Implant retention in a rabbit model of fracture-related infection

Similar infection clearance but impaired bone healing in delayed compared to early infection

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Aims

Fracture-related infection (FRI) is commonly classified based on the time of onset of symptoms. Early infections (< two weeks) are treated with debridement, antibiotics, and implant retention (DAIR). For late infections (> ten weeks), guidelines recommend implant removal due to tolerant biofilms. For delayed infections (two to ten weeks), recommendations are unclear. In this study we compared infection clearance and bone healing in early and delayed FRI treated with DAIR in a rabbit model.

Methods

Staphylococcus aureus was inoculated into a humeral osteotomy in 17 rabbits after plate osteosynthesis. Infection developed for one week (early group, n = 6) or four weeks (delayed group, n = 6) before DAIR (systemic antibiotics: two weeks, nafcillin + rifampin; four weeks, levofloxacin + rifampin). A control group (n = 5) received revision surgery after four weeks without antibiotics. Bacteriology of humerus, soft-tissue, and implants was performed seven weeks after revision surgery. Bone healing was assessed using a modified radiological union scale in tibial fractures (mRUST).

Results

Greater bacterial burden in the early group compared to the delayed and control groups at revision surgery indicates a retraction of the infection from one to four weeks. Infection was cleared in all animals in the early and delayed groups at euthanasia, but not in the control group. Osteotomies healed in the early group, but bone healing was significantly compromised in the delayed and control groups.

Conclusion

The duration of the infection from one to four weeks does not impact the success of infection clearance in this model. Bone healing, however, is impaired as the duration of the infection increases.

Article focus

- Comparative efficacy of debridement, antibiotics, and implant retention (DAIR) procedure in early versus delayed infection in a rabbit model of fracture-related infection with *Staphylococcus aureus*.

Key messages

- DAIR procedure clears infection with *S. aureus* infection in this rabbit model of early (one week) and delayed (four weeks) infection.
- Bone healing is disturbed after the presence of an infection for four weeks, but not after one week.

Strength and limitations

- This is the first study that trials the concept of early and delayed DAIR treatment in a rabbit model of fracture-related infection.
- The effects of other bacterial species and longer infection duration (late infection) were not tested.

Introduction

Fracture-related infection (FRI) is a major burden for patients, physicians, and healthcare systems.[1-3] Treatment failure rates can reach up to 50%, especially after complex lower limb open fractures and multiple revision surgeries.[4-6] The formation of a mature biofilm is considered the main reason for treatment failure, as it allows bacteria to evade antibiotic action and host immune responses. The first stage of biofilm formation occurs within the first hours after implant colonization. After the initial attachment, bacteria start producing an extracellular matrix and continue growing three-dimensionally, forming a mature biofilm, which becomes tolerant to antibiotic treatment.[7] The point at which the biofilm is mature, or unlikely to respond to antibiotic therapy in patients with FRI, is not clearly defined.[8] Previously, treatment strategies classified FRI based on the time elapsed since the onset of symptoms, which may also reflect maturity of the biofilm. The most widely used classification for FRI is that of Willenegger and Roth,[9] which categorize infections into early (< two weeks), delayed (two to ten weeks), and late (> ten weeks). Importantly, early FRI may be treated with debridement, antibiotics, and implant retention (DAIR) after confirming that the osteosynthesis is stable, the reduction is adequate, and the soft-tissues are intact. Implant retention is a desirable treatment approach as it involves fewer surgeries, minimizes the risk of losing reduction in compound fractures or multifragmentary joint fractures, and is associated with lower costs. Late infections, by contrast, are believed to have a mature biofilm on the implant, and therefore treatment guidelines generally recommend complete implant removal or exchange.[10] The application of this classification in clinical practice was described by Kuehl et al[11] in a cohort of 229 patients with FRI. In the group with early FRI, 85.7% (42/49) of patients underwent DAIR compared to just 9.8% (9/92) of patients with late FRI. Delayed infections, however, fall between these two options and are considered a grey zone, where no clear recommendations exist.[10] A systematic review by Morgenstern et al,[12] which includes the prospective cohort of Kuehl et al,[11] suggests that delayed infections could be treated similarly to early infections with a DAIR procedure.

The comparative outcome of a DAIR approach between early and delayed FRI may be most appropriately assessed in the first instance in a controlled preclinical study. In this study, we investigated whether early and delayed FRIs respond differently to a DAIR procedure in terms of infection clearance and bone healing in an established rabbit model of FRI.

