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Histological score for degrees of severity in an implant-associated infection model in mice

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Abstract

Introduction Several scores were introduced to diagnose and to classify osteomyelitis in practice. Mouse models are often used to study the pathophysiology of bone infection and to test therapeutic strategies. Aim of the present study was to design a score to diagnose and quantify implant-associated infection in a murine experimental model.

Materials and methods Four independent parameters were developed: existence of callus, consolidation of the fracture, structural changes of the medullary cavity and number of bacteria. The score was assessed in a standardized implant-associated mouse model with 35 BALB/c-mice. The left femur was osteotomized, fixed by a titanium locking plate and infection was induced by inoculation of *Staphylococcus aureus* into the fracture gap. For the sham group, the procedure was performed without inoculation of bacteria. The score was assessed on days 7, 14 and 28. Each item of the score showed lower values for the infection group compared to the controls after 4 weeks.

Results Regardless of the assessed time point, the overall total score was significantly higher in the control group compared to the infection group ($p < 0.0001$). Analysis revealed a sensitivity of 0.85, specificity of 1.0, negative predictive value of 0.67 and positive predictive value of 1.0.

Conclusion The proposed score assessing severity of fracture-related infection in an implant-associated murine model was easy to access, feasible to diagnose and estimate bone healing and infection in a murine bone infection with a high sensitivity. Therefore, this score might be a useful tool to quantify infection-related changes after fracture in further future preclinical studies.

Keywords Mouse model · Fracture-related infection · Score · *Staphylococcus aureus*

Introduction

Fracture-related infections (FRI) are one of the major challenges in orthopedic and trauma surgery. Long-term antibiotic uses, multiple surgical treatments, including debridement and implant removal, as well as high health-care costs and poor functional outcome for the concerned patients are often associated with implant-associated infections [1–3]. However, FRI is a severe complication following musculoskeletal trauma with a prevalence of 0.5–3% for closed and up to 25–30% for open fractures. However, post-traumatic infection is a severe complication following bone fracture with a prevalence of 0.5–3% for closed fractures and up to 25–30% after open extremity fractures [1, 2, 4, 5]. Reconstruction of fractures using implants is an additional risk factor for the development of FRI [6–8]. About 10–30% might chronify [9]. *Staphylococcus aureus* (SA) is the most common bacteria proven in posttraumatic infections and responsible for 50–70% of posttraumatic bone infections [10, 11]. Several clinical studies tried to establish scores for a definition of osteomyelitis [12, 13]. Interestingly, most of these studies take into account neither the presence of a fracture nor the application of an implant. This seems surprising

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because fracture stabilization is one of the most risk factors for developing a FRI [6–8]. Studies concerning diagnosis and therapy of FRI are various, but a definition is still missing. A universally accepted definition of implant-associated infections after fracture fixation is yet not established. There is still a lack of clear definition [2, 14, 15]. For this reason, an expert group recently developed a consensus definition for FRI [15]. Furthermore, histopathology studies in FRI are limited. Followed up to the consensus definition, Morgenstern et al. investigate the value of histological analysis in the diagnosis of FRI by measuring number of neutrophil granulocytes [16]. Scores, taking into account fracture consolidation as well as number of bacteria and histopathological alterations, are not established yet. Most insights into the pathophysiological mechanism of bone infections are gained from animal experiments. Animal models have not only been shown to be useful in studies on drug safety but transgenic models have also allowed for the study of complex pathophysiological pathways in great detail. Animal models are used to resemble the inflammatory pathophysiological conditions occurring in humans during infection. Therefore, similar FRI classification systems may simplify comparability of experimental and clinical conditions and improve transfer "from bench to bedside" in future studies. Therefore, similar classification systems in experimental and clinical FRI may simplify comparability of the experimental and the clinical condition and simplify the transfer "from bench to bedside" in further studies. However, such scores cannot not always be used in experimental settings as features such as "clinical history and risk factors" are not available for experimental animals. On the other hand, other features of the human classifications, such as microbiological or histopathological examinations, are routinely assessed in experimental bone infection models. However, this well-established scores assessing severity of infection cannot be used in the experimental situation as features such as "clinical history and risk factors" are not feasible for experimental animals. However, other features of the human classifications, such as microbiological or histopathological examinations, are routinely assessed in experimental bone infection models. Aim of the present study was to design a score to diagnose and quantify infection in a murine experimental implant-associated infection model. Following further clinical scores, the new classification system should involve diagnostic imaging, microbiology and histopathology.

Materials and methods

Ethical statement

The present animal experiment was approved by the local institutional committee on animal care ("Landesamt für Naturschutz, Umwelt und Verbraucherschutz" of the federal state of North Rhine–Westphalia, Germany—file number: 87-51.04.2010.A375) and was in line with the European Communities Council Directive (86/609/EEC). Specific effort was made to minimize the number of animals. Reporting of the results of the present study adheres to the "Animals in Research: Reporting in vivo Experiments" criteria (ARRIVE criteria).

Animals

Female non-transgenic BALB/c-mice with an average weight of 21 g were used for the present study. The age ranged between 10 and 12 weeks. Mice were kept in the local animal research institution in standard polycarbonate (Makrolon type II) cages under a conventional 12-h light–dark cycle (7:00 a.m./p.m.). A total of six mice were kept in one cage. Mice had free access to food and water.

