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SUPPLEMENT ARTICLE

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

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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 potentially mortality and present formidable challenges in surgical and antimicrobial treatment.

Methods: In June 2018, we searched the literature using PuEbMed and EMBASE for published studies on the diagnosis of diabetic foot infection. On the basis of predetermined criteria, we reviewed prospective controlled, as well as noncontrolled, studies in any language, seeking translations for those not in English. We then developed evidence statements on the basis of the included papers.

Results: From the 4242 records screened, we selected 35 papers that met our inclusion criteria. The quality of all but one 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

Abbreviations: IWGDF, International Working Group on the Diabetic Foot; PAD, peripheral artery disease; PICO, population (or patients), intervention, control (or comparator), outcome(s); PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized controlled trial; SIGN, Scottish Intercollegiate Guidelines Network.

scheme correlates with the patient's likelihood of ulcer healing, of lower extremity amputation, and risk of death. Elevated levels of selected serum inflammatory markers are supportive, but not diagnostic, of soft tissue or bone infection. In patients with suspected diabetic foot osteomyelitis, both a positive probe-to-bone test and an elevated erythrocyte sedimentation rate are strongly associated with its presence. 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. Plain X-ray remains the first-line imaging examination when there is suspicion of diabetic foot osteomyelitis, but advanced imaging methods help in cases when either the diagnosis or the localization of infection is uncertain.

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

KEYWORDS

diabetes mellitus, diabetic foot, diagnosis, foot ulcer, imaging studies, inflammatory markers, osteomyelitis, probe-to-bone, systematic review

1 | INTRODUCTION

Foot infections are frequent complications of diabetes mellitus that are associated with high morbidity, occasional mortality, and heavy resource utilization, 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 (DFI) result in amputation,[3] making this 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 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 therapeutic strategies for patients with a DFI.[5,6]

Although it is a more difficult process than performing a systematic review on treatment, the editorial board of the International Working Group on the Diabetic Foot (IWGDF) asked the working group on DFI to conduct a systematic review looking at all available publications on diagnosis of DFI. We sought publications that contained original research information on 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 IWGDF guideline on diagnosis and treatment of DFIs also published in this issue of Diabetes/Metabolism Research and Reviews.

2 | METHODS

We performed the literature search for this systematic review on June 30, 2018 on the basis of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[7] On October 28, 2018, we prospectively registered the systematic review in the PROSPERO database for systematic reviews, which assigned it the number CRD42018102682.

2.1 | Creation of population (or patients), intervention, control (or comparator), outcome(s)

We began by defining the population (patients) of interest (P), interventions (I) performed and outcomes (O) assessed, and thereby formulated the clinical questions (population (or patients), intervention, control (or comparator), outcome(s) [PICOs]) we would attempt to address. The IWGDF editorial board and 12 external experts (not members of the guideline working group) from various geographical regions worldwide then reviewed these questions and PICOs for their clinical relevance. Using their input, we revised the PICOs to their final form for this review.

2.2 | Literature review

With our oversight, two medical librarians performed electronic database searches using the databases of MEDLINE (PubMed), EMBASE, and Scopus, using a combination of MeSH and keyword terms. The search terms we used were ((((((((diagnostic imaging[MeSH Terms]) OR diagnosis[MeSH Subheading]) OR microbiology[MeSH Subheading]) AND diabetic foot[MeSH Terms])) OR ((((foot disease* [MeSH Terms]) OR osteomyelitis[MeSH Terms]) AND diabetes mellitus[MeSH Terms]) AND diagnosis[MeSH Subheading])) OR (((diabetic foot/blood[MeSH Terms]) OR diagnosis[MeSH Subheading]) AND diabetic foot infection[Title])) OR (((diabetic foot [Title/Abstract]) AND diagnosis[Title/Abstract]) AND ("2017/01/01"[Date - Publication]: "3000"[Date - Publication]))) AND humans[MeSH Terms].

2.3 | Selection criteria

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 Infectious Diseases Society of America (IDSA)/IWGDF classifications.[8,9] These two organizations have independently developed a classification scheme for defining the presence and severity of DFI, but the committees share the same chair and several members, and the two schemes are nearly identical. We selected studies using the following criteria: enrolled patients had a diagnosis of DFI on the basis of the IDSA or IWGDF classification and, in case of osteomyelitis, on the results of a bone specimen examination (ie, microbiological and/or histological evaluation); and they presented primary research involving clinical findings, microbiological assessment, biomarkers, or imaging techniques. The infection working group agreed that acceptable study designs could include meta-analyses, systematic reviews, randomized 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 evaluation of the diabetic population was not individualized, and studies that included fewer than 15 patients with diabetes.

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 (ie, diagnosis of DFI) that had to be identified in the literature search. Our search terms identified all 20 publications. 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 working group members, 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)). After the two members of each team reached consensus on which papers met the criteria, they obtained and independently reviewed the full paper of all potentially eligible publications using the same key criteria to determine final eligibility for inclusion. The two reviewers then independently performed an extraction of the data from each included paper by using a form on the basis of the QUADAS-2 tool for the quality assessment of studies.[10] For the initial review, the final inclusion decision, and the data extraction, the two reviewers compared their opinions and reached consensus when necessary.

