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Risk Factors for Infection in Patients with Chronic Leg Ulcers: A Survival Analysis

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Disclosures

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Abstract

Aim:

This study aimed to validate the relationships between possible predictive factors and clinically diagnosed infection in adult patients with chronic leg ulcers.

Methods:

This study used a sample of 636 adult participants whose ulcers were diagnosed as either venous, arterial or mixed aetiology leg ulcers and had no clinical signs of infection at recruitment. Data were extracted from recruitment to 12 weeks from six longitudinal prospective studies from 2008 to 2015. Survival analysis was used to investigate mean time-to-infection, including the Kaplan-Meier method and the Cox proportional-hazards regression model.

Results:

The sample included 74.7% venous, 19.6% mixed and 5.7% arterial leg ulcers. There were 101 (15.9%) participants diagnosed with infection at least once within 12 weeks of follow-up.

Mean time-to-infection was 10.89 weeks (95%CI= 10.66 - 11.12). After adjustment for potential confounders, a Cox proportional hazards regression model found that depression, using walking aids, calf ankle ratio <1.3, wound area ≥ 10 cm^2 and ulcers with slough tissue at recruitment were significant risk factors for wound infection.

Conclusion:

This study has validated the predictive ability of factors which have been found in a cross-sectional study to be significantly associated with infection in patients with leg ulcers, including venous leg ulcers, arterial leg ulcers and mixed aetiology leg ulcers. Results showed that patients with chronic leg ulcers, who either presented with depression, used walking aids, had a calf ankle ratio <1.3, a wound area ≥ 10 cm^2 or an ulcer with slough tissue, had greater likelihood of developing infection compared to those without these factors.

What's known

There is evidence of significant relationships between some factors, such as wound with a duration ≥ 2 years or peripheral vascular disease and the likelihood of infection in chronic wounds.

A cross-sectional study has found seven factors, including depression, chronic pulmonary disease, taking anti-coagulants, calf-ankle circumference ratio <1.3, ulcers area ≥10 cm^2, ulcers with slough tissue and ulcers with heavy exudate, associated with clinical diagnosed infection in chronic leg ulcers.

What's new

Five patient-related factors were identified as predictors of the development of infection in patients with chronic leg ulcers including: depression, using walking aids, calf ankle circumference ratio <1.3, wound area ≥ 10 cm^2 and ulcer with slough tissue.

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Introduction

Almost 3.6% of people aged 65 and over are living with chronic leg ulcers.[1] In the UK, three types of leg ulcers, including arterial, venous and mixed leg ulcers account for almost 14% of the annual prevalence of different wound types and consume significant wound care costs.[2, 3] In Australia, these chronic leg ulcers also play a significant role in healthcare costs.[4] While a chronic leg ulcer is considered as a defect in the skin below the knee showing no positive signs of healing after four weeks of appropriate treatment,[5] the severity of the ulceration and the actual healing time can be significant and influenced by many factors,[6-8] such as psychological factors[9] and ulcer-related factors.[10, 11] Chronic wound infection was identified in 3% of patients, who presented to the Emergency Department, and required hospitalisation.[12] Infection is identified as one of the most important contributors for delayed healing, wound complications, hospitalisation, increasing healthcare costs and resource consumption.[13, 14]

Infection occurs when there is an imbalance between the host's ability to defend and the bacteria's ability to cause harm in the favour of the microbe.[15-19] Many host-related factors can result in a decrease in the host's ability to defend, including psychosocial factors, physical and local factors.[20-22] In fact, much research has investigated risk factors associated with non-wound-related infection[23-25] and in surgical wounds.[26-30] Numerous studies have investigated factors associated with diabetic foot ulcers,[31, 32] and others types of chronic wounds such as pressure ulcers.[33, 34] Recently, Dissemond and colleagues suggested a wound classification to identify wounds at risk of infection, in which authors explicitly discussed about the important roles of endogenous and exogenous factors in the development of wound infection.[19] Although, little work has been done to identify risk factors associated with infection in chronic leg ulcers (e.g. chronic venous, arterial and mixed aetiology leg ulcers).[35-38] the authors' recent study has identified several factors associated with infection in chronic leg ulcers.[39] However, this was a cross-sectional study which focused on only examining the relationships between possible risk factors and infection at one point in time, which, despite the limitations may assist in understanding the risk factors for chronic leg ulcer infection. In addition, current literature related to infection in chronic leg ulcers highlighted a need of not only identifying factors associated with leg ulcer infection but investigating more in-depth factors that may influence

the development of infection in these leg ulcers. Thus, this study aimed to extend knowledge of factors influencing development of infection.