Methods

The study was approved by and registered at the ethical committee of the Canton of Grisons in Switzerland (approval number 06_2016). All procedures were performed in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facility and performed in accordance with Swiss animal protection law and ARRIVE guidelines 2.0.[13] We have included an ARRIVE checklist to show that we have conformed to these guidelines.

Animals

A total of 17 skeletally mature specific pathogen-free (SPF) female New Zealand White rabbits (Charles River Laboratories, Germany)aged between 40 and 44 weeks and with a mean body weight of 4.6 kg (standard deviation (SD) 0.8) were included in the study. All animals were screened prior to entry into the study and found to be healthy after a standard clinical examination. Approved animals were then allowed to acclimatize to their surroundings for two weeks prior to the start of the study. During this time, they were group-housed with a 12-hour dark/12-hour light cycle, and fed with hay, lettuce, and supplemental feed for rabbits (Biomill, Switzerland). After surgery, the animals were single-housed until the end of the observation period.

Surgical procedure

The rabbit humerus model of plating osteosynthesis described by Arens et al[14] was used in this study. In short, after anaesthesia a mid-diaphyseal transverse osteotomy of a rabbit humerus was created with a 0.44 mm Gigly saw (RISystem, Switzerland), and fixed with a 52 mm seven-hole Locking Compression Plate (LCP) and six 2 mm locking bicortical screws, made of 316 L stainless steel (Depuy Synthes, USA). The osteotomy was located directly underneath the unused central combi-hole.

Bacteria inoculum preparation

Staphylococcus aureus strain (JAR 060131) is a clinical isolate from a patient with an infected hip prosthesis.[15] The strain is broadly antibiotic-susceptible, including nafcillin, rifampin, and levofloxacin. It is available from the Swiss Culture Collection, with accession number CCOS 890. *S. aureus* was chosen in this model, as it is the most common FRI pathogen in human patients.[11,16] Bacterial inocula were individually prepared in phosphate-buffered saline solution (PBS; MilliporeSigma, Switzerland) for each surgery as previously described.[17] Inoculation was then performed by pipetting 34 µl bacterial inocula onto the central screw hole overlying the osteotomy and to the adjacent proximal and distal screw holes (102 µl in total, the total number of bacteria was measured and recorded, as described below). Quantitative culture of each inoculum was performed immediately after preparation to check the accuracy of the prepared inoculum. The target colony-forming unit (CFU) count was 2.0×10^6, with an acceptable range of 9.0×10^5 to 3.0×10^6 CFU.

