Diagnosis of infection in the foot of patients with diabetes: a systematic review

Senneville, E.; Albalawi, Z.; Asten, S.A. van; Abbas, Z.G.; Allison, G.; Aragón-Sánchez, J.; ... ; Peters, E.J.G.

Received: 26 July 2023 | Accepted: 29 August 2023

DOI: 10.1002/dmrr.3723

RESEARCH ARTICLE

WILEY

Diagnosis of infection in the foot of patients with diabetes: A systematic review

Éric Senneville[1,2] | Zaina Albalawi[3] | Suzanne A. van Asten[4] | Zulfiqarali G. Abbas[5] | Geneve Allison[6] | Javier Aragón-Sánchez[7] | John M. Embil[8] | Lawrence A. Lavery[9] | Majdi Alhasan[10] | Orhan Oz[11] | Ilker Uçkay[12] | Vilma Urbančič-Rovan[13] | Zhang-Rong Xu[14] | Edgar J. G. Peters[15,16,17]

[1]Department of Infectious Diseases, Gustave Dron Hospital, Tourcoing, France

[2]Univ-Lille, Lille, France

[3]Department of Medicine, Division of Endocrinology, Memorial University, St. John's, Newfoundland, Canada

[4]Department of Medical Microbiology, Leiden University Medical Centre, Leiden, The Netherlands

[5]Abbas Medical Centre, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[6]Department of Medicine, Tufts Medical Center, Boston, Massachusetts, USA

[7]Department of Surgery, La Paloma Hospital, Las Palmas de Gran Canaria, Spain

[8]Alberta Public Laboratories, University of Alberta Hospital, Edmonton, Alberta, Canada

[9]Department of Plastic Surgery, Southwestern Medical Center, Dallas, Texas, USA

[10]Department of Medicine, Prisma Health-Midlands, Columbia, South Carolina, USA

[11]Department of Plastic Surgery, UT Southwestern Medical Center, Dallas, Texas, USA

[12]Department of Infectious Diseases, Balgrist University Hospital, Zurich, Switzerland

[13]Faculty of Medicine, University Medical Centre, University of Ljubljana, Ljubljana, Slovenia

[14]Diabetes Centre, The 306th Hospital of PLA, Beijing, China

[15]Section of Infectious Diseases, Department of Internal Medicine, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands

[16]Amsterdam Movement Sciences, Rehabilitation and Development, Amsterdam, The Netherlands

[17]Amsterdam Infection & Immunity, Infectious Diseases, Amsterdam, The Netherlands

Correspondence
Éric Senneville, Infectious Diseases Department, Gustave Dron Hospital, 135 rue du Président Coty, Tourcoing 59200, France.
Email: senneric670@gmail.com

Abstract

Background: Securing an early accurate diagnosis of diabetic foot infections and assessment of their severity are of paramount importance since these infections can cause great morbidity and potential mortality and present formidable challenges in surgical and antimicrobial treatment.

Methods: In June 2022, we searched the literature using PubMed and EMBASE for published studies on the diagnosis of diabetic foot infection (DFI). On the basis of pre-determined criteria, we reviewed prospective controlled, as well as non-controlled, studies in English. We then developed evidence statements based on the included papers.

Abbreviations: CRP, C-reactive protein; DFI, diabetes-related foot infection; DFO, diabetes-related osteomyelitis of the foot; DFU, diabetes-related foot ulcer; ESR, erythrocyte sedimentation rate; HBOT, hyperbaric oxygen therapy; IDSA, Infectious Diseases Society of America; IWGDF, International Working Group on the Diabetic Foot; MRI, magnetic resonance imaging; PACO, population assessment control outcome diabetes-related; PCR, polymerase chain reaction; PCT, procalcitonin; PET, positron emission tomodensitometry; SPECT, single photon emission computed tomography.

Results: We selected a total of 64 papers that met our inclusion criteria. The certainty of the majority of the evidence statements was low because of the weak methodology of nearly all of the studies. The available data suggest that diagnosing diabetic foot infections on the basis of clinical signs and symptoms and classified according to the International Working Group of the Diabetic Foot/Infectious Diseases Society of America scheme correlates with the patient's likelihood of the need for hospitalisation, lower extremity amputation, and risk of death. Elevated levels of selected serum inflammatory markers such as erythrocyte sedimentation rate (ESR), C-reactive protein and procalcitonin are supportive, but not diagnostic, of soft tissue infection. Culturing tissue samples of soft tissues or bone, when care is taken to avoid contamination, provides more accurate microbiological information than culturing superficial (swab) samples. Although non-culture techniques, especially next-generation sequencing, are likely to identify more bacteria from tissue samples including bone than standard cultures, no studies have established a significant impact on the management of patients with DFIs. In patients with suspected diabetic foot osteomyelitis, the combination of a positive probe-to-bone test and elevated ESR supports this diagnosis. Plain X-ray remains the first-line imaging examination when there is suspicion of diabetic foot osteomyelitis (DFO), but advanced imaging methods including magnetic resonance imaging (MRI) and nuclear imaging when MRI is not feasible help in cases when either the diagnosis or the localisation of infection is uncertain. Intra-operative or non-per-wound percutaneous biopsy is the best method to accurately identify bone pathogens in case of a suspicion of a DFO. Bedside percutaneous biopsies are effective and safe and are an option to obtain bone culture data when conventional (i.e. surgical or radiological) procedures are not feasible.

Conclusions: The results of this systematic review of the diagnosis of diabetic foot infections provide some guidance for clinicians, but there is still a need for more prospective controlled studies of high quality.

KEYWORDS
diabetes mellitus, diabetes-related foot infection, diagnosis, foot ulcer, imaging studies, inflammatory markers

1 | INTRODUCTION

Foot infections are frequent complications of diabetes mellitus that are associated with high morbidity, occasional mortality, and heavy resource utilisation, including antibiotic therapy and surgical procedures.[1–3] The yearly incidence of diabetic foot ulcers (DFUs) is about 2% with a lifetime incidence between 19% and 34%,[4] and about half of these ulcers become infected. Approximately 20% of moderate and severe diabetic foot infections (DFIs) result in amputation,[3] making it the most common proximate cause of lower extremity amputation in most countries.

There are three main issues regarding the diagnosis of DFI: (a) how to define the presence or absence of infection; (b) how to classify infection severity; and (c) how to determine whether infection involves the soft tissue, bone (osteomyelitis), or both. Determining the answers to these questions can greatly enhance the management of a DFI. Because an uninfected DFU should not be treated with antibiotic therapy, defining the presence or absence of DFI should help clinicians decide when they should prescribe antimicrobial therapy or consider surgical resection of infected tissues. Furthermore, determining the classification of the infection severity should help clinicians choose the most appropriate additional diagnostic examinations and management strategies for patients with a DFI.[5]

In 2019, we performed a systematic review of these topics, which was published in this journal in 2020.[6] We conducted an update of this systematic review by looking at all available publications on the diagnosis of DFI from June 2018 to June 2022. We sought publications that contained original research information on the

diagnosis or classification of infection of the foot in persons with diabetes mellitus. The aim of this systematic review was to review, evaluate, and report the available data on the diagnosis of DFIs that could help inform the working group in developing recommendations for the intersocietal guideline on the diagnosis and treatment of DFI, produced by the International Working Group on the Diabetic Foot (IWGDF) and the Infectious Disease Society of America (IDSA).[7]

2 | METHODS

We performed the literature search for this systematic review on 30 June 2022, for any publication published between June 2018 and the search date. We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[8] On 10 May 2022, we prospectively registered the systematic review in the PROSPERO 2022 database for systematic reviews (CRD42022324795).[9] We began by defining the clinical questions we wished to address and subsequently the population (patients) of interest (P), assessments (A) performed, and outcomes (O) (PACOs) we would attempt to address. Three members (OO, GA and MA) were designated by the IDSA to be part of the working group in addition to 3 other members (EJPGP, JME and ES) who had already worked as experts in the panel of the 2012 IDSA guidelines doc ument. The IWGDF editorial board and 10 external experts (not members of the guideline working group) from various geographical regions worldwide then reviewed these questions and PACOs for their clinical relevance. With this input, we revised the PACOs to their final form for this systematic review. The clinical questions focusing on the treatment of DFIs are answered in another systematic review published in parallel.[10]

2.1 | PACO definitions

The population of interest for this systematic review was people over the age of 18 years with diabetes mellitus and a foot infection. As defined by the IWGDF/IDSA classifications, DFI is defined clinically as the presence of manifestations of an inflammatory process in any tissue below the malleoli in a person with diabetes mellitus that involves the skin, soft tissue, bone, or other structures, caused by any microorganism.[11,12]

As assessment, all types of diagnostic tests were considered, including clinical findings (e.g., history, physical examination, probe-to-bone test), laboratory tests (e.g., blood leucocyte count, erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], procalcitonin [PCT]), and imaging studies (e.g., plain X-ray, MRI, and radionuclide scans). We looked at the diagnostic accuracy (specificity, sensitivity, positive predictive value, negative predictive value, positive and negative likelihood ratios), the timing of diagnostic tests, and financial costs of the selected tests. We focused on the correlation of clinical findings with other diagnostic tests suggestive of infection, the comparison of different types of culture techniques (e.g., swab vs. tissue), of a positive culture of aseptically collected bone to other diagnostic tests for osteomyelitis (e.g., blood tests for inflammatory markers, various imaging studies), and the comparison of histological evidence of bone infection (presence of inflammatory cells, necrosis) to other diagnostic tests for osteomyelitis (e.g., blood tests for inflammatory markers, various imaging studies).

2.2 | Inclusion and exclusion criteria

We selected studies using the following criteria: enroled patients had a diagnosis of DFI based on the IDSA/IWGDF classification and, in case of osteomyelitis, on the results of a bone specimen examination (i.e., microbiological and/or histological evaluation); and they presented primary research involving clinical findings, microbiological assessment, biomarkers, or imaging techniques. The DFI had to be diagnosed clinically based on the presence of local and systemic signs and symptoms of inflammation. The severity of infection had to be determined using classification schemes such as the IWGDF/IDSA classification system.

The infection working group agreed that acceptable study designs could include meta-analyses, systematic reviews, randomised controlled trials (RCTs), non-RCTs, case-control studies, and prospective cohort studies. We excluded papers that were conducted on nonhuman subjects, review articles, retrospective studies, studies in which the reported data on the evaluation of the diabetic population was not individualised, and studies that included fewer than 15 patients with diabetes. We excluded studies with insufficient data to calculate sensitivity and specificity.

2.3 | Search strategy

The literature search covered studies in English. Please see Appendix A for a detailed description of the search string. With our oversight, a medical librarian performed electronic database searches using the databases of MEDLINE (PubMed), EMBASE, and Scopus, using a combination of MeSH and keyword terms.[9] To test the search terms we intended to employ, we first created a set of 20 key publications that we knew should be in the scope of the systematic review (i.e., diagnosis of DFI) that had to be identified in the literature search. Our search terms identified all 20 publications.

2.4 | Eligibility, data extraction and quality assessment

After conducting the actual search, we divided the papers retrieved and assigned one-sixth of the papers to one of six infection working group teams of two members each. These assessors, working independently, reviewed their assigned publications by title and abstract to determine eligibility on the basis of the presence of the criteria listed above (appropriate population, study design, outcome[s] measurement, and diagnostic intervention[s]) using the COVIDENCE online

software (https://app.covidence.org). After the two members of each team reached a consensus on which papers met the criteria, it was included for the next stage-full paper review and the same pairs of assessors independently screened all included full text articles for potential inclusion. Any disagreements between assessors were discussed until consensus was reached, with a third assessor being involved if needed. The two assessors then independently performed an extraction of the data from each included paper using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool and used this also for the quality assessment of the study (i.e., risk of bias).[13]

After appropriate data were extracted from each included paper, these were summarised in a standardised evidence table that included study design, risk of bias, setting, follow-up, study population and characteristics, the variable or condition assessed, the index test and reference test examined, results of analyses, and an open field for comments. Through both electronic communications and an in-person meeting, each member of the working group reviewed and discussed the content of the evidence tables. Working group member (s) did not participate in the selection or the discussion of a paper if they were (co)-author of that paper.

In the results, the risk of bias assessment and evidence tables are shown of studies found in the updated search. For those details on earlier studies, we refer to our previous systematic review.[6] In the description of the results and in the evidence statements, we used the information from studies identified in both the previous and this updated search.

2.5 | Evidence statements

Based on the strength of the available evidence, we formulated evidence statements with the accompanying assessment of the quality of the evidence, according to the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) methodology. The authors rated the certainty of the evidence for each formulated evidence statement as 'high', 'moderate', 'low' or 'very low' in regard to the strength of confidence in estimates of the effect of a prognostic test on patient-important outcomes.[14] GRADE defines 'high' as 'We are very confident that the true effect lies close to that of the estimate of the effect'; 'moderate' as 'We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different'; 'low' as 'Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect', and 'very low' as 'We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect'. The rating was determined based on the level of evidence, the risk of bias, (in)consistency of results, (in)precision, (in)directness, publication bias, and effect size. Each evidence statement was phrased in accordance with the methods described by GRADE. All authors discussed these evidence statements until consensus was reached. The next sections will start with each PACO, followed by a summary of evidence and with an evidence statement as a conclusion.

3 | RESULTS

The PRISMA flowchart with the study selection process is shown in Figure 1. The risk of bias assessment of each paper can be found in Table 1. The full evidence table can be found in Appendix A, Table A1.

3.1 | PACO 1

In a person with diabetes in the clinical diagnosis of a foot infection, do the International Working Group of the Diabetic Foot/Infectious Diseases Society of America (IWGDF/IDSA) severity criteria better correlate with the need for hospitalisation, lower extremity amputation, and mortality than other current classifications?

