Use of Circulating Bacteriophage DNA to Rule Out Staphylococcal Infections

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INTRODUCTION: Diagnosing periprosthetic joint infections (PJI) is challenging, often requiring invasively obtained specimens to identify the offending pathogen. We previously reported on using bacteriophage sequences in cell-free DNA (cfDNA) in plasma to diagnose bacterial pathogens in sepsis. We hypothesize that bacteriophage cfDNA in plasma can diagnose and track clearance of PJI.

METHODS:

Bacteriophage sequences were identified in cfDNA from plasma of 3 distinct patient cohorts: patients with a current *Staphylococcal* PJI, prior PJI, and no PJI. The overall phageome was described in all 3 cohorts, and the proportion of *Staphylococcus* bacteriophage was compared among the 3 cohorts. The presence of *Staphylococcus* bacteriophage as a binary variable was also analyzed for its diagnostic performance in identifying Staphylococcal PJI. ESR, CRP, *Staphylococcus* phage proportion and diversity were found to have non-normal distributions, making the Kruskal-Wallis test with multiple comparisons was most appropriate for evaluating differences between three groups, and Mann-Whitney U Test for comparisons between two groups. Fisher's exact test was used in the contingency table analyses as it is more precise in calculations in smaller sample sizes. Alpha error was set to 0.5, and all analyses were done in GraphPad Prism 10.2.3.

RESULTS:

Thirty-five plasma samples (10 current PJI, 12 prior PJI, 13 no PJI) were included in the analysis. There were no differences in the distribution of bacterial cfDNA among the three cohorts (p=0.597). The current PJI cohort had a significantly higher proportion of *Staphylococcus* bacteriophage compared to no PJI (12% vs 5%, p-value=0.040) but there was no difference in proportion of *Staphylococcus* bacteriophage between current PJI and prior PJI (12% vs 2%, p-value=1.00) or between prior PJI and no PJI (2% vs 5%, p-value=0.147). ESR did not reach statistical significance between the three groups; however, CRP levels were higher in the current PJI group compared to prior PJI (Kruskal-Wallis, p=0.036) and no PJI (Kruskal-Wallis, p<0.001), and no significant difference was observed between prior PJI and no PJI (Kruskal-Wallis, p=0.278) (Figure 1). *Staphylococcus* phage was present in 6/10 (60%) samples in current PJI, 7/12 (58%) samples in prior PJI, and 1/13 (8%) samples in no PJI (Figure 2). The difference in proportion was significant between current PJI and no PJI (Fishers Exact test, p=0.019, Figure 2) and between prior PJI and no PJI (Fishers Exact test, p=0.011, Figure 2), but there was no difference between current PJI and prior PJI (Fishers Exact test, p=1.00, Figure 2). The presence of *Staphylococcus* phage had a sensitivity and specificity of 60% and 92%, respectively, for identifying a Staphylococcal PJI.

DISCUSSION AND CONCLUSION:

We observed a disruption in the plasma phageome with an increase in proportion of *Staphylococcus* bacteriophage in patients with Staphylococcal PJI. The presence of *Staphylococcus* bacteriophage persisted in patients with prior PJI, which may indicate subclinical persistent infection that is not detected through conventional diagnostic methods. Bacteriophage cfDNA may be a promising diagnostic tool to non-invasively diagnose Staphylococcal PJI.

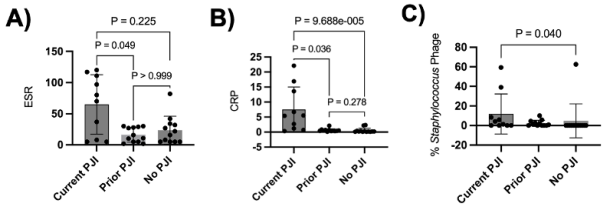

Figure 1: Distribution of (A) ESR (mm/h), (B) CRP (mg/dL), and (C) percentage of *Staphylococcus* phage by PJI status suggesting that circulating plasma cfDNA of patients with a current *Staphylococcus* infection are comprised of a larger proportion of *Staphylococcus* phages.

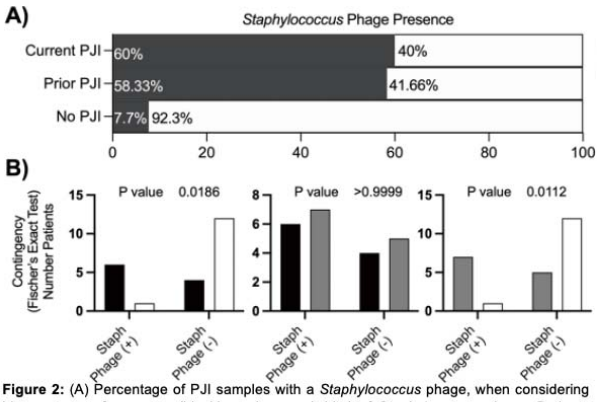

Figure 2: (A) Percentage of PJI samples with a *Staphylococcus* phage, when considering binary status of presence (black) or absence (white) of *Staphylococcus* phage. Patients with a current or prior PJI had a similar percent of *Staphylococcus* phage, whereas those with no history of PJI were less likely to have *Staphylococcus* phage, in their circulating cell-free DNA (cfDNA). (B) Contingency graphs showing correlation between circulating *Staphylococcus* phage cfDNA and the diagnosis of a PJI (black), prior PJI (grey), and no PJI (white). Fischer's Exact test demonstrates a correlation with presence of circulating *Staphylococcus* phage cfDNA and the presence of an active of prior PJI.