Animal surgery/the implant-associated osteitis model

A well-characterized and standardized implant-associated infection model in mice was used as described before [17, 18]. In detail, general anesthesia was induced by intraperitoneal injection of ketamine (100 mg/kg bodyweight) and xylazine (2%, 5 mg/kg body weight). Osteotomy of the left femur was performed in its middle third with a Gigli saw (0.22 mm in diameter) after sterile preparation. The femur was reconstructed by a 4-hole stable-angle plate and screw combination (length 7.75 mm, width 1.5 mm, thickness 0.7 mm and each 2 two self-cutting screws at the proximal and distal fragments; AO Foundation, Research Implants Systems, Davos, Suisse). The femur osteotomy and position of the implants were controlled by X-ray radiography on days 7, 14 and 28. For the infection group, infection was induced by inoculation of the osteotomy gap with 1×10^3 SA in 1 µl PBS (type ATCC 29213; 10^3 colony-forming units, CFU). Mice were kept on a heating pad during surgery and the postoperative period to maintain a body temperature of 37 °C. Meloxicam (5 mg/kg s.c., directly after surgery and five more days every 24 h) was used for postoperative analgesia. Mice were re-anesthetized on the 7th, 14th and 28th days and killed by cervical dislocation on day 28. Radiographic examinations were done on the 7th, 14th and 28th days. Mice were visited and checked once a day. The right femur was sterilely removed and fixated by formalin solution. In total, 35 BALB/c-mice were randomly allocated to treatment groups. The infection group included 27 mice (day 7:5 mice, day 14:4 mice and day 28:18 mice) and the control group included 8 mice (4 mice each on day 7 and day 28). All experiments were performed in the local animal research institution. Mice were euthanized by cervical dislocation when the following termination criteria were existing: (1) pain within 1 week (no use of the impeller), (2) refusal to eat with weight loss of overall 20% or 15% in 12 h, (3) unsuccessful fracture stabilization and (4) infections, which are not under control with the surgical treatment.

Experimental outcomes

Femurs were decalcified with EDTA solution for 7 days and irrigated for additional 12 h at 36° C. Decalcified samples were embedded in paraffin, cut by a microtome and stained using hematoxylin–eosin (HE) or Giemsa. Microscopy was performed at 10-, 40- and 100-fold magnification.

Score

Based on our clinical experience, we suggested a score assessing severity of FRI, which involved four independent histological parameters:

1. Existence of callus;
2. Consolidation of the osteotomy;
3. Structural changes of the medullary cavity;
4. Number of bacteria.

The definition of each parameter and its weighting are summarized in Table 1.

Existence of callus was evaluated in the HE staining of the femur. Therefore, the quotient between callus and bony cortex was estimated. A quotient > 1.6 was considered as good (Fig. 1a), a quotient between 1.1 and 1.6 as poor (Fig. 1b) and quotient < 1.1 as no callus formation (Fig. 1c). Consolidation of the osteotomy was also evaluated in the histological examinations: a complete healing (complete radiological healing of the osteotomy site, Fig. 2a) was distinguished from an incomplete (Fig. 2b) healing and destruction (atrophy of the bony cortex, Fig. 2c). Physiological structural changes of the medullary cavity were defined as an organized cell structure and the absence of polymorphonuclear neutrophil granulocytes (PMN) as the standard of immunocompetent cells (Fig. 3a). Further, a disorganized cell structure with an accumulation of PMN (Fig. 3b) or—more severe—necrosis with devitalized tissue within the medullary cavity was considered as changes due to osteomyelitis (Fig. 3c). Quantification of bacteria was evaluated by Giemsa staining. The number of bacteria was assessed by the hotspot method in 20 visual fields at 100-fold magnification [19]. Low number of bacteria was defined as less than 4 bacterial colonies (Fig. 4a), a moderate number as 4–10 bacterial colonies (Fig. 4b), and a high number of bacteria as > 10 bacterial colonies per 20 visual fields (Fig. 4c). Using this score, maximally 27 and minimally 4 points could be achieved. A lower total score was suggested to correlate with a higher probability for a post-procedural FRI.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, Inc., 7825 Fay Avenue, Suite 230, La Jolla, CA 92037 USA). Data are presented as the median ± standard deviation (SD). *P* values of 0.05 and below were considered significant. The Mann–Whitney *U* test was used to assess significance. To analyze the diagnostic value of the evaluated score, a receiver operating characteristics (ROC) curve was generated and analyzed. The area under the curve (AUC), the optimal sensitivity, specificity, negative (NPV) and positive predictive value (PPV) were assessed.

Table 1 A score to assess severity of osteitis in mice—definition of each parameter

Parameter	Score points	Description	Definition
Existence of callus	6	Large callus formation	Ratio callus/bony cortex > 1.6
	3	Large callus formation	Ratio callus/bony cortex 1.1–1.6
	1	No callus formation	Ratio callus/bony cortex < 1.1
Consolidation of the fracture	12	Consolidation	Complete radiological healing of the osteotomy
	6	Incomplete consolidation	Incomplete radiological healing of the osteotomy
	1	Destruction	Atrophy of the bony cortex
Structural changes of the medullary cavity	6	Physiological conditions	Well-organized cell structure
	3	Disorganized cell structure	Disorganized cell structure with accumulation of immune cells
	1	Necrosis	Devitalized tissue
Number of bacteria	3	Low number	<4/20 visual fields at 100-fold magnification
	2	Moderate number	4–10/20 visual fields at 100-fold magnification
	1	High number	<10/20 visual fields at 100-fold magnification

