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MINIREVIEW

Diagnosing Fracture-Related Infections: Where Are We Now?

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ABSTRACT Accurate diagnosis of fracture-related infection (FRI) is critical for preventing poor outcomes such as loss of function or amputation. Due to the multiple variables associated with FRI, however, accurate diagnosis is challenging and complicated by a lack of standardized diagnostic criteria. Limitations with the current gold standard for diagnosis, which is routine microbiology culture, further complicate the diagnostic and management process. Efforts to optimize the process rely on a foundation of data derived from prosthetic joint infections (PJI), but differences in PJI and FRI make it clear that unique approaches for these distinct infections are required. A more concerted effort focusing on FRI has dominated more recent investigations and publications leading to a consensus definition by the American Orthopedics (AO) Foundation and the European Bone and Joint Infection Society (EBJIS). This has the potential to better standardize the diagnostic process, which will not only improve patient care but also facilitate more robust and reproducible research related to the diagnosis and management of FRI. The purpose of this minireview is to explore the consensus definition, describe the foundation of data supporting current FRI diagnostic techniques, and identify pathways for optimization of clinical microbiology-based strategies and data.

KEYWORDS fracture-related infection, osteitis, osteomyelitis, culture-negative infection

Over three million extremity fractures are reported in the United States each year (1). Infection risk following fracture ranges from 0 to 55% depending on individual patient comorbidities, the type of open fracture, anatomical location, environmental conditions, extent of soft tissue damage, and other infection risk factors (2, 3). Fracture-related infections (FRI) can potentially lead to a delay in union or nonunion in over a third of cases, which can result in loss of function or need for amputation (4). Complications related to infection reduce quality of life (5) and are associated with significant increases in health care costs related to additional surgeries and increases in length-of-stay and readmission rates (6). Therefore, prevention and a timely, accurate diagnosis of infection after fracture are critical for preventing these devastating complications.

Due to the multiple variables associated with FRI, accurate diagnosis is challenging and complicated by the lack of a standardized criteria for diagnosis. Until recently, the principles of diagnosis and management for FRI have relied on data derived from prosthetic joint infections (PJI). Due to substantial differences in host factors, anatomical locations, causative organisms, and implant characteristics, however, it is clear that the diagnosis and treatment of FRI would deviate significantly from PJI. Thus, a more concerted effort focusing on FRI has dominated more recent investigations and publications leading to a consensus definition by the American Orthopedics (AO) Foundation and the European Bone and Joint Infection Society (EBJIS) (7).

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PATHOGENESIS OF FRACTURE-RELATED INFECTION

Fracture characteristics and risk factors. Variables in fracture classification include the bone, location, and morphology of the fracture (i.e., simple, wedge, or comminuted) (8) and whether or not the bone is exposed to the outside environment due to soft tissue injury or loss (open fracture). These variables are important considerations not only for the management of the fracture but also for the subsequent risk of complications. Infections following fracture are typically described based on the time of onset: early versus delayed versus late onset or as acute versus chronic infection. Early infections present within 2 weeks of fracture fixation and exhibit the classic signs of infection (fever, erythema, cellulitis, and drainage), while delayed and late onset infections are more indolent and present less than or greater than 10 weeks, respectively (9). Acute infections occur less than 6 weeks of fracture fixation, and chronic infections occur after 6 weeks of fracture fixation. There is not enough evidence to favor one classification system over the other, but it is important to note that both emphasize the role of biofilm maturation during the 2 to 6 weeks after implantation of fixation to help with management decisions in FRI (10).

Late infections are more frequently associated with compromised fracture healing. Distinguishing between septic and aseptic nonunion is critical because management strategies for the two scenarios differ drastically—septic or infected nonunions often require a multistaged surgical approach that includes initial eradication of the infection (radical resection of dead and infected tissues, including bone), prolonged antibiotic therapy, followed by repair of the nonunion (bone grafting and repeat fixation) (11). Early infections typically result from inoculation at the time of injury, during the course of management of open wounds, or during the surgical procedure. Due to the high degree of variability in causative organisms, targeted and strategic antimicrobial therapy is essential for timely clearance of pathogenic organisms while limiting selective pressure for resistance (10, 12). The failure to promptly and accurately identify infections and their etiologic agents results in both inappropriate surgical and pharmaceutical interventions, both of which can lead to poor outcomes.

Common FRI pathogens. The most common microorganism associated with FRI is *Staphylococcus aureus*, followed by coagulase-negative staphylococci (CoNS), *Enterobacteriaceae*, anaerobes, and streptococci (13, 14). A variety of factors can alter the distribution of the most frequently isolated pathogens, however. For example, in a multiinstitutional study that included level 1 regional referral trauma centers located in each of the seven climatic regions of the continental United States, researchers found that the most commonly encountered etiologic agent of infection after open fracture varied not only by region but also by season (15). While methicillin-resistant *S. aureus* (MRSA) was the most prevalent pathogen isolated in general, methicillin-sensitive *S. aureus* (MSSA), CoNS, *Enterobacter* spp., *Acinetobacter* spp., *Enterococcus* spp., and *Pseudomonas aeruginosa* were most commonly isolated at some point in different regions (15). The timing of infection relative to implantation may also be associated with variations in pathogen prevalence. For example, in a single institution study of internal fixation-associated infections, which were defined based on criteria established for PJI, investigators found that *S. aureus* and *Enterobacteriaceae* were most prevalent in early infections (0 to 2 weeks following implant), while CoNS, streptococci, and anaerobes increased in prevalence for delayed and late infections (3 to 10 weeks and >10 weeks following implant, respectively) (14). Finally, polymicrobial culture results are also common in the context of FRI, reported in 20 to 67% of cases (14, 16, 17).