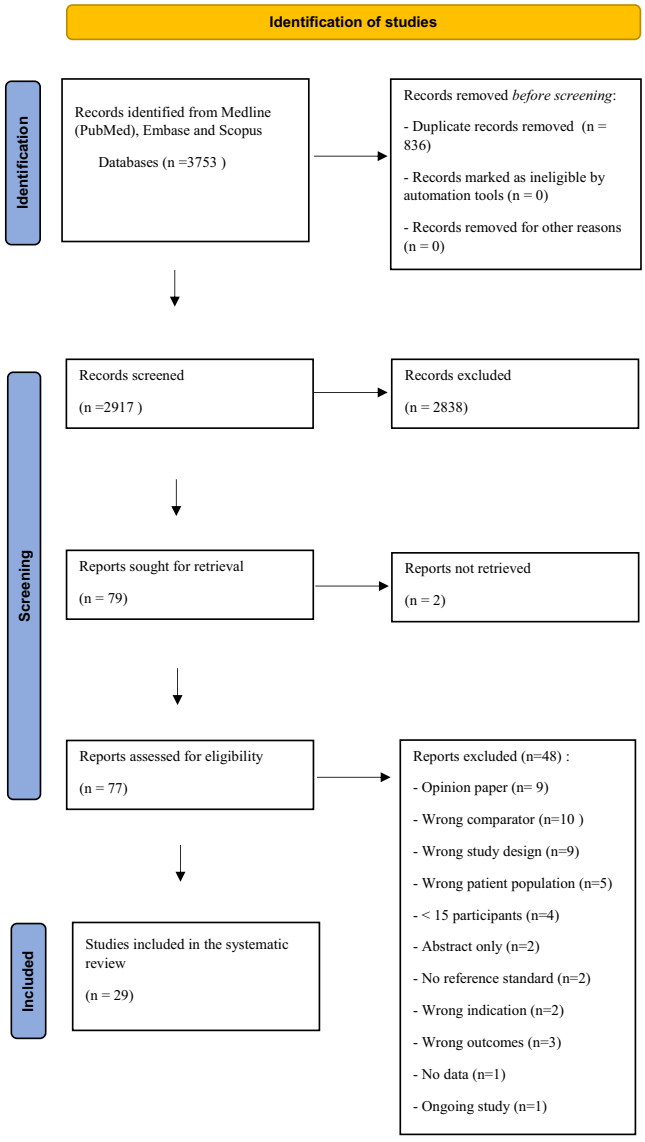
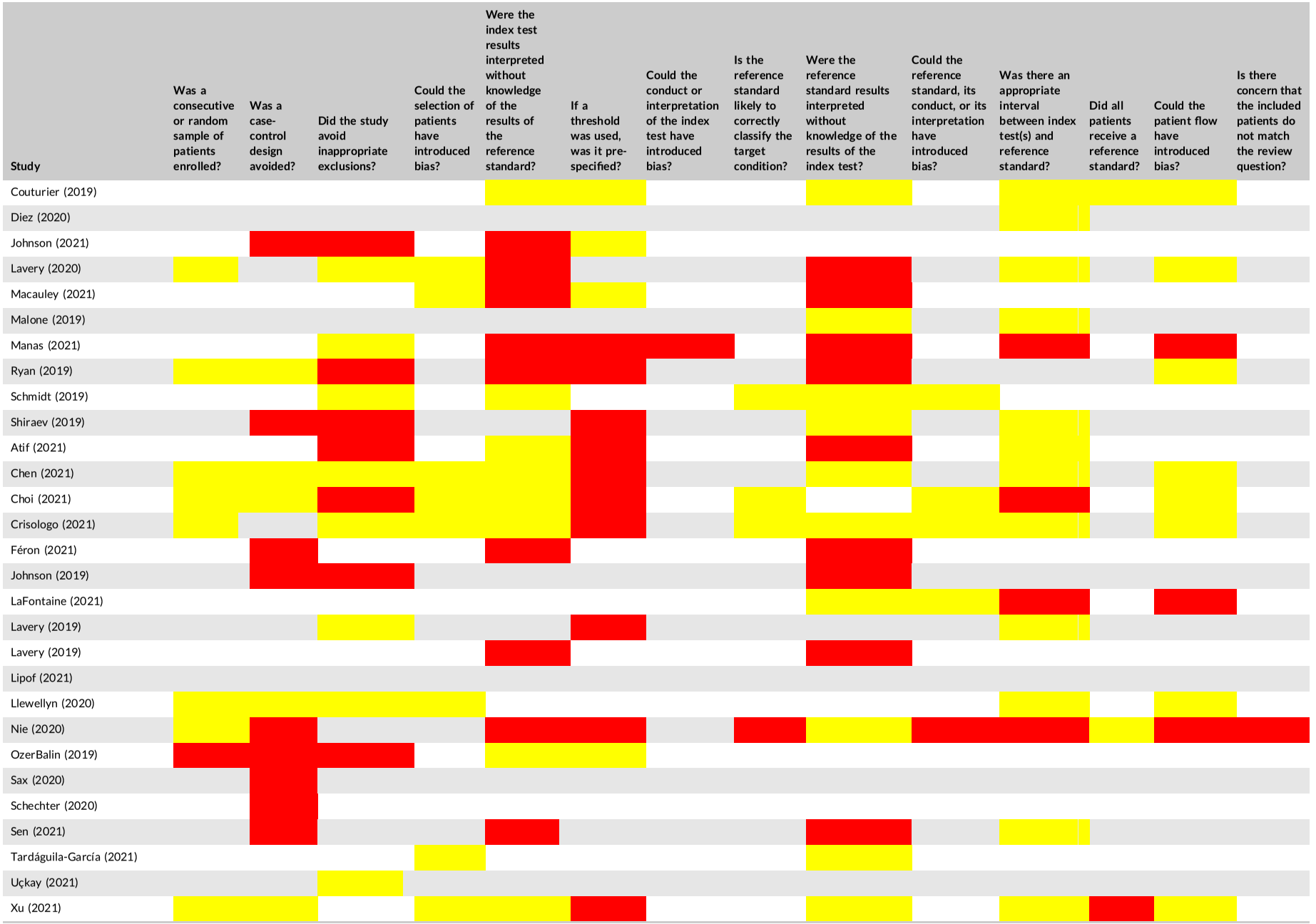


FIGURE 1 Flow diagram for 2023 systematic review of the diagnosis of diabetic foot infection.

TABLE 1 Risk of bias assessment of 29 included studies in the 2023 systematic review.



Note: Green box, yes; red box, no; yellow box, unclear; white box, not applicable.

3.1.1 | Classification of infection and outcomes

Summary of the literature

Given several different clinical presentations of infectious complications of the foot, the IWGDF/IDSA classification schemes for DFI include four classes based on the presence and severity of infection.[11] In the prospective study by Abbas et al. there was a correlation between the IWGDF/IDSA class and the need for hospitalisation, as well as for lower extremity amputation.[15] These results are consistent with a previous prospective cohort study of 1666 diabetic patients who participated in a disease management programme.[16] During a 27-month evaluation period, 248 subjects developed a foot ulcer, 151 (61%) of whom were treated for infection, including 30 diagnosed with osteomyelitis. The authors observed a trend with increasing IDSA infection classification severity towards an increased risk of lower extremity amputation, higher anatomic levels of lower extremity amputation, and lower extremity-related hospitalisations. Wukich et al. validated part of the IWGDF/IDSA classification by comparing clinical outcomes in a prospective cohort study of patients with a moderate versus a severe DFI and found that patients with greater than or equal to two findings of systemic inflammatory response syndrome (i.e., severe infection) had worse clinical outcomes, including more and higher level lower extremity amputations.[17] The 2019 IWGDF/IDSA infection classification system no longer includes osteomyelitis as one of the criteria for making an infection class 3 but rather designates its presence in any class 3 or 4 infection by adding 'O' to the classification noting that this modified classification has not yet been externally validated.[12] A newer version of this classification has been suggested by Lavery et al. in a retrospective study in which moderate infection was subdivided into moderate DFIs with or without bone infection.[18] Although reporting association measures only, it did provide data to support an increasing risk of non-healing and amputation with moderate infection complicated by bone involvement compared with moderate soft tissue infection alone. Another study from the same group suggests that the IWGDF/IDSA classification scheme better reflects outcomes if risk categories are stratified by skin and soft tissue infections or osteomyelitis and moderate and severe infections are not separated.[19] This retrospective study concludes that individuals with underlying osteomyelitis have a worse outcome with more surgeries and amputations, longer hospitalisations and higher rates of recurrent infection and readmissions for infection than patients with moderate and severe soft tissue infections without osteomyelitis. In this study, the authors propose modifying the IDSA classification scheme to include the following categories: no infection, mild soft tissue infection, moderate/severe soft tissue infections, and moderate/severe foot infections with osteomyelitis. The authors also did not find a difference in those who had moderate and severe skin and soft tissue infections, except for readmission related to infection and acute kidney injury. Although the study was well conducted and raises the question of whether the IWGDF/IDSA guidelines should be modified, the authors do acknowledge that the study was retrospective and, therefore, there may have been potential measurement and selection bias. Other authors have attempted to predict the risk of adverse outcomes for persons with diabetes and foot infections.[20–22] An example is the consideration of the Laboratory Risk Indicator for Necrotising Fasciitis (LRINEC). This indicator has been proposed as a potential scoring system to predict both amputation and mortality in persons with diabetic foot infection but has not been found to be associated with a difference in outcome (other than longer hospital stay) for individuals with moderate and severe diabetic foot infection.[21,22]

Finally, the current IWGDF/IDSA scheme is part of the Wound Ischaemia and foot Infection (WIfI) classification system which includes assessment of ischaemia, IWGDF/IDSA infection severity and wound depth which results in an overall risk category.[23] This WIfI classification may help to predict several outcomes such as healing, LEA, hospital admissions and costs in persons with a diabetes-related foot ulcer, as concluded in the recent IWGDF systematic review of classification systems that is published in parallel in this journal.[24]

Evidence statement In people with diabetes and an infected foot, the IDSA/IWGDF classification system score may help predict clinical outcome (healing, LEA, hospital admission[s] and length of stay) but not mortality.

Certainty of the evidence Low, based on external validation studies.

References Lavery 2007[16] and 2020,[18] Wukich 2013,[17] Ryan 2019,[19] Sen 2021,[21] Johnson 2021.[22]

3.2 | PACO 2

In a person with diabetes and infection of the foot, which test best predicts the presence of the causative pathogen(s) as identified by tissue biopsy results, and results in more tailored use of antibiotics, with less extended-spectrum antibiotic consumption and associated cost savings?

3.2.1 | Tissue samples versus swabs

Summary of the literature

Infection of a DFU differs from contamination by microorganisms in that it represents an invasion of the host tissues. The concordance between the culture results of tissue samples and superficial swabs has been compared by different authors. In a prospective study, Huang et al. compared swabs versus tissue (punch) biopsies in patients with an infected DFU classified as grade 2 ($n = 10$), grade 3 ($n = 29$), or grade 3 ($n = 17$), according to the IWGDF classification.[25] The concordance between swab and biopsy results was high in grade 2 infections (90%) but decreased in grades 3 (41.4%) and 4 (41.2%) infections. The authors also observed that the concordance for gram-negative bacilli was lower than gram-positive cocci and concluded that swabs should not be used for cultures of grade 3 or 4 DFIs.46 Mutluoglu et al. compared the

culture results of 89 pairs of swabs versus deep tissue specimens in 54 patients with DFUs, 47 (87%) of which were infected.[26] In comparison with deep tissues, swabs identified at least one additional microorganism and missed at least one microorganism in, respectively, 11% and 9% of the cases. The authors established that the overall accuracy of swabs in these settings was 73%.[26] In another study, the results of swab versus tissue sample culture were compared in patients with neuropathic ($n = 28$) and neuro-ischaemic ($n = 22$) DFUs.[27] The number of isolates was higher on swabs versus deep tissues in neuropathic DFUs (1.71 vs. 1.21) and in neuro-ischaemic DFUs (1.32 vs. 1.05) but was only significant ($p = 0.033$) in neuropathic DFUs.[49] The results of these studies, on the basis of small population size, were confirmed by a recent large prospective multicentric (CODIFI) study in which 400 patients with infected DFUs were included. This study showed that cultures of swab specimens were both less sensitive and specific compared with tissue samples (obtained with a sterile dermal curette or scalpel).[28]

Evidence statement Tissue sample culture is likely more appropriate than superficial swabs for the identification of the pathogen(s) involved in infected DFUs.

Certainty of the evidence Moderate, based on prospective and retrospective studies of variable quality.

References Huang 2016,[25] Mutluoglu 2012,[26] Demetriou 2013,[27] Nelson 2017.[28]

3.2.2 | Non-culture microbiological diagnosis of diabetic foot infections

Summary of the literature

We found 5 studies that compared the diagnostic accuracy of non-culture techniques versus culture methods to identify pathogen(s) in diabetic foot infections including osteomyelitis.[29–34] All 5 were single-centre prospective studies that reported data about the use of different non-culture methods including metagenomic new generation sequencing (mNGS),[29–31] coupled loop-mediated isothermal amplification, and clustered regularly interspaced short palindromic repeats (LAMP-CRISPR)[30] for the quick and specific detection of *S. aureus* including methicillin-resistant strains (MRSA), quantitative Reverse Transcription Polymerase Chain reaction (qRT-PCR)[30] DNA sequencing,[32,33] 16S ribosomal ribonucleic gene sequencing[34] and immunoassay.[31] Data about the coexistence of peripheral artery disease in the included patients and whether patients received antibiotics prior to tissue sampling (except in Choi's study[29]) were not provided in these studies. Three studies[29–31] assessed mNGS in skin structure DFIs. Two others assessed mNGS in diabetic foot osteomyelitis (DFO).[33,34] All tissue samples, including bone, were obtained intraoperatively in the 5 studies. Staphylococci and streptococci were the most prevalent pathogens identified by standard culture in the 5 studies despite the various geographical origins of the participants.

In the prospective cohort study from Lipof et al. that include 30 patients with moderate to severe DFIs, mNGS and culture were highly correlated with *S. aureus* ($r = 0.86$) and *S. agalactiae* ($r = 1.0$).[31] In the same study, the authors found a low correlation between the results of an immunoassay for newly synthesised anti-bodies (NSA) to *S. aureus* and *S. agalactiae*, and standard culture ($r = 0.18$ for *S. aureus*) and $r = 0.67$ for *S. agalactiae*. In another prospective cohort study, mNGS revealed 5.1 (1–11) pathogens whereas standard culture revealed 2.6 (1–6) per sample with a polymicrobial infection identified in 19 (65.6%) versus 25 (86.2%) cases in standard culture versus mNGS, respectively.[29] mNGS detected all organisms identified on the culture in 14 cases (46.7%), there was partial overlap of organisms identified by mNGS and culture in 8 cases (26.7%), and a complete discordance between the two techniques was recorded in 8 other cases (26.7%). Similar results were reported by Noor et al.[32]

Molecular techniques were compared to standard culture methods for the microbiological documentation of DFO. In an observational prospective study, van Asten et al. found in a series of 34 patients with suspected bone infection on the basis of the IWGDF/IDSA classification a higher prevalence with a 16S ribosomal ribonucleic gene sequencing compared with the conventional technique for anaerobic pathogens (86.9% vs. 23.1%, $p = 0.001$), gram-positive bacilli, especially *Corynebacterium* spp. (78.3% vs. 3.8%, $p < 0.001$), and polymicrobial infections (91.3% vs. 64.0%, $p = 0.125$).[34] Of note is that 3 bone samples positive on conventional culture were negative by the molecular technique.[34] Malone et al. found that all 14 infected bones positive by mNGS had positive corresponding proximal bone margins, while 8 out of 14 (57%) proximal bone margins were negative by standard culture and positive by mNGS.[33] The significance of the patterns mNGS ± standard culture- remains, however, unclear. In a cross-sectional study involving 18 tissue samples of infected diabetic foot ulcers, the LAMP-CRISPR assay was shown to have an agreement of 100% with qRT-PCR and 94.4% with standard culture results for the detection of *S. aureus* and MRSA.[30] The assay sample-to-result time of LAMP-CRISPR was lower compared to Real-time PCR, mNGS, and culture (1 h, 1–2 h, 2 days, and 3–7 days, respectively).

Overall, in comparison to standard cultures, molecular techniques provide a wider range of pathogens per sample. However, non-culture techniques such as mNGS and LAMP-CRISPR assay for the identification of pathogens in both skin structure DFIs and DFOs do not inform on the metabolic status of the bacteria (i.e., planktonic vs. stationary phase) and therefore are unable to help guide the choice of antibiotic agents to treat positive bone margins (like standard cultures). No studies have reported any significant impact of the use of non-culture techniques on post-surgical antibiotic therapy.

Evidence statement Non-culture (molecular) techniques likely identify more bacteria from soft-tissues and bone samples than standard cultures, although the clinical relevance of this difference is unclear.

Certainty of the evidence Moderate, on the basis of 5 single-centre prospective cohort studies of variable quality.

References Choi 2021,[29] Chen 2021,[30] Lipof 2021,[31] Noor 2018,[32] Malone 2019,[33] van Asten 2016.[34]

3.2.3 | Bone culture versus soft tissue culture or swabs

Summary of the literature

One retrospective study reported that results of a swab culture compared with a culture of a percutaneous bone biopsy specimen had an overall concordance between isolates of only 22.5%, suggesting that superficial swab cultures do not reliably identify bone bacteria.[35] The same group reported in a prospective study that a deep soft tissue needle puncture had identical microbiological results to those of a contemporaneously collected transcutaneous bone biopsy in only 10 (32.3%) of 31 patients.[36] These results were confirmed in a prospective study that compared the data from 134 bone biopsies and 140 superficial swabs.[37] While the mean number of isolates per sample was similar, bone pathogens were identified in the corresponding swab culture in only 55 cases (38.2%) with the highest concordance for *S. aureus*.[37] A systematic review and meta-analysis with heterogeneity and some inconsistency reported concordance rate values between percutaneous bone biopsy and superficial swabs that ranged between 2.8% and 17.4%.[38] The highest rates were observed for *S. aureus* ranging from 42.8% to 82.3%. More recently, the low concordance between deep swab specimens and surgical bone biopsy cultures was confirmed with an overall κ value between the two methods of 0.302 with the best values obtained for *S. aureus* ($\kappa = 0.571$) and MRSA ($\kappa = 0.644$).[39] In this study, however, patients had received antibiotics for >4 weeks prior to biopsy in 40% of the cases which may have had an impact on the results, as discussed below.

Evidence statement Bone biopsy and non-bone sample cultures show low correlation rates.

