrococcus lylae (301)* *Staphylococcus cohnii (47)* *Staphylococcus epidermidis (129)* *Staphylococcus haemolyticus (127)* *Staphylococcus hominis (102)*	Infection determined not to be in joint
65	Knee	Infection	*Candida parapsilosis*	2 stage	<0.7	44	800	30	No	*Candida parapsilosis*	*Candida parapsilosis*	No organism detected	Diagnosis inconclusive
72	Hip	Soft tissue reaction - Gram stain positive	Negative	2 stage	2.4	51	n/a	n/a	No	No growth	n/a	No organism detected	Infection determined not to be in joint
73	Hip	Infection	*Propionibacterium acnes*	2 stage	<0.7	27	n/a	n/a	*Propionibacterium acnes*	*Propionibacterium acnes*	*n/a*	Did not report	cfDNA assay did not report results for this organism at the time of this study

STable 1. Clinical findings related to infection workup and the microbial cfDNA sequencing results per patient.

† Joint specimens collected around time of surgery grew no organism, yet specimens from weeks prior were positive.
§ *Streptococcus mitis* group demonstrate phenotypic overlap difficult to discern by culture, thus species in this group are considered matches[1].
‖ Cases where no more than 1 of 6-8 joint specimens grew a microorganism; the specimen considered a contaminant.
* Pathogens identified by blood microbial cfDNA sequencing after relaxation of clinical thresholds for sepsis.
◊ *P. propionicum* considered species match to *A. massiliensis* due to taxonomic similarities; *P. propionicum* prior member of *Actinomyces*[2]. n/a Sample not collected or assay not run.

[1]Jensen, A., Scholz, C.F., Kilian, M. 2016. *Int J Syst Evol Microbiol* 66:4803. [2]Kononen, E., Wade, W.G. 2015. *Clinical microbiology reviews* 28:419.

