The Foot 38 (2019) 1–3

Contents lists available at ScienceDirect

The Foot

journal homepage: www.elsevier.com/locate/foot

Original Article

Bacteriologic analysis of bone biopsy from diabetic foot infections within a VA patient population

Charles Parks, Sandy Nguyen*

California School of Podiatric Medicine, University of California, San Francisco , United States

ABSTRACT

Diabetic patients with foot infections were evaluated over a 5-year period from April 2005 to March 2010. Cultures were obtained from 92 patients after surgical debridement. All of the patients were classified as "severe" diabetic foot infections (DFIs) and PEDIS grade 4. Wound specimens were collected by bone biopsy and sent to San Francisco VA Medical Center Microbiology Department for aerobic and anaerobic cultures. Among the 92 cases, the study resulted in a total of 410 pathogens, which 203 pathogens were from bone cultures and 207 pathogens were from soft tissue cultures. 74% of cases were polymicrobial and 26% had growth of a single organism. *Staphylococcus aureus* presented in 49.35% of bone cultures and 55.38% of soft tissue cultures, *Streptococcus* species presented in 44.16% and 36.92% respectably and roughly 33% of both bone and soft tissue had staphylococcus coagulase negative present. Gram-negative organisms occurred in 25% of all cultures taken. *Pseudomonas* accounted for 15% of soft tissue infections but only 1.3% bone cultures. MRSA was found in 17.39% of cultures and VRE was seen in 4.34% of all culture results. *Staphylococcus aureus* was the most prevalent organisms seen. This study presents a comprehensive microbiological survey of diabetic patients with DFIs within the San Francisco VA Medical Center and this study will help guide physicians to improve clinical outcomes of DFIs by using proper antibiotics.

1. Introduction

Diabetic foot ulcer (DFU) is one of the most common complications of diabetes with infection occurring in over half of foot ulcers [1]. The lifetime risk of a foot ulcer for patients with diabetes may be as high as 25%. In 2012, 21 million Americans had diabetes, making diabetes the 7th leading cause of death. Impaired wound healing, vascular disease, foot deformity and loss of sensation are the main causes of DFUs [2]. These ulcers frequently become infected, leading to reduced physical and mental quality of life, need for health care provider visits, antimicrobial treatment and lower extremity amputations [3].

DFUs with osteomyelitis are a major cause of lower extremity amputations [4–6]. Osteomyelitis is inflammation of the bone caused by an infectious organism. Often, osteomyelitis requires multidisciplinary approach due to its difficult clinical problem to diagnose and treat [7]. According to the International Working Group on the Diabetic Foot, a leg is lost to diabetes somewhere in the world every 30 s, with 50% of amputations secondary to infection. DFUs are a major cause of morbidity and mortality, accounting for approximately two-thirds of all non-traumatic amputations preformed in the United States. Chronic foot ulcers precede lower-limb amputation 70–85% of the time in patients with diabetes [8–10]. In order to adequately manage and treat diabetic foot infections (DFIs), debridement and understanding common flora is necessary to initiate definitive antibiotic [5].

Historically, research papers have proven that DFIs are polymicrobial with the most common pathogen *Staphylococcus aureus* and beta-hemolytic Streptococci. Others have reported a predominance of Gram-negative aerobes. Over the last few decades, many studies have contraindicated one another possibly due to differences in the causative organisms occurring over time, geographic variations or the types of severity of infection [11]. This information is important because starting empiric antibiotics for pathogen coverage prior to results of Culture and Sensitivities is often necessary.

The aim of this study is to compare the culture analysis from DFIs at the San Francisco VA Medical Center with the published literature. Management of bone and soft tissue infections requires debridement and antibiotic therapy. Knowledge of common bacterial pathogens in DFIs is critical in order to initiate appropriate antibiotic therapy. Between April 2005 to March 2010, 92 diabetic patients with DFIs were retrospectively evaluated. Deep bone and soft tissue cultures and bone biopsy were obtain from patients with DFIs under sterile conditions in the operating room and sent for Culture and Sensitivity, performed at San Francisco VA Medical Center Microbiology Department protocol and recorded. All patients were classified as "severe" DFI, PEDIS grade 4, according to the Infectious Disease Society of America (IDSA) guidelines for the diagnosis and treatment of DFI [12].