A particularly important consideration in the pathogenesis of FRI is the formation of biofilm and its association with fracture fixation. Biofilm (an adherent consortium of microorganisms surrounded by a complex extracellular matrix) begins to appear within hours of inoculation. As the biofilm matures, it creates an environment in which

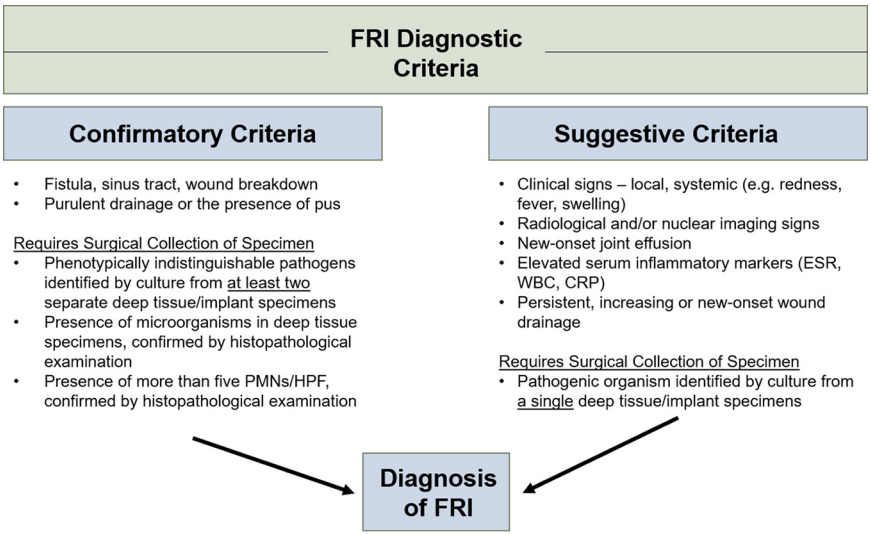

FIG 1 FRI diagnostic criteria. For confirmatory criteria, any single criterion is sufficient for a confirmatory diagnosis of FRI. For suggestive criteria, identification of any of these criteria should prompt further investigation, which may lead to identifying confirmatory criteria. FRI, fracture-related infection; PMNs, polymorphonuclear neutrophils; HPF, high-power field (×400 magnification); ESR, erythrocyte sedimentation rate; WBC, white blood cell; CRP, C-reactive protein. (Adapted from reference 66, which is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International licence [https://creativecommons.org/licenses/by-nc/4.0/].)

stationary-growth-phase bacteria that are more resistant to antimicrobial therapy may reside (9, 18). This is a shared concern for any orthopedic infection involving an implant and must be considered from both a diagnostic and management perspective.

CONSENSUS DEFINITION FOR FRI DIAGNOSIS

Standardization within case definitions and laboratory protocols are essential, not only for improved patient care but also for a more robust and reproducible comparison of different diagnostic processes, thus maximizing the conclusions that can be drawn from various studies (19). Once standardization is achieved, it is possible to evaluate how the application of novel diagnostic techniques and algorithms may influence the management and outcomes associated with FRI.

A 1996 review (20) explored this lack of universal definitions regarding the diagnosis of fracture-related infection: terms like osteitis and osteomyelitis were often used interchangeably, variability in fracture classification made it difficult to generate universal terminology, and infection was not historically studied as a primary outcome for research. Ultimately, this made it difficult to standardize the approaches for identifying risk factors and diagnosing infections because of differences in clinical presentation based on the overall severity and depth of tissue damage associated with infection. A recently published systematic review noted that only 2% of randomized clinical trials using FRI as an outcome cited a validated definition, 28% contained a definition generated by the trial's authors, and 70% did not reference a definition (10).

Recent guidelines published by the American Orthopedics Foundation and the European Bone and Joint Infection Society, which were based on a combination of evidence-based and expert opinion recommendations, stipulated two levels of confidence in the diagnosis of FRI: confirmatory and suggestive (Fig. 1). If a single confirmatory criterion is met, then an infection is considered to be definitively diagnosed, independent of the presence or absence of other criteria. Confirmatory criteria for an FRI include the following: (i) presence of a fistula, sinus tract, or wound breakdown with communication to the bone or implant; (ii) presence of pus during surgery or purulent discharge from the wound; (iii) phenotypically indistinguishable pathogens identified via culturing done from

greater than or equal to two deep tissue or fluid samples collected during surgery;, and (iv) presence of microorganisms in deep tissue samples collected during surgery and confirmed via histopathology (21). While the presence of polymorphonuclear neutrophils (PMNs) at a certain threshold per high-power field (HPF) was not included in the original confirmatory criteria, as it is for PJI (22), it has since been added to the definition as the fifth confirmatory sign of FRI for chronic/late onset cases (7). This decision was based on recent data that demonstrated a complete absence of PMNs had a very high correlation with aseptic nonunion, while the presence of $>$5 PMNs/HPF (at $\times$400 magnification) was always associated with infection (23).

If none of the confirmatory criteria are met but there are certain features present that are often associated with an FRI, then the diagnosis is considered suggestive, and further investigation is required, if possible. Suggestive criteria for an FRI include the following: (i) clinical signs such as pain, local swelling, local increased temperature, local redness, or fever; (ii) radiological signs such as bone lysis, presence of a nonunion, implant loosening, or sequestration; (iii) a pathogenic organism identified from one deep tissue/implant specimen; (iv) elevated levels of serum inflammatory markers; (v) wound drainage beyond the first few days postsurgery; and (vi) presence of joint effusion in fracture patients (21).

CURRENT FRI DIAGNOSTIC TOOLS

The diagnosis of FRI involves a multidisciplinary approach that includes clinical evaluations, intraoperative findings, various imaging modalities, and laboratory medicine techniques. However, direct detection of pathogens using microbiology cultures and histology is the current gold standard for diagnosis of FRI.