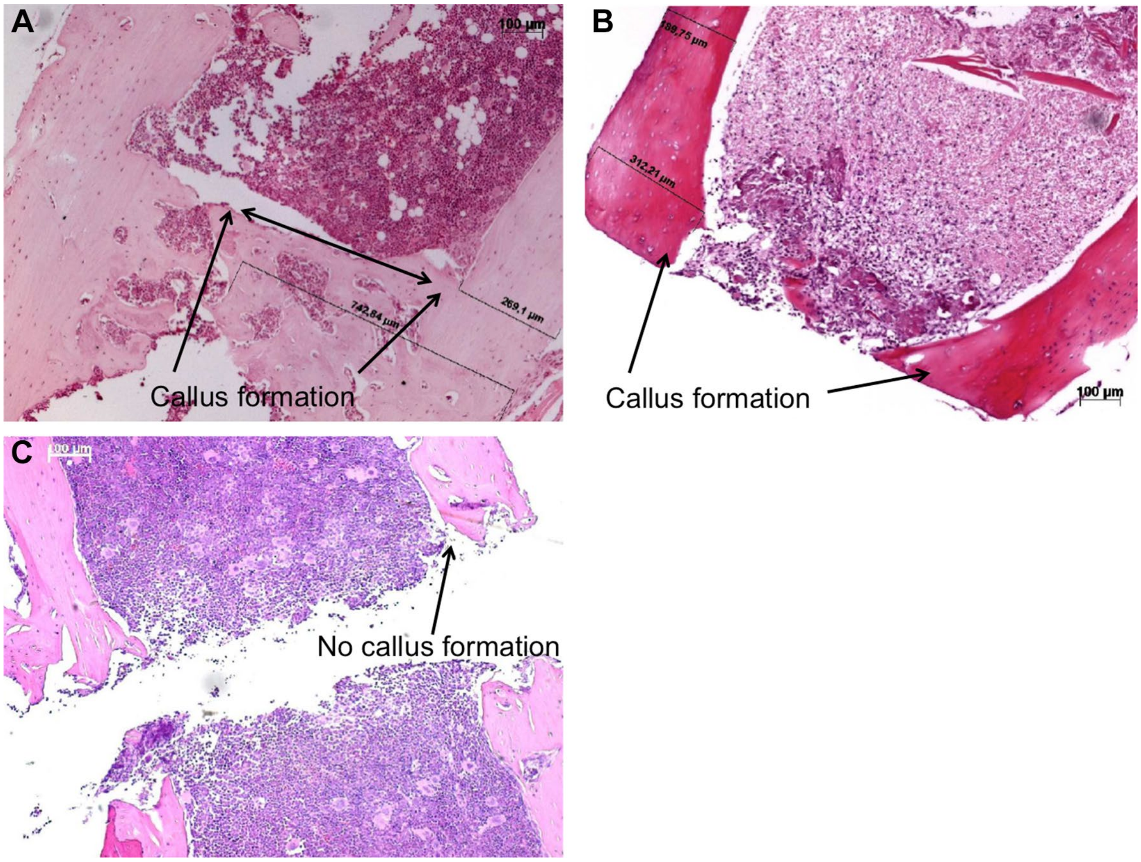

Fig. 1 Callus formation. A quotient > 1.6 was considered as good (**a**) a quotient between 1.1 and 1.6 as poor (**b**) and a quotient < 1.1 as no callus formation (**c**)

Results

Baseline data

Osteotomy and reconstruction of the femur were performed on 35 mice (27 in the osteitis model and 8 in the control group) as described above. We observed no case fatalities due to surgery or anesthesia (mortality rate 0/35*).*

Callus formation

Callus formation was observed in the control group 1 week after osteotomy. According to the proposed score, mice in the control group reached a median of 3 ± 1.8 pts. 1 week and 6 ± 1.3 pts. 4 weeks after osteotomy (Fig. 5a). Mice assigned to the infection group showed comparable callus formation within the first week (3 ± 1 pts.) but not thereafter (1 ± 1.2 pts. 4 weeks after osteotomy).

Bone consolidation

Bone consolidation is a sign of a physiological fracture healing and should be distinguished from callus formation. Complete osteotomy consolidation was only observed in one mouse in the control group after 4 weeks (1/4 mice, 25%, Fig. 5b). All control mice in the 1-week group and 3/4 mice in the 4-week group showed a partial osteotomy consolidation with a persisting definable fracture gap (score 6 ± 0 pts. at 1 week; 6 ± 2.6 at 4 weeks). Comparably, mice designated to the implant-associated infection model showed no complete osteotomy consolidation. Infection mice showed a progressive destruction of the fracture gap and the cortical bone. The median score for the infection mice was 6 ± 0 pts. after 1 week, 6 ± 2.2 after 2 weeks and 1 ± 3.2 after 4 weeks.

Structural changes of the medullary cavity

All control mice showed neither a disorganized cell structure nor an accumulation of PMN within the medullary cavity after 1 or 4 weeks (median score: 6 ± 0 pts., each 1 and 4 weeks after fracture). In contrast, infection mice showed changes of the medullary cavity with a disorganized cell structure at 1 and 2 weeks after fracture (median score: 3 ± 2.2 after 1 week and 2.2 ± 2 after 2 weeks, Fig. 5c). Infection mice additionally showed an invasion of PMN