2. Materials and methods

Ninety two diabetic patients with DFI were retrospectively

* Corresponding author.
E-mail addresses: Charles.Parks@UCSF.edu (C. Parks), Sandy.Nguyen@samuelmerritt.edu (S. Nguyen).

https://doi.org/10.1016/j.foot.2018.10.004
Received 28 May 2018; Received in revised form 3 October 2018; Accepted 8 October 2018
0958-2592/ Published by Elsevier Ltd.

evaluated over a five-year period resulting in 203 total pathogens. This retrospective review took culture data from diabetic male and female adults who had clinically infected wounds, which were classified as severe in nature. Deep cultures were taken from both soft tissue and bone from patients with a DFI in the operating room. The specimen was taken utilizing sterile technique and placed into sterile containers and transported to the VA San Francisco Microbiology department following OR protocol, where they were tested for culture and sensitivity. To avoid the isolation of colonizing flora, rather than pathogenic flora, no cultures were obtained from superficial swab or superficial debris. All cultures were taken following IDSA practice guidelines for taking proper accurate cultures with thorough irrigation and debridement prior to taking the specimen for culture from the ulcer floor (Lipsky). All patients were classified as "severe" DFI PEDIS grade 4, according to the IDSA guidelines for the diagnosis and treatment of DFI [13]. Cultures were collected from clinically infected bone and soft tissue and were sent for sensitivity testing.

In 2003, PEDIS classification was introduced by the International Working Group of the Diabetic Foot (IWGDF) to categorize DFU objectively and facilitate communication between health-care providers [14]. The PEDIS classification is broken up into 5 different categories: perfusion, extent (size), depth (tissue loss), infection and sensation (neuropathy). Perfusion is determined by noninvasive vascular studies, such as ankle-brachial index, toe brachial index, transcutaneous oxygen pressure and ankle/toe pressures and physical examination. Extent is measured by calculating the diameters of the wound and depth is determined by using a blunt probe. Clinical signs and symptoms of infection as well as lab tests assist in the diagnosis of infection. Sensation is evaluated by the use of a 10 g monofilament and 128 Hz tuning fork for vibratory sensation [14]. The PEDIS classification allows providers to predict the outcome of DFU through a semi-quatitative gradation for severity of each of the categories [13].

3. Results

There were 92 cases that were retrospectively reviewed, which resulted in a total of 410 pathogens, 203 pathogens from bone culture and 207 pathogens from soft tissue samples. Table 1 shows results of the soft tissue and bone pathogens and percentage of growth. Of the 92 total cases reviewed for C&S, 74% of cases were polymicrobial and 26% had growth of a single organism. *Staphylococcus aureus* presented in 49.35% of bone cultures and 55.38% of soft tissue cultures, *Streptococcus* species 44.16% and 36.92% respectably. ~33% of both bone and soft tissue had staphylococcus coagulase negative present. Gram negative organisms occurred in ¼ of all cultures taken. *Pseudomonas* accounted for 15% of soft tissue infections but only 1.3% bone cultures. *Staphylococcus* was the most prevalent organisms seen. MRSA was found in 17.39% of cultures and VRE seen in 4.34% of all culture results.

Table 1
Bacterial pathogens from soft tissue and bone specimens.

Microorganism(s)	Bone		Soft tissue		Both	
	(n = 77)	%	(n = 65)	%	(n = 92)	%
Aerobic Gram-positive cocci						
Staphylococcus aureus	38	49.35	36	55.38	74	80.43
MSSA	27	35.06	31	47.69	58	63.04
MRSA	11	14.29	5	7.69	16	17.39
Staphylococcus coagulase negative	26	33.77	21	32.32	47	51.09
Enterococcus species	11	14.29	20	30.77	31	33.70
Vanco resistant	2	2.60	2	3.08	4	4.34
Not Vanco resistant	9	11.69	18	27.69	27	29.34
Streptococcus species	34	44.16	24	36.92	58	63.04
Corynebacterium species	17	22.08	11	16.92	28	30.43
Aerobic Gram-negative Bacilli						
Enterobacteriaceae	4	5.19	6	9.23	10	10.87
Pseudomonas species	1	1.30	10	15.38	11	11.96
Other Gram negative rod species	17	22.08	21	32.31	38	41.30
Obligate anaerobes						
Peptococci and Peptostreptococci	6	7.79	2	3.08	8	8.70

*Note total adds up to > 100 because of polymicrobial infections.

