Acta Dermatovenerol Croat 2005;13(3):168-172 CLINICAL ARTICLE

Bacterial Flora of Leg Ulcers in Patients Admitted to Department of Dermatology, Poznań University of Medical Sciences, during the 1998-2002 Period

Maria Żmudzińska, Magdalena Czarnecka-Operacz, Wojciech Silny

University Department of Dermatology and Allergic Diseases Diagnostic Center, University of Medical Sciences, Poznań, Poland

Corresponding author:

Maria Żmudzińska, MD

University Department of Dermatology

Allergic Diseases Diagnostic Center

University of Medical Sciences

ul. Przybyszewskiego 49

60-355 Poznań

Poland

m.zmudinska@wp.pl

Received: August 30, 2004.

Accepted: December 10, 2004.

SUMMARY Venous leg ulcers are an important cause of morbidity in a significant percentage of the world population. The percentage of leg ulceration, either active or healed, in the European population is about 1%-2%. The aim of this study was to analyze the rate of colonization and qualitative composition of the bacterial flora isolated from leg ulcers in patients admitted to Dermatology Department, Poznań University of Medical Sciences, during the 1998-2002 period, with special reference to the infection risk factors. Bacteriological diagnosis of 175 wound swabs was performed in compliance with compulsory laboratory methods. In 173 positive results, the predominant culture composition included *Staphylococcus aureus* (56.57%), *Pseudomonas aeruginosa* (37.14%), *Enterococcus faecalis* (22.29%), *Proteus mirabilis* (13.71%) and *Escherichia coli* (12.57%). There was a significant increase in the incidence of *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*, along with a decrease in the incidence of *Staphylococcus aureus* isolation during the study period. The rate of yeast-like fungi strains, mainly *Candida albicans*, recorded in culture composition showed a systematic increase. Changes in the qualitative and quantitative composition of bacterial flora, presence of multiple isolates, and concomitant diseases that may influence the characteristics of leg ulcer disease were closely monitored.

KEY WORDS leg ulcers, bacterial flora, wound healing

INTRODUCTION

Chronic venous leg ulcers are an important cause of morbidity in a significant percentage of the world's population. The prevalence of lower extremity ulceration is estimated to be approximately 1% in the European population. Up to 80% of all leg ulcers are caused by venous disease (1-4).

Leg ulcers appearing in the course of chronic venous insufficiency frequently recur after healing. The pathophysiology of venous ulceration is multifactorial and complex. The bacterial influence on the pathogenesis of chronic venous ulcers is unclear (5,6). Chronic wound surface can become

Acta Dermatovenerol Croat
2005;13(3):168-172

a moist medium for bacterial growth. Very often the microorganisms cultured from chronic ulcers are of endogenous origin, mainly fecal, oral and cutaneous. In most cases, the nature of chronic venous ulceration is polymicrobial (5-10). Various reports indicate that 80% to 100% of chronic ulcers are at some point colonized with bacteria. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are predominantly isolated from leg ulcers (5-8).

Delayed wound healing has been associated with aerobic or facultative pathogens (5-7,9). The presence of *Proteus mirabilis* and *Pseudomonas aeruginosa* in wound swabs has been linked to poorly healing ulcers (11). Other authors suggest a positive correlation between prolonged wound healing and higher quantitative bacterial count or the presence of four and more bacterial groups (7,9,11).

In this study, the rate of colonization and qualitative composition of leg ulcer bacterial flora were analyzed according to characteristic features of the leg ulcer syndrome and concomitant diseases.

MATERIAL AND METHODS

Patients suffering from leg ulcers admitted to Dermatology Department of Poznań University of Medical Sciences between 1998 and 2002 were included in the study.

Microbiological examination of wound swab samples was performed at the Laboratory of Bacteriology, Clinical Hospital No. 2 in Poznań. A total of 175 wound swabs were obtained from patients aged 34-93 prior to any antimicrobial therapy.

Columbia agar with 5% sheep blood, Chapman medium, Sabouraud medium with antibacterial agent, medium for gram-negative bacteria culture, coccosal medium and enriched broth were inoculated for the isolation of aerobic bacteria. The incubation took place at 37° C for 48 h. For anaerobes, isolation specimens were plated onto Columbia agar with 5% sheep blood, selective medium for anaerobic gram-negative bacteria and multiplying broth. The plate media were incubated at 37° C in anaerobic conditions. The identification of both aerobic and anaerobic organisms was performed by use of the ATB Plus bioMerieux computer method.