	Major criteria		Minor criteria						
Patient	Sinus tract	2 cultures of same organism	Single positive culture	Elevated CRP	Elevated ESR	Elevated joint fluid WBC	Elevated SF PMN%	Positive Histology	MSIS diagnosis
1	–	✓	–	✓	✓	✓	✓	–	PJI
2	–	✓	–	✓	✓	✓	✓	✓	PJI
4	✓	✓	–	–	–	✓	✓	✓	PJI
5	–	✓	–	✓	✓	✓	✓	✓	PJI
7	–	✓	–	✓	✓	n/a	n/a	✓	PJI
8	–	✓	–	✓	✓	–	–	✓	PJI
9	–	✓	–	n/a	n/a	✓	✓	–	PJI
10	–	✓	–	✓	✓	✓	✓	✓	PJI
11	✓	✓	–	✓	✓	✓	✓	✓	PJI
12	–	✓	–	–	–	✓	✓	–	PJI
13	✓	✓	–	✓	✓	✓	✓	✓	PJI
15	–	✓	–	✓	✓	✓	✓	–	PJI
16	–	✓	–	–	–	✓	✓	–	PJI
17	–	✓	–	✓	✓	✓	✓	–	PJI
19	✓	✓	–	✓	–	✓	✓	✓	PJI
20	–	✓	–	✓	–	✓	✓	✓	PJI
21	–	✓	–	✓	✓	✓	✓	✓	PJI
22	–	–	✓	n/a	n/a	n/a	n/a	✓	n/a
24	–	✓	–	✓	✓	✓	✓	✓	PJI
25	–	✓	–	✓	✓	✓	✓	✓	PJI
26	–	✓	–	✓	✓	✓	✓	✓	PJI
27	–	✓	–	✓	✓	✓	✓	✓	PJI
28	–	✓	–	✓	✓	✓	–	✓	PJI
29	✓	–	–	✓	✓	✓	✓	–	PJI
30	–	✓	–	✓	✓	✓	✓	✓	PJI
32	–	✓	–	✓	✓	✓	✓	✓	PJI
35	–	–	✓	✓	✓	✓	✓	✓	PJI
36	✓	✓	–	✓	✓	✓	✓	✓	PJI
37	–	✓	–	✓	✓	n/a	n/a	–	PJI
39	✓	✓	–	✓	✓	n/a	n/a	✓	PJI
42	–	✓	–	✓	✓	✓	✓	✓	PJI
43	–	✓	–	✓	✓	✓	✓	–	PJI
45	–	✓	–	✓	✓	✓	✓	✓	PJI
46	–	✓	–	✓	✓	✓	–	✓	PJI
47	–	✓	–	✓	✓	✓	✓	✓	PJI
50	–	–	–	✓	✓	n/a	n/a	✓	n/a
52	✓	✓	–	✓	✓	✓	✓	✓	PJI
53	✓	✓	–	✓	✓	✓	✓	✓	PJI
54	–	✓	–	✓	✓	✓	✓	✓	PJI
55	✓	✓	–	✓	✓	✓	✓	✓	PJI
56	–	✓	–	✓	✓	✓	✓	✓	PJI
57	–	✓	–	✓	–	✓	✓	✓	PJI
60	✓	✓	–	✓	–	n/a	n/a	✓	PJI
61	✓	–	–	✓	✓	n/a	n/a	✓	PJI
62	✓	✓	–	✓	✓	n/a	n/a	✓	PJI
63	–	✓	–	✓	✓	n/a	n/a	✓	PJI
64	–	–	✓	✓	✓	n/a	n/a	✓	PJI
66	–	✓	–	✓	✓	✓	✓	n/a	PJI
67	–	✓	–	✓	✓	✓	✓	✓	PJI
68	✓	–	–	✓	✓	✓	✓	✓	PJI
69	✓	✓	–	✓	✓	n/a	n/a	✓	PJI
70	–	–	✓	✓	✓	✓	–	✓	PJI
71	✓	✓	–	✓	✓	✓	✓	✓	PJI
Excluded patients									
3	–	–	–	✓	n/a	✓	✓	–	Not infected
6	–	–	–	✓	✓	n/a	n/a	n/a	n/a
14	–	–	–	✓	–	✓	✓	–	Not infected
18	–	–	–	n/a	n/a	n/a	n/a	–	n/a
23	–	–	–	✓	✓	✓	✓	✓	PJI
31	–	–	–	✓	✓	✓	–	–	Not infected
33	✓	–	–	✓	✓	✓	✓	✓	PJI
34	–	–	–	✓	✓	–	✓	–	Not infected
38	–	✓	–	✓	✓	✓	✓	–	PJI
40	–	–	–	–	✓	n/a	n/a	–	Not infected
41	–	–	–	✓	✓	–	–	✓	Not infected
44	✓	–	–	✓	✓	n/a	n/a	✓	PJI
48	–	–	–	✓	✓	✓	✓	✓	PJI
49	–	–	–	✓	–	–	–	✓	Not infected
51	–	–	–	–	–	–	–	–	Not infected
58	–	–	✓	–	–	✓	–	–	Not infected
59	✓	–	✓	✓	✓	–	–	✓	PJI
65	✓	✓	–	–	✓	✓	–	✓	PJI
72	✓	–	–	✓	✓	✓	✓	✓	PJI
73	✓	✓	–	–	✓	n/a	n/a	✓	PJI

STable 2. Musculoskeletal infection society (MSIS) prosthetic joint infection criteria scores for each patient. MSIS criteria features used were from the 2014 international consensus group[1] with thresholds from 2017[2]. Major criteria include the presence of at least 2 joint specimens culturing the same organism or the presence of a sinus tract. Either of these 2 criteria qualifies the case as an infection. Minor criteria include a single-positive culture, elevated serum CRP (>1 mg/dL), ESR (>30 mm/hr), synovial fluid white blood cell (WBC) count (>3000 cells/microliter), synovial fluid percentage of polymorphonuclear cells (PMN%, neutrophils, >80% of cells) and positive histological analysis of neutrophils in joint tissue. Histology scores were based on clinical reports from musculoskeletal pathologists who examine for at least 5 neutrophils in at least 5 high-powered field (400x). An infection is considered present if 3 of 5 minor criteria are positive. Final clinical diagnosis was made by the treating surgeon and an infectious disease physician.