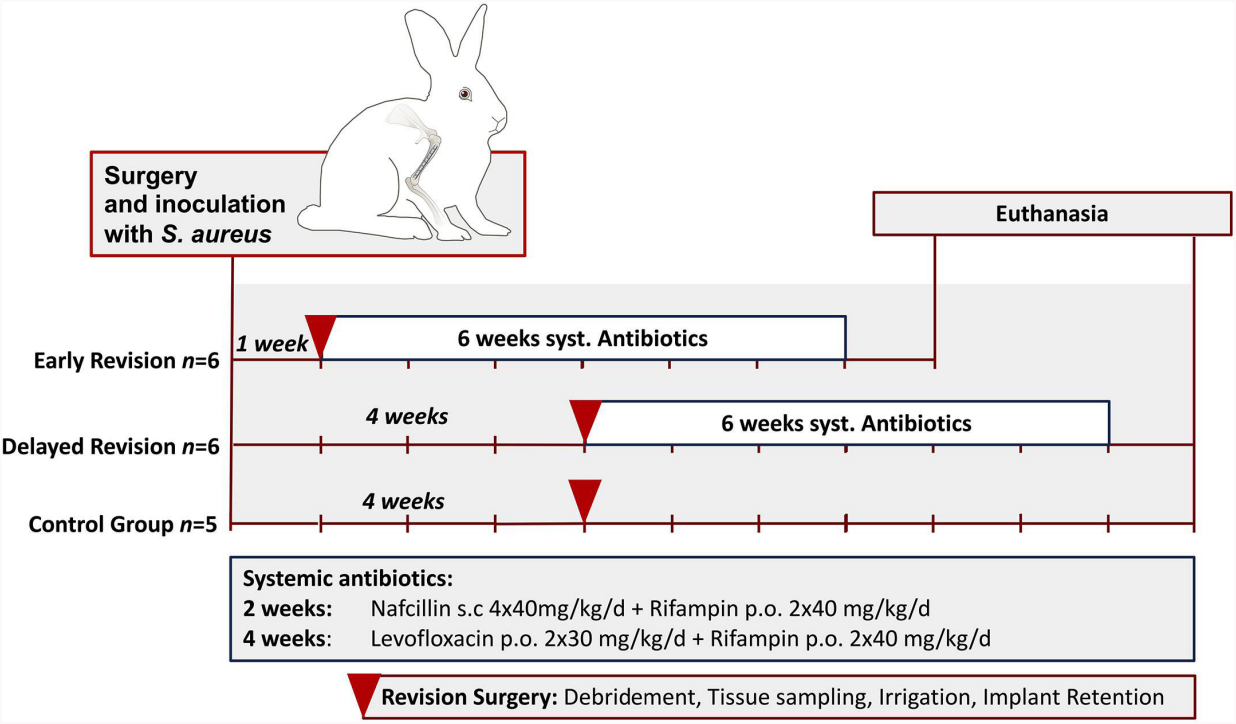

Fig. 1
Overview of the design of the in vivo infection study in the rabbit humerus model of plate osteosynthesis. Revision surgery was either after one week (early revision group, n = 6) or after four weeks (delayed revision, n = 6 and control group, n = 5). Systemic antibiotic treatment was administered for six weeks in both intervention groups. No systemic antibiotic was administered to the animals in the control group. *S. aureus*, *Staphylococcus aureus*.

In order to reduce the number of animals needed in the study and to better discriminate between the effectiveness of treatment regimens, this inoculum was chosen based on previous studies in order to achieve a 100% infection rate at revision surgery.[14]

Study plan and group distribution

After the initial surgery and inoculation, the animals were randomized to either one week (n = 6) or four weeks (n = 6) to allow the infection to develop before revision surgery (early revision and delayed revision, respectively). Veterinarians in charge were aware of the group allocation. A control group (n = 5) also received revision surgery four weeks after bacterial inoculation, but no further antibiotic therapy. This group served to determine whether debridement and the animal's own host responses are able to clear the infection and heal the osteotomy in the absence of antibiotic therapy. An overview of the study design is shown in Figure 1.

DAIR procedure

The revision surgery comprised debridement, irrigation, and retention of the implant followed by systemic antibiotic treatment (DAIR procedure). It was performed in a standardized manner (Supplementary Figure a). Each layer from the subcutaneous tissue to fascia and muscle down to the implant and bone surface was debrided systematically in a clockwise manner around the whole circumference with sharp curettes and rongeurs. All necrotic tissue was removed, with only viable tissue that was red and elastic, with capillary bleeding and intact contractility remaining. All debrided tissue was placed in sterile tubes for immediate microbiological processing. Irrigation was performed with standard saline solution (NaCl 0.9%) and low pressure using a bulb syringe (600 ml). The irrigation fluid was recovered by suction, stored in sterile containers, and immediately processed for microbiological culture. Wound closure was performed in standard manner in layers finishing with an intracutaneous suture.

Clinical observations, exclusion criteria, and euthanasia

Blood samples were taken before surgery, three days after surgery, and weekly thereafter until the end of the observation period for white blood cell (WBC) count (Vet ABC; Scil animal care, Germany) and CRP (Rabbit CRP ELISA Kit; Immunology Consultants Laboratory (ICL), USA). Weight was measured at surgery and weekly thereafter as a criterion for early exclusion. Body temperature was measured daily. Exclusion criteria were set as described by Arens et al[14] at a weight loss exceeding 15% of the initial body weight within two weeks, local infection with severe lameness, persistent swelling and discharge, or signs of systemic infection such as fever, depression, and anorexia. After the observation period, all animals were euthanized using intravenously administered pentobarbital (Esconarkon; Streuli Pharma AG, Switzerland).

