

Microbiome Analysis for Assessments of Treatment Response and Salvage Prognosis in Infected Diabetic Foot Ulcers

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Category: Basic Sciences/Biologics,Diabetes

Keywords: Diabetic foot ulcer, Diabetic foot infection, Microbiome

Introduction/Purpose: Diabetic foot ulcers (DFUs) contribute to 80% of non-traumatic lower-extremity amputations. Surgeons are often forced to make surgical decision without adequate prognostic information. DFU infections are often polymicrobial, representing complex microbial communities. A microbiota is the ecological community of various microorganisms that share body space. Currently, the methods of detecting an active infection, identifying the pathogenic bacteria within the microbiome, measuring the response to therapy, and assessing prognosis are limited. Using a molecular genomic technique of 16S rRNA sequencing, our goals are to assess the pathogenic bioburden of DFUs and to monitor the bacterial community changes in response to antibiotic treatment. Our hypothesis is that the microbiome in DFUs responding to debridement and antibiotics treatment is distinct from those that fail to respond.

Methods: Patients with type I or II diabetes who presented with an infected DFU were enrolled. Infections were identified using clinical signs. The DFU size was measured and classified using the Wagner classification. Enrolled patients were initially managed with foot salvaging therapy (FST): irrigation and debridement followed by wet-to-dry dressings and 6 weeks of intravenous antibiotic treatment. Superficial and deep DFU samples were obtained and evaluated by 16S rRNA microbiome analysis and qPCR for bacterial abundance. This was repeated at 4, 8, and 12 weeks following the initiation of FST. At 12 weeks, patients were divided into two groups, healed and non-healed, based on the change in the size of the wound and absence or presence of 12 secondary signs of infection. Alpha- and beta-diversity were measured by the Shannon index and Bray-Curtis dissimilarity index to evaluate changes in the microbiome between the healed and non-healed groups.

Results: From July 2015 to August 2016, 21 patients were enrolled and 3 deceased due to medical comorbidities. Of the 18 patients available for follow-up, 10 failed FST and 8 healed. The qPCR and microbiome analysis revealed that the bacterial abundance and diversity of the bacterial community were substantially reduced following debridement and intravenous antibiotic treatment. At the initial enrollment, those group that healed versus non-healed showed significant differences in microbiome composition, with the healed group enriched with Actinomycetales and Staphylococcus, and the non-healed group enriched with Bacteroidales and Streptococcus. At week 4, such differences disappeared and bacterial abundance significantly decreased. New differences were evident at week 8: the healed group was enriched with Actinomycetales and non-healed group with Bacilli.

Conclusion: Abundant presence of Bacteroidales and Streptococcus at the initial presentation of infected DFU maybe a poor prognostic sign for healing with FST. Through molecular analysis of the wound microbiome, we can identify pathogens of prognostic value at the initial cultures and assess response to therapy with significant differences at 8 weeks after. Our study provides useful information for counseling patients of treatment prognosis and determining to pursuit further foot salvage versus amputation.

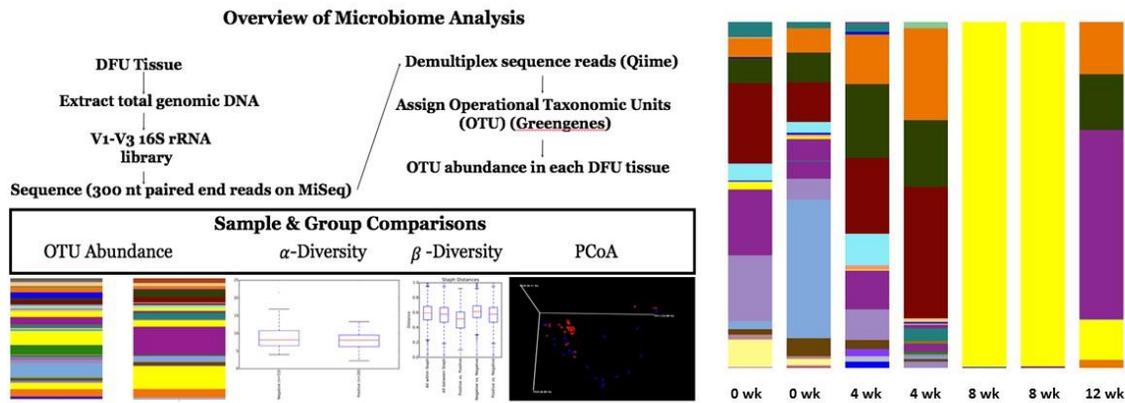


Figure 1. Patient is *S. aureus* confirmed positive by microbiome and immunoassay at enrollment, but negative by standard culture. The microbiome analysis shows high diversity and relatively large *S. aureus* abundance (purple band) at the initial presentation. As patient underwent 6 weeks course of antibiotics treatment, decreased abundance of *S. aureus* is noted at 4 weeks. Significantly decreased diversity with absence of *S. aureus* is noted at 8 weeks. With persistent infection, resurgence of *S. aureus* is noted at 12 weeks.