Methods

Study design and setting

This is a secondary analysis using a combined data set from a series of longitudinal observation studies. The aim of this study was to validate the previously identified risk factors' ability to predict the likelihood of developing infection.[39]

The research questions included:

1. Did infection occur, and if so, what was the length of time after baseline when infection occurred in patients, who were not diagnosed with infection at recruitment to the study, but presented with the identified risk factors?
2. Did any other additional risk factors exist that were associated with the development of infection in patients with chronic venous leg ulcers, chronic arterial leg ulcers and chronic mixed leg ulcers who were subsequently clinical diagnosed as infected?

The sample included all adult patients who were diagnosed with either chronic venous leg ulcers, chronic arterial leg ulcers or mixed leg ulcers. Patients were from outpatient clinics or community settings who had been previously recruited for six longitudinal studies in Australia, from 2004 to 2015.

Sample selection

The sample for this study was extracted from six data sets from longitudinal studies. The aims of these studies included: (1) developing and validating a risk assessment tool for delayed healing in venous leg ulcers,[40] (2) identifying the relationship between biochemical markers and wound healing in chronic venous leg ulcers treated with compression therapy, (3) determining effective care pathways for chronic wounds,[41, 42] (4) evaluating wound healing outcomes in the Wound Healing Community Outreach Service, (5) investigating the effects of different levels of compression on biochemical changes and wound healing progress, and (6) investigating wound fluid characteristics in chronic wounds. Patients in these previous studies were recruited if they had an ulcer below the knee for more than 4 weeks, resided in the community, were able to understand English, able to attend either community-based clinics or outpatient clinics, or received community home nursing services.

Patients from previous studies were included in the current study if they: were 18 years or over, had at least one ulceration below the knee which had been present for more than 4 weeks, had an ulceration primarily either arterial, venous or mixed, were not diagnosed with infection at recruitment and were followed up to week 12 in the contributing studies. Patients were excluded if they: had other types of ulceration than the three ulceration types mentioned above, had cognitive impairment or malignancy in the leg and were diagnosed with infection at recruitment. The total sample was 636 patients (See Figure *1).* The data for this study included all follow-up data from recruitment to week twelve of the contributing studies. The sample for this study was completely separated from the sample from the previous cross-sectional study conducted by our group[39]. In the previous study, all patients, including with and without being diagnosed with infection at recruitment were selected, and the analysis was focused on examine the relationships between factors and infection at recruitment only.[39] The diagnosis of chronic leg ulcer infection in the previous studies was purely based on clinical signs and symptoms of infection as recommended in national and international guidelines at the time of recruitment.[7, 22, 43-45] Wounds were clinically diagnosed as infected or non-infected by clinicians in charge, who were either medical practitioners, vascular physicians, nurse practitioners or registered nurses. These clinicians specialised in wound management and had extensive experience and expertise in wound care.

Data collection

On admission to the contributing studies, the following information was collected: sociodemographic factors (gender, age, living arrangement, marital status), medical history (comorbidities, medications, and vascular history), smoking status, and whether they required walking aids (walking aids referred to any types of devices patients reported using to help them moving around which included canes or sticks, wheelie walkers, wheelchairs and mobility scooters). With regards to patients' comorbidities, such as depression, patients were asked if they had been diagnosed with depression (yes or no). Patients' medical records were also retrieved to validate patient reported comorbidities and obtain any missing information.

Patients were also assessed for their ulcer and surrounding tissue characteristics, ulcer infection status including details of clinical signs of infection. Clinical diagnosis of infection was provided by either registered nurses, nurse practitioners, vascular physicians, or medical practitioners who had experience and expertise in wound care. The diagnosis of infection was based on their clinical experience and national and international guidelines recommended at

the time of recruitment.[7, 22, 43-45] Other measurements at recruitment included: level of pain on a Numerical Pain Rating Scale ranging from 1 to 10, Ankle Brachial Pressure Index (ABPI) and Calf and Ankle Circumference ratio

The follow-up data included: ulcer and surrounding ulcer characteristics, infection status, pain level and related treatment information (types of dressings and medications). Patients were followed either weekly or every two weeks. However, due to variation in the length of follow-up between previous studies and missing data caused by participant loss to follow-up or participants who failed to attend the clinics, the data did not have the same number of participants at each week of completed data. Data for this study were only included up to week 12 from baseline from the previously existing data.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 25. Descriptive analyses were performed for all variables to check frequencies on categorical variables and means, SDs or medians, and ranges for continuous variables. Survival analysis was used to identify the time-to-infection in patients with chronic leg ulcers, including the Kaplan-Meier method.[46] Specific risk factors for infection were assessed using the Cox regression proportional hazards model. Hazard ratios and 95% Confidence Intervals were calculated. It was hypothesised that the time-to-infection would be shorter in patients with identified risk factors compared to those without identified risk factors.