[1]Parvizi J, Gehrke T. 2014. *Journal of Arthroplasty.* 29: 1331.
[2]Shahi AM, Kheir MM, Tarabichi M, Hosseinzadeh HR, Tan TL, Parvizi J. 2017. *JBJS.* 99: 1419.

mcfDNA-seq detected microorganism	PJI causing organism?
Actinomyces massilensis	Yes
Bacteroides thetaiotaomicron	Yes, rare
Corynebacterium afermentans	Yes, rare
Enterobacter clocae complex	Yes
Enterococcus faecalis	Yes
Enterococcus faecium	Yes
Escherichia coli	Yes
Haemophilus parainfluenzae	Yes, rare
Helicobacter pylori	Yes
Klebisella pneumoniae	Yes
Proteus mirabilis	Yes
Pseudomonas aeruginosa	Yes
Staphylococcus aureus	Yes
Staphylococcus cohnii	Yes
Staphylococcus epidermidis	Yes
Staphylococcus haemolyticus	Yes
Staphylococcus hominis	Yes
Staphylococcus lugdunensis	Yes
Staphylococcus warneri	Yes
Streptococcus agalacticae	Yes
Streptococcus dysgalacticae	Yes
Streptococcus gordonii	Yes
Streptococcus mitis	Yes
Streptococcus oralis	Yes
Streptococcus pneumoniae	Yes
Streptococcus salivarius	Yes
Streptococcus sanguinis	Yes
Streptococcus tigurinus	Yes

mcfDNA-seq detected microorganism	PJI causing organism?
Bacteroides uniformis	No
Herpes simplex virus type 1	No
Kytococcus sedentarius	No
Lactobacillus fermentum	No
Lactobacillus gasseri	No
Micrococcus lylae	No
Rothia dentocariosa	No
Rothia mucilaginosa	No
Saccharomyces cervisiae	No

STable 3. Microorganisms detected by blood microbial cfDNA sequencing are classified here as common or improbable prosthetic joint infection-causing pathogens. Tande AJ, Patel R. 2014. *Clinical Microbiology Reviews.* 27:302.

Surgical site

	Pathogen detected	No detection	Total		
Knee	23	12	35	Odds ratio	0.96, 95%CI = [0.23, 3.67]
Hip	12	6	18	Significance	p = 1.00
Total	35	18	53		

Previous antibiotic use

	Pathogen detected	No detection	Total*		
Long term	13	10	23	Odds ratio	0.44, 95%CI = [0.04, 3.23]
Short term	6	2	8	Significance	p = 0.43
Total*	19	12	31		

Previous antibiotic use

	Pathogen detected	No detection	Total		
Antibiotics	19	12	31	Odds ratio	0.60, 95%CI = [0.14, 2.22]
No antibiotics	16	6	22	Significance	p = 0.56
Total*	35	18	53		

Infection onset

	Pathogen detected	No detection	Total		
Chronic	20	13	33	Odds ratio	0.51, 95%CI = [0.12, 2.00]
Acute	15	5	20	Significance	p = 0.37
Total	35	18	53		

Infectious pathogen

	Pathogen detected	No detection	Total		
Staph	15	11	26	Odds ratio	0.48, 95%CI = [0.13, 1.75]
Non-staph	20	7	27	Significance	p = 0.25
Total	35	18	53		

