Predictors for limb loss among patient with diabetic foot infections: an observational retrospective multicentric study in Turkey

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Abstract

We aimed to investigate the predictors for limb loss among patients with diabetes who have complicated skin/soft-tissue infections. In this observational study, consecutive patients with diabetic foot infection (DFI) from 17 centres in Turkey, between May 2011 and May 2013 were included. The Turkish DFI Working Group performed the study. Predictors of limb loss were investigated by multivariate analysis. In total, 455 patients with DFI were included. Median age was 61 years, 68% were male, 65% of the patients were hospitalized, 52% of the patients had used antibiotics within the last month, and 121 (27%) had osteomyelitis. Of the 208 microorganisms isolated, 92 (44.2%) were Gram-positive cocci and 114 (54.8%) were Gram-negative rods (GNR). The most common GNR was *Pseudomonas*; the second was *Escherichia coli*, with extended spectrum β-lactamase positivity of 33%. Methicillin-resistant *Staphylococcus* species were found in 14% (29/208). Amputations were performed in 126/455 (28%) patients, 44/126 (34%) of these were major amputations. In multivariate analysis, significant predictors for limb loss were, male gender (OR 1.75, 95% CI 1.04–2.96, p 0.034), duration of diabetes >20 years (OR 1.9, 95% CI 1.18–3.11, p 0.008), infected ulcer versus cellulitis (OR 1.9, 95% CI 1.11–3.18, p 0.019), history of peripheral vascular disease (OR 2, 95% CI 1.26–3.27, p 0.004), retinopathy (OR 2.25, 95% CI 1.19–4.25, p 0.012), erythrocyte sedimentation rate >70 mm/hr (OR 1.6, 95% CI 1.01–2.68, p 0.05), and infection with GNR (OR 1.8, 95% CI 1.08–3.02, p 0.02). Multivariate analysis revealed that, besides the known risk factors such as male gender, duration of diabetes >20 years, infected ulcers, history of peripheral vascular disease and retinopathy, detection of GNR was a significant predictor of limb loss.

Keywords: Antimicrobial resistance, diabetic foot, Gram-negative, infections, limb loss
Original Submission: 10 March 2014; **Revised Submission:** 8 March 2015; **Accepted:** 20 March 2015
Editor: M. Paul
Article published online: 8 April 2015

http://dx.doi.org/10.1016/j.cmi.2015.03.018

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Introduction

Diabetes mellitus (DM) is a common chronic disease that has become a global health problem with an alarming increase in incidence [1]. In Turkey, the prevalence of DM has been reported as 7.2%, indicating approximately five million patients with the disease [2]. Among different complications, diabetic foot infection (DFI) is a significant cause of morbidity and mortality among patients with diabetes and has a life-time incidence of 4–7% [3]. It is one of the primary reasons for diabetes-related hospitalizations and limb loss [4]. Infection in diabetic foot ulcers may progress rapidly and often leads to lower limb amputation so it is critical to determine the presence of infection [5]. Timely diagnosis, early identification of the need for hospitalization and institution of proper patient monitoring protocols are important for follow up and survival of the patients. Adequate antimicrobial therapy should be started as early as possible based on criteria such as possible pathogens, depth of the infection, presence of ischaemia and presence of any systemic symptoms [6].

Here we report a multicentre observational retrospective study. Our objective was to investigate the predictors for limb loss among patients with diabetes who have skin/soft-tissue infections and osteomyelitis.

Patients and methods

In this retrospective observational study, consecutive patients with DFI from 17 centres in Turkey were included between 15 May 2011 and 30 May 2013. All the inpatients and outpatients were followed up and assessed by members of the Turkish Diabetic Foot Study Group of the Turkish Society of Clinical Microbiology and Infectious Diseases.