Medical imaging. Clinicians utilize medical imaging to determine fracture healing, implant stability, and anatomical details that suggest infection not detected on physical exam (7). Plain radiographs (X-ray), computed tomography (CT), magnetic resonance imaging (MRI), three-phase bone scan (BS), white blood cell (WBC) scintigraphy, and fluorodeoxyglucose positron emission tomography (FDG-PET) are the most common modalities used to diagnose FRI—each with its own distinct advantages and disadvantages (24). X-ray is typically used to assess overall alignment, fracture healing, and implant condition, with CT scans used only if further detail of these issues is needed. MRI is used to interrogate the soft tissues and medullary space of long bones. Nuclear techniques such as BS, WBC scintigraphy, and FDG-PET are used to increase the sensitivity when distinguishing between infected and noninfected tissues (24). While individually these various techniques contribute to the diagnostic process, there is no commonly accepted algorithm on when to employ each one; a prospective Dutch study is under way in hopes of identifying the most accurate imaging strategy for diagnosis FRI (25).

Diagnostic biomarkers. A variety of inflammatory markers are also used as part of the diagnostic process in FRI, including C-reactive protein (CRP), leukocyte count (LC), and erythrocyte sedimentation rate (ESR). While nonspecific elevation of these biomarkers occurs normally during the acute phase following trauma and surgery, higher than normal levels further along in the recovery process may be indicative of infection. Depending on the specific biomarker, sensitivity and specificity can range from 22.9% to 100% and 34.3% to 85.7%, respectively (26). Interestingly, even when the utility of these biomarkers is evaluated in the context of a more standardized definition of FRI, the sensitivity and specificity of CRP were only 67% (95% confidence interval [CI], 52% to 80%) and 61% (95% CI, 47% to 99%), respectively. Despite this relatively low sensitivity and specificity, serum CRP performs better than leukocyte count, percentage of neutrophils, and neutrophil-to-lymphocyte ratio (27). Although other biomarkers such as interleukin-6 (IL-6), D-dimer, interferon alpha, and procalcitonin are used in the diagnosis of PJI, there is currently no support for their use in FRI, and in some cases (like IL-6), they are inferior (28). Additional information for these biomarkers and others is needed, however.

Histopathology. There is limited data supporting specific guidelines for the interpretation of histopathology results in the context of FRI, but a comparison to PJI suggests that similar protocols may serve as an appropriate foundation for defining confirmatory histopathological criteria for FRI. Using formalin-fixed, paraffin-embedded tissue specimens, at least 10 high-power fields (×400 magnification) should be examined in each inflamed area and the number of PMNs recorded. The presence of greater than or equal to five polymorphonuclear neutrophils per high-power field (PMNs/HPF) in fractures is recommended as the appropriate threshold for a confirmed diagnosis of FRI (23).

Microbiology culture. Microbiology cultures are currently the gold standard for the identification of pathogens and the diagnosis of FRI. Suboptimal clinical sensitivity of culture-based approaches, however, can lead to false-negative culture results; it is estimated that 10% of FRI are culture negative (29). Thus, missed infections are potentially treated inappropriately, or more commonly, empirical antibiotic treatment will be prescribed to cover a suspected infection in culture-negative cases. Without an identified pathogen, empirical therapy provides broad coverage of the most likely organisms, increasing the risk of adverse drug event and antimicrobial resistance. Therefore, optimization of routine cultures for FRI is essential for improved individual patient care and public health outcomes. From specimen collection, transport, processing, incubation, and interpretation, all parts of the culture process play an important role in determining the diagnostic yield of this testing for FRI. Much of this process, and strategies to improve it, are based on PJI research; some techniques have been studied specifically in the context of fractures, but others would benefit from more intentional application and evaluation specific to FRI.

Swab cultures are generally accepted to have an even lower sensitivity and higher risk of contamination compared to tissue, and thus, tissue cultures are preferred; this has been demonstrated for both PJI and osteomyelitis cases such as FRI (10, 30, 31). To optimize sensitivity of culture for intraoperative tissue specimens, it is recommended that at least five samples be collected from individual, representative sites within the infected area (7). This recommendation is consistent with the related field of PJI where research has found that the greatest accuracy was observed when four specimens were obtained using conventional periprosthetic tissue culture techniques (91% accuracy; 95% CI, 77% to 100%) (32). Submitting multiple tissue samples for culture can introduce increased economic and diagnostic burden within the laboratory setting, so efforts should be made to continue to evaluate and optimize processes so that an appropriate balance of cost-effectiveness can be achieved, similar to what has been explored in related fields (33).

To ensure optimal specificity, these specimens should be collected at the beginning of the procedure minimizing the risk of specimen contamination. Since clinical significance is often determined by the detection of phenotypically indistinguishable organisms in at least two different specimens (21), minimizing the risk of cross-contamination during specimen collection is critical. Ideally, separate instruments should be used for each sample collected without touching any other area of the patient with either the sample or instrument (7). In a study evaluating the "no touch" technique, a significantly higher rate of contamination was observed when samples were taken using reused instruments compared to fresh, previously unused instruments (34). Immediately after collection, samples should then be placed in sterile saline and transferred promptly to the laboratory; loss of viability due to delayed specimen transport is thought to contribute to culture negativity in PJI cases (35). Using standardized specimen collection and processing approaches that incorporate these best practices has been shown to enhance culture yield for the diagnosis of FRI (16). There is a paucity of data related to other optimization techniques for FRI cultures, such as specialized transport media or pooling of tissue samples; more research is required in this area.

