International Conference
on Clinical Metagenomics



Assessment of host response using meta-transcriptomics for periprosthetic joint infection diagnosis

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Arthroplasty





Arthroplasty failure





Causes of arthroplasty failure leading to revision

Breakdown of hip revision		11 = 1010
Failure Mode	Total Number of Failures	Percent of Total Failures
Aseptic Loosening	305	23%
Dislocation	295	22%
Infection	291	22%
Instability	85	6%
Mechanical Complications	82	6%
Bone Fracture	50	4%
Component Fracture	25	2%
Pain	22	2%
Wear	11	1%
Other	149	11%

Non-infectious arthroplasty failure (NIAF) | Periprosthetic joint infection (PJI)



Adapted from: Kenney C, Dick S, Lea J, Liu J, Ebraheim NA. 2019. A systematic review of the causes of failure of Revision Total Hip Arthroplasty. *J Orthop*.16(5):393-395.

Breakdown of hin revision

n = 1315

Periprosthetic joint infection (PJI)

- 2-4% of patients undergoing arthroplasty surgery
 - Increased cost
 - Decreased quality of life
 - Loss of implanted device
 - Loss of limb

- Often treated via IDCR
 - Implant Debridement
 - <u>Component Resection</u>
- Other (3%) Enterococcus species (3%) Anaerobic bacteria (4%) Staphylococcus aureus (27%) Aerobic Gram-negative bacilli (6%) Culture negative (7%) Streptococcus species (8%) -Polymicrobial (15%) Coagulase-negative Staphylococcus (27%)
- Microbes often antimicrobial resistant
- Underlying immune response relatively unknown

Tande AJ, Patel R. 2014. Prosthetic joint infection. *Clin Microbiol Rev* 27:302-45. Del Pozo JL, Patel R. 2009. Infection associated with prosthetic joints. *NEJM* 361:787-794. Higgins E, Suh GA, Tande AJ. 2022. Enhancing diagnostics in orthopedic infections. *J Clin Microbiol.* 60(6):e0219621

PJI diagnosis techniques



Traditional Bacterial Culture

- Culture-negative PJI (7-15%, 42% reported)
- Low sensitivity
- Impacted by prior antibiotic usage



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Next Generation Sequencing (16S, WGS)

- Low bacterial product abundance
- High contamination rate
- Impacted by prior antibiotic usage
- Bioinformatically time consuming



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Host Biomarkers

- Limited (α -defensin, CRP, WBC counts, etc.)
- Can be expensive

MAYO CLINIC Tande AJ, Patel R. 2014. Prosthetic joint infection. Clin Microbiol Rev 27:302-45. Del Pozo JL, Patel R. 2009. Infection associated with prosthetic joints. NEJM 361:787-794. Higgins E, Suh GA, Tande AJ. 2022. Enhancing diagnostics in orthopedic infections. J Clin Microbiol. 60(6):e0219621

Synovial fluid alpha-defensin FDA approved for PJI diagnosis aid

FDA NEWS RELEASE

FDA permits marketing of first diagnostic test to aid in detecting prosthetic joint infections



For Immediate Release: May 23, 2019

Today, the U.S. Food and Drug Administration permitted marketing of the Synovasure Lateral Flow Test Kit as an aid for the detection of periprosthetic joint infection (infection around a joint replacement) in the synovial (lubricant) fluid of patients being evaluated for revision surgery, which is surgery performed to replace or compensate for a failed implant.



www.fda.gov/news-events/press-announcements/fda-permits-marketing-first-diagnostic-test-aid-detecting-prosthetic-joint-infections

PJI - a unique and complex disease to diagnose and to treat





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Patient characteristics	Periprosthetic joint infection (n=53)	Non-infected arthroplasty failure (n=40)
Age (yr)	66	63
Male	30 (57%)	15 (38%)
First prosthetic revision	25 (47%)	24 (60%)
Knee revision	28 (53%)	39 (98%)
Hip revision	25 (47%)	1 (2%)
Infectious indication		
Staphylococcal	29 (55%)	NA
Staphylococcus aureus	8 (28%)	NA
Staphylococcus capitis	1 (3%)	NA
Staphylococcus epidermidis	15 (52%)	NA
Staphylococcus lugdunensis	5 (17%)	NA
Non-staphylococcal*	24 (45%)	NA
Acute infection (<90 days)	3 (6%)	NA
Chronic infection (>90 days)	50 (94%)	NA
Non-infectious indication		
Aseptic loosening**	NA	6 (15%)
Instability	NA	23 (58%)
Stiffness	NA	6 (15%)
Miscellaneous	NA	5 (12%)