4. Discussion

Foot infections are a major and increasing problem worldwide for people with diabetes. In the United States about 25% of diabetic patients develop foot ulcerations in their lifetime. DFIs typically begin in a wound secondary to neuropathic ulceration with loss of protective sensation and vascular disease. Clinical signs and symptoms of DFUs include pain, erythema, edema, purulent exudate and warmth. Identification of localized infection in DFUs is essential to prevent amputation [15].

Many studies have cited that DFIs are polymicrobial, with aerobic gram-positive cocci. Most common causative organisms are staphylococci [11,13]. The emergence of antibiotic resistant pathogens has been a challenging aspect of treating patient's DFIs. To avoid increasing resistance to antibiotics, most authorities advocate treatment only for clinically infected wounds and the use of the narrowest-spectrum therapy possible. Our study shows that in diabetic patients with severe DFIs, the infections are generally polymicrobial, with mixed Gram-positive and Gram-negative species. Although, gram positive cocci has been reported as the most common pathogenic agent in the USA and Europe, Gram negative species were the most frequent causative pathogens reported in Asian studies [16]. This information helps guide first line antibiotic therapy so potentially limb-threatening infections can result in the best possible outcomes.

With a total of 410 species grown, 203 pathogens grew from bone culture and 204 pathogens from soft tissue samples. 74% of cases were polymicrobial, compared to Citron who reported 84% in 2007. *Staphylococcus* and *Streptococcus* species were the most prevalent organisms seen. These findings were consistent with previously published literature. Gram-negative aerobes account for approximately ¼ of pathogens. MRSA was found in 17.39% of the cases, which is an increase from the published 9.3% from Citron in 2007, with Citron's samples from soft tissue samples with varying degree of sterility. MRSA has been a pathogen of concern in patients with DFI for almost 3 decades; in fact, the first two isolates of Vancomycin-resistant MRSA strains were isolated from diabetic patients with foot infection [17].

According to Saltoglu et al., the most common microorganisms found were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* with a MRSA rate of 31% (791 patients), associated with an increase in rehospitalization rate. Meanwhile, Chen et al. reported 57% of the total 141 patients DFUs yielded MRSA pathogens and their patients had a 2 year cumulative probability of invasive systemic infections (31%) and an all-cause mortality rate (13%). Comparing our study to earlier and most recent studies, it shows an increase in incidence rate of MRSA over the years. This is problematic for patients as well as health care providers because MRSA causes increase in treatment failure, increase in the time required for healing of ulcerations and rate of amputations [16].

As reported in Table 2, *Enterobacteriaceae* was the most common pathogen found in cultures or DFUs in Wheat et al. and Bamburger et al. studies and third most common in Lavery et al., Newman et al. and Tan et al., while fourth in Citron et al. [5,18–20]. These values are significantly higher compared to 10.87% in our study. With a total of 465 cases in Beijing area, Li et al. most frequent isolated organisms were E*nterobacteriaceae.* Studies have shown that older patients are more likely to suffer from gram negative rod infections and patients with chronic wounds or recently had antibiotic treatments [21].

Table 2
Comparative table of bacterial pathogens from classic diabetic foot culture literature.

Microorganism(s)	Wheat (n = 27)	Bamberger (n = 51)	Lavery (n = 36)	Newman (n = 26)	Tan (n = 38)	Citron (n = 258)
Aerobic Gram-positive cocci						
Staphylococcus aureus	40	43	47	31	32	46.00
MSSA						36
MRSA						9.3
Staphylococcus coagulase negative						10
Enterococcus species	30	45	28	8	8	32.60
Vanco resistant						
Not Vanco resistant						
Streptococcus species	30	35	61	27	32	42.60
Corynebacterium species	10	16	n/a	4	n/a	27.90
Aerobic Gram-negative Bacilli						
Enterobacteriaceae	50	47	45	20	29	32.90
Pseudomonas species	10	10	11	15	0	9.30
Other Gram negative rod species						
Obligate anaerobes						
Peptococci and Peptostreptococci	20	27	3	n/a	34	n/a

Considering the abundant data on various microbials found in DFUs, the literature remains equivocal. The authors encourage proper specimen technique to result in the most appropriate and accurate pathogen identified. The most repeatable and accurate technique is to avoid superficial swab or debris cultures, and to obtain the specimens post irrigation and debridement under a sterile field. Our findings were cultured from inter-operative bone and tissue taken under sterile technique post irrigation and debridement. The results were consistent with previously reported studies and we hope that clinician will use broad spectrum antibiotics to cover a polymicrobial diabetic foot infection until their culture and susceptibility results help drive and tailor their antibiotic treatment choices.