Pathology result for infection

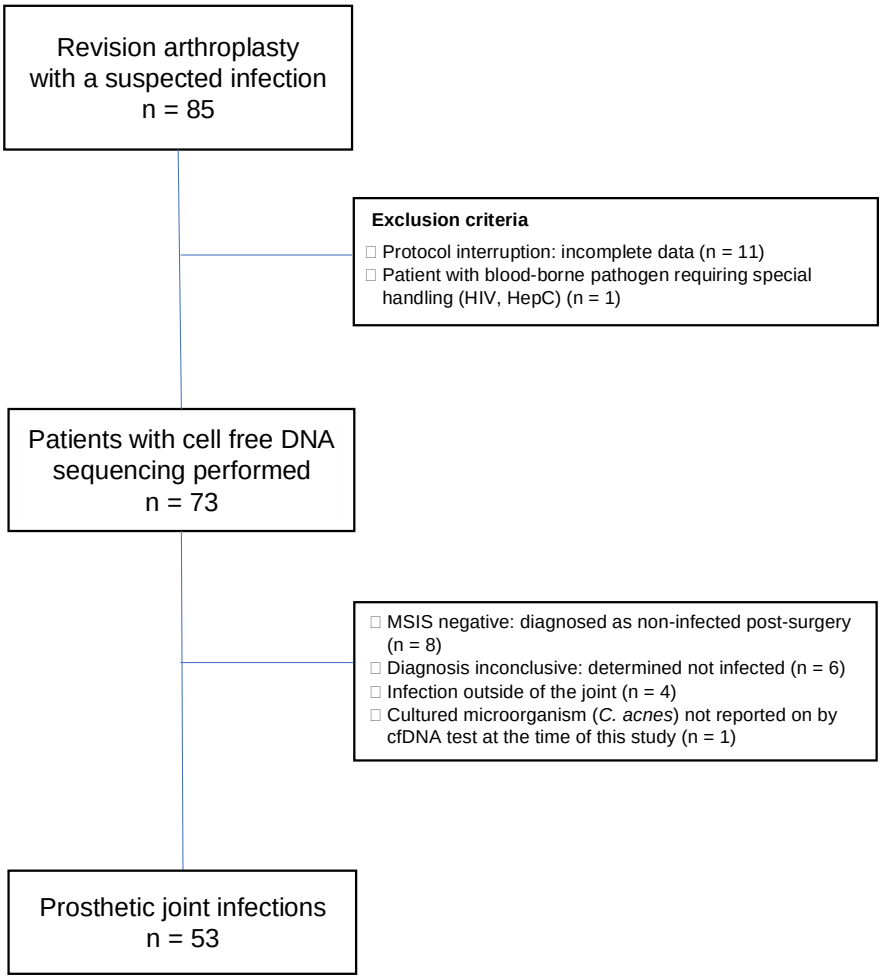
	Pathogen detected	No detection	Total*		
Positive	30	11	41	Odds ratio	11.59, 95%CI = [1.98, 126.88]
Negative	2	9	11	Significance	p = 0.001
Total*	32	20	52		

STable 4. Post-hoc analysis of potential confounding variables and their relation to pathogen identification blood microbial cfDNA sequencing. Fisher exact tests were used to calculate odd's ratios to account for cells with low frequencies. An alpha of 0.05 was chosen for significance.

Patient	Pathology report summary
1	Tissue Reaction to Particulate Implant Material - Soft Tissue: -- Methyl Methacrylate Cement Debris, Small Particulate -- with Chronic and Acute Hematoma
2	Acute Inflammation Consistent with Implant Infection Tissue Reaction to Particulate Implant Material
4	Acute and Chronic Inflammation without Identifiable Implant debris Consistent with Implant Infection
5	Acute Inflammation with implant debris, Consistent with Implant Infection
7	Tissue Reaction to particulate implant material, histologic features are Consistent with Ongoing Infection
8	Tissue Reaction to Particulate Implant Material - soft tissue: Consistent with Ongoing Infection
9	Chronic Proliferative and Exudative Synovitis with Perivascular and Interstitial Lymphoplasmacytic Inflammation with Binucleated Forms and Russell's Bodies with Neutrophilic Component, Consistent with Psoriatic Arthritis. Infection cannot be Excluded.
10	Acute Inflammation without Identifiable Implant Debris, consistent with Implant Infection Replacement Device: Knee, Components: -- Metallic and Plastic, Cemented
11	Extensive Necrosis and Acute Inflammation consistent with implant infection
12	Tissue Reaction to Particulate Implant material - soft tissue - Negative for infection
13	Acute and Chronic Inflammation and Granulation Tissue without identifiable implant debris consistent with implant infection
15	Chronic Tissue reaction without identifiable implant material, soft tissue. No Evidence of Infection
16	Chronic Tissue Reaction without Implant Material, Soft Tissue -- No Evidence of Infection Dermal Scar (Skin Scar) with Foreign Body Granulomas
17	Chronic Tissue Reaction without Implant Material, Soft Tissue Replacement Device: Knee, Components: Metallic and Polyethylene Tibial Components
19	Neo-synovium with Acute Inflammation and Giant Cell Reaction to Large Particulate Polymethyl Methacrylate Orthopedic Cement, consistent with Implant Infection
20	Degenerative Joint Disease, Hip, Supero-Lateral
21	Tissue Reaction to Particulate Implant Material, Bone: No Evidence of Infection:
22	Acute and Chronic Inflammation and Granulation Tissue, Implant Infection Cannot Be Ruled Out
24	Necrotizing Inflammation with Implant Debris Consistent with Implant Infection Replacement Device: Knee, Component: Semi-constrained polyethylene tibial liner with metallic bushing and metallic holder.
25	Acute and Chronic Inflammation and Granulation Tissue consistent with Implant Infection
26	Acute and Chronic Inflammation and Granulation Tissue, Consistent with Implant Infection
27	Acute and Chronic Inflammation and Granulation Tissue consistent with Implant Infection
28	Tissue Reaction to Particulate Implant Material - Soft Tissue: - Consistent with Infection
29	Acute Inflammation with Implant Polymethyl Methacrylate Orthopedic Cement Debris, consistent with Implant Infection
30	Acute and Chronic Inflammation and Granulation Tissue consistent with Implant Infection
32	Consistent with On-going Infection
35	Acute and Chronic Inflammation and Granulation Tissue consistent with Implant Infection
36	Skin with Subcutaneous Acute Inflammation, consistent with Infection
37	Sclerosis and Degeneration of Soft Tissue Elements, Consistent with Traumatic Injury
39	Acute Inflammation consistent with Implant Infection
42	Acute and Chronic Inflammation and Granulation Tissue with Implant Debris, consistent with Implant Infection
43	Tissue Reaction to Particulate Implant Material - Soft Tissue: -- Methyl Methacrylate Cement Debris, Small Particulate -- Polyethylene Debris, Small Particulate
45	Granulation Tissue with Acute and Lymphocytic Inflammation, consistent with Implant Infection
46	Acute and Chronic Inflammation and Granulation Tissue with Implant Debris consistent with Implant Infection
47	Acute and Chronic Inflammation and Granulation Tissue with Implant Debris, consistent with Implant Infection
50	Acute Inflammation consistent with Implant Infection
52	Acute and Chronic Inflammation consistent with Implant Infection
53	Necrotizing Acute and Chronic Inflammation and Granulation Tissue, Consistent with Implant Infection -- with Deposition of Calcium Pyrophosphate, Incidental
54	Consistent with On-going Infection
55	Acute and Chronic Inflammation, Consistent with Implant Infection
56	Granulation Tissue with Acute and Lymphocytic Inflammation and without Identifiable Implant Debris, consistent with Implant Infection
57	Acute and Chronic Inflammation and Granulation Tissue consistent with Implant Infection -- with Polyethylene Spacer
60	Chronic Inflammation and Granulation Tissue, with Neutrophils, Consistent with Implant Infection
61	Acute Inflammation and Granulation Tissue Consistent with Implant Infection
62	Acute Inflammation with Implant Debris, Suggestive of Infection
63	Acute Inflammation consistent with Implant Infection
64	Acute Inflammation consistent with Implant Infection
66	n/a