Certainty of the evidence Moderate, based on one meta-analysis with and prospective and retrospective cohort studies, downgraded because of heterogeneity and inconsistency.

References Senneville 2006[35] and 2009,[36] Elamurugan2011,[37] Schechter 2020,[38] Manas 2021.[39]

3.2.4 | Bone histology versus culture for the diagnosis of DFO

Summary of the literature

Bone biopsy for either culture or histology is still a matter of debate in the management of patients with DFO. A matched case-control concluded that a positive bone culture combined with a negative histological result was just as likely as a negative microbiological and positive histological result, suggesting that the two methods were equally useful diagnostically.[40] However, there is still debate about the reliability of histopathology for the diagnosis of DFO.[41] The difference between favourable and unfavourable opinions seems to have been related to the use of standardised histopathologic definitions, which is likely to increase the reliability of the results.[42] Two recent studies addressed this question.[43,44] In a series of 52 bone samples taken intraoperatively reported by Tardaguila et al., 69% had positive bone culture results, and 90.4% had positive histology results ($p = 0.013$).[43] Features compatible with acute and chronic osteomyelitis were recorded in 25.5% and 74.5% of the samples with positive histology. The sensitivity, specificity, and positive/negative predictive value of the microbiologic bone culture were 0.70, 0.40, 0.92/0.13, respectively. Histologic examinations could be used to accurately identify DFO, especially in chronic DFO without clinical signs of infection. It is concluded that patients with a suspicion of DFO could be underdiagnosed because of false-negative results provided by bone culture. In another series of 36 bone samples taken by either a percutaneous bone biopsy ($n = 10$) or intraoperative surgical cultures ($n = 25$), Lavery et al. found that standard cultures might identify more cases of osteomyelitis than histology (respectively, 68.6% vs. 45.7%, $p = 0.06$, odds ratio [OR] 2.59, 95% CI 0.98–6.87) but the difference was not significant.[44] Histology was positive in all the cases with positive microbiology using either traditional culture or genetic sequencing. Biomolecular techniques (16S ribosomal RNA testing) identified significantly more cases of osteomyelitis than histology (82.9% vs. 45.7%, $p = 0.002$, OR 5.74, 95% CI 1.91–17.28). Of note, however, is that only the culture provides information about the causative pathogen and its antibiotic susceptibilities.

Evidence statement Culture and histology of bone samples may result in similar diagnostic accuracy with standardised reporting of the results for the diagnosis of DFO.

Certainty of the evidence Low, based on prospective single site small studies and retrospective studies with high risk of bias.

References Weiner 2011,[30] Meyr 2018,[41] Cecilia-Matilia 2013,[42] Tardáguila-García 2021,[43] Lavery 2019.[44]

3.2.5 | Percutaneous versus surgical bone biopsy

Summary of the literature

In Feron et al.'s prospective non-randomised study, the diagnostic accuracy of bedside percutaneous bone biopsies was compared to conventional surgical or radiological procedures.[45] While the microbiological data were comparable in both procedures including the proportion of polymicrobial infections, a lower proportion of culture-positive specimens was recorded for bedside than conventional procedures (i.e., 50.6% vs. 77.3%; $p < 0.01$).[45] No patient had received antibiotics within 2 weeks prior to the biopsy. Of note, in this study, histology and bone culture were combined in all patients

to define the presence of osteomyelitis. This study also shows the safety of percutaneous bone biopsy even performed at the bedside after marking the area to be biopsied with no complications such as bleeding, biopsy-related ulcer, infection, or necrosis. Three patients (7%) of the patients experienced pain at the biopsy site that resolved within 24h.

The benefit of performing the percutaneous bone biopsy via healthy skin versus through the ulcer was established by Couturier et al. who reported in a retrospective single centre study an overall concordance between the two procedures of 58.4% and the highest concordance observed for *S. aureus* (79.2%).[46] The impact of bone culture results on the selection of antibiotic therapy was analysed in this study and showed that using the per-wound biopsy results, too broad, incompletely effective, and totally ineffective antimicrobial therapy would have been prescribed ,respectively, 26.3%, 13%, and 18.5% of the cases. In another study, the impact of culturing a bone specimen taken at the margin of an amputation of the foot on the guided antimicrobial therapy was assessed by Shiraev et al.[47] All patients with negative bone margin received no further antibiotic treatment versus 5% in patients with positive bone margin, suggesting a benefit in the antibiotic use.

Evidence statement (Bedside) Percutaneous bone biopsy avoiding the ulcer can safely identify bone pathogens and help guide the antibiotic therapy.

Certainty of the evidence Moderate, based on one prospective study, and two retrospective studies.

References Feron 2021,[45] Couturier 2019,[46] Shiraev 2019.[47]

3.2.6 | Previous antibiotic therapy and bone biopsy

Summary of the literature

The negative effect of previous antibiotic therapy on the positivity of bone culture was suggested in the retrospective study at high risk of bias of Manas et al. without any significant association to the antibiotic therapy duration ($r = -0.358$, $p = 0.005$).[39] In a retrospective analysis of a series of 40 consecutive patients who underwent a surgical debridement for a moderate to severe DFI without ischaemia, Macauley et al. compared microbiological reports of tissue samples obtained from the podiatrist at initial patient contact with those of resection specimen or marginal samples after surgical debridement.[48] The duration of antibiotics received from initial presentation to debridement was 16.2 ± 5.5 days when pathogenic bacteria were identified in per-operative samples versus 53 ± 21.0 days in cases where per-operative sample cultures were negative. According to the authors, these results suggest that a longer duration of targeted preoperative antimicrobial therapy may reduce residual marginal infection by pathogenic bacteria.

Evidence statement Antibiotic therapy prior to biopsy may increase the risk of negative bone culture.

Certainty of the evidence Low, based on two retrospective cohort studies.

References Manas 2021,[39] Macauley 2021.[48]

3.2.7 | Persistent osteomyelitis following foot amputation

Summary of the literature

The negative impact on the patient outcome of having a positive histopathologic margin during various types of amputation for DFO has been reported in previous studies.[49,50] Atway et al. reported a retrospective observational study of 27 patients who underwent a forefoot amputation for DFO.[49] Eleven (40.7%) had a residual osteomyelitis according to the results of bone margins culture results. Negative outcomes including wound dehiscence, re-ulceration, re-amputation, or death were recorded in 25% (4/16) of patients with a negative bone margin and 81.8% (9/11) of patients with a positive bone margin ($p = 0.0063$). In a similar retrospective study, Kowalsky et al. included 111 patients of whom 39 (35.14%) had a positive bone margin pathological examination.[50] The median total duration of antibiotic treatment was longer in patients with positive bone margins (19 [range 10–134] days vs. 14 [range 2–63]; $p = 0.01$). While infection relapse at the proximal amputation site did not differ between patients with or without positive bone margins, the need for more proximal amputation was recorded in 17 of 39 (43.59%) patients with positive margins and 11 of 72 (15.28%) patients with negative margins ($p = 0.001$). These initial results were questioned in a prospective observational study that included 72 patients who underwent a forefoot amputation for a DFO.[51] In this study, proximal bone margins with no histopathological signs of osteomyelitis were recorded in 63 out of 72 cases (87.5%) with a strong inter-observer reliability of the results. Residual osteomyelitis resulted in readmission 2.6 times more often and more postoperative complications.[51] Another retrospective study analysed the outcome of 66 patients who underwent a below-ankle amputation for a DFO according to the results of the histology of the bone margin.[52] At the 12-month follow-up, 39 (59%) remission of osteomyelitis was recorded with no difference among cases with a negative initial histopathologic margin 29/48 (60.4%) compared with 10/18 (55.6%) cases with a positive histopathologic margin ($p = 0.72$). Relapsing DFO is a frequent event, and in the literature, most studies do not use a bone biopsy for diagnosing this complication. In a cross-sectional study, Crisologo et al. compared the results of biopsy-proven DFO and common surrogate markers for treatment success (i.e. failure of wound healing, re-ulceration, re-admission for DFI at the same site, and amputation at the same site after discharge).[53] The authors did not find any differences for these surrogate markers between patients with DFO and

soft-tissue DFIs suggesting that DFO remission is not directly related to these surrogate measurements. Overall, studies that assessed the relation between persisting osteomyelitis at the margin site after bone resection, including either bone resection (conservative surgery) or amputation and the outcomes (re-infection, re-admission and additional surgery) are retrospective and have a risk of bias and inconsistency in their results.

Evidence statement Persistence of osteomyelitis following amputation for a DFO may result in poor outcome.

Certainty of the evidence Low, based on 4 studies (3 retrospective and one prospective observational).

References Atway 2012,[49] Kowalski 2011,[50] Schmidt 2019,[51] Johnson 2019,[52] Crisologo 2021.[53]

3.2.8 | Clinical assessment of the presence of *Pseudomonas aeruginosa* in infected diabetic foot ulcers

Summary of the literature

One single-centre study compared clinical signs (e.g., macerated skin and green colour) made by 13 experienced physicians with tissue culture results for the diagnosis of *Pseudomonas* spp. involvement in moderate to severe diabetic foot infections.[54] In 221 instances of diabetic foot infections in a total of 88 patients, sensitivity, specificity, and positive and negative predictive values of the clinical assessment, the visual (blue-green colour) and olfactory (grape-fruit-like smell) performance of experienced health care workers in predicting *Pseudomonas* spp. involvement in DFI were established at 0.32, 0.84, 0.18, and 0.92, using culture as a referent test. The use of these clinical signs seems more helpful for ruling out than ruling in the involvement of *Pseudomonas* spp. in soft-tissue DFIs. The main potential benefit of using these clinical signs in daily care is the reduction of unnecessary prescription of broad-spectrum antibiotics with activity against *Pseudomonas* spp. when all clinical findings are negative in a population with a low pre-test probability of a *Pseudomonas* spp. infection.

Evidence statement In a patient with moderate to severe diabetic foot infection, clinical assessment to determine the presence of *Pseudomonas* spp. may have a high negative predictive value but a low positive predictive value.

Certainty of the evidence Low, based on one prospective cohort study.

Reference Uçkay 2021.[54]

3.3 | PACO 3

In a person with diabetes and suspected bone and/or joint infection of the foot, do the available tests for (i) diagnosing osteomyelitis and/or septic arthritis and (ii) monitoring of osteomyelitis (including residual/post-operative osteomyelitis) correlate with bone biopsy results?

3.3.1 | Serum markers for soft-tissue DFIs

Summary of the literature

Most available studies assessed the value of blood tests, especially white blood cell counts (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and procalcitonin (PCT), by comparing them to results of IWGDF/IDSA criteria for infection.[55–66]

In a US multicentre retrospective study involving 338 consecutive admissions for DFI, the mean white blood cell count (WBC) on admission was $11.9 \pm 5.4 \times 103$ cells/mm^3. WBC was within normal limits in 56% (189 out of 338) of the cases.[55] A similar study from Sweden involving 223 consecutive patients hospitalised for DFI found that about 50% of patients lacked a sedimentation rate >70 mm/hour, and WBC >10 G/L.[56] In a prospective monocentre study that included 93 patients with a DFU and no antimicrobial treatment in the preceding 6 months, patients were classified according to the IWGDF/IDSA criteria for DFI.[57] A two-fold increase in PCT and a fourfold increase in CRP level were recorded in grade 2 patients in comparison with grade 1 patients. CRP as a single marker had the highest sensitivity and specificity without any additional diagnostic accuracy of using high-sensitivity CRP. The combination of CRP and PCT provided the most relevant formula ([0.162 × CRP mg/L] + [17.437 × PCT mg/L]) with a cut-off value of 4 for distinguishing between grade 1 and grade 2 ulcers with a sensitivity/specificity/positive predictive value/negative predictive value and positive likelihood ratio of 0.909/0.826/0.833/0.905 and 4.2, respectively.[57]

In most studies, ESR, CRP, and PCT values have been higher in patients with an infected diabetic foot ulcer (IDFU) compared with a non-IDFU. ESR values can be affected by various co-morbidities (e.g., anaemia, azotaemia) and may not be elevated in acute infections due to the relatively slow response of this inflammatory biomarker. Compared to ESR, CRP levels tend to rise more quickly with infection and fall more quickly with the resolution of infection.[58] In a recent meta-analysis with high heterogeneity due to the inclusion of studies with a wide range of the sample size and the use of different cut-off values,[59–65] CRP and PCT were shown as the best markers for the diagnosis of grade 2 and grade 3 infected DFUs, respectively.[66]

Evidence statement Inflammatory serum biomarker (WBC, ESR CRP, and PCT) values may not correlate with the presence or absence of a DFI.

Certainty of the evidence Low, based on studies including one meta-analysis with important inconsistency.

References Armstrong 1996,[55] Eneroth 1997,[56], Jeandrot 2008,[57] Uzun 2007,[59], Al-Shammaree 2017,[60] Korkmaz 2018,[61] Umapathy 2018,[62] Zakariah 2020,[63] Todorova 2021,[64] Park 2017,[65] Sharma 2022.[66]

3.3.2 | Serum markers for DFO

Summary of the literature

In a meta-analysis that included 8 studies of which 6 examined ESR and other biomarkers for the diagnosis of DFO, ESR was found to be the best biomarker to identify patients with DFO with bivariate pooled sensitivity and specificity of 0.81 (95% CI 0.71–0.88) and 0.90 (95% CI 0.75–0.96), respectively.[67] The same authors conducted a small-size prospective cohort study comparing biomarkers in patients hospitalised for a moderate and severe DFI.[68] Twenty-four patients were diagnosed with DFO and 11 without DFO, according to bone examination in all cases. PCT was found to be the best blood test to distinguish DFO from non-DFO ($p = 0{\cdot}049$). There were no significant differences between the two groups in the levels of the other markers. CRP, ESR, PCT and IL-6 levels significantly declined in the group with osteomyelitis after starting therapy, while MCP-1 increased ($p = 0{\cdot}002$).[68]

In a small-size case-control study of 27 patients, wherein16 had DFO based on bone examination and 11 controls had soft-tissue infections, were assessed on the serum procollagen type 1 N propeptide (P1NP) values, WBC, platelets, ESR and CRP.[69] Mean serum P1NP levels were significantly higher in the DFO group (10.5 ± 5.2 mg/L vs. 3.1 ± 2.8 mg/L; $p = 0.001$). P1NP showed a sensitivity/specificity of 86.7%/80% compared to 70.6%/80%, 56.2%/45.4%, and 50%/37% for CRP, WBC and platelets, respectively. Patients were not graded for the infection severity and control patients had no bone examination.[69] The combined ESR/CRP values were assessed for distinguishing DFO from non-DFO.[70] The likelihood of DFO was high in case of ESR >60 mm/hr and CRP >7.9 mg/dL (LR + 2.38, LR − 0.65), while with an ESR >60 mm/hr with a CRP ≤7.9 mg/L, the authors suggested that another test may be needed to establish a DFO diagnosis. If the ESR was between 30 and 60 mm/hr, then DFO was an equivocal diagnosis and other testing modalities were recommended. The risk of DFO was lower with an ESR <30 mm/hr, justifying other testing modalities if clinical suspicion for DFO remains high. Nie et al., compared in a case-control single-centre China-based study with high risk of bias the values of the logarithmic ratio of triglyceride to high-density lipoprotein–cholesterol concentration (AIP) in patients with soft-tissue DFO and DFOs.[71] Patients with higher AIP values tended to be at higher risk of having a DFO 1.81 (95% confidence interval [CI] 0.71–4.61) and 4.70 (1.88–11.75). Of note, not only patients with neuropathic ulcers but also patients taking statins in the last 3 months were excluded from the study population and given the relatively small increases in the odd's ratio, the certainty of the evidence is low.

Evidence statement Elevated inflammatory serum biomarker (ESR, CRP, and PCT) values may correlate with the presence or absence of a DFO; combined ESR/CRP tests may increase the diagnostic accuracy of DFO.

Certainty of the evidence Low, based on one meta-analysis and cohort studies, all with high risk of bias and inconsistency.