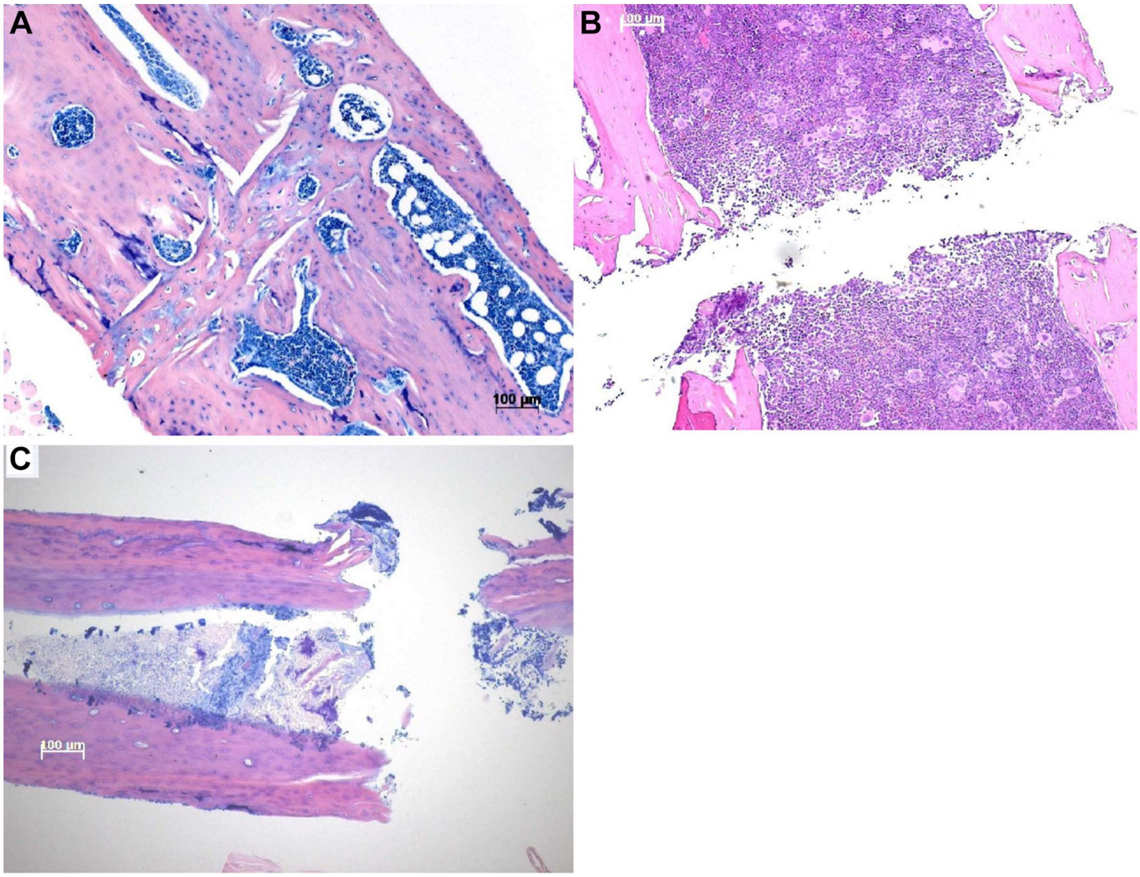

Fig. 2 Osteotomy consolidation. A complete healing osteotomy (**a**) was distinguished from an incomplete (**b**) healing osteotomy and a destruction/atrophy of the bony cortex (**c**)

within the medullary cavity after 4 weeks (median score: 3 ± 1.4).

Bacteria

As a high number of bacteria within the fracture gap indicate an infection, number of bacteria in the histopathological slices was quantified using the hotspot method in 20 visual fields at 100-fold magnification [19]. As expected, only single bacteria were found in control mice at 1 and 4 weeks after initial surgery (median score: 3 ± 0 pts., each at week one and four, Fig. 5d). The infection group showed with 4/5 mice (80%) a high number of bacteria at 1 week (> 10 bacterial colonies per 20 visual fields; median score: 2 ± 0.8 pts.). A high number of bacteria in the fracture gap were also observed 2 (score: 2 ± 1 pts.) and 4 weeks (score: 2 ± 0.8 pts.) after osteotomy.

Total score

The total score was calculated by addition of the scores based on the analyzed four independent histological parameters. We assumed that a lower total score correlates with a higher probability for a post-procedural FRI. For the control group, median total score was 18 ± 1.8 pts. at one and 21 ± 1.3 pts. at 4 weeks (Fig. 6). The higher score at 4 weeks might reflect the physiological re-constitution of the bone. For mice assigned to the osteitis group, the total score was 13 ± 2.3 pts. at 1 (vs. control group: $p = 0.03$), 10.5 ± 4 pts. at 2 and 8.5 ± 4.8 pts. at 4 weeks (vs. control group: $p = 0.002$).

Regardless of the assessed time point, the overall total score was significantly higher in the control group (21 ± 2.4 pts.) compared to the osteitis group (11 ± 4.6 pts.; $p < 0.0001$). For a further prediction of the diagnostic value of the proposed score, we performed a ROC analysis. Using the ROC, the cutoff value for detecting an osteitis was 15 pts. Using 15 pts. as cutoff value, ROC analysis revealed an AUC of 0.97, sensitivity of 0.85, specificity of 1.0, NPV of 0.67 and PPV of 1.0 for the group of 35 studied animals (Fig. 7).