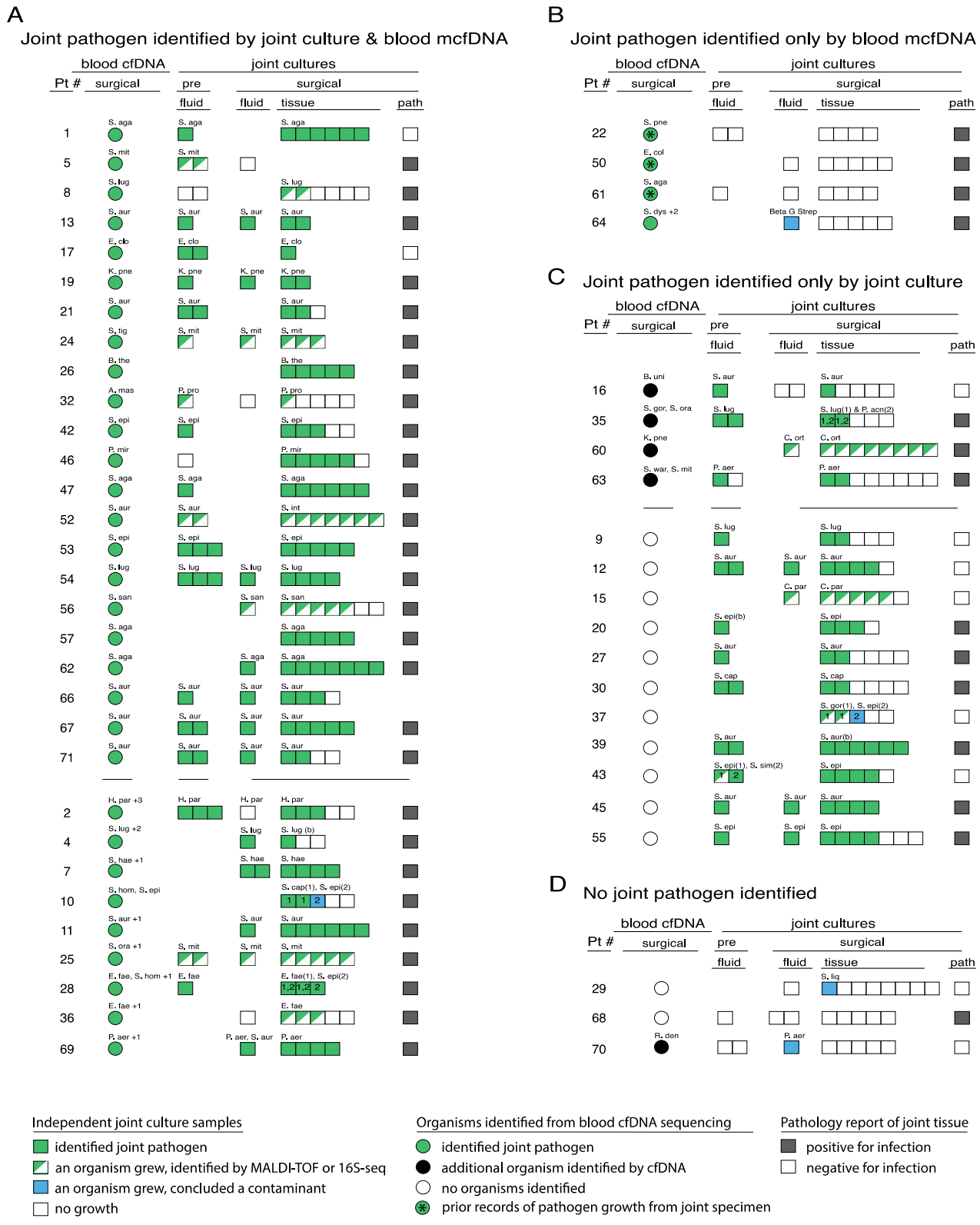
67	Acute and Chronic Inflammation and Granulation Tissue consistent with Implant Infection
68	Consistent with On-going Infection
69	Sinus Tract with Acute Infection
70	Chronic Tissue Reaction without Identifiable Implant Material, Soft Tissue. No Evidence of Infection
71	Granulation Tissue with Acute and Lymphocytic Inflammation and without Identifiable Implant Material, consistent with Implant Infection