RESULTS

Microorganisms were cultured in 173 (99%) of 175 samples. Women accounted for 80% of study patients. The predominant pathogens were *Staphylococcus aureus* (56.57%), *Pseudomonas aeruginosa* (37.14%), *Enterococcus faecalis* (22.29%), *Proteus mirabilis* (13.71%), *Escherichia coli* (12.57%) and *Candida* spp. (6.86%) (Fig. 1).

A significant increase was recorded in the rate of *Pseudomonas aeruginosa* from 27.78% in 1998 to 42.42% in 2002, *Enterococcus faecalis* from 8.33% in 2000 to 39.39% in 2002, *Escherichia coli* from 11.11% in 2000 to 18.18% in 2002, *Staphylococcus haemolyticus* from 2.78% in 2000 to 15.15% in 2002, *Candida* spp. from 2.78 in 2000 to 12.12% in 2002, and *Enterobacter cloacae* from 2.78% in 2000 to 6.06% in 2002. An interesting pattern was observed in the concurrent decrease in the incidence of *Staphylococcus aureus* isolation from 69.44% in 2000 to 48.48% in 2002 (Fig. 2).

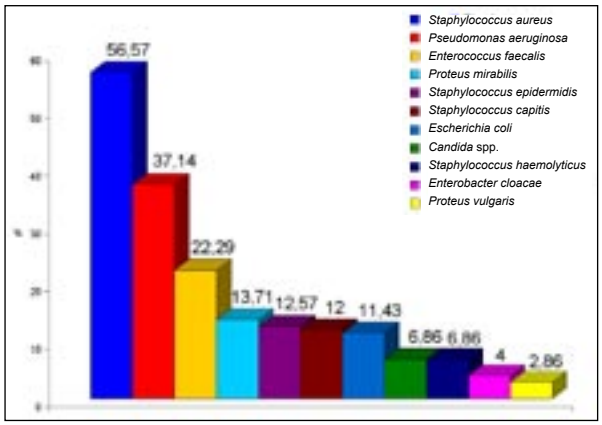

Figure 1. Percentage composition of predominant bacterial strains cultured between 1998 and 2002.

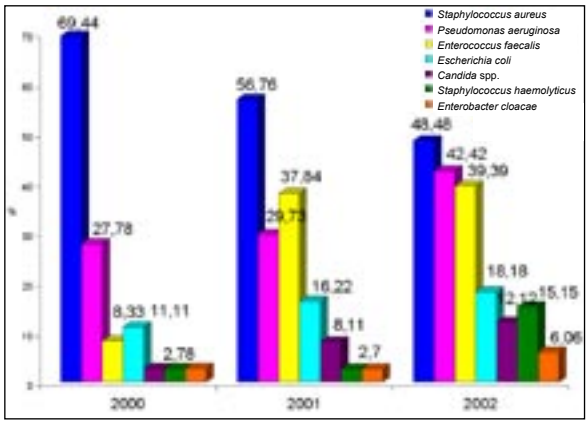

Figure 2. Variation in the incidence of bacterial isolation during the 1998-2002 period.

Acta Dermatovenerol Croat
2005;13(3):168-172

The number of isolates per specimen varied from one to six. Two and three microorganisms were most frequently cultured from one ulcer (Fig. 3). The number of polymicrobial specimens increased with the duration of ulcer disease. The presence of three or four organisms was observed in ulcers non-healing for 10 or more years. Single or double isolates were predominant in the samples cultured form ulcers lasting for less than 5 years (Fig. 4).

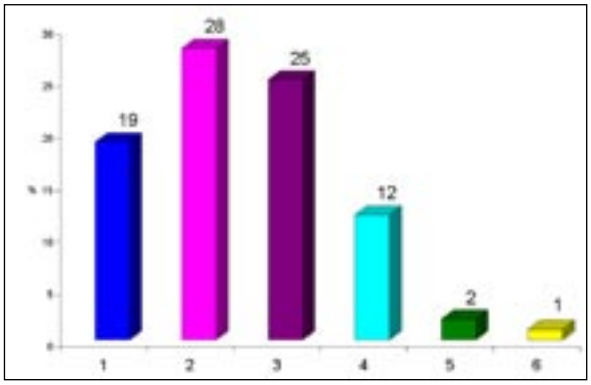

Figure 3. Percentage of polymicrobial isolates.

