Δ Proteomic Analyses of Peripheral Blood from Patients with Fracture-Related Infection Reveals Systemic Activation of the Complement and Coagulation Cascades

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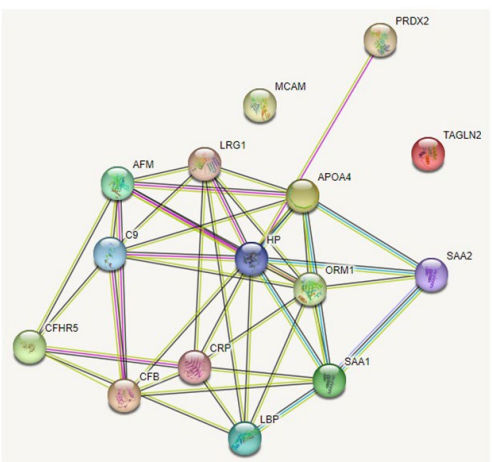
Purpose: Fracture-related infection (FRI) can be difficult to diagnose and contributes to decreased patient quality of life and increased societal economic costs. Definitive diagnosis of FRI centers around detection and identification of pathogenic microorganisms, which demands procurement of multiple deep tissue samples for microbiological testing amid surgical debridement. Alternative diagnostic approaches based on biomarkers are needed to improve patient care. We hypothesized that liquid chromatography-mass spectrometry (LC-MS)-based proteomics of plasma would identify potential biomarkers for FRI.

Methods: 27 patients with confirmed FRI were matched based on age (±15 years), time after index surgery (±2 weeks), and fracture region against 27 patients who remained infection free for ≥6 months after treatment with a retained orthopaedic implant. Peripheral blood was drawn from patients in the FRI group ≤48 hours from their infection procedure preoperatively or from patients in the control group at standard of care clinical visits. Quantitative tandem mass tag (TMT)-LC-MS was utilized to identify protein abundances in plasma. Principal component analyses (PCAs) were used to determine the optimal number of components to decrease the data dimensions and determine linear algebraic combinations for discriminant analysis. Additionally, proteins in the first 3 PCs were entered into Reactome pathway analysis to identify biologic functions.

Results: The 2 groups were not significantly different in terms of age, sex, body mass index, alcohol use, diabetes, and tobacco use. There were significantly more (P = 0.001) open fractures in the FRI group (13/27) compared to the control group (1/27). 73 proteins were found to be significantly increased or decreased in FRI patients compared to the matched controls (unadjusted t-test P≤0.05). 32 of these proteins were found in all 54 patient samples and underwent subsequent PCA and pathway analyses. A 3-component PCA accounted for 46% of the variation and was 88.9% specific for the diagnosis of FRI. Reactome pathway analysis of these 3 components revealed activation of the complement (see Figure 1) and coagulation cascades.



Reactome Pathways

pathway	description	count in network	strength	▲ false discovery rate
HSA-168249	Innate Immune System	9 of 1025	1.06	2.51e-05
HSA-166658	Complement cascade	4 of 57	1.96	0.00012
HSA-977606	Regulation of Complement cascade	3 of 47	1.92	0.0038

Conclusion: Proteomic analyses of plasma from FRI patients demonstrates systemic activation of the complement cascade in a highly specific manner. Further investigation along these lines may help to better understand the systemic response to FRI and improve diagnostic strategies.

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