2.4 | Classifying study design and risk of bias

We classified the design of each included study using the Scottish Intercollegiate Grouping Network (SIGN) algorithm (https://www.sign.ac.uk/assets/study_design.pdf). The SIGN levels of evidence were level 1 for RCTs and level 2 for case-control and cohort studies. Risk of bias was scored (using SIGN) for each study as ++ (*very low risk of bias*), + (*low risk of bias*), or − (*high risk of bias*).

2.5 | Developing an evidence table

After appropriate data were extracted from each included paper, they were summarized in a standardized 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 performance statistics, and an open field for comments. By both electronic communications and at an in-person meeting, each member of the working group reviewed and discussed the content of the evidence tables. On the basis of the data in this evidence table, we formulated evidence statements. Working group member(s) did *not* participate in the selection or the discussion of a paper if they were (co)-author of that paper.

3 | RESULTS

The risk of bias assessment of each paper can be found in Table 1. The full evidence table can be found in Appendix S1. The PRISMA flowchart with study selection process is shown in Figure 1.

PICO: In a person with diabetes and a foot infection, do increasing levels of severity of the IWGDF/IDSA criteria correlate with increasing rates of adverse outcomes (eg, need for hospitalization, failure to resolve infection, and lower extremity amputation)?

Summary of the literature: In a study[11] at a diabetic foot referral centre in Tanzania, 252 diabetic patients presenting with 375 foot ulcers were examined prospectively using the following classifications: Meggitt/Wagner; University of Texas; Sepsis, Arteriopathy, and Denervation [S(AD)SAD]; and, Perfusion, Extension, Depth, Infection, Sensation (PEDIS IWGDF classification scheme). The infection is scored as described above using criteria similar to those of the IDSA. Using the χ^2 test for trend, the results of the classifications correlated strongly with wound healing or ulcer resolution, assessed by either the patient undergoing lower extremity amputation or dying. Specifically, there was a significant trend between a

TABLE 1 Risk of bias of included publications

Reference	Study Groups Defined	Selection Bias Avoided/ Excluded	Outcome clearly Defined	Outcome Assessed Blind for Exposure	Selective Loss to Follow-up Excluded	Major Confounders/ Prognostic Factors Identified and Controlled	Selective Reporting Ruled Out?	Free From Commercial Interest?	Score
Abbas et al[11]	+	+	+	–	+	+	+	+	7/8
Abbas et al[12]	+	+	+	–	+	–	+	+	6/8
Abdel Razek and Samir[43]	+	+	+	–	+	+	+	+	7/8
Aslangul et al[44]	+	+	+	–	+	–	–	–	4/8
Blume et al[40]	+	+	+	–	–	–	+	–	4/8
Ertugrul et al[35]	+	?	+	–	?	?	+	+	4/8
Fleischer[29]	+	–	+	–	?	+	?	?	3/8
Hayes et al[33]	+	–	+	+	?	–	–	+	4/8
Hazenberg et[17]	+	+	+	–	?	–	+	+	5/8
Ingram et al[14]	+	+	+	+	+	+	+	–	7/8
Johnson et al[36]	+	+	+	+	?	?	+	+	6/8
Lam et al[25]	+	+	+	?	?	?	+	+	5/8
Lauri et al[34]	+	+	+	?	?	–	+	+	5/8
Lavery et al[13]	+	+	+	–	+	?	+	+	6/8
Löffler et al[15]	+	+	+	+	–	–	–	+	5/8
Morales-Lozano et al[30]	+	+	+	–	+	+	+	–	6/8
Mutluoglu et al[48]	+	+	+	–	+	+	+	–	6/8
Nawaz et al[37]	+	+	+	+	?	–	–	+	5/8
Nelson et al[50]	+	+	–	–	?	–	+	+	4/8
Newman et al[38]	+	+	+	+	–	–	–	+	5/8
Ottolino-Perry et al[64]	+	–	+	+	?	–	–	–	3/8
Poosapadi Arjunan et al[75]	–	–	+	?	?	–	+	+	8/8
Poirier et al[45]	+	+	+	–	+	+	+	+	7/8
Rastogi et al[42]	+	+	+	+	–	+	+	+	7/8
Senneville et al[22]	+	+	+	+	?	+	+	+	7/8
Senneville et al[23]	+	+	+	+	?	+	+	+	7/8
Shagos et al[39]	+	+	+	–	?	–	+	+	5/8-
Sotto et al[65]	+	+	+	+	+	+	+	+	8/8
Treglia et al[41]	+	+	+	?	?	–	+	+	5/8
van Asten et al[51]	+	+	+	?	?	–	+	+	5/8
van Asten et al[31]	+	+	+	–	+	?	+	+	6/8
van Asten et al[32]	+	+	+	+	+	–	+	+	7/8
Weiner et al[24]	+	+	+	+	?	–	+	+	6/8
Wukich et al[54]	+	+	+	–	+	–	–	–	4/8

Note. Green +, yes; red –, no; and amber, unclear.

