Chapter

Diabetic Foot Osteomyelitis: Frequent Pathogens and Conservative Antibiotic Therapy

Nicolas Vogel, Tanja Huber and Ilker Uçkay

Abstract

Chronic diabetic foot osteomyelitis (DFO) is a frequent complication in adult polyneuropathy patients with long-standing diabetes mellitus. Regarding the conservative therapy, there are several crucial steps in adequate diagnosing and approaches. The management should be performed in a multidisciplinary approach following the findings of recent research, general principles of antibiotic therapy for bone; and according to (inter-)national guidance. In this chapter we emphasize the overview on the state-of-the-art management regarding the diagnosis and antibiotic therapy in DFO. In contrast, in this general narrative review and clinical recommendation, we skip the surgical, vascular and psychological aspects.

Keywords: Diabetic foot osteomyelitis, remission, microbiology, diagnosis, antibiotic therapy

1. Introduction

Patients with diabetes mellitus are at risk of complications of several organ systems and immunological problems of the cellular and humoral pathways [1]. Frequent clinical complications are diabetic foot infections, including acutely the soft tissues, or chronically the bone: diabetic foot osteomyelitis (DFO). In adult patients there is a lifetime risk of 25% for foot infections and a 15 times higher risk of lower limb amputation. The latter is associated with a high associated mortality risk of 50% within five years [1, 2]. Understandably, these infections are leading to massive healthcare costs and antibiotic consumption [3]. In this chapter, we provide an overview over the current conservative (antibiotic) approach to chronic DFO; emphasizing the state-of-the-art of diagnostic procedures and antibiotic regimens for the conservative, internist management. To keep this chapter as short as possible, we skip the discussion of the different surgical procedures, diabetic foot soft tissue infections [4], treatment of necrosis and gangrene [5], the management of angiopathy, topical antibiotic use of ulcers, implant-related DFO, non-infectious complications in the diabetic foot [6], podiatry, or off-loading, for which a broader literature is available.

2. Pathophysiology

Several underlying mechanisms are leading to a chronic foot infection in adult diabetic patients [1, 7]. Of course, the immunological impairments are crucial

for development of all sorts of infections, but there are more important factors contributing to the appearance of DFO, of which the neuropathy and vasculopathy are the most important cornerstones. In general, and as first step, foot ulcers are induced by pressure and further maceration of the skin [8]. Additionally, there might be a peripheral (microangiopathic) arterial disease (PAD), for which diabetes is an independent risk factor [9]. Wound healing may be impaired if blood flow is reduced. Data show the presence of PAD in about 30% of diabetic foot ulcers [10].

3. Diagnostic process

3.1 Clinical assessment

In general, the diagnosis and treatment of DFO should be embedded in a standardized multimodal and multidisciplinary approach. The first step is the clinical assessment in terms of the visual presence of infection: new induration, new warmth, new redness, tenderness, purulence and/or altered pain are the main findings. Besides the local signs of infection, there might be systemic repercussions with shivering, lymphangiopathy, and sepsis. Possible additional signs are delayed healing or granulation, putrid smell, or wound vulnerability. These latter symptoms are unspecific and can also occur in other differential diagnoses such as ischemia, acute gout or activated Charcot neuro-arthropathy [11, 12]. The only pathognomonic clinical sign for the external and visual diagnosis of DFO is the presence of fragments of bone discharging from a wound. This is only possible in advanced infections related to ulcers; and it is rare. Often, a DFO is suspected and later confirmed. Large, deep or chronic wounds (persisting for ≥3 months) or red and swollen toes ("sausage toe") raise the suspicion of DFO. Another simple diagnostic approach is the probe-to-bone test. The clinician uses a sterile blunt metal probe to determine, whether bone can be palpated through the diabetic foot ulcer. A negative test does not completely rule out DFO, while a positive test has an acceptable predictive value for deep bone infection [13, 14]. Although needle puncture of deep soft tissue does not reliably predict the results of bone cultures, puncture of the bone itself may be an easy way to obtain bone culture on the ward [15]. When DFO is suspected, two separate positive deep bony microbiological samples showing the same bacteria may sometimes additionally confirm DFO [16]. One or two weeks of "antibiotic-free window", before biopsy or surgery, are recommended to avoid false-negative results [17]. In contrast, the microbiological confirmation of DFO is not necessary when the infected area is amputated in toto [18]. All blood tests have no independent values in the mere confirmation of DFO, but might determine the initial, clinical severity of disease on admission.