NA: not applicable

*Includes (n): Actinomyces naeslundii (1), Bacteroides fragilis (1), Citrobacter koseri (1), Corynebacterium jeikeium (1), Cutibacterium acnes (3), Enterobacter cloacae (1), Enterococcus faecalis (2), Escherichia coli (1), Granulicatella adiacens (1), Prevotella bivia (1), Pseudomonas aeruginosa (2), Streptococcus agalactiae (4), Streptococcus anginosus (1), Streptococcus dysgalactiae (1), and viridans group Streptococcus species (3)

**Non-metalosis and non-particle-induced osteolytic loosening



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Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeq-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. *JBJS. Accepted.*

Sonicate fluid bulk RNA-seq



^e Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, MN, United States



Sonicate fluid bulk RNA-seq methods



MAYO CLINIC Masters TL, Bhagwate AV, Dehankar MK, Greenwood-Quaintance KE, Abdel MP, Mandrekar JN, Patel R. 2022. Human transcriptomic response to periprosthetic joint infection. *Gene*. 825:146400

Differentially expressed transcripts between PJI and NIAF sonicate fluids



Masters TL, Bhagwate AV, Dehankar MK, Greenwood-Quaintance KE, Abdel MP, Mandrekar JN,

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Patel R. 2022. Human transcriptomic response to periprosthetic joint infection. Gene. 825:146400

Using ROC curves to determine predictive accuracy



Altered from: Zhang C, Zhao J, Zhu Z, Li Y, Li K, Wang Y and Zheng Y. 2022. Applications of artificial intelligence in myopia: Current and future directions. *Front Med.* 9:840498.

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Transcripts most predictive of PJI via ROC curve analysis

Gene marker	Gene product	AUC	P value
PI3 (SKALP)	Peptidase inhibitor 3	0.996	0.0019
IL5RA	Interleukin 5 receptor subunit alpha	0.990	0.0002
F7	Coagulation factor VII	0.973	0.0004
IL1B	Interleukin 1 beta	0.967	<.0001
FCRL4	Fc receptor-like 4 (B cell receptor)	0.962	<.0001
IL8	Interleukin 8	0.958	0.0007
IL1A	Interleukin 1 alpha	0.918	<.0001
CCL20	C-C motif chemokine 20	0.897	<.0001
FCGR1B	Fc Fragment Of IgG Receptor Ib	0.883	<.0001
UMOD	Uromodulin	0.858	0.0002
IL6	Interleukin 6	0.856	0.0003
CSF3	Colony stimulating factor 3	0.752	0.0024
BPI	Bactericidal/permeability increasing protein	0.748	0.0585

*AUC of 1.0 = 100% sensitivity and specificity; >0.9 = highly predictive

Q.

F7, FCRL4, and CCL20 are novel potential biomarkers for PJI detection

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IL1B, IL8, and PF4V1 differentiated S. aureus PJI and S. epidermidis PJI

Gene marker	Gene product	AUC	P value
IL1B	Interleukin 1 beta	0.80	0.02
IL8	Interleukin 8	0.75	0.053
PF4V1	Platelet factor 4	0.78	0.03

*AUC of 1.0 = 100% sensitivity and specificity; >0.9 = highly predictive



Masters TL, Bhagwate AV, Dehankar MK, Greenwood-Quaintance KE, Abdel MP, Mandrekar JN, Patel R. 2022. Human transcriptomic response to periprosthetic joint infection. *Gene*. 825:146400

Bulk RNA-seq conclusions

- Bulk RNA-seq analysis found ~2800 PJI vs NIAF differentially expressed transcripts
 - Confirmed 28 previously reported PJI biomarkers
 - 7 transcripts highly predictive of PJI (ROC curve AUC > 0.9)
 - 3 potential novel PJI biomarkers (F7, FCRL4, CCL20)
 - IL1B, IL8, and PF4V1 predictive of S. aureus from S. epidermidis PJI



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 - IL1B, IL8, and PF4V1 predictive of *S. aureus* from *S. epidermidis* PJI

What types of cells make up the sonicate fluid transcriptome?