Radiography

Radiographs of the operated limb were taken in two planes postoperatively and once a week thereafter for the rest of the study. A contact radiograph (full thickness) was taken of the operated limb post-euthanasia using high-resolution technical film (D4 Structurix DW ETE; Agfa, Belgium) and a cabinet radiograph system (Faxitron X-Ray Corporation, USA). Bone healing on radiographs was analyzed using a modifica-

tion of the radiological union scale in tibial fractures (mRUST) as published by Litrenta et al.[18] This is a radiological scoring system assessing bone healing in a standardized manner on conventional radiographs in two planes, originally developed for tibia fractures[19] and later validated for the humerus.[20] Each cortex on the anteroposterior and lateral radiograph is scored as: 1 = no callus; 2 = callus present; 3 = bridging callus; and 4 = remodelled. All scores are summed up, resulting in a sum score ranging from 4 to 16. A mRUST score ≥ 11 is considered as healed (green area), a score < 9 is considered as not healed (red area), and a score from 9 to 10 is considered neither union nor definite nonunion, according to Leow et al.[21] The blinded assessment of the score was performed by one of the authors (JP).

Antibiotic administration

The antibiotic regimen in this study was based on recommendations for implant-related infections in human medicine and adapted to the rabbit model.[22] An overview is depicted in Figure 1. During the first two weeks, rabbits received Nafcillin and Rifampin in dosages that were proven safe and resemble the clinical situation in human medicine.[23-25] Nafcillin manufactured for injection in humans was administered subcutaneously to all rabbits in a dosage of 4 × 40 mg/kg/d. The subcutaneous route was chosen as intravenous catheters in rabbits are not tolerated well over this long period, and intravenous puncture for every administration would create an undue burden for the animal. Rifampin was administered orally 2 × 40 mg/kg/d. It was mixed with food supplement (Critical Care; Oxbow Animal Health, USA) so that the oral application was accepted by the rabbits. Similar to the clinical situation, antibiotic treatment was converted after two weeks to an oral administration with levofloxacin in a dosage of 2 × 30 mg/kg/d for another four weeks. Rifampin was continued at the above-described dosage throughout the whole period. Antibiotic treatment ended after six weeks in total. All rabbits were then euthanized after an additional week to give enough time for antibiotic washout in order to prevent false-negative culture results.

Quantitative bacteriology

Post-revision quantitative cultures were performed separately on all visibly infected or necrotic tissues removed during debridement (subcutaneous tissue, muscle/fascia, bone/implant surface). Additionally, the irrigation fluids were collected separately (four suction bags of 150 ml each). After sonication for three minutes and thoroughly shaking the bags by hand for 20 seconds, 200 µl of the undiluted samples were spread on blood agar (BA) plates and incubated overnight at 37°C. If no growth was observed on the BA plates after 24 hours, 100 ml of irrigation fluid samples, which were stored overnight at 4°C, was filtered through a sterile membrane filter and the membrane was then incubated on a BA plate for 24 hours. Thus, the lower limit of detection (LOD) was 1.5 CFU per sample.

Post-mortem quantitative bacterial cultures were also performed for the soft-tissue adjacent to the plate, for the implant (after sonication) and bone separately according to the protocol previously described.[14] Bacterial colonies were confirmed to be *S. aureus* using the latex agglutination test (Staphaurex, Thermo Fisher Scientific, Switzerland).

The soft-tissue adjacent to the plate was removed using a sterile scalpel, and placed in a sterile receptacle containing PBS. Then they were roughly cut into pieces no larger than 0.5 cm using sterile scissors, and homogenized using an Omni-TH hand-held homogeniser (LabForce AG, Switzerland) with sterile Omni-tip plastic probes. The screws and the plate were completely submerged in sterile receptacles containing PBS. Then they were vortexed for 20 seconds followed by sonication for three minutes at 35 kHz in an ultrasonicating water bath (Bandelin Sonorex Super 10 P; Bandelin, Germany). The bone samples were roughly cut into small fragments no larger than 0.5 cm using a sterile luer and immediately homogenized using a Polytron PT3100 (Kinematica AG, Switzerland). All homogenized tissue samples and sonicated implant samples were then immediately serially diluted in PBS and plated onto BA plates. BA plates were prepared using Blood Agar Base (Oxoid AG, Thermo Fisher Scientific), containing 5% defibrinated horse blood. Agar plates were incubated at 37°C and colonies counted at 24 hours and 48 hours.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9.4.0 for macOS (GraphPad Software, USA). Normality was tested with Shapiro-Wilk test. Groups were compared using Mann-Whitney U test for continuous data with non-normal distribution and the independent-samples *t*-test for normally distributed data. All p-values were two-sided and intended to be exploratory, therefore no adjustment for multiplicity was made. P-values ≤ 0.05 were considered statistically significant. In descriptive analysis, continuous variables are reported as median (interquartile range (IQR)) in case of non-normal distribution, and as mean (SD) in case of normally distributed data.