Time to event variable was coded as: Time zero was when participants entered the study – Baseline. Time-to-infection was measured from baseline to the week of being clinically diagnosed as infected for the first time. Censoring times were measured either from baseline to the week that the participant left the study (if they had not had an infection before that time) or at 12 weeks if infection did not occur. For those whose ulcer healed before the 12-week study period and infection did not occur before healing, the censoring time was entered as 12 weeks.

Six assumptions for Kaplan Meier methods[47] were checked and the data met all these six assumptions. Kaplan-Meier method was used for binary variables and Cox-Proportional Hazards Regression method was used for continuous variables.[46] Chi-square Log Rank Test was then used to make the decision of whether there was a difference between the group of patients with identified factors and those without these factors, if Chi-square > 3.84, H_0 was rejected, p-value ≤ 0.05 was the significance level.[48] Cumulative Incidence Curves were

generated to show the cumulative probabilities of experiencing a clinically diagnosed infection event.

All variables from the binary tests that had a p value < 0.1 were simultaneously entered in a Cox-proportional hazards regression model. Backwards stepwise (Wald) method was used to form the final parsimonious model, p value < 0.1 with the entry criteria and p-value <0.1 as the removal criteria.

The ethics application for this study was approved by Queensland University of Technology's Human Research Ethics Committee ID number 1500001146.

Results

Characteristics of the study sample

A total of 636 patient cases were eligible for analysis with 50% males and a mean age ± SD of 71.1 ± 14.35 years at admission. One third of patients were younger than 65 years old and 35% used some type of mobility aid to assist their mobility (Table 1). Almost 16% patients were diagnosed with infection at least once within the 12-week period (Table *2*).

The probability for patients to survive for 12 weeks from recruitment was 0.79. Overall, mean survival time ± SD was 10.88 ± .12 weeks, 95%CI = 10.66 - 11.11.

Bivariate tests

The data were first tested for the difference in time-to-infection in the sample participants with and without individual factors including: demographic factors, medical conditions, medications, and ulcer characteristics. Fourteen specific variables from the list above were assessed using Kaplan-Meier and Cox-regression. Four factors, using walking aids, calf ankle ratio <1.3, ulcer area ≥ 10 cm^2 and ulcer with slough tissue were identified as risk factors for infection (p<0.05) (Table *3*).

Cox-proportional hazards regression model

In the bivariate tests, eight factors had p<0.1, however, as being diagnosed with diabetes and taking diabetic medications were highly correlated, only a diagnosis of diabetics was entered in the model. Thus, seven factors with p<0.1 from bivariate tests were initially entered to the Cox regression model, including gender, depression, diabetes, using walking aids, calf ankle ratio <1.3, ulcer area ≥ 10 cm^2 and slough ulcer bed tissue. There were 77 cases with missing values, thus the total sample for the Cox regression model was 559 cases. The final

model (n=559) was significant p <0.001, Chi-square = 59.7 and consisted of five factors, including depression, using walking aids, calf ankle ratio <1.3, ulcer area ≥ 10 cm^2 and ulcers with slough tissue (see Table 4). For example, in the unadjusted Kaplan-Meier analysis, the mean time-to-infection in patients with calf-ankle circumference (CAC) ratio <1.3 was 10.92 weeks compared to 8.87 weeks in those with CAC ratio ≥ 1.3 (log rank test p = 0.006). In the multi-variable model, the result remained statistically significant with an adjusted hazard ratio of 2.69 (p = 0.008, 95% CI = 1.29 - 5.58) and the probability of being without an infection at 12 weeks was 0.67 for those with CAC < 1.3 and 0.83 for those with CAC ≥ 1.3 (see Figure *2*).

Discussion

The early recognition of patients at risk of developing infection in chronic wounds can enable timely and appropriate management and therefore improved patient outcomes and minimised infection-related costs in wound care. While evidence-based care is recommended for all patients with chronic wounds, identifying patients at high risk of infection is essential to enhance optimal practice as the strategies in wound management for these patients may differ from those without high risk factors.[19, 22]

A previous cross-sectional study identified depression, calf ankle ratio <1.3, ulcer area ≥ 10 cm^2 and ulcer with slough tissue as risk factors associated with clinically diagnosed infection in patients with either arterial, venous or mixed leg ulcers[39]. These findings were confirmed by the current study.