more severe infection classification (using the PEDIS criterion of infection) with higher risk of ulcer nonhealing (χ^2 37.927; $P < .001$) and greater ulcer depth (χ^2 70.558; $P < .001$).[11] The conclusions that can be drawn from this study are limited by the fact that only 3.7% of the total population had a grade 4 infection according to PEDIS, and about 20% of the included patients were lost to follow-up. In

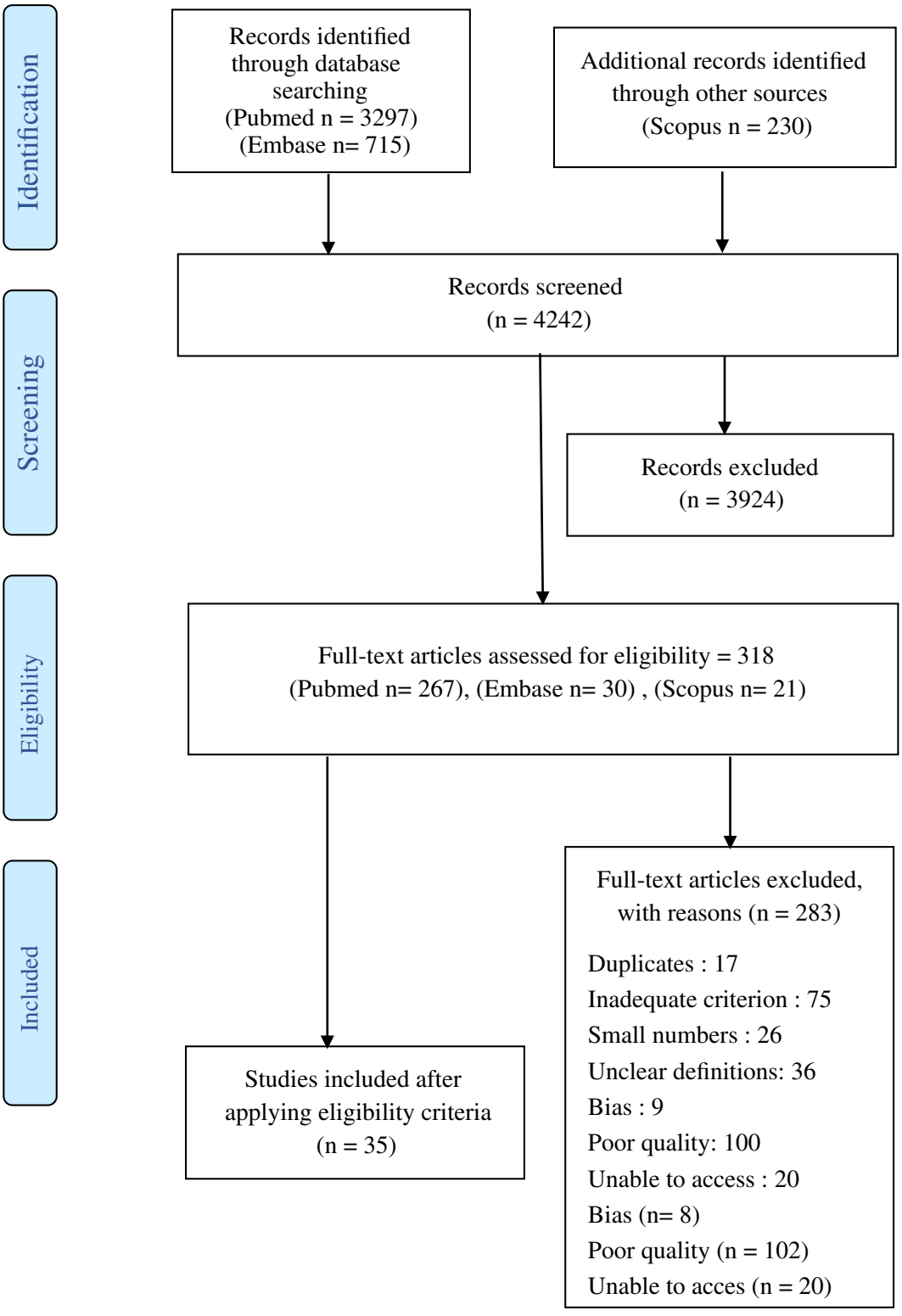

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

addition, the mean age of the population was relatively young (54.7 years), and evidence of peripheral arteriopathy was detected clinically in only 44 (11.7%), which is considerably lower than in diabetic population suffering from infected DFUs reported from high income countries.[8,9,11]

Evidence statement 1: IWGDF/IDSA classification of infection of the diabetic foot correlates with ulcer healing and both the likelihood of lower extremity amputation and risk of death.

Quality of the evidence: Low. On the basis of a single, non-controlled study.

Reference: Abbas et al[11]

PICO: Which persons presenting with diabetes and foot infection should be hospitalized for management of infection?

Summary of literature: In the prospective study by Abbas et al,[11] there was a correlation between the IWGDF/IDSA class and the need for hospitalization, as well as for lower extremity amputation. These results are consistent with a previous study by Lavery et al[13] on the basis of results of their prospective cohort study of 1666 diabetic patients who participated in a disease management programme. 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. They observed a trend with increasing IDSA infection classification severity towards an increased risk for lower extremity amputation, higher anatomic levels of lower extremity amputation, and lower extremity-related hospitalizations. In patients with grade 4/severe infections, about 80% were hospitalized, and 90% underwent lower extremity amputation. In this study, the need for amputation and for hospitalization did not differ between those who had no infection compared with mild infection. This may reflect either the frequent difficulty in distinguishing clinically uninfected vs mildly infected DFUs or the fact that that the large subgroup of patients with a mild DFI do not need hospitalization and rarely require amputation.