Results

Exclusion of rabbits

All rabbits in this study survived both the initial surgical procedure and revision surgery. However, one rabbit in the early group had a wound dehiscence after revision surgery and was therefore euthanized earlier and excluded from the study. One rabbit in the control group and one rabbit in the delayed group did not have any signs of infection and were culture-negative at revision surgery, and were therefore excluded from the analysis. Those two rabbits were replaced in order to achieve a group size of six rabbits in the intervention groups. For the control group, we did not replace the one excluded rabbit, as the other five rabbits showed homogenous results. Thus, 17 rabbits were included in the final analysis. Rabbits tolerated the antibiotic course well. Three animals had mild-to-moderate diarrhoea, but no further symptoms that would require early euthanasia.

Clinical observations

The body temperature and body weight in all rabbits were within the normal range during the whole study period, with no differences between the groups (data not shown). CRP levels increased after the initial surgery to a peak at three days and then trended downwards, indicating that the infection was limited to the operated limb and did not spread systemically (Supplementary Figure ba). The increase within the first

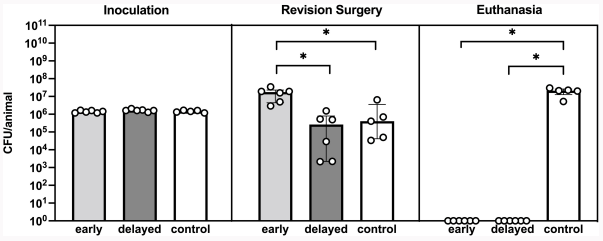

Fig. 2
Quantitative microbiology of the three study groups: *Staphylococcus aureus* colony-forming unit (CFU) count at inoculation, revision surgery, and euthanasia. *p < 0.05, Mann-Whitney U test. Bars indicate the median, and error bars indicate the interquartile range. *p < 0.05, Mann-Whitney U test.

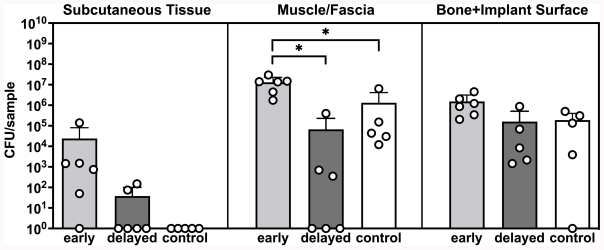

Fig. 3
Quantitative microbiology of the different samples collected during revision surgery. *Staphylococcus aureus* colony-forming unit (CFU) counts in subcutaneous tissue, muscle/fascia, and bone and implant surface in the early, delayed, and control groups. Bars indicate the mean, and error bars indicate the standard deviation. *p < 0.05, independent-samples *t*-test.

days after surgery was similar, as expected, in all groups. The WBC count was not significantly different between the three groups at any time and displayed large variability within each group and timepoint (Supplementary Figure bb).

Microbiology

The prepared inocula ranged from 1.2×10^6 to 2.1×10^6 CFU (median 1.6×10^6 CFU (IQR 1.3×10^6 to 1.7×10^6); acceptable range: 9.0×10^5 to 3.0×10^6) (Figure 2), without significant differences between the three groups. The debridement material and irrigation fluid taken at the revision surgery were culture-positive for *S. aureus* after both one and four weeks (prior to any treatment). The sum of the CFU count of all samples (subcutaneous tissue, muscle/fascia, bone/implant surface) during revision surgery was significantly higher in the early group after one week compared to the delayed and control groups after four weeks (CFU median: early: 1.8×10^7 (IQR 4.4×10^6 to 2.3×10^7); delayed: 2.6×10^5 (IQR 2.2×10^3 to 7.9×10^5); control: 4.1×10^5 (IQR 4.2×10^4 to 3.6×10^6); early vs delayed: p = 0.002; early vs control: p = 0.017, Mann-Whitney U test). No difference was observed between the delayed group and the control group at revision surgery, as expected, since both groups were identical at this time. At euthanasia, animals from the control group that received debridement, but no antibiotic therapy, were still infected and almost all samples had high bacterial counts (CFU median: 2.1×10^7 (IQR 1.3×10^7 to 2.6×10^7)). Rabbits receiving DAIR in the early and delayed groups did not show any bacterial growth in any sample after euthanasia (early vs control: p = 0.002; delayed vs control: p = 0.002, Mann-Whitney U test).