FUTURE DIRECTIONS FOR CLINICAL MICROBIOLOGY FRI DIAGNOSTICS

While certain criteria allow for confirmed or suggestive FRI diagnosis in the absence of culture positivity, the identification of a pathogen is still critical for the optimal

management of disease. A 2018 meta-analysis of all published literature on diagnostic validation of FRI specimens found just nine studies that evaluated the accuracy of laboratory techniques, with only two of these studies including molecular testing (19). Therefore, efforts to continue to improve the microbiology diagnostic testing options for FRI need to be a priority.

Optimized culture techniques. The paucity of research related to laboratory diagnostics for FRI contrasts with the work that has been accomplished for PJI. There are multiple publications evaluating modified specimen processing techniques, culture conditions, and the application of various pathogen detection techniques. In fact, there has even been a multicenter study aimed at determining how culture conditions in totality, including sample number, culture medium type, and incubation periods, can be optimized to balance diagnostic yield and feasibility for prosthetic joint infection cultures (33). Ostensibly, work such as this may be extrapolated to guide optimization efforts in the field of FRI diagnosis and management as well. Beyond altering incubation conditions, alternative processing techniques have also been shown to be effective for improving PJI culture yield. For example, sonication or dithiothreitol treatment of explanted prostheses from PJI cases prior to inoculation of culture plates improves the sensitivity and diagnostic yield by releasing bacteria from biofilm (36–40). Furthermore, more complete homogenization of periprosthetic tissue followed by incubation in blood culture bottles or incubation in liquid enriched broth rather than solid agar has also been shown to improve the diagnostic yield and time to detection of culture-based approaches for PJI (41, 42).

Combined, these data suggest that using alternative tissue processing techniques and enhanced culture conditions may be similarly beneficial for FRI diagnosis, especially for cases at highest risk for culture negativity. In a single study that compared sonication to conventional tissue cultures, diagnostic yield and sensitivity improved from 57% to 90% (43). A separate study examined the diagnostic yield of sonication of explanted prosthetic or fracture fixation devices and found that sensitivity improved from 87% to 100% when sonification fluid was placed in blood culture bottles (44). Additional work to validate these findings, and to evaluate the impact of other optimization strategies, is needed. Furthermore, procedures must be optimized and results interpreted carefully to reduce the risk of contamination and lower specificity of cultures utilizing these alternative processing techniques. For example, studies demonstrate that while prosthesis vortexing/sonication in a solid container improves culture sensitivity compared to tissue culture without decreasing specificity (45), sonication within bags is associated with a risk of contamination due to bag leakage (46).

Molecular diagnostics. Despite the volume of work focusing on improving culture yields for PJI, up to 15% of PJI cases remain culture negative (47, 48). Because bacterial nucleic acid has been found in over 90% of synovial fluid samples that were culture negative (49), molecular diagnostics-based techniques have been evaluated as a means of improving the diagnostic yield of laboratory testing (50). The performance of molecular diagnostics for FRI has not been fully evaluated yet, but studies of PJI demonstrate that there are many factors that must be considered. For example, molecular diagnostics can entail a range of approaches that have varied in performance and feasibility when studied for PJI, including targeted PCR for the most common etiologic agent(s) (51, 52), multiplex panels that detect a number of potential pathogens with or without antimicrobial resistance markers (53–59), targeted metagenomics with identification to the genus/species level (60), or shotgun metagenomics with appropriate sequencing depth of coverage to allow for detection of not only species-level identifications but also antimicrobial resistance genes (61, 62). Not only do these approaches allow for a range of bacterial pathogen identification but they may also detect fungi as well, thus potentially expanding the range of pathogen identification in a single test. As with culture, however, detection of any potential bacterial or fungal pathogen that also represents commensal flora must be interpreted with caution due to risk of contamination; these risks are increased with molecular detection techniques, as both host and contaminating nucleic acid can have a critical impact on downstream results and analysis (62).

In addition to the type of molecular diagnostic approach used, specimen "type" is of critical importance when using molecular testing; 16S rRNA PCR analysis performed on swabs of fracture implant surfaces had lower diagnostic yield compared to conventional tissue culture (63). Diagnostic yield improved on needle aspirates or surgical biopsy specimens, followed by 16S rRNA PCR-based techniques (64). Finally, the routine application of some molecular techniques, such as metagenomic analysis, has been limited even for the diagnosis of PJI due to considerations of cost, reimbursement, and turnaround time (65). In PJI studies, the cost of metagenomic analysis was calculated at several hundred U.S. dollars per sample, compared to the few dollar supply costs of standard culture techniques, leading the authors to conclude that metagenomic analysis currently is not economically justifiable for application in every case (62). It is possible that more targeted molecular approaches may be more pragmatic while still improving diagnostic yield. Therefore, not only will it be critical to fully evaluate the accuracy of molecular detection techniques for FRI cases, but it will also be important to consider appropriate utilization from a cost-effectiveness perspective.

CONCLUSIONS

The need for a standardized diagnostic and treatment plan for FRI patients has become abundantly clear. The rise in antibiotic-resistant bacteria due to the empirical use of broad-spectrum antibiotics as the preferred treatment has highlighted the need for personalized treatment approaches. This can be achieved by identifying pathogens in culture, but it poses many issues due to the chance of a patient having a culture-negative FRI. This lack of standardization causes issues in all aspects of the diagnostic process. The recently published consensus guidelines for defining FRI have the potential to better standardize the diagnostic process, which will not only improve patient care but also facilitate more robust and reproducible research related to the diagnosis and management of FRI. Within this context, future work dedicated to the evaluation of testing strategies that are novel when applied to FRI, such as optimized specimen processing, broth-based culture incubation, and molecular detection techniques, can be performed. Due to their effectiveness in the related field of PJI, it is likely that these strategies will improve the diagnostic yield of FRI laboratory testing and thus reduce the risk of diagnostic error and mismanagement.