Infection was diagnosed clinically by a trained physician according to the validated system for infection severity defined by the International Working Group on the Diabetic Foot (also abbreviated as Diabetic Foot) PEDIS classification. A DFI severity score was determined as mild, moderate or severe following the guidelines of the Infectious Diseases Society of America. Accordingly, patients were also identified by clinical description for soft-tissue infection (cellulitis, infected ulcer, necrotizing infection) or osteomyelitis [7]. All patients with severe infection, selected patients with a moderate infection with complicating features, and any patient unable to comply with the required outpatient regimen for psychological or social reasons were hospitalized initially. Specimens for culture included samples from deep tissue obtained by biopsy or curettage after the wound had been cleansed and debrided. Wound swabs were avoided. The following patient information was recorded using a structured electronic form: age, sex, duration of diabetes, diabetes type and type of diabetic treatment, presence of chronic renal failure, cardiac failure, retinopathy, history of dialysis, history of previous foot infection, hospital admissions, medical and surgical treatments, history of amputation and presenting complaints, infection severity, time spent before hospitalization, microorganisms and antimicrobial resistance, clinical and laboratory findings, diagnostic procedures used, antimicrobial and other treatments and treatment outcomes for the current admission.

The primary outcome was limb loss. Definition of limb loss includes toe amputation, metatarsal (ray) amputation, below-knee amputation (BKA) and above-knee amputation (AKA). Both BKA and AKA were considered as major amputations. In our study, the decision for amputation was taken by the Diabetic Foot Council of the respective hospitals. The patients were followed up after the operations by regular visits. Duration of follow up was 3–6 months.

An independent professional data manager from a Contract Research Organization (CRO) visited the centres and recorded the data into an electronic form.

The study was approved by the Ethical Committee of Cerrahpaşa Medical Faculty of Istanbul University.

Statistical analysis

For comparison of continuous variables a Kruskal–Wallis test was performed, and for categorical variables a chi-square test was performed. Multivariate analysis was performed for detection of the predictors of limb loss. The independent variables for multivariate analysis were selected according to statistical significance in univariate analysis, collinear variables were excluded. The performance of multivariate analysis was estimated by calculation of the area under the receiver operating characteristics curve, and compared with the performance of a previously published model (4). All the patients were scored according to an already defined risk score (4), and the score performance was assessed. Statistical significance was set as $p < 0.05$, and STATA (version 11; StataCorp, College Station, TX, USA) was used.

TABLE 1. Demographic characteristics, risk factors, and comordities of patients related to limb loss

	Limb loss (*n* = 126)	No limb loss (*n* = 329)	p
Demographic characteristics			
Male gender, *n* (%)	96 (76)	214 (65)	0.022
Median age (IQR)	62 (57–70)	61 (54–69)	0.113
Age ≥50 years, *n* (%)	119 (94)	279 (85)	0.005
Diabetes mellitus, type II, *n* (%)	126 (100)	326 (99)	NA
Insulin use	112 (89)	239 (73)	<0.001
Risk factors			
Median DM duration (years, IQR)	19 (10–25)	14 (7–20)	0.001
Median body mass index (IQR)	28 (25–29)	28 (25–31)	0.198
History of hospitalization, *n* (%)	95 (75)	202 (61)	0.005
History of recurrent infection, *n* (%)	9 (7)	26 (8)	0.785
History of osteomyelitis, *n* (%)	46 (37)	75 (23)	0.003
History of debridement, *n* (%)	54 (43)	101 (31)	0.014
History of amputation, *n* (%)	43 (34)	67 (20)	0.002
History of current smoking, *n* (%)	40 (32)	84 (26)	0.183
History of vascular surgery, *n* (%)	20 (16)	51 (16)	0.922
Antibiotic use within the last 30 days, *n* (%)	73 (58)	164 (50)	0.122
Nasal carriage of *Staphylococcus aureus*, *n* (%)	2 (1.5)	11 (3.3)	0.314
Rate of methicillin-resistant *Staphylococcus aureus*, *n* (%)	2 (1.5)	5 (1.5)	0.958
Dialysis, *n* (%)	18 (14)	32 (10)	0.164
Comorbidities			
Chronic renal disease, *n* (%)	43 (34)	79 (24)	0.029
Coronary artery disease, *n* (%)	26 (21)	46 (14)	0.082
Retinopathy, *n* (%)	25 (20)	32 (10)	0.004

Results

Among 455 DFI patients treated in 17 centres during the study period, 310 (68%) were male. Median age of the patients was 61 years, with a range of 29–90 years (Table 1). Seventy-five per cent of the patients were hospitalized and 52% of the patients had used antibiotics within the last month (Table 1). All the patients with severe infection and 248 patients (88%) with moderately severe infections were hospitalized. Among the patients, 287 (63%) had complicated skin/soft-tissue infections, and 121 (27%) had osteomyelitis. Peripheral arterial disease was identified in 210 (46.1%) patients (Table 2).