Regarding specific tissue samples collected during revision surgery, the results showed a lower number of bacteria in the delayed and control groups and mainly in the subcutaneous tissue and adjacent muscle/fascia tissue (Figure 3). Four of six samples in the delayed group and all five samples in the control group from the subcutaneous tissue were culture-negative at revision surgery compared to one of six in the early group. The CFU count in the control and delayed groups was significantly lower for the muscle/fascia samples compared to the early group (CFU mean: early group: 1.3×10^7 (SD 9.9×10^6); delayed group: 6.7×10^4 (SD 1.6×10^5); early vs delayed, p = 0.009; control group: 1.3×10^6 (SD 2.8×10^6); early vs control, p = 0.031, independent-samples *t*-test). Differences between the groups regarding the samples from the bone and implant surface were not significant.

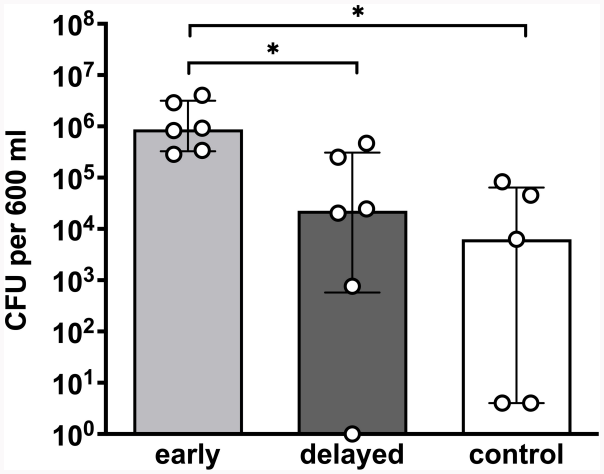

Fig. 4
Staphylococcus aureus colony-forming unit (CFU) count in irrigation fluid (600 ml saline per rabbit) at revision surgery in the early, delayed, and control groups. Bars indicate the median, and error bars indicate the interquartile range. *p < 0.05, Mann-Whitney U test.

During revision surgery using the DAIR approach, the wound was irrigated with a total amount of 600 ml saline (four times 150 ml from a bulb syringe). This reduced the number of bacteria in all groups in the last portion to 10% (mean 10.6% (SD 10.9%)) compared to the CFU count in the first portion. The summarized number of bacteria that were flushed out of the wound was significantly higher in the early group compared to the delayed and control groups (median: early group: 8.7×10^5 (IQR 3.3×10^5 to 3.2×10^6); delayed group: 2.2×10^4 (IQR 5.7×10^2 to 3.1×10^5); early vs delayed, p = 0.009; control group: 6.3×10^3 (IQR 4.0×10^3 to 6.4×10^4), early vs control, p = 0.004; Mann-Whitney U test) (Figure 4).

Radiological evaluation

Plain radiographs of the operated humeri were taken in two planes weekly, and contact radiographs were taken after euthanasia. All plate osteosyntheses were radiologically free from signs of instability until the end of the observation period, as reflected by the fact that the rabbits were fully weightbearing on their forelegs. Callus formation appeared more irregular and voluminous in the delayed and control groups as revealed by contact radiographs compared to the

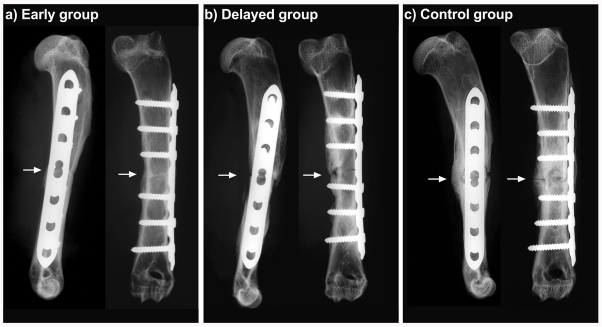

Fig. 5
Representative contact radiographs in anteroposterior and lateral view of the rabbit humerus after euthanasia, with arrows marking the osteotomy site in: a) the early group at eight weeks; b) the delayed group at 11 weeks; and c) the control group at 11 weeks. All rabbits received a plate osteosynthesis of a humeral osteotomy and were inoculated with *Staphylococcus aureus*. Debridement and irrigation with implant retention was performed after one week (early) or after four weeks (delayed and control). The early and delayed groups received systemic antibiotics for six weeks, and the control group received no systemic treatment.

