Risk Factors for Infection with *Pseudomonas aeruginosa* in Diabetic Foot Infections

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Background: Selecting empirical therapy for a diabetic foot infection (DFI) requires knowing how likely infection with *Pseudomonas aeruginosa* is in a particular patient. We designed this study to define the risk factors associated with *P aeruginosa* in DFI.

Methods: We performed a preplanned microbiological subanalysis of data from a study assessing the effects of treatment with intralesional epidermal growth factor for diabetic foot wounds in patients in Turkey between January 1, 2012, and December 31, 2013. Patients were screened for risk factors, and the data of enrolled individuals were recorded in custom-designed patient data forms. Factors affecting *P aeruginosa* isolation were evaluated by univariate and multivariate logistic regression analyses, with statistical significance set at $P < .05$.

Results: There were 174 patients enrolled in the main study. Statistical analysis was performed in 90 evaluable patients for whom we had microbiological assessments. Cultures were sterile in 19 patients, and 89 bacterial isolates were found in the other 71. The most frequently isolated bacteria were *P aeruginosa* (n = 23, 25.8%) and *Staphylococcus aureus* (n = 12, 13.5%). Previous lower-extremity amputation and a history of using active wound dressings were the only statistically significant independent risk factors for the isolation of *P aeruginosa* in these DFIs.

Conclusions: This retrospective study provides some information on risk factors for infection with this difficult pathogen in patients with DFI. We need prospective studies in various parts of the world to better define this issue. (J Am Podiatr Med Assoc 107(6): 000-000, 2017)

Foot infections are among the most frequent and morbid consequences of diabetes. Determining the causative pathogens in a diabetic foot infection (DFI) is crucial in selecting the optimal antibiotic therapy. Although there have been many studies published on the bacteriology of DFIs during the past 25 years, the reported results have been varied and often contradictory.[1] These discrepancies could potentially be explained by changes in the causative organisms over time, geographic variations in pathogens at different study sites, or the type and severity of the infection seen at each site.[1] The predominant microbial causes of DFIs in North American and European countries are aerobic gram-positive cocci, especially *Staphylococcus aureus*. Studies from warm climates in Asia and Africa have demonstrated that aerobic gram-negative organisms, particularly *Pseudomonas aeruginosa*, are far more common than in Western countries. The reasons for these differences in microbial pathogens are not clear, but the substantial difference in the microbiological profile of DFIs in Western (North American/European) compared with Asian countries may be attributable to several cultural, geographic, and climatic factors.[2]

Often, *P aeruginosa* isolates are resistant to commonly used antibiotics, requiring the use of specially selected (sometimes costly) agents. This organism, similar to several others, is commonly

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associated with biofilm infections, and because of its resistance to many commonly used antibiotics, its presence in a DFI may be a risk factor for lower-extremity amputation. Selecting empirical therapy for a DFI, therefore, requires knowing the likelihood of infection with *P aeruginosa*, particularly in patients in warm climates such as in Asia and Africa. Because very few studies have evaluated the risk factors for infection with *P aeruginosa*, we designed this study to assess this issue in a group of patients with a DFI.

Materials and Methods

We performed a preplanned microbiological subanalysis of the data we obtained from a study assessing the effects of treatment with intralesional epidermal growth factor for diabetic foot wounds in patients in Turkey. The study was approved by the ethical committee of the University of Adnan Menderes, School of Medicine (Aydin, Turkey). Between January 1, 2012, and December 31, 2013, we identified patients seen at any of 25 Turkish medical centers with a newly diagnosed diabetic foot wound, a recurrent infection after apparent cure, or a history of amputation below the metatarsus and enrolled them in this retrospective study. All of the patients were screened for risk factors known to be associated with lower-extremity wounds, eg, advanced age, male sex, long duration of diabetes, previous hospitalization, previous lower-extremity amputation, previous foot infection (especially osteomyelitis), presence of peripheral neuropathy or peripheral vascular disease, deep wound depth, and midfoot or hindfoot ulcer localization. We recorded the data on enrolled patients in custom-designed patient data forms. A trained physician assessed all of the patients for the presence of any foot abnormality according to methods and recommendations of the International Working Group on the Diabetic Foot (PEDIS) classification.[3]