In total, 208 microorganisms were isolated from 455 patients, 92 (44.2%) were Gram-positive cocci and 114 (54.8%) were Gram-negative rods (GNR) (Table 3). Limb loss occurred in 126 (28%) patients: amputation of the big toe in 35 (28%), amputation of other toes in 32 (25%), ray amputation in 15 (12%), BKA in 31 (24%), and AKA in 13 (10%) patients. Of all the amputations, one-third were major amputations. During follow up, 32 (7%) patients required recurrent surgery and re-infection occurred in 35 (7.7%) patients. The death rate during follow up was 4.8% (22 patients). Length of hospital stay was 21 days (range: 3–170 days) for moderate DFI and 24 days (3–120) for severe DFI (p 0.190).

On univariate analysis, history of amputation was more common among the patients with limb loss (34% versus 20%, p 0.002, Table 1). Limb loss rates were higher for patients who had osteomyelitis—with osteomyelitis 78 (61.9%) versus without osteomyelitis 48 (38.1%), p < 0.001. BKA rates were higher for patients with osteomyelitis compared with patients with complicated skin/soft-tissue infection—osteomyelitis 12 (9.9%) versus skin/soft-tissue infection 14 (4.1%), p 0.020. Leucocyte counts were significantly higher in amputated compared with non-amputated patients (13 000 versus 11 000, respectively; p 0.004). We found significantly high rates of GNR (*Escherichia coli* and *Psuedomonas aeruginosa*) in amputated patients (p 0.02). Rates of limb loss were similar in patients from whom extended-spectrum β-lactamase (ESBL) were isolated

TABLE 2. Characteristics of infection according to limb loss

	Limb loss (*n* = 126)	No limb loss (*n* = 329)	p
PEDIS Peripheral artery disease (PAD)*			
Stage 1:No PAD sign, palpable dorsopedal or posterior tibial artery, *n* (%)	41 (33)	193 (59)	<0.001
Stage 2: Signs of PAD and claudication, but no severe ischaemia, *n* (%)	51 (40)	96 (29)	0.021
Stage 3: Severe ischaemia, *n* (%)	32 (25)	31 (9)	<0.001
Median wound width (cm^2), (IQR)	6 (3–15)	6 (3–15)	0.313
Site of wound			
Big toe, *n* (%)	57 (95)	83 (85)	0.048
Other toes, *n* (%)	60 (48)	73 (22)	<0.001
Metatarsal, *n* (%)	49 (32)	54 (16)	<0.001
Back foot, *n* (%)	17 (13)	49 (15)	0.704
Heel, *n* (%)	14 (11)	54 (16)	0.156
PEDIS Grade and IDSA infection severity score (*lit.7)			
PEDIS Grade 2 (Mild infection) (%)	23 (18)	116 (35)	<0.001
PEDIS Grade 3 (Moderate infection) (%)	77 (57)	191 (51)	0.553
PEDIS Grade 4 (Severe infection = SIRS) (%)	26 (21)	22 (7)	<0.001
Osteomyelitis (%)	73 (58)	48 (15)	<0.001
Charcot joint (%)	4 (3)	10 (3)	0.227
Median day of hospitalization	30 (4–170)	17 (3–90)	<0.001
Median leucocyte count (10^3 µ/L)	13,000	11,000	0.004
Median C-reactive protein level (mg/dl)	26	16	0.009
Median erythrocyte sedimentation rate (mm/h) ±standard deviation	87	70	0.001
HA1C level > 7 (%)	94 (75)	236 (72)	0.539
Hyperbaric O_2 therapy (%)	38 (30)	66 (20)	0.001
Outcome measures			
Exitus (%)	7 (6)	15 (5)	0.658
Recurrent infection (%)	9 (7)	26 (8)	0.785
Re-hospitalization (%)	15 (12)	27 (8)	0.01

IDSA, Infectious Diseases Society of America.
*PEDIS stage, grade and severity of infection were determined according to the literature [7].