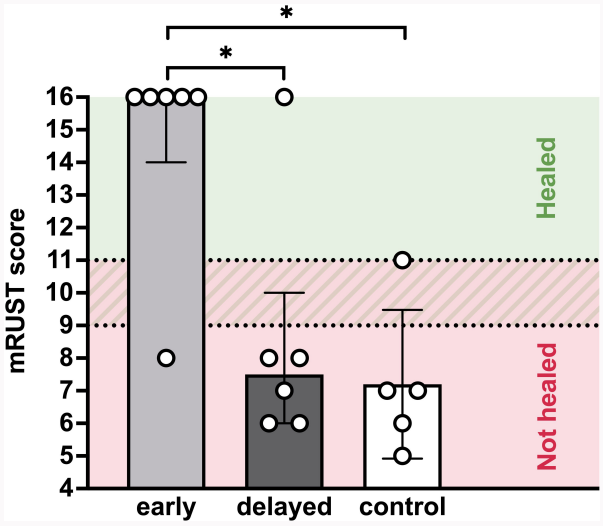

Fig. 6
Quantification of bone healing in the study groups using the modified radiological union scale in tibial fractures (mRUST) score in anteroposterior and lateral radiographs of the rabbit humerus at eight weeks (early group) and 11 weeks (delayed group and control group). A mRUST score ≥ 11 was considered a healed osteotomy (green area), a mRUST score < 9 was considered a non-healed osteotomy (red area), and a mRUST score of 9 to 10 was considered neither union nor definite nonunion.[21] Bars indicate the median, and error bars indicate the interquartile range. *p < 0.05, Mann-Whitney U test.

early group (Figure 5). The mRUST score revealed significantly better bone healing in the early group compared to the delayed and control groups (CFU median: early: 16 (IQR 14 to 16); delayed: 7.5 (IQR 6 to 10); control: 7 (IQR 5.5 to 9); early vs delayed: p = 0.041; early vs control, p = 0.007, Mann-Whitney U test). The early group showed complete bone healing in five out of six rabbits (at week 8), while the osteotomy gap was not united in the delayed group in five out of six rabbits and four out of five rabbits in the control group (at week 11) (Figure 6).

Discussion

In our study of the standardized rabbit infection model, we could show that an infection period of four weeks allows a successful infection clearance with a DAIR procedure, since no viable bacteria were detected in the soft-tissues, in the bone, or on the sonicated implants at the time of euthanasia. These results are in line with a clinical review by Morgenstern et al,[12] which included six studies with a total of 276 patients. They came to the cautious conclusion, based on heterogeneous data, that infections in the delayed time interval up to ten weeks can be treated with a DAIR procedure with a success rate of over 80%. For a shorter interval of less than three weeks, the success rate was as high as 86% to 100%. A recent clinical study by Kuehl et al[11] classified 229 cases of FRI according to Willenegger and Roth,[9] and found similar failure rates after DAIR in subgroups for early and delayed infections (14% and 12%), while late infections of more than ten weeks had a higher failure rate (33%). Although the subgroups were small, these data suggest that DAIR is also a viable option for delayed infections. McNally et al[4] performed a multicentre study with 433 patients, and did not find the time from injury until DAIR to be a risk factor for failure. Our results in a controlled preclinical model support these clinical observations; however, our animal data are limited to a four-week period. The current literature on the treatment of FRI does not provide a clear cut-off point at which DAIR is no longer advisable, but rather suggests a continuum of decreasing success rate.[3,26]

The infection period of four weeks showed a lower bacterial load in the superficial layers and in the irrigation fluid compared to the early group (after one week), which implies that the infection retreats at later stages to the hard-to-reach areas such as the bone and implant surface. It is likely that the immune system has easier access in the subcutaneous tissue due to more vascularization. Recent findings have shown that *S. aureus* is able to invade deep into the bone via the osteocyte lacunocanalicular network, thus evading immune responses.[27] The standardized revision surgery in our model does not reach all infected areas, such as the intramedullary canal or the implant-bone contact area. Therefore, the overall number of bacteria present in the wound after four weeks might be even higher compared to the bacterial load after one week. This highlights the need for a radical debridement of the bone if an infection had time to spread deep into the tissue.