Can predicted cellular profiling be used to differentiate PJI and NIAF?

Masters TL, Bhagwate AV, Dehankar MK, Greenwood-Quaintance KE, Abdel MP, Mandrekar JN, Patel R. 2022. Human transcriptomic response to periprosthetic joint infection. *Gene*. 825:146400

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- + Direct sampling of infected area
- Loss of cellularity











CIBERSORTx cellular deconvolution

Bulk RNA-seq





Altered from: Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. 2018. Profiling Tumor Infiltrating Immune Cells with CIBERSORT. *Methods Mol Biol.* 1711:243-259.

PJI and NIAF sonicate fluid samples have different predicted cellularity profiles





Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeq-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. JBJS. Accepted.

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Predicted cellular fractions (specifically neutrophils and activated mast cells) are highly sensitive and specific for differentiating <u>PJI</u> from <u>NIAF</u> samples



*AUC of 1.0 = 100% sensitivity and specificity; >0.9 = highly predictive



Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeq-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. *JBJS*. Accepted.

Predicted cellularity profiling is unable to differentiate <u>staphylococcal PJI</u> from <u>non-staphylococcal PJI</u>, though predicted monocytes are differentially abundant





Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeq-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. *JBJS. Accepted.*

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Total granulocyte fraction elevated in <u>*S. aureus* PJI</u>, while total macrophage/monocyte fraction elevated in <u>*S. epidermidis* PJI</u>



Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeq-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. JBJS. Accepted.

Total granulocyte fraction elevated in <u>S. aureus PJI</u>, while total macrophage/monocyte fraction elevated in S. epidermidis PJI



Interesting inflammatory differences caused by S. aureus vs S. epidermidis?

MAYO CLINIC Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeg-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. JBJS. Accepted.

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Like staphylococcal vs non-staphylococcal PJI, predicted cellularity profiling is unable to differentiate <u>S. aureus</u> and <u>S. epidermidis PJI</u>



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Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeq-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. JBJS. Accepted.

Predicted cellularity profiling is unable to adequately differentiate <u>causes of NIAF</u>, though differences in individual predicted cellular fractions are present





Fisher CR, Krull JE, Bhagwate A, Masters T, Greenwood-Quaintance KE, Abdel MP, Patel R. 2022. Predicted cellularity using RNASeq-based cellular deconvolution differentiates periprosthetic joint infection from non-infectious arthroplasty failure. JBJS. Accepted.

Cellular deconvolution conclusions

- Cellular deconvolution via CIBERSORTx can differentiate PJI and NIAF, but not individual causes thereof:
 - Staphylococcal vs non-staphylococcal PJI
 - S. aureus vs S. epidermidis PJI
 - Causes of NIAF
- Predicted neutrophil and activated mast cell fractions highly predictive of PJI



Host-based meta-transcriptomic profiling provides potential targets for future investigation to improve clinical diagnosis and better understand the complex immune response during PJI and NIAF



Limitations

- Sample cohort:
 - Samples from incontrovertible PJI cases
 - All had two or more positive cultures of the same microorganism
 - No polymicrobial, no culture-negative
 - Limited patient co-morbidities (no underlying rheumatic or neoplastic disorders)
 - Low sample sizes in many of the PJI and NIAF subgroups



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 - Limited patient co-morbidities (no underlying rheumatic or neoplastic disorders)
 - Low sample sizes in many of the PJI and NIAF subgroups
- Cellular deconvolution:
 - "Predicted" cellularity fractions
 - Dependent in cell-types included in signature matrix
 - Signature matrix primarily from cancerous tissues
 - Activated mast cells?
 - Limited CIBERSORTx use in bacterial infections
 - Heavy bioinformatic/ analytical component
 - Robust subgroup analyses could not be conducted due to low subgroup size



A future for multi-omics approaches of infectious disease diagnosis?



Multi-omics analysis





Metagenomics















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Acknowledgements

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Thank you! Questions?

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