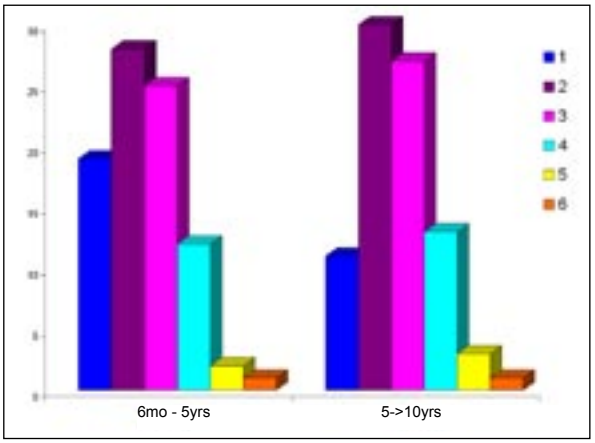

Figure 4. Correlation between polymicrobial samples and duration of venous leg ulcer

The presence of *Proteus mirabilis* and *Pseudomonas aeruginosa* in wound swabs could be associated with delayed wound healing. Proteus mirabilis and *Pseudomonas aeruginosa* were mainly cultured from patients suffering from leg ulcers for 10 years and more. This correlation was more evident for *Proteus mirabilis* than for *Pseudomonas aeruginosa*, where positive cultures were also frequently obtained from patients with a shorter history of leg ulcer disease (Fig. 5).

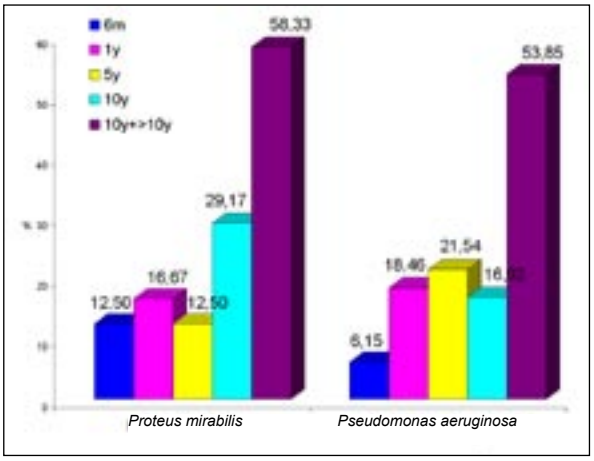

Figure 5. Correlation between the presence of *Proteus mirabilis* and *Pseudomonas aeruginosa,* and duration of leg ulcer disease.

The coexisting diseases such as diabetes mellitus or erysipelas did not alter the percentage of isolation and had no effect on either quantitative or qualitative composition of the bacterial flora. Surprisingly, diabetes mellitus was not a factor increasing the incidence of *Candida* spp. colonization.

DISCUSSION

Venous leg ulcers are associated with medical, social and emotional burden, thus reducing the patient's quality of life. Chronic wounds very often present a non-healing and recurrent medical problem.

Our study confirmed results reported elsewhere on *Staphylococcus aureus* as the predominant microorganism cultured from leg ulcers swabs. On the other hand, we observed a decrease in the incidence of *Staphylococcus aureus* isolation, along with an increase in the rate of *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* during the study period. The increasing number of *Candida* spp. isolates was seen in patients without diabetes mellitus as the concomitant disease. Polymicrobial results of swab cultures were obtained from patients suffering from leg ulcers for many years.

Patients diagnosed with chronic venous insufficiency suffer from impaired wound healing in the affected extremity. The mechanism of delayed healing is unknown. The risk factors for the development of chronic venous insufficiency include age, female sex, obesity, lack of physical activity, and family history (1-5). A chronic, long-lasting

venous insufficiency may lead to premature fibroblast aging, which is strongly connected with impairment of the wound healing processes. In addition, insufficient blood supply is present due to the pathophysiologic mechanisms of chronic venous insufficiency in lower extremities (12).

The following mechanisms should be taken in consideration when discussing the bacterial role in the wound healing process: contamination, where the wound has non-replicating organisms on its; colonization takes place when bacteria capable of replicating on the ulcer surface inhibit non-viable tissue in the wound in the absence of immune response. Clinically significant infection is defined as a sudden onset of pain or increased pain, spreading erythema, swelling, cellulitis, purulent exudation and odor (13-16). These situations can take place in leg ulcers.