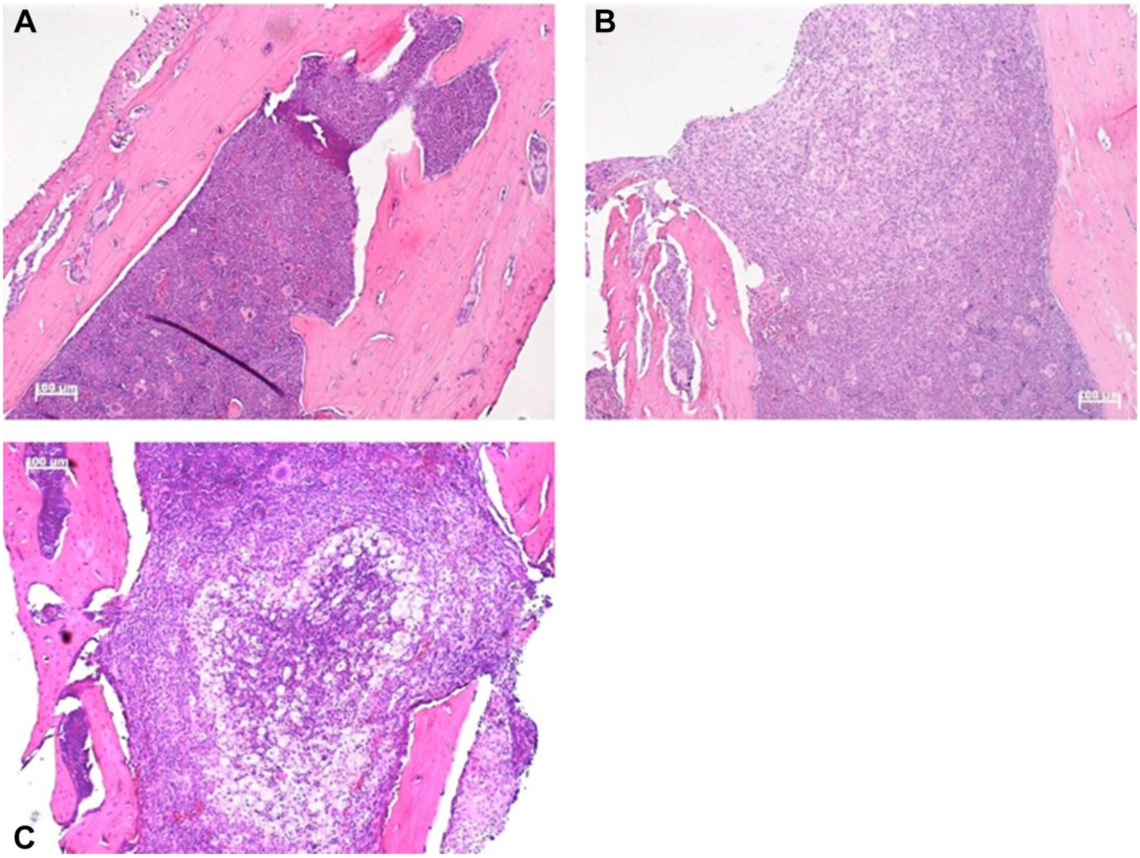

Fig. 3 Structural changes of the medullary cavity. This figure demonstrates the structural changes of the medullary cavity following bone infection. A physiological configuration (**a**) has to be differentiated from a disorganized cell structure (**b**) and necrotic tissue (**c**)

Discussion

The present study aimed to design a score to diagnose and quantify infection in murine experimental FRI. The proposed score involving diagnostic imaging, microbiology and histopathology was (1) easy to access, (2) feasible to estimate bone healing and infection with a high sensitivity and (3) might, therefore, be a useful tool to quantify infection-related changes after FRI in further studies.

In humans, FRI leads to an impairment of fracture consolidation and callus formation as well as invasion of immune-competent cells into the medullary cavity [20–22]. These structural changes are used to diagnose and classify bone infections in clinical practice and are, e.g. integral parts of the classification score of osteomyelitis by Schmidt et al. [13]. However, such scores were not yet established for murine FRI models. Introduction of comparable scores in a murine model might facilitate the comparability in the human situation. The presently proposed score involving imaging, microbiological and histopathological features was based on a previous clinical score [13]. Evaluation of a well-established implant-associated infection model revealed an impaired callus formation and consolidation of the fracture, a disorganized cell structure of the medullary cavity with invasion of PMN and a high number of bacteria in osteitis animals compared to sham animals. These changes are well known from the human situation after osteitis. Recent studies tried to fulfill the lack of a clear definition concerning FRI [15, 16]. Experts developed a consensus definition. Therefore, they determined two different levels of certainty: first, confirmatory items and second suggestive items. Infection is confirmed if a sinus fistula or a wound infection is present, there is intraoperative purulence, cultures identify phenotypically indistinguishable pathogens from at least two separate deep-tissue or implant specimens, or microorganisms are identified in deep-tissue specimens on specific staining. This definition has a high impact on clinical diagnosis of FRI, but the expert group was unable to include histology as a criterion for the diagnosis of FRI. Morgenstern et al. recognized this missing part in definition and investigated the role of quantitative histological analysis in the diagnosis of FRI [16]. This study confirms our own