References van Asten 2016[67] and 2017,[68] Hayes 2018,[69] Lavery 2019,[70] Nie 2020.[71]

3.3.3 | Probe-to-bone test for the diagnosis of DFO

Summary of the literature

The probe-to-bone test (PTB) test consists of inserting a sterile metal blunt probe into an ulcer. PBT is considered positive if a hard, gritty surface is felt inside. In a systematic review that included 1017 patients from seven studies that used culture and/or histopathology of bone specimens to confirm osteomyelitis, the PTB test had a pooled sensitivity of 87% (95% CI, 75%–93%), specificity of 83% (CI, 65%–93%), PPV of 98%, and NPV of 70%.[72] Two studies we identified were not included in this systematic review. One was a case-control study of 54 patients with diabetes which found that the PTB test had a sensitivity of 85% but a specificity of only 47% for diagnosing osteomyelitis (confirmed by histology).[73] The other, a prospective cohort study that combined the PTB test with undefined clinical signs of osteomyelitis, concluded that this combination had a sensitivity of 64.8%, specificity of 77.8%, PPV of 91.9%, and NPV of 36.2%.[74] In the aforementioned study with a low risk of bias in Chinese patients, the diagnostic accuracy was improved by combining ESR and PTB in parallel, if either test was positive, with sensitivity, specificity, +PV, −PV, of respectively, 96%, 65.7%, 0.78, 0.93, and a +LR and −LR of respectively, 2.8, and 0.06, respectively.[75] When the result of the PTB test was combined with that of plain X-rays, the sensitivity increased to 88.6%, specificity fell to 66.7%, PPV remained similar at 91.2%, and NPV increased to 60% for the combination. In a large, single-centre study that included 111 patients with DFO and 86 without DFO, Xu et al. defined optimal cut-offs for the combination of ESR and PTB to distinguish DFO from non-DFO.[76] The best cut point of ESR was >43 mm/hr with sensitivity, specificity, positive predictive value (+PV), and negative predictive value (−PV) of 82.9%, 70.5%, 0.78, and 0.77, respectively In a study at low risk of bias, the authors pointed out that ESR>70 mm/hr established in previous studies was a rare event in their population of Chinese patients and suggested that this cut-off is too high for this population.

Evidence statement Probe-to-bone test assessment can help to confirm or reject the diagnosis of DFO; by combining this test with either ESR or plain X-ray, its diagnostic accuracy improves.

Certainty of the evidence Low, based on one meta-analysis and cohort studies, all with high risk of bias.

References Lam 2016,[72] Fleischer 2009,[73] Morales-Lozano 2010,[74] Xu 2020.[76]

3.3.4 | Imaging in the diagnosis of DFO

Summary of the literature

The suspicion of osteomyelitis complicating an infected DFU is based on assessing clinical findings (e.g., deep ulcer over a bony prominence, visible exposed bone, positive PTB test, and 'sausage toe' appearance), laboratory tests (e.g., serum biomarkers), and imaging studies (e.g., cortical disruption, sequestrum, involucrum, marrow oedema, or tracer uptake) while positive bone culture and/or histology is considered as the definite diagnosis of DFO.[11,12] Among the numerous imaging modalities available for the diagnosis of DFIs, plain X-ray is the most readily and widely available, least expensive, and most easily interpreted (at least preliminarily) by non-radiologists. In addition to plain-X ray, magnetic resonance imaging (MRI) is the most widely available advanced imaging technique to confirm the suspicion of DFO with moderate costs and wide availability (in high-income countries). Besides being used as a diagnostic tool, MRI gives a good overview of the anatomy of both soft tissue and bone, which can be of aid when considering surgical interventions. In addition to MRI, nuclear imaging techniques have been used to establish the diagnosis of DFO, especially when plain-X ray, clinical findings and inflammatory biomarkers are not conclusive. We identified one high-quality meta-analysis that compared MRI, WBC scintigraphy, and fluorodeoxyglucose (FDG)-positron emission tomography (PET)/compute tomography (CT) for the detection of diabetic foot osteomyelitis.[76] The authors included only studies that used the results of histopathological review or culture of a specimen of affected bone (collected by surgical or percutaneous biopsy) as a criterion standard in pooled estimation of diagnostic performance metrics. Among the studies, 13 with a total of 421 patients investigated the diagnostic value of MRI, 9 studies with 206 patients investigated 111In-oxine-WBC scintigraphy, 10 studies with 206 patients studied.

99mTc-HMPAO WBC scintigraphy, and 6 studies with 254 patients investigated FDG-PET/CT. While the pooled sensitivity of all the different imaging modalities was comparable (89%–93%), the specificity of MRI (75%, 63%–84%) and 111In-oxine WBC scintigraphy (75%, 66%–82%) were considerably lower than that of 99mTc-HMPAO WBC scintigraphy (92%, 78%–98%) and 18F-FDG-PET/CT (92%, 85%–96%). Five studies[77–81] identified in our search were included in this meta-analysis. We found one small, prospective preliminary study not included in the meta-analysis which reported similar results for 99mTc-HMPAO WBC imaging in diagnosing diabetic foot osteomyelitis (sensitivity 90% and specificity 86%).[82] Another meta-analysis with low risk of bias that examined 18F-FDG PET and 18F-FDG-PET/CT for assessing osteomyelitis in the diabetic foot found a relatively low sensitivity (74%; 95% CI, 60%–85%) related to the fact that one of the four included studies reported a sensitivity of 29% but a high specificity (91%; 95% CI, 85%–96%).[83] The presence of osteomyelitis, documented in 6 studies, ranged from 10% to 54% (average approximately 30%) of the enrolled cases. One prospective study not included in this analysis also found that for diagnosing osteomyelitis in patients with Charcot neuro-arthropathy, 18F-FDG labelled leucocyte PET/CT had the same sensitivity (83.3%) as contrast to enhanced MRI, but its specificity was 100%, compared with 63.6%.[84] One small prospective study of low quality found that diffusion-weighted MRI for the diagnosis of diabetic foot osteomyelitis had low performance characteristics (sensitivity 64.6% and accuracy 63.7%).[85] However, when using a calculated apparent diffusion coefficient, two reviewers were able to differentiate diabetic osteo-arthropathy from osteomyelitis with an accuracy of 94% and 93%, with excellent inter-observer agreement. A bedside strategy to avoid unnecessary antibiotic use was assessed in one prospective cohort study using coupled 67 Ga single-photon emission computerised tomography (SPECT) imaging and bedside percutaneous bone puncture in patients with suspected bone infection of the foot.[86] Among 55 patients, those with a positive scan ($N = 40$) underwent bedside percutaneous bone puncture. The sensitivity and specificity of this combined method were 88.0% and 93.6%, respectively, and the PPV and NPV were 91.7% and 90.7%. Another prospective study found that the combination of 99mTc-HMPAO leucocyte scintigraphy and 99mTc-MDP bone scintigraphy in 75 patients with suspected bone infection had a sensitivity of 92.6% and a specificity of 97.6%.[87]

A systematic review and meta-analysis with low risk of bias of total 36 imaging studies (8 at high risk of bias) evaluated the diagnostic accuracy of imaging tests to diagnose osteomyelitis in people with diabetic foot ulcers.[88] Diagnostic accuracy was estimated for X-rays, scintigraphy, MRI, single photon emission computed tomography (SPECT) and positron emission tomography (PET) using bivariate meta-analyses. While X-rays and scintigraphy showed generally inferior diagnostic accuracy, MRI had high diagnostic accuracy (22 studies: 96.4% sensitivity; 83.8% specificity) as well as PET-CT (6 studies: 84.3% sensitivity; 92.8% specificity) and possibly also SPECT-CT, but the number of studies was small (3 studies: 95.6% sensitivity, 55.1% specificity). Based on the results of this comprehensive review, both MRI and PET-CT have very good diagnostic accuracy, while the evidence of SPECT-CT remains limited. Two additional single-center studies, identified in our searches, retrospectively aimed to improve the accuracy of MRI findings with two different methods.[89,90] In the study from La Fontaine et al., a blinded expert musculoskeletal radiologist reviewed the MRI findings of 58 patients with biopsy-proven DFO.[89] After the second reading of the specificity, the negative predictive value, as well as the overall accuracy (84%), improved considerably compared to the first read. In a study at low risk of bias, Sax et al. evaluated the risk factors to predict the development of osteomyelitis in 60 pedal ulcers with an initial MRI with bone marrow oedema without a corresponding T1 signal.[90] Increasing the bone marrow region of interest signal/joint fluid ratios on T2/STIR images were the strongest risk factors for developing osteomyelitis as identified on a second MRI (therefore, helping an early diagnosis of DFO), while ulcer size and depth were weaker predictors.

Imaging techniques with contrast enhancement and leucocyte labelling with semi-quantitative assessment have been assessed in a small prospective study with low risk of bias but with imprecision. In this study, the diagnostic accuracy of diffusion-weighted and dynamic

contrast-enhanced MRI was compared with ^{18}F-FDG PET/CT to differentiate osteomyelitis from Charcot neuro-osteoarthropathy (CNO) in the foot of persons with diabetes.[91] Each bone lesion was evaluated individually by comparing the results of the different techniques. A total of 18 bone lesions in 18 patients met the criteria for DFO and 50 bone lesions in 14 patients for CNO. The visual assessment of ^{18}F-FDG PET/CT showed significantly higher accuracy (AUC 0.924) than MRI parameters, while semi-quantitative parameters did not provide a significant improvement over visual analysis noting that only 31 patients were included in this single-center study. Data on the use of 18F-FDG PET/CT as a discriminating tool between DFO and CNO still remain limited. Another study with low risk of bias showed 99% positive predictive value, and 57% negative predictive value with 94% diagnostic accuracy of 99mTc labelled Ubiquicidin SPECT/CT for the detection of osteomyelitis in patients with diabetes-related foot ulcers.[92]

Evidence statement MRI, WBC scintigraphy, and 18F-FDGPET/CT are likely useful for the diagnosis of osteomyelitis in cases where doubt persists after assessing the results of clinical findings and plain X-ray of the foot.

Certainty of the evidence Moderate, based on two meta-analyses that included some prospective and mostly retrospective studies, downgraded because of inconsistency and imprecision.

References Lauri 2017,[76] Ertugrul 2006,[77] Johnson 1996,[78] Nawaz 2010,[79] Newman 1992,[80] Shagos 2015,[81] Blume 1997,[82] Treglia 2013,[83] Rastogi 2016,[84] Abdel Razek 2017,[85] Aslangul 2013,[86] Poirier 2002,[87] Llewellyn 2020,[88] LaFontaine 2021,[89] Sax 2020,[90] Diez 2020,[91] Atif 2021.[92]

4 | DISCUSSION

We report the results of an updated search for publications that investigated any means of diagnosis of any type of foot infection in persons with diabetes. The 64 studies included in the present review comprised 11 retrospective cohort studies, 38 prospective cohort studies, 8 cross-sectional studies, 2 systematic reviews and 5 meta-analyses. Although the IWGDF/IDSA consensus definition and classification of DFIs have now been implemented in many (inter)national guidelines, we found some studies that used the Wagner classification to define and score the severity of infection. However, this classification is not optimal to assess the presence and severity of the infection and prevented us from including these papers in our systematic review. We used either microbiology or histology bone examination as the reference for the assessment of diagnostic tools of DFO. This might be seen as a too strict inclusion criterion, especially as bone biopsy is limited by false positive and negative results. However, it is currently seen as the reference for the diagnosis of osteomyelitis in any other area of the body and we did not find data that argued against this generally admitted statement. In addition, we only included studies that enroled at least 15 evaluable patients with diabetes and a clinical diagnosis of infection of the foot. The strict selection of the papers in this systematic review precluded formulating evidence statements for some of the questions of interest in the field.

We focused our systematic review related to classification systems on studies that addressed the validity of definitions and classification systems of DFU infections for predicting the risk of hospitalisation, lower extremity amputation, and death but not ulcer healing. Given several different clinical presentations of infectious complications of the foot, the IWGDF/IDSA classification schemes for DFI include four classes based on the presence and severity of infection.[11,12] The simplicity of the IWGDF/IDSA scheme, compared with the other existing classifications, is one of its major advantages. The IWGDF/IDSA infection classification scheme no longer includes osteomyelitis as one of the criteria for making an infection class 3, but rather designates its presence in any class 3 or 4 infection by adding 'O' to the classification. Among the currently available DFU classification systems, Meggitt-Wagner, University of Texas, WIfI, PEDIS and SINBAD, PEDIS (in which DFU infection is defined and classified by the means of the IWGDF/IDSA criteria) has the largest amount of evidence.[93] Of note, SINBAD and WIfI both use the IWGDF/IDSA criteria for the definition and classification of DFU infection. The IWGDF/IDSA criteria showed in our review a significant correlation with the risk of hospitalisation, minor or major amputation but not death. Of note, in a prospective study, PEDIS score $\geq$2 was associated with a significant risk of both major and minor amputations, but the unique independent predictors of major amputation during the whole follow-up was an ankle-brachial index <0.26 which may be related to the participants' characteristics recruited in a vascular surgery unit where they were amputated for 85% of hem.[94] An additional advantage is that the IWGDF/IDSA classification system is easy to use in the daily care, especially for differentiating infected from non-infected ulcers.

Since 2019, studies on the input of molecular techniques have emerged, especially mNGS for the microbiological diagnosis of soft-tissue DFIs and DFOs. These studies reported a limited number of patients who were included while receiving antibiotics, which is likely to favour non-culture versus conventional culture methods. On the other hand, these results also highlight the higher sensitivity of non-culture methods for the microbiological diagnosis of DFI in cases where patients had received antibiotics before debridement and subsequent culture, which is a frequent situation in daily practice. Nevertheless, a major caveat of non-culture methods is the risk of unjustified prescription of broad-spectrum antibiotics since non-culture results do not distinguish between living and dead microorganisms or pathogenic versus non-pathogenic strains. Avoiding false-negative microbiological results is of paramount importance as the absence of data is likely to result in the over-prescription of broad-spectrum antibiotics. The superiority of tissue versus swab samples as established in the CODIFI study has not been questioned in these recent studies.[28]

The likelihood of osteomyelitis complicating an infected DFU is based on assessing clinical findings (e.g., deep ulcer over a bony prominence, visible exposed bone, positive PTB test, and 'sausage toe' appearance) and imaging studies (e.g., cortical disruption, sequestrum, involucrum, marrow oedema, or tracer uptake) for bone abnormalities. Besides ESR, a potential role of PCT and CRP for distinguishing soft-tissue DFIs from DFOs has been suggested.[66] Higher values of these inflammatory markers were associated with DFO in our review of the literature, but we were unable to define specific cut-off values. The combination of a positive PTB with raised inflammatory marker(s) is likely to improve the diagnostic accuracy of each separate test. However, MRI and nuclear medicine imaging show the best diagnostic accuracy when repeated plain X-rays remain inconclusive. MRI, indeed, does not only provide a complete assessment of the bone and joint structures but also of the surrounding soft tissues. Among the other imaging techniques, PET-CT seems to have a better specificity but a lower sensitivity and the data on DFIs are still limited. PET-CT may be of interest in differentiating DFO from acute Charcot neuro-osteoarthropathy. Although considerably less available than plain X-ray, both PET-CT and SPECT-CT have the advantage of being easier to carry out compared to bone scintigraphy when leucocyte-labelled techniques are used in the latter.

As infection differs from contamination in that it represents an invasion of the host tissues, samples for the culture of tissues are likely to provide more accurate data than superficial swabs. Current guidelines recommend against using swabs to collect wound specimens for microbiological assessments[8,9] and the results of the recent CODIFI study demonstrate the greater accuracy of tissue than swab specimens.[28] In addition, the results of studies assessing quantitative bioburden are mitigated by the fact that there is no widely accepted or validated definition of 'wound bioburden.' The detection of pathogenic virulence genes is an interesting avenue of research that may help differentiate colonisers from pathogens, but studies to date have only included monomicrobial *S. aureus* DFIs. Although we lack evidence, we believe that new molecular real-time bacterial identification (including determination of virulence genes and antibiotic resistance profiles) may overcome the delay in obtaining culture results and help clinicians administer earlier and more appropriate antibiotic therapy, especially for severe infections. A cautionary note is that the identification of a greater number of types of microorganisms when using molecular techniques compared with cultural techniques may lead to prescribing an unnecessarily broad-spectrum antibiotic regimen. Furthermore, molecular techniques are not currently available to most clinicians in their routine practice. Given the availability and low cost of this technique, Gram staining offers a solution to guide the empirical antibiotic treatment in patients with infected DFUs, particularly in the settings of low-income countries.