In contrast to the debridement samples at revision surgery, the analysis at euthanasia includes the total infected area (whole humerus, adjacent soft-tissues, and sonicated implants). Thus, the hard-to-reach areas are also covered. The hypothesis that the bacterial load in this model increases over time is supported by the fact that, despite the standardized debridement after four weeks, the CFU count at euthanasia in the control group was increased compared to the initial inoculum. The persistence of infection in the control group demonstrates the importance of additional antibiotic treatment after surgical debridement in order to clear the infection.

One of the primary concerns in FRI is bone healing. It has been shown in several in vivo models that regular bone healing is disrupted in the presence of an infection.[14,28-30] Therefore, it is important to consider bone healing in addition

to infection clearance in this study. Radiographs showed that the delayed group and the control group had worse bone healing than the early group despite the longer study period (11 vs 8 weeks). The callus in the delayed group and in the control group was more voluminous and irregular compared to the early group, and it formed at a notable distance from the osteotomy site. These radiological phenomena are known from chronic infected nonunions in the clinical setting. Bone healing phases in the rabbit are comparable to human bone healing, although they happen faster in smaller animals and normally union can be expected after four to six weeks.[28,29,31]

Infection disrupts early callus formation and impairs osteogenic responses.[28,32,33] However, mild inflammatory responses at the periphery also stimulate osteogenesis by resembling the immediate physiological bone healing response after fracture.[34,35] This could explain the observed voluminous callus formation distant to the infected osteotomy site that was seen in the delayed and control groups in our model. Interestingly, in our study bone healing failed to occur if the infection had persisted for four weeks, even if no viable bacteria were eventually cultured in the delayed group. However, regular bone healing occurred after an infection period of one week. Therefore, it seems plausible that the process of bone healing was effectively disturbed in the period from the first to the fourth week. Whether this process was permanently disturbed or whether the osteotomy would have healed after 11 weeks cannot be conclusively assessed in this study.

Since no union score for humeral osteotomies with plate fixation exists for rabbit models, we adapted the criteria of the mRUST score to quantify bone healing.[18] Due to the missing validation of the score, these results should be interpreted with caution. Future preclinical studies should also investigate bone healing with biomechanical, imaging, and histological assessment to substantiate the observation of impaired bone healing after longer infection duration. Further limitations of the study include the use of only a single bacterial species and the fact that no infection duration longer than four weeks was studied. In addition, the generally faster bone healing in rabbits compared with humans also raises the question of the extent to which the time periods of the early, delayed, and late infection classification may be translated to the human situation.[28] Furthermore, since this study involved healthy rabbits and clinical reality often involves elderly and sick patients,[36,37] the success of the DAIR procedure in delayed infections could be overestimated by the results from our study and thus cannot be directly translated to human medicine. Nevertheless, the controlled conditions of an experimental animal study offer advantages over the highly variable patient factors that often complicate clinical studies, and the trends observed in this study can be considered to be indicators of the relative contribution of infection duration to fracture healing outcomes.

Furthermore, we acknowledge that bacteria can be difficult to culture due to their ability to enter a viable but non-culturable state, especially after antibiotic treatment. Thus, determining 'eradication of the infection' is challenging with conventional culturing methods alone. We therefore use the term 'infection clearance' as this describes the removal of growing bacteria, acknowledging that some remaining bacteria may not be culturable but could be detected with molecular methods. We refrained from the use of additional methods as this would have required operating on additional animals without substantially improving or changing the outcome or interpretation of our data. In addition, these methods are not applied on a regular basis in clinical practice.

In clinical practice, the question often arises whether the fracture ends should be freshened in FRI or whether potential fibrous callus tissue should be preserved. The value of freshening the bone ends in our model by re-osteotomy with the Gigli saw should be investigated in future studies, as it seems plausible that restarting the process of bone healing at the time of revision surgery could improve bone healing in the delayed group.

In conclusion, the duration of the infection in this model does not affect the success of infection clearance within four weeks. This result thereby supports the hypothesis that delayed FRI up to four weeks can be successfully cleared with a DAIR procedure. However, the prolonged duration of infection appears to have disrupted the process of bone healing to such an extent that even after clearance of the infection, bone healing no longer proceeds.

Supplementary material

Figures showing representative images of the revision surgery of the infected rabbit humerus model, and blood markers including CRP and white blood cell count over time in the three study groups.

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ICMJE COI statement

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Data sharing

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Ethical review statement

The study was approved by and registered at the ethical committee of the Canton of Grisons in Switzerland (approval number 06_2016).

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