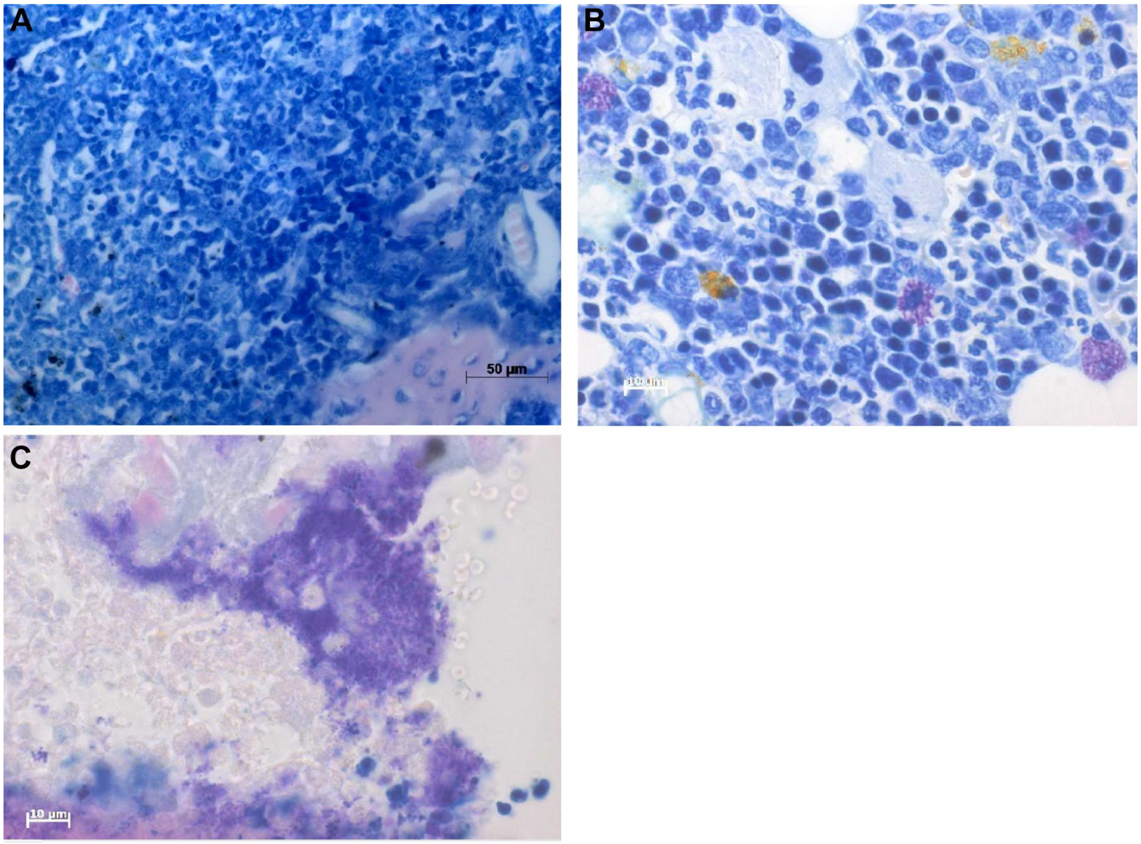

Fig. 4 Quantification of bacteria. Low number of bacteria was defined as less than 4 bacterial colonies (**a**), moderate number as 4–10 bacterial colonies (**b**), and high number of bacteria as > 10 bacterial colonies per 20 visual fields (**c**)

results: histology can be used as a diagnostic tool for FRI. Therefore, we established a score including diagnostic imaging, microbiology as well as histopathology. Combining all four items and calculating an overall score allowed diagnosing FRI with high sensitivity and specificity. The proposed score enables to estimate severity of induced infection, to compare infection in different studies and might, therefore, be a useful tool for further studies addressing pathomechanisms and new therapeutic approaches to FRI.

Limitations

Finally, we acknowledge different limitations of the present study: (1) recently, it was questioned if and how animal models reflect the pathophysiological and molecular changes occurring in humans after infection. Genomic responses after acute inflammatory stress were mentioned to poorly mimic the human inflammatory condition [23]. However, similar genomic responses in mouse models and human inflammatory diseases were found in a later study using the same data set [24]. Analysis of genomic or molecular responses of osteitis was beyond the scope of the present study. However, the imaging, microbiological and histopathological changes observed after murine osteitis are well known from the human situation. (2) Our present score was evaluated in a limited number of mice allocated to osteitis and sham group. However, higher numbers of animals might not be reasonable for animal protection reasons. (3) The diagnostic reliability of newly introduced tests is usually assessed by comparing the new test with gold standard method. To our best knowledge, this is the first test to diagnose osteitis in mice by combining imaging, microbiological and histopathological features. Therefore, a comparison to the gold standard method was not possible.

Conclusion

In the present study, we designed a score to diagnose and quantify FRI in an implant-associated murine model by combining diagnostic imaging, microbiological and histopathological features. The proposed score was easy to

