

Prevalence of mycoses in chronic lower limb wounds of people with onychomycosis and/or tinea pedis

Crutchfield L • Frescos N • Duncan GJ • Weller C • Woodward M

Chronic lower limb wounds are constantly exposed to bacterial and fungal organisms and are inevitably contaminated with several organisms. The aim of the pilot study was to investigate the prevalence of fungi in chronic lower limb wounds in people with onychomycosis and/or tinea pedis.

Consecutive, consenting patients with a chronic lower limb wound and a clinical diagnosis of onychomycosis and/or tinea pedis were recruited from two wound management clinics over a five-week period following ethics approval for the relevant institutions.

Wound swabs, toenail clippings and/or skin scrapings were collected from each participant and subjected to mycological examination. Wound swabs were analysed for fungal presence by culture, while both microscopy and culture techniques analysed toenail and skin samples.

In four participants, wound cultures tested positive for the opportunistic fungal *Candida* species. All four participants also had onychomycosis confirmed in their toenails by either a microscopy or culture analysis. Statistical analyses did not generate any significant ($p > 0.05$) relationships between fungal presence in wounds and wound aetiology, wound duration or positive toenail and skin samples, however fungi-positive wounds and wound location were found to be statistically significantly related ($p < 0.05$).

Crutchfield L, Frescos N, Duncan GJ, Weller C & Woodward M. Prevalence of mycoses in chronic lower limb wounds of people with onychomycosis and/or tinea pedis. *Primary Intentions* 2005; 13(2): 61-67.

Lauren Crutchfield

BPod (Hons)
Podiatrist
c/o Department of Podiatry
La Trobe University VIC

*Nicoletta Frescos

BAppSci (Pod) MPH
Clinical Education Coordinator/Lecturer
Department of Podiatry
La Trobe University
Bundoora, VIC 3086
Tel: (03) 9479 5832
Fax: (03) 9479 5784
e-mail: N.Frescos@latrobe.edu.au

Gregory J Duncan

BPharm MPH
Research Fellow
Department of Pharmacy Practice
Monash University VIC

Carolina Weller

BN MEd(Research)
Senior Lecturer
School of Nursing
La Trobe University VIC

Assoc.Prof Michael Woodward

MBBS FRACP
Director, Aged Care Services
Austin Health,
Heidelberg Reapatriation Hospital, VIC

* Correspondence to Nicoletta Frescos

Work is attributed to:
Department of Podiatry
Faculty of Health Sciences
La Trobe University
Bundoora VIC 3086

Introduction

Wounds are a serious public health concern because of the potential for associated morbidity and the costs that wound management imposes on both the patient and the community. This concern increases when wounds fail to heal and become chronic, with patients facing decreased quality of life, possible infection, loss of a limb or even loss of life¹.

Chronic lower limb wounds are constantly exposed to bacterial and fungal organisms and are inevitably contaminated with several organisms^{2, 3}. Wound micro-organisms do not

necessarily cause clinical infection but are known to delay healing in addition to being associated with pathophysiological processes such as diabetes, peripheral vascular disease and hypertension^{2, 4, 5-8}. The contaminating organisms are primarily bacteria, but fungi may be also found and may adversely affect wound healing³.

Fungi often cause superficial infections that occur on the feet. Onychomycosis (fungal infection of the toenails) and tinea pedis (fungal infection of the skin on the feet) are contagious conditions and are among the most common fungal foot infections⁹. While onychomycosis and tinea pedis are not usually a problem among the healthy population, the potential complications, including the spread of infection to other parts of the body and secondary bacterial infection for 'at risk' individuals, pose a more serious threat.

