A Novel Sequencing Technique to investigate Orthopaedic Prosthetic Joint Infection

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INTRODUCTION: Prosthetic joint infection (PJI) can affect up to 2% of all orthopaedic arthroplasty surgeries in the UK and is associated with increased morbidity, mortality and healthcare costs, with revision surgery for PJI costing around 3 times that of surgery for non-infected aseptic revision[1,2]. The diagnosis of PJI remains a challenge, international consensus meetings have updated diagnostic criteria, however, there remains no unified diagnosis or test[3,4]. Microbiology culture identifying an infective organism remains the gold standard for diagnosis and for rationalisation of antimicrobial treatment, however a cohort of 'culture negative' patients remain a diagnostic challenge. Novel gene sequencing techniques are coming to the forefront of diagnostics across all medical specialties including orthopaedics in the investigation of PJI, particularly the culture negative cohort. Next generation and metagenomic sequencing can detect all host and microbial DNA in a sample and provide antimicrobial resistance genes, however, the high sensitivity can lead to detection of pathogens with unclear clinical importance. Furthermore, transforming raw sequence data into clinically applicable information requires technical infrastructure, robust data analysis and cost. In this study we present a novel, extraction free, sequencing technique which switches the diagnostic strategy from pathogen identification to individual host immune response to infection. We sequenced tissue and blood on a unique immune response panel to identify gene signatures with genus level bacterial identification.

METHODS: Blood in PAXGene ® tubes and tissue in sterile RPMI were collected from 24 patients undergoing revision arthroplasty surgery for aseptic revision and PJI. Patient data from electronic hospital records was collected relating to microbiology, histopathology and blood biochemistry. All patient samples and clinical data were collected with informed patient consent and with institutional review board approval. Tissue was formalin fixed paraffin embedded (FFPE) and cut in 5µm sections stained with H+E to assess tissue cellularity or transferred to HTG molecular diagnostics along with blood tubes for NGS. Quantitative analysis of a pre-defined immune gene panel was performed using NGS and a nuclease protection assay (HTG Molecular diagnostics). Data was provided as raw read counts and analysed with statistical significance set at $p<0.05$. Clinical patient data was used to identify the infective organism and to stratify samples as PJI or aspetic and further subdivide into acute or chronic PJI based on MSIS criteria[4].

RESULTS SECTION: *FFPE tissue sequencing*: Annotating sample as PJI or aseptic showed unique gene signatures separating these two groups, with significantly increased expression of genes involved in response to infection in the PJI group. Further analysis with samples annotated as acute, chronic or aseptic showed two distinct signature patterns. Chronically infected tissue samples clustered with aseptic control with enriched gene sets, that identify biological activity related to these genes, did not fit clinically with response to infection. Acutely infected tissue was driving signature 2 and included clinically relevant genes involved in response to bacterial infection (figure 1). Blood sequencing: Annotation of samples based on bacterial strain (*staphylococcal*, *streptococcal*) identified 98 genes differential expressed between the two bacterial. Hierarchal clustering of these differentially expressed genes showed separation in to two groups, suggesting an underlying unique gene signature for *staphylococcal* vs *streptococcal* (figure 2) *Staphylococcal* infection significantly upregulated genes involved in a T cell response compared with *streptococcal* infection which upregulated macrophage markers.

DISCUSSION: FFPE tissue sequencing showed proof of concept for identifying a unique immune signature differentiating PJI from aseptic tissue. Chronically infected PJI tissue has a reduced immune response, similar to aseptic tissue. Prior clinical studies have shown that traditional immune markers for infection/inflammation have low diagnostic value in the chronic setting. Sequencing from blood identified unique immune gene signatures for *staphylococcal* and *streptococcal* bacteria. Protein correlation of sequencing data and further assessment of this immune gene panel in a larger cohort with more bacterial strains including polymicrobial infection, often seen in PJI, is warranted.

SIGNIFICANCE/CLINICAL RELEVANCE: Switching diagnostic rationale from pathogen identification to host immune repose to bacteria has demonstrated unique gene signatures in PJI tissue and blood stratifying acute from chronic infection and staphylococcal from streptococcal bacteria, furthering our knowledge of implant related immune modulation and providing genus level bacterial information for PJI diagnosis from blood.

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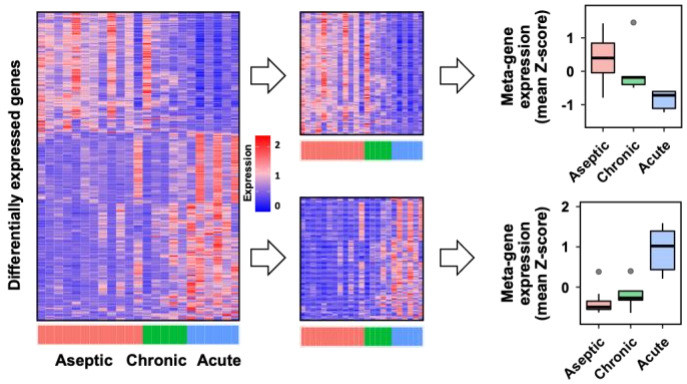

Figure 1: *FFPE tissue sequencing* heatmap and meta-gene signatures of differentially expressed genes comparing acute and chronic PJI with aseptic control.

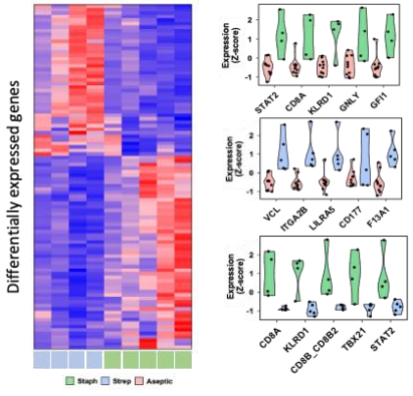

Figure 2: *Blood sequencing*: heatmap and summary violin plots of differentially expressed genes comparing staphylococcal, streptococcal and aseptic control.