TABLE 3. Microorganisms isolated from patients with diabetic foot infection (*n* = 208)

	n (%)
Gram-negative bacteria	114 (54.8)
Pseudomonas aeruginosa	36 (17.3)
Escherichia coli	30 (14.4)
Enterobacter	11 (5.3)
Klebsiella	10 (4.8)
ESBL producing Enterobacteriaceae	10 (19.6)
Proteus	13 (4.8)
Acinetobacter	10 (4.8)
Morganella	4 (1.9)
Gram-positive bacteria	92 (44.2)
Methicillin-sensitive *Staphylococcus aureus*	37 (17.8)
Methicillin-resistant *Staphylococcus aureus*	11 (5.3)
Methicillin-resistant coagulase negative *Staphylococcus*	18 (8.6)
Streptococcus	14 (6.7)
Enterococcus	12 (5.8)
Anaerobic bacteria	2 (1)

TABLE 4. Predictors of limb loss

	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Chronic renal disease	1.64	1.05–2.56	0.03	0.84	0.5–1.41	0.520
Age >50 years	3	1.34–6.91	0.008	2.41	0.98–5.88	0.053
Male gender	1.71	1.07–2.74	0.023	1.75	1.04–2.96	0.034
Duration of DM > 20 years	2.3	1.53–3.54	<0.001	1.9	1.18–3.11	0.008
Infected ulcer versus cellulitis	2.6	1.61–4.16	<0.001	1.9	1.11–3.18	0.019
History of amputation	2	1.28–3.19	0.002	1.23	0.72–2.09	0.447
History of peripheral vascular disease	3	1.99–4.71	<0.001	2	1.26–3.27	0.004
Retinopathy	2.29	1.29–4.06	0.004	2.25	1.19–4.25	0.012
Leucocyte count >11 000/mm^3	1.8	1.16–2.67	0.008	1.4	0.88–2.25	0.150
Erythrocyte sedimentation rate >70 mm/hr	1.75	1.13–2.71	0.011	1.6	1.01–2.68	0.05
Gram-negative bacteria	1.8	1.15–2.86	0.01	1.8	1.08–3.02	0.022

versus patients without ESBL. Rates of limb loss were similar in patients undergoing dialysis (18/50) versus patients not undergoing dialysis (108/405), p 0.164.

On multivariate analysis, we found the following independent parameters as significant: male gender (OR 1.75, 95% CI 1.04–2.96, p 0.034), duration of DM > 20 years (OR 1.9, 95% CI 1.18–3.11, p 0.008), infected ulcer versus cellulitis (OR 1.9, 95% CI 1.11–3.18, p 0.019), history of peripheral vascular disease (OR 2, 95% CI 1.26–3.27, p 0.004), retinopathy (OR 2.25, 95% CI 1.19–4.25, p 0.012), erythrocyte sedimentation rate >70 mm/h (OR 1.6, 95% CI 1.01–2.68, p 0.05), and infection with Gram-negative bacteria (OR 1.8, 95% CI 1.08–3.02, p 0.02). (Table 4). The area under the receiver operating characteristics curve was 0.754 (95% CI 0.7–0.8). Another multivariate analysis was performed including the independent variables indicated by Lipsky *et al.* [4]. The independent variables included were chronic renal disease, male gender, age >50 years, history of amputation, leucocyte count >11 000, peripheral vascular disease and infected ulcer versus cellulitis. Out of these independent variables, age >50 years, peripheral vascular disease and infected ulcer versus cellulitis were found to significantly predict limb loss. This model's area under the receiver operating characteristics curve was 0.722 (95% CI 0.67–0.77).

We scored our patients according to the scoring system suggested by Lipsky *et al.* (4), and this scoring system revealed good predictive performance of limb loss (Fig. 1).

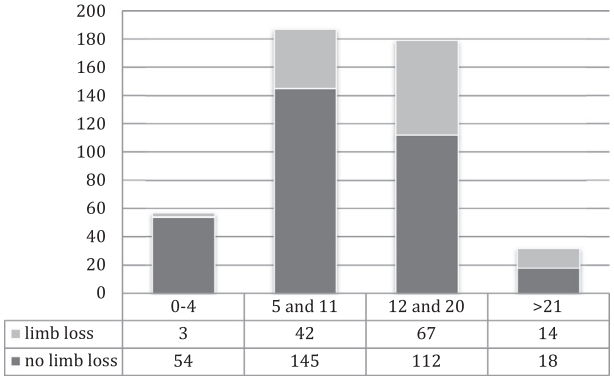

FIG. 1. Distribution of the cases according to Lipsky *et al.*'s risk score and limb loss rates.