Onychomycosis and tinea pedis are recognised as the most common superficial diseases⁹. Superficial fungal infections are reportedly increasing, particularly for individuals over 60 years of age and with a compromised health status¹⁰⁻¹². Data on prevalence of onychomycosis and tinea pedis in the literature are conflicting. The climate and environmental surround influence the pathogenicity of organisms and make it difficult to generalise study findings to the worldwide population¹³⁻¹⁵. Onychomycosis is the most common disease of nails and accounts for between 18% and 40% of all nail disorders^{13, 16}. The prevalence rates for tinea pedis in the general population ranged between 4%¹⁷ and 10%¹⁸. Further, there are estimates that over 70% of the population worldwide are affected by tinea pedis during their lifetime¹⁷.

A comparative study between diabetic and non-diabetic populations by testing abnormal appearing nails by Gupta *et al.*¹⁶ showed that the prevalence of onychomycosis for the non-diabetic population was 40% while that of the diabetic population was 57%. The researchers generalised that onychomycosis was present in approximately one-third of the diabetic population with or without abnormal-appearing nails. Furthermore, their results indicated that onychomycosis was significantly correlated with increasing age.

Onychomycosis and tinea pedis have the potential to cause further morbidity such as wounds, cellulitis and secondary bacterial infection, particularly for individuals with a compromised health status^{15, 19, 20}. Wounds are constantly exposed to micro-organism invasion and, inevitably, harbour bacteria and fungi⁷. Micro-organisms found in chronic wounds are commonly bacterial, although fungi may be

present to a lesser extent⁷. Most pathogenic organisms in chronic wounds are bacterial^{2, 7}. Documentation of the role of fungi in chronic lower limb wounds is limited. However, fungi in burns-related wounds are recognised to have the potential to initiate complications during healing, therefore causing delayed healing²¹.

Bamberg *et al.*² found fungus (species not specified) was isolated in 5.3% of wound samples collected by various wound clinicians but did not indicate whether the organisms isolated were pathogenic or contaminants, or if the wound was acute or chronic, or if there were any classical signs of infection. Dow *et al.*³ isolated *Candida* species in chronic wounds with a polymicrobial infection, although the fungi demonstrated low-grade virulence in the wound. Ocariz *et al.*²² described onychomycosis in chronic leg wounds stating that chronic venous insufficiency can contribute to nail changes.

A literature review found no research that described the prevalence of fungal infections in people who have both chronic wounds and fungal infections in their feet. Further, no study was sourced that had investigated whether a relationship exists between fungal infections of the foot and lower limb chronic wounds, and whether the presence of the infection is a contributing factor to the delay in healing of the wound. An exploration of the role of fungi in chronic wounds would therefore contribute to current knowledge about microflora in the wounds. If fungal presence was identified to have a significant association with delayed healing, wound practices would need to include consideration of anti-fungal therapies.

This pilot study was a preliminary investigation into the prevalence of fungal presence in the chronic wound of people who also have a fungal infection of the foot. The researchers hypothesised that wound clinic patients with laboratory confirmed onychomycosis and/or tinea pedis would have fungal elements present in their wound.

Methods

Ethics approval was granted by the Austin Health Human Research Ethics Committee and the La Trobe University Human Ethics Committee. The Austin Health Wound Clinic and the Austin Health High Risk Foot Clinic gave their permission for the study to be conducted in these clinics.

Participants were recruited over a five-week period from the patient population at two metropolitan wound management clinics located in Melbourne, Australia. Consecutive patients

with a chronic lower limb wound and a clinical diagnosis of a fungal infection of the nails and/or skin of their feet on the same limb, were invited to participate in the study. Informed consent was obtained from all participants prior to entering the study.

A lower limb wound was defined by the researchers as any wound occurring on the leg from below the knee joint including the foot and digits. A wound was deemed chronic when it had been present for at least six weeks. When the participant had more than one wound present on the limb being assessed, the wound studied was that which was least responsive to therapy according to wound tracings, medical notes and wound clinicians. The inclusion criteria included presence of a chronic lower limb wound and clinical suspicion of onychomycosis and/or tinea pedis on the same limb as the wound.