REFERENCES

1. Rui P, Kang K. 2017. National hospital ambulatory medical care survey: 2017 emergency department summary tables. National Center for Health Statistics, Hyattsville, MD.
2. Bezstarosti H, Van Lieshout EMM, Voskamp LW, Kortram K, Obremskey W, McNally MA, Metsemakers WJ, Verhofstad MHJ. 2019. Insights into treatment and outcome of fracture-related infection: a systematic literature review. Arch Orthop Trauma Surg 139:61–72. https://doi.org/10.1007/s00402-018-3048-0.
3. Carsenti-Etesse H, Doyon F, Desplaces N, Gagey O, Tancrède C, Pradier C, Dunais B, Dellamonica P. 1999. Epidemiology of bacterial infection during management of open leg fractures. Eur J Clin Microbiol Infect Dis 18:315–323. https://doi.org/10.1007/pl00015012.
4. Mills L, Tsang J, Hopper G, Keenan G, Simpson AH. 2016. The multifactorial aetiology of fracture nonunion and the importance of searching for latent infection. Bone Joint Res 5:512–519. https://doi.org/10.1302/2046-3758.510.BJR-2016-0138.
5. Lerner RK, Esterhai JL, Jr, Polomano RC, Cheatle MD, Heppenstall RB. 1993. Quality of life assessment of patients with posttraumatic fracture nonunion, chronic refractory osteomyelitis, and lower-extremity amputation. Clin Orthop Relat Res 1993:28–36.
6. Chitnis AS, Vanderkarr M, Sparks C, McGlohorn J, Holy CE. 2019. Complications and its impact in patients with closed and open tibial shaft fractures requiring open reduction and internal fixation. J Comp Eff Res 8:1405–1416. https://doi.org/10.2217/cer-2019-0108.
7. Govaert GAM, Kuehl R, Atkins BL, Trampuz A, Morgenstern M, Obremskey WT, Verhofstad MHJ, McNally MA, Metsemakers WJ, Fracture-Related Infection (FRI) Consensus Group. 2020. Diagnosing fracture-related infection: current concepts and recommendations. J Orthop Trauma 34:8–17. https://doi.org/10.1097/BOT.0000000000001614.
8. Meinberg EG, Agel J, Roberts CS, Karam MD, Kellam JF. 2018. Fracture and dislocation classification compendium−2018. J Orthop Trauma 32(Suppl 1):S1–S170. https://doi.org/10.1097/BOT.0000000000001063.
9. Steinmetz S, Wernly D, Moerenhout K, Trampuz A, Borens O. 2019. Infection after fracture fixation. EFORT Open Rev 4:468–475. https://doi.org/10.1302/2058-5241.4.180093.
10. Metsemakers WJ, Kuehl R, Moriarty TF, Richards RG, Verhofstad MHJ, Borens O, Kates S, Morgenstern M. 2018. Infection after fracture fixation: current surgical and microbiological concepts. Injury 49:511–522. https://doi.org/10.1016/j.injury.2016.09.019.
11. Kanakaris NK, Tosounidis TH, Giannoudis PV. 2015. Surgical management of infected non-unions: an update. Injury 46(Suppl 5):S25–S32. https://doi.org/10.1016/j.injury.2015.08.009.
12. Foster AL, Moriarty TF, Trampuz A, Jaiprakash A, Burch MA, Crawford R, Paterson DL, Metsemakers WJ, Schuetz M, Richards RG. 2020. Fracture-related infection: current methods for prevention and treatment. Expert Rev Anti Infect Ther 18:307–321. https://doi.org/10.1080/14787210.2020.1729740.
13. Depypere M, Morgenstern M, Kuehl R, Senneville E, Moriarty TF, Obremskey WT, Zimmerli W, Trampuz A, Lagrou K, Metsemakers WJ. 2020. Pathogenesis and management of fracture-related infection. Clin Microbiol Infect 26:572–578. https://doi.org/10.1016/j.cmi.2019.08.006.
14. Kuehl R, Tschudin-Sutter S, Morgenstern M, Dangel M, Egli A, Nowakowski A, Suhm N, Theilacker C, Widmer AF. 2019. Time-dependent differences in management and microbiology of orthopaedic internal fixation-associated