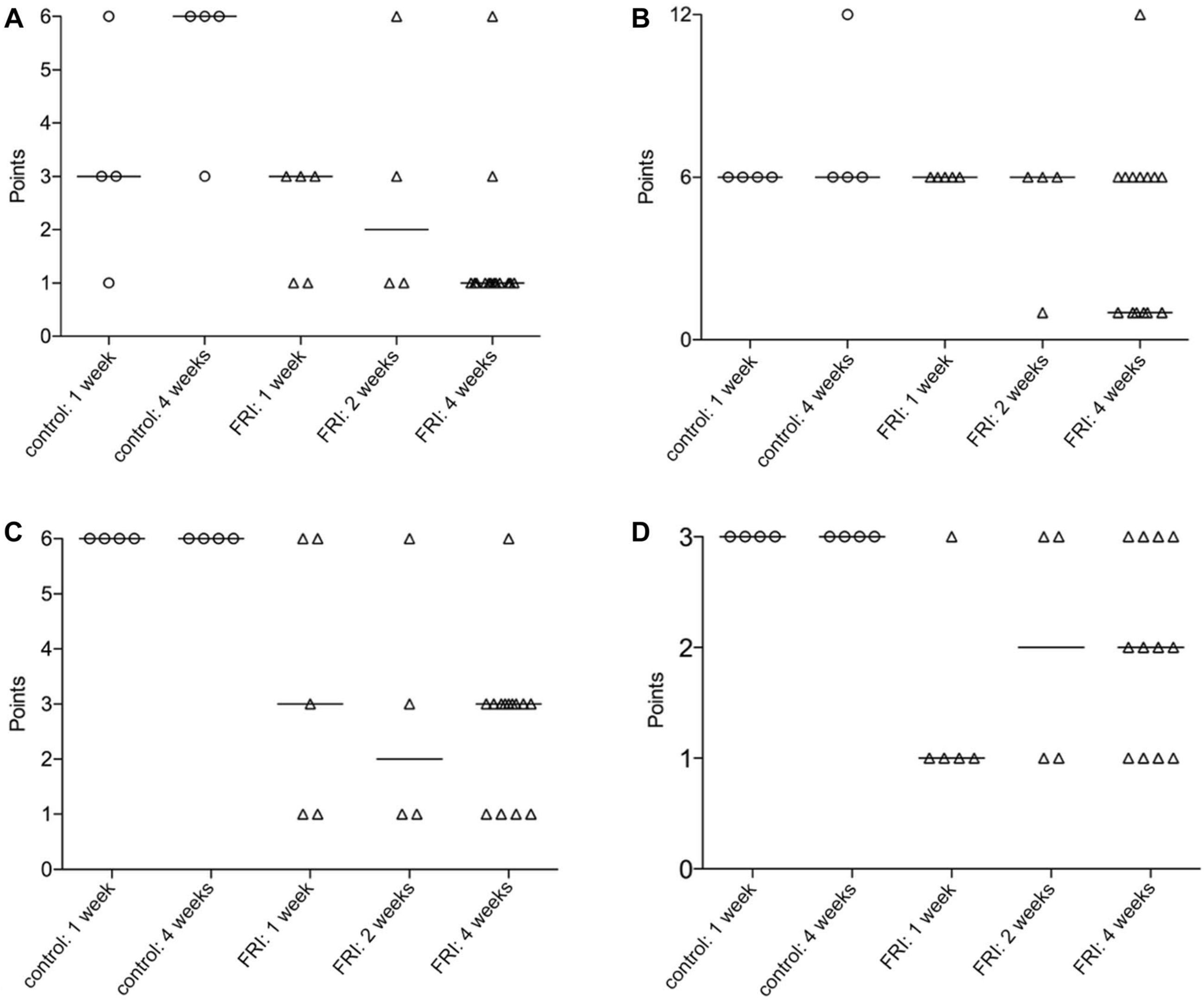

Fig. 5 Distribution of values for each parameter of the score assessing osteitis. This figure shows the assessed values and median values (black lines) in the control and FRI (fracture-related infection) groups for callus formation (**a**), consolidation of the fracture (**b**), structural changes of the medullary cavity (**c**) and number of bacteria (**d**)

access, feasible to diagnose and estimate bone healing and infection with a high sensitivity. Therefore, this score might be a useful tool to quantify infection-related changes after fracture fixation in future preclinical studies. In the present study, we design a score to diagnose and quantify osteitis and osteomyelitis in murine experimental osteitis by combining diagnostic imaging, microbiological and histopathological features. The score was easy to access; feasible to diagnose and estimate bone healing and infection in a murine osteitis with a high sensitivity. Therefore, this score might be a useful tool to quantify infection-related changes after osteitis in further studies and to compare the results of different studies.

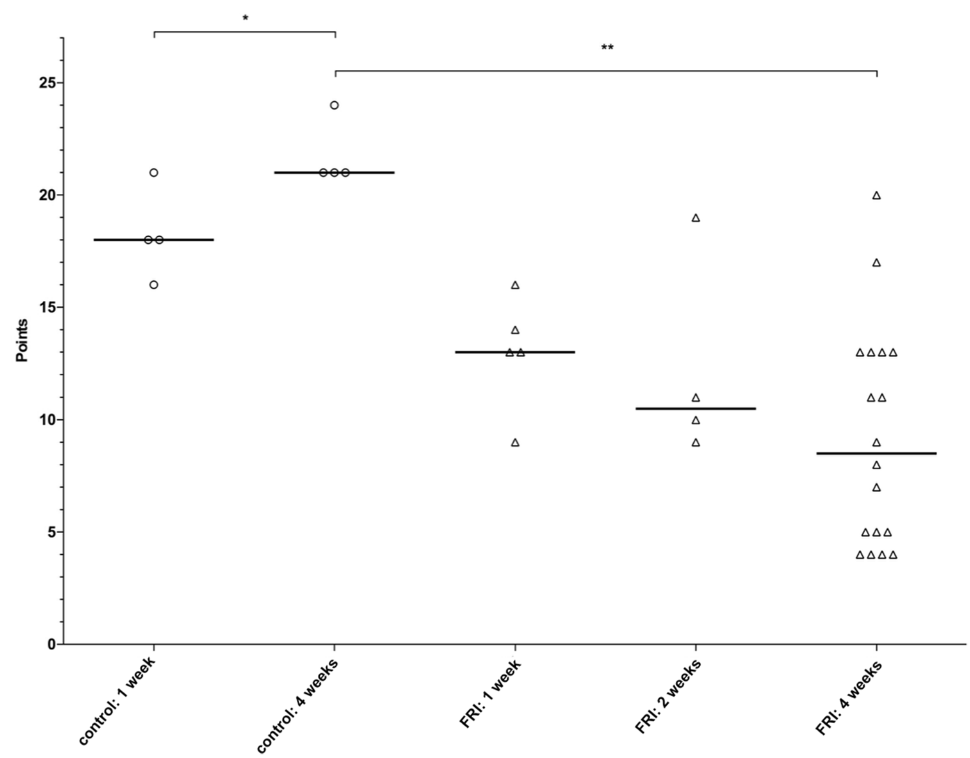

Fig. 6 Total score. This figure summarizes the values of the total score for each group. The higher score in the control group at 4 weeks might reflect the physiological re-constitution of the bone. Mice assigned to the FRI (fracture-related infection) group showed significantly lower median score values at one and 4 weeks after induction of the infection