Discussion

Despite the improvements in diagnosis and management, DFI remains a significant challenge [8]. In this large cohort study conducted in Turkey, we studied the predictors of limb loss among inpatients and outpatients with DFI, and described the bacteriological findings and clinical outcomes. On multivariate analysis the following independent parameters were found to be significant for the prediction of limb loss: male gender, duration of DM > 20 years, infected ulcer versus cellulitis, history of peripheral artery disease, erythrocyte sedimentation rate >70 mm/h, and infection with Gram-negative bacteria. The predictive estimate of our model 0.754, 95% CI 0.7–0.8 was similar to that of a model suggested by Lipsky *et al.*, 0.722, 95% CI 0.67–0.77 [4].

Lipsky *et al.* suggested the following risk factors for lower limb amputation in patients with diabetes: surgical site infection, vasculopathy, amputation history and high leucocyte count >11 000/mm^3 [4]. We added a few more variables to this suggested model, such as erythrocyte sedimentation rate >70, Gram-negative infection and retinopathy. In another study, a wound severity score and leucocyte counts were found to be related to wound infection [9]. Faglia *et al.* reported that major amputation rates were higher for patients with white blood cell count >10 000/mm^3, dialysis and osteomyelitis [10]. In our study, the majority of isolated pathogens (55%) were GNR. The most frequently isolated GNR was *P. aeruginosa* followed by *E. coli*. *Pseudomonas aeruginosa* has been previously reported as the most common bacteria among patients with severe DFI in Turkey [11,12]. One-third of *E. coli* isolated in our study were ESBL positive. Recently, ESBL-producing *Enterobactericeae* species have been reported as a global problem [13]. Therefore, the presence of ESBL-producing bacteria should be considered in Turkey and similar countries in the empiric treatment for severe DFI.

Among the isolated bacteria 44% were found to be Gram-positive. Approximately one-quarter of patients from whom *S. aureus* was isolated were found to be methicillin-resistant. Nather *et al.* [14] and Fejfarová *et al.* [15] found methicillin-resistant *Staphylococcus aureus* (MRSA) to be one of the

significant predictors for limb loss. We did not find MRSA to be a predictive factor for limb loss, but MRSA was found to be significantly associated with recurrent surgery.

In our study, the majority of patients (87%) were aged >50 years and had been diabetic for a long time (mean 15 years). Moreover we found that the duration of diabetes was one of the predictive factors for limb loss. Our findings are in agreement with some studies, such as those by Resnick *et al.* [16] and Lehto *et al.* [17]; however, a few studies had different findings [14,18]. Osteomyelitis negatively affects both the outcome and the treatment of DFI. We found a significant association between osteomyelitis and overall and major amputation, similar to Armstrong *et al.* [19]. However, we did not include osteomyelitis in the multivariable model to avoid collinearity. Duration of antibiotic treatment, length of hospital stay, and the duration of infection before initiation of treatment were longer in the 121 cases with osteomyelitis in our study compared with other patients. Mutluoğlu *et al.* compared patients with and without osteomyelitis; and found that length of hospital stay and antibiotic treatment, time before initiation of treatment and wound healing times were longer and more surgical interventions were required for patients with osteomyelitis [20].

Often, the specific pathogen cannot be isolated in suspected or probable osteomyelitis. One of the limitations of our study regarded the diagnosis of osteomyelitis, which was based mostly on clinical, laboratory and radiological imaging results. Diagnosis was established by magnetic resonance imaging (99 patients), probe test (52 patients), three-dimensional bone scintigraphy (seven patients), histopathological finding (six patients); bone culture rates (35 patients) were lower. Anaerobic cultures were not sufficiently performed at the study centres. Another limitation lies in the design of the current study, which was retrospective. Therefore, we missed some individual parameters such as reasons for amputation. The strengths of our multicentre study were the high number of participants and the inclusion of both inpatients and outpatients. Also, all the patients were followed up by diabetic foot councils for about 3–6 months.

Conclusions

Foot infections among patients with diabetes are increasing, especially in the elderly population. DFIs result in potential limb loss and fatal outcome. Emergence of antibiotic resistance is a significant problem in the treatment of DFI. Multivariate analysis revealed that detection of Gram-negative infections predicted the limb loss significantly. Early diagnosis and case management in DFI are crucial. Surveillance of isolates and their susceptibility profile should be monitored. The management of patients should be optimized. Follow up of the patients by diabetic foot councils would be useful to decrease the rate of amputation. To promote this, our research group initiated a multidisciplinary national guide for DFI in 2013.