Depression has been recognised as a risk factor for numerous types of infection. In a prospective population-based study Anderson and colleagues[23] found depression was associated with different types of infection, such as sepsis infection and skin infection. Patients with depression had a higher risk of developing wound infection after undergoing cardiothoracic surgery.[24] The significant relationship between depression and healing of chronic wounds was also noted previously.[9] While infection is one factor for delayed healing, the role and mechanism of depression on infection in chronic wounds is yet to be thoroughly investigated. The authors' previous study established a relationship between depression and infection in chronic leg ulcers in a cross-sectional analysis.[39] The current finding supports a greater understanding on how depression may increase the likelihood of developing infection in adults with leg ulcers compared to those without depression.

Despite being regarded as endogenous risk factors for wound infection, diabetes mellitus[19,37] and advanced age[19] were not found to be risk factors for infection in this group of patients.

Calf-ankle circumference ratio < 1.3 was found to be a risk factor for infection in this sample. A decrease in the function of the calf muscle pump was found related to severity of the venous leg ulcer in an early study of Araki et al..[6] The calf-ankle circumference ratio indicates the severity of the calf muscle pump impairment[11], which was previously found to be significantly related to delayed healing in venous leg ulcers.[10] This finding supports evidence suggesting that a significant calf muscle pump dysfunction increases the risk of infection in patients with chronic leg ulcers.[39]

This study found ulcer area $\geq 10cm^2$ and ulcers with slough tissue were two other predictors of infection, confirming the findings from previous studies.[34, 39] This finding further corroborates the ideas of Dissemond at al.,[19] who suggested that on a scale of > 3 points, for a wound to be considered at risk of infection, wounds with dimension > 10 cm^2 should be scored one. Slough is described as a non-viable tissue which contains proteinaceous tissue, fibrin, neutrophils and bacteria[49, 50] and is suggested to be related to biofilm.[50] However, the impact of biofilm on the healing process in chronic wounds is yet to be understood.

Importantly, using walking aids to mobilise was identified as another risk for infection in patients with chronic leg ulcers. Decreased mobility has been shown to be associated with urinary tract infection[25] and wound infection in postoperative patients[29, 30]. Reduced mobility was also found significantly associated with delayed healing[8], however, such association has not previously been investigated or found in relation to infection in patients with leg ulcers. This is the first study to find patients who used walking aids to mobilise were more likely to develop infection than those mobilising independently. This important finding suggests further well-designed research to gain a better understanding in this area.

These findings will assist clinicians to actively tailor management strategies when patients are recognised as at high risk of developing infection and can be used to better inform patients about their treatment and to be more aware about their prognosis. Also, it highlights the importance of a collaborative approach between health professions in managing patients with chronic wounds, suggesting greater connections and/or communication channels among all healthcare professionals is essential to successfully heal chronic leg ulcers. Further research targeting patients at high risk of infection should be conducted to extend current knowledge and improve patient outcomes.

One of the study limitations was the diagnosis of infection was made clinically by clinicians. However, the diagnoses made by the medical doctors and nurse practitioners who had many years' experience in wound care and were experts in the area.

Conclusion

This study has validated the predictive ability of five factors which have been previously found to be significantly associated with chronic leg ulcer infection. These factors included depression, using walking aids to mobilise, having a calf-ankle circumference ration < 1.3, ulcer area ≥ 10cm^2 and ulcer with slough tissue. Patients with chronic leg ulcer, who had these risk factors, had a greater risk of developing infection.

Author contributions

Ut T Bui: Research design, analysis and drafting paper. Helen Edwards: research design and revising critically. Kathleen Finlayson: analysis and revising critically. All authors: approval of final version.