Onychomycosis was suspected when discolouration of the nail plate was accompanied by another feature such as thickening or debris affecting the nail plate or nail bed. This was based on the Zaias classification of onychomycosis²³. Suspicion of tinea pedis was based on presenting signs and symptoms as described by Brooks and Beder¹⁷ and Zuber and Baddam²⁴. Onychomycosis and tinea pedis were clinically differentiated from other nail and skin conditions by consideration of the patient's age, medical history and previous skin and nail health.

Wound location was also observed and classified into one of seven regions of the lower limb (above gaiter, gaiter, malleoli, plantar rear foot, plantar midfoot, plantar forefoot and digits). Wound aetiology was classified as arterial, venous, mixed arterial and venous, infectious, metabolic or miscellaneous (classification adapted from Carville, 1998²⁵).

Toenail clippings and/or skin scrapings, together with wound swabs, were collected by either the principal researcher or a podiatry consultant, and sent for laboratory analysis to confirm or exclude the presence of a fungal infection. Prior to collection of skin and nail samples, the affected area was cleaned with a 70% v/v isopropyl alcohol wipe and toenail clippings and debris were taken from the proximal portion of the most affected toenail(s). Skin scrapings were collected from the advancing border of the affected area.

Standardised wound swabs were taken following routine wound debridement and cleansing using saline or tap water to decrease surface contaminants. All mycological testing for the collection specimens was conducted at a laboratory experienced in fungal analysis. The analysis of the skin and

toenails consisted of microscopy and culture. The wound swabs were analysed by culture only.

Statistical analysis involved the use of descriptive statistics with a more detailed analysis of data using Pearson chi-square, Mann Whitney U and Cronbach's alpha test. Data analysis was conducted using the computer program package 'SPSS for Windows' (Version 11.5).

Results

Twenty-eight participants were recruited in the study of which 16 (57%) were male. Participant ages ranged from 27 years to 85 years (average was 71 years \pm 14 years). Twenty-five participants (89%) had at least one medical condition that could impair their health status. Cardiovascular disease and Type 2 diabetes mellitus were the most common medical conditions. No participant had Type 1 diabetes mellitus. The most common locations for wounds were the plantar forefoot, malleoli and digits. The total length of time that wounds were present varied greatly with a range of 9 weeks to 387 weeks (average of 55 weeks + 82 weeks). There was no documented evidence that any of the wounds had previous fungal infection identified.

Toenail and skin samples

Thirty four wounds were sampled on a total of 28 participants. Of these wounds 31 were on participants diagnosed clinically to have onychomycosis and 3 on those with tinea pedis. Microscopy or culture analysis confirmed that 19 participants (68%) had onychomycosis and one participant had tinea pedis. There were no statistically significant values generated ($p > 0.05$) between laboratory analysis of nail and skin samples and participant variables such as age, gender and wound duration. Microscopy or culture analysis identified four wounds (from four separate participants) with fungi-positive results (Table 1). The only variable found to be statistically significant with fungi-positive wounds was wound location ($p < 0.5$), three wounds were located on digits, which also had co-existing onychomycosis.

Discussion

The average age (71 years) was reflective of that in which chronic wounds⁸ and onychomycosis^{10, 12, 13, 14, 16, 26}, are more frequent. The majority of the study population (89%) had a medical condition that could impair their overall health status and increase susceptibility to infections^{4, 5, 27}. While Type 2 diabetes mellitus is a co-morbidity known to delay wound healing the lack of a significant association with fungi-positive wounds and onychomycosis ($p > 0.05$)

in this study was possibly due to the small sample size. The traditional and commonly accepted view has been that people with diabetes have an increased risk of developing infection anywhere in the body; however this view has been questioned by more recent studies²⁸⁻³⁰. Furthermore, certain opportunistic organisms have been found to infect people with diabetes with higher frequency than the general population^{29 30}.

The polymicrobial environment in chronic wounds is created by organisms from the surrounding skin or by external contamination^{7, 31}. If onychomycosis or tinea pedis was capable of spreading fungal elements into a wound, it would be reasonable to suggest that wounds closer to the superficial fungal infection would be more likely to experience fungal contamination. Wound location was the only factor significantly associated with positive wound cultures ($p < 0.05$), although the small sample size requires this significance to be interpreted with caution. Three of the fungi-positive wounds were located on the digits that were the same toes where onychomycosis was detected. The nature of dressings and their retention may also have contributed to the association.