infections: an observational prospective study with 229 patients. Clin Microbiol Infect 25:76–81. https://doi.org/10.1016/j.cmi.2018.03.040.
15. Sagi HC, Donohue D, Cooper S, Barei DP, Siebler J, Archdeacon MT, Sciadini M, Romeo M, Bergin PF, Higgins T, Mir H, Center for Bone and Joint Infection. 2017. Institutional and seasonal variations in the incidence and causative organisms for posttraumatic infection following open fractures. J Orthop Trauma 31:78–84. https://doi.org/10.1097/BOT.0000000000000730.
16. Hellebrekers P, Rentenaar RJ, McNally MA, Hietbrink F, Houwert RM, Leenen LPH, Govaert GAM. 2019. Getting it right first time: the importance of a structured tissue sampling protocol for diagnosing fracture-related infections. Injury 50:1649–1655. https://doi.org/10.1016/j.injury.2019.05.014.
17. Trampuz A, Zimmerli W. 2006. Diagnosis and treatment of infections associated with fracture-fixation devices. Injury 37:S59–S66. https://doi.org/10.1016/j.injury.2006.04.010.
18. Depypere M, Kuehl R, Metsemakers WJ, Senneville E, McNally MA, Obremskey WT, Zimmerli W, Atkins BL, Trampuz A, Fracture-Related Infection (FRI) Consensus Group. 2020. Recommendations for systemic antimicrobial therapy in fracture-related infection: a consensus from an international expert group. J Orthop Trauma 34:30–41. https://doi.org/10.1097/BOT.0000000000001626.
19. Onsea J, Depypere M, Govaert G, Kuehl R, Vandendriessche T, Morgenstern M, McNally M, Trampuz A, Metsemakers WJ. 2018. Accuracy of tissue and sonication fluid sampling for the diagnosis of fracture-related infection: a systematic review and critical appraisal. J Bone Jt Infect 3:173–181. https://doi.org/10.7150/jbji.27840.
20. Arens S, Hansis M, Schlegel U, Eijer H, Printzen G, Ziegler WJ, Perren SM. 1996. Infection after open reduction and internal fixation with dynamic compression plates–clinical and experimental data. Injury 27(Suppl 3):SC27–SC33. https://doi.org/10.1016/0020-1383(96)89029-2.
21. Metsemakers WJ, Morgenstern M, McNally MA, Moriarty TF, McFadyen I, Scarborough M, Athanasou NA, Ochsner PE, Kuehl R, Raschke M, Borens O, Xie Z, Velkes S, Hungerer S, Kates SL, Zalavras C, Giannoudis PV, Richards RG, Verhofstad MHJ. 2018. Fracture-related infection: a consensus on definition from an international expert group. Injury 49:505–510. https://doi.org/10.1016/j.injury.2017.08.040.
22. Parvizi J, Tan TL, Goswami K, Higuera C, Della Valle C, Chen AF, Shohat N. 2018. The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. J Arthroplasty 33:1309–1314.e2. https://doi.org/10.1016/j.arth.2018.02.078.
23. Morgenstern M, Athanasou NA, Ferguson JY, Metsemakers WJ, Atkins BL, McNally MA. 2018. The value of quantitative histology in the diagnosis of fracture-related infection. Bone Joint J 100-B:966–972. https://doi.org/10.1302/0301-620X.100B7.BJJ-2018-0052.R1.
24. Glaudemans AWJM, Bosch P, Slart RHJA, IJpma FFA, Govaert GAM. 2019. Diagnosing fracture-related infections: can we optimize our nuclear imaging techniques? Eur J Nucl Med Mol Imaging 46:1583–1587. https://doi.org/10.1007/s00259-019-04378-5.
25. Govaert G, Hobbelink M, Reininga I, Bosch P, Kwee TC, de Jong PA, Jutte PC, Vogely HC, Dierckx R, Leenen L, Glaudemans A, Ij F. 2019. The accuracy of diagnostic Imaging techniques in patients with a suspected Fracture-related Infection (IFI) trial: study protocol for a prospective multicenter cohort study. BMJ Open 9:e027772. https://doi.org/10.1136/bmjopen-2018-027772.
26. van den Kieboom J, Bosch P, Plate JDJ, IJpma FFA, Kuehl R, McNally MA, Metsemakers W-J, Govaert GAM. 2018. Diagnostic accuracy of serum inflammatory markers in late fracture-related infection: a systematic review and meta-analysis. Bone Joint J 100-B:1542–1550. https://doi.org/10.1302/0301-620X.100B12.BJJ-2018-0586.R1.
27. Sigmund IK, Dudareva M, Watts D, Morgenstern M, Athanasou NA, McNally MA. 2020. Limited diagnostic value of serum inflammatory biomarkers in the diagnosis of fracture-related infections. Bone Joint J 102-B:904–911. https://doi.org/10.1302/0301-620X.102B7.BJJ-2019-1739.R1.
28. Wang S, Yin P, Quan C, Khan K, Wang G, Wang L, Cui L, Zhang L, Zhang L, Tang P. 2017. Evaluating the use of serum inflammatory markers for preoperative diagnosis of infection in patients with nonunions. Biomed Res Int 2017:9146317. https://doi.org/10.1155/2017/9146317.
29. Gitajn IL, Heng M, Weaver MJ, Ehrlichman LK, Harris MB. 2016. Culture-negative infection after operative fixation of fractures. J Orthop Trauma 30:538–544. https://doi.org/10.1097/BOT.0000000000000618.
30. Aggarwal VK, Higuera C, Deirmengian G, Parvizi J, Austin MS. 2013. Swab cultures are not as effective as tissue cultures for diagnosis of periprosthetic joint infection. Clin Orthop Relat Res 471:3196–3203. https://doi.org/10.1007/s11999-013-2974-y.