On admission, after cleansing and debridement, we obtained specimens of the wound for culture by swab or curette of the ulcer base, needle aspiration, or biopsy, depending on the wound depth and characteristics. Wounds were considered to be clinically infected if they have at least two classic symptoms or signs of inflammation (erythema, warmth, tenderness, pain, or induration) or purulent secretions; we also sought additional or secondary signs suggestive of infection (eg, nonpurulent secretions, friable or discolored granulation tissue, undermining of wound edges, foul odor).[3] Investigators diagnosed the presence of osteomyelitis based on the results of bone biopsy, radiography, magnetic resonance imaging, or nuclear scintigraphy. Peripheral neuropathy was diagnosed by checking for protective sensation using a monofilament. Glycemic control was assessed by the serum hemoglobin A_{1c} value. Amputations at the level of the metatarsals or toes were classified as minor, and those above the ankle were classified as major.

Statistical Analysis

We conducted proportional comparisons for categorical variables using the χ^2 test and determined the normal distribution of constant variables using the Kolmogorov-Smirnov test. We performed the Student t test for variables with a normal distribution and the nonparametric Mann-Whitney U test for variables without a normal distribution. We then sought to identify what factors might be associated with growth of *P aeruginosa* from wound cultures. Using univariate, then multivariate logistic regression analyses, we compared the prevalence of factors in patients with, compared with those without, a *P aeruginosa* isolate from their foot wound. Statistical significance was set at $P < .05$.

Results

We identified 174 patients for enrollment in the main study on which this substudy is based. Only 90 of these patients, most of whom had type 2 diabetes, had microbiological assessments, and we used these patients in the present analysis (Fig. 1). The demographic and clinical characteristics of these patients are shown in Table 1. Most patients were late middle-aged men who had had their foot ulcer for approximately 2.5 months; most of them had a

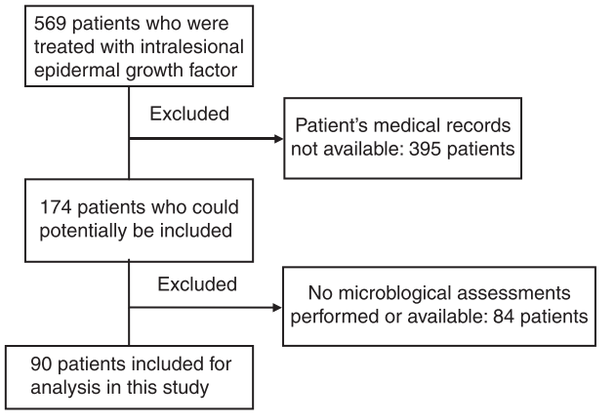

Figure 1. Patient flow scheme.