The implementation of bone biopsy in the management of DFO is still a matter of debate. Bone biopsy is, however, a unique method for both ruling the diagnosis in or out and for reliably identifying the causative microorganisms. Our review identified one systematic review and meta-analysis plus 3 additional papers that provide useful information about the weak concordance between bone and non-bone culture results and its safety whether it is performed percutaneously or intraoperatively. Two recent studies suggest that percutaneous bone biopsies can be performed at the bedside and even during the consultation of outpatients.[45,95] Another matter of debate is the need for an antibiotic-free period prior to the bone biopsy to limit the risk of false-negative cultures. Recent studies suggest that previous antibiotic therapy can negatively affect the identification of the causative microorganism(s), but how long this treatment should be stopped prior to the biopsy is still not known.[39,48] Data about the beneficial effect of performing a bone biopsy on the outcome of patients with a suspicion of DFO results are still limited and are studied in an RCT that is currently ongoing in the Netherlands.[96] The impact of a positive culture of bone margins on the outcome of patients operated on with either an amputation or conservative surgery is still debated. Two recent papers we identified continue to provide conflicting data on this point.[43,44] Overall, these studies confirm that non-bone samples are not appropriate to reliably identify bone pathogens in patients with diabetes and a suspicion of DFO. The highest concordance observed with *S. aureus* does not mean that the identification of *S. aureus* in non-bone culture may suffice for affirming this pathogen is responsible for the DFO. Given the complexity of antibiotic therapy in such patients, all should be done to obtain a bone culture like in any other case of chronic osteomyelitis.

5 | CONCLUSIONS

This updated systematic review of the diagnosis of diabetic foot infections has revealed that the use of the IWGDF/IDSA is by far not systematically recorded in the papers we reviewed, even in the most recent ones. Even if the overall data provided by the studies published since 2019 do not globally differ from those from the 2019 systematic review, they provide, however, confirmation of some debated questions, especially regarding the use of a clinical definition of infection based on the IWGDF/IDSA classification system, the absence of a clear impact of non-culture microbiological diagnostic tools, the place of sophisticated imaging techniques and the reliability of bone biopsy and the possibility of performing this procedure safely transcutaneous at the beside. However, all these topics need to be studied further in future comparative studies of sufficient quality and using more standardised techniques in order to obtain robust data that help to define optimal clinical care.

AUTHOR CONTRIBUTIONS

Éric Senneville, Edgar J. G. Peters, Suzanne A. van Asten, and Zaina Albalawi participated in the writing of the document, and all the working group members (Zulfiqarali G. Abbas, Geneve Allison, Javier Aragón-Sánchez, John M. Embil, Lawrence A. Lavery, Majdi Alhasan, Orhan Oz, Ilker Uçkay, Vilma Urbančič-Rovan, Zhang-Rong Xu)

participated in the literature search, the evaluation of the content and quality of the papers selected for the analysis, and review of the final document.

ACKNOWLEDGEMENTS

We thank Jaime Lora-Tamayo (independent external expert) for his review of the article, especially the statistical issues. We thank Nicolaas Schaper and Jaap Van Netten for their peer review of the manuscript. We thank Mrs Orvie Dingwall, BA, MLIS, AHIP, MHI-KNET Librarian, Head, WRHA Virtual Library and Outreach Neil John Maclean Health Sciences Library, University of Manitoba for her help in the literature search.

CONFLICT OF INTEREST STATEMENT

Full conflict of interest statements of all authors can be found online at www.iwgdfguidelines.org.

ETHICS STATEMENT

None.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

ORCID

Éric Senneville https://orcid.org/0000-0002-5720-8908

REFERENCES

1. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA. Risk factors for foot infections in individuals with diabetes. *Diabetes Care*. 2006;29(6):1288-1293. https://doi.org/10.2337/dc05-2425
2. Hao D, Hu C, Zhang T, Feng G, Chai J, Li T. Contribution of infection and peripheral artery disease to severity of diabetic foot ulcers in Chinese patients. *Int J Clin Pract*. 2014;68(9):1161-1164. https://doi.org/10.1111/ijcp.12440
3. Lazzarini PA, Pacella RE, Armstrong DG, van Netten JJ. Diabetes-related lower-extremity complications are a leading cause of the global burden of disability. *Diabet Med*. 2018;35(9):1297-1299. https://doi.org/10.1111/dme.13680
4. Armstrong DG, Boulton AJ, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med*. 2017;376(24):2367-2375. https://doi.org/10.1056/nejmra1615439
5. Peters EJ, Lipsky BA, Aragón-Sánchez J, et al. International Working Group on the Diabetic Foot. Interventions in the management of infection in the foot in diabetes: a systematic review. *Diabetes Metab Res Rev*. 2016;32(Suppl 1):S145-S153. https://doi.org/10.1002/dmrr.2706
6. Senneville É, Lipsky BA, Abbas ZG, et al. Diagnosis of infection in the foot in diabetes: a systematic review. *Diabetes Metab Res Rev*. 2020;36(Suppl 1):e3281. https://doi.org/10.1002/dmrr.3281
7. Senneville E, Albalawi Z, van Asten S, et al. IWGDF/IDSA guidelines on the diagnosis and treatment of diabetes-related foot infections (IWGDF 2023 update). *Diabetes Metab Res Rev*. 2023:XX.
8. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. PRISMA group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol*. 2009;62(10):1006-1012. https://doi.org/10.1016/j.jclinepi.2009.06.005
9. Malone M, Senneville E, Peters E, et al. A Systematic review of diagnosis of infection of the diabetic foot (soft tissue and bone): update. *PROSPERO*. 2022:CRD42022324795.
10. Peters EJG, Albalawi Z, van Asten SA, et al. Interventions in the management of diabetes-related foot infections: a systematic review. *Diabetes Metab Res Rev*. 2023:XX.
11. Lipsky BA, Berendt AR, Cornia PB, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis*. 2012;54(12):132-173. https://doi.org/10.1093/cid/cis346
12. Lipsky BA, Senneville É, Abbas ZG, et al. International Working Group on the Diabetic Foot (IWGDF). Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev*. 2020;36(Suppl 1):e3280.
13. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529-536. https://doi.org/10.7326/0003-4819-155-8-201110180-00009
14. Schünemann HJ, Brozek J, Guyatt G, Oxman A. GRADE Handbook 2013; 2013. https://gdt.gradepro.org/app/handbook/handbook.html
15. Abbas ZG, Lutale JK, Game FL, Jeffcoate WJ. Comparison of four systems of classification of diabetic foot ulcers in Tanzania. *Diabet Med*. 2008;25(2):134-137. https://doi.org/10.1111/j.1464-5491.2007.02308.x
16. Lavery LA, Armstrong DG, Murdoch DP, Peters EJ, Lipsky BA. Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. *Clin Infect Dis*. 2007;44(4):562-565. https://doi.org/10.1086/511036
17. Wukich DK, Hobizal KB, Brooks MM. Severity of diabetic foot infection and rate of limb salvage. *Foot and Ankle Int*. 2013;34(3):351-358. https://doi.org/10.1177/1071100712467980
18. Lavery LA, Ryan EC, Ahn J, et al. The infected diabetic foot: Re-evaluating the Infectious Diseases Society of America diabetic foot infection classification. *Clin Infect Dis*. 2020;70(8):1573-1579. https://doi.org/10.1093/cid/ciz489
19. Ryan EC, Crisologo PA, Oz OK, La Fontaine J, Wukich DK, Lavery LA. Do SIRS criteria predict clinical outcomes in diabetic skin and soft tissue infections? *J Foot Ankle Surg*. 2019;58(6):1055-1057. https://doi.org/10.1053/j.jfas.2019.06.001
20. Ozer Balin S, Sagmak Tartar A, Ugur K, et al. Pentraxin-3: a new parameter in predicting the severity of diabetic foot infection? *Int Wound J*. 2019;16(3):659-664. https://doi.org/10.1111/iwj.13075
21. Sen P, Demirdal T. Predicitive ability of LRINEC score in the prediction of limb loss and mortality in diabetic foot infection. *Diagn Microbiol Infect Dis*. 2021;100(1):115323. https://doi.org/10.1016/j.diagmicrobio.2021.115323
22. Johnson LJ, Crisologo PA, Sivaganesan S, Caldwell CC, Henning J. Evaluation of the Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score for detecting necrotizing soft tissue infections in patients with diabetes and lower extremity infection. *Diabetes Res Clin Pract*. 2021;171:108520. https://doi.org/10.1016/j.diabres.2020.108520
23. Mills JL, Sr, Conte MS, Armstrong DG, et al. The society for vascular surgery lower extremity threatened limb classification system: risk stratification based on wound, ischemia, and foot infection (WIfI). *J Vasc Surg*. 2014;59(1):220-234. e1-2. https://doi.org/10.1016/j.jvs.2013.08.003
24. Monteiro-Soares M, Hamilton EJ, Russell DA, et al. Classification of foot ulcers in people with diabetes: a systematic review. *Diabetes Metab Res Rev*. 2023:e3645. https://doi.org/10.1002/dmrr.3645
25. Huang Y, Cao Y, Zou MX, et al. Comparison of tissue versus swab culturing of infected diabetic foot wounds. *Int J Endocrinol*. 2016;2016:1-6. https://doi.org/10.1155/2016/8198714

26. Mutluoglu M, Günalp U, Turahn V, Gorenek L, Ay H, Lipsky B. How reliable are cultures of specimens from superficial swabs compared with those of deep tissue in patients with diabetic foot ulcers? *J Diabetes Complications*. 2012;26(3):225-229. https://doi.org/10.1016/j.jdiacomp.2012.03.015
27. Demetriou M, Papanas N, Panopoulou M, Papatheodorou K, Bounovas A, Maltezos E. Tissue and swab culture in diabetic foot infections: neuropathic versus neuroischemic ulcers. *Int J Low Extrem Wounds*. 2013;12(2):87-93. https://doi.org/10.1177/1534734613481975
28. Nelson A, Wright-Hughes A, Ross Backhouse M, et al. CODIFI (Concordance in Diabetic Foot Ulcer Infection): a cross-sectional study of wound swab versus tissue sampling in infected diabetic foot ulcers in England. *BMJ Open*. 2018;8(1):e019437. https://doi.org/10.1136/bmjopen-2017-01943
29. Choi Y, Oda E, Waldman O, Sajda T, Beck C, Oh I. Next-generation sequencing for pathogen identification in infected foot ulcers. *Foot Ankle Orthop*. 2021;6(3):24730114211026933. https://doi.org/10.1177/24730114211026933
30. Chen Y, Shi Y, Zhu W, et al. Combining CRISPR-Cas12a-based technology and metagenomics next generation sequencing: a new paradigm for rapid and full-scale detection of microbes in infectious diabetic foot samples. *Front Microbiol*. 2021;12:742040. https://doi.org/10.3389/fmicb.2021.742040
31. Lipof JS, Jones CMC, Daiss J, Oh I. Comparative study of culture, next-generation sequencing, and immunoassay for identification of pathogen in diabetic foot ulcer. *J Orthop Res*. 2021;39(12):2638-2645. https://doi.org/10.1002/jor.25001
32. Noor S, Raghav A, Parwez I, Ozair M, Ahmad J. Molecular and culture-based assessment of bacterial pathogens in subjects with diabetic foot ulcer. *Diabetes Metab Syndr*. 2018;12(3):417-421. https://doi.org/10.1016/j.dsx.2018.03.001
33. Malone M, Fritz BG, Vickery K, et al. Analysis of proximal bone margins in diabetic foot osteomyelitis by conventional culture, DNA sequencing and microscopy. *APMIS*. 2019;127(10):660-670. https://doi.org/10.1111/apm.12986
34. van Asten SA, La Fontaine J, Peters EJ, Bhavan K, Kim PJ, Lavery LA. The microbiome of diabetic foot osteomyelitis. *Eur J Clin Microbiol Infect Dis*. 2016;35(2):293-298. https://doi.org/10.1007/s10096-015-2544-1
35. Senneville E, Melliez H, Beltrand E, et al. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. *Clin Infect Dis*. 2006;42(1):57-62. https://doi.org/10.1086/498112
36. Senneville E, Morant H, Descamps D, et al. Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot. *Clin Infect Dis*. 2009;48(7):888-893. https://doi.org/10.1086/597263
37. Elamurugan TP, Jagdish S, Kate V, Chandra Parija S. Role of bone biopsy specimen culture in the management of diabetic foot osteomyelitis. *Int J Surg*. 2011;9(3):214-216. https://doi.org/10.1016/j.ijsu.2010.11.011
38. Schechter MC, Ali MK, Risk BB, et al. Percutaneous bone biopsy for diabetic foot osteomyelitis: a systematic review and meta-analysis. *Open Forum Infect Dis*. 2020;7(10):ofaa393. https://doi.org/10.1093/ofid/ofaa393
39. Manas AB, Taori S, Ahluwalia R, et al. Admission time deep swab specimens compared with surgical bone sampling in hospitalized individuals with diabetic foot osteomyelitis and soft tissue infection. *Int J Low Extrem Wounds*. 2021;20(4):300-308. https://doi.org/10.1177/1534734620916386
40. Weiner RD, Viselli SJ, Fulkert KA, Accetta P. Histology versus microbiology for accuracy in identification of osteomyelitis in the diabetic foot. *J Foot Ankle Surg*. 2011;50(2):197-200. https://doi.org/10.1053/j.jfas.2010.12.001
41. Meyr AJ, Seo K, Khurana JS, Choksi R, Chakraborty B. Level of agreement with a multi-test approach to the diagnosis of diabetic foot osteomyelitis. *J Foot Ankle Surg*. 2018;57(6):1137-1139. https://doi.org/10.1053/j.jfas.2018.05.010
42. Cecilia-Matilla A, Lazaro-Martinez JL, Aragon-Sanchez J. Statistical reliability of bone biopsy for the diagnosis of diabetic foot osteomyelitis. *J Foot Ankle Surg*. 2013;52(5):692. https://doi.org/10.1053/j.jfas.2013.05.003
43. Tardáguila-García A, Sanz-Corbalán I, García-Morales E, García-Álvarez Y, Molines-Barroso RJ, Lázaro-Martínez JL. Diagnostic accuracy of bone culture versus biopsy in diabetic foot osteomyelitis. *Adv Skin Wound Care*. 2021;34(4):204-208. https://doi.org/10.1097/01.asw.0000734376.32571.20
44. Lavery LA, Crisologo PA, La Fontaine J, Bhavan K, Oz OK, Davis KE. Are we misdiagnosing diabetic foot osteomyelitis? Is the gold standard gold? [published correction appears in J Foot Ankle Surg. 2020 may - Jun;59(3):646]. *J Foot Ankle Surg*. 2019;58(4):713-716. https://doi.org/10.1053/j.jfas.2018.12.010
45. Féron F, de Ponfilly GP, Potier L, et al. Reliability and safety of bedside blind bone biopsy performed by a diabetologist for the diagnosis and treatment of diabetic foot osteomyelitis. *Diabetes Care*. 2021;44(11):2480-2486. https://doi.org/10.2337/dc20-3170
46. Couturier A, Chabaud A, Desbiez F, et al. Comparison of microbiological results obtained from per-wound bone biopsies versus transcutaneous bone biopsies in diabetic foot osteomyelitis: a prospective cohort study. *Eur J Clin Microbiol Infect Dis*. 2019;38(7):1287-1291. https://doi.org/10.1007/s10096-019-03547-6
47. Shiraev TP, Lipsky BA, Kwok TMY, Robinson DA. Utility of culturing marginal bone in patients undergoing lower limb amputation for infection. *J Foot Ankle Surg*. 2019;58(5):847-851. https://doi.org/10.1053/j.jfas.2018.12.012
48. Macauley M, Adams G, Mackenny P, et al. Microbiological evaluation of resection margins of the infected diabetic foot ulcer. *Diabet Med*. 2021;38(4):e14440. https://doi.org/10.1111/dme.14440
49. Atway S, Nerone VS, Springer KD, Woodruff DM. Rate of residual osteomyelitis after partial footamputation in diabetic patients: a standardized method for evaluating bone margins with intraoperative culture. *J Foot Ankle Surg*. 2012;51(6):749-752. https://doi.org/10.1053/j.jfas.2012.06.017
50. Kowalski TJ, Matsuda M, Sorenson MD, Gundrum JD, Agger WA. The effect of residual osteomyelitis at the resection margin in patients with surgically treated diabetic foot infection. *J Foot Ankle Surg*. 2011;50(2):171-175. https://doi.org/10.1053/j.jfas.2010.12.009
51. Schmidt BM, McHugh JB, Patel RM, Wrobel JS. Prospective analysis of surgical bone margins after partial foot amputation in diabetic patients admitted with moderate to severe foot infections. *Foot Ankle Spec*. 2019;12(2):131-137. https://doi.org/10.1177/1938640018770285
52. Johnson MJ, Shumway N, Bivins M, Bessesen MT. Outcomes of limb-sparing surgery for osteomyelitis in the diabetic foot: importance of the histopathologic margin. *Open Forum Infect Dis*. 2019;6(10):ofz382. https://doi.org/10.1093/ofid/ofz382
53. Crisologo PA, Malone M, La Fontaine J, et al. Are surrogate markers for diabetic foot osteomyelitis remission reliable? *J Am Podiatr Med Assoc*. 2021;111(5). https://doi.org/10.7547/20-147
54. Uçkay I, Holy D, Schöni M, et al. How good are clinicians in predicting the presence of Pseudomonas spp. in diabetic foot infections? A prospective clinical evaluation. *Endocrinol Diabetes Metab*. 2021;4(2):e00225. https://doi.org/10.1002/edm2.225
55. Armstrong DG, Perales TA, Murff RT, Edelson GW, Welchon JG. Value of white blood cell count with differential in the acute diabetic foot infection. *J Am Podiatr Med Assoc*. 1996;86(5):224-227. https://doi.org/10.7547/87507315-86-5-224