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References

1. Agale SV. Chronic leg ulcers: Epidemiology, aetiopathogenesis, and management. *Ulcers*. 2013;2013:1-9.
2. Guest JF, Ayoub N, McIlwraith T, et al. Health economic burden that wounds impose on the National Health Service in the UK. *BMJ Open*. 2015;5:e009283.
3. Guest JF, Ayoub N, McIlwraith T, et al. Health economic burden that different wound types impose on the UK's National Health Service. *Int Wound J*. 2017;14:322-330.
4. Whitlock E, Morcom J, Spurling G, et al. Wound care costs in general practice: A cross-sectional study. *Aust Fam Physician*. 2014;43:143-146.
5. Mekkes JR, Loots MAM, Van Der Wal AC, et al. Causes, investigation and treatment of leg ulceration. *Br J Dermatol*. 2003;148:388-401.
6. Araki CT, Back TL, Padberg FT, et al. The significance of calf muscle pump function in venous ulceration. *J Vasc Surg*. 1994;20:872-879.
7. Australian Wound Management Association. *Bacterial impact on wound healing: From contamination to infection*. Position document version 15: AWMA 2011.
8. Gohel MS, Taylor M, Earnshaw JJ, et al. Risk factors for delayed healing and recurrence of chronic venous leg ulcers-An analysis of 1324 legs. *Eur J Vasc Endovasc Surg*. 2005;29:74-77.
9. Cole-King A, Harding KG. Psychological factors and delayed healing in chronic wounds. *Psychosom Med*. 2001;63:216-220.
10. Milic DJ, Zivic SS, Bogdanovic DC, et al. Risk factors related to the failure of venous leg ulcers to heal with compression treatment. *J Vasc Surg*. 2009;49:1242-1247.
11. Flour M. New research and innovations in venous leg ulcer management. *Wounds Int*. 2010;1:15-18.
12. Talan DA, Salhi BA, Moran GJ, et al. Factors associated with decision to hospitalize emergency department patients with skin and soft tissue infection. *West J Emerg Med*. 2015;16:89-97.
13. Guo S, DiPietro LA. Factors affecting wound healing. *J Dent Res*. 2010;89:219-229.
14. Leaper D, Assadian O, Edmiston CE. Approach to chronic wound infections. *Br J Dermatol*. 2015;173:351-358.
15. Edwards R, Harding KG. Bacteria and wound healing. *Curr Opin Infect Dis*. 2004;17:91-96.
16. Myers BA. *Wound management: Principles and practice*. Upper Saddle River, N.J: Pearson 2012.
17. Scalise A, Bianchi A, Tartaglione C, et al. Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors. *Semin Vasc Surg*. 2015;28:151-159.
18. Tomkovich S, Jobin C. Microbiota and host immune responses: a love–hate relationship. *Immunology*. 2016;147:1-10.
19. Dissemond J, Assadian O, Gerber V, Kingsley A, Kramer A, Leaper DJ, Kramer A, Leaper DJ, Mosti G, Piatkowski de Grzymala A, Riepe G, Risse A, Romanelli M, Strohal R, Traber J, Vasel-Biergans A, Wild T, Eberlein T. Classification of wounds at risk and their antimicrobial treatment with polihexanide: A practice-oriented expert recommendation. *Skin Pharmacol Physiol*. 2011;24:245-255.
20. Siddiqui AR, Bernstein JM. Chronic wound infection: Facts and controversies. *Clin Dermatol*. 2010;28:519-526.
21. Swanson T, Angel D, Sussman G, et al. *Wound infection in clinical practice : principles of best practice*. International Wound Infection Institute 2016.
22. World Union of Wound Healing Societies. *Principles of best practice: Wound infection in clinical practice. An international consensus*. London: MEP Ltd 2008.