Table 1. Demographic and Clinical Characteristics of the 90 Enrolled Patients

Characteristic	Value
Age (mean ± SD [years])	61.11 ± 11.75
Male sex (No. [%])	64 (71.1)
Duration of diabetes (median [25%–75%] [years])	9 (5–19)
Type 2 diabetes (No. [%])	84 (93.3)
Renal failure (No. [%])	25 (27.8)
Receiving renal dialysis (No. [%])	19 (21.1)
Smoking (active or history) (No. [%]) (n = 81)	37 (45.7)
HbA_{1c}, median (25%–75%) (n = 74)	8 (6–9)
Previous hospitalization (No. [%]) (n = 89)	66 (74.2)
Duration of diabetic foot ulcer (median [25%–75%] [days])	74 (42–180)
Previous foot ulcer at any site (No. [%])	47 (52.2)
Previous foot osteomyelitis at any site (No. [%])	25 (27.8)
Previous debridement (soft tissue) (No. [%]) (n = 89)	41 (46.1)
Previous lower-extremity amputation (ipsilateral or contralateral) (No. [%])	22 (24.4)
Previous vascular surgery (No. [%])	22 (24.4)
Previous hyperbaric oxygen therapy (No. [%]) (n = 86)	17 (19.8)
Previous negative pressure wound therapy (No. [%]) (n = 87)	21 (24.1)
Previous active wound dressing (No. [%]) (n = 88)	13 (14.7)
Antibiotic drug administration within the past 30 days (No. [%])	56 (62.2)
Peripheral vascular disease (No. [%])	
Grade 1	36 (40.0)
Grade 2	37 (41.1)
Grade 3	17 (18.9)
Wound depth (No. [%])	
Grade 1	20 (22.2)
Grade 2	35 (38.9)
Grade 3	35 (38.9)
Neuropathy (No. [%])	68 (75.6)
Ulcer localizations (No. [%])	
Great toe	14 (15.6)
Other toes	11 (12.2)
Metatarsal	12 (13.3)
Dorsal foot	14 (15.6)
Plantar foot	16 (17.8)
Heel	23 (25.6)
Infection (International Working Group on the Diabetic Foot classification) (No. [%])	
Grade 1	14 (15.6)
Grade 2	19 (21.1)
Grade 3	49 (54.4)
Grade 4	8 (8.9)
Osteomyelitis (No. [%]) (n = 89)	31 (34.8)
Wound size (median [25%–75%] [cm^2])	15 (6–24)
Leukocyte count (median [25%–75%] [/mm^3]) (n = 61)	9,000 (7,000–13,000)
C-reactive protein (median [25%–75%] [mg/dL]) (n = 56)	35 (9.5–109)
Erythrocyte sedimentation rate (median [25%–75%] [mm/h]) (n = 50)	55 (24–76)

Abbreviation: HbA_{1c}, hemoglobin A_{1c}.

grade 3 or 4 infection according to the PEDIS classification (63.3%).

Wound cultures were negative in 19 patients (21.1%); in the other 71 patients, 89 bacteria were isolated. The percentage of patients whose wound culture yielded a gram-negative aerobic bacillus (n = 49, 55.1%) was higher than for those who had a gram-positive coccus (n = 36, 40.4%).The most frequently isolated microorganism was *P aeruginosa* (n = 23, 25.8%). Others common isolates were *S aureus* (n = 12, 13.5%) and coagulase-negative staphylococci (n = 10, 11.2%) (Table 2). The rate of methicillin-resistant isolates among *S aureus* was low (n = 4, 4.5%). Seventeen patients had monomicrobial infection due to *P aeruginosa*.

Of the 174 included patients, 32 (18.4%) under-

Table 2. Microorganisms Isolated from foot Wound Culture

Causative Bacteria	No. (%)
Gram-positive aerobic cocci	
Staphylococcus aureus	12
Methicillin resistant	4
Coagulase-negative staphylococcus	10
Methicillin resistant	6
Streptococcus spp	8
Enterococcus spp	6
Subtotal	36 (40.4)
Gram-negative aerobic bacilli	
Pseudomonas aeruginosa	23
Escherichia coli	7
Klebsiella pneumoniae	6
Proteus spp	5
Morganella morgagni	4
Enterobacter spp	2
Serratia marcescens	1
Stenotrophomonas maltophilia	1
Subtotal	49 (55.1)
Obligate anaerobes	3 (3.4)
Candida parapsilosis	1 (1.1)
Total	89 (100)

went a minor amputation and five (2.9%) had a major amputation. Culture results were positive in all of the patients with major amputation, and *P aeruginosa* was isolated in two of the five. Culture results were positive in 16 of 32 patients who underwent a minor amputation, and *P aeruginosa* was isolated in five of them. There was no significant difference between isolates cultured from those having a major versus a minor amputation ($P > .05$).

We looked for factors that might potentially be associated with isolation of *P aeruginosa* by comparing patients who did, versus those who did not, have that isolate on wound cultures. By univariate analysis we found nine factors that were statistically significantly different between the two groups (Table 3). On multivariate analysis, however, the only statistically significant independent risk factors for the growth of *P aeruginosa* on wound culture were having had a previous lower-extremity amputation and having previously used an active wound dressing (eg, one containing iodine or silver) (Table 4).