Evidence statement 2: Hospitalization should be considered in many patients with IWGDF/IDSA grade 3/moderate and all with 4/severe in view of their high risk of lower extremity complications, such as amputations.

Quality of the evidence: Low. On the basis of noncontrolled study.

Reference: Abbas et al[11] and Lavery et al[13]

PICO: In a person with diabetes and a suspected foot infection, how well do the IWGDF/IDSA clinical criteria for diagnosing soft tissue infection correlate with other diagnostic tests?

3.1 | Biomarkers

Summary of the literature: One study of 27 mild/class 2 infected DFUs and 34 noninfected DFUs from patients in a community setting prospectively assessed the performance characteristics of the following biomarkers of inflammation: white blood cell (WBC) count, C-reactive protein (CRP), procalcitonin (PCT), and calprotectin (CPT), a new specific marker for infection.[14] All enrolled patients had not been treated with any antibiotics for at least 2 weeks before inclusion in the study. PCT levels in 41 out of 59 samples, including 21 from 29 infected DFU, were undetectable. The authors devised a likelihood score for infection using the three other biomarkers, but it failed to demonstrate any benefit in distinguishing infected from noninfected DFUs. When CPT was replaced by a clinical sign (ulcer area) in a new likelihood score, its sensitivity was 0.64, specificity 0.81, positive predictive value (PPV) 0.73, and negative predictive value (NPV) 0.75. These results suggest that WBC, as well as CRP, PCT, and CPT are of limited value in assisting with the diagnosis of mild/class 2 DFI in patients seen in the community setting.[14] One other study[15] compared the lactate concentrations in wound fluid, collected by the Levine technique,[16] from infected and noninfected DFUs. Overall median wound fluid lactate concentration was 21.03 mM (5.58-80.40 mM), but it was significantly higher in infected vs noninfected DFU [27.18 mM (7.14-80.40 mM) vs 18.38 mM (5.58-50.34 mM); $P = .001$].

Evidence statement 3: Inflammatory serum biomarkers, such as CRP, PCT, and wound lactate concentration, show relatively little correlation with the presence of a DFI.

Quality of the evidence: Low. On the basis of two cohort studies.

References: Ingram et al[14] and Löffler et al[15]

PICO: In a person with diabetes and a suspected foot infection, do the IDSA/IWGDF criteria for diagnosing soft tissue infection correlate with results of skin temperature measurement or quantitative microbiology?

Summary of the literature: The validity and reliability of assessing DFI using photographic foot imaging and infrared thermography were reported in a convenience sample of 38 consecutive patients with diabetes who presented with a foot infection or were admitted to an inpatient clinic with a foot-related complication. The authors suggested that foot infection could be suspected on the basis of a temperature difference between the affected and the unaffected foot of greater than 2.2°C, which they defined as a "hotspot."[17] Two independent observers compared "live" clinical assessment using the PEDIS classification with photographs (assessed for the presence of erythema and ulcers) taken at study inclusion and at 2 and 4 weeks later. The specificity of photographs for the diagnosis of infection was greather than 85%, but the sensitivity was greater than 60%. On the other hand, diagnosis of infection on the basis of hotspots present has a sensitivity greater than 90% but a specificity of less than 25%. By combining photographic and temperature assessments using a parallel strategy, the sensitivity was 61-70% and specificity 79-80%, resulting in positive and NPVs of 80-83% and 53-65%, respectively. Intraobserver agreement between photographic assessments was good (Cohen $\kappa = 0.77$) and moderate (0.52) between the two observers.[17]

In a systematic review of the diagnostic performance of clinical examination and wound sampling with classic microbiological analysis for infected DFUs, O'Meara et al[18] identified three eligible studies.[19-21] Soft tissue infection was defined by growth on culture of greater than 10^5 colony-forming units (CFUs) per gram of tissue for tissue biopsy specimens and greater than 10^5 CFU per square centimetre for swab specimens. In a cross-sectional study, patients with chronic wounds of various aetiologies, including two with DFUs, underwent punch biopsy culture as the reference test, while the index test consisted of the use of a clinical signs and symptoms checklist containing 11 items (pain, erythema, oedema, heat, purulent exudate, serous exudate plus concurrent inflammation, delayed healing, discoloration of granulation tissue, friable granulation tissue, foul odour, and wound breakdown).[19] The highest sensitivity values (around 80% for friable granulation and delayed healing) were associated with specificity values of 76% and 64%, respectively. Another study of the diagnosis of infection in 38 patients with chronic wounds, of which 10 were

DFUs, used a positive culture of a punch biopsy as the reference test and wound swab with quantitative analysis (ie, greater than 10^5 CFU per gram of tissue for biopsy and 10^5 CFU per square centimetre for swabs) as the index test.[20] The estimated sensitivity for quantitative analysis of a wound swab was 79% and the specificity was 60%, as compared with the tissue biopsy results. In a third culture study of chronic wounds, in which 29 of 124 patients had DFUs, results of specimens for culture assessed by semiquantitative analysis were compared with those using quantitative analysis as the reference standard.[21] By using four different thresholds (from highest to lowest inoculum) of bacterial growth on four sequential quadrants of an agar plate by semiquantitative analysis, the best values of sensitivity/specificity (ie, 79/90%) were obtained for the third threshold, corresponding to observed bacterial growth in the first three of the four quadrants.[21]

Evidence statement 4a: Results of neither infrared thermography nor photography examinations alone correlate with the IDSA/IWGDF criteria of infection; while the two combined may offer satisfactory specificity, sensitivity was low and thermography currently has limited availability.