STable 5. Summary of Pathology reports.



SFigure 1. Post-enrollment patient exclusions

http://dx.doi.org/10.2106/JBJS.20.02229



SFigure 2. Sequencing microbial cfDNA from the blood detects pathogens in prosthetic joint infections. For patients undergoing surgical procedures for a prosthetic joint infection, plasma purified from a single blood sample was analyzed by microbial cfDNA sequencing (Blood cfDNA); green circles indicate a species match with the joint culture organism, a yellow circle indicates an organism not cultured from the joint. Standard-of-care joint fluid and tissue speci- mens collected at a pre-surgical visit (pre) and during the surgery (surgical) were assayed in microbiologic cultures; each independent joint sample is depicted as a square. Filled squares indicate growth; green indicates more than one culture grew the same species, which was deemed the joint pathogen, while blue indicates a single culture grew the organism, which was considered a contaminant. Half green and half white squares indicate that a subsequent molecular analysis (MALDI-TOF or 16S-seq) was performed on the culture growth to identify the species. Surgical tissue was histologically scored by a pathologist (path); a grey box indicates signs of infection. A. Thirty-one infections for which blood microbial cfDNA sequencing and joint cultures identified the joint pathogen. B. Four infections where blood cfDNA sequencing iden- tified the causative organisms, yet joint specimens around the time of surgery did not grown an organism. Prior records of joint cultures confirmed the identification of the joint pathogen (*, inside green circle). C. Fifteen infections where joint cultures identified a pathogen, but that species was not identified by the blood cfDNA test. D. Three infections in which neither joint cultures nor blood cfDNA sequencing identified a pathogen.

1. Blauwkamp TA, Thair S, Rosen MJ, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nat Microbiol.* 2019;4(4):663-674.
2. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* 2014;30(15):2114-2120.
3. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nature methods.* 2012;9(4):357-359.
4. Camacho C, Coulouris G, Avagyan V, et al. BLAST+: architecture and applications. *BMC Bioinformatics.* 2009;10:421.