23. Andersson NW, Goodwin RD, Okkels N, et al. Depression and the risk of severe infections: prospective analyses on a nationwide representative sample. *Int J Epidemiol*. 2016;45:131-139.
24. Leitenburger A, Schoen J, Shafer E, et al. Depression increased risk for developing sternal wound infection. *Infectious Disease News*. Thorofare: Slack Incorporated 2014:27.
25. Rogers MAM, Fries BE, Kaufman SR, et al. Mobility and other predictors of hospitalization for urinary tract infection: a retrospective cohort study. *BMC Geriatr*. 2008;8:31-31.
26. Aziz F, Bohr T, Lehman EB. Wound disruption after lower extremity bypass surgery is a predictor of subsequent development of wound infection. *Ann Vasc Surg*. 2017;43:176-187.
27. Hollenbeak CS, Lave JR, Zeddies T, et al. Factors associated with risk of surgical wound infections. *Am J Med Qual*. 2006;21(Suppl 6):29S-34S.
28. Sanger PC, van Ramshorst GH, Mercan E, et al. A prognostic model of surgical site infection using daily clinical wound assessment. *J Am Coll Surg*. 2016;223:259-259.
29. Rath S, Schreuders TAR, Stam HJ, et al. Early active motion versus immobilization after tendon transfer for foot drop deformity: A randomized clinical trial. *Clin Orthop Relat Res*. 2010;468:2477-2484.
30. Shakil-ur-Rehman S, Sheikh SI, Danish KF. The role of early mobilization in the prevention of post operative wound infection after lower extremity orthepedic surgeries. *J Islam Int Med Coll*. 2015:63-66.
31. Adam KM, Mahmoud SM, Mahadi SI, et al. Extended leg infection of diabetic foot ulcers: Risk factors and outcome. *J Wound Care*. 2011;20:440-444.
32. Lavery LA, Armstrong DG, Wunderlich RP, et al. Risk factors for foot infections in individuals with diabetes. *Diabetes Care*. 2006;29:1288-1293.
33. Cukjati D, Robnik-Sikonja M, Rebersek S, et al. Prognostic factors in the prediction of chronic wound healing by electrical stimulation. *Med Biol Eng Comput*. 2001;39:542-550.
34. Jones J. Exploring the link between the clinical challenges of wound exudate and infection. *Br J Nurs*. 2013;22(Sup3):P8-P12.
35. De Souza JM, Vieira ÉC, Cortez TM, et al. Clinical and microbiologic evaluation of chronic leg ulcers: A cross-sectional study. *Adv Skin Wound Care*. 2014;27:222-227.
36. Harris LS, Luck JE, Atherton RR. Suboptimal identification of patient-specific risk factors for poor wound healing can be improved by simple interventions. *Int Wound J*. 2017;14:138-141.
37. Siaw-Sakyi V. Early wound infection identification using the WIRE tool in community health care settings: An audit report. *Br J Community Nurs*. 2017;22(Sup12):S20-S27.
38. Tosh PK, Agolory S, Strong BL, et al. Prevalence and risk factors associated with vancomycin-resistant Staphylococcus aureus precursor organism colonization among patients with chronic lower-extremity wounds in Southeastern Michigan. *Infect Control Hosp Epidemiol*. 2013;34:954-960.
39. Bui U, Edwards H, Finlayson K. Identifying risk factors associated with infection in patients with chronic leg ulcers. *Int Wound J*. 2017;1-8.
40. Parker CN. Predicting the likelihood of non-healing : a venous leg ulcer risk assessment tool [PhD thesis]. Queensland University of Technology 2014. http://eprints.qut.edu.au/78680/1/Christina_Parker_Thesis.pdf
41. Edwards H, Finlayson K, Courtney M, et al. Health service pathways for patients with chronic leg ulcers: Identifying effective pathways for facilitation of evidence based wound care. *BMC Health Serv Res*. 2013;13:86-86.

42. Edwards H, Finlayson K, Maresco-Pennisi D, et al. The long and winding road: Health services for clients with chronic leg ulcers in the community. *Wound Practice & Research*. 2014;22:226-233.
43. Cutting KF, Harding KG. Criteria for identifying wound infection. *J Wound Care*. 1994;3:198-201.
44. Cutting KF, White R. Defined and refined: criteria for identifying wound infection revisited. *Br J Community Nurs*. 2004;9:S6-15.
45. Gardner SE, Frantz RA, Troia C, et al. A tool to assess clinical signs and symptoms of localized infection in chronic wounds: development and reliability. *Ostomy Wound Management*. 2001;47:40-47.
46. Liu X. *Survival analysis: models and applications*. Chichester, West Sussex, United Kingdom: Wiley 2012.
47. Pallant JF. *SPSS survival manual: a step by step guide to data analysis using SPSS*. Crows Nest, N.S.W: Allen & Unwin 2007.
48. Sullivan L. *Survival analysis* Available: http://sphweb.bumc.bu.edu/otlt/MPH-Modules/BS/BS704_Survival/BS704_Survival_print.html. Accessed March 13, 2017.
49. Grey JE, Enoch S, Harding KG. Wound assessment. *BMJ*. 2006;332:285-288.
50. Percival SL, Suleman L. Slough and biofilm: removal of barriers to wound healing by desloughing. *J Wound Care*. 2015;24:498-510.

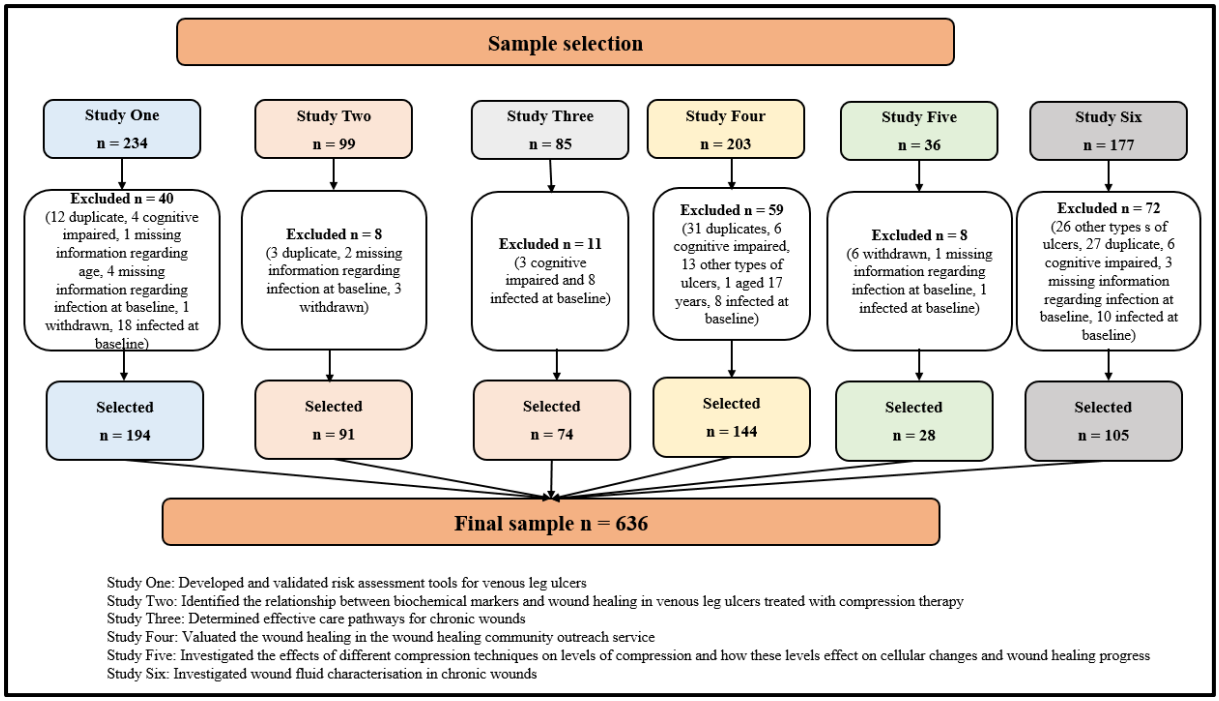

Figure 1: Sample selection

Table 1: Characteristics of study sample (N = 636)

Characteristics	N (%)
Gender	
Male	318 (50.0%)
Female	318 (50.0%)
Age	
<65 years	181 (28.5)
≥ 65 years	455 (71.5%)
Age, mean ± SD	71.1 ± 14.35
Live alone	208 (32.7%)
Walking aids	220 (34.6%)
Smoker	57 (9.0%)
Depression	50 (7.9%)
Diabetics	115 (18.1%)
Chronic Pulmonary Disease	91 (14.4%)

Table 2: Ulcer characteristics

Characteristics at recruitment	N (%)
Type of ulcer	
Venous	475 (74.7%)
Arterial	36 (5.7%)
Mixed	125 (19.7%)
Ulcer duration ≥ 24 weeks	273 (44.2%)
Ulcer Area ≥ 10cm^2	118 (18.8%)
Tissue type	
Epithelial	149 (23.8%)
Granulation	301 (48.1%)
Slough	169 (27.0%)
Necrotic	7 (1.1%)
Level of exudate	
Small	161 (25.3%)
Moderate	256 (40.3%)
Heavy exudate	209 (33.4%)
Clinically diagnosed infection at least once within 12 weeks	101 (15.9%)
Calf Ankle Circumference Ratio <1.3	23 (4.0%)

Table 3: Mean time-to-infection according to demographic, medical and ulcer factors

Demographic and medical characteristics	Mean time-to-infection (weeks)	95%CI	Log-rank test	p-value
Age ≥ 65 years old				
No	11.15	10.76-11.53		
Yes	10.78	10.50-11.05	.67	.411
Gender				
Male	11.11	10.81-11.39		
Female	10.67	10.31-11.01	3.68	.055
Using walking aids				
No	11.28	11.05-11.51		
Yes	10.13	9.65-10.61	20.91	<.001
Smoker				
No	10.85	10.61-11.09		
Yes	10.98	10.63-11.75	1.034	.309
Live alone				
No	10.98	10.72-11.25		
Yes	10.64	10.21-11.07	1.871	.171
CPD				
No	10.93	10.69-11.17		
Yes	10.55	9.87-11.24	2.360	.124
Depression				
No	10.94	10.71-11.17		
Yes	10.22	10.22-9.18	2.92	.088

