Cutting and breaking bones are not equivalent fracture-related infection models: *In vivo* Comparison of traumatic fractures mimicking features of the clinical condition with simple osteotomies

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INTRODUCTION: Fracture-related infection (FRI) represents a significant challenge in orthopedic trauma surgery, with a growing research interest in FRI prevention and treatment requiring clinically relevant preclinical models. However, only 6% of described preclinical models combine all the main clinical features of FRI: a fracture, soft tissue damage, and delay in treatment.[1] Hence, current preclinical models of FRI significantly differ from the clinical situation in humans, which may impact the obtained results. This study aimed to evaluate the combined effect of etiology and severity of bone discontinuity and traumatic soft tissue injury on the muscle damage and bacterial burden of clinically relevant FRI preclinical model in rats. We hypothesize that a trauma resulting in a fracture and soft tissue trauma will increase bacterial burden compared with an osteotomy without trauma.

METHODS: The study was ethically approved by relevant authorities (Cantonal authorities in Graubünden, Switzerland: Permission # 13E/2022) and performed in a facility accredited by the AAALAC. Twenty-two healthy and skeletally mature (older than 20 weeks) female rats were enrolled in the study. Under general anesthesia and balanced analgesia, all rats underwent femoral bone discontinuity creation by the mean of an osteotomy (group A) or traumatic short oblique or transverse fracture with concurrent soft tissue injury using an apparatus creating a blunt impact from a weight dropped from a height (group B). Afterward, open reduction and internal fixation with bone plate and screws were undertaken for all rats (**Fig 1**), followed by bacterial inoculation with 10^4 Staphylococcus epidermidis colony forming units (CFU). Animal burden was evaluated twice daily using a study-specific scoring system. The impact of osteotomy versus blunt impact was described by the severity of soft tissue trauma from Creatinine Kinase (CK) and Aspartate Aminotransferase (AST) serum levels at 1, 3, and 5 days postoperatively and the severity of the infection determined by the bacterial burden measured using total CFU counts from surgical sites and implants at euthanasia. The impact of the bone discontinuity etiology on CK and AST serum levels and the individual and combined harvested tissue and implant CFU counts were determined using a one-way repeated measures multivariate analysis of variance (one-way MANOVA) and one-way MANOVA combined with univariate logistic regression models, respectively. A p-value ≤ 0.05 was considered significant.

RESULTS SECTION: From the 22 enrolled rats, 16 completed the study and were included in the analysis (n=8/group). Five rats from group B were euthanized under general anesthesia without fracture fixation because of inadequate fracture configuration, and one rat from group A was euthanized at 2 days postoperatively because of severely reduced general behavior due to pica. The measured CK and AST serum levels were significantly increased at 1 day postoperatively compared to before surgery (282±124 vs. 144±77 U/L and 372±172 vs. 153±78 U/L, respectively; p=0.001) and decreased to values similar to baseline at day 3 (p=0.808, 0.168) without being affected by the study group (p=0.059). The severity of the infection (all harvested CFU as combined outcomes) was significantly impacted by the study group ($p = 0.012$; $F(3.0, 10) = 6.253$; Wilk's $\Lambda = 0.348$, partial $\eta 2 = .652$). In addition, the rats that underwent traumatic fracture creation had significantly increased CFU counts from the soft tissues ($8.4\pm2.5 \times 10^6$ vs $4.8\pm1.9 \times 10^6$ CFU; p=0.002) and total CFU counts harvested from the operated limb ($1.5\pm0.4 \times 10^6$ vs $1.0\pm0.3 \times 10^6$ CFU; p=0.043; **Fig 2**).

DISCUSSION: It is generally accepted that the outcomes of fractures and FRI in human depends not only on the fracture itself but also on the combined soft tissue injury and stability of the fixation. This study confirmed that this concept also applies to animal experiments mimicking FRI. Before this study, there was only one description of a preclinical model using rats that included all FRI clinical features by creating a femoral fracture using a blunt trauma followed by a surgical site inoculated with a bacterial suspension before surgical stabilization using an intramedullary K-wire.[2-5] While this type of surgical stabilization might be less technically challenging, the lack of stability was a significant limitation as it is known to impact infection rates and does not reflect current clinical recommendations for fracture fixation. The FRI preclinical model described here overcomes this limitation by using appropriate plate and screw fixation. Although preclinical research using animal models must represent current clinical problems,[6] it is also essential to use highly reproducible models with the most minor severity and burden on animal welfare for the studied outcomes. While this study provides a good rationale for modifying currently used FRI preclinical models, the severity and burden on animal welfare of traumatic fractures should be thoroughly investigated to justify this change (*currently under analysis by the same study group; gait analysis and grimace scale results pending*). From an ethical point of view, the increased number of animals needed to have a reproducible fracture and the resulting expected additional burden for the animal must be described so that an appropriate balance of goods can be conducted for ethical project applications.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is the first to prove the importance of the type of bone injury and soft tissue trauma with regards to infection severity in animal models and to describe an animal model for FRI research with appropriate consideration of soft tissue injury in fractures achieving stable fixation. The use of preclinical FRI models with an osteotomy instead of a traumatic fracture creation have an impact on study results and may limit the clinical translation of new prevention and treatment strategies for FRI depending on the research question.

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IMAGES AND TABLES:

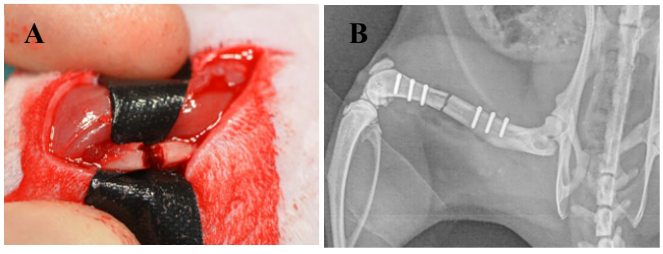

Figure 1 - Intraoperative (A) and radiographic (B) images of a rat from group B that underwent traumatic fracture creation and repair with peek plate (radiolucent) and screws.

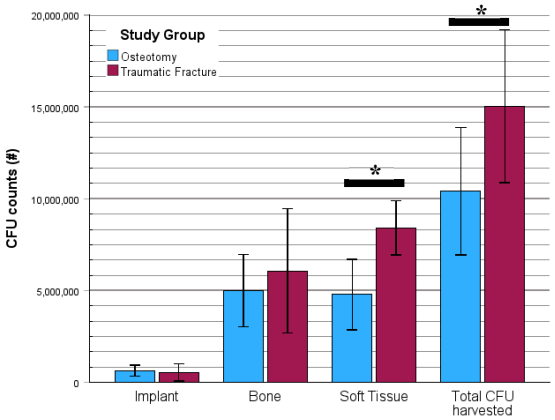

Figure 2 - CFU counts differences between the fracture and osteotomy groups for each sampling locations
** indicates a p value ≤ 0.05*