Quality of evidence rating: Low. On the basis of only one prospective study with high risk of bias.

References: Hazenberg et al[17]

Evidence statement 4b: Wound bioburden assessed by either quantitative or semiquantitative analysis of the microbial load does not correlate well with the presence of an infection.

Quality of the evidenceb: Low. On the basis of case-control or retrospective series of patients, most of relatively low quality and some with a small number of patients.

Reference: Gardner et al,[22] Bill et al,[19], and Ratliff and Rodeheaver[20]

PICO: In a person with diabetes and suspected bone infection of the foot, which diagnostic tests best correlate with the presence of osteomyelitis, as diagnosed on the basis of culture and/or histopathology of a bone specimen?

Summary of literature: Two prospective studies from Senneville et al compared the results of bone with soft tissue cultures in the same patient. One 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.[21] The other study[23] reported 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. In a matched case-control study, 44 bone specimens from diabetic patients the authors analysed, then compared, the value of histology vs microbiology for making the diagnosis of osteomyelitis.[24] They concluded that finding a positive microbiological and negative histological result was just as likely as a negative microbiological and positive histological result, suggesting that the two methods were equally useful diagnostically. Of note, however, is that only the culture provides information about the causative pathogen and its antibiotic susceptibilities.

3.2 | Clinical findings

The main clinical technique evaluated in the published literature for diagnosing diabetic foot osteomyelitis has been the probe-to-bone test (PTB test). One recent systematic review[25] included seven studies with a cumulative total of 1017 patients. All seven studies used culture and/or histopathology of bone specimens to confirm osteomyelitis; in six, it was the sole method while one also used magnetic resonance imaging (MRI). The analysis demonstrated that 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%. As predicted by Bayes theorem for any diagnostic test, the PPV increased when the PTB test was used in clinical settings or among populations with high pretest probability of osteomyelitis, while the NPV increased in settings or among populations with a low pretest probability. On the basis of the pooled sensitivity and specificity, we calculated that the positive and negative likelihood ratios of the PTB test in diagnosing or excluding diabetic foot osteomyelitis would be 5.1 and 0.16, respectively. While three cohort studies[26-28] were eligible for inclusion in this systematic review, two others that we identified were not.[29,30] One of the excluded papers was a case-control study of 54 patients with diabetes seen in a tertiary-care hospital[30] that found that the PTB test had a sensitivity of 85% but a specificity of only 47% for diagnosing osteomyelitis (confirmed by histology). Among the 30 clinical and laboratory characteristics reviewed in this study, the most accurate tests to differentiate osteomyelitis from cellulitis were ulcer depth > 3 mm (univariate odds ratio 10.4; $P = .001$) and a CRP > 3.2 mg/dL (univariate odds ratio 10.8; $P < .001$). The combination of ulcer depth with elevated serum inflammatory markers [CRP > 3.2 mg/dL or erythrocyte sedimentation rate (ESR) > 60 mm/h] proved most useful in detecting bone infection (sensitivity 100%). The other excluded paper, a prospective cohort study[31] 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%. Of note, different indices were assessed and if positive, the reference test was performed; this may have underestimated the number of false negative cases and thus overestimated the sensitivity of the test. In addition, the clinical signs that led the authors to suspect an osteomyelitis were not described. 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. Of the 132 feet with clinical suspicion of infection studied, 105 (79.5%) were diagnosed as osteomyelitis on the basis of histopathology. A correct diagnosis was made by the combination of PTB test and plain X-rays in 98.4% of the neuropathic ulcers compared with 88% of the neuro-ischaemic ulcers. A multivariate analysis that included the ulcer type (neuro-ischaemic or neuropathic) and its duration marginally improved the PPV of the PTB test to 94.5%.[30] Thus, in addition to the performing clinician's experience and the ulcer's anatomic location, the accuracy of the PTB test may vary with the aetiology of the ulcer.

3.3 | Biomarkers

A systematic review published in 2016[31] investigated the value of using serum inflammatory markers to diagnose diabetic foot osteomyelitis. ESR, with a pooled sensitivity of 0.81 and a specificity of 0.90, was the most useful of the tests in distinguishing osteomyelitis from cellulitis (soft tissue infection). Unfortunately, there was insufficient data to draw conclusions about the other markers studied [CRP, PCT, interleukins (IL) 2, 6, and 8, and tumour necrosis factor alpha (TNFα)]. Other factors limiting the ability to compare studies were the low methodological quality of the available studies, small patient populations, and high variability of reference tests and cut-off values of the markers used.

