Identification Of MicroRNA Biomarkers Associated With Staphylococcal Fracture-related Infection.

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Disclosures: No disclosure

INTRODUCTION: The pathogenesis of fracture related infection (FRI) is related to microorganisms growing in biofilms, which render these infections difficult to treat. Most infections are caused by staphylococci and are acquired during trauma (in penetrating injuries) or subsequent fracture-fixation procedures. A combination of clinical, laboratory, histopathology, microbiology, and imaging studies are usually needed to accurately diagnose infection. In this context miRNAs have been extensively researched as possible biomarkers in different fields. MiRNAs are a class of non-coding RNAs that play key roles in the regulation of gene expression. Acting at the post-transcriptional level, they fine-tune the expression of as much as 30% of all mammalian protein-encoding genes. In addition to their important roles in physiologic processes, microRNAs have also been implicated in the development and progression of several diseases including a broad range of cancers, cardiovascular disease, and neurological disorders. Therefore, the aim of this study was to identify miRNAs in the serum of patients with staphylococcal fracture related infection (FRI) and use miRNAs as biomarkers for infections.

METHODS: Serum samples from patients with confirmed staphylococcal FRI after open fracture were collected from the University Hospital Muenster and Berlin (Germany). In total *n*=36 patients were included in the study, *n*=10 patients with *Staphylococcus* (*S.*) *aureus* FRI, *n*=10 with *S. epidermidis* FRI and *n*=16 age-matched controls (open fracture, no infection). Samples were selected according to time post-trauma in the control and test groups. RNA isolation and miRNA sequencing was performed by Qiagen according to standard protocols.

RESULTS SECTION: We used the non-biased, global microRNA analysis using RNA-seq to generate data from a population of 36 human patients with orthopedic device related infection and FRI, caused by either *S. aureus* (*n*=10) or *S. epidermidis* (*n*=10). We then compared this data to the 16 uninfected patients with orthopedic devices (controls, uninfected). Analyses of patient samples with both types of infection showed a significant number of miRNA sequences enriched in the serum relative to uninfected patients (Figure 1). In the *S. aureus* cohort 43 miRNAs were differentially expressed compared to the uninfected patients, while in the *S. epidermidis* group 125 miRNAs.
Of the large number of significant targets in both types of infection, top miRNA targets were selected for further validation based on a high absolute fold-change in expression in infected samples relative to uninfected samples, with a high level of consistency (p-value<0.05). Eight miRNA targets were common to both *S. aureus* and *S. epidermidis* infections: hsa-miR-483-5p, hsa-miR-1246, hsa-miR-10b-3p, hsa-miR-320c, hsa-miR-1290, hsa-miR-320d, hsa-miR-4508, hsa-miR-294-3p.
Real time-PCR of serum samples using the selected miRNA targets confirmed 5 miRNAs as differentially expressed using a cutoff of p-value < 0.05 when comparing the *S. aureus* infected group to the uninfected group using a t-test: hsa-miR-483-5p, hsa-miR-1246, hsa-miR-1290 were upregulated while hsa-miR-23a-3p and hsa-miR-148b-3p were downregulated. Instead, when comparing the *S. epidermidis* infected group to the uninfected group, only 1 miRNA was confirmed to be downregulated: hsa-miR-1-3p.

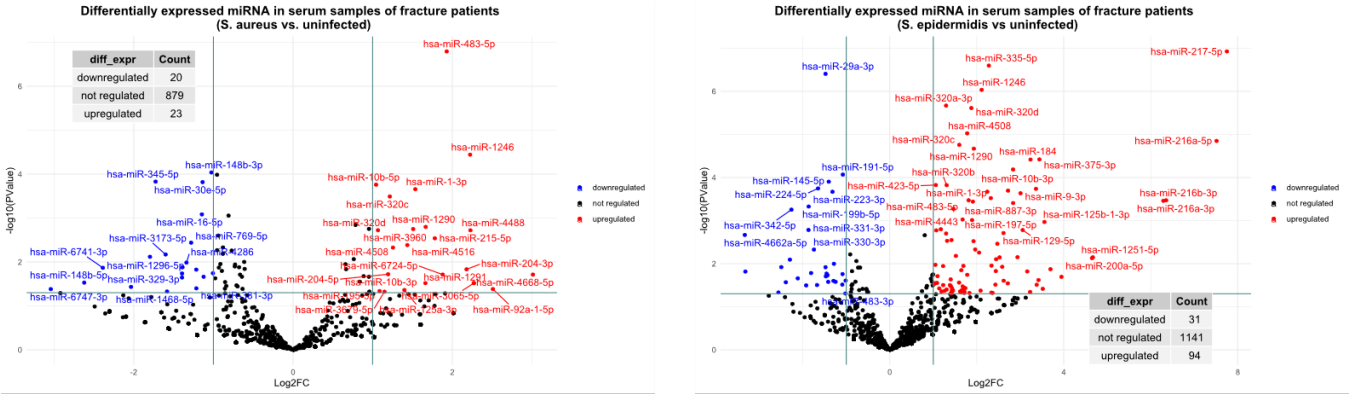

Figure 1. Volcano plots of differentially expressed miRNAs in serum samples of patients with *S. aureus* (left) and *S. epidermidis* (right) FRI.

DISCUSSION: It is clear from the unsupervised analysis that the samples cluster according to their biological group infected *vs* uninfected, indicating that the underlying biology of the samples is causing the largest variation between the samples. Interestingly, *S. epidermidis* infections led to a more significant miRNA upregulation level relative to that of *S. aureus* infections, which are typically considered more aggressive. The miRNAs identified in this study can now be tested in a larger cohort of staphylococcal FRI and evaluate if they are similarly expressed also in FRI caused by different microbials.

SIGNIFICANCE/CLINICAL RELEVANCE: Expanding knowledge about the role of microRNAs in biology and their dysregulation in many diseases has prompted scientists to investigate their potential use in disease therapy. The role of miRNAs in FRI is almost unknown. Analyses of samples from FRI patients showed that it is possible to compare baseline expression patterns typically associated with normal fracture healing versus that of infected fractures. This study opens the possibility to further investigate miRNAs in FRI as a diagnostic approach, but potentially also a theragnostic approach.

ACKNOWLEDGEMENTS: This project was founded by AO Trauma.