56. Eneroth M, Apelqvist J, Stenstrom A. Clinical characteristics and outcome in 223 diabetic patients with deep foot infections. *Foot Ankle Int*. 1997;18(11):716-722. https://doi.org/10.1177/107110079701801107
57. Jeandrot A, Richard JL, Combescure C, et al. Serum procalcitonin and C-reactive protein concentrations to distinguish mildly infected from non-infected diabetic foot ulcers: a pilot study. *Diabetologia*. 2008;51(2):347-352. https://doi.org/10.1007/s00125-007-0840-8
58. Reeves G. C-reactive protein. *Aust Prescr*. 2007;30(3):74-76. https://doi.org/10.18773/austprescr.2007.043
59. Uzun G, Solmazgul E, Curuksulu H, et al. Procalcitonin as a diagnostic aid in diabetic foot infections. *Tohoku J Exp Med*. 2007;213(4):305-312. https://doi.org/10.1620/tjem.213.305
60. Al-Shammaree SAW, Abu ABA, Salman IN. Procalcitonin levels and other biochemical parameters in patients with or without diabetic foot complications. *J Res Med Sci*. 2017;22(1):95. https://doi.org/10.4103/jrms.jrms_906_16
61. Korkmaz P, Kocak H, Onbasi K, et al. The role of serum procalcitonin, interleukin-6, and fibrinogen levels in differential diagnosis of diabetic foot ulcer infection. *J Diabetes Res*. 2018;2018:7104352--7104357. https://doi.org/10.1155/2018/7104352
62. Umapathy D, Dornadula S, Rajagopalan A, et al. Potential of circulatory procalcitonin as a biomarker reflecting inflammation among South Indian diabetic foot ulcers. *J Vasc Surg*. 2018;67(4):1283-1291.e2. https://doi.org/10.1016/j.jvs.2017.02.060
63. Zakariah NA, Bajuri MY, Hassan R, et al. Is procalcitonin more superior to hs-CRP in the diagnosis of infection in diabetic foot ulcer? *Malays J Pathol*. 2020;42(1):77-84.
64. Todorova AS, Dimova RB, Chakarova NY, et al. Comparative evaluation of the diagnostic value of procalcitonin and hsCRP for the presence of mild-to-moderate diabetic foot infections. *Int J Low Extrem Wounds*. 2023;22(2):353-359. https://doi.org/10.1177/15347346211011849
65. Park JH, Suh DH, Kim HJ, Lee YI, Kwak IH, Choi GW. Role of procalcitonin in infected diabetic foot ulcer. *Diabetes Res Clin Pract*. 2017;128:51-57. https://doi.org/10.1016/j.diabres.2017.04.008
66. Sharma H, Sharma S, Krishnan A, et al. The efficacy of inflammatory markers in diagnosing infected diabetic foot ulcers and diabetic foot osteomyelitis: systematic review and metaanalysis. *PLoS ONE*. 2022;17(4):e0267412. https://doi.org/10.1371/journal.pone.0267412
67. van Asten SA, Peters EJG, Xi Y, Alfred Lavery L. The role of biomarkers to diagnose diabetic foot osteomyelitis; a meta-analysis. *Curr Diabetes Rev*. 2016;12(4):396-402. https://doi.org/10.2174/1573399811666150713104401
68. van Asten SA, Nichols A, La Fontaine J, Bhavan K, Peters EJ, Lavery LA. The value of inflammatory markers to diagnose and monitor diabetic foot osteomyelitis. *Int Wound J*. 2017;14(1):40-45. https://doi.org/10.1111/iwj.12545
69. Hayes OG, Vangaveti VN, Malabu UH. Serum procollagen type 1 N propeptide: a novel diagnostic test for diabetic foot osteomyelitis – a case-control study. *J Res Med Sci*. 2018;30:23-39.
70. Lavery LA, Ahn J, Ryan EC, et al. What are the optimal cutoff values for ESR and CRP to diagnose osteomyelitis in patients with diabetes-related foot infections? *Clin Orthop Relat Res*. 2019;477(7):1594-1602. https://doi.org/10.1097/corr.0000000000000718
71. Nie X, Gao L, Wang L, Wang J. Atherogenic index of plasma: a potential biomarker for clinical diagnosis of diabetic foot osteomyelitis. *Surg Infect*. 2020;21(1):9-14. https://doi.org/10.1089/sur.2019.020
72. Lam K, van Asten SA, Nguyen T, La Fontaine J, Lavery LA. Diagnostic accuracy of probe to bone to detect osteomyelitis in the diabetic foot: a systematic review. *Clin Infect Dis*. 2016;63(7):944-994. https://doi.org/10.1093/cid/ciw445
73. Fleischer AE, Didyk AA, Woods JB, Burns SE, Wrobel JS, Armstrong DG. Combined clinical and laboratory testing improves diagnostic accuracy for osteomyelitis in the diabetic foot. *J Foot Ankle Surg*. 2009;48(1):39-46. https://doi.org/10.1053/j.jfas.2008.09.003
74. Morales-Lozano R, González Fernández ML, Martinez Hernández D, Beneit Montesinos JV, Guisado Jiménez S, Gonzalez Jurado MA. Validating the probe-to-bone test and other tests for diagnosing chronic osteomyelitis in the diabetic foot. *Diabetes Care*. 2010;33(10):2140-2145. https://doi.org/10.2337/dc09-2309
75. Xu J, Cheng F, Li Y, Zhang J, Feng S, Wang P. Erythrocyte sedimentation rate combined with the probe-to-bone test for fast and early diagnosis of diabetic foot osteomyelitis. *Int J Low Extrem Wounds*. 2021;20(3):227-231. https://doi.org/10.1177/1534734620923278
76. Lauri C, Tamminga M, Glaudemans AWJM, et al. Detection of osteomyelitis in the diabetic foot by imaging techniques: a systematic review and meta-analysis comparing MRI, white blood cell scintigraphy, and FDG-PET. *Diabetes Care*. 2017;40(8):1111-1120. https://doi.org/10.2337/dc17-0532
77. Ertugrul MB, Baktiroglu S, Salman S, et al. The diagnosis of osteomyelitis of the foot in diabetes: microbiological examination vs. magnetic resonance imaging and labelled leucocyte scanning. *Diabet Med*. 2006;23(6):649-653. https://doi.org/10.1111/j.1464-5491.2006.01887.x
78. Johnson JE, Kennedy EJ, Shereff MJ, Patel NC, Collier BD. Prospective study of bone, indium-111-labeled white blood cell, and gallium- 67 scanning for the evaluation of osteomyelitis in the diabetic foot. *Foot Ankle Int*. 1996;17(1):10-16. https://doi.org/10.1177/107110079601700103
79. Nawaz A, Torigian DA, Siegelman ES, Basu S, Chryssikos T, Alavi A. Diagnostic performance of FDG-PET, MRI, and plain film radiography (PFR) for the diagnosis of osteomyelitis in the diabetic foot. *Mol Imaging Biol*. 2010;12(3):335-342. https://doi.org/10.1007/s11307-009-0268-2
80. Newman LG, Waller J, Palestro CJ, et al. Leukocyte scanning with 111In is superior to magnetic resonance imaging in diagnosis of clinically unsuspected osteomyelitis in diabetic foot ulcers. *Diabetes Care*. 1992;15(11):1527-1530. https://doi.org/10.2337/diacare.15.11.1527
81. Shagos GS, Shanmugasundaram P, Varma AK, Padma S, Sarma M. 18-F fluorodeoxyglucose positron emission tomography-computed tomography imaging: a viable alternative to three phase bone scan in evaluating diabetic foot complications? *Indian J Nucl Med*. 2015;30(2):97-103. https://doi.org/10.4103/0972-3919.152946
82. Blume PA, Dey HM, Daley LJ, Arrighi JA, Soufer R, Gorecki GA. Diagnosis of pedal osteomyelitis with Tc-99m HMPAO labeled leukocytes. *J Foot Ankle Surg*. 1997;36(2):120-126. https://doi.org/10.1016/s1067-2516(97)80057-9
83. Treglia G, Sadeghi R, Annunziata S, et al. Diagnostic performance of fluorine-18-fluorodeoxyglucose positron emission tomography for the diagnosis of osteomyelitis related to diabetic foot: a systematic review and a meta-analysis. *Foot*. 2013;23(4):140-148. https://doi.org/10.1016/j.foot.2013.07.002
84. Rastogi A, Bhattacharya A, Prakash M, et al. Utility of PET/CT with fluorine-18-fluorodeoxyglucose-labeled autologous leukocytes for diagnosing diabetic foot osteomyelitis in patients with Charcot's neuroarthropathy. *Nucl Med Commun*. 2016;37(12):1253-1259. https://doi.org/10.1097/mnm.0000000000000603
85. Abdel Razek AAK, Samir S. Diagnostic performance of diffusion weighted MR imaging in differentiation of diabetic osteoarthropathy and osteomyelitis in diabetic foot. *Eur J Radiol*. 2017;89:221-225. https://doi.org/10.1016/j.ejrad.2017.02.015
86. Aslangul E, M'bemba J, Caillat-Vigneron N, et al. Diagnosing diabetic foot osteomyelitis in patients without signs of soft tissue infection by coupling hybrid 67Ga SPECT/CT with bedside percutaneous bone puncture. *Diabetes Care*. 2013;36(8):2203-2210. https://doi.org/10.2337/dc12-2108

87. Poirier JY, Garin E, Derrien C, et al. Diagnosis of osteomyelitis in the diabetic foot with a 99mTc-HMPAO leucocyte scintigraphy combined with a 99mTc-MDP bone scintigraphy. *Diabetes Metab*. 2002; 28:485-490.
88. Llewellyn A, Kraft J, Holton C, Harden M, Simmonds M. Imaging for detection of osteomyelitis in people with diabetic foot ulcers: a systematic review and meta-analysis. *Eur J Radiol*. 2020;131:109215. https://doi.org/10.1016/j.ejrad.2020.109215
89. La Fontaine J, Bhavan K, Jupiter D, Lavery LA, Chhabra A. Magnetic resonance imaging of diabetic foot osteomyelitis: imaging accuracy in biopsy-proven disease. *J Foot Ankle Surg*. 2021;60(1):17-20. https://doi.org/10.1053/j.jfas.2020.02.012
90. Sax AJ, Halpern EJ, Zoga AC, Roedl JB, Belair JA, Morrison WB. Predicting osteomyelitis in patients whose initial MRI demonstrated bone marrow edema without corresponding T1 signal marrow replacement. *Skelet Radiol*. 2020;49(8):1239-1247. https://doi.org/10.1007/s00256-020-03396-x
91. Diez AIG, Fuster D, Morata L, et al. Comparison of the diagnostic accuracy of diffusion-weighted and dynamic contrast-enhanced MRI with ^{18}F-FDG PET/CT to differentiate osteomyelitis from Charcot neuro-osteoarthropathy in diabetic foot. *Eur J Radiol*. 2020;132: 109299. https://doi.org/10.1016/j.ejrad.2020.109299
92. Atif M, Hussain F, Dar Z, Khatoon J, Ajmal S, Adil M. Diagnostic accuracy of 99mTc labelled Ubiquicidin (29-41) SPECT/CT for diagnosis of osteomyelitis in diabetic foot. *Pak Armed Forces Med J (PAFMJ)*. 2021;71(Jun. 2021):1015-1019. https://doi.org/10.51253/pafmj.v71i3.4102
93. Schaper NC. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabetes Metab Res Rev*. 2004;20(Suppl 1):S90-S95. https://doi.org/10.1002/dmrr.464
94. Bravo-MolinaLinares-Palomino AJP, Lozano-Alonso S, Asensio-García R, Ros-Díe E, Hernández-Quero J. Influence of wound scores and microbiology on the outcome of the diabetic foot syndrome. *J Diabetes Complications*. 2016;30(2):329-334. https://doi.org/10.1016/j.jdiacomp.2015.11.001
95. Kosmopoulou OA, Dumont IJ. Feasibility of percutaneous bone biopsy as part of the management of diabetic foot osteomyelitis in a 100% neuropathic, grade 3 IDSA/IWGDF population on an outpatient basis. *Int J Low Extrem Wounds*. 2020;19(4):382-387. https://doi.org/10.1177/1534734620902609
96. Gramberg MCTT, Lagrand RS, Sabelis LWE, et al. Using a BonE BiOPsy (BeBoP) to determine the causative agent in persons with diabetes and foot osteomyelitis: study protocol for a multicentre, randomised controlled trial. *Trials*. 2021;22(1):517. https://doi.org/10.1186/s13063-021-05472-6

How to cite this article: Senneville É, Albalawi Z, van Asten SA, et al. Diagnosis of infection in the foot of patients with diabetes: a systematic review. *Diabetes Metab Res Rev*. 2024; e3723. https://doi.org/10.1002/dmrr.3723

APPENDIX A

TABLE A1 Table of evidence: 2023 update systematic review of the diagnosis of diabetic foot infection.

Reference: Atif (2021) Title: Diagnostic accuracy of 99 mtc labelled ubiquicidin (29–41) spect/ct for diagnosis of osteomyelitis in diabetic foot	SIGN: 2 Cross-sectional study Single centre study	Aim: To determine the diagnostic accuracy of 99mTc labelled Ubiquicidin (29–41) SPECT/CT for detection of osteomyelitis in diabetic foot patients by taking bone biopsy as the gold standard Specific reference test: Inclusion criteria: Patients with a suspicion of DFO Total participants: 122	Results: The 99mTc–UBI (29–41) scan showed 94.69% Sensitivity 94.7% Specificity 88.9% Positive predictive value 99.1% Negative predictive value 57.1% Diagnostic accuracy 94.2%	
Reference: Chen (2021) Title: Combining CRISPR-Cas12a-Based technology and metagenomics next generation sequencing: A new paradigm for rapid and full-scale detection of microbes in infectious diabetic foot samples	SIGN: 2 Cross sectional study Single centre study	Aim: To assess the concordance between loop-mediated isothermal amplification (LAMP) and trans cleavage of Cas12a with standard methods, including bacterial culture, qRT-PCR, and metagenomics new generation sequencing (mNGS) Specific reference test: Standard methods (culture and PCR) Inclusion criteria: Patients with DFI admitted at an emergency unit	Results: The specificity of the LAMP-CRISPR assay for *S. aureus* PCR identification was 100%, without cross-reactions with non-*S. aureus* strains. Evaluating assay performance with 18 samples from DFI patients showed that the assay had 94.4% agreement (17/18 samples) with clinical culture results. The results of mNGS for 8/18 samples were consistent with those	Comments: Small size Definition of type of DFI, unclear no data about antibiotics use incidence of PAD?