One prospective cohort study from the same group[32] investigated the levels of ESR, CRP, PCT, IL-6, IL-8, TNFα, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1 alpha (MIP1α) at three different time points in 35 patients with a DFI. In this study, the level of PCT best distinguished osteomyelitis from cellulitis at baseline (0.26 ng/mL ± 0.45 [mean ± SD] vs 0.07 ng/mL ± 0.07; P = .049), closely followed by CRP (10.08 mg/dL vs 5.44 mg/dL; P = .054). The conclusions from this study are limited by the fact that the inflammatory markers were not normally distributed, and the authors used the nonparametric Wilcoxon rank test. After starting antibiotic therapy in the group with osteomyelitis, the CRP, ESR, PCT, and IL-6 levels all significantly declined, suggesting that these biomarkers might also be useful for ascertaining the effectiveness of therapy during follow-up.

One recent case-control study from Australia[33] found significantly higher serum levels of procollagen type 1 N propeptide (P1NP), a bone formation turnover marker, in 16 patients with diabetic foot osteomyelitis compared with 11 patients with DFUs without osteomyelitis (10.5 ± 5.2 ng/mL vs 3.1 ± 2.8 ng/mL; P = .001). The mean serum P1NP levels were significantly higher in the diabetic foot osteomyelitis than the control group (10.5 ng/mL vs 3.1 ± 2.8 ng/mL; P = .001). An elevated P1NP level had a sensitivity of 86.7% and a specificity of 80% compared with 70.6% and 80% for CRP.

3.4 | Imaging

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.[34] 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 a 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 ^{111}In-oxine-WBC scintigraphy, 10 studies with 206 patients studied ^{99m}Tc-HMPAO WBC scintigraphy, and 6 studies with 254 patients investigated FDG-PET/CT. While the pooled sensitivity of all the different imaging modalities were comparable (89-93%), the specificity of MRI (75%, 63-84%) and ^{111}In-oxine WBC scintigraphy (75%, 66-82%) were considerably lower than ^{99m}Tc-HMPAO WBC scintigraphy (92%, 78-98%) and ^{18}F-FDG-PET/CT (92%, 85-96). Five studies[35-39] identified in our search were included in this meta-analysis. We found one small, prospective preliminary study[40] that was not included in the meta-analysis that reported similar results for ^{99m}Tc-HMPAO WBC imaging in diagnosing diabetic foot osteomyelitis (sensitivity 90% and specificity 86%).

Another meta-analysis with low risk of bias[41] that examined ^{18}F-FDG PET and ^{18}F-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 studies included reported a sensitivity of 29% but a high specificity (91%; 95% CI, 85-96%). Although this systematic review assessed nine studies, only four were included in the meta-analysis. The presence of osteomyelitis, documented in six studies, ranged from 10-54% (average approximately 30%) of the enrolled cases. One prospective study not included in this analysis[42] also found that for diagnosing osteomyelitis in patients with Charcot neuroarthropathy, ^{18}F-FDG-labelled leukocyte PET/CT had the same sensitivity (83.3%) as contrast to enhanced MRI, but its specificity was 100%, compared with 63.6%. One small prospective study of low quality[43] found that diffusion-weighted MRI for the diagnosis of diabetic foot osteomyelitis had low performance characteristics (sensitivity 64.6% and accuracy 63.7%). However, when using a calculated apparent diffusion coefficient, two reviewers were able to differentiate diabetic osteoarthropathy from osteomyelitis with an accuracy of 94% and 93%, with excellent interobserver agreement. Aiming to develop a simple bedside strategy and to avoid unnecessary antibiotic use, one prospective cohort study[44] coupled ^{67}Ga single-photon emission computerized tomography (SPECT) imaging and bedside percutaneous bone puncture in patients with suspected bone infection of the foot. Among 55 patients who underwent hybrid ^{67}Ga SPECT and X-ray CT (SPECT/CT) imaging, those with a positive scan (N = 40) then 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[45] found that the combination of ^{99m}Tc-HMPAO leukocyte scintigraphy and ^{99m}Tc-MDP bone scintigraphy in 75 patients with suspected bone infection had a sensitivity of 92.6% and a specificity of 97.6%. This combination of imaging tests, with its high spatial resolution, seemed to be very helpful in differentiating bone infection from soft tissue infection, especially in patients with Charcot neuroarthropathy.

Evidence statement 5a: Bone (as opposed to soft tissue) sample analysis by microbiological or histological methods is the most appropriate way to confirm bone involvement complicating a DFI, as well as to identify clinically relevant bone microorganisms.

References: Senneville et al[21], Senneville, Morant, et al[23], and Weiner et al[24]

Quality of evidence: Moderate.

Evidence statement 5b: The PTB test is useful to help diagnose diabetic foot osteomyelitis in high-risk patients when it is positive and

rule it out in settings with a low pretest probability when it is negative.

Quality of the evidence: Low. On the basis of cohort studies with methodological biases.