3.2 Imaging

Upon the clinical suspicion of soft tissue infection and/or chronic ulcer in the polyneuropathy diabetic foot, the clinicians should also always exclude an underlying DFO; at minimum with an X-ray and once at the initial assessment. The X-ray can also be repeated if the lesions are not improving despite adequate therapy, local wound care and off-loading. In a usual approach, the X-ray should be the first imaging, in which signs of DFO can be detected such as osteopenia, periosteal reactions or erosions of the osseous borders [19, 20]. However, the overall sensitivity of the

plain radiography in diagnosing DFO is low. One review cited a pooled sensitivity of 0.54 and a specificity of 0.68 [21]. If the X-ray cannot provide a definitive radiological diagnosis, guidelines frequently recommend MRI for diagnosing DFO with a specificity of 79% and a sensitivity of 93% [22, 23]. However, the MRI is no guarantee of correct radiological diagnosis of DFO [24]. In the MRI, we may find focal signs on T2-weighted images and a loss of signal intensity on T1-weighted images. Furthermore, there is the potential to use short tau inversion recovery sequences (STIR), in which we see high bone signal [25–27]. What is truly beneficial by using the MRI, is the possibility to detect bone marrow edema within 1–2 days after beginning of the bone infection [28, 29]. The MRI is a diagnostic tool for a more accurate diagnostic of DFO. However, we lack clinical data revealing that DFO diagnosed by MRI would have a better outcome than those diagnosed by X-ray and by clinical impression alone. For this latter question, we prospectively followed 390 DFO episodes in 186 adult patients for a median of 2.9 years and performed 318 standard conventional X-rays (median costs 100 Swiss Francs; 100 US$) and with 47 (12%) MRI scans (median costs 800 US$). Among them, 18 episodes were associated with positive findings in the MRI only, but lacked bone lesions in the previous X-ray two to three days ago. In the database, the median duration of systemic antibiotics was 28 days for MRI-only episodes and 30 days for X-ray-positive cases and we achieved overall remission in 25% of the MRI-managed cases compared to 27% of the cases with only a standard X-ray imaging on admission. When adjusting for the large case-mix, DFO episodes diagnosed by the MRI had no different remission rates [30].

3.3 Microbiological diagnostic

The microbiological diagnostic relies on specimens for culturing the involved pathogens. No expert recommends superficial wound swabs, because there is always an (inconstant) microbiome of multiple organisms in chronically open wounds. These superficial probes are frequently misleading, since they often represent colonizing species or contaminations [12] (unless the swab originates from mere pus). Clinicians should always aim for several deep samples of infected (intraoperative) tissues or bone. An optimal specimen would be deep, infected, and still vital tissue, with or without pus, to catch the anaerobic pathogens [12, 31]. The microbiological gold standard for DFO relies on a bone biopsy, which is also feasible outside of the operating theater; especially in patients with polyneuropathy, who feel almost no pain during the bed-side sampling [25]. The accuracy of the results is increased by taking at least two separate bone probes. If they show the same pathogen, we usually identify the pathogen of DFO [16]. Histology has no widely-accepted criteria for DFO. Characteristic findings are aggregates of inflammatory cells, bone lesions, fibrosis, and/or reactive bone formation. As with other orthopedic infections the results depend on the care with which intraoperative samples are obtained (to avoid contamination) and whether the patient was under active antibiotic therapy [32]. While newer molecular laboratory methods identify more pathogens from DFO, the IWGDF guidelines suggest sticking with conventional culture methods for the first-line identification [33]. This is because of their lower cost, the lack of evidence of any benefit to covering the additional isolates identified and the potential for incurring the adverse effects of unnecessarily broad-spectrum antibiotic therapy. Practically, the only serology with a theoretical use for diagnosing DFO are anti-streptolysin antibodies for beta-hemolytic streptococci. If they are positive [34], the clinicians can (retrospectively) diagnose a streptococcal infection, which might be more useful in acute and severe soft tissue diabetic foot infections than in chronic DFOs.

4. Microbiological therapy

4.1 Pathogens of DFO

Beside possible surgical intervention there is need for an antibacterial treatment. Choosing an active agent is always first empirical, and subsequently targeted to the microbiological culture results. Knowledge of the possible microorganisms is a precondition to an empirical therapy [35]. Dependent on the country, most isolates of DFO are *Staphylococcus aureus*, β-hemolytic streptococci, coagulase-negative staphylococci and Gram-negative pathogens such as *Pseudomonas aeruginosa* [36, 37]. Interestingly, the location of the infection is critical as well, so are calcaneal infections associated with *P. aeruginosa* in diabetic patients [19]. Unfortunately, despite the advocated greenish color of superficial *Pseudomonas* infections and a presumed characteristic smell of the infected wound, even long-standing clinicians cannot predict the presence of *P. aeruginosa* by visual and olfactory means alone. The microbiological laboratory assessment is still necessary [38]. Multi-resistant pathogens in DFO are increasing in frequency worldwide [39] such as extended-spectrum beta-lactamase-producing Gram-negative rods (ESBL). Compared to DFI without involvement of the bone a meta-analysis found a three times higher chance in DFO for isolating a multi-resistant pathogen [40]. Fungi are rarity. Enterococci are equally rare but relatively more prevalent in the infected diabetic foot compared to other osteoarticular infections in the body such as steptococci and staphylococci [41, 42]. It is important to recognize that in the DFO patient, we may retrieve any bacteria, including avirulent coagulase-negative staphylococci and corynebacteria. Unlike to other infections such as pneumonia or endocarditis, the causative pathogens can also change during the current therapy of DFO, by selection of new (more resistant) pathogens by the therapeutic antibiotics and iterative surgeries during treatment. Therefore, if ever there is surgery during ongoing systemic antibiotic treatment, we recommend to re-sample again. The incidence of such a new microbiological finding can be as high as 10% [43].