Discussion

Selecting antibiotic therapy for a DFI is largely based on the causative pathogen(s). Empirical therapy requires the clinician to make the best guess at the likely organism(s), and then to modify therapy based on the results of culture and sensitivity tests of wounds and other specimens.[3] Because isolation of *P aeruginosa* from a wound requires using antibiotic agents that are different (and generally more broad spectrum and expensive) than those for treating aerobic gram-positive cocci, it is important for clinicians to be aware of the likelihood of infection with this organism in their location. A recent concern has been that empirical antibiotic therapy directed at *P aeruginosa* may be overused in Western countries, where it is a relatively uncommonly isolated pathogen.[3]

The rates of isolation of various types of bacteria from DFIs differ among countries and continents. A large multicenter study of mild DFIs from the United States in 2008 revealed that gram-positive

Table 3. Univariate Analysis Comparing Factors Potentially Associated with the Isolation of *Pseudomonas aeruginosa* from Wound Culture

	P aeruginosa on culture (No.)		
Factor	Negative	Positive	*P* Value
Antibiotics received within the previous 30 days			
No (n = 34)	31	3	.005
Yes (n = 56)	36	20	
Previous foot ulcer at any site			
No (n = 43)	40	3	<.001
Yes (n = 47)	27	20	
Previous foot osteomyelitis at any site			
No (n = 65)	57	8	<.001
Yes (n = 25)	10	15	
Previous debridement (soft tissue)			
No (n = 48)	41	7	.009
Yes (n = 41)	25	16	
Previous lower-extremity amputation (ipsilateral or contralateral)			
No (n = 67)	59	8	<.001
Yes (n = 23)	8	15	
Previous active wound dressing history			
No (n = 75)	60	15	.002
Yes (n = 13)	5	8	
Wound depth			
Grade 1 (n = 20)	20	0	<.001
Grade 2 (n = 35)	29	6	
Grade 3 (n = 35)	18	17	
Neuropathy			
No (n = 22)	21	1	.01
Yes (n = 68)	46	22	
Infection (International Working Group on the Diabetic Foot classification)			
Grade 1 (n = 14)	12	2	.025
Grade 2 (n = 19)	18	1	
Grade 3 (n = 49)	32	17	
Grade 4 (n = 8)	5	3	

Table 4. Logistic Regression Analysis of Factors Potentially Associated with Isolation of *Pseudomonas aeruginosa* from Wound Culture

Variable	*P* Value	Odds Ratio	95% Confidence Interval
Previous lower-extremity amputation (ipsilateral or contralateral)	<.001	12,865	3,865–42,439
Previous active wound dressing	.018	5,993	1,364–26,328

aerobes were isolated in 77%, and gram-negative aerobes were isolated in 21.2%; specifically, *P aeruginosa* was isolated in only 6.9%.[4] On the other hand, in the same study, because many of these infections were mixed, the investigators could not be sure which were the causative or pathogenic bacteria. In another large, US, multicenter study that enrolled patients with moderate or severe DFIs, the rate of isolation of gram-positive cocci was 57.1%, whereas the rate of isolation of *P aeruginosa* was only 2.5%.[1] In another study performed in France with patients with Wagner grade 3 to 5 wounds, the same rates were found to be 44.5% and 10.8%, respectively.[5] In a recently published study from the United States, *P aeruginosa* was noted to be "an uncommon pathogen," found in only 4.5% of DFIs.[6] However, studies from less developed countries, especially those in Asia and Africa, report that, using standard microbiological methods, aerobic gram-negative bacilli, especially *P aeruginosa*, are more often pathogens in DFIs.[7] A study of 440 patients with a DFI in Kuwait found that 51.2 % were caused by aerobic gram-negative organisms, whereas aerobic gram-positive organisms caused only 32.3%.[8] Similarly, in a study from Malaysia, gram-negative bacteria were found in 52% of DFIs, with *P aeruginosa* isolated in 25%.[9] Studies of DFI from Kuwait and India reported rates of *P aeruginosa* isolates of 17.5% and 27.05%, respectively.[10,11]

