Cysteine Depletion in Bacterial Infection a Diagnostic Biomarker for FRI

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Disclosures: nothing to disclose

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. In case of acute FRI, standard blood markers such as white blood cell count, or C-reactive protein aid in the diagnosis of infection, however, in case of low-grade infection, diagnosis can be more challenging. The identification of biomarkers that diagnose FRI would be of enormous value for early intervention. Ideally, blood-based biomarkers would be the best option as they would require a minimally invasive procedure.
The field of metabolomics holds great promise for improvements in precision medicine, characterization of metabolic derangements that underlie disease, discovery of new therapeutic targets, and biomarkers. Metabolomics studies have successfully identified a wide range of candidate biomarkers, including biomarkers for bone diseases such as osteoporosis and treatment response in patients with rheumatoid arthritis. To date, metabolomics has rarely been employed for bacterial infection and has never been explored for FRI diagnosis. Against this background, we applied metabolomics for the first time on serum obtained from a sheep model of *S. aureus* FRI and from human patients with *S. epidermidis* infection.

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=21), 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 2, 3 or 5 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. Samples were stored at -20°C until preparation.
Human samples from *S. epidermidis* infected patients (n=26) were collected at revision surgery from the Hospital in Münster and in Berlin, Germany under ethical local approval. Patient included those with any orthopedic device related infection (ODRI), not exclusively FRI. Patients undergoing implant removal without any sign of infection were used as control (n=18). Serum samples were stored at -70°C until preparation.
Metabolites were extracted using a methanol-based extraction protocol. Metabolite analysis was performed using a mass spectrometry-based workflow. For metabolite detection, a Vanquish Core HPLC system coupled with an 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 performed 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: In both *S. aureus* infected sheep samples and *S. epidermidis* infected human samples, several metabolites show a decreased or an increased serum concentrations at revision compared to baseline values (pre-OP, sheep samples) or compared to control (human samples).
Specifically, *S. aureus* infected animals showed reduction of L-Cystine (p = 0.009) and increased levels of 4-Hydroxyproline (p<0.0001) compared to pre-OP values. *S. epidermidis* infected human patients show significant reduced levels of L-Cystine compared to controls (p<0.0001) as well as tryptophane, leucine and citrulline (p<0.0001). Hydroxyproline did not differ between infected patients and controls (p=0.11).

DISCUSSION: The discovery of metabolic markers of FRI would allow patients to receive a more promptly appropriate infection-based therapy. The metabolites identified in the preclinical and clinical data set indicate that L-cysteine depletion is a common characteristic of infection since both *S. aureus* and *S. epidermidis* infection caused a downregulation of this metabolite. L-cysteine is a semi-essential thiol-containing amino acid, and it is an essential source of sulfur for the synthesis of diverse cellular factors that play important biological functions in maintaining redox homeostasis, enzyme catalysis, and electron transfer. Cysteine is partitioned into various downstream metabolic pathways including glutathione (GSH) synthesis, one of the major antioxidant defenses against high oxidative stress. The thiol group (-SH) of cysteine is a major substrate for the synthesis of GSH within cells. Thus, cysteine thiols play a crucial role in oxidative stress and studies indicated that bacteria could generate antioxidant molecules from cysteine. Further studies could focus on understanding if cysteine depletion in bacterial infection might indicate an involvement of the host metabolism and a new mechanism of bacterial defense against oxidative stress.

SIGNIFICANCE/CLINICAL RELEVANCE: To date, few studies have investigated the role of metabolites in bacterial infection, and none have focused on FRI. The discovery of metabolic markers of specific conditions, such as FRI, could facilitate the development of specific assays and allow a prompter diagnosis and intervention. Incorrect or delayed diagnosis of infection can prolong treatment and increase healthcare costs. Early and reliable diagnosis could be a game changer for patient care. However, further investigation is required to fully understand the role of the metabolites identified in this study and the interplay between bacterial infection and metabolites. This work can serve as a starting point for metabolomics-based diagnosis for clinical use.

ACKNOWLEDGEMENTS: This study was funded by the AO Trauma. The authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 857287 (BBCE – Baltic Biomaterials Centre of Excellence).