4.2 Antibiotic therapy

The systemic antibiotic therapy is – next to a possible surgery, iterative professional debridement, (podiatric) wound care, enhancement of the patient's compliance, and off-loading – always required, if the goal is the healing of DFO. A clear recommendation for a specific antimicrobial agent, or the general administration route, cannot be made. A lot of studies and meta-analyses failed to show a superiority of one specific drug against the others [44]. During the initial empirical treatment, we recommend to cover *S. aureus*. If the therapy fails to achieve a proper reduction of local inflammatory symptoms, then the therapy should be broadened to include (aerobic and anaerobic) Gram-negative bacteria [45]. In severe infections, (sub)tropical regions, or sepsis, a relatively broad empirical coverage targeting the local epidemiology of Gram-negative pathogens could be chosen [46] from the start. Further there is few data supporting parenteral therapy [47]. Of note, the microbiological culture results can lead to necessity of parenteral agents due to resistant pathogens.

Ideally, the DFO therapy is accompanied by professional debridement, or the resection of necrotic and infected bone (total amputation). A study of 50 patients with chronic toe DFO showed that patients with surgical resections had a significantly lower relapse rate. This was also witnessed in single-center survey with partial amputations [48]. In well-selected patients and neuropathic DFO cases without progressive ischemia, other studies report successful treatment

without surgery, with selected remission rates of 60–70% [49, 50]. When surgery is not necessary for various reasons, a strictly conservative antibiotic therapy is very reasonable. Of note, the proportion of antibiotic-related side effects in randomized-controlled DFO trials may compromise up to 20–30% of all systemic antibiotic DFO regimens [49–55].

4.2.1 Biofilms

Clinicians often neglect the substantial role of bacterial biofilm in various infections, including the diabetic foot [35, 51]. Biofilm-forming bacteria are more refractory to host response and medical treatment and may be responsible for chronicity and complications. The proportion of biofilm-forming bacteria in DFO has been estimated at 30–60% [51]. In a clinical and microbiological study from Turkey [52], the assessed proportion of suspected biofilms among 339 diabetic foot wound isolates occurred in 34%. The multivariate regression analysis revealed two variables to be significant factors associated with biofilm: MDR micro-organism and XDR micro-organism [52]. New strategies are required in the management of wounds with biofilm to effectively destroy and even to prevent its formation.

One antibiotic might be associated with better outcomes in treating DFO biofilms: rifampin [53]. In analogy to implant-related staphylococcal infections, the antibiotic combination with rifampin may reveal a superior outcome. For example, Senneville et al. published a non-randomized observational study in 17 DFO patients treated with ofloxacin-rifampicin and achieved a remission in 88% of the cases [54]. Many other examples, especially from the US and France, are reported. We need the confirmation of the benefit of rifampicin use in DFO in future prospective-randomized trials.

4.3 Duration of antibiotic therapy

Because of a substantial risk for clinical failures according to every day's clinical experience, many physicians treat DFOs, on purpose, with a very long course of antimicrobial therapy, although guidelines limit the overall antibiotic prescription to 4–6 weeks only [33, 55]. Of note, this official guidance never had advocated a prolonged course. A retrospective evaluation with 1018 episodes of soft tissue infections and DFO failed to determine an optimal duration of systemic antibiotic administration regarding the remission, or failure, of diabetic foot infection [48]. A randomized controlled trial found that 6 weeks, compared with 12 weeks, of targeted antibiotic DFO therapy produced similar results [56]. This opinion is shared by other research groups [57, 58]. Today, a maximal duration of 6 weeks is the standard. If the is no remission after this period, clinicians should consider a new approach, which is surgical in the majority of cases. Maybe, the actual standard of 6 weeks might equally be too long for usual DFO cases. Recently, we published our experience of a randomized, controlled (RCT) pilot trial investigating shorter antibiotic administrations for DFO [55]. In this trial, a systemic antibiotic therapy of 3-weeks gave similar (and statistically non-inferior) incidences of remission and adverse events to a course of 6 weeks [55]. We also started the confirmatory RCT with 400 planned episodes in the Balgrist University Hospital in Zurich [59], by using a streamlined surgical approach, an initial radiological examination by magnetic resonance imaging and stratification between surgical versus totally antibiotic treatment approaches. If we confirm our pilot findings, the clinical implications, especially for improved antibiotic stewardship of in the field of DFO [60] might be substantial. Until further results are present, we agree with recommendations of up to six weeks of antibiotic therapy when residual infected bone is suspected or proven [25, 61–66].