31. Zuluaga AF, Galvis W, Jaimes F, Vesga O. 2002. Lack of microbiological concordance between bone and non-bone specimens in chronic osteomyelitis: an observational study. BMC Infect Dis 2:8. https://doi.org/10.1186/1471-2334-2-8.
32. Peel TN, Spelman T, Dylla BL, Hughes JG, Greenwood-Quaintance KE, Cheng AC, Mandrekar JN, Patel R. 2017. Optimal periprosthetic tissue specimen number for diagnosis of prosthetic joint infection. J Clin Microbiol 55:234–243. https://doi.org/10.1128/JCM.01914-16.
33. Bémer P, Léger J, Tandé D, Plouzeau C, Valentin AS, Jolivet-Gougeon A, Lemarié C, Kempf M, Héry-Arnaud G, Bret L, Juvin ME, Giraudeau B, Corvec S, Burucoa C, Centre de Référence des Infections Ostéo-articulaires du Grand Ouest (CRIOGO) Study Team. 2016. How many samples and how many culture media to diagnose a prosthetic joint infection: a clinical and microbiological prospective multicenter study. J Clin Microbiol 54:385–391. https://doi.org/10.1128/JCM.02497-15.
34. Makki D, Abdalla S, El Gamal TA, Harvey D, Jackson G, Platt S. 2018. Is it necessary to change instruments between sampling sites when taking multiple tissue specimens in musculoskeletal infections? Ann R Coll Surg Engl 100:563–565. https://doi.org/10.1308/rcsann.2018.0097.
35. Parikh MS, Antony S. 2016. A comprehensive review of the diagnosis and management of prosthetic joint infections in the absence of positive cultures. J Infect Public Health 9:545–556. https://doi.org/10.1016/j.jiph.2015.12.001.
36. Drago L, Romanò CL, Mattina R, Signori V, De Vecchi E. 2012. Does dithiothreitol improve bacterial detection from infected prostheses? A pilot study. Clin Orthop Relat Res 470:2915–2925. https://doi.org/10.1007/s11999-012-2415-3.
37. Drago L, Signori V, De Vecchi E, Vassena C, Palazzi E, Cappelletti L, Romanò D, Romanò CL. 2013. Use of dithiothreitol to improve the diagnosis of prosthetic joint infections. J Orthop Res 31:1694–1699. https://doi.org/10.1002/jor.22423.
38. Sambri A, Cadossi M, Giannini S, Pignatti G, Marcacci M, Neri MP, Maso A, Storni E, Gamberini S, Naldi S, Torri A, Zannoli S, Tassinari M, Fantini M, Bianchi G, Donati D, Sambri V. 2018. Is treatment with dithiothreitol more effective than sonication for the diagnosis of prosthetic joint infection? Clin Orthop Relat Res 476:137–145. https://doi.org/10.1007/s11999.0000000000000060.
39. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. 1999. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am 81:672–683. https://doi.org/10.2106/00004623-199905000-00008.
40. Wouthuyzen-Bakker M, Benito N, Soriano A. 2017. The effect of preoperative antimicrobial prophylaxis on intraoperative culture results in patients with a suspected or confirmed prosthetic joint infection: a systematic review. J Clin Microbiol 55:2765–2774. https://doi.org/10.1128/JCM.00640-17.
41. Peel TN, Dylla BL, Hughes JG, Lynch DT, Greenwood-Quaintance KE, Cheng AC, Mandrekar JN, Patel R. 2016. Improved diagnosis of prosthetic joint infection by culturing periprosthetic tissue specimens in blood culture bottles. mBio 7:e01776-15. https://doi.org/10.1128/mBio.01776-15.
42. Senneville E, Savage C, Nallet I, Yazdanpanah Y, Giraud F, Migaud H, Dubreuil L, Courcol R, Mouton Y. 2006. Improved aero-anaerobe recovery from infected prosthetic joint samples taken from 72 patients and collected intraoperatively in Rosenow's broth. Acta Orthop 77:120–124. https://doi.org/10.1080/17453670610045795.
43. Yano MH, Klautau GB, da Silva CB, Nigro S, Avanzi O, Mercadante MT, Salles MJ. 2014. Improved diagnosis of infection associated with osteosynthesis by use of sonication of fracture fixation implants. J Clin Microbiol 52:4176–4182. https://doi.org/10.1128/JCM.02140-14.
44. Portillo ME, Salvadó M, Trampuz A, Siverio A, Alier A, Sorli L, Martínez S, Pérez-Prieto D, Horcajada JP, Puig-Verdie L. 2015. Improved diagnosis of orthopedic implant-associated infection by inoculation of sonication fluid into blood culture bottles. J Clin Microbiol 53:1622–1627. https://doi.org/10.1128/JCM.03683-14.
45. Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelo J, Osmon DR, McDowell A, Patrick S, Steckelberg JM, Mandrekar JN, Fernandez Sampedro M, Patel R. 2009. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. J Clin Microbiol 47:1878–1884. https://doi.org/10.1128/JCM.01686-08.
46. Trampuz A, Piper KE, Hanssen AD, Osmon DR, Cockerill FR, Steckelberg JM, Patel R. 2006. Sonication of explanted prosthetic components in bags for diagnosis of prosthetic joint infection is associated with risk of contamination. J Clin Microbiol 44:628–631. https://doi.org/10.1128/JCM.44.2.628-631.2006.

