Detecting differences in serum metabolites to diagnose fracture related infection (FRI) in a sheep model

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INTRODUCTION: Fracture related infection (FRI) represents one of the major complications in orthopedic and trauma surgery. FRIs are reported to occur between 1 and 30% depending on the nature of the injury. The most prevalent pathogen is *Staphylococcus aureus*, with reported rates up to 40% compared to other microorganisms. The identification of biomarkers that diagnose FRI, would be of enormous value for early intervention. In case of acute FRI, standard blood markers such as white blood cell count or C-reactive protein can clearly identify the presence of infection. But in case of low-grade infection which lack clear clinical or radiological signs the classical analyses do not show robust and reproducible results. Ideally, blood-based biomarkers would be the best option as they would require a minimally invasive procedure.
Metabolomics enables to profile a large number of metabolites, hence providing a comprehensive coverage of biological processes and metabolic pathways. Changes in metabolite concentrations is often directly related to pathogenic mechanisms. Metabolomics technologies have enabled a more rapid discovery of biomarkers used as early indicators of diseases, such as in pancreatic cancer, type 2 diabetes, and many other conditions. To date, metabolomics has rarely been employed for bacterial infection and has never been explored for FRI diagnosis.

METHODS: In this study, metabolomics analyses were performed on serum samples obtained from a sheep model of FRI. This study was approved by cantonal Ethical authorities in Chur, Switzerland. Briefly, Swiss Alpine sheep (n=7), of 2-6 years age and weight 50-80 kg received a 2 mm defect in the tibia and fixed with a 10-hole 5.5 mm steel plate (DePuySynthes Inc.). Subsequently, the defect was inoculated with 10^6 CFU/mL of *Staphylococcus aureus* MSSA (ATCC 25923) After 3 weeks, revision surgery was performed, and systemic antibiotic therapy started for two weeks with flucloxacillin and then for four weeks with rifampicin and cotrimoxazole. After 2 weeks of antibiotic flush-out, the animals were euthanized. Serum samples were collected every week. Samples from specific time points were used for metabolomic analyses: preoperative, at revision surgery, 6 weeks post revision (end of antibiotic treatment) and at euthanasia. Metabolites were extracted using methanol-based extraction protocol. Targeted metabolite analysis was performed using mass spectrometry-based workflow. For metabolite detection Vanquish Core HPLC system coupled with and Orbitrap Exploris 120 mass spectrometer was employed. Metabolites were analyzed using a full MS scan mode (m/z 50 to 600, resolution 30000). Data processing was done using Trace Finder 4.1 software (Thermo Scientific) with a seven-point linear calibration curve and 1/x weighing for quantification of metabolites.

RESULTS SECTION: Several metabolites show a decreased serum concentrations during the infection period and an increase back to baseline values after the antibiotic treatment and infection resolution. A more detailed analyses focused only on the preoperative phase *vs* infection (revision surgery), highlighted significant differences between the two conditions. A total of 11 metabolites were downregulated during infection, while only one metabolite was upregulated. Among those, L-Cysteine (p-value = 0.000002) and 4-hydroxyproline (p-value = 0.02), are significantly down and up- regulated, respectively.

DISCUSSION: Hydroxyproline is a non-essential amino acid found in collagen and few other extracellular animal proteins. Elevated levels of hydroxyproline have been reported in several disorders. Deficiency of L-cysteine has been reported in other infectious diseases like Covid-19 or HIV. The discovery of metabolic markers of specific conditions, such as FRI, could facilitate the development of specific assays and should be followed up in future studies.

SIGNIFICANCE/CLINICAL RELEVANCE: To date, few studies have investigated the role of metabolites in bacterial infection and none in FRI. Further investigation is required to fully understand their role and the interplay between bacterial infection and metabolites. This work can serve as a starting point for metabolomics-based diagnosis for clinical use.

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