TABLE A1 (Continued)

		Exclusion criteria: NR Total participants: 18	of the reference culture and LAMP-CRISPR assay Conclusion: Combining the LAMP-CRISPR assay and mNGS provides an advanced platform for molecular pathogen diagnosis of DFI	
Reference: Choi (2021) Title: Next-generation sequencing for pathogen identification in infected foot ulcers	SIGN: 2 Prospective cohort study Single centre study	Aim: To assess the concordance of results between NGS and conventional cultures of intraoperative bone samples Specific reference test: Standard culture Inclusion criteria: Patients with DFI (IDSA definition) that necessitated operative intervention (irrigation and debridement or amputations) Exclusion criteria: Severe ischaemia, venous stasis ulcer, immunodeficiency, malnutrition, pregnant, or unconscious Total participants: 30	Results: Polymicrobial in =19 (65.6%) versus 25 (86.2%) cases in NGS - Complete concordance: 14 cases (46.7%) - Partial concordance: 8 cases (26.7%) - Discordance (NGS and culture identified completely different bacteria without any overlap): 8 cases (26.7%) Conclusions: NGS frequently provides different profiles of microorganisms along with antibiotic-resistant gene information compared to conventional culture in polymicrobial foot infection	Comments: Patients with severe infections requiring surgery peripheral artery disease (PAD)?
Reference: Couturier (2019) Title: Comparison of microbiological results obtained from per-wound bone biopsies versus transcutaneous bone biopsies in diabetic foot osteo-myelitis: a Prospective cohort study	SIGN: 2 Prospective cohort study Single centre study	Aim: To correlate the microbiological results obtained from per-wound bone biopsies with bone biopsy through healthy skin for the diagnosis of DFO Specific reference test: Bone culture results obtained by healthy skin Inclusion criteria: Clinical signs suggestive of infection and DFO Exclusion criteria: Indication for immediate surgical management and unwillingness or inability to provide informed consent active Charcot's disease Total participants: 46	Results: Overall concordance = 48.4%; *S. aureus*: 79.2% Impact on the antibiotic therapy by using the results of per-wound biopsy: Appropriate AT in 68%, too broad in 10%, too narrow in 13% and inappropriate treatment in 18.5% Conclusions: per-wound bone biopsy cultures are not sufficiently accurate to identify the causative organisms in patients with an infected diabetic foot ulcer, except in the case of *S. aureus*	Comments:
Reference: Crisologo (2021) Title: Are surrogate markers for diabetic foot osteomyelitis remission reliable?	SIGN: 2 Cross sectional study Single centre study	Aim: To compare outcomes of patients with DF osteomyelitis using different definitions of DFO remission Specific reference test: - Ulcer failed to heal within 12 months - Reulceration within 12 months - Readmission for diabetic foot infection at the same site - Additional surgery at the same site after discharge	Results: No differences in outcomes between OM vs. skin and soft- tissue infections diagnosed at baseline: - OM reinfection (16.7% vs. 45.5%; $p = 0.07$) - Wounds that failed to heal (8.3% vs. 9.1%; $p = 0.94$), - reulceration (20.8% vs. 27.3%; $p = 0.67$) - Readmission for skin and soft-tissue infection at the index	Selection of the patients unclear

(Continues)

TABLE A1 (Continued)

		- Additional amputation at the same site after discharge Inclusion criteria: Patients of 21 years or older and a moderate to severe diabetic foot skin and soft-tissue infection based on the IDSA classification with suspicion of underlying DFO Total participants: 35 24 patients with OM all had bone biopsy 11 patient with DFI (clinical signs of infection according top IDSA)	site (16.7% vs. 36.4%; $p = 0.20$) - Amputation at the index site after discharge (12.5% vs. 36.4%; $p = 0.10$)	
Reference: Diez (2020) Title: Comparison of the diagnostic accuracy of diffusion-weighted and dynamic contrast-enhanced MRI with ^{18}F-FDG PET/CT to differentiate osteomyelitis from Charcot neuro-osteoarthropathy in diabetic foot	SIGN: 2 Cross sectional study Single centre study	Aim: To compare the diagnostic accuracy of DWI (apparent diffusion coefficient and high-b-value signal pathological-to-normal bone ratio) and MRI parameters versus time intensity curve using two different ratio of interest sizes-ROIs-, and 18F-FDG PET/CT to differentiate DFO from Charcot neuro-osteoarthropathy (CN) Specific reference test: Bone biopsies in bones with lesion in conventional MR scans Inclusion criteria: Patients with grade 3 (moderate) or grade 4 (severe) infected foot ulcers and suspected DFO according to the IDSA/IWGDF; patients with procedures and imaging techniques (DCE-MRI in patients with adequate renal function and DWI) and (18F-FDG PET/CT) obtained in the same week Exclusion criteria: The presence of bone fractures or a pattern of osteonecrosis, absence of bone lesions, lack of adequate images, or no confirmed diagnosis of DFO Total participants: 31	Results: MRI parameters showed better diagnostic accuracy (AUC = 0.8140.830) and reliability (ICC >0.9) for large than for small ROIs (AUC = 0.7360.750) Visual assessment of 18F- FDG PET/CT demonstrated a significantly higher accuracy (AUC = 0.924) than MRI parameters Semi-quantitative 18F-FDG PET/CT parameters did not provide significant improvement over visual analysis (AUC = 0.8480.903) Conclusion: MRI parameters allowed reliable differentiation of DFO and CN, particularly for large ROIs.Visual assessment of 18F-FDG PET/CT was the most accurate technique for differentiation	
Reference: Feron (2021) Title: Reliability and safety of bedside blind bone biopsy performed by a diabetologist for the diagnosis and treatment of diabetic foot osteomyelitis	SIGN: 2 Prospective cohort study Multicenter study	Aim: To compare the proportion of positive results of bedside versus standard percutaneous bone biopsy (PBB) in patients with suspicion of DFO Specific reference test: standard (either radiological or surgical PBB) Inclusion criteria: Consecutive inpatients with suspected DFO	Results: The proportion of patients with positive BB was lower in bedside versus standard BB (40 of 79, 50.6% vs. 34 of 44, 77.3%, $p < 0.01$). No difference in the rate of culture contamination between both procedures Conclusions: Bedside BB is a simple, safe, and efficient	Comments: The explanation for the lower proportion of positive bedside BB unclear; differences in the patients population: More T2D, nephropathy and retinopathy in bedside BB group

TABLE A1 (Continued)

		Exclusion criteria: Antibiotic within 2 weeks prior to the biopsy Total participants: Standard = 44; bedside = 79	procedure for the diagnosis of DFO	
Reference: Fontaine (2021) Title: Magnetic resonance imaging of diabetic foot osteomyelitis: Imaging accuracy in biopsy-proven disease	SIGN: 2 Cross-sectional study Single centre study	Aim: To assess the accuracy of MRI readings in biopsy-proven diabetic foot osteomyelitis with a second read done by a blinded, expert musculoskeletal radiologist Specific reference test: Bone biopsy culture and/or histology Inclusion criteria: Adult (aged 18–100 years) patients with known diabetes mellitus, clinically suspected osteomyelitis, completed MRI, and a bone biopsy within a clinically relevant time frame from imaging, defined as 8 weeks Total participants: 58	Results: In 17 of 58 patients (29.3%), the diagnosis of DFO by MRI was not confirmed by bone biopsy There were 12 false positives and 5 false negatives. After the second expert read, there were 5 false positives and 4 false negatives The sensitivity and specificity of MRI were 87% and 37%, and the positive and negative predictive values were 74% and 58%, respectively, for the official read The sensitivity and specificity of MRI were 90% and 74%, and the positive and negative predictive values were 88% and 78%, respectively, for the second read. The overall accuracy was 84% for the second read	The time interval that was selected between the obtained bone sample and imaging (max 8 weeks)
Reference: Johnson (2019) Title: Outcomes of limb-sparing surgery for osteomyelitis in the diabetic foot: Importance of the histopathologic margin	SIGN: 2 Retrospective cohort study Single centre study	Aim: To assess the association of a positive histopathologic margin with the outcome of osteomyelitis in the diabetic foot Specific reference test: Remission with those who experienced recurrence in the 12 months of follow-up, Inclusion criteria: Total participants: 39 cases	Results: Osteomyelitis of the diabetic foot at the histopathologic margin of resection was not associated with increased risk of treatment failure. Resection at the level of the digit was associated with a lower risk of failure than at the metatarsal level	
Reference: Johnson (2021) Title: Evaluation of the laboratory risk indicator for necrotising fasciitis (LRINEC) score for detecting necrotising soft tissue infections in patients with diabetes and lower extremity infection	SIGN: 2 Prospective cohort study Single centre study	Aim: To assess the laboratory risk indicator for necrotising fasciitis (LRINEC), a scoring system for necrotising soft tissue infections, to diagnose necrotising soft tissue infections of the lower extremity in patients with diabetes Specific reference test: Necrotising soft-tissue infection Inclusion criteria: Patients with diabetes mellitus type 2 who presented to the Emergency Department at the University of Cincinnati Medical Center with lower extremity infections that	Results: The LRINEC was associated with necrotising soft tissue infection diagnosis in patients with diabetes ($p = 0.01$). Sensitivity 100% Specificity 69% Positive predictive value 16.6% Negative predictive value 100%	

(Continues)

TABLE A1 (Continued)

		required surgical intervention Total participants: 69		
Reference: Lavery (2019) Title: What are the optimal cutoff values for ESR and CRP to diagnose osteomyelitis in patients with diabetes-related foot infections?	SIGN: 2+ Retrospective cohort study Single centre study	Aim: 1. Define optimal cutoff values for ESR and CRP in the diagnosis of osteomyelitis in DFU 2. Create a diagnostic algorithm using ESR CRP cutoffs to improve diagnosis of osteomyelitis in setting of diabetic foot infections Specific reference test: 1. Osteomyelitis group: was established by either bone biopsy or histology 2. Soft tissue infection group: Negative MRI, negative SPECT/CT, or negative results on bone biopsy to identify patients without a bone infection Inclusion criteria: Patients age 18–89 treated from 2010 to 2017 Exclusion criteria: 1. Patients without ESR or CRP tested within 72 h of admission 2. Patients without pathology results or radiology (SPECT or MRI) 3. Patients 'with comorbidities that could affect baseline ESR and CRP levels, such as autoimmune diseases' 4. Patients without diabetes according to ADA Total participants: 1843	Results: Cut-off values: ESR >60 and CRP >7.9 sensitivity 0.36 Specificity 0.91 OR 4.55 CI 2.74–7.55	
Reference: Lavery (2019) Title: Are we misdiagnosing diabetic foot osteomyelitis? Is the gold standard gold?	SIGN: 2 Prospective cohort study Single centre study	Aim: To compare the incidence of osteomyelitis (suspicion on clinical presentation including a positive probe-to-bone test or a deep infection near bone or joint, radiographic changes, or magnetic resonance imaging (MRI) findings consistent with osteomyelitis). Based on different operational definitions Specific reference test: 5 definitions for osteomyelitis: (1) traditional culture, (2) histology, (3) genetic sequencing, (4) traditional culture and histology, and (5) genetic sequencing and histology Inclusion criteria: Patients from July to Oct 2015 who met	Results: The incidence of osteomyelitis was 68.6% Traditional cultures vs. histology: 68.6% vs. 45.7%, $p = 0.06$, OR 2.59, 95% CI 0.98–6.87 In every case that histology reported osteomyelitis, the bone standard culture and genetic methods were positive The 16S rRNA testing vs. histology: 82.9% versus 45.7%, $p = 0.002$, OR 5.74, 95% CI 1.91–17.28 Genetic sequencing vs. standard culture: 82.9% versus 68.6%, ($p = 0.17$, OR 2.22, 95% CI 0.71–6.87) Using both histology and traditional culture or	Very small patient population, underpowered statistical analysis

TABLE A1 (Continued)

		the criteria of ≥21 years of age and a moderate to severe infection Exclusion criteria: Exclusion criteria included patients with other acute infectious diseases, previously diagnosed osteomyelitis of the foot, organ or haematologic malignancy, or end-stage renal disease requiring dialysis or patients who were on immunosuppressive therapies Total participants: 35	histology versus genetic sequencing: 68.6% versus 82.9% = NS	
Reference: Lavery (2020) Title: The infected diabetic foot: Re-evaluating the infectious diseases society of America diabetic foot infection classification	SIGN: 2 Retrospective cohort study Single centre study	Aim: To retrospectively review the data of osteomyelitis patients to determine whether the IDSA classification better reflects outcomes if risk categories are stratified by soft tissue infection or osteomyelitis and moderate and severe infections are not categorised separately Specific reference test: IWGDF/IDSA definition and classification of DFI Inclusion criteria: Patients admitted to hospital over an 8-year period with moderate and severe diabetic foot infections Total participants: 353 (137 patients with soft tissue infections and 157 patients with osteomyelitis who met inclusion criteria and had at least a 12-month follow-up documented in the electronic medical records)	Results: Osteomyelitis patients had a greater antibiotic duration (32.5 ± 46.8 vs. 63.8 ± 55.1 days; $p < 0.01$) Surgery frequency (55.5% vs. 99.4%; $p < 0.01$) Number of surgeries (2.1 ± 1.3 vs. 3.3 ± 2.3; $p < 0.01$), amputations (26.3% vs. 83.4%; $p < 0.01$) Reinfection (38.0% vs. 56.7%; $p < 0.01$) Length of stay (14.5 ± 14.9 vs. 22.6 ± 19.0 days; $p < 0.01$) There were no differences in moderate and severe STI outcomes except for infection readmissions (46.2% vs. 25.0%; $p = 0.02$), and acute kidney injury (31.2% vs. 50.0%; $p = 0.03$)	
Reference: Lipof (2021) Title: Comparative study of culture, next-generation sequencing, and immunoassay for identification of pathogen in diabetic foot ulcer study design: Cohort study	SIGN: 2 Prospective cohort study Single centre study	Aim: To assess the concordance between NGS, and newly specific antibodies immunoassay (NSA) for the detection of *S. aureus* and *S. agalactiae* Specific reference test: Standard culture Inclusion criteria: Infected DFU and underwent surgical intervention (irrigation and debridement or amputations) Total participants: 30	Results: Concordance between culture and NGS in 21 cases (70%) and discordance in 9 (30%) cases - NGS versus culture for *S. aureus* ($\kappa = 0.86$) and *S. agalactiae* ($\kappa = 1.0$) - NSA versus culture *S. aureus* ($\kappa = 0.18$) and *S. agalactiae* ($\kappa = 0.67$) Conclusions: There is high concordance between culture and NGS in identifying the dominant pathogen in DFU	Comments: Small sample size ($N = 30$). No uninfected group
Reference: Llewellyn (2020)	SIGN: 1	Aim: To compare imaging (MRI, X-ray, SPECT, scintigraphy,	Results:	Eight out of 36 studies at high risk of bias

(Continues)

TABLE A1 (Continued)

Title: Imaging for detection of osteomyelitis in people with diabetic foot ulcers: A systematic review and meta-analysis	Systematic review and meta-analysis	ultrasound, PET to the specific reference standard for diagnosis of DFO) Specific reference test: The preferred reference standard was histopathology or microbiology from bone biopsy or pus aspiration. Surgery was also accepted as reference standard Inclusion criteria: Diabetic patients with concern for DFO Total studies included for analysis: 36	MRI: 22 studies: 96.4% sensitivity (95% CI 90.7–98.7); 83.8% specificity (76.0–89.5) PET scans: (6 studies: 84.3% sensitivity [52.8–96.3]; 92.8% specificity [75.7–98.2]) SPECT: 3 studies: 95.6% sensitivity (76.0–99.3); 55.1% specificity (19.3–86.3) Scintigraphy: 17 studies: 84.2% sensitivity (76.8–89.6); 67.7% specificity (56.2–77.4) X-rays: 16 studies: 61.9% sensitivity (50.5–72.1); 78.3% specificity (62.9–88.5)	
Reference: Macauley (2021) Title: Microbiological evaluation of resection margins of the infected diabetic foot ulcer	SIGN: 2 Retrospective cohort study Single centre study	Aim: To evaluate the impact of surgical debridement on the microbiology of resection margins of an infected diabetic foot ulcer and to compare the use of marginal sampling as a guide for antimicrobial therapy Specific reference test: Intraoperative tissue sample culture results Inclusion criteria: Patients with diabetic foot infection, and at least one palpable foot pulse to the ulcer requiring surgical debridement between Feb 2017 and April 2019 Exclusion criteria: Severe peripheral arterial disease with no foot pulses Total participants: 40	Results: The duration of antibiotics received from initial presentation to debridement was 16.2 ± 5.5 days when pathogenic bacteria were identified in per-operative samples versus 53 ± 21.0 days in cases where per-operative sample cultures were negative The results suggest that a longer duration of targeted preoperative antimicrobial therapy may reduce residual marginal infection of pathogenic bacteria	
Reference: Malone (2019) Title: Analysis of proximal bone margins in diabetic foot osteomyelitis by conventional culture, DNA sequencing and microscopy	SIGN: 2 Prospective cohort study Single centre study	Aim: To compare intraoperative bone specimens ('infected' and proximal 'clean' margins) using conventional culture and next-generation DNA sequencing Specific reference test: Standard culture Inclusion criteria: PEDIS grade 3 or 4 infection (IDSA)/ (IWGDF) with a high clinical suspicion of underlying DFO Exclusion criteria: Patients requiring revision surgery of previously diagnosed DFO; the presence of gangrene or necrotising soft tissue infection; or evidence of Charcot neuropathic osteoarthropathy Total participants: 14	Results: 8 of 14 (57%) proximal bone margins had no growth by culture all 14 infected and margin bones were positive on NGS Conclusions: NGS is more sensitive than conventional culture ? Significance of culture-negative–NGS positive findings and implications for clinical outcomes	Comments: Very small size population; incidence of PAD?