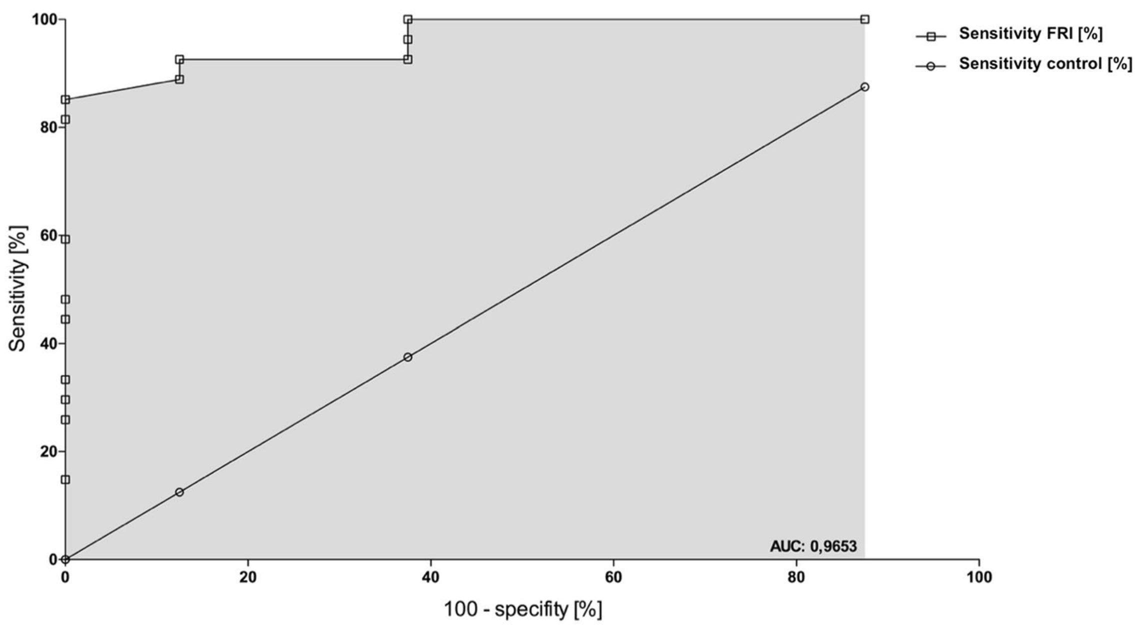

Fig. 7 Receiver operating characteristics (ROC) for the total score. *FRI* fracture-related infection

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Author contributions All authors have read and approved the final submitted manuscript. CB: literature search, study design, data collection, data analysis, data interpretation and writing. MH: study design, data collection, data analysis, data interpretation. TL and CDW: study design, data collection, data analysis, data interpretation and critical revision. JW: critical revision.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

1. Sabaté BM, O'Mahony L, Zeiter S et al (2017) Influence of fracture stability on Staphylococcus epidermidis and *Staphylococcus aureus* infection in a murine femoral fracture model. Eur Cell Mater 34:321–340
2. Metsemakers WJ, Kuehl R, Moriarty TF et al (2018) Infection after fracture fixation: current surgical and microbiological concepts. Injury 49(3):511–522
3. Willey M, Karam M (2016) Impact of infection on fracture fixation. Orthop Clin 47(2):357–364
4. Uçkay I, Hoffmeyer P, Lew D, Pittet D (2013) Prevention of surgical site infections in orthopaedic surgery and bone trauma: state-of-the-art update. J Hosp Infect 84(1):5–12
5. Papakostidis C, Kanakaris NK, Pretel J et al (2011) Prevalence of complications of open tibial shaft fractures stratified as per the Gustilo-Anderson classification. Injury 42(12):1408–1415
6. Geipel U, Herrmann M (2004) Das infizierte Implantat. Orthopäde 33(12):1411–1428
7. Schmelz A, Kinzl L, Einsiedel T (2006) Osteitis. Chirurg 77(10):943–962
8. Walter G, Hoffmann R (2009) Implantat-assoziierte Infektionen in Orthopädie und Unfallchirurgie. Krankenh-Hyg Infekt 31(1):8–14
9. Seybold D, Schildhauer T (2010) Knocheninfekte. Orthopädie und Unfallchirurgie essentials: Intensivkurs zur Weiterbildung. Georg Thieme-Verlag, Stuttgart, pp 135–142
10. Birt MC, Anderson DW, Bruce Toby E, Wang J (2017) Osteomyelitis: recent advances in pathophysiology and therapeutic strategies. J Orthop 14(1):45–52
11. Montanaro L, Speziale P, Campoccia D et al (2011) Scenery of *Staphylococcus* implant infections in orthopedics. Futur Microbiol 6(11):1329–1349
12. Cierny GI, Mader JT, Penninck JJ (2003) The classic: a clinical staging system for adult osteomyelitis. Clin Orthop 414:7–24
13. Schmidt HGK, Tiemann AH, Braunschweig R et al (2011) Zur definition der diagnose osteomyelitis—osteomyelitis-diagnose-score (ODS). Z Für Orthop Unfallchirurgie 149(04):449–460
14. Metsemakers W, Kortram K, Morgenstern M et al (2018) Definition of infection after fracture fixation: a systematic review of randomized controlled trials to evaluate current practice. Injury 49(3):497–504
15. Metsemakers W, Morgenstern M, McNally MA et al (2018) Fracture-related infection: a consensus on definition from an international expert group. Injury 49(3):505–510
16. Morgenstern M, Athanasou NA, Ferguson JY et al (2018) The value of quantitative histology in the diagnosis of fracture-related infection. Bone Jt J 100B(7):966–972
17. Windolf CD, Meng W, Lögters TT et al (2013) Implant-associated localized osteitis in murine femur fracture by biofilm forming *Staphylococcus aureus*: a novel experimental model. J Orthop Res 31(12):2013–2020
18. Windolf CD, Lögters T, Scholz M, et al. (2014) Lysostaphin-coated titan-implants preventing localized osteitis by *Staphylococcus aureus* in a mouse model. PLoS One 9(12) (cited 9 Aug 2015). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4275259/
19. Swiderska Z, Korzynska A, Markiewicz T, et al. (2015) Comparison of the manual, semiautomatic, and automatic selection and leveling of hot spots in whole slide images for Ki-67 quantification in meningiomas. Anal Cell Pathol (cited 1 Oct 2018). https://www.hindawi.com/journals/acp/2015/498746/abs/
20. Tiemann AH, Braunschweig R, Hofmann GO (2012) Knocheninfektionen. Unfallchirurg 115(6):480–488
21. Lew DP, Waldvogel FA (2004) Osteomyelitis. The Lancet 364(9431):369–379
22. Ciampolini J, Harding KG (2000) Pathophysiology of chronic bacterial osteomyelitis. Why do antibiotics fail so often? Postgrad Med J 76(898):479–483
23. Seok J, Warren HS, Cuenca AG et al (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci 110(9):3507–3512
24. Takao K, Miyakawa T (2015) Genomic responses in mouse models greatly mimic human inflammatory diseases. Proc Natl Acad Sci 112(4):1167–1172