Conflict of interest

No conflict of interest.

References

[1] Chen Y, Ding H, Wu H, Chen H. The relationship between osteomyelitis complications and drug resistant infection risk in diabetic foot ulcer: a meta analysis. Int J Low Extrem Wounds 2017;916:183–90.
[2] Blume P, Wu Stephanie. Updating the diabetic foot treatment algorithm: recommendations on treatment using advanced medicine and therapies. Wounds 2018;30(2):29–35.
[3] Wu WX, Liu D, Wang Y, Wang C, Yanf C, Liu X, et al. Empirical antibiotic treatment in diabetic foot infection: a study focusing on the culture and antibiotic sensitivity in a population from southern china. Int J Low Extrem Wounds 2017;16:173–82.
[4] Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA 2005;293:217–28.
[5] Lavery L, Sariaya M, Hisham A, Harkless L. Microbiology of osteomyelitis in diabetic foot infections. J. Foot Ankle Surg 2016;34(1):61–4.
[6] Lipsky B. Osteomyelitis of the foot in diabetic patients. Clin Infect Dis 1997;25:1318–26.
[7] Mandell J, Khurana B, Smith J, Czuczman G, Ghazikhanian V, Smith S. Osteomyelitis of the lower extreimity: pathophysiology, imaging, and classification with an emphasis on diabetic foot infection. Am Soc Emerg Radiol 2017.
[8] Palumbo PJ, Melton III LJ. Peripheral vascular disease and diabetes. Diabetes in America: diabetes data compiled 1984. Washington, D.C: Government Printing Office; 1985. August:XV-1–XV-21. (NIH publication. 85–1468).
[9] Ollendorf D, Kotsanos J, Wishner W. Potential economic benefits of lower-extremity amputation prevention strategies in diabetes. Diabetes Care 1998;21:1240–5.
[10] Sanders LJ. Diabetes mellitus: prevention of amputation. J Am Podiatric Assoc 1994;84:322–8.
[11] Citron D, Goldstein E, Merriam V, Lipsky B, Abramson M. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. J Clin Microbiol 2007(September):2819–28.
[12] Schaper NC. International ulcer classification for research purposes by the International Working Group on the Diabetic Foot. Proceedings of the 4th International Symposium on the Diabetic Foot. Diab Metab Res Rev 1988.
[13] Lipsky B, Berendt A, Cornia P, Pile J, Peters, Armstrong D, et al. 2012 Infectious Disease Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2012;54(12):132–73.
[14] Chuan F., Tang K., Jiang P, Zhou B., He X. Reliability and validity of the PEDIS classification system and score in patients with diabetic foot ulcer. 2015.
[15] Gardner S, Hillis S, Frantz R. Clinical signs of infection in diabetic foot ulcers with high microbial load. Biol Res Nurs 2009;11:119–28.
[16] Saltoglu, et al. Influence of multidrug resistant organisms on the ouctome of diabetic foot infection. Int J Infect Dis 2018;70(May):10–4.
[17] Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, et al. Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. N Engl J Med 2003;348:1342–7.
[18] Bamberger D, Gerding D. Osteomyelitis in the feet of diabetic patients. Am J Med 1987;83:653–60.
[19] Tan J, Friedman N, Hazelton-Miller C, Flanagan P, File T. Can aggressive treatment of diabetic foot infections reduce the need for above-ankle amputation. Clin Infect Dis 1996;23:286–91.
[20] Wheat J, Allen S, Henry M, Kernek C, Siders J, Kuebler T, et al. Diabetic foot infections. Arch Intern Med 1986;146:1935–40.
[21] Li X, Qi X, Yuan G, Ju S, Yu Z, Deng W, et al. Microbiological profile and clinical characteristics of diabetic foot infection in northern China: a retrospective multicentre survey in the Beijing area. J Med Microbiol 2018.