TABLE A1 (Continued)

Reference: Manas (2021) Title: Admission time deep swab specimens compared with surgical bone sampling in hospitalised individuals with DFO and soft tissue infection	SIGN: 2 Retrospective cohort study Single centre study	Aim: To compare the concordance between deep wound swab (DWS) cultures and surgical bone cultures Specific reference test: Standard (radiological or surgical) percutaneous bone biopsy cultures Inclusion criteria: Patients admitted to hospital with a severe limb-threatening DFI with DFO Exclusion criteria: NR Total participants: 63	Results: Overall concordance: $\kappa = 0.302$; for *S. aureus*: $\kappa = 0.571$ for MRSA: $\kappa = 0.644$ correlation: +LHR overall: 5.81; *S.aureus*: 18.7; gram-negative bacilli: 11.9 Prior antibiotic therapy ($p = 0.03$) and duration of ulceration <8 weeks ($p = 0.025$) predictive of negative growth on bone samples Conclusions: Only a fair concordance between deep wound swabs and surgical bone specimens in patients presenting with a severe DFI and DFO except for *S. aureus*	Comments: Confirmation of previous reports
Reference: Nie (2020) Title: Atherogenic index of plasma: A potential biomarker for clinical diagnosis of diabetic foot osteomyelitis	SIGN: 2 Retrospective cohort study	Aim: To examine the relation between atherogenic index of plasma, routine blood lipid concentrations (atherogenic index of plasma [AIP], the logarithmic ratio of triglyceride TG) to (high-density lipoprotein–cholesterol [HDL-C] concentration), and diabetic foot osteomyelitis Specific reference test: At least three criteria among: 1) The presence of cellulitis in ulcer wounds 2) Exposure of bone 3) Positive pathogenic bacteria culture in deep bone tissue 4) Radiologic or radio-nuclide scanning images suggestive of inflammatory manifestations of bone tissue 5) Bone histopathologic biopsy diagnosis and neutrophils or chronic inflammation cell invasion define the presence of OM Inclusion criteria: Patients with DFU admitted to hospital The subjects have osteomyelitis ($n = 165$) control cases are patients without osteomyelitis ($n = 275$) Total participants: 440	Results: - Mean AIP in the 165 patients with OM = 0.23 ± 0.24, versus 0.11 ± 0.39 in the controls, OR 1.81 (95% CI 0.71–4.61) At a cut-off of 0.184: • Sensitivity: 84.3% • Specificity: 83.1%	Applicability in patients with diabetes and statins treatment?
Reference: Ozer Balin (2019) Title: Pentraxin-3: A new parameter in predicting the severity of diabetic foot infection?	SIGN: 2 Prospective cohort study	Aim: To evaluate the diagnostic and prognostic values of pentraxin-3 (PTX-3) in patients with infected diabetic foot ulcers (IDFU)	Results: A negative correlation between plasma PTX-3 and glucose levels was found: Healthy controls: 5.83 (3.41–20) ng/mL	First study on the subject

(Continues)

TABLE A1 (Continued)

		as well as to assess the association between PTX-3 levels and IDFU severity Specific reference test: IWGDF/IDSA definition an classification of DFI Inclusion criteria: 60 patients with IDFU (group 1), 45 patients with type 2 DM without diabetic foot ulcers (non-DFU, group 2), and 45 age-matched and sex-matched healthy individuals as controls (group 3) Total participants: 60	Group 1: 1.47 (0.61–15.13) ng/mL Group 2: 3.26 (0.67–20) ng/mL The PTX-3 level in patients who did or did not undergo amputation was 4.1 (0.8–13.7) versus 1 (0.6–15.1) ng/mL, respectively	
Reference: Ryan (2019) Title: Do SIRS criteria predict clinical outcomes in diabetic skin and soft tissue infections?	SIGN: 2 Retrospective cohort study Signe centre study	Aim: To evaluate clinical outcomes of DFI patients with skin and soft tissue infections based on SIRS criteria. Hypothesis: SIRS criteria is a strong predictor of outcomes or adverse events Specific reference test: Need for surgical intervention Lower-extremity amputation Hospital length of stay Wound healing Reinfection Readmission Mortality Inclusion criteria: Patients admitted for severe skin and soft tissue complicating a diabetic foot from 2009 to 2017 Total participants: 137 44 severe DFI I/DSA 93 moderate DFI/IDSA	Results: Severe versus moderate DFI: - The need for surgery (47.7% vs. 59.1%, $p = 0.27$), - The number of surgeries (median 1.0 ± 1.5 vs.1.2 ± 1.4, $p = 0.24$) - Any amputation (20.5% vs. 29.0%, $p = 0.29$) - Minor amputations (13.6% vs. 21.5%, $p = 0.27$) - Major amputations (6.8% vs. 7.5%, $p = 0.34$) - Duration of antibiotics (median 34.1 ± 46.5 vs. 31.9 ± 47 days, $p = 0.28$) - Wound healing within 1 year (68.2% vs. 66.7%, $p = 1.00$) - Time to heal (median 132.3 ± 102.0 vs. 136.5 ± 103.3 days, $p = 0.74$) - Foot-related readmissions to the hospital were more common in patients with moderate infections (46.2% vs. 25.0%, $p = 0.02$) - Length of hospital stay was the only outcome variable that was significantly higher in patients with severe infections (median 12.7 ± 11.9 vs. 7.8 ± 5.8 days, $p < 0.01$)	Selection of the patients unclear
Reference: Sax (2020) Title: Predicting osteomyelitis in patients whose initial MRI demonstrated bone marrow oedema without corresponding T1 signal marrow replacement	SIGN: 2 Retrospective cohort study Single centre study	Aim: To determine which characteristics of diabetic ulcers portend the strongest risk for osteomyelitis inpatients whose initial T1-weighted imaging was normal Specific reference test: Inclusion criteria: Inpatients with suspected DFO. Exclusion criteria: Exclusion criteria included metal or other artefacts, and recent surgery precluding	Results: 34 (56.7%) progressed to DFO ROI ratios >53% had a 6.5-fold increased risk of osteomyelitis, $p < 0.001$ Ulcers greater than 3 cm^2 has a 2-fold increase in the risk of osteomyelitis, $p = 0.04$ Conclusion Increasing bone marrow ROI signal/joint fluid ratios on T2/STIR images were the strongest risk factors for devel-oping osteomyelitis,	

TABLE A1 (Continued)

		evaluation of the bone marrow signal. Total participants: 60 patients (60 pedal ulcers)	while ulcer size and depth are weaker predictors
Reference: Schechter (2020) Title: Percutaneous bone biopsy (PBB) for diabetic foot osteomyelitis: A systematic review and meta-analysis	SIGN: 1 Systematic review and meta-analysis	Aim: Systematic review and meta-analysis on percutaneous bone biopsy (PBB) for DFO; report of the proportion of PBB obtained from patients with suspected DFO that were culture-positive Specific reference test: NA Inclusion criteria: Articles in any language published up to 31 December 2019, reporting the frequency of culture-positive PBBs. Exclusion criteria: NR Total included: 780 patients with 837 PBBs from 11 studies	Results: The proportion of PBBs and wound swabs with identical culture results = 2.8%–17.4% with the highest proportion for *S. aureus* was the bacteria with the highest proportion of concordant results (42.8%) Conclusions: High rates of culture-positive PBBs among included studies. PBBs could be of great benefit for patients with DFO because they could help establish a microbiological diagnosis if proven safe and reliable
Reference: Schmidt (2019) Title: Prospective analysis of surgical bone margins after partial foot amputation in diabetic patients admitted with moderate to severe foot infections	SIGN: 2 Prospective cohort study Single centre study	Aim: To examine the effect of obtainment of proximal surgical margins on patients undergoing ablative diabetic foot surgery for DFO and which patient characteristics are associated with the 'clean,' or noninfected margin Specific reference test: Patient outcomes (wound dehiscence, re-ulceration, re-amputation, death) following surgical intervention for 1 year. Reliability of agreement among board-certified pathologist diagnosis using the kappa coefficient for both distal-to and proximal-to surgical bone margins Inclusion criteria: Patients with either type 1 or type 2 diabetes mellitus who underwent forefoot ablative surgery for suspected bone infection Total participants: 72	Results: Residual osteomyelitis resulted in readmission 2.6 times more often and more postoperative Complications: Strong interobserver reliability: (Kappa coefficient for distal margin = 0.76; for proximal margin = 0.91)
Reference: Sen (2021) Title: Predictive ability of LRINEC score in the prediction of limb loss and mortality in diabetic foot infection	SIGN: 2 Retrospective cohort study Signe centre study	Aim: To assess the predictive ability of LRINEC score in predicting lower limb amputation and in-hospital mortality in patients with diabetic foot infection Specific reference test: Mortality, amputation Inclusion criteria: Patients who were hospitalised with the	Results: Predictive ability of LRINEC score in predicting lower limb amputation and in-hospital mortality in patients with diabetic foot infection Median LRINEC score of patients who underwent amputation was higher than median LRINEC score of

(Continues)

TABLE A1 (Continued)

		diagnosis diabetic foot infections Total participants: 416	patients without amputation ($p < 0.001$) Median LRINEC score of deceased patients was higher than median LRINEC score of patients who survived ($p = 0.022$)	
Reference: Shiraev (2019) Title: Utility of culturing marginal bone in patients undergoing lower limb amputation for infection	SIGN: 2 Retrospective cohort study Single centre study	Aim: Impact of culturing marginal bone on antibiotic use or further surgical intervention, Specific reference test: No bone biopsy Inclusion criteria: Patients with any type of lower limb extremity because of infection on the background of diabetes and or arterial insufficiency Exclusion criteria: No exclusion criteria Total participants: 132 including 79 (59,8%) with diabetes	Results: Patients with a positive bone margin are more likely to undergo further surgical intervention (overall, 20,0% vs. 6,1% $p = 0,47$ without significant difference in the need for further intervention in pts with or without diabetes 13.4% vs. 10.1%); 8/8 pts with neg. bone margin received further AT versus 45/50 (95%) pts with pos. bone margin Conclusions: A positive bone margin culture is associated with the need for further surgical intervention. The benefit of this procedure is unclear	Comments:
Reference: Tardáguila-García (2021) Title: Diagnostic accuracy of bone culture versus biopsy in diabetic foot osteomyelitis	SIGN: 2 Cross-sectional study Single centre study	Aim: To compare the diagnostic accuracy of bone culture (microbiology) and biopsy (histology) in patients with acute or chronic diabetic foot osteomyelitis (DFO) based on clinical suspicion of DFO was established through a combination of the probe-to-bone (PTB) test and a plain X-ray examination. Two experienced clinicians preformed PTB and reviewed X-ray individually. Both tests had to be positive per both clinicians for inclusion in study Specific reference test: Bone culture Inclusion criteria: Exclusion criteria: The exclusion criteria were critical limb ischaemia, Charcot foot, indication of medical treatment for DFO pregnancy, and breastfeeding Total participants: 52	Results: Thirty-six patients (69.2%) had positive bone culture results; 28 samples (77.7%) were monomicrobial, and 8 samples polymicrobial (22.3%). Of 42 that tested positive histologically for osteomyelitis, 36 (76.5%) were also microbiologically positive. The results show that bone culture had a sensitivity of 0.70, specificity of 0.40, PPV of 0.92, and NPV of 0.13; the LR + was1.20, and LR−was 0.86	
Reference: Uçkay (2021) Title: How good are clinicians in predicting the presence of *Pseudomonas* spp. in diabetic foot infections? A	SIGN: 2 Prospective cohort study Single centre study	Aim: Compare clinical physicians' predictions of DFI-about the presence of *Pseudomonas* spp. in intraoperative tissues	Results: Sensitivity: 0.32 Specificity: 0.84 PPV: 0.18 NPV: 0.92	Comments: No data about the type and severity of DFIs

TABLE A1 (Continued)

prospective clinical evaluation		Specific reference test: Results tissue cultures and/or clinical remission of DFI achieved with antibiotic agents that did not cover *Pseudomonas* spp. Inclusion criteria: Consecutive patients admitted for DFI Exclusion criteria: NR Total participants: 88 (221 episodes of DFI)	No impact of gram-negative rods on gram staining Conclusions: Further investigations needed to determine if negative prediction can prevent the use of anti-Pseudomonas spp. agents in patients administered for DFI	
Reference: Xu (2021) Title: Erythrocyte sedimentation rate combined with the probe-to-bone test for fast and early diagnosis of diabetic foot osteomyelitis	SIGN: 2 Retrospective cohort study Single centre study	Aim: o Find the optimal cutoff of ESR for Chinese patients and assess the value of ESR combing with PTB to fast diagnosis DFO in early stage Specific reference test: Bone biopsy (culture and/or histopathology) (if suspicion of DFO) Inclusion criteria: Patients admitted to hospital for a DFI Exclusion criteria: Patients with other infectious diseases or rheumatic immune diseases, hepatic insufficiency (alanine transaminase >100 IU/L), cardiac function level 4 (New York Heart Association), end-stage renal disease requiring dialysis, and diabetic foot patients with severe peripheral artery disease (ankle brachial index <0.5) before revascularisation Total participants: 204 patients 111 patients with DFO (3 patients refused bone biopsy) 86 with non-DFO DFI	Results: 1) The best cutoff value of DFO was ESR >43 mm/h: Se = 82.9%, Spe = 70.5%, + = 0.78, −PV = 0.77, + LR = 2.8, −LR = 0.24 2) ESR >50 mm/h: Se = 47.7%, Spe = 88.6%, + PV = 0.84, −PV = missing data, + LR = 4.2, −LR = 0.59 3) PTB only: Se = 76.6%, Spe = 93.2%, + PV = 0.93, −PV = 0.76, + LR = 11.26, −LR = 0.25 4) ESR >43 mm/h with PTB-positive (both positive to diagnosis of DFO): Se = 63.56%, Spe = 98%, + PV = 0.97, −PV = 0.67, + LR = 31.75, −LR = 0.37 5) ESR >43 mm/h, PTB-positive (any 1 test positive to diagnosis of DFO: Se = 96%, Spe = 65.7%, + PV = 0.78, −PV = 0.93, + LR = 2.8, −LR = 0.06	The provided data only apply to the Chinese population