4.3.1 Serum inflammatory parameters during the follow-up control of therapy

Clinicians frequently control serum C-reactive protein (CRP) levels during the therapy of DFO. This routine practice should be abandoned. There is often no immediate benefit. On the contrary, surprisingly high CRP blood levels usually trigger unnecessary exams (X-rays, angiology exams, superficial wound swabs, urinary cultures); even in absence of clinical indications. The worst consequence would be a prolongation of the scheduled antibiotic therapy, only basing on these CRP level. In our prospectively collected database [55], routine serum CRP samples, at different time points during ongoing antimicrobial therapy for (operated) DFO, failed to predict future clinical failures [58].

4.3.2 Duration of antibiotic therapy after surgical resection of DFO

After a complete surgical resection of all infected and necrotic bone, many experts only warrant a short very duration of antimicrobial therapy (2–5 days) to finish with a remaining soft tissue infection [18, 59, 60]. However, surgeons frequently doubt about the clinical absence of residual bone infection in the proximal amputation stump [63–65]. The IWGDF recommends sampling the marginal, remaining bone for evidence of residual infection; and advocates a up to 6 weeks of a consecutive, targeted antimicrobial therapy if the residual bone samples return with positive microbiological results [33]. This recommendation is cautious. We reported 482 DFO episodes with a median follow-up of two years after presumably curative total amputation [18]. According to this experience, neither the duration of the postsurgical antibiotic use, nor its immediate discontinuation, predicted future clinical failure [18]. Other research groups advocate that 5 days of a post-surgical antibiotic continuation are sufficient for a potential residual bone infection after amputation [64]. The residual cultures may also be false-negative, when receiving antibiotics, or false-positive when the samples are contaminated [33]. For example, colleagues from Basel, Switzerland, suggested that positive cultures, without a visual clinical confirmation of osteomyelitis and without concomitant histological confirmation, might overestimate the true rate of residual osteomyelitis, because of contamination at the time of surgery [66].

4.4 Administration route of antibiotic therapy

During the last decades, clinicians used a weeks'-long parenteral antibiotic therapy for all severe or moderate cases of DFO, usually with a switch to oral administration the hospitalized patient has been improving [67]. Today, we are living a change of paradigm in daily clinical life and start to consider oral regimens as efficacious as intravenous therapy in chronic DFO [48, 55, 67]. Therapy with oral antibiotic drugs is effective in non-bacteremic mild and moderate DFOs. A review of 93 DFO cases strongly supports the possibility of oral antibiotic regimens right from the start [68]. The same principle applies for other forms and localizations of chronic osteomyelitis [67]. Additional retrospective and prospective studies demonstrated the non-inferiority of oral antibiotic medication for DFO [60]. In our single-center cohort with a defined clinical diabetic foot infection pathway, oral β-lactam therapy did not alter the incidence of remission [67]. Spanish researchers conducted a prospective-randomized trial in DFO patients; with a strictly conservative antibiotic treatment of ninety days versus an approach with surgery plus antibiotics of ninety days. In the conservative arm, oral antibiotics were given very early in the course. Practically, the outcomes were equivalent [61]. The authors of this chapter ignore the existence of a prospective-randomized trial in favor of a long

initial parenteral treatment for chronic, non-septic, DFO in adult patients. Finally, topical antibiotics have no place in the treatment of unresected, deep DFO [69].

4.5 Antibiotic stewardship and clinical pathways

DFO's are probably among the most frequent diseases leading to antibiotic overuse [60]. We think that the principles of antibiotic stewardship should also concern DFOs. We reviewed the literature on DFO [60] to assess the value of antibiotic stewardship in the management of DFO. According to this review, the three most effective measures could be: correct diagnosis of bone infection; use of antibiotic regimens with the narrowest spectrum; and, limiting the duration of antimicrobial treatment. Clinical pathways have been instituted for DFOs [70]. A multidisciplinary management regularly showed a significant reduction in amputation risks [71]. However, these multidisciplinary teams have also their limitations: 1) it is difficult to bring the team members together; 2) the number of patients requiring evaluation often exceeds the capacity of fixed regular meetings; 3) the meetings are time-consuming and key members may be absent. Theoretically, order-sets (especially if they are embedded within interactive electronic websites) [12] are tools to implement "bundles" of approaches and, hopefully, improve outcomes. However, the academic experience of these order sets must be further evaluated, especially in resource-poor settings. There are also many administrative approaches that might improve antibiotic stewardship in DFO. Governments can initiate diabetic foot centers [72], or regular workshops and public educational lectures. The access to regional or international guidelines must be encouraged [60].