Turkey is geographically located between, and links, the European and Asian continents. A recent study from Turkey found that aerobic gram-positive and gram-negative organisms were isolated with almost equal frequency from DFIs, both in the entire period of 1989–2011 (48.4% versus 48.4%) and in just the most recent years of 2007–2011 (49.9% versus 48.8%).[2] This study also specifically noted a high rate of infection with *P aeruginosa* in these patients: 13.7% between 1989 and 2011 and 14.9% between 2007 and 2011.[2] In the present study, the frequency of *P aeruginosa* is even higher, with a rate of 25.8%. Other studies from Turkey have demonstrated a frequency of *P aeruginosa* isolation from DFIs varying between 20% and 30%.[12-15]

The causative bacteria in DFIs may be related to the severity of the infection. In the early stages of DFI, especially in patients who have not recently received antibiotic drug therapy, gram-positive bacteria are usually predominant; gram-negative bacteria are more frequent in chronic infections, especially after treatment with antimicrobial therapy or with water-based treatments (eg, soaking or irrigation).[7] In a study from Spain,[16] the presence of a severe foot infection and a high white blood cell count were found to be risk factors for isolating gram-negative bacteria in diabetic patients with foot osteomyelitis. The results of the present study are compatible with this observation: 63.3% of the microbiologically assessed patients had advanced grade (3 and 4) foot infections according to the PEDIS classification (Table 3).

Serious infections caused by *P aeruginosa* are often nosocomial, and therapeutic options are increasingly limited due to the continued emergence and spread of antimicrobial-resistant strains.[17] For *P aeruginosa* infections that involve sites other than the diabetic foot, risk factors for *P aeruginosa* infection include previous hospitalizations, intensive care unit stay, immunocompromised status, previous antibiotic therapy, history of *P aeruginosa* isolates, and open wounds.[18-20] These risk factors emphasize that *P aeruginosa* infection are often health care related. There are, however, few published studies analyzing the risk factors for *P aeruginosa* in DFI. In a recent US study that enrolled 112 patients with DFIs, the authors reviewed many factors, including patient age, glycosylated hemoglobin level, tobacco use, presence of osteomyelitis, a prescription for antibiotic drugs in the preceding 3 months, and type of operative procedure and found that none were associated with *P aeruginosa* infection.[6] Despite the high rate of patients with moderate or severe infection (98.2%), they found a low rate of *P aeruginosa* infection (4.5%).We found a high rate of *P aeruginosa* (25.8%), however, despite a relatively low rate of patients with moderate or severe infections (63.3%). Because we had a sufficient number of patients presenting with *P aeruginosa* infection, we had the chance to evaluate various risk factors for this pathogen. In this study, most of the risk factors found to be statistically

significantly associated with isolation of *P aeruginosa* on univariate analysis, including previous antibiotic drug therapy within the past month; a history of foot ulcer, osteomyelitis, or lower-limb amputation; and use of an active wound dressing, suggest that these patients had been exposed to a health-care setting. This is also true of the only two factors that remained statistically significantly associated with *P aeruginosa* infection by multivariate analysis, ie, a history of a previous lower-extremity amputation and previous treatment with an active wound dressing. On the other hand, the relationship between active wound dressings and *P aeruginosa* is a surprising result. There are many types of wound dressings, such as gauze, hydrogels, foams, alginates, and hydrocolloids, available on the market today. Gauze is described as an inactive dressing, and dressings containing iodines, silver, honey, or antimicrobials are known as active wound dressings. In daily practice, the main reasons to apply active dressings are to maintain the moisture of the wound and to gain antimicrobial effectiveness. It is known that high levels of moisture in a wound facilitate the growth of *P aeruginosa*. This finding would need to be validated by further studies.

The main limitation of this work is that it is a retrospective study. Therefore, we did not have the opportunity to obtain data about the patient's daily life practices and socioeconomic status, and we could not evaluate the relationship of these factors and the presence in the wound of *P aeruginosa*.- With the relatively recent recognition of the importance of *P aeruginosa* as a pathogen in diabetic patients with foot infection, it will be important to further investigate the risk factors for infection with this difficult pathogen. We hope others will further investigate this issue in prospective studies in various parts of the world.

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