References: Lam et al,[25] Lavery et al,[26] Mutluoglu et al,[27] Zaiton et al,[28] Fleischer et al,[29] and Morales-Lozano et al[30]

Evidence statement 5c: In a person with diabetes and suspected bone infection of the foot, elevation of the ESR is the most useful of the available laboratory (inflammatory marker) tests to diagnose osteomyelitis.

References: van Asten et al[31] and Hayes et al[33]

Level of evidence: Low. On the basis of retrospective cohort studies.

Evidence statement 5d: MRI, WBC scintigraphy, and ^{18}F-FDG-PET/CT may be 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.

Quality of evidence: Moderate. On the basis of two meta-analyses and prospective cohort studies.

References: Lauri et al,[34] Ertugrul et al,[35] Johnson et al,[36] Nawaz et al,[37] Newman et als,[38] Shagos et al,[39] Blume et al,[40] Treglia et al,[41] Rastogi et al,[42] Abdel Razek and Samir,[43] Aslangul et al,[44] and Poirier et al[45]

PICO: In a person with diabetes and a foot infection, do specimens of wound tissue (obtained by curettage or biopsy) provide more clinically useful information on growth of pathogens or avoidance of contaminants than wound swabs?

Summary of literature: In a prospective study, Huang et al[46] compared swabs vs 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.[46] 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] These data are consistent with those reported by Gardner et al,[47] who reported that in uninfected ulcers, a longer duration was associated with a shift in the type of bacteria colonizing the ulcer. Mutluoglu et al[48] compared the culture results of 89 pairs of swabs vs deep tissue specimens in 54 patients with DFUs, 47 (87%) of which were infected. 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 swabs in these settings was 73%.[48] In another study, the results of swab vs tissue sample culture were compared in patients with neuropathic (n = 28) and neuro-ischaemic (n = 22) DFUs.[49] The number of isolates was higher on swabs vs 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 = .033) in neuropathic DFUs.[49] The results of these studies, on the basis of small size populations, 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).[50]

Evidence statement 6: Deep tissue sample cultures provide more accurate information than do swabs for the microbiological documentation of infected DFUs.

Quality of evidence: Moderate. On the basis of one well-designed prospective study and low-level cohort studies.

References: Huang et al,[46] Gardner et al,[47] Mutluoglu et al,[48] Demetriou et al,[49] and Nelson et al[50]

PICO 5: In a person with diabetes and a foot infection, do the results of molecular (genotypic) microbiological tests better distinguish likely clinically relevant pathogens requiring antibiotic therapy than standard (phenotypic) cultures?

Summary of the literature: An observational prospective study compared the results of conventional culture of bone samples taken from 34 patients with suspected bone infection on the basis of the IWGDF/IDSA classification with results obtained using a 16*S* ribosomal ribonucleic gene sequencing approach.[51] Bone samples were obtained by percutaneous biopsy through noninfected skin and processed by histopathological and microbiological examination. While three bone samples that were positive on conventional culture were negative by the molecular technique, there was an overall higher prevalence with the molecular compared with the conventional technique of anaerobic pathogens (86.9% vs 23.1%, P = .001), gram-positive bacilli, especially *Corynebacterium* spp. (78.3% vs 3.8%, P < .001), and polymicrobial infections (91.3% vs 64.0%, P = .125). The study did not provide a comparison of the results of the two culture techniques according to the IWGDF/IDSA grade of the infection.

Evidence statement 7: The clinical usefulness of the additional information provided by molecular (genotypic) compared with standard (phenotypic) microbiological tests for diagnosing diabetic foot osteomyelitis in the daily practice is currently unknown.

Quality of the evidence: Low. On the basis of one prospective cohort study.

Reference: van Asten et al[51]

4 | DISCUSSION

This article presents the first systematic review conducted by the IWGDF on diagnosis of DFI and is based on the results of our search for publications that investigated any means of diagnosis of any type of foot infection in persons with diabetes. We only included studies that used specific definitions of infection for enrolled subjects: (a) the IWGDF/IDSA classification scheme to define the presence of an infection and (b) examining a bone sample (by microbiological culture and/or histopathology) to define a bone infection. Among the 4242 papers returned by our search string, only 35 met our criteria for inclusion in this review; these consisted of 28 prospective cohort studies, three meta-analyses, two systematic reviews, and two case-control studies. As the IWGDF/IDSA consensus definition and classification of DFIs were not published until 2004,[52,53] and we used these

recommendations as a basis for the selection of the papers, we chose not to refer to studies published before this date, except for four[36,38,40,45] in which the diagnostic criteria of infection used were consistent with the IWGDF/IDSA recommendations. On the other hand, even during these last 15 years, not all published studies used this classification to describe infection in the enrolled patients. This issue contributes to the relatively low number of reviewed papers included in this systematic review. Another limitation is that we decided to only include studies that enrolled at least 15 evaluable patients with diabetes and to require bone tissue examination (to establish a diagnosis) for studies on diabetic foot osteomyelitis. The strict selection of the papers in this systematic review precluded proposing evidence statements for some of the questions of interest in the field.