5. Possible future research

We need many prospective, clinical trials targeting the reduction of unnecessary (systemic and topical) antibiotic use, assessing the value of antibiotic stewardship programs, and developing evidence-based guidance. We should also be interested in microbiomes and new therapies. We want to target unanswered questions and advance research in all aspects of DFO. For example, regarding neo-vascularization, one future hope lies in stem cells. Knowing that a subset of human monocytes expresses TIE-2, we could enhance neovascularization, since ischemia is a major concomitant problem to chronic DFO. We successfully extracted high numbers of proangiogenic TIE-2 monocytes from venous blood of diabetic patients without ischemia [73]. Likewise, current scientific achievements confirm the feasibility of amplifying adipose stem cells for angiogenesis from the abdominal fat of ischemic patients [74]. The implication of these findings in terms of autologous injections for therapeutic neo-angiogenesis will require further studies. DFO will remain a "never-ending challenge" [75].

6. Conclusions

We can treat DFO conservatively by (targeted) systemic antibiotic administration, proper wound debridement (if necessary), and adequate off-loading. With the conservative therapy, the progressive destruction of underlying bone can be arrested in probably 60–70% of episodes in well-selected, compliant patients without major bone destruction or advanced concomitant ischemia; at least for a short follow-up time. A clinical regular and close follow-up by specialized health-care workers is paramount, since clinical failures on the long-term are frequent;

especially in the reason for the initial chronic DFO has not been reversed. The secondary prevention of further infection episodes is important. Any systemic antimicrobial agent is suitable, and very probably in oral administration form from the start (unless there is a concomitant severe clinical systemic inflammation, bacteremia or sepsis). The duration of antibiotic therapy is currently fixed to six weeks, but further trials and evaluations reducing the overall duration to lesser time spans are under way [59].

Conflict of interest

The authors declare no conflict of interest.

Author details

Nicolas Vogel[1,2*], Tanja Huber[3] and Ilker Uçkay[4]

1 Internal Medicine, Spital Zollikerberg, Zollikerberg, Switzerland

2 Internal Medicine, Balgrist University Hospital, Zurich, Switzerland

3 Hospital Pharmacy, Balgrist University Hospital, Zurich, Switzerland

4 Infectiology and Infection Control, Balgrist University Hospital, Zurich, Switzerland

*Address all correspondence to: nicolasvogel@yahoo.com

References

[1] Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 Diabetes and its Impact on the Immune System. Curr Diabetes Rev. 2020;16(5):442-449.

[2] Yammine K, Hayek F, Assi C. A meta-analysis of mortality after minor amputation among patients with diabetes and/or peripheral vascular disease. J Vasc Surg. 2020;72(6): 2197-2207.

[3] Association AD. Economic Costs of Diabetes in the U.S. in 2017. Diabetes Care. 2018;41(5):917-928.

[4] Tobalem M, Uçkay I. Images in clinical medicine. Evolution of a diabetic foot infection. N Engl J Med. 2013;369(23):2252.

[5] Al Wahbi A. Operative versus non-operative treatment in diabetic dry toe gangrene. Diabetes Metab Syndr. 2019;13(2):959-963.

[6] Gariani K, Waibel FWA, Viehöfer AF, Uçkay I. Plantar Fasciitis in Diabetic Foot Patients: Risk Factors, Pathophysiology, Diagnosis, and Management. Diabetes Metab Syndr Obes. 2020;13:1271-1279.

[7] Khanolkar MP, Bain SC, Stephens JW. The diabetic foot. QJM. 2008;101(9):685-695.

[8] Vinik AI, Nevoret ML, Casellini C, Parson H. Diabetic neuropathy. Endocrinol Metab Clin North Am. 2013;42(4):747-787.

[9] Association AD. Peripheral arterial disease in people with diabetes. Diabetes Care. 2003;26(12):3333-3341.

[10] Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, et al. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. Diabetes Care. 1999;22(1):157-162.

[11] Berli M, Vlachopoulos L, Leupi S, Böni T, Baltin C. Treatment of Charcot Neuroarthropathy and osteomyelitis of the same foot: a retrospective cohort study. BMC Musculoskelet Disord. 2017;18(1):460.

[12] Uçkay I, Gariani K, Pataky Z, Lipsky BA. Diabetic foot infections: state-of-the-art. Diabetes Obes Metab. 2014;16(4):305-316.

[13] Butalia S, Palda VA, Sargeant RJ, Detsky AS, Mourad O. Does this patient with diabetes have osteomyelitis of the lower extremity? JAMA. 2008;299(7):806-813.

[14] Aragón-Sánchez J, Lipsky BA, Lázaro-Martínez JL. Diagnosing diabetic foot osteomyelitis: is the combination of probe-to-bone test and plain radiography sufficient for high-risk inpatients? Diabet Med. 2011;28(2):191-194.

[15] Senneville E, Morant H, Descamps D, Dekeyser S, Beltrand E, Singer B, 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.

[16] Bernard L, Assal M, Garzoni C, Uçkay I. Predicting the pathogen of diabetic toe osteomyelitis by two consecutive ulcer cultures with bone contact. Eur J Clin Microbiol Infect Dis. 2011;30(2):279-281.

[17] Govaert GAM, Kuehl R, Atkins BL, Trampuz A, Morgenstern M, Obremskey WT, et al. Diagnosing Fracture-Related Infection: Current Concepts and Recommendations. J Orthop Trauma. 2020;34(1):8-17.

[18] Rossel A, Lebowitz D, Gariani K, Abbas M, Kressmann B, Assal M, et al. Stopping antibiotics after surgical amputation in diabetic foot and ankle infections-A daily practice cohort. Endocrinol Diabetes Metab. 2019;2(2): e00059.

[19] Waibel FWA, Uçkay I, Sairanen K, Waibel L, Berli MC, Böni T, et al. Diabetic calcaneal osteomyelitis. Infez Med. 2019;27(3):225-238.

[20] Álvaro-Afonso FJ, Lázaro-Martínez JL, Aragón-Sánchez J, García-Morales E, Cecilia-Matilla A, Beneit-Montesinos JV. Interobserver and intraobserver reproducibility of plain X-rays in the diagnosis of diabetic foot osteomyelitis. Int J Low Extrem Wounds. 2013;12(1):12-15.

[21] Newman LG, Waller J, Palestro CJ, Schwartz M, Klein MJ, Hermann G, et al. Unsuspected osteomyelitis in diabetic foot ulcers. Diagnosis and monitoring by leukocyte scanning with indium in 111 oxyquinoline. JAMA. 1991;266(9): 1246-1251.

[22] Lauri C, Tamminga M, Glaudemans AWJM, Juárez Orozco LE, Erba PA, Jutte PC, 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.

[23] Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, 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):e132-e173.

[24] 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.

[25] Lipsky BA, Peters EJ, Senneville E, Berendt AR, Embil JM, Lavery LA, et al. Expert opinion on the management of infections in the diabetic foot. Diabetes Metab Res Rev. 2012;28 Suppl 1:163-178.

[26] Gholamrezanezhad A, Basques K, Batouli A, Matcuk G, Alavi A, Jadvar H. Clinical Nononcologic Applications of PET/CT and PET/MRI in Musculoskeletal, Orthopedic, and Rheumatologic Imaging. AJR Am J Roentgenol. 2018;210(6):W245-WW63.

[27] Donovan A, Schweitzer ME. Use of MR imaging in diagnosing diabetes-related pedal osteomyelitis. Radiographics. 2010;30(3):723-736.

[28] Jaramillo D. Infection: musculoskeletal. Pediatr Radiol. 2011;41 Suppl 1:S127-S134.

[29] Lee YJ, Sadigh S, Mankad K, Kapse N, Rajeswaran G. The imaging of osteomyelitis. Quant Imaging Med Surg. 2016;6(2):184-198.

[30] Gariani K, Lebowitz D, Kressmann B, Gariani J, Uçkay I. X-Ray Versus Magnetic Resonance Imaging in Diabetic Foot Osteomyelitis: A Clinical Comparison. Curr Diabetes Rev. 2021;17(3):373-377.

[31] Charles PG, Uçkay I, Kressmann B, Emonet S, Lipsky BA. The role of anaerobes in diabetic foot infections. Anaerobe. 2015;34:8-13.

[32] Al-Mayahi M, Cian A, Lipsky BA, Suvà D, Müller C, Landelle C, et al. Administration of antibiotic agents before intraoperative sampling in orthopedic infections alters culture results. J Infect. 2015;71(5):518-525.

[33] Lipsky BA, Senneville É, Abbas ZG, Aragón-Sánchez J, Diggle M, Embil JM, et al. 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.

[34] Uçkay I, Ferry T, Stern R, Ambrosioni J, Gamulin A, Andrey D, et al. Use of serum antistreptolysin O titers in the microbial diagnosis of orthopedic infections. Int J Infect Dis. 2009;13(4):421-424.

[35] Ertuğrul B, Uçkay I, Schöni M, Peter-Riesch B, Lipsky BA. Management of diabetic foot infections in the light of recent literature and new international guidelines. Expert Rev Anti Infect Ther. 2020;18(4):293-305.

[36] Dang CN, Prasad YD, Boulton AJ, Jude EB. Methicillin-resistant Staphylococcus aureus in the diabetic foot clinic: a worsening problem. Diabet Med. 2003;20(2):159-161.

[37] Bowler PG, Davies BJ. The microbiology of infected and noninfected leg ulcers. Int J Dermatol. 1999;38(8):573-578.

[38] Uçkay I, Holy D, Schöni M, Waibel FWA, Trache T, Burkhard J, 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.

[39] Varaiya AY, Dogra JD, Kulkarni MH, Bhalekar PN. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in diabetic foot infections. Indian J Pathol Microbiol. 2008;51(3):370-372.

[40] Chen Y, Ding H, Wu H, Chen HL. The Relationship Between Osteomyelitis Complication and Drug-Resistant Infection Risk in Diabetic Foot Ulcer: A Meta-analysis. Int J Low Extrem Wounds. 2017;16(3):183-190.

[41] Tumminello A, Dominguez D, Lebowitz D, Bartolone P, Betz M, Hannouche D, et al. Staphylococcus aureus versus streptococci in orthopaedic infections. Infect Dis (Lond). 2017;49(9):716-718.

[42] Uçkay I, Pires D, Agostinho A, Guanziroli N, Öztürk M, Bartolone P, et al. Enterococci in orthopaedic infections: Who is at risk getting infected? J Infect. 2017;75(4):309-314.

[43] Wuarin L, Abbas M, Harbarth S, Waibel F, Holy D, Burkhard J, et al. Changing perioperative prophylaxis during antibiotic therapy and iterative debridement for orthopedic infections? PLoS One. 2019;14(12):e0226674.

[44] Glaudemans AW, Uçkay I, Lipsky BA. Challenges in diagnosing infection in the diabetic foot. Diabet Med. 2015;32(6):748-759.

[45] Hatipoglu M, Mutluoglu M, Uzun G, Karabacak E, Turhan V, Lipsky BA. The microbiologic profile of diabetic foot infections in Turkey: a 20-year systematic review: diabetic foot infections in Turkey. Eur J Clin Microbiol Infect Dis. 2014;33(6): 871-878.

[46] Lipsky BA. Empirical therapy for diabetic foot infections: are there clinical clues to guide antibiotic selection? Clin Microbiol Infect. 2007;13(4):351-353.

[47] Li HK, Agweyu A, English M, Bejon P. An unsupported preference for intravenous antibiotics. PLoS Med. 2015;12(5):e1001825.

[48] Gariani K, Lebowitz D, von Dach E, Kressmann B, Lipsky BA, Uçkay I. Remission in diabetic foot infections: Duration of antibiotic therapy and other possible associated factors. Diabetes Obes Metab. 2019;21(2):244-251.

[49] Pittet D, Wyssa B, Herter-Clavel C, Kursteiner K, Vaucher J, Lew PD. Outcome of diabetic foot infections

treated conservatively: a retrospective cohort study with long-term follow-up. Arch Intern Med. 1999;159(8):851-856.

[50] Jeffcoate WJ, Lipsky BA. Controversies in diagnosing and managing osteomyelitis of the foot in diabetes. Clin Infect Dis. 2004;39 Suppl 2:S115-S122.

[51] Malik A, Mohammad Z, Ahmad J. The diabetic foot infections: biofilms and antimicrobial resistance. Diabetes Metab Syndr. 2013;7(2):101-107.

[52] Vatan A, Saltoglu N, Yemisen M, Balkan II, Surme S, Demiray T, et al. Association between biofilm and multi/extensive drug resistance in diabetic foot infection. Int J Clin Pract. 2018;72(3):e13060.

[53] Wilson BM, Bessesen MT, Doros G, Brown ST, Saade E, Hermos J, et al. Adjunctive Rifampin Therapy For Diabetic Foot Osteomyelitis in the Veterans Health Administration. JAMA Netw Open. 2019;2(11):e1916003.

[54] Senneville E, Yazdanpanah Y, Cazaubiel M, Cordonnier M, Valette M, Beltrand E, et al. Rifampicin-ofloxacin oral regimen for the treatment of mild to moderate diabetic foot osteomyelitis. J Antimicrob Chemother. 2001;48(6):927-930.

[55] Gariani K, Pham TT, Kressmann B, Jornayvaz FR, Gastaldi G, Stafylakis D, et al. Three versus six weeks of antibiotic therapy for diabetic foot osteomyelitis: A prospective, randomized, non-inferiority pilot trial. Clin Infect Dis. 2020.

[56] Tone A, Nguyen S, Devemy F, Topolinski H, Valette M, Cazaubiel M, et al. Six-week versus twelve-week antibiotic therapy for nonsurgically treated diabetic foot osteomyelitis: a multicenter open-label controlled randomized study. Diabetes Care 2015;38:302-307. Diabetes Care. 2015;38(4):735.

[57] Lazzarini L, Lipsky BA, Mader JT. Antibiotic treatment of osteomyelitis: what have we learned from 30 years of clinical trials? Int J Infect Dis. 2005;9(3):127-138.

[58] Pham TT, Wetzel O, Gariani K, Kressmann B, Jornayvaz FR, Lipsky BA, et al. Is routine measurement of the serum C-reactive protein level helpful during antibiotic therapy for diabetic foot infection? Diabetes Obes Metab. 2021;23(2):637-641.

[59] Waibel F, Berli M, Catanzaro S, Sairanen K, Schöni M, Böni T, et al. Optimization of the antibiotic management of diabetic foot infections: protocol for two randomized controlled trials. Trials. 2020;21(1):54.

[60] Uçkay I, Berli M, Sendi P, Lipsky BA. Principles and practice of antibiotic stewardship in the management of diabetic foot infections. Curr Opin Infect Dis. 2019;32(2):95-101.

[61] Lázaro-Martínez JL, Aragón-Sánchez J, García-Morales E. Antibiotics versus conservative surgery for treating diabetic foot osteomyelitis: a randomized comparative trial. Diabetes Care. 2014;37(3):789-795.

[62] Lesens O, Desbiez F, Theïs C, Ferry T, Bensalem M, Laurichesse H, et al. Staphylococcus aureus-Related Diabetic Osteomyelitis: Medical or Surgical Management? A French and Spanish Retrospective Cohort. Int J Low Extrem Wounds. 2015;14(3):284-290.

[63] 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.

[64] Saltoglu N, Yemisen M, Ergonul O, Kadanali A, Karagoz G, Batirel A, et al. Predictors for limb loss among patient with diabetic foot infections: an observational retrospective multicentric

study in Turkey. Clin Microbiol Infect. 2015;21(7):659-664.

[65] 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.

[66] Mijuskovic B, Kuehl R, Widmer AF, Jundt G, Frei R, Gürke L, et al. Culture of Bone Biopsy Specimens Overestimates Rate of Residual Osteomyelitis After Toe or Forefoot Amputation. J Bone Joint Surg Am. 2018;100(17):1448-1454.

[67] Gariani K, Lebowitz D, Kressmann B, von Dach E, Sendi P, Waibel F, et al. Oral amoxicillin-clavulanate for treating diabetic foot infections. Diabetes Obes Metab. 2019;21(6):1483-1486.

[68] Embil JM, Rose G, Trepman E, Math MC, Duerksen F, Simonsen JN, et al. Oral antimicrobial therapy for diabetic foot osteomyelitis. Foot Ankle Int. 2006;27(10):771-779.

[69] Uçkay I, Kressmann B, Malacarne S, Toumanova A, Jaafar J, Lew D, et al. A randomized, controlled study to investigate the efficacy and safety of a topical gentamicin-collagen sponge in combination with systemic antibiotic therapy in diabetic patients with a moderate or severe foot ulcer infection. BMC Infect Dis. 2018;18(1):361.

[70] Catho G, De Kraker M, Waldispühl Suter B, Valotti R, Harbarth S, Kaiser L, et al. Study protocol for a multicentre, cluster randomised, superiority trial evaluating the impact of computerised decision support, audit and feedback on antibiotic use: the COMPuterized Antibiotic Stewardship Study (COMPASS). BMJ Open. 2018;8(6): e022666.

[71] Darbellay P, Uçkay I, Dominguez D, Mugnai D, Filtri L, Lew D, et al. Diabetic foot infection: a multidisciplinary approach. Rev Med Suisse. 2011;7(292):894-897.

[72] Cawich SO, Islam S, Hariharan S, Harnarayan P, Budhooram S, Ramsewak S, et al. The economic impact of hospitalization for diabetic foot infections in a Caribbean nation. Perm J. 2014;18(1):e101-e104.

[73] Uçkay I, Imhof BA, Kressmann B, Lew D, Lipsky BA, Sidibe A. Characterization of Proangiogenic Monocytes from Blood in Patients with Chronic Ischemic Diabetic Foot Ulcers and Controls. Stem Cells Dev. 2020;29(14):911-918.

[74] Uçkay I, Baquié M, Mosser S, Hervé MP, Bruyere-Cerdan P, Roux-Lombard P, et al. Regenerative Secretoma of Adipose-Derived Stem Cells from Ischemic Patients. J Stem Cell Res Ther 2019, 9:5.

[75] Peter-Riesch B. The Diabetic Foot: The Never-Ending Challenge. Endocr Dev. 2016;31:108-134.