Fungi were isolated in approximately 14% of the study participants with a chronic lower limb wound. There is little research investigating fungi in such wounds, so the prevalence rate from this study population could not be readily compared with the results of other work. One study that made reference to fungal analysis in wounds² found that the fungi organisms were isolated in 5.3% of the wound cultures investigated.

The four fungi-positive wounds each grew *Candida*, which is the only type of fungi reported to inhabit a chronic wound³. Two of the fungi-positive wounds grew *C.albicans* although the same fungus was not grown in the corresponding toenail culture. The remaining two fungi-positive wound cultures and the respective toenail cultures grew non-specific *Candida*. The laboratory looked specifically for *C.albicans*, as it is the species often implicated in fungal infections of the nails^{15, 32}. All other candidal growth was reported generically as 'Candida'.

All of the fungi-positive wounds had corresponding toenail samples of laboratory-confirmed onychomycosis. However, only one of the fungi-positive wounds contained the same species of fungus (*T.mentagrophytes var. interdigitale*) as the respective toenail culture. This wound was located on the digit from which toenail clippings were collected. This

toenail was considered the likely source for the presence of *T.mentagrophytes var.interdigitale*, as dermatophytes are not regarded normal skin flora³³. This dermatophyte was, therefore, not likely to have spread from the surrounding skin, the area nominated in the literature as the usual source of microbial contamination^{7, 32}.

Limitations

The sample population in this study was small, impacting on the statistical significance of results. Financial restraints limited the collection of swabs to one wound only per limb. Repeat testing of wound, nail and skin samples or even biopsy and histological examination would improve the reliability of the results^{10, 14}. However, the cost and the invasiveness of biopsy excluded these methods of diagnosis for this pilot study.

Conclusion

Fungal presence was not common in wounds from participants with clinically diagnosed or laboratory-confirmed onychomycosis and tinea pedis. It was found that 14% of participants from the study population with fungi-positive wounds had co-existing laboratory confirmed onychomycosis. While each of the four fungi-positive wounds shared several characteristics, wound location was the only variable statistically significantly associated with fungal presence. Three fungi-positive wounds were located on the same toe that had laboratory-confirmed onychomycosis, which supports the literature that states micro-organisms in wounds are usually from the wound site periphery.

This pilot study was able to describe the presence of mycoses in the wounds, toenails and skin of the population, in a small sample size. Further research exploring the role of fungi will contribute to the understanding and practices related to microflora in wounds and more adequately describe the potential relationship between delayed healing in chronic lower limb wounds and onychomycosis or tinea pedis.

Acknowledgements

The researchers would like to acknowledge Professor David Ellis and the Mycology Unit at the Adelaide Women's and Children's Hospital in Adelaide, and Novartis Pharmaceutical Company for their generous support and contribution towards the study.

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Table 1: Wound and toenail characteristics of the four participants with fungi-positive wounds.

PARTICIPANTS				
Gender	male	female	male	male
Age	80 years	77 years	85 years	82 years
Wound location	Digits (apex)	Digits (apex)	Malleoli	Digits (apex)
Wound aetiology	Miscellaneous	Miscellaneous	Mixed arterial and venous	Arterial
Wound duration	13 weeks	9 weeks	22 weeks	9 weeks
Wound culture	<i>T. mentagrophytes var. interdigitale</i> <i>C.albicans</i>	Candida ^a	Candida ^a	<i>Candida albicans</i>
Nail microscopy	Dermatophyte	Dermatophyte	Dermatophyte	<i>Malessezia furfur</i>
Nail culture	<i>T. mentagrophytes var. interdigitale</i>	Candida ^a	Candida ^a	No fungi present.

^a The laboratory did not specify which Candida species was present as cultures were not specific for species other than *C.albicans*.