Differentiation between the infected and non-infected leg ulcers may pose a dilemma and is a matter of much discussion, because treatment is determined by the diagnosis. In our retrospective study, we were not able to correlate our data with detailed clinical characteristics of the examined leg ulcers. Some authors suggest that the presence of bacteria in chronic wounds can be identified as a secondary infection, which can also result in impaired wound healing. The presence of more than 10^5 bacteria per gram of tissue may impede wound healing and epithelial regeneration (17). Most leg ulcers are characterized by complex bacterial population. It is suggested that microbial interactions may contribute to the pathogenesis of chronic wound infections, leading to deterioration of such wounds (6).

Moreover, it should be emphasized that infected ulcers may not only become a serious and dangerous source of nosocomial infections but chronic wounds may also be colonized with nosocomial bacterial strains causing systemic complications in elderly patients with impaired immunity system.

CONCLUSIONS

Staphylococcus aureus was the main bacterial factor cultured from leg ulcer swabs obtained from patients admitted to Dermatology Department of Poznań University of Medical Sciences between 1998 and 2002. The study period was characterized by a significant increase in the incidence of *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli* and *Candida* spp. isolation. The finding of *Pseudomonas aeruginosa*, *Proteus mirabilis* and polymicrobial samples in patients with chronic leg ulcer disease may confirm the potential role of bacterial synergism in delayed healing of chronic wounds.

References

1. Adhikari A, Criqui MH, Wooll V, Denenberg JO, Fronek A, Langer RD, *et al.* The epidemiology of chronic venous diseases. Phlebology 2000;15:2-18.
2. Zapalski S, Oszkinis G. Ambulatoryjne leczenie chorób żył kończyn dolnych. Via Medica 2001.
3. Zimmet SE. Venous leg ulcers: modern evaluation and management. Dermatol Surg 1999;25:236-41.
4. Heit JA, Rooke TW, Silverstein MD, Mohr DN, Lohse CM, Petterson TM, *et al.* Trends in the incidence of venous stasis syndrome and venous ulcer: a 25-year population-based study. J Vasc Surg 2001;33:1022-7.
5. Brook I, Frazier EH. Aerobic and anaerobic microbiology of chronic venous ulcers. Int J Dermatol 1998;37:426-8.
6. Bowler PG, Davies BJ. The microbiology of infected and noninfected leg ulcers. Int J Dermatol 1999;38:573-8.
7. Halbert AR, Stacey MC, Rohr JB, Jopp-McKay A. The effect of bacterial colonization on venous ulcer healing. Aust J Dermatol 1992;33:75-80.
8. Harker J. The effect of bacteria on leg ulcer healing. Br J Community Nurs 2001;6:126-34.
9. Madsen SM, Westh H, Danielsen L, Rosdahl VT. Bacterial colonization and healing of venous leg ulcers. APMIS 1996;104: 895-9.
10. Daltrey DC, Rhodes B, Chattwood JG. Investigation into the microbial flora of healing and non-healing decubitus ulcers. J Clin Pathol 1981;34:701-5.
11. Trengove NJ, Stacey MC, Mc Gechie DF, Mata S. Quantitative bacteriology and leg ulcer healing. J Wound Care 1996;5:227-80.
12. Stanley A, Osler T. Senescence and the healing rates of venous ulcers. J Vasc Surg 2001;33:1206-10.
13. Dow G, Browne A, Sibbald RG. Infections in chronic wounds: controversies in diagnosis and treatment. Ostomy/Wound Manag 1999;45:23-40.

14. Kunimoto BT. Assessment of venous ulcers an in-depth discussion of a literature-guided approach. Ostomy/Wound Manag 2001;47: 38-52.
15. Seneczko F, Kaszuba A, Kozłowska M, Spinek A, Mordaka R, Pakula R, *et al.* Flora bakteryjna owrzodzeń goleni w przebiegu przewlekłej niewydolności obwodowego krążenia żylnego. Część I. częstość izolacji i skład jakościowy flory bakteryjnej. PdiA 2003;XX, 1:15-21.
16. McDaniel HB, Marston WA, Farber MA, Mendes RR, Owens LV, Young ML, *et al.* Recurrence of chronic venous ulcers on the basis of clinical, etiological, anatomical, and pathophysiological criteria and air plethysmography. J Vasc Surg 2002;35:723-8.
17. Valencia IC, Falabella A, Kirsner RS, Eaglstein WH. Chronic venous insufficiency and venous ulceration. J Am Acad Dermatol 2001;44:401-21.

You can have nice hands inspite of house cleaning with Nivea cream!
From the Nivea collection of Zlatko Puntijar