Distinct Host Metabolic Signatures as Biomarker Candidates in *S. aureus* Versus *S. epidermidis* Fracture-Related Infections

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INTRODUCTION: Fracture-related infections (FRIs) are serious complications in orthopedic and trauma surgery, affecting 1-30% of cases depending on injury type. *Staphylococcus aureus* is the leading pathogen, identified in up to 40% of FRIs, while low-grade infections such as those caused by *S. epidermidis* are more difficult to diagnose. Standard blood markers like white blood cell count and C-reactive protein help detect acute infections but lack sensitivity for low-grade cases. Identifying specific, minimally invasive biomarkers, ideally blood-based, would greatly improve early diagnosis and treatment. Metabolomics offers such potential, enabling the discovery of biomarkers through profiling metabolic changes. While applied successfully in bone diseases and treatment responses in rheumatoid arthritis, metabolomics has been rarely used in bacterial infections and not yet explored for differentiating FRIs caused by *S. aureus* versus *S. epidermidis*.

METHODS: In this study, metabolomics analyses were conducted on human serum samples collected from patients infected with *S. aureus* ($n = 36$) and *S. epidermidis* ($n = 31$) during revision surgeries at hospitals in Münster and Berlin, Germany. The study was approved by local ethics committees. The patients included those with any orthopedic device-related infections (ODRIs), not limited to just fracture-related infections (FRIs). A control group comprised patients undergoing implant removal without any signs of infection ($n = 26$). The serum samples were stored at -70°C until they were prepared for analysis. 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: Metabolomic profiling revealed distinct differences between FRI patients (*S. aureus* and *S. epidermidis*) and controls. Several metabolites were significantly upregulated in the FRI group, including lactic acid, malic acid, succinic acid, glutamic acid, fumarate, and erythrose 4-phosphate, with fold changes ranging from moderate (log2FC ~1) to strong (log2FC >2). These findings indicate enhanced activity of pathways related to energy metabolism and the tricarboxylic acid (TCA) cycle. Conversely, a subset of metabolites was markedly reduced in FRI patients compared to controls. Notably, pyruvate, butyrylcarnitine, aminoadipic acid, and serotonin were significantly downregulated, suggesting impaired glycolytic flux, fatty acid metabolism, and neurotransmitter balance. Comparison of metabolite profiles between *S. aureus* and *S. epidermidis* infections revealed marked differences in amino acid and collagen-related metabolites. Patients with *S. aureus* infection showed significantly higher levels of 4-hydroxyproline ($p = 0.015$), methylhistidine ($p = 0.027$), and hydroxylysine ($p = 0.041$), suggesting enhanced collagen turnover and protein catabolism in this group. In contrast, alpha-ketoglutarate was notably decreased in *S. aureus* patients ($p = 0.049$), pointing to altered TCA cycle activity. Collectively, these findings indicate that *S. aureus* infection is associated with a stronger perturbation of amino acid metabolism and extracellular matrix remodeling compared with *S. epidermidis* infection.

DISCUSSION: The observed metabolic alterations in FRI patients suggest that infection induces a profound remodeling of host energy metabolism. The consistent upregulation of TCA intermediates such as succinic acid, malic acid, fumarate, and glutamic acid points to an enhanced mitochondrial respiration, processes frequently linked to immune cell activation and the inflammatory response. Elevated lactate further supports a metabolic shift toward aerobic glycolysis, a hallmark of activated immune and stromal cells in infected tissue. Concurrent reductions in pyruvate, butyrylcarnitine, serotonin, and aminoadipic acid suggest impaired glycolytic input, fatty acid oxidation, and amino acid balance, reflecting diversion of substrates toward immune-metabolic demands. Together, these findings indicate that FRI is associated with a coordinated reprogramming of central carbon metabolism, where host cells prioritize biosynthetic and immunometabolic demands at the expense of energy efficiency.
The distinct metabolic profile observed in *S. aureus*-infected patients highlights its more aggressive pathogenicity compared to *S. epidermidis*. The pronounced elevation of collagen-derived metabolites such as hydroxylysine, 4-hydroxyproline, and methylhistidine suggests increased extracellular matrix degradation, consistent with the known tissue-destructive nature of *S. aureus*. This pattern likely indicates increased proteolytic activity and collagen turnover in infected bone and soft tissue. In contrast, the significant decrease in alpha-ketoglutarate suggests a disruption in central energy metabolism. These changes underscore a metabolically demanding and tissue-destructive response of the host to *S. aureus*, unlike the more gradual alterations observed with *S. epidermidis*.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings highlight the potential of metabolic profiling to improve diagnosis and management of FRI. Elevated TCA intermediates and lactate may serve as biomarkers of infection and immune activation, while collagen-derived metabolites could help distinguish aggressive *S. aureus* infections from indolent *S. epidermidis* cases. Such markers may support earlier, more targeted interventions and guide personalized treatment strategies.

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