Electronic Thesis and Dissertation Repository

10-26-2015 12:00 AM

Evaluation and Treatment of Lower Extremity Wounds in a Vascular Surgery Patient Population

Christine Anne Murphy, *The University of Western Ontario*

Supervisor: Pamela E Houghton, *The University of Western Ontario*
A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Health and Rehabilitation Sciences
© Christine Anne Murphy 2015

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Part of the Other Medicine and Health Sciences Commons

Recommended Citation

EVALUATION AND TREATMENT OF LOWER EXTREMITY WOUNDS IN A VASCULAR SURGERY PATIENT POPULATION: CLINICAL SIGNS OF INFECTION AND THE IMPACT OF LOW FREQUENCY (22.5kHz) CONTACT ULTRASOUND DEBRIDEMENT ON WOUND HEALING OUTCOMES

(Thesis format: Integrated Article)

by

Christine Anne <u>Murphy</u>

Graduate Program in Health and Rehabilitation Sciences

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

© Christine Anne Murphy 2015

Abstract

Purpose

To determine in a population with lower extremity wounds associated with vasculopathy if: (1) previously proposed clinical signs of infection are valid indicators of wound infection; (2) low frequency (22.5 kHz) contact ultrasound debridement (LFCUD) is well tolerated and feasible to apply in a nurse-led vascular wound clinic; (3) healing outcomes are improved for patients receiving LFCUD in comparison to patients receiving usual care (UC).

Methods

A total of 80 patients were in the study. First, a pilot group of ten patients were followed to determine tolerability, feasibility and wound response of 4 weekly LFCUD treatments. Then 70 patients were randomly allocated into LFCUD plus UC ($n= 33$), or UC ($n = 37$). Clinical signs of wound infection were compared to tissue culture and physician evaluation. Outcomes included mean percentage decrease in wound surface area (%WSA), change in wound appearance (revised Photographic Wound Assessment Tool [revPWAT]), and change in pre- to post-treatment pain scores by Visual Analogue Scale (VAS).

Results

No individual clinical sign was both highly sensitive and specific to indicate infection. The presence of three combined signs yielded the highest positive likelihood ratio (7.2), but absence of signs was uninformative. LFCUD is well tolerated and feasible for nurse-application. After 4 treatments the between-group change in %WSA was not statistically significant. The LFCUD group showed a significant linear trend in WSA reduction with each treatment visit ($p = < 0.01$), and a significant improvement in wound appearance for the LFCUD group (4.36 revPWAT points, 2.07-6.66, 95% CI, $p = 0.01$) compared to UC. There was no significant decrease in wound infections between groups. There was a significant decline in VAS pain score of 16.56mm (± 32.5, $t_{(31)} = 2.89$, $p = 0.007$, 95%

CI) in the LFCUD group but this was not significant in the UC group. There were no treatment-related adverse effects.

Conclusions

Clinical signs of infection are specific, but inadequately present for screening the vascular population. LFCUD is well tolerated and resulted in superior wound appearance with consistent trending of WSA reduction. It was not determined that LFCUD reduces infection, improves healing times or supports wound closure.

Keywords

Co-Authorship Statement

This thesis work was performed under the supervision of Dr. Pamela E. Houghton, and advisory committee members Dr. Dianne Bryant and Dr. Gregory W. Rose who will be co-authors on publication of Chapters 2, 3, and 4 of this paper.

Additionally Dr. Tim Brandys was on-site clinical supervisor and vascular surgery advisor, and will be co-author of these chapters.

Acknowledgments

I would like to thank my supervisory committee members, Dr. Pamela Houghton, Dr. Dianne Bryant and Dr. Gregory Rose for their support and guidance through this process.

Dr. Houghton has been an inspiration since before my graduate studies began, and I am honoured to have had the opportunity to study under her guidance. As a founding member of the Canadian Association of Wound Care and an internationally recognized researcher in the field of wound healing, I have been inspired and driven by her influence to become a new researcher in this fascinating field. Her kindness, patience and calm guidance have made this journey possible for me.

Dr. Bryant and Dr. Rose have provided critical feedback and unlimited support in their specialty areas, without which I could not have completed this project. Their insights have provided critical context that will stay with me for future research endeavours.

Additionally I would like to acknowledge the vascular surgeons of The Ottawa Hospital for their unwavering support and enthusiasm for this research. Dr. Tim Brandys, Dr. Sudhir Nagpal, Dr. Andrew Hill, Dr. George Hajjar, Dr. Prasad Jetty and Dr. Dalibor Kubelik have been keenly interested in the aspects of this trial, provided specialty insight, and actively engaged patients so that successful recruitment was attained.

I would like to thank research assistants Amy Chesley, Ellie Gee and Deepa Iyer, without whose support this research could not have been possible. Thank you to Carmen Gervais,

Dedication

Finally and particularly, I dedicate this dissertation to my children, Sarah, Lauren and Ryan who have endured this lengthy process with patience, love and humour.

Table of Contents

List of Tables

List of Figures

List of Plates

List of Appendices

List of Abbreviations

ABPI	Ankle Brachial Pressure Index
BMI	Body Mass Index
CI	Confidence Interval
CSSC	Clinical Signs and Symptoms Checklist
ET RN	Enterostomal Therapy Registered Nurse
FN	False Negative
FP	False Positive
ID	Infectious Diseases
IDSA	Infectious Diseases Society of America
LEAD	Lower Extremity Arterial Disease
LFCUD	Low Frequency Contact Ultrasound Debridement
LR	Likelihood Ratio
MDROs	Multi-Drug Resistant Organisms
NPWT	Negative Pressure Wound Therapy
OR	Odds Ratio
PVD	Peripheral Vascular Disease
REB	Research Ethics Board
revPWAT	Revised Photographic Wound Assessment Tool (Wound appearance)
RPN	Registered Practical Nurse
SENS	Sensitivity
SD	Standard Deviation
SPEC	Specificity
STSG	Split Thickness Skin Graft

TCOM	Trans-Cutaneous Oxygen Measurement
TN	True Negative
TP	True Positive
UC	Usual Care
VAS	Visual Analogue Scale (Pain)
WSA	Wound Surface Area

1   Chapter 1: Thesis Introduction

The aim of this doctoral research is to improve wound care treatment for patients with vascular disease. This research, comprised of three comprehensive and clinical studies, responds to a growing need in Canada to address the needs of a particular population with challenging health needs. With a view to improving access to care and improving healing outcomes for patients with vascular disease, this study aims to better identify and address signs of wound infection, and treat wound infection using ultra sound assisted debridement.

The alarming cost of caring for wounds in Canada is estimated at $3.9 billion per year or around three percent of national healthcare expenditures, and due to an aging population this figure is expected to increase by 30% by 2020.[1] In Ontario alone, the cost of lower extremity wound care is estimated at $511 million annually,[2] and around 50% of home care nursing visits are related to some aspect of wound care.[3] Notably, when vascular disease is present, the time to heal a wound extends considerably.[4] For example, without vascular surgery, just 25% of lower extremity wounds will be healed at six months, and 50% at one year.[5] Extended healing times reduce quality of life, require more health care resources, and allow greater opportunity for complications, including infection, to occur.

Furthermore, the prevalence of peripheral vascular disease (PVD) is thought to be increasing.[6] This is because PVD is strongly linked to other prevalent conditions such as diabetes, increased age, hypertension, dyslipidemia, and chronic renal failure.[7] Patients requiring surgery to address PVD have readmission rates three times higher when a wound is present prior to vascular surgery, and in the United States, the cost of each vascular readmission is estimated to add $12,400 US dollars to the cost of care.[8] Further, given that any improvement in post-operative blood flow may have limited durability,[9] effective wound therapies are needed within a window of opportunity after surgery.

A diagnosis of diabetes is a common predictor of PVD and interferes with healing considerably. Patients with diabetes have an increased risk of lower extremity arterial disease by two to four times, and increased wound prevalence of almost six times. Diabetes impairs the ability to fight bacteria in the wound with

diabetes-related soft tissue infection or bone infections (osteomyelitis) estimated to be responsible for around 25% of all hospital admissions for persons with diabetes.[10]

For patients with diabetic foot ulcers, infection has been implicated in non-healing wounds when arterial disease is present, but is not thought to prevent healing if perfusion is normal.[4] Despite the fact that infection may lead to serious consequences including advancing illness, amputation and death,[11] few studies have focused on identifying and treating infections particular to patients with vascular disease. Given that limited vascular supply is known to starve the wound tissue of basic needs and decrease the amount of antibiotic and other treatments that will be delivered to a wound,[12] further research is required to guide the prompt diagnosis of infection and to determine which treatments are most effective for this vulnerable population.

Not only is there a need for better recognizing and addressing infection, there is also a need to explore effective forms of wound treatment for this high risk population. At present, there are few effective methods to support wound healing in patients with vascular disease. Advanced wound products and biophysical therapies are known to address micro-imbalances in the wound environment and can be effective in advancing chronic wounds towards healing.[13] However, skilled and meticulous surface preparation is also required, which, in clinical practice, may be frequently unavailable. Therefore the true clinical potential of these therapies may be underachieved. Taken further, the vascular population is often excluded from advanced wound therapy trials, and so the effectiveness of these promising therapies on this population is yet to be determined.

One possible solution could be to improve the quality of, and access to, debridement procedures for patients with vascular disease. These procedures prepare, cleanse and stimulate the wound surface, and are an essential part of preparing the wound for healing.[14-16] A debridement method that is easy to apply, safe, and comfortable for the patient could improve access to care for the vascular patient, and possibly improve their healing outcomes. However, at present, the availability of any debridement procedure

remains a barrier since the procedure requires a supported clinical environment, specific sterile equipment, clinicians with advanced clinical skills, and well-developed policies (that are, to date, underdeveloped).[16]

Responding to the needs of a growing, yet under-researched population, the purpose of this study is to examine whether currently accepted clinical signs of infection are reliable indicators in a population with PVD. We also evaluate a method of ultrasound assisted debridement that might improve access to care, and possibly produce better healing outcomes. We explore whether accurately identifying infection and then offering aggressive and timely wound therapy in the outpatient setting could potentially reduce some vascular and diabetes-related acute care admissions, morbidity, days of in-hospital stay and associated costs.

The purpose of this thesis is to evaluate clinical signs of infection noted in patients with vasculopathy, and to investigate a potential treatment method for this high risk population. The body of work is presented in three chapters as follows.

In Chapter 1 of this thesis, we investigate the applicability of previously proposed signs of infection for a vasculopathic population. This chapter responds to the need to research patients using a vascular surgery service, who may have disease that are more advanced and have more advanced wound pathologies. At present, it is unknown how well signs of infection perform as vascular disease advances. Responding to this need, we explore whether the classical signs of infection (e.g., purulent drainage, heat, redness, swelling pain)[17] occur in patients with vasculopathic wounds, who are prone to having ineffective inflammatory responses.[18] Additionally, we explore other previously proposed subtle signs of infection, which are used in composite tools to identify infections in other populations.[19]

Chapter 2 presents a small prospective pilot study focused on the weekly application of a newer, low frequency contact ultrasound (LFCUD) system of debridement. Ultrasound treatments have been previously found to stimulate mechanisms of healing[20, 21; 22, 23] and to have antibacterial properties. In this pilot study, ten patients received four weekly LFCUD treatments that were applied by a nurse specialist. Attending to patient tolerance

and feasibility, we measure pain, adverse events, changes in wound appearance, and changes in wound surface area (WSA). We find LFCUD is attractive for this population, because it selectively targets the dead (necrotic) tissue, and reportedly causes minimal pain and bleeding. [24-26] We propose LFCUD could be applied by a specialist nurse in a supported environment without need for operating room space.

In Chapter 3, we describe our randomized controlled trial that took place after our pilot study to determine treatment effects. Patients were assigned to the LFCUD plus usual care (UC) group or to the UC group. All patients were followed by the vascular surgery department for issues related to lower extremity wounds. As a part of our study, we measured treatment effects on wound contraction, wound appearance, wound closure, and other complications.

This goal of this thesis is to provide new knowledge regarding infection appearances and treatment responses in a population with advancing vascular disease. Since patients with vascular disease are prone to infection and the development of necrotic debris, we explore the effect of a novel treatment for vascular wounds. We explore if debridement assisted by ultrasound can address particular healing barriers that these patients often face. While this population is under-researched in previous clinical trials due to their complicated health presentation, we believe that investigating the particular needs of this population is critical since the complicated nature of their disease may alter response. Additionally, given the microcellular imbalances that occur with advancing vascular pathology, patients with vascular disease may have more to benefit than a general population from emerging advanced wound therapies which may address cellular response.

References

1. Canada Wound Care Alliance. National stakeholder round-table. 27th Jun 2012, Mississauga, Canada 27 June 2012.

2. Shannon RJ. A cost-utility evaluation of best practice implementation of leg and foot ulcer care in the Ontario community. Wound Care Canada 2007;5:S53-6.

3. MacIsaac C. Outcomes research. Closing the gap between evidence and action: How outcome measurement informs the implementation of evidence-based practice. Wounds 2007;19(11):299-309.

4. Prompers L, Schaper N, Apelqvist J, Edmonds M, Jude E, Mauricio D, Uccioli L, Urbancic V, Bakker K, Holstein P, et al. Prediction of outcome in individuals with diabetic foot ulcers: Focus on the differences between individuals with and without peripheral arterial disease. the EURODIALE study. Diabetologia 2008 May;51(5):747-55.

5. Risk factors associated with healing chronic diabetic foot ulcers: The importance of hyperglycemia [Internet]United States; c2006 [cited 52 3].

6. Mills JL S. Update and validation of the society for vascular surgery wound, ischemia, and foot infection threatened limb classification system. Semin Vasc Surg 2014 Mar;27(1):16-22.

7. Bonham PA, Flemister BG. Guideline for management of wounds in patients with lower-extremity arterial disease. Mt Laurel, NJ: Wound, Ostomy and Continence Nurses' Society: WOCN Clinical Practice Guideline series; 2014. ID: 2819; M1: Book, Whole.

8. Xenos ES, Lyden JA, Korosec RL, Davenport DL. Ninety-day readmission risks, rates and costs after common vascular surgeries. Am J Manag Care 2014;20(10):e432-8.

9. Norgen L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FGR. Inter-society consensus for the management of peripheral arterial disease (TASC II). Eur J Vasc Endovasc Surg 2007;33:S1-S75.

10. CIHI Report. Compromised wounds in Canada. Canadian Institute for Health Information; 2013:ID: 2811.

11. Diehm C, Lange S, Darius H, Pittrow D, Stritzky Bv, Tepohl G, Haberl RL, Allenberg JR, Dasch B, Trampisch HJ. Association of low ankle brachial index with high mortality in primary care. Eur Heart J 2006 Jul;27(14):1743-9.

12. Lipsky BA. A report from the international consensus on diagnosing and treating the infected diabetic foot. Diabetes, Metabolism Research and Reviews 2004;20:S68-77.

13. Woo K, Ayello EA, Sibbald RG. The edge effect: Current therapeutic options to advance the wound edge. Adv Skin Wound Care 2007;20(2):99-116.

14. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. diabetic ulcer study group. J Am Coll Surg 1996 Jul;183(1):61-4.

15. Kirshen C, Woo K, Ayello EA, Sibbald RG. Debridement: A vital component of wound bed preparation. Adv Skin Wound Care 2006;19(9):506-17.