Demographic and medical characteristics	Mean time-to-infection (weeks)	95%CI	Log-rank test	p-value
Diabetes				
No	10.79	10.53-11.05		
Yes	11.29	10.87-11.69	3.31	.069
Taking diabetic drugs				
No	10.79	10.54-11.04		
Yes	11.53	11.17-11.89	3.53	.060
Taking anticoagulant drugs				
No	10.92	10.64-11.24		
Yes	10.83	10.49-11.16	.818	.366
Calf-Ankle ratio < 1.3				
No	10.92	10.68-11.16		
Yes	8.87	7.06-10.67	7.59	.006
Ulcer area ≥ 10 cm^2				
No	11.12	10.89-11.34		
Yes	9.81	9.11-10.51	22.04	<.001
Ulcer Duration > 24 weeks				
No	10.86	10.54-11.17		
Yes	10.84	10.62-11.08	673	.412
Slough tissue				
No	11.29	10.89-11.37		
Yes	10.15	9.59-10.69	18.12	<.001
Heavy exudate				

Demographic and medical characteristics	Mean time-to-infection (weeks)	95%CI	Log-rank test	p-value
No	10.90	10.63-11.18		
Yes	10.87	10.40-11.21	.079	.779

CPD=Chronis Pulmonary Disease, CI=Confidence Interval

Table 4: Incidence rate of infection - Unadjusted Cox regression model (N = 559)

Factor	**Coefficient**	**HR**	**95%CI**	**P-value**
Using walking aids	.72	2.06	1.36 - 3.10	.001
Depression	.58	1.79	.97 - 3.28	.062
Calf Ankle Ratio < 1.3	.99	2.69	1.29 - 5.58	.008
Ulcer area ≥10cm^2	.93	2.54	1.64 - 3.93	<.001
Slough tissue	.50	1.66	1.09 - 2.52	.019

HR = Hazard Ratio, CI = Confidence Interval

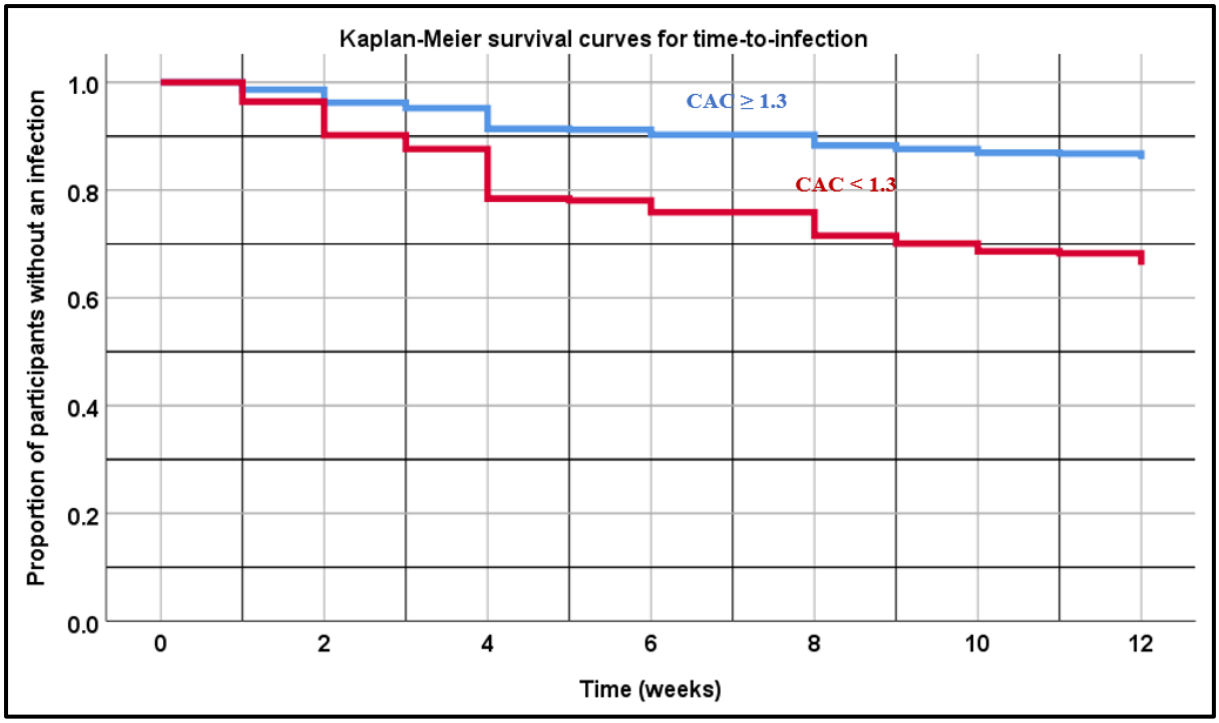

Figure 2: Kaplan-Meier survival plots of patients with calf-ankle circumference (CAC) ratio <1.3 compared with patients with CAC ratio ≥1.3 adjusted for four other factors (using walking aids, depression, ulcer ≥ 10cm^2, and ulcers with slough tissue)