47. Berbari EF, Marculescu C, Sia I, Lahr BD, Hanssen AD, Steckelberg JM, Gullerud R, Osmon DR. 2007. Culture-negative prosthetic joint infection. Clin Infect Dis 45:1113–1119. https://doi.org/10.1086/522184.
48. Palan J, Nolan C, Sarantos K, Westerman R, King R, Foguet P. 2019. Culture-negative periprosthetic joint infections. EFORT Open Rev 4:585–594. https://doi.org/10.1302/2058-5241.4.180067.
49. Bereza P, Ekiel A, Augustciak-Duma A, Aptekorz M, Wilk I, Kusz D, Wojciechowski P, Sieroń AL, Martirosian G. 2016. Comparison of cultures and 16S rRNA sequencing for identification of bacteria in two-stage revision arthroplasties: preliminary report. BMC Musculoskelet Disord 17:138. https://doi.org/10.1186/s12891-016-0991-1.
50. Tande AJ, Patel R. 2014. Prosthetic joint infection. Clin Microbiol Rev 27:302–345. https://doi.org/10.1128/CMR.00111-13.
51. Yang F, Choe H, Kobayashi N, Tezuka T, Oba M, Miyamae Y, Morita A, Abe K, Inaba Y. 2021. An automated real-time PCR assay for synovial fluid improves the preoperative etiological diagnosis of periprosthetic joint infection and septic arthritis. J Orthop Res 39:348–355. https://doi.org/10.1002/jor.24959.
52. Cazanave C, Greenwood-Quaintance KE, Hanssen AD, Patel R. 2012. Corynebacterium prosthetic joint infection. J Clin Microbiol 50:1518–1523. https://doi.org/10.1128/JCM.06439-11.
53. Graue C, Schmitt BH, Waggoner A, Laurent F, Abad L, Bauer T, Mazariegos I, Balada-Llasat JM, Horn J, Wolk D, Jefferis A, Hermans M, Verhoofstad I, Butler-Wu S, Butler-Wu S, Umali-Wilcox M, Murphy CN, Cabrera BJ, Esteban J, Macias-Valcayo A, Craft D, von Bredow B, Leber A, Everhart K, Bard JD, Mestas J, Daly J, Barr R, Kensinger B, Pons B, Jay C. 2020. 322. Evaluation of the BioFire Bone and Joint Infection (BJI) panel for the detection of microorganisms and antimicrobial resistance genes in synovial fluid specimens. Open Forum Infect Dis 7:S233–S234. https://doi.org/10.1093/ofid/ofaa439.518.
54. Pons B, Jay C, Martin T, Sothier I, Savelli H, Kensinger B, Laurent F, Abad L, Murphy C, Craney A, Schmitt B, Waggoner A, Butler-Wu S, Costales C, Bien-Bard J, Mestas J, Esteban J, Salar-Vidal L, Harrington A, Collier S, Leber A, Everhart K, Balada-Llasat JM, Horn J, Magro S, Bourzac K. 2018. 2290. Identification of pathogens in synovial fluid samples with an automated multiplexed molecular detection system. Open Forum Infect Dis 5:S678–S679. https://doi.org/10.1093/ofid/ofy210.1943.
55. Aamot HV, Johnsen BO, Skråmm I. 2019. Rapid diagnostics of orthopedic implant-associated infections using Unyvero ITI implant and tissue infection application is not optimal for Staphylococcus species identification. BMC Res Notes 12:725. https://doi.org/10.1186/s13104-019-4755-5.
56. Lafeuille E, Jauréguiberry S, Devriese F, Sadowski E, Fourniols E, Aubry A, CRIOAC Pitié-Salpêtrière. 2021. First evaluation of the automated-multiplex-PCR Unyvero ITI G2 cartridge for rapid diagnosis of osteo-articular infections. Infect Dis Now 51:179–186. https://doi.org/10.1016/j.medmal.2020.09.010.
57. Malandain D, Bémer P, Leroy AG, Léger J, Plouzeau C, Valentin AS, Jolivet-Gougeon A, Tandé D, Héry-Arnaud G, Lemarié C, Kempf M, Bret L, Burucoa C, Corvec S, Centre de Référence des Infections Ostéo-articulaires du Grand Ouest (CRIOGO) Study Team. 2018. Assessment of the automated multiplex-PCR Unyvero i60 ITI(®) cartridge system to diagnose prosthetic joint infection: a multicentre study. Clin Microbiol Infect 24:83.e1–83.e6. https://doi.org/10.1016/j.cmi.2017.05.017.
58. Sigmund IK, Windhager R, Sevelda F, Staats K, Puchner SE, Stenicka S, Thalhammer F, Holinka J. 2019. Multiplex PCR Unyvero i60 ITI application improves detection of low-virulent microorganisms in periprosthetic joint infections. Int Orthop 43:1891–1898. https://doi.org/10.1007/s00264-018-4136-z.
59. Zannoli S, Sambri A, Morotti M, Tassinari M, Torri A, Bianchi G, De Paolis M, Donati DM, Sambri V. 2021. Unyvero ITI(®) system for the clinical resolution of discrepancies in periprosthetic joint infection diagnosis. Musculoskelet Surg 105:39–42. https://doi.org/10.1007/s12306-019-00626-x.
60. Chen MF, Chang CH, Chiang-Ni C, Hsieh PH, Shih HN, Ueng SWN, Chang Y. 2019. Rapid analysis of bacterial composition in prosthetic joint infection by 16S rRNA metagenomic sequencing. Bone Joint Res 8:367–377. https://doi.org/10.1302/2046-3758.88.BJR-2019-0003.R2.
61. Yan Q, Wi YM, Thoendel MJ, Raval YS, Greenwood-Quaintance KE, Abdel MP, Jeraldo PR, Chia N, Patel R. 2019. Evaluation of the CosmosID bioinformatics platform for prosthetic joint-associated sonicate fluid shotgun metagenomic data analysis. J Clin Microbiol 57:e01182-18. https://doi.org/10.1128/JCM.01182-18.
62. Ivy MI, Thoendel MJ, Jeraldo PR, Greenwood-Quaintance KE, Hanssen AD, Abdel MP, Chia N, Yao JZ, Tande AJ, Mandrekar JN, Patel R. 2018. Direct detection and identification of prosthetic joint infection pathogens in synovial fluid by metagenomic shotgun sequencing. J Clin Microbiol 56:e00402-18. https://doi.org/10.1128/JCM.00402-18.
63. Omar M, Suero EM, Liodakis E, Reichling M, Guenther D, Decker S, Stiesch M, Krettek C, Eberhard J. 2016. Diagnostic performance of swab PCR as an alternative to tissue culture methods for diagnosing infections associated with fracture fixation devices. Injury 47:1421–1426. https://doi.org/10.1016/j.injury.2016.04.038.
64. Fenollar F, Roux V, Stein A, Drancourt M, Raoult D. 2006. Analysis of 525 samples to determine the usefulness of PCR amplification and sequencing of the 16S rRNA gene for diagnosis of bone and joint infections. J Clin Microbiol 44:1018–1028. https://doi.org/10.1128/JCM.44.3.1018-1028.2006.
65. Greninger AL. 2018. The challenge of diagnostic metagenomics. Expert Rev Mol Diagn 18:605–615. https://doi.org/10.1080/14737159.2018.1487292.
66. McNally M, Govaert G, Dudareva M, Morgenstern M, Metsemakers WJ. 2020. Definition and diagnosis of fracture-related infection. EFORT Open Rev 5:614–619. https://doi.org/10.1302/2058-5241.5.190072.