Transparency declaration

The authors declare that they have no conflicts of interest.

References

[1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047–53.
[2] Satman I, Yilmaz T, Sengül A, Salman S, Salman F, Uygur S, et al. Population-based study of diabetes and risk characteristics in Turkey: results of the turkish diabetes epidemiology study (TURDEP). Diabetes Care 2002;25:1551–6.
[3] Peters EJ, Lipsky BA. Diagnosis and management of infection in the diabetic foot. Med Clin North Am 2013;97:911–46.
[4] Lipsky BA, Weigelt JA, Sun X, Johannes RS, Derby KG, Tabak YP. Developing and validating a risk score for lower-extremity amputation in patients hospitalize for a diabetic foot infection. Diabetes Care 2011;34:1695–700.
[5] Wukich DK, Armstrong DG, Attinger CE, Boulton AJ, Burns PR, Frykberg RG, et al. Inpatient management of diabetic foot disorders: a clinical guide. Diabetes Care 2013;36:2862–71.
[6] Caravaggi C, Sganzaroli A, Galenda P, Bassetti M, Ferraresi R, Gabrielli L. The management of the infected diabetic foot. Curr Diabetes Rev 2013;9:7–24.
[7] Lavery LA, Armstrong G, Murdoch DP, Peters EJ, Lipsky BA. Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. Clin Infect Dis 2007;44:562–5.
[8] Papanas N, Mani R. Advances in infections and wound healing for the diabetic foot: the die is cast. Intern J Lower Ext Wounds 2013;12:83–6.
[9] Lipsky BA, Sheehan P, Armstrong DG, Tice AD, Polis AB, Abramson MA. Clinical predictors of treatment failure for diabetic foot infections: data from a prospective trial. Int Wound J 2007;4:30–8.
[10] Faglia E, Clerici G, Caminiti M, Curci V, Somalvico F. Influence of osteomyelitis location in the foot of diabetic patients with transtibial amputation. Foot Ankle Int 2013;34:222–7.
[11] Saltoglu N, Dalkiran A, Tetiker T, Bayram H, Tasova Y, Dalay C, et al. Piperacillin/tazobactam versus imipenem/cilastatin for severe diabetic foot infections: a prospective, randomized clinical trial in a university hospital. Clin Microbiol Infect 2010;16:1252–7.
[12] Ertugrul BM, Oncul O, Tulek N, Willke A, Sacar S, Tunccan OG, et al. A prospective, multi-center study: factors related to the management of diabetic foot infections. Eur J Clin Microbiol Infect Dis 2012;31:2345–52.
[13] Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 2006;29:1727–32.
[14] Nather A, Bee CS, Huak CY, Chew JL, Lin CB, Neo S, et al. Epidemiology of diabetic foot problems and predicitve factors for limb loss. J Diabetes Complications 2008;22:77–82.

[15] Fejfarová V, Jirkovská A, Skibová J, Petkov V. Pathogen resistance and other risk factors in the frequency of lower limb amputations in patients with the diabetic foot syndrome. Vnitr Lek 2002;48:302–6.

[16] Resnick HE, Carter EA, Sosenko JM, Henly SJ, Fabsitz RR, Ness FK, et al. Strong Heart Study Incidence of lower-extremity amputation in American Indians: the Strong Heart Study. Diabetes Care 2004;27:1885–91.

[17] Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Risk factors predicting lower extremity amputations in patients with NIDDM. Diabetes Care 1996;19:607–12.

[18] Gürlek A, Bayraktar M, Savaş C, Gedik O. Amputation rate in 147 Turkish patients with diabetic foot: the Hacettepe University Hospital experience. Exp Clin Endocrinol Diabetes 1998;106:404–9.

[19] Armstrong DG. An overview of foot infections in diabetes. Diabetes Technol Ther 2011;13:951–7.

[20] Mutluoglu M, Sivrioglu AK, Eroglu M, Uzun G, Turhan V, Ay H, et al. The implications of the presence of osteomyelitis on outcomes of infected diabetic foot wounds. Scand J Infect Dis 2013;45:497–503.