Given the several different clinical presentations of infectious complications of the foot, the IWGDF/IDSA classification schemes for DFI include four classes on the basis of the presence and severity of infection.[8,9] The simplicity of the IWGDF/IDSA scheme, compared with the other existing classifications, is one of its major advantages. Wukich et al[54] validated part of the IWGDF/IDSA classification by comparing clinical outcomes in a prospective cohort study of patients with a moderate vs a severe DFI and found that patients with greater than or equal to two findings of systemic inflammatory response syndrome (ie, severe infection) had worse clinical outcomes, including more and higher level lower extremity amputations.[54] Results of a more recent prospective study from the multicentre Eurodiale group that included 575 patients with a DFI also demonstrated that the IWGDF/IDSA severity predicted the need for lower extremity amputation.[55] Of note is that as of the 2019 guideline, the IWGDF 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.

Studies have investigated the role of various inflammatory biomarkers measured in blood for diagnosing infection and in distinguishing skin/soft tissue infection from osteomyelitis in diabetic patients with a foot ulcer.[56-63] Most of the published studies have addressed the value of WBC, ESR, CRP, or PCT by comparing serum levels with the presence of clinical signs of infection.[56,58,61,63] About half of the studies we included investigated the value of the WBC for assessing infection of a DFU, and these generally found no significant correlation between the WBC counts and the infection severity.[56,58,61,63] All[56-62] but two studies[13,63] of serum PCT values reported that they were significantly higher in infected than noninfected DFU; in one other study, PCT was found significantly higher in patients with osteomyelitis.[32] No published study has reported a correlation between the level of PCT and the severity of infection according to the IDSA/IWGDF classification. Of note is that in most countries, the PCT test is more expensive and less widely available than the CRP.

As infection differs from contamination in that it represents invasion of the host tissues, samples for culture of tissues are likely to provide more accurate data than superficial swabs. In the study by Ottolino-Perry et al,[64] real-time autofluorescence was found to be useful for both assessing the "wound bioburden" and improving the sensitivity of swab cultures.[64] In addition to the fact that current guidelines recommend against using swabs to collect wound specimens for microbiological assessments[8,9] and the results of the recent Concordance in Diabetic Foot Ulcer Infection (CODIFI) study demonstrate the greater accuracy of tissue compared with swab specimens,[50] the findings of the Ottolino-Perry et al's[62] study 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 colonizers from pathogens, but studies to date have only included monomicrobial *Staphylococcus aureus* DFIs.[65-67] 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[68-70] 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 suspicion of osteomyelitis complicating an infected DFU is based on assessing clinical findings (eg, deep ulcer over a bony prominence, visible exposed bone, positive PTB test, and "sausage toe" appearance), laboratory tests (eg, serum biomarkers, tissue histology, and microbiology), and imaging studies (eg, cortical disruption, sequestrum, involucrum, marrow oedema, or tracer uptake) for bone abnormalities.[8,9] In one study, the combination of two relatively simple, widely available, and inexpensive tests—the PTB test and plain X-rays—was quite accurate with a reported sensitivity/specificity of 97%/93% in a population of high-risk patients and provided better results for diagnosing osteomyelitis than when the tests were considered separately.[71] Another series examined patients in whom diabetic foot osteomyelitis was suspected who were assessed with the combination of PTB test, plain X-rays, MRI, and culture and histology of a bone sample taken per operatively.[72] Levels of interrater agreement between the five diagnostic tests were low (range 42-62%), but the highest was between MRI and plain X-ray. Bone examination (culture and histology) allows a clinician to either confirm or exclude osteomyelitis, provided the biopsy has been performed properly (especially avoiding contamination). A recent study showed that the results of culture from a per wound bone biopsy specimen did not correlate well with those of a transcutaneous bone biopsy specimen via an uninfected skin route, demonstrating the need to limit the risk of contamination of the bone sample by colonizing bacteria.[73]

For skin and skin structure infection, imaging studies can potentially provide information about whether infection extends to deeper structures (eg, abscess, myositis, and gangrene) that cannot be easily assessed by clinical examination. When imaging is used to assess for possible bone infection, it plays a major role in determining management, including the potential need for surgical resection and duration

of antibiotic therapy (see the systematic review on interventions in the management of DFI in this journal). Among the numerous imaging modalities available for the diagnosis of DFIs, plain X-ray is the first to consider. It is the most readily and widely available, least expensive, and most easily interpreted (at least preliminarily) by nonradiologists. Some reports suggest that new tracers (including radioisotopes and optical imaging dyes) in nuclear medicine imaging may play useful roles in more accurately evaluating DFIs.[74] In the near future, bacteria-specific imaging could assist in the evaluation of DFIs, as they may provide an early and accurate diagnosis of infection, potentially including the recognition of antibiotic-resistant bacterial cells.[74]

5 | CONCLUSIONS

This systematic review of the published literature on diagnosis of DFI is the first to be reported by the IWGDF. The overall results show that due to a limited number of high-quality studies, the number of evidence statements we can propose and the strength of our recommendation are limited by the low-quality data available. We hope that the increasingly frequent use of the currently recommended definition and classification of DFIs will help standardize future comparisons of various diagnostic studies in this field. This is especially compelling given the arrival of new techniques in imaging and microbiology likely to improve the evaluation of these infections.

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CONFLICT OF INTEREST

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

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AUTHOR CONTRIBUTIONS

ES, EJP, SAV, and BAL participated in the writing of the document, and all the working group members 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.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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