16. Rodd-Nielsen E, Harris CL. Conservative sharp wound debridement: An overview of Canadian education, practice, risk, and policy. J Wound Ostomy Continence Nurs 2013 Nov-Dec;40(6):594-601.

17. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJG, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. 2012 infectious Diseases Society of America Clinical Practice Guideline for the diagnosis and treatment of diabetic foot infections. Clinical Infectious Diseases 2012;54(12):132-73.

18. Cutting KF, Harding KG. Criteria for identifying wound infection. J Wound Care 1994;3(4):198-201.

19. Woo KY, Sibbald RG. A cross-sectional validation study of using NERDS and STONEES to assess bacterial burden. Ostomy Wound Manage 2009 Aug 1;55(8):40-8.

20. Dyson M, Pond JB, Joseph J, Warwick R. The stimulation of tissue regeneration by means of ultrasound. Clin Sci 1968;35(2):273-85.

21. Dyson M, Luke DA. Induction of mast cell degranulation in skin by ultrasound. IEEE Trans Ultrason Ferroelectr Freq Control 1986;33(2):194-201.

22. Roper J, Harrison A, Bass MD. Induction of adhesion-dependent signals using low-intensity ultrasound. J Vis Exp 2012 May 8;(63):e4024. doi(63):e4024.

23. Harvey W, Dyson M, Pond JB, Grahame R. The 'in vitro' stimulation of protein synthesis in human fibroblasts by therapeutic levels of ultrasound. Excerpta Medica, 363 1975:10-21.

24. Tan J, Abisi S, Smith A, Burnand KG. A painless method of ultrasonically assisted debridement of chronic leg ulcers: A pilot study. European Journal of Vascular & Endovascular Surgery 2007 Feb;33(2):234-8.

25. Schoenbach SF, Song IC. Ultrasonic debridement: A new approach in the treatment of burn wounds. Plastic & Reconstructive Surgery 1980 Jul;66(1):34-7.

26. Rediske AM, Hymas WC, Wilkinson R, Pitt WG. Ultrasonic enhancement of antibiotic action on several species of bacteria. J Gen Appl Microbiol 1998 Aug;44(4):283-8.

2   Chapter 2: Validity of clinical signs of wound infection in patients with vascular disease

2.1   Introduction

People with vascular disease are an under-researched population who are vulnerable to the effects of wound infection. It has been estimated that over one in 20 ambulatory people over 50 years of age across Canada are affected by vascular pathology, which results in poorly functioning blood vessels.[1] Types of vascular pathology include occlusive arterial disease, microcirculatory pathologies, diabetic neuropathic changes, and venous insufficiency. Symptoms are associated with the causative factor, and include pain or fatigue in the legs, skin breakdown, fluid accumulation in the legs (edema) and poor wound healing.[2-5]

Vascular disease influences wound healing in part because adequate blood flow is essential to produce an efficient inflammatory response necessary to promote early wound healing. Critically, the cells that are the basic building blocks of tissue rely on blood flow to deliver essential life-support elements such as oxygen and nutrients.[6]

Oxygen plays a significant role in tissue repair. Chronically low oxygen (referred to as chronic hypoxia), occurs with arterial, venous and cardio-respiratory pathologies, and critically affects tissue repair.[7] Importantly, an oxygen gradient (between well-oxygenated blood and the damaged central wound tissue) is essential to drive the formation of new tissue, which is known as granulation tissue.[7, 8] However, people with vascular disease have a lower oxygen gradient, since there is less oxygen available from central blood supply, in addition to less oxygen available in the wound environment. Chronic hypoxia in people with vascular disease may be attributed to a combination of vascular changes including occlusive arterial disease, microcirculatory pathology, and the presence of edema and venous insufficiency.[8]

Any interruption of oxygen and nutrient supply may rapidly lead to cellular demise. This results in an extending mass of necrotic material which becomes a food source for bacteria and increases the risk of infection. Furthermore, a hypo-perfused wound permits

the spread of bacteria or the development of infections since counter measures, including the availability of leukocytes, are impaired.[9] Therefore, infection is a frequent and serious problem for patients with vascular disease, and the threat of advancing wound infection can necessitate surgical procedures and even amputation as life-saving procedures. Unfortunately, the prognosis following major amputation is poor. For example, after two years, an estimated 40% of patients will achieve mobility, 30% will die, 15% will convert to above knee amputation, 15% will undergo amputation of the other leg, and only 60% will heal the surgical amputation wound by primary closure.[10] Therefore, early identification of infection in patients with vascular disease is highly desirable to avert poor outcomes.

When blood vessels are diseased, revascularization procedures such as bypass and angioplasty are commonly used for the vascular surgery population to restore blood flow. That said, while these procedures may improve local perfusion and healing potential, the underlying systemic pathology is not resolved.[11] Calcified vessel wall deposits and atherosclerotic plaques may affect the movement, function and communicative abilities of components such as white blood cells, growth factors, and proteases that are essential for tissue restructure. To date, it is unknown to what extent the inflammatory response may be restored after varying degrees of re-perfusion. Further, it is unknown if patients who have received vascular procedures demonstrate inflammatory signs of infection that may be noted in other populations.

2.1.1   Wound Infection

Wound infection occurs when microorganisms overwhelm the host defense system and invade tissue, causing a local and systemic response.[12] Wound infection is traditionally marked by the presence of purulence and/or by the presence of two inflammatory signs such as pain, erythema, heat or edema.[13] However, it is widely acknowledged that inflammatory signs may be diminished in immunocompromised patients and so these signs may be unreliable.[12]

Testing for bacteria by culturing sample obtained from the wound is the most available and objective method to measure bacterial burden. However, using culture alone is not

suitable to identify wound infection as methods of obtaining and analyzing cultures may be inconsistent and the area sampled may not fully reflect the wound environment.[14] There is also an ongoing debate about the best method to obtain the culture. While obtaining a portion of tissue for sampling is viewed as the gold standard,[12] wound surface swabs such as the Levine technique[15] are more widely available, more comfortable for the patient, and correlate well with aerobic and anaerobic tissue culture.[16] After 30 days, a tissue culture may provide more accurate information about persistent and resistant strains of bacteria that remain active in the wound.[17] It is possible that this late result occurs from the deeper tissue samples since a surface swab may not access non-planktonic and persistent biofilm bacteria.[18] These persistent types of bacteria are hard to identify, but have been estimated to be present in 60% of chronic wounds.[19]

While there have been various developments, recent genetic methods of identifying bacteria suggest only a small percent of bacteria may be culturable at all.[18] Further, while the information gained from the culture is essential for guiding antibiotic therapy, the objective result does not measure the degree of host insult or ability to defend against the bacteria. Therefore, the diagnosis of wound infection, particularly for vulnerable patients with vascular disease, requires a comprehensive and clinical evaluation.

Identifying wound infection by appearance is an appealing option since culture only provides partial information and requires a time-consuming laboratory analysis. While identifying particular clinical signs would ideally identify a patient reaction to the bacteria, this approach assumes a well-functioning inflammatory response. Unfortunately, patients with vascular disease may lack the physiological capabilities for that needed response.[12] Therefore, to date, researchers are uncertain if the signs of infection that have been associated with other populations are applicable to members of the acute care vascular surgery population.

2.1.2   Subtle Signs of Infection

Interestingly, many chronic wounds of various etiologies do not seem to show the classic inflammatory signs of infection that form the basis of the traditional Infectious Diseases Society of America (IDSA) infection classification.[20] Previous authors have proposed

there are additional and subtle signs of wound infections including delayed healing, discoloration, friable granulation tissue (e.g., tissue that bleeds easily), unexpected pain or tenderness, pocketing of the wound base, bridging of the epithelium or soft tissue, and abnormal smell.[21] To validate these signs for chronic wounds, a Clinical Signs and Symptoms Checklist was compiled, and each sign was compared against a sample of cultured tissue to achieve equal or greater than 10^5 organisms per gram of wound tissue of bacterial growth, which is considered the gold standard measure of infection.[22] The study consistently found that pain was absent in all patients without infection, meaning that the presence of pain would be highly likely to indicate infection. However, the study also found that no single sign was present in all infected wounds, meaning no one sign could be used to screen patients for infection.

2.1.3   Composite of Signs of Infection

Since subtle signs were more frequently found yet no single sign represented infection, a composite method made of a combination of signs was proposed. Subsequently, it was found that a composite list of signs performed better than individual signs, when comparing positive tissue results (greater than 10^6 organisms per gram of tissue) in diabetic foot ulcers.[23] Pain was, again, found to be absent in all uninfected patients.

Building on the approach of identifying composite signs, other researchers developed and validated a mnemonic format of the signs termed "NERDS and STONEES" to increase the clinical applicability and to support clinicians in identifying the clinical signs.[24] The mnemonic, "NERDS," refers to characteristics of superficial bacterial burden: Non-healing; Exudate; Redness (friability); Debris; and Smell. "STONEES" refers to characteristics of deep tissue infections: Size bigger; Temperature; Os (probes to bone); New areas of breakdown; Exudate increase; Erythema with Edema; and Smell. When comparing the semi-quantitative cultures of wound fluid swabs, the study found the presence of three signs associated with NERDS or STONEES was indicative of superficial or deeper bacterial growth, respectively. Interestingly, wounds that had debris, increased serous exudate, and friable granulation tissue were five times more likely to have scant or light culture growth which they termed "superficial critical colonization." Additionally, wounds with an elevated temperature were eight times more likely to be

infected as evidenced by moderate or heavy culture growth. However, this study excluded patients with advancing vascular disease (because of possible effects on wound appearance and progress). These authors have since suggested a new and similar composite tool, "UPPER and LOWER", which is described in detail later, but is yet to be validated. This, again, serves as an impetus for examining the individual and composite signs of wound infection among the vascular surgery population.

Further complicating the issue, there is a global demand to reduce the indiscriminate use of antibiotics that are responsible for the spread of multi drug resistant organisms (MDROs).[25] Despite this need to reduce antibiotic prescriptions and curb what is referred to as a global antibiotic crisis, precise, point-of-care diagnostic tests for wound infection are not yet available. Given that the under treatment of wound infections can have serious consequences for vulnerable patients (as described above), clinicians are therefore faced with a dilemma. They must choose between aggressive antibiotic prescription practices that may encourage MDRO development or less aggressive approaches that may inadequately address a latent infection. Not only is there a need for an improvement in diagnostic techniques, there is also a need for population-specific approaches that account for and respond to particular patient characteristics.[26] This study aims to develop a method to identify wound infection in patients with common characteristics of vascular disease while offering a comprehensive and clinical approach to these demands.

2.2   Research Purpose

While introducing customized assessment measures for specific populations may seem unwieldy, there is a need to confirm clinical tools are transferable to specific and vulnerable populations. To date, the impact of advancing illness, including vascular disease and associated compounding health problems, on infected wound appearance is not well understood. The aim of this study is to examine the validity of classical and subtle signs of wound infection in patients with peripheral vascular disease (PVD), who often require surgical intervention and may have a poor healing potential due, in part, to the high risk of wound infection. This study asks the following questions about patients with vasculopathy:

1. Are there individual classical and subtle signs that are associated with the presence of wound infection, as identified by an Infectious Disease physician and/or by a positive tissue culture?

2. Are there composite signs (that incorporate combined signs of infection) that are associated with presence of wound infection, as identified by an Infectious Disease physician positive diagnosis and/or by a positive tissue culture?

3. Is there an improved combination of signs that might serve the vasculopathic population better than existing composite methods?

This study was approved by the Ottawa Hospital Research Institute Research Ethics Board, and the Western University Research Ethics Board (See Appendix 1). The study involved 78 participants with PVD and lower extremity wounds. Bringing together the expertise of medical professionals, including an Enterostomal Therapy Registered Nurse (ET RN), a team of vascular surgeons and an Infectious Disease (ID) physician, this project aims to identify wound infections in patients with vascular disease and improve diagnostic measures.

2.3   Methods

2.3.1   Recruitment

Patients were recruited at an outpatient clinic and in-patient ward of a vascular surgery service provided in in Ottawa, Canada. All patients who met inclusion criteria were approached consecutively by one of six vascular surgeons. Patients were eligible if they were English speaking adults (18 years or older), and had a full thickness wound of determined etiology that was below the knee and measured greater than $1cm^2$. Patients were excluded if they had severe arterial insufficiency which was defined as absence of palpable pedal pulses combined with ankle brachial pressure index (ABPI) less than or equal to 0.3; toe pressure less than or equal to 20mmHg; or transcutaneous oxygen measure less than or equal to 20 mmHg. Patients were also excluded if they: had an acute limb or life-threatening infection; had an exposed vascular graft, blood vessel, bone or tendon in the base of the wound; were medically unstable or had a condition that

reduced life expectancy; were receiving hyperbaric oxygen; had a cardiac pacemaker or defibrillator; were immunosuppressed; or had malignancy in the wound.

2.3.2   Perfusion Assessment

Perfusion was determined by ABPI, toe pressure , transcutaneous oxygen measure, computed tomography angiography or conventional digital subtraction angiography, as determined by the vascular surgeon and in keeping with comprehensive vascular assessment.[27] In the circumstances where more invasive tests had been performed, ABPI and toe pressure testing were not employed since perfusion status was already determined and this population has a high incidence of unobtainable Doppler tests due to calcified blood vessels. One of six vascular surgeons evaluated patients and interpreted tests to determine if perfusion was optimized, and if patients were eligible to participate.

2.3.3   Measuring Individual and Composite Signs

Clinical signs recorded in this study were recorded by a single assessor, who was an ET RN with graduate education in the field of wound care. One wound per patient was assessed and the presence or absence of individual and composite signs (e.g., heat, increasing pain, erythema, edema, purulent exudate, increasing wound size, delayed healing, wound breakdown, odour, serous exudate, debris, friable granulation, induration) was noted as dichotomous data. See Table 1 for an overview of the individual and composite signs measured.

Table 1. *Definitions Used for Signs of Infection*

Signs	Definition
Heat	Heat was defined as wound temperature $> 3^o$ F from a point approximately 10 cm at the distal aspect of the peri-wound skin.
Increasing pain	Increasing pain was the new elevation of pain > 50mm on the Visual Analogue Scale (VAS), and/or patient described increased pain since last assessment.
Erythema	Erythema was redness >2 cm beyond the wound edge.
Edema	Edema was presence of swelling within 4cm of the wound edge that demonstrated pitting indentation with locally applied pressure.
Purulent exudate	Purulent exudate was the visible appearance of creamy-yellow exudate.
Increasing wound size	Increasing wound size was larger wound since last since the last visit.
Delayed healing	Delayed healing was determined by less than 20% to 40% wound area contraction in the previous two to four weeks (as retrieved from medical documents or by patient description if data were unavailable).
Wound breakdown	Wound breakdown was small open areas in newly formed epithelial tissue that were not caused by re-injury or trauma.
Odour	Odour was noticeable unpleasant smell after dressing removal.
Serous exudate	Serous exudate was $> 50\%$ surface area of removed dressing saturated clear wound fluid.
Debris	Debris was the presence of slough, eschar, necrotic or non-viable tissue.
Friable granulation	Friability was determined by observation of bleeding with gentle touch of sterile instrument.
Induration	Induration was the presence of hard mass with loss of tissue pliability in the peri-wound region.

Abbreviations: ^{o}F = degrees Fahrenheit; cm = centimeter; VAS = Visual Analogue Scale (in millimetres out of a total 100mm).

2.3.4   Measuring Wound Size and Temperature

After taking the individual and composite measurements, wound size was determined by tracing the wound edge on clear acetate which was then measured by planimetry (Visitrak®, Smith & Nephew, UK). Wound photographs were taken with a ruler in the image using a Canon Rebel 300D EOS, 8 megapixel resolution, 60mm macro lens digital camera with a ring flash and automatic focus. Wound temperature was measured with an infrared thermometer (Thermotrace®, Prizm Medical, Dunwoody, GA) at the midpoint of the wound. It was then compared to the area approximately 10cm distal to the wound on the ipsilateral limb.

2.3.5   Analyzing Tissue Sample

One ID physician estimated each patient's wound for infection. After cleansing with physiological saline solution, the tissue sample was retrieved by either a 3mm punch biopsy or scalpel. In small wounds where biopsy was not attainable, the physician used a scalpel to obtain the tissue sample from the most central part of the wound that was accessible. The ID physician comprehensively evaluated the wound, measured patient symptoms, and determined whether the wound was infected or not-infected. Subsequently, when the laboratory analysis of the tissue sample was available, both the ID physician's evaluation and the tissue culture result were used to determine whether or not the wound was infected. This reference standard was chosen since it is commonly used in clinical practice to determine whether antibiotic treatment is required. The tissue sample was sent by the ID physician immediately to a local laboratory in a sterile container without transport media. The species and quantity of bacteria were determined through a clinical laboratory semi-quantitative analysis method. As a part of the process, samples were weighed, homogenized, serially diluted then smeared onto plated standard media and cultured under aerobic and anaerobic conditions. A positive culture was defined as laboratory-reported moderate or heavy bacterial growth detected by isolation in three or four quadrants.

2.3.6   Prescribing Antibiotic Therapy

Lastly, after receiving the laboratory results, the same ID physician prescribed antibiotic therapy as per the Infectious Diseases Society of America's (IDSA) Practice Guidelines for Skin and Soft Tissue Infection[28] and Diabetic Foot Infection.[13] In many cases, this physician had to decide whether to continue or extend current antibiotic therapy.

2.3.7   Individual and Composite Signs

Individual and composite signs were designated as present or absent, and were calculated as dichotomous data. Specifically, for the IDSA combination of signs, the presence of two or more signs of inflammation (e.g., pain, erythema, heat or edema) and/or purulent exudate was considered indicative of infection. Using the aforementioned NERDS and STONEES combination, we considered cases with two, three or four signs present as indicative of a wound infection. We also used the mnemonic UPPER and LOWER, which had not yet been validated, to examine the two, three or four signs indicative of infection. The signs in "UPPER" refer to superficial bacterial burden: Unhealthy tissue; Pain; Poor healing; Exudate; and Reek. The signs in "LOWER" are: Larger in size; Osseous tissue; Warmth, Edema and Redness.

2.3.8   Sensitivity and Specificity

Sensitivity was calculated by dividing the true positive scores by all the actually infected wounds (true positive/ true positive + false negative). Specificity was determined by dividing the true negative scores by all truly non-infected wounds (true negative/ true negative + false positive). See Table 2 for definitions of the terms.

Table 2. *Presence of Individual and Composite Signs: Definitions for Results*

Result	Definition
True positive (TP)	True positive means that the sign was present when the wound was infected.
False Positive (FP)	False positive means that the sign was present, but the wound was not infected.
False negative (FN)	False negative means that the sign was absent, but the wound was infected.
True negative (TN)	True negative means that the sign was absent and the wound was not infected.

2.3.9   Likelihood Ratio

The Likelihood Ratio (LR) is the proportion of probability of having a test result that correctly identifies the condition compared to the probability of returning a false result. Thus, formulae for LRs are derived from the sensitivity and specificity data as follows:

LR+   = $\dfrac{\text{True Positive Rate}}{\text{False Positive Rate}}$ = $\dfrac{\text{Sensitivity}}{1 - \text{Specificity}}$

LR −   = $\dfrac{\text{False Negative Rate}}{\text{True Negative Rate}}$ = $\dfrac{1 - \text{Sensitivity}}{\text{Specificity}}$

The LR is used to determine the extent that the result of a test will increase or decrease a pre-test probability of the target disorder.[26] The translation of the pre-test to post-test probability was accomplished using a nomogram calculator,[29] which involves placing a ruler from the pre-test probability across the LR to reveal the post-test probability. LRs were not calculable when the numerator or denominator was zero.

2.3.10   Odds Ratio

We also calculated the odds ratio (OR). The OR describes the odds of infection when sign is present compared to odds of infection when a sign is absent.[26] The OR was calculated as (TP x TN)/ (FP x FN) and presented with 95% confidence intervals.

2.3.11   Statistical Analysis

Data were entered into a computer research data base (Empower Health Research, London, Canada), and exported directly to the statistical software package (SPSS, Version 23.0, Armonk, NY) for analysis. The difference in demographics and physical characteristics of patients assigned to groups that had infected or non-infected wounds was determined by the Independent Student's t-Test for continuous variables and X^2 Test for categorical variables. The Fisher's Exact Test was used when any categorical count was equal to or less than five. Statistical significance was considered at $p = {<}0.05$. A positive Likelihood Ratio of greater than five or negative Likelihood Ratio of less than

0.2 was considered the threshold of clinical importance since it would alter the post-test probability sufficiently to affect treatment.[26]

2.4   Results

The study involved 78 participants, who consented to participate and were recruited between December 2013 and May 2015. The characteristics of the 78 subjects are presented in Table 3. Of the participants, 22 (28.2%) were diagnosed as having a wound infection using a combination of the ID physician's assessment and/or a positive culture result. Sixty-five patients (83.3%) had wounds with light growth or no growth on culture, and 13 (16.7%) had moderate or heavy growth. Nine patients were identified as infected by the ID physician without moderate or heavy growth on the culture. Patients diagnosed with infection tended to have had longer duration diabetes, wounds on the heel or leg, and fewer re-vascularization procedures, but these differences were not statistically significant. However, patients with infection had higher Body Mass Index (BMI) which was the only characteristic that was statistically significant, ($p = 0.010$).

Table 3. *Patient Demographics by Wound Bacterial Burden from Semi-quantitive Culture*

	Total Sample	Infected	Non-infected
	(*n* = 78)	(*n* = 22)	(*n* = 56)
Age (Years)	65.76 ± 10.36	62.41 ± 6.67	67.07 ± 11.27
Male	78.2 (61)	81.8 (18)	76.8 (43)
BMI	26.80 ± 6.19	29.63 ± 7.71	25.68 ± 5.15
Wound Duration (Months) (*n*= 76)	14.13 ± 25.78	9.68 ± 11.79	15.95 ± 29.57
Wound Location			
Toe/ Toe Amputation Site	16.6 (13)	13.6 (3)	17.9 (10)
Mid-Foot/Plantar	26.9 (21)	18.2 (4)	30.3 (17)
Heel	20.5 (16)	27.3 (6)	17.9 (10)
Malleolar	6.4 (5)	4.5 (1)	7.1 (4)
Leg	29.5 (23)	36.4 (8)	26.8 (15)
Diabetes	69.2 (54)	77.3 (17)	66.1 (37)
Duration Diabetes (Years) (*n*= 52)	20.63 ± 12.45	24.71 ± 13.66	18.66 ± 11.5
Anti-coagulant Medication	65.4 (51)	63.6 (14)	66.1 (37)
Antibiotic Medication	62.8 (49)	72.7 (16)	58.9 (33)
Hemoglobin (*n* = 77)	114.13 ± 18.66	109.50 ± 21.09	115.98 ± 17.46
Albumin (*n* = 70)	31.29 ± 5.69	30.33 ± 6.19	31.69 ± 5.48
Arterial Insufficiency	(*n=67*)	(*n=19*)	(*n=48*)
Previous Angioplasty	55.2 (37)	47.4 (9)	58.3(28)
LE Bypass Graft	32.8 (22)	26.3 (5)	35.4(17)
Previous Amputation			
Major (Transtibial/ Transfemoral)	13.4 (9)	10.5 (2)	14.6 (7)
Distal (Transmetatarsal/ Digital)	29.9 (20)	26.3 (5)	31.3 (15)
Pedal Pulse Palpable	dp 17.9(12) pt 16.4(11)	dp 15.8(3) pt 15.8(3)	dp 18.8(9) pt 16.1(8)

Unless otherwise stated, values expressed as mean ± Standard Deviation with range in parentheses, or percentage (*n*).
Abbreviations: BMI = Body Mass Index; dp = Dorsalis Pedis; pt = Posterior Tibial

Representative of the population, patients were predominantly male and the mean age was 65.76 (range 28-90) years. Wound presentations were typical of a vasculopathic population, and included chronic ulcerations, non-healing surgical sites and open amputation wounds with underlying arterial, venous, diabetic or combined etiology. The mean wound duration was 14.13 ± 25.8 months (as recorded in 76 out of 78 patients).

Most patients were taking an anti-coagulant medication and the majority were prescribed antibiotic therapy. Many patients had long term diabetes and were insulin dependent. Two patients could not recall the time since diabetes diagnosis, and this information was not available from the medical record. As might be anticipated with a vasculopathic population, 28% of foot wounds were not associated with diabetes, and 33.3% of wounds of patients with diabetes were not on the foot.

ABPI was not obtainable in many patients mainly because of incompressible arteries. The low inclusion threshold of 0.3 may not be suitable for debridement in usual circumstances, but since subjects were evaluated by the vascular surgeon, was chosen to ensure no patients amenable to wound response were excluded. Interestingly, although the ABPI cut-off was 0.3, the 29 patients who were able to have the test had a mean ABPI of greater than 0.9, which is defined as the threshold of peripheral arterial disease.[10] As expected, these normal ABPI results were reflective of the post revascularization status rather than degree of vascular disease as 67 out of 78 (85.9%) patients had clear evidence of arterial pathology. This included patients with previous lower extremity bypass graft, angioplasty or amputations. Fifty-seven of 68 people in the study had no palpable pedal pulse.

2.4.1   Individual Signs of Infection

The True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) results with the sensitivity and specificity of individual clinical signs are presented in Table 4. The most frequent sign was edema, which was present in 55 patients. Seventeen (55%) patients with edema had a wound infection. Many patients in this study also had delayed healing ($n = 53$ patients), debris ($n = 52$), and friable granulation tissue ($n = 51$).

Notably, signs that were rarely seen in this population included induration ($n = 2$), wound breakdown ($n = 4$), increased wound size ($n = 4$), and odour ($n = 6$). The two clinical signs with the highest sensitivity scores were the presence of edema (0.77) and delayed healing (0.73). Most other signs had moderate to low sensitivity which means that many people, who were considered to have an infection, lacked these individual signs.

Table 4 *Sensitivity and Specificity of Individual Clinical Signs*

Sign	TP	FN	TN	FP	SENS	SPEC	OR (95% CI)	LR+
Heat	6	16	51	5	0.27	0.91	3.83 (1.03 – 14.22)	3.0
Increasing Pain	5	17	39	17	0.23	0.70	0.68 (0.21 – 2.13)	0.8
Erythema	12	10	44	12	0.55	0.82	4.40 (1.53 – 12.63)	3.1
Edema	17	5	18	38	0.77	0.32	1.61 (0.51 – 5.06)	1.1
*Edema/Erythema	11	11	48	8	0.50	0.86	6.00 (1.95 – 18.42)	3.6
Purulent exudate	3	19	50	6	0.14	0.89	1.32 (0.30 – 5.80)	1.3
Increasing wound Size	2	20	54	2	0.09	0.96	2.70 (0.36 – 20.48)	2.3
Delayed healing	16	6	19	37	0.73	0.34	1.36 (0.46 – 4.07)	1.1
Wound breakdown	3	19	55	1	0.14	0.98	8.68 (0.85 – 88.59)	7.0
Odour	3	19	53	3	0.14	0.95	2.79 (0.52 – 15.03)	2.8
Serous exudate	4	18	51	5	0.18	0.91	2.27 (0.54 – 9.38)	2.0
Debris	14	8	18	38	0.64	0.32	0.83 (0.30 – 2.33)	0.9
Friable granulation	15	7	20	36	0.68	0.36	1.19 (0.42 – 3.40)	1.1
Induration	2	20	56	0	0.09	1.00	3.80 (2.61 – 5.54)	nc

*Woo and Sibbald reported edema and erythema combined, not individually reported.

Abbreviations: TP = True Positive; FN = False Negative; TN = True Negative; FP = False Positive; Sens = Sensitivity; Spec = Specificity; OR (95% CI) = Odds ratio with 95% confidence interval in parentheses; LR+ = Positive Likelihood Ratio.

Many signs showed relatively high (greater than 0.90) specificity. Heat, increasing wound size, wound breakdown, odour, and serous exudate were not present in non-infected wounds over 90% of the time. As such, the absence of one of these signs was viewed as highly suggestive that a wound infection was not present. Induration was not seen in non-infected wounds at all. Further, several signs occurred in the absence of bacterial growth (e.g. delayed healing, friable tissue, and debris), and therefore were poorly specific for infection in wounds. That is, the signs that occurred for reasons other than bacterial growth could not be used to identify an infected wound.

The odds ratio (OR) with 95% confidence interval (CI) for each sign is reported in Table 4. The 95% CI reveals that a large possible range of OR exists for most signs and therefore the estimate is yet imprecise. Induration produced the most precisely elevated OR at 3.8 (2.61 − 5.54, 95% CI). This means that considering the 95% CI, the true odds of having an infection when induration is present is elevated by 2.6 and 5.5 times. Interestingly, the odds of having infection crossed the "no difference" threshold (OR = 1) of one for a sign that was previously thought to be important (increased pain). In fact, the OR of most individual signs included CI's that included the number one. Collectively, the odds ratios did not suggest that the presence of any individual sign was strongly associated with wound infection.

Wound breakdown produced the highest positive likelihood ratio (LR+) of seven. Pre-test probability of infection was estimated at 31%,[22] which means 79% of patients with wound breakdown were likely to have an infection. All other individual signs had a LR+ of < 5 which has a small effect on the post-test probability. Unfortunately, negative likelihood ratios (LR-) for individual signs were all above 0.2 (between 0.5 − 1.36), which means the absence of any one of these signs does not necessarily mean the wound is free of infection.

2.4.2   Comparison to Previously Validated Signs

Table 5 presents values for sensitivity and specificity in cases where the infection was confirmed by a combination of the clinical diagnosis by the ID physician and the tissue culture results, which were expressed semi-quantitatively. Our results (Murphy et al,

2015) are compared to previous studies performed by Gardner and colleagues[23], who defined infection by yielding bacterial counts greater than 10^6 per gram of tissue, and Woo and Sibbald,[24] who identified moderate to heavy bacterial growth in cultured wound fluid collected by wound swab using Levine's method.[15] As noted in Table 5, the values for sensitivity and specificity discovered in this study are quite different than those reported previously. We found several signs were more specific than previously reported, but the sensitivity of signs examined by Woo and Sibbald[24] were not attained. Along the same lines as Gardner and colleagues,[23] our study found most signs had better specificity that sensitivity. That said, the few signs that they marked as sensitive were not the same ones that we found to be sensitive in our study. This is important since it shows that wound infections of people with vascular disease may fail to show signs of infection that are typically used for screening in other populations.

Table 5 *Sensitivity and Specificity in Current and Previously Published Studies*

Sign	Murphy et al 2015		Gardner et al[23] 2009		Woo & Sibbald[24] 2009	
	n = 78		*n* = 64		*n* = 112	
	SENS	SPEC	SENS	SPEC	SENS	SPEC
Heat	0.27	0.91	0.12	0.85	0.76	0.71
Increasing Pain	0.23	0.70	0.12	1.00	-	-
Erythema	0.55	0.82	0.32	0.77	*	*
Edema	0.77	0.32	0.20	0.77	*	*
*Edema/Erythema	0.50	0.86	-	-	0.87	0.44
Purulent exudate	0.14	0.89	0.26	0.65	0.70	0.64
Increasing wound Size	0.09	0.96	-	-	0.50	0.83
Delayed healing	0.73	0.34	0.48	0.54	-	-
Wound breakdown	0.14	0.98	-	-	-	-
Odour	0.14	0.95	0.20	0.87	0.37	0.86
Serous exudate	0.18	0.91	0.88	0.21	-	-
Debris	0.64	0.32	-	-	-	-
Friable granulation	0.68	0.36	0.00	0.77	-	-
Induration	0.09	1.00	-	-	-	-

*Woo and Sibbald reported edema and erythema combined, not individually reported. Some other items not reported in published articles. Abbreviations: MD = Doctor of Medicine; SQ = Semi-Quantitive; Q = Quantitive; Sens = Sensitivity; Spec = Specificity.

Woo and Sibbald[24] found different values for sensitivity than those derived from the current study. In particular, they found the presence of both erythema and edema together were highly sensitive to the presence of infection in the wound (0.87). That said, these signs were not very specific. Our results were the opposite in that most people who did not exhibit either of these clinical signs did not have infection. In other words, we found the combination of these signs was more specific, and so their combined absence was indicative of a non-infected wound.

2.4.3　Composite Signs

We combined two or more clinical signs (as defined by Woo and Sibbald[24] and the IDSA[13] previously) to obtain the NERDS, STONEES, UPPER, LOWER and IDSA values for sensitivity, specificity and likelihood ratios of these composite scores (as presented in Table 6). A dummy code variable was used for calculations based on the threshold of 2, 3 and 4 signs. Adding the number of individual signs together produced a better balance between specificity and sensitivity with the combination of three clinical signs included in STONEES and LOWER scales producing a rather high LR+ (greater than 7.0). Despite this finding, we did not achieve the relatively high sensitivity and specificity in any one sign found by Woo and Sibbald. That said, combining the clinical signs defined by IDSA produced moderate sensitivity and good specificity. Our specificity results (0.91) were stronger than those reported previously by Gardner and colleagues,[23] who had specificity results of 0.46. However, sensitivity was not improved with the IDSA combination of signs which suggests using composite signs may not be suitable as a screening method for patients with vascular disease, since these signs are often absent in infected wounds.

Table 6 *Composite Signs of Infection in Current and Previous Studies: Sensitivity, Specificity, and Likelihood Ratios*

Sign	Woo & Sibbald[24] 2009 ID MD + Culture			Murphy et al 2015: ID MD + Culture		
	SENS	SPEC	LR+	SENS	SPEC	LR+
IDSA signs	-	-	-	0.41	0.91	4.55
NERDS (2)	0.85	0.33	1.27	0.83	0.23	1.08
NERDS (3)	0.73	0.81	3.84	0.31	0.54	0.67
NERDS (4)	0.38	1.00	nc	0.11	0.69	0.35
STONEES (2)	0.95	0.50	1.9	0.59	0.82	3.28
STONEES (3)	0.90	0.69	2.9	0.36	0.95	7.2
STONEES (4)	0.53	0.92	6.6	0.14	0.98	7.0
UPPER (2)				0.83	0.23	1.08
UPPER (3)				0.43	0.46	0.80
UPPER (4)				0.22	0.69	0.71
LOWER (2)	-	-	-	0.59	0.80	2.95
LOWER (3)	-	-	-	0.36	0.95	7.2

Abbreviations: Sens = Sensitivity; Spec = Specificity; LR+ = Positive Likelihood Ratio; IDSA = Infectious Diseases Society of America; NERDS = Composite infection tool (Non-healing, Exudate, Red friable tissue, Debris, Smell); STONEES = Composite infection tool (Size bigger, Temperature increased, Osseous tissue, New areas of breakdown, Erythema and Edema combined, Smell)[30]; UPPER = Composite infection tool (Unhealthy granulation tissue, Pain, Poor healing, Exudate, Reek) and LOWER = Composite infection tool (Larger in size, Osseous tissue, Warmth, Edema, Redness)[31]. Murphy et al = current study. Composite tools described with (2) = 2 signs present; (3) = 3 signs present; (4) = 4 signs present. nc = Not Calculable. IDSA, STONEES and LOWER compared against ≥moderate to heavy semi-quantitative growth; NERDS AND UPPER compared against < moderate culture growth.

2.5   Discussion

The purpose of this study was to examine the validity of classical and subtle signs of wound infection in patients with extensive peripheral vascular disease, who often require surgical intervention. We have shown that several individual signs are specific for wound infection in a vasculopathic population. Our study found most signs are poorly sensitive which means when infection is present, clinicians cannot be confident these clinical signs will appear. Yet when infection is absent, the presence of these signs rarely occurs. Since there is poor balance between sensitivity and specificity, clinical signs alone are not a suitable diagnostic test for wound infection in vascular patients.

Recent studies have used different criteria to define wound infection.[22-24] Following Gardner and colleagues, we used a tissue biopsy as the sample for culture. However, we were unable to use a quantitative culture method to define an infection threshold. Like Woo and Sibbald, we used a semi-quantitative scale. However, their samples were obtained by culturing wound fluid, collected using the Levine method, which invariably represents bacteria from more superficial layers. Using this approach, Woo and Sibbald have quite a high rate of samples that cultured a light amount of bacteria.[24]

In order to provide the greatest diagnostic value, a balance between strong positive values of specificity and sensitivity is required. In our study, the best balance between specificity and sensitivity was obtained when several signs were combined. In particular, the presence of three or four signs associated with previously defined STONEES yielded positive likelihood ratios of greater than 7.0, which is considered clinically important.[26] Confirming the results of Woo and Sibbald,[24] our findings suggest a composite is more informative in detecting infection than individual signs.

The different clinical signs observed in this study occurred non-uniformly. There were a few signs that were seldom observed including induration, elevated peri-ulcer temperature, odour and exudate. This finding was expected as the majority of patients had diabetes and peripheral vascular disease, which is known to blunt inflammatory response.[32] There were a few signs that were commonly seen in our patients, including:

edema, delayed healing, and fragile granulation tissue. A high false positive rate with these signs is likely due to the characteristics of the patients under study.

The inability to achieve a 20% to 40% decrease in wound size within four weeks (defined as delayed healing) was often associated with infection (True Positive = 16). That said, delayed healing was also common in patients without infection (False Positive = 37). It might be expected that delayed healing may occur in a population with vasculopathy given the impoverished wound environment. Additionally, the high prevalence of friable granulation tissue in the wound base may have occurred since 65.4% of patients in the study took anticoagulant medication which is a common treatment for a vascular population

Given that elevated wound temperature depends largely on perfusion, the infrequency of this finding was expected. In this study, we detected elevated wound temperature using slightly different techniques than previously.[24] We opted not to compare temperatures to the same location on the contralateral limb since limb temperature is affected by perfusion and several of our patients had undergone unilateral vascular surgery or angioplasty. Instead, we used the same infrared thermometry method to detect wound temperature that was three degrees higher than the surrounding skin located 10cm distally on the same limb. Using this approach, we found heat to informative since it was highly specific (0.91), but poorly sensitive (0.27) as it was an infrequent finding.

We found, as Gardner, and colleagues[23] did, that no single sign was strongly associated with the presence of infection. Additionally, we did not find that increasing pain or wound breakdown to be 100% specific. This may be because patients with arterial disease were excluded from that study, since pain and wound breakdown may be associated with ischemia.[33] Contrary to their research, our study found increased pain was only moderately specific for infection. That said, we also found that presence of pain did not necessarily suggest infection was present. This difference may be because patients with vasculopathy include those with diminished and altered pain experiences often complicated by ischemia, edema and/or neuropathy.[34] It is therefore expected that vascular disease influences pain response with and without the presence of infection. The

addition of a debridement procedure may have promoted anticipation of pain which may have further muted this sign.

We found a composite of signs for infection to be more specific for our population than in the two previous studies. Conversely, we did not confirm previous findings that suggested certain signs including non-healing, exudate increase, friability, debris in the wound and odour, to be associated with the presence of localized, superficial critical colonization.[24] This previous study showed more signs with a stronger sensitivity for infection than our study. When comparing values for the positive likelihood ratio, we confirmed the results of Woo and Sibbald, showing STONEES and LOWER with three signs performed best as a specific measure of infection. That said, we did not find the NERDS or UPPER composites achieved likelihood ratios that were informative when compared with less than moderate culture growth. It is possible that our deeper tissue culture method affected the results of the superficial bacterial burden assessment.

Although previous studies have focused on non-arterial populations, the ABPI inclusion criterion is frequently 0.5, which is within current definitions of peripheral arterial disease.[10] Interestingly, Gardner and colleagues had defined arterial insufficiency as being ABPI <0.5.[22] Our population has a mean ABPI of around 0.90 but had a strong representation of peripheral vascular procedures. It may be useful for future studies to report previous vascular surgical history in addition to ABPI in order to gain an improved sense of arterial pathology.

2.5.1 Limitations

Patients with PVD are known to be at very high risk of wound infection, and often require hospital admission for vascular surgery and even amputation. However, the incidence of bacterial invasion into the tissue biopsies taken in this study was fairly low. The high standard of care provided by an experienced interprofessional team working at a tertiary care hospital may have blunted our chances of determining whether clinical signs are very sensitive at detecting new or emerging infections.

Clinical signs recorded in this study were recorded by a single assessor who was the ET RN. While specific criteria were used for each sign, these findings were not confirmed by a second assessor and no test of reliability of the results produced by this assessor was conducted. Furthermore, some criteria used to detect the presence of certain clinical signs, previously defined by other groups (e.g. increased temperature) had to be modified to fit this population. It is therefore very possible some signs of infection were missed or mislabeled. However, using a highly trained nurse who has several years' experience with this patient population is advantageous in that this is the type of clinician who would most benefit from a valid diagnostic test that is both sensitive and specific to infection in this high risk population.

In our study, the culture results were combined with a clinical diagnosis of infection by an ID physician. Therefore, the methods used to confirm the presence of infection were not the same as in the described previous studies which used culture alone, not the medical professional's evaluation as the reference standard. In those studies, the sensitivity, specificity and LRs of individual and composite signs were only calculated against positive culture results. In our study, there was no increase in the sensitivity and/or the specificity of individual signs when compared to culture alone. Additionally, composite signs had reduced LR+s when compared to culture result alone. We also compared clinical signs to culture results from a sample of tissue taken after extensive wound debridement. As such, the tissue samples used represent a deeper layer of the wound than surface swabs, which may have contributed to a lower rate of moderate to heavy growth of bacteria. By combining these methods, we are confident that we accurately assigned patients to either the infected or not infected groups.

We used a consecutive convenience sampling method for a patient group that was serviced by a group of vascular surgeons. As a result, the patient group included in this study was quite varied and included a subgroup of people with a long history of diabetes. Several patients had recently undergone angioplasty and/or bypass grafting and some had a wound at an unhealed amputation site. Furthermore, only 75% of those attending the vascular service either consented or were eligible to participate in the study. Whether or

not these results can be generalized to all patients with peripheral vascular disease is unknown.

We specifically excluded those patients that had immunosuppressive or palliative conditions that could prevent healing, bleeding disorders, malignancy in the region or excessive pain. Therefore, our results may not pertain to patients with these conditions.

The results of this study were also influenced by the assessment practices and standards of the health care team managing these patients. For example, the physicians and surgeons prescribed anti-coagulant and antibacterial medication in 65% and 63% of patients, respectively. While these medications are considered limb saving by this health care team, they invariably affect our results. Certain clinical signs such as induration were not seen at all, and other signs (e.g., friable granulation tissue) occurred in most patients regardless of whether infection was present.

The use of topical and systemic antimicrobials in the study may have reduced the appearance of subtle signs of infection and resulted in a relatively small sample of patients with overt infection. One previous group excluded patients on systemic antibiotics when examining the change in clinical signs of infection. This exclusion was due to concern that the host response would be affected by the medication, lessening clinical signs.[31] Given that our patient population had a very high requirement for antibiotic therapy, excluding patients who were on antibiotics would have prevented us from conducting this study.

The exclusion of patients with exposed bone limited the number of possible signs to select that have formerly be noted to be indicative of deep infection in other populations. It is therefore unknown if the addition of probe to bone sign would improve sensitivity or specificity of the composite tools.[24]

2.6   Conclusion

Based on the results of this study there is a strong likelihood infection exists when three or four signs associated with deeper infection (e.g., wound breakdown, increasing wound size, odour, heat, serous exudate) are present. Importantly, we have also found that the

presence of any of one or more clinical signs cannot be used to confirm that an infection exists. As a part of this study, we found signs of superficial infection or critical colonization were not strongly associated with a smaller growth of bacteria or the subclinical diagnosis of infection. These results are influenced by the specific characteristics of the vasculopathic patient group tested, the rigorous method of the ID physician used to confirm the presence of infection, and the subjective nature of detecting clinical signs.

2.6.1   Clinical Implications and Future Research

Our results suggest that it is not suitable to use clinical signs of infection as a screening method, when determining the presence of infection for patients with vascular disease. Although certain signs of infection may occur, they may be a late and unreliable indication of infection.

Importantly, in light of the drive to reduce unnecessary antibiotic prescriptions, wound infection may fail to overtly present in this vulnerable population. Notably, the findings of this study show that most signs are less likely to be present in a population with vasculopathy. In our analysis, many signs that were more frequently seen when infection was present were also noted when infection was absent, and so were of little clinical value. Therefore, if the wound is not healing at a reasonable rate and without complications, it may be prudent to have a low threshold to send a sample for bacterial analysis.

Validating a clinical tool to identify infection for people with PVD and poor healing potential is important since little is known about this population often excluded from clinical trials. Further, determining a group of clinical signs that represent a very high likelihood of wound infection in PVD patients is critical since wound infection can have grave consequences including amputation and even death. Our study found that no sign either individually or in combination was sensitive enough to detect infection, which means visual inspection of a wound cannot be used to rule out infection. Therefore, the use of both tissue biopsy and clinical exam by an ID physician may be warranted to screen this patient group for infection.

There is urgent need for future research to develop more objective point-of-care diagnostic tests for wound infection in the vascular population as well as for other groups. Additionally, future research using newer precise genetic methods of bacterial identification as the gold standard is warranted. Future studies are also needed to address the challenges of this population, and to better understand assessment and management practices.

References

1. Makowsky M, McMurty MS, Elton T, Rosenthal M, Gunther M, Percy M, Wong K, Fok J, Sebastianski M, Tsuyuki R. Prevalence and treatment patterns of lower extremity peripheral arterial disease amongst patients at risk in ambulatory health settings. Can J Cardiology 2011;27(3):389,e11-8.

2. Rooke TW, Hirsch AT, Misra S, Sidawy AN, Beckman JA, Findeiss LK, Golzarian J, Gornik HL, Halperin JL, Jaff MR, et al. 2011 ACCF/AHA focused update of the guideline for the management of patients with peripheral artery disease (updating the 2005 guideline): A report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines: Developed in collaboration with the Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society for Vascular Medicine, and Society for Vascular Surgery. Catheter Cardiovasc Interv 2012 Mar 1;79(4):501-31.

3. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WRC, Olin JW, Puschett JB, et al. ACC/AHA guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic). Journal of Vascular and Interventional Radiology 2006 9;17(9):1383-98.

4. Registered Nurses' Association of Ontario (RNAO). Assessment and management of venous leg ulcers: Guideline supplemement; Update 2007:ID: 298.

5. Mills JL S. Update and validation of the society for vascular surgery wound, ischemia, and foot infection threatened limb classification system. Semin Vasc Surg 2014 Mar;27(1):16-22.

6. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: Mechanisms, signaling, and translation. Sci Transl Med 2014 Dec 3;6(265):265sr6.

7. Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science 1983;221(4617):1283-5.

8. Lokmic Z, Darby IA, Thompson EW, Mitchell GM. Time course analysis of hypoxia, granulation tissue and blood vessel growth, and remodeling in healing rat cutaneous incisional primary intention wounds. Wound Repair and Regeneration 2006;14(3):277-88.

9. Attinger CE, Evans KK. Operative management of diabetic foot wounds and infections: Maximizing length and optimizing biomechanics. In: A. N. Sidawy, editor. Philadelphia: Lippincott, Williams and Wilkins; 2006. ID: 823; M1: Book, Section.

10. Norgen L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FGR. Inter-society consensus for the management of peripheral arterial disease (TASC II). Eur J Vasc Endovasc Surg 2007;33:S1-S75.

11. Abularrage CJ, Sidawy AN, White PW, Weiswasser JM, Arora S. Abnormalities of microcirculation in diabetes. In: A. N. Sidawy, editor. Philadelphia, PA: Lippincott, Williams and Wilkins; 2006. ID: 3727; M1: Book, Section.

12. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001;14(2):244-69.

13. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJG, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. 2012 Infectious Diseases Society of America Clinical Practice Guideline for the diagnosis and treatment of diabetic foot infections. Clinical Infectious Diseases 2012;54(12):132-73.

14. Reddy M, Gill SS, Wu W, Kalkar SR, Rochon PA. Does this patient have an infection of a chronic wound? JAMA 2012 Feb 8;307(6):605-11.

15. Levine NS, Lindberg RB, Mason AD, Pruitt BA. The quantitive swab culture and smear: A quick simple method for determining the number of viable aerobic bacteria on open wounds. J Trauma 1976;16(2):89-94.

16. Smith ME, Robinowitz N, Chaulk P, Johnston K. Comparison of chronic wound culture techniques: Swab versus curetted tissue for microbial recovery. Br J Community Nurs 2014;19(90):S22-6.

17. Pellizzer G, Strazzabosco M, Presi S, Furlan F, Lora L, Benedetti P, Bonato M, Erle G, de Lalla F. Deep tissue biopsy vs. superficial swab culture monitoring in the microbiological assessment of limb-threatening diabetic foot infection. Diabet Med 2001 Oct;18(10):822-7.

18. Stewart EJ. Growing unculturable bacteria. J Bacteriol 2012;194(16):4151-60.

19. James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J, Costerton JW, Stewart PS. Biofilms in chronic wounds. Wound Repair Regen 2008 Jan-Feb;16(1):37-44.

20. Lavery LA, Fontaine J, Thakral G, Kim PJ, Bhavan K, Davis KE. Randomized clinical trial to compare negative-pressure wound therapy approaches with low and high pressure, silicone-coated dressing, and polyurethane foam dressing. Plastic and Reconstructive Surgery 2014;133:722-6.

21. Cutting KF, Harding KG. Criteria for identifying wound infection. J Wound Care 1994;3(4):198-201.

22. Gardner SE, Frantz RA, Doebbeling BN. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair Regen 2001 May-Jun;9(3):178-86.

23. Gardner SE, Hillis Sl, Franz RA. Clinical signs of infection in diabetic foot ulcers with high microbial load. Biol Res Nurs 2009(11):119-28.

24. Woo KY, Sibbald RG. A cross-sectional validation study of using NERDS and STONEES to assess bacterial burden. Ostomy Wound Manage 2009 Aug 1;55(8):40-8.

25. World Health Organization. Worldwide country situation analysis: Response to antimicrobial resistance. WHO Press; 2015.

26. Furukawa TA, Strauss S, Bucher HC, Guyatt G. Diagnostic tests. In: G. Guyatt, D. Rennie, M. O'Meade, D. J. Cook, editors. New York, NY: McGraw Hill; 2002. ID: 3716; M1: Book, Section.

27. Kalra M, Gloviczki P. Distal bypass to inframalleolar vessels. In: A. N. Sidawy, Editor. 1; 1 ed. Lippincott, Williams and Wilkins; 2006. .

28. Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJC, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clinical Infectious Diseases 2005;41:S395-519.

29. Fagan TJ. Letter: Nomogram for Bayes theorem. N Engl J Med 1975 Jul 31;293(5):257.

30. Sibbald RG, Woo K, Ayello EA. Increased bacterial burden and infection: The story of NERDS and STONES. Adv Skin Wound Care 2006;19:447-61.

31. Woo KY, Coutts PM, Sibbald RG. A randomized controlled trial to evaluate an antimicrobial dressing with silver alginate powder for the management of chronic wounds exhibiting signs of critical colonization. Adv Skin Wound Care 2012 Nov;25(11):503-8.

32. Kim E, Tolhurst AT, Cho S. Deregulation of inflammatory response in the diabetic condition is associated with ischemic brain injury. Journal of Neuroinflammation 2014;11(83):1-9.

33. RNAO Nursing Best Practice Guideline. Assessment and management of foot ulcers for people with diabetes. 2nd ed. Toronto, ON: Registered Nurses' Association of Ontario; 2013. .

34. Bonham PA, Flemister BG. Guideline for management of wounds in patients with lower-extremity arterial disease. Mt Laurel, NJ: Wound, Ostomy and Continence Nurses' Society: WOCN clinical practice guideline series; 2014. ID: 2819; M1: Book, Whole.

3 Chapter 3: The UltraHeal Pilot study: Tolerability and feasibility of 22.5 kHz low frequency contact ultrasound debridement (LFCUD) for patients with lower extremity wounds and vascular pathology

3.1 Introduction

There are few effective methods to support wound healing in patients with vascular disease. Lower extremity wounds complicated by arterial and venous pathologies can be extremely challenging to heal, and these non-healing wounds are associated with recurrent infections, limb amputation and death.[1] Therefore, more effective methods are needed to support healing in this vulnerable population. One approach is to improve the quality of, and access to, debridement procedures. These procedures improve the wound environment and are an essential part of preparing the wound for healing.[2-4] Despite the need for this step in wound preparation, debridement is not consistently available for these patients. A method of ultrasound debridement that might be safely and comfortably administered in a nurse-led wound clinic is intriguing as it may improve access to care and possibly improve outcomes.

3.1.1 Peripheral Vascular Disease

Peripheral vascular disease (PVD), which is associated with the development of lower extremity wounds is an increasingly prevalent health problem.[5] PVD includes similar, yet not equivalent, forms of blood vessel pathology such as peripheral arterial disease (PAD), peripheral arterial occlusive disease, venous insufficiency, and lower extremity arterial disease (LEAD). All of these conditions affect blood flow within the lower extremities. While the prevalence of these various vascular pathologies is difficult to determine since many patients are asymptomatic, the prevalence of LEAD is thought to be increasing. LEAD is linked to increased age and other conditions of growing prevalence including diabetes, hypertension, dyslipidemia, and chronic renal failure.[5] It has been estimated that LEAD is present in 30% of the population over 70 years of age,[6] and 40 % of the population over 80 years.[7] Poor arterial blood supply is the hallmark of LEAD and can result in death of individual cells, which can lead to the development of

lower extremity wounds. Therefore, the occurrence of non-healing wounds is often a symptom of advancing LEAD. A recent Canadian study found the highest prevalence of compromised wounds was found in patients with PVD, and in acute care settings, patients with generalized cardiovascular disease had the highest wound prevalence.[8]

Patients receiving lower extremity revascularization procedures have readmission rates three times higher when an open wound is present prior to vascular surgery.[9] However, vascular surgery is often critical for providing sufficient blood flow to permit healing to occur.[10] Patients with diabetes frequently have lower leg tibial vessel disease, which may be suitable for arterial reconstruction procedures such as arterial bypass or angioplasty.[11] Further, given that post-operative blood flow may be improved for an unknown period of time, effective wound therapies such as LFCUD could be especially beneficial within a limited window of opportunity.

A diagnosis of diabetes has been reported to increase the risk of LEAD by two to four times, and to increase compromised wound prevalence by almost six times.[8] Since diabetes is now considered a global pandemic, it is predicted that the incidence of LEAD-related wounds will continue to escalate.[12] Wounds that are related to soft tissue infections and osteomyelitis are responsible for around 25% of all hospital admissions for persons with diabetes.[8] Responding to this growing need for effective wound therapies in a vulnerable population, this study evaluates a therapy that might improve wound outcomes. We examined if offering more aggressive and targeted wound therapy in the outpatient setting could potentially reduce some vascular and diabetes-related acute care admissions, days of in-hospital stay and associated costs.

3.1.2   Debridement

The procedure of debridement involves the removal of non-viable material, foreign bodies and poorly healing tissue from a wound.[13] Debridement that activates a bleeding response usually involves cutting away the dead tissue with a scalpel and attempting to avoid damage to the healthy cells. Although higher rates of wound closure are associated with an increased frequency of debridement episodes,[14] there is a requirement for particular clinical skills and often a surgical environment to offer sufficiently thorough

debridement to affect wound progress. Clinicians are often apprehensive about performing aggressive debridement on patients with vascular disease because of the risk of causing further tissue damage. Additionally, patients are often anti-coagulated and may bleed excessively. Sharp debridement may enlarge the wound when there is limited ability to heal since determination of the edge between healthy and unhealthy tissue is visual and imprecise. This study investigates if low frequency contact ultrasound debridement (LFCUD), a form of debridement that selectively targets dead tissue whilst providing a stimulant effect of ultrasound energy, may offer a solution to this dilemma.

3.1.3   Ultrasound

Ultrasound is defined as acoustic energy which is above the level of human hearing (greater than 20 kHz).[15] It has been used for various therapeutic applications since around 1950,[16] and is considered to be a safe, effective and relatively easy to use mode of wound therapy with few adverse effects.[17] Ultrasound energy is used in many dissimilar therapeutic devices, which encompass a wide range of frequencies and transmission methods. Different devices have potentially variable degrees of biological effect including tissue response[18, 19] and antibacterial properties.[20-22] Ultrasound frequency (f) is measured in Hertz (Hz) which are units that describe the number of times a molecule subjected to ultrasound is displaced and recovers in cycles per second. Variations of frequency are known to affect the depth of tissue penetration, cellular response, heat generation and dispersal of ultrasound into superficial tissue (known as attenuation).[16]

The frequency range of LFCUD devices spans from 22.5 kHz to 35 kHz, and this range is known as long wave or low frequency ultrasound.[15] Differences in the amount of ultrasound delivered and the tissue response will occur depending on the probe design, on the ultrasound intensity (measured in Watts per centimetre squared), on the treatment duration, and on whether or not the device is in pulsed or continuous mode. For an in-depth review of a variety of ultrasound therapies, see Kloth and Niezgoda.[15]

Several clinical reports have documented the beneficial effects of a combined ultrasound and debridement system, similar to the treatment used in the current study, in different

populations.[17, 23, 24] These reports have found 25 kHz LFCUD to be well tolerated in a diverse group of patients suffering from a variety of chronic non-healing wounds. The focus of this paper is the effect of the lowest frequency device (22.5 kHz) ultrasound that involves direct contact of the probe to the base of the wound.

3.2 Research Purpose

Patients in the vascular surgery wound clinic are a vulnerable population, who frequently have a high frequency of wound complications including infection, accumulating necrotic debris, poor granulation response and slow or absent wound closure. The purpose of this pilot study is to determine the tolerability and feasibility of using 22.5 kHz LFCUD in a nurse-led vascular surgery wound clinic. The anticipated goal of LFCUD treatments is to reduce complications and improve clinical outcomes among patients with vascular insufficiency, who may also have diabetic neuropathy and/or wound infection. This study examines whether or not LFCUD, a selective method of debridement, stimulates wound healing, impacts wound size or improves wound appearance. The following research questions guide the study:

1) Is there a reduction in wound size after four weekly LFCUD treatments plus usual care? If so, by what amount?

2) Is there an improvement in wound appearance after four weekly LFCUD treatments as determined by validated assessment tool (revPWAT)?

3) Is LFCUD well tolerated? Are there minimal adverse reactions that are unrelated and related to treatment?

4) Is LFCUD feasible to apply in a nurse-led vascular surgery wound clinic?

3.3 Methods

This prospective single arm observational study compared wound appearance and size before and after a series of four weekly 22.5 kHz LFCUD treatments (see Figure 1). A consecutive sample of patients with lower extremity wounds, who were referred from an acute care vascular service, were recruited. Wound assessments were completed at a baseline visit (Week 0) as well as one week after the last ultrasound treatment (Week 5). Patients also returned for follow up visit at Week 12. This study involved: (1) securing

approval from the university and hospital Research Ethics Boards (REBs); (2) recruiting patients; (3) administering treatments (e.g., usual care, negative pressure wound therapy, initial debridement, LFCUD); (4) evaluating patients (e.g., for wound size, wound appearance, pain, skin grafting, adverse events); and, lastly (5) analyzing the data.

Figure 1 *Study flow diagram*

Screening
Screening
Screen Form (ET RN)
Vascular Assessment

Recruitment

Pre-treatment(Week 0 - Baseline visit)
Infection Assessment (ID)
Pain (VAS)
WSA (Planimetry)
Wound Appearance (revPWAT)

Week 1 - LFCUD Treatment 1
WSA
Pain Pre and Post Treatment
Adverse Events

Week 2 - LFCUD Treatment 2
WSA
Pain Pre and Post Treatment
Adverse Events

Week 3 - LFCUD Treatment 3
WSA
Pain Pre and Post Treatment
Adverse Events

Week 4 -LFCUD Treatment 4
WSA
Pain Pre and Post Treatment
Adverse Events

Week 5 - Post-treatment
Infection Assessment (ID)
Pain (VAS)
WSA (Planimetry)
Wound Appearance (revPWAT)

Week 12 - Follow-up visit
Wound Closure
Pain (VAS)
WSA (Planimetry)
Complications
Hospital Visits Pain (VAS)
WSA (Planimetry)

Abbreviations: ET RN = Enterostomal Registered Therapy Nurse; ID = Infectious Diseases physician; VAS = Visual Analogue Scale; revPWAT = revised photographic wound assessment tool; LFCUD = low frequency ultrasound debridement; WSA = wound surface area

3.3.1   Study Approvals

The methodological quality and study protocol was reviewed and approved by both the university and clinical Research Ethics Boards (see Appendices 1 and 2), and was monitored by an external Data Safety Monitoring Board. Further, the Vascular Surgery Department designed and activated a Medical Directive, and implemented a Delegated Medical Act to ensure that all appropriate permissions and skill competencies were reviewed and approved by the Nursing Professional Practice Department and the Department of Vascular Surgery.

Training on the Sonic One® device was provided and certification was awarded to the Enterostomal Therapy Nurse (ET RN) on the ultrasound debridement technique by the manufacturer (Misonix, Farmingdale, USA). This training was conducted in coordination with the Program Director of Vascular Surgery at the study centre.

3.3.2   Patient Recruitment

Patients were recruited to represent a typical and varied population that receives wound care from a vascular surgery department. Patients who had a lower extremity wound, and were either attending the hospital outpatient vascular surgery clinic or admitted to the vascular surgery in-patient unit, were approached by one of six vascular surgeons or their delegates during their routine clinic or hospital visit. Subjects included were those with treated infections, diabetes, peripheral vascular disease, neuropathy and various other comorbidities. Patients were at least 18 years of age and were willing to commit to the weekly treatment schedule. Additionally, patients were eligible if they had a lower extremity wound greater than 1cm^2 wound surface area (WSA), had ankle brachial pressure index (ABPI) of greater than 0.3 and did not have a vascular intervention planned (or deemed necessary) in the upcoming weeks. Patients were excluded if they had any conditions that could be aggravated by LFCUD (e.g., acute deep vein thrombosis or acute coagulopathy, malignancy in the region of therapy, or excessive pain). Patients with pacemakers or internal cardiac defibrillator devices were also excluded since it was not feasible to verify with all possible manufacturers that these devices would not be compromised. We also excluded patients if they had untreated infections, exposed

vascular graft or bone, were immunosuppressed, medically unstable or unlikely to complete the study. Patients with severe arterial insufficiency that was likely to prevent healing (i.e. pre-operative or palliative status as determined by the vascular surgeon) were excluded from the study.

Patients who agreed to be contacted were then approached by the researcher. The study was explained and an approved letter of information was provided in plain language that described the study protocol and all risks. Patients were asked to take the information home and consider the information for at least 24 hours. Those who elected to participate then contacted the administrative assistant to arrange the first visit. Each patient was assured that their choice regarding trial enrollment would not affect their access to ongoing wound care.

3.3.3   Treatment

Ten patients were recruited to receive weekly 22.5 KHz LFCUD treatment over the course of four weeks. The LFCUD was applied by the Enterostomal Therapy Registered Nurse (ET RN) in the setting of an acute care vascular surgery wound clinic or in a private room in the in-patient vascular surgery ward (see Plate 2). The LFCUD treatment was applied until necrotic debris was removed and the wound surface was lightly bleeding. Patients taking oral analgesic to manage the pain for dressing changes continued to do so. If the patient suggested that the analgesia was insufficient, a local lidocaine injection was offered as an anaesthetic. At each visit, patients were encouraged tell the ET RN should they become uncomfortable during LFCUD treatment. Further, all patients were advised that local anaesthetic was available and that treatments could be stopped at any time upon their request.

3.3.4   Ongoing Usual Care

All patients in the study continued to receive ongoing wound care as is provided in the vascular surgery wound clinic or ward. Typically, their treatment included moist wound therapy, pressure offloading or compression wrap as warranted. Their treatment also involved monitoring for infection as per the Clinical Infectious Diseases Practice Guidelines.[25, 26] Patients were provided with the same antimicrobial dressing (Silvercel,

Acelity, San Antonio, Tx) or Negative Pressure Wound Therapy (NPWT) based on wound characteristics. Patients with large, deep or structurally unstable wounds received NPWT as this is a usual treatment method to support healing for this patient group. NPWT was provided in one of two available formats: the Vacuum-Assisted Closure Device (VAC®, Acelity, San Antonio, USA) and Renasys® (Smith and Nephew, London, UK). The format was selected by the community nursing agency based on availability. Both NPWT systems incorporated a foam layer that was directly placed on the wound bed without an interface dressing. NPWT was achieved by placing an occlusive dressing over the foam layer, and then attaching tubing to a negative pressure machine that creates a gentle suction on the wound bed. Patients received intermittent NPWT therapy (five minutes on and two minutes off) with the aim of promoting granulation response and supporting local perfusion.

3.3.5   Initial Debridement

At the initial visit (at Week 0) an Infectious Diseases (ID) physician evaluated the patient for wound infection and provided systemic antimicrobial therapy as needed. This physician also provided an initial removal of all necrotic tissue using a standard sharp debridement technique. This debridement ensured all patients had a standardized baseline wound status. After cleansing with physiological saline solution, a tissue sample was taken for a standard bacterial culture and antimicrobial susceptibility analysis. The sample was immediately sent to a clinical laboratory for semi-quantitative bacterial analysis in aerobic and anaerobic conditions. Regardless of whether or not necrotic debris was visible, the wound bed was debrided to produce active bleeding.

3.3.6   Low Frequency Contact Ultrasound Debridement (LFCUD)

The Sonic One® (Misonix, Farmingdale, USA) device was used for all LFCUD treatments (see Appendix 4). This device consists of a generator with an application probe containing a piezoelectric crystal which converts electrical energy into mechanical ultrasound energy as the crystal oscillates at 22,500 times per second. As a part of this process, the rapid contraction and expansion of the crystal causes acoustic streaming and cavitation. Streaming refers to micro currents that form in the saline irrigation couplant

which occur in addition to a violent collapse of microbubbles (cavitation). By these mechanisms, selective emulsification and removal of necrotic debris occurs, and viable tissue remains intact until a clean, lightly bleeding wound surface is revealed (see Appendix 5). The Sonic One® device maintains a constant intensity and frequency, and delivers more or less energy depending on the type of tissue and force applied by the clinician.

The Sonic One® device, which is operated with a pedal switch, was prepared for each use with an autoclaved hand piece and removable probe with sterile disposable tubing set for saline delivery. Personal protective equipment for aerosol generating procedure was utilized during each treatment application (as per local infection control practices). Sterile gloves were donned to handle the sterile components during treatment, and sterile touch technique was used for all wound care and dressing applications (as per local clinical practice). A pre-treatment and post-treatment wound rinse of 0.05% chlorhexidine was provided with each treatment to remove any bacteria including those that may have been released from a chronic biofilm during treatment.

In most instances, the LFCUD was applied with the standard gold probe (Figure 2). A green probe (see Appendix 7) was selected if patients were apprehensive or concerned about pain as it provides a more gentle application with wider ultrasound energy dispersion. Ultrasound was applied in a continuous mode, which means there was no interruption in ultrasound dosing during treatment. The probe was applied to the wound bed at a setting of Amplitude 5, which is the maximum probe head movement setting (see Appendix 6) and provides the most aggressive debridement. Physiological saline was used as the coupling and cooling agent. The sterilized probe was placed in contact with the wound bed and applied with a gentle circular motion to all areas of the wound surface. This treatment continued until light bleeding was visible and no necrotic debris remained. The time of application and type of probe type were recorded for each LFCUD treatment. The mean treatment time was 3.5 minutes.

Figure 2. *A sample of Sonic One ® probes*

From left to right: Blue probe (for deep tunneled areas); Gold probe (for regular use), front and side views; Magenta probe (for adherent debris)

3.3.7   Evaluation

At each visit, the ET RN performed an overall wound assessment as is usual clinic practice. The number of wounds that closed and re-opened were recorded, as well as the incidence of wound-related pain and wound infection. Any complication, emergency room or physician visit, or hospital admission that occurred during the 12 week observation period was also documented. As a part of this clinical trial, the following outcomes were evaluated: (1) wound size; (2) wound appearance; (3) wound pain; and (4) adverse reactions. All study data were entered into a secure research database (Empower Health Research, Inc.).

3.3.8   Wound Size

As a part of the evaluation, an independent assessor, who was a Registered Practical Nurse (RPN), measured the wounds at each visit by tracing the wound edges onto an acetate film, recording the planimetry data and entering these measurements into the electronic database. The measurements were determined by the Visitrak® planimeter (Smith & Nephew, London, UK), which is an instrument that has been previously shown

to accurately determine WSA in centimeters squared.[27] Each measurement was taken three times with the mean of the three results recorded to reduce error of measurement. Measurements were taken after the initial debridement to document the baseline WSA, and before the LFCUD treatment at each weekly visit.

3.3.9   Wound Appearance

To measure wound appearance, the RPN took a photograph of the wound at each appointment. The photography was taken using a Canon Rebel 300D EOS, 8 megapixel resolution, 60mm macro lens digital camera with a ring flash, automatic focus and consistent ambient lighting. This photograph was then used by the ET RN to score the wound progress using a previously validated revised Photographic Wound Assessment Tool (RevPWAT). The revPWAT is an eight item pen and paper tool that assigns a decreasing numerical value to aspects of wound healing.[28] With a maximum score of 32, a decreasing score illustrates that a wound that is improving. Scoring of the photographs was done with photos out of sequence so that the previous patient score was unknown. The revPWAT tool allowed us to consider changes in wound size, depth, necrotic tissue type/ amount, granulation tissue type/amount, wound edges, and periwound skin viability.

3.3.10   Wound Pain

To measure wound pain, the RPN asked patients to rate their pain intensity before LFCUD treatment using the previously validated Visual Analogue Scale (VAS). The VAS uses millimetre increments with maximum pain possible at 100mm (VAS ruler, Molnlycke, Gothenburg, Sweden).[29] As a part of the evaluation, the RPN asked each patient to move a marked plastic tab on the VAS ruler to indicate their wound pain level. For each measurement, the RPN requested that the patient distinguish wound pain from limb or other pain. The ET RN also asked patients to rate their pain after treatment and recorded the number of patients who required local anaesthetic.

3.3.11   Adverse Events

Adverse events were noted to be present or absent at each visit, and all adverse events were reported to the Data Safety Monitoring Board following the requirements of the REBs. The vascular surgeon and ID physician were notified with every concern, to determine if any event was related to the treatment and if further medical care was required. The number of major and distal lower extremity amputations as well as the number of emergency room visits and related hospital admissions were also noted for the duration of the study. All ten patients who received treatment were included in the statistical analysis. One subject missed Week 5 due to transport issues, and another subject missed the Week 12 follow up visit due to a scheduling error.

3.3.12   Skin Grafting

To ensure optimal patient care, patients were referred to the plastic surgery service as soon as their wounds were granulating well and appeared appropriate for skin grafting (regardless of whether or not the four LFCUD treatments had been completed). The decision to close by grafting and the timing of the procedure was determined by the plastic surgeon. Patients who were grafted received a split thickness skin graft (STSG) retrieved from the anterior thigh or abdomen. The two patients who received STSGs were receiving NPWT, and continued to receive NPWT for five uninterrupted days post graft as is local usual procedure to support graft success.

3.3.13   Data Analysis

All data was collected and stored in a secure research database (EmPower Health Research, Inc., London, Ontario, Canada), and analyzed using SPSS v.23 (SPSS, IBM Corporation, Armonk, NY). All subjects completed the 12 week observation period, however, one subject missed the final visit, and one subject had already received a STSG. For conservative estimation, the last outcome carried forward was used to impute these missing data. The change in wound surface area before and after the LFCUD treatment, and between Week 0 (baseline) and Week 12 was calculated and compared using the Paired Student's t-Test. The mean percentage change in wound surface area was also calculated. To determine the change in wound tissue quality before and after the

treatment period (Week 0 and Week 5), the change in mean revPWAT score was calculated and compared using the Paired Student's t-Test. The mean value ± Standard Deviation of the total revPWAT score was calculated before, and after LFCUD treatment period (at Week 0 and Week 5). Given the relatively small sample size, data were tested for normal distribution by a visual inspection of histogram and Shapiro-Wilks test to verify that the parametric method was appropriate. Additionally, the revPWAT data was subjected to a nonparametric analysis (Wilcoxian Rank Test) without a change in results. Demographics and related medical information were also recorded. Mean values and standard deviations were calculated for continuous variables (e.g. age, wound duration), with frequency and range used to express dichotomous variables (e.g. gender, diabetes).

3.4   Results

Ten patients were recruited in the study from December 2013 until April 2014. Fourteen consecutive patients were invited to participate in the study, but three subjects were found to be ineligible at first debridement due to either depth of wound ($n = 2$), or untreated osteomyelitis ($n = 1$). One subject was withdrawn just prior to starting LFCUD treatments due to rapid decline in health status. Data are presented for the ten eligible patients who participated in and completed the study.

3.4.1   Patient Demographics

Patient demographics are reported in Table 7. Eight of the ten patients were male. The mean age of the participants was 66.1 years (SD = 9.9, range = 55-85 years). The average mean duration of wound was ten months, and seven of the ten participants' wounds were located somewhere on the foot. Seven out of ten patients had advanced arterial disease, two patients had painful venous leg ulcers of more than two years in duration, and one patient had occluded micro vessels in the foot after a sepsis event. Seven patients had diabetes with concurrent neuropathy and were being treated for infection. Three of the patients had previous limb amputation including one major, one distal, and one distal followed by major amputation procedure. Lastly, four patients had undergone previous re-vascularization procedures ($n = 4$ angioplasty; $n = 2$ bypass procedure prior to angioplasty).

Table 7 *Characteristics of Patients Enrolled in UltraHeal Pilot Study*

Characteristic	*M* SD (range [-] or distribution (*n* = 10)
Age (Years)	66.1 ± 9.9 (55-85)
Sex (Male: Female)	10 (8:2)
Wound Location:	
Toe/ Toe amputation site	2
Foot (Plantar, Dorsal)	3 (1,2)
Heel	2
Leg	3
Wound Duration (months)	10.1 ± 11.4 (2 – 36)
Initial Wound Surface Area (cm^2)	30.34 ± 26.2 (1.93 – 63.8)
Diabetes :	7
Neuropathy	7
Antibiotic therapy	7
Previous Lower Limb Amputation:	3
Distal only (pedal)	1
Major only (trans-femoral /trans-tibial)	1
Distal AND Major	1
Previous vascular intervention affected limb	4
Angioplasty	4
Bypass Graft:	2
(Angioplasty AND Bypass graft)	2

Abbreviations: cm^2 = centimetres squared

Values expressed as frequency distribution or mean ± Standard Deviation with maximum and minimum values or distribution in parentheses.

3.4.2   Wound Size

The Wound Surface Area (WSA) significantly declined over the course of the treatment (see Figure 3). In nine of ten cases, the wound size decreased progressively with weekly treatments of low frequency ultrasound. The tenth person had unchanged wound surface but it was later suspected that there may have been repetitive trauma occurrences from a wheelchair foot rest with transfers. The initial mean WSA was $30.35cm^2$. After four LFCUD treatments, the mean decrease in WSA observed over six weeks was 15.52 cm^2 ($2.73-28.32cm^2$ 95% CI), which was statistically significant ($p=0.023$). The average percentage area reduction during this treatment period was 39.4% (SD $\pm$ 29.3). None of the wounds enlarged. Wounds continued to decrease after LFCUD stopped but at a slower rate. The total percentage WSA reduction from Week 5 to Week 12 was slower than it had been during the treatment phase at 25.9%. One patient had a small re-opening at Week 12 and it was later determined that a vascular graft occlusion had occurred. This wound was also colonized with *Pseudomonas Aeruginosa* and despite these barriers, we were pleased to note that the wound later achieved closure with a successful skin graft.

Figure 3 *Change in wound surface area by planimetry from baseline (week 0) to post-treatments (week 5), and at 12 week follow-up visit*
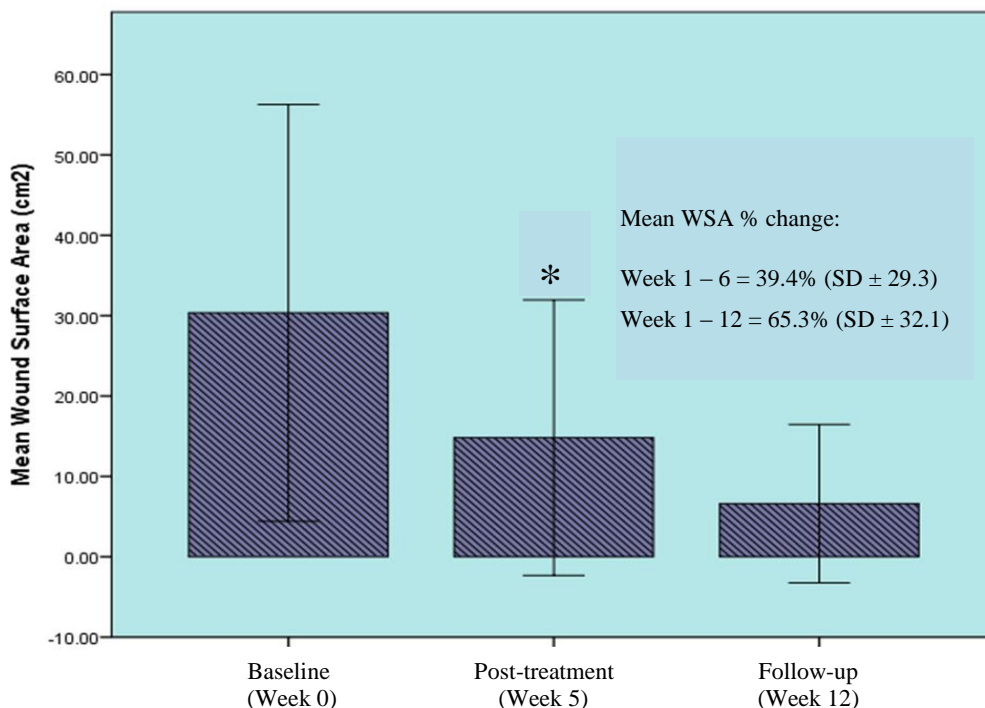
Error bars = ± 1 Standard Deviation (SD). Mean Wound Surface (WSA) area measured using acetate tracing and planimetry at baseline (Week 0), and after 4 weekly LFCUD treatments (Week 5). The follow up visit (Week 12) is shown for comparison. Asterisk denotes statistical significance, ($p = 0.023$)

3.4.3   Wound Appearance

Wound appearance significantly improved from baseline following the weekly LFCUD treatments (Figure 4). In nine of ten cases, the wound appearance improved progressively with four weekly treatments of LFCUD. The tenth person had an unchanged wound appearance, which, as previously mentioned, might be attributed to repetitive trauma occurrences from the wheelchair foot rest with transfers. The mean revPWAT score derived from digital images taken at baseline prior to commencing LFCUD treatment was 22.6 (SD ± 2.95). After treatments concluded (Week 5), there was a decrease in mean revPWAT score of 8.3 out of the possible 32 points (SD ± 5.03, 4.7- 11.9 95% CI, $p=<0.01$), indicating a significant improvement in wound appearance. This decrease in

revPWAT score was primarily due to the improved appearance of healthy granulation tissue. Eight out of ten wounds had lower revPWAT scores after LFCUD treatments, suggesting they each demonstrated better tissue health. For the other two patients, one had the missed visit and the other had been newly skin grafted, which meant the health of the wound base could not be determined. In both cases, data were imputed using the last outcome carried forward as a conservative measure.

Figure 4 *Change in wound appearance by revPWAT score from baseline (week 0) to post-treatments (week 5)*
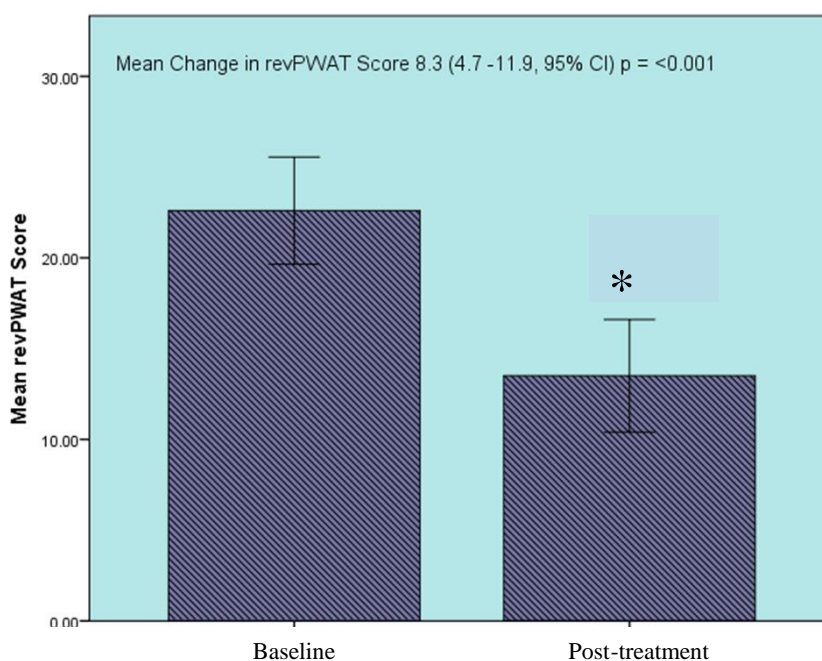
Error bars = ± 1 Standard Deviation (SD). Mean revPWAT Scores determined from digital images taken prior to LFCUD (baseline), at baseline (Week 0), and after 4 weekly LFCUD treatments (Week 5). Asterisk denotes statistical significance, (p= <0.01).

3.4.4 Wound Pain

Wound pain was found to decrease over the course of the weekly LFCUD treatments. The mean decrease in VAS pain scores form Week 0 to Week 5 was 6mm (-6.7 to 18.7, 95% CI). No patients refused treatment based on concerns about pain, and all treatment

sessions were attended. One patient required local anaesthetic at the first two visits, but did not require it at the final two visits as the wound improved. Most patients who had experience with sharp debridement commented that this form of debridement was much more comfortable. Patients stated that the treatment sensation they experienced was more of a vibration sensation than a painful stimulus, noting they were pleasantly surprised with their treatment experience. In fact, pre-treatment and post treatment pain measures demonstrated that LFCUD treatments were well tolerated and did not induce much discomfort.

3.4.5   Adverse Reactions

There were no LFCUD treatment-related adverse reactions. Three patients suffered from adverse events unrelated to the treatment. One patient reported a failure of his NPWT device that had occurred for three days continuously prior to the visit. The device had been left in place during the pump failure, and the patient presented with a new localized wound infection. At the study visit, the patient was examined by the ID physician, and antibiotics were prescribed with resolution. Successful skin grafting was performed the following week as scheduled. A second patient was admitted to the hospital for congestive heart failure and renal failure. This patient also suffered a rash that may have been related to an antibiotic medication. An adjustment of medications was required, and this patient was discharged after ten days. The wound continued to improve during this period. A third patient, who had a proximal dorsal foot wound, incurred a toe infection on the same foot. The toe required amputation and the infection resolved. A study team meeting was called and all members agreed that the toe infection was not related to the treatment since the infection was not near the treated area and the study wound had continued to improve during this time. The Data Safety Monitoring Board was also notified of these events as were the individual vascular surgeons who were overseeing each case.

3.5   Discussion

This single-arm prospective pilot study has demonstrated for the first time that, in a vascular clinic population, 22.5 kHz LFCUD is tolerable, feasible to apply, and unlikely

to cause adverse events. We found that weekly LFCUD treatments could be provided as a reasonable treatment schedule, and were easily incorporated into an out-patient wound clinic setting. In this current study, in nine of ten cases, the wound appearance improved and wound size decreased progressively with weekly treatments of LFCUD. For the person with unchanged wound status, it was suspected that he may have suffered repetitive trauma that may have affected his results.

We were particularly pleased to find that overall there was minimal pain with treatments. While the patient who required local anaesthesia did find the treatment uncomfortable at first, this was no longer the case at later sessions when local anaesthetic was administered and when wound acuity decreased. Patients tolerated the procedures well, which has been noted previously in one study, which found that 19 patients with recalcitrant venous leg ulcers reported negligible pain that did not require local anaesthesia after an average of five treatments administered every two to three weeks.[24] Similarly, Herberger found LFCUD was well tolerated compared to conventional surgical debridement procedures.[17] This is an important point since pain may be a perceived barrier to debridement in some clinical settings. The mean pain score declined between Week 0 to Week 5 (post-treatment visit) and tolerability of the treatment was also reflected in our 90% study completion rate.

We had surmised that treatment might stimulate an initial healing response in this challenging population, and were pleased to note a significant improvement in wound appearance in this sample of patients. This result follows a previous study, which treated 17 patients with a variety of chronic wound presentations and found that 53% either healed in eight months or similar to our population, had healthy granulation suitable enough for skin grafting.[30] We found 30% of our patients had wound appearance that improved enough to be eligible for split thickness skin graft (STSG),(see Plates 3 and 4). This is an important finding since patients in the vascular surgery wound clinic have high incidence of poor quality granulation tissue and are therefore not commonly prime candidates for STSG. One of these patients achieved a successful STSG despite having a positive culture for *Pseudomonas Aeruginosa,* which is a bacterium known to contribute to STSG failure.[31]

Patients in this pilot study demonstrated a significant decrease in wound size. We did not find this surprising, since several past studies have reported applications of ultrasound energy to have a positive effect on wound healing.[24,30] It is widely accepted that wounds that fail to heal may be delayed in the inflammatory phase. It is possible that ultrasound debridement assists conversion to the proliferative phase by stimulating growth factor release. Growth factors are known to influence the growth of new blood vessels (angiogenesis) needed to repair damaged tissue,[32] and are a critical part of producing healthy granulation tissue.[33, 34] Debridement to bleeding acts as a trigger for growth factor release since the blood clot material provides multiple growth factors as it degrades.[34] Growth factors then summon fibroblast cells, which produce new collagen to provide structure and strength to the new tissue. Ultrasound energy is known to boost the ability of fibroblasts to produce collagen, and to stimulate new blood vessel growth to support new granulation tissue.[35] Ultrasound is also known to have a blood vessel dilation effect which opens up blood flow to the wound.[36] Therefore, LFCUD could provide several enhanced attributes that may be beneficial to patients with vascular disease.

Several studies have investigated the effect of various types of ultrasound on wound healing outcomes. These include indirect ultrasound applications through water or vapor, and at higher frequencies (MHz). One study employing a 30 kHz foot bath was found to significantly reduce venous leg ulcer areas at three and eight weeks compared to a control group.[37] Subsequently, a case-series study of 23 patients found 69% of patients with chronic, previously non-healing lower extremity wounds were able to achieve healing with 40 kHz ultrasound applied through a saline vapor. We were pleased to find a 39.4% WSA decrease over four weeks in a challenging population, since this rate is in line with populations without vascular disease.[24, 30] Importantly, the findings of our study show LFCUD did not result in the deterioration of any of the patients in a vulnerable vascular population.

3.5.1   Limitations

This study had a few key limitations. This trial was an uncontrolled pilot study and therefore it is not possible to determine whether LFCUD improves healing since there

was no comparison group. Further investigation through parallel group randomized-controlled trial is warranted to determine these outcomes.

Additionally, the sample size was small at ten participants. We determined that ten patients was a pragmatic number as it allowed us to verify treatment scheduling and logistical issues for a future trial while also providing sufficient familiarity with the device for consistent application method in the future trial.

Since we selected patients who did not have exposed bone and tendon, we may have excluded a large proportion of the vascular surgery wound clinic population, who could benefit from this treatment. However, we decided to limit our study since osteomyelitis and associated intractable infection could be more likely with exposed deep structures, and that these patients may fail to show similar signs of healing progression expected of a usual vascular population. That said, such patients should be involved in future trials to better understand this possible effect.

Patients with a variety of lower extremity wound types were included in this study. It is possible that differences in underlying wound etiology may have affected the consistency of our results. However, this group of patients is typical of those seen by a vascular surgery wound clinic, and we considered underlying vascular disease to be the most important common factor since it was of interest to determine feasibility in clinical practice.

The duration of the study was limited to 12 weeks so it was not possible to determine an effect on complete wound closure. However, we believed this to be a reasonable observation period, since patients with PVD frequently suffer setbacks due to complicated health issues. With that, we were encouraged to find that none of the wounds enlarged during the study period.

It is likely that patients under the care of specialized teams would show improvement even without additional therapies. That said, even with specialist care this population typically faces frequent deterioration in wound appearance. As such, our finding that no patient deteriorated is unexpected and encouraging.

3.6   Conclusion

The results of this study suggest that LFCUD administered by an ET RN in an out-patient setting to a small group of high risk patients with PVD is feasible to apply weekly, is safe, and is well tolerated. Wound pain was not a barrier over the course of the LFCUD treatments. Wound size decreased and wound appearance improved from the baseline status. Further, the LFCUD treatment did not bring about any treatment-related adverse reactions.

Additionally, from the results of this trial we are able to calculate a sample size for a randomized controlled trial to determine a possible treatment effect of LFCUD compared to a control group (see Appendix 3). We also determined the schedule and design of this pilot study is suitable for subsequent trials.

3.6.1   Clinical Implications

Our results showed that LFCUD could be applied safely in a supported out-patient setting by an ET RN. Given that there was no need for additional operating room resources or medical/surgical personnel, nurse-applied LFCUD could improve access to debridement procedures. Additionally, making LFCUD more widely available in the supported out-patient clinic may reduce the burden on those critical resources.

A benefit of LFCUD is that it targets necrotic tissue specifically, and, as such, may be particularly valuable for those considered borderline for debridement. Although caution is necessary and each patient must be considered in context, failure to remove necrotic debris and biofilm is not without risk, since this approach may contribute to further deterioration of the vascular wound. Therefore, an interprofessional team approach provides the best and safest approach for the use of advanced wound therapies such as LFCUD.

People with PVD often experience challenges with delayed wound healing, and are at high risk for limb threatening infections and lower extremity amputations. Additionally, there may be a limited window of opportunity for healing after a revascularization procedure since vascular disease represents systemic pathology, and blood flow that is

restored post-operatively may not be permanently or fully restored. Moving forward, it is critical that future available and effective methods are implemented to support healing during the immediate post-operative period. Future research is also warranted to determine if LFCUD may promote healing in a population with vascular disease and limited options.

References

1. Diehm C, Lange S, Darius H, Pittrow D, Stritzky Bv, Tepohl G, Haberl RL, Allenberg JR, Dasch B, Trampisch HJ. Association of low ankle brachial index with high mortality in primary care. Eur Heart J 2006 Jul;27(14):1743-9.

2. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic ulcer study group. J Am Coll Surg 1996 Jul;183(1):61-4.

3. Kirshen C, Woo K, Ayello EA, Sibbald RG. Debridement: A vital component of wound bed preparation. Adv Skin Wound Care 2006;19(9):506-17.

4. Rodd-Nielson E. Evidence-based recommendations for conservative sharp debridement. Ottawa: Canadian Association for Enterostomal Therapy; 2011. ID: 824; M1: Book, Whole.

5. Bonham PA, Flemister BG. Guideline for management of wounds in patients with lower-extremity arterial disease. Mt Laurel, NJ: Wound, Ostomy and Continence Nurses' Society: WOCN clinical practice guideline series; 2014. ID: 2819; M1: Book, Whole.

6. Bozkurt AK, Tasci I, Tabak O, Gumus M, Kaplan Y. Peripheral artery disease assessed by ankle-brachial index in patients with established cardiovascular disease or at least one risk factor for atherothrombosis--CAREFUL study: A national, multi-center, cross-sectional observational study. BMC Cardiovasc Disord 2011 Jan 19;11:4,2261-11-4.

7. Bergiers S, Vaes B, Degryse J. To screen or not to screen for peripheral arterial disease in subjects aged 80 and over in primary health care: A cross-sectional analysis from the BELFRAIL study. BMC Fam Pract 2011 May 23;12:39,2296-12-39.

8. CIHI Report. Compromised wounds in Canada. Canadian Institute for Health Information; 2013:ID: 2811.

9. Xenos ES, Lyden JA, Korosec RL, Davenport DL. Ninety-day readmission risks, rates and costs after common vascular surgeries. Am J Manag Care 2014;20(10):e432-8.

10. Niezgoda JA, Kindwall EP. Oxygen therapy: Management of the hypoxic wound. In: J. M. McCulloch, L. C. Kloth, editors. Philadelphia, PA: Davis, E.A; 2010. ID: 3685; M1: Book, Section.

11. Akbari CM, Sidawy AN. Overview of the diabetic foot and limb salvage. In: Philadelphia: Lippincott, Williams and Wilkins; 2006. ID: 215; M1: Book, Section.

12. Association. CD. An economic tsunami: The cost of diabetes in Canada. 2009.

13. Steed DL. Debridement. Am J Surg 2004 May;187(5):71S-4S.

14. Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. Plast Reconstr Surg 2006;117(7):143S-51S.

15. Kloth LC, Niezgoda JA. Ultrasound for wound debridement and healing. In: J. M. Muccullogh, L. C. Kloth, editors. Philadelphia, PA: F.A Davis; 2010. ID: 2814; M1: Book, Section.

16. Sussman C, Bates-Jensen BM. Wound healing and physiology: Acute and chronic. In: C. Sussman, B. M. Bates-Jensen, editors. Philadelphia, PA: Lippincott, Williams & Williams; 2007. ID: 3751; M1: Book, Section.

17. Herberger K, Franzke N, Blome C, Kirsten N, Augustin M. Efficacy, tolerability and patient benefit of ultrasound-assisted wound treatment versus surgical debridement: A randomized clinical study. Dermatology 2011;222(3):244-9.

18. Ennis WJ, Valdes W, Gainer M, Meneses P. Evaluation of clinical effectiveness of MIST ultrasound therapy for the healing of chronic wounds. Adv Skin Wound Care 2006 Oct;19(8):437-46.

19. Franek A, Chmielewska D, Brzezinska-Wcislo L, Slezak A, Blaszczak E. Application of various power densities of ultrasound in the treatment of leg ulcers. The Journal of Dermatological Treatment 2004 Dec;15(6):379-86.

20. Qian Z, Sagers RD, Pitt WG. The effect of ultrasonic frequency upon enhanced killing of P. aeruginosa biofilms. Ann Biomed Eng 1997 Jan-Feb;25(1):69-76.

21. Pitt WG, McBride MO, Lunceford JK, Roper RJ, Sagers RD. Ultrasonic enhancement of antibiotic action on gram-negative bacteria. Antimicrob Agents Chemother 1994 Nov;38(11):2577-82.

22. Schoenbach SF, Song IC. Ultrasonic debridement: A new approach in the treatment of burn wounds. Plastic & Reconstructive Surgery 1980 Jul;66(1):34-7.

23. Singh A. Usage of ultrasound in wound management. Masters Thesis Paper - Unpublished. 2006:1-106.

24. Tan J, Abisi S, Smith A, Burnand KG. A painless method of ultrasonically assisted debridement of chronic leg ulcers: A pilot study. European Journal of Vascular & Endovascular Surgery 2007 Feb;33(2):234-8.

25. Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJC, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clinical Infectious Diseases 2005;41:S395-519.

26. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJG, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. 2012 infectious diseases society of america clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clinical Infectious Diseases 2012;54(12):132-73.

27. Sugama J, Matsui Y, Sanada H, Konya C, Okuwa M, Kitagawa A. A study of the efficiency and convenience of an advanced portable wound measurement system (VISITRAK). J Clin Nurs 2007 Jul;16(7):1265-9.

28. Thompson N, Gordey L, Bowles H, Parslow N, Houghton P. Reliability and validity of the revised photographic wound assessment tool on digital images taken of various types of chronic wounds. Adv Skin Wound Care 2013 Aug;26(8):360-73.

29. Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 1983 Sep;17(1):45-56.

30. Breuing KH, Bayer L, Neuwalder J, Orgill DP. Early experience using low-frequency ultrasound in chronic wounds. Ann Plast Surg 2005 Aug;55(2):183-7.

31. Hogsberg T, Bjarnsholt T, Thomsen JS, Kirketerp-Moller K. Success rate of split-thickness skin grafting of chronic venous leg ulcers depends on the presence of pseudomonas aeruginosa: A retrospective study. PLoS One 2011;6(5):e20492.

32. Zhang J, Cao R, Zhang Y, Jia T, Cao Y, Wahlberg E. Differential roles of PDGFR-alpha and PDGFR-beta in angiogenesis and vessel stability. Faseb Journal 2009;23:1-11.

33. Krishnamoorthy L, Harding KG. Specific growth factors and the healing of chronic wounds. J Wound Care 2001;10(5):173-7.

34. Krishnamoorthy L, Morris HL, Harding KG. A dynamic regulator: The role of growth factors in tissue repair. J Wound Care 2001;10(5):173-8.

35. Young SR, Dyson M. The effect of therapeutic ultrasound on angiogenesis. Ultrasound Med Biol 1990;16(3):261-9.

36. Sugita Y, Mizuno S, Nakayama N, Iwaki T, Murakami E, Wang Z, Endoh R, Furuhata H. Nitric oxide generation directly responds to ultrasound exposure. Ultrasound Med Biol 2008 Mar;34(3):487-93.

37. Weichenthal M, Mohr P, Stegmann W, Breitbart EW. Low-frequency ultrasound treatment of chronic venous ulcers. Wound Repair and Regeneration : Official

Publication of the Wound Healing Society [and] the European Tissue Repair Society
1997 Jan-Mar;5(1):18-22.

4 The UltraHeal Randomized Controlled Trial: Effect of 22.5 kHz low frequency contact ultrasound debridement (LFCUD) on lower extremity wound healing for patients followed by a vascular surgery service

4.1 Introduction

Patients with wounds complicated by vascular disease are challenging to heal and face serious health risks. In particular, people who are followed in the vascular surgery wound clinic include those with arterial disease, venous disease and diabetic neuropathic disease, which often occur concurrently. Advancing disease results in complicated wounds which may occur spontaneously as a result of relatively minor injuries or after elective surgical procedures. These wounds may be considered symptoms of advancing vascular disease since healing and immune functions are lessened by advancing vascular pathology.

Peripheral vascular disease (PVD) is surprisingly common and has been described as under-appreciated, under-diagnosed and under-treated.[1] Peripheral arterial disease (PAD) is one form of PVD that involves the vessels that transport blood to the tissues. PAD is estimated to be present in 29% of the people older than 70 years, and in 29% of people between 50 and 69 years old who have diabetes or use tobacco.[2] Risk factors for PAD include diabetes, advancing age, cholesterol issues, hypertension, chronic renal insufficiency, smoking, and family history of cardiovascular disease.[3] Patients with PAD often present with an array of complex medical challenges.

Notably, the presence of diabetes contributes to the development of vascular PVD and increases the risk of the serious outcomes of infection, amputation and death. Atherosclerosis is the underlying pathology of PVD and is the factor responsible for most deaths and morbidity for patients with diabetes.[4] Given an increasing elderly population and what has been described as the economic tsunami of diabetes,[5] the number of people with PAD-related wounds can be expected to increase. In 2010, diabetes affected 7.6% of the Canadian population, and that figure is estimated to grow to 10.8% by 2020.[6] Unfortunately, diabetes also contributes to foot wounds that are the leading cause of non-

traumatic lower limb amputation.[7] To date, there are few conservative treatment alternatives for people with wounds related to PVD.

Once a wound develops, vascular disease impairs wound healing, and alterations in blood flow interfere with the delivery and transport of factors essential for cellular function and healing. Oxygen is particularly important to the performance of cells such as macrophages and fibroblasts cells that critically influence tissue repair.[8] Impaired blood flow also affects the delivery of systemic medications needed to address illness and infections.[9] A poorly perfused wound environment mutes the inflammatory response, increasing excess bacterial growth and increasing the risk of infection.[10] The repair of damaged tissue may be so dysfunctional that cell death occurs. This leads to an accumulation of devitalized tissue (necrosis) in the wound which becomes a food source for bacteria.[19] With poor perfusion, the risk of systemic infection, and even limb amputation, is increased.

Debridement refers to the removal of any necrotic or unhealthy tissue from the wound and wound margins,[11] and is widely accepted as a fundamental treatment required to improve the wound environment and promote healing.[12-14] Although several forms of debridement exist, the complete removal of all necrotic tissue leaving a fresh, bleeding wound base is considered the gold standard for chronic or non-healing wounds as it can restart acute cellular repair processes. Debridement to bleeding tissue also removes old and inactive cells to refresh advancing wound edges.[15, 16]

Sharp debridement is thought to reduce wound bacteria, including those present in biofilms.[17] This is important because biofilm bacteria are present in at least 60% of chronic wounds.[18] Bacteria that develop in protective biofilm colonies are problematic since they are notoriously hard to remove or destroy, are very difficult to detect and are believed to be the root cause of recurring wound infections.[19]

Although debridement to bleeding may have several benefits, these procedures are demanding of clinician time, skills and resources. As such, debridement that induces bleeding is not readily available in many clinical settings.[20] In many areas inadequate funding models may be in place to reimburse physicians for the time required for

debridement. Non-physician clinical staff may have insufficient education or experience, varying levels of competencies, and lack of clear policies which form barriers to performing debridement.[21] Alternative options, such as low frequency contact ultrasound debridement (LFCUD) are of interest as they could improve access to care for the vascular population.

4.1.1   Benefits of Ultrasound

Ultrasound is acoustic energy in the form of sound waves above the range of human hearing (greater than 20,000 kHz).[22] There are several variations of therapeutic ultrasound used to treat wounds including indirect and direct contact methods that may be directed either to the wound or peri-wound area. These applications incorporate a range of frequencies, and formats of ultrasound delivery that may be delivered in continuous or pulsed (intermittent) modes. Higher frequency (MHz) applications are applied using a transducer to the peri-ulcer skin and coupled via aqueous gel, or through a water bath medium. Lower frequency therapeutic systems (kHz) are available that deliver ultrasound by probes or through saline vapor. The lower frequencies produce a longer wavelength which penetrates tissue more deeply and generates less heat compared to higher (MHz) frequencies.[22] Although these various modes are all based on ultrasound energy, it is inappropriate to compare them directly since indications, dosage and delivery methods are not equivalent. The focus of this study is to explore the effect of LFCUD, which is a direct wound contact application of ultrasound that immediately and visibly removes necrotic debris and causes a light bleeding response.

4.1.2   Physiological Effects on Healing

Ultrasound has been shown to promote cellular response, including, fibroblast activity, collagen deposition and new blood vessel growth to induce tissue repair.[23-26] Ultrasound has also been shown to induce blood vessel dilation[27] and improve the quality of granulation tissue.[25, 28] More recently, there is emerging evidence that ultrasound promotes migration mechanisms and promotes cell adhesion, which is necessary for tissue repair to occur.[29] Interestingly, increase of local blood flow, oxygen uptake and tissue regeneration in embryo tissues have all been noted with ultrasound application.[30]

These particular attributes are of interest for a vascular population. In light of these benefits, our study examines the combination of ultrasound and debridement.

4.1.3   Bactericidal Effects

It is also possible that ultrasound may help treat infection since it aids to dismantle, remove and damage bacteria, including those in the aforementioned biofilms. As early as 1980, Schoenbach and Song found that five minutes of low frequency ultrasound (20 kHz) applied indirectly by water bath decreased *Pseudomonas Aeruginosa* bacteria in rats with septic burn wounds.[31] The ultrasound permitted survival and wounds progressed to epithelization and healing. In comparison, 25% of the control group died from complications associated with sepsis, and wounds in this group appeared ulcerated and covered with necrotic eschar.

The effectiveness of antibiotic therapy appears to be enhanced with ultrasound. One study found that pseudomonas biofilm was more susceptible to the antibiotic gentamicin when ultrasound frequencies of between 70 kHz and 10 MHz were applied. The lowest frequency ultrasound (70 kHz) produced the most pronounced synergistic effect of ultrasound and antibiotics.[32] Another study found the same low frequency ultrasound dismantled the protective blocking effect of *Pseudomonas Aeruginosa*, and more than doubled the transport of gentamicin through *Escherichia Coli* biofilms.[33] This synergistic effect has been described between ultrasound and several classes of antibiotics, producing a declining bacterial effect of several orders of magnitude.[34] Interestingly, a similar anti-fungal effect was noted in an in vitro study.[35] Ultrasound is thought to work in part by making biofilm bacteria vulnerable to antibiotic penetration and speeding up bacterial metabolism. This results in a greater uptake and processing of antibiotics by the bacteria, which, in turn, promotes bacterial death.[36]

LFCUD devices incorporate potentially beneficial ultrasound energy to precisely remove debris while causing minimal disturbance of viable tissue.[37] There is emerging evidence that LFCUD may support healing in several challenging wound applications. For example, LFCUD has been used to support skin graft patients with various wound types,[38] and to permit successful closure after prosthetic vascular graft infection.[39]

LFCUD is also thought to shorten the time to secondary closure of infected sterno-cutaneous fistulae in cardiac surgery patients.[40] A similar form of LFCUD has been used to remove plaque biofilm in dentistry while also reducing damage to gum tissue from instrumentation.[41] While early wound studies using LFCUD are promising, the healing response of a population with vascular disease has yet to be explored.

4.1.4   Study Rationale

The vascular population is very susceptible to delayed wound healing and wound infection which can be difficult to eradicate and can delay wound closure. Ineffective wound healing and advancing infection may result in serious consequences that include limb amputations, extended hospital stays and even death.

Debridement is the gold standard to remove necrotic tissue and restart an acute healing response. Ultrasound and debridement may have a synergistic effect to support tissue repair. Ultrasound includes bactericidal effects that potentially disrupt biofilms and boost the effectiveness of antibiotics. However, since LFCUD is a newer technology, it has not yet been studied in a controlled clinical trial to investigate patients with PVD.

4.2   Research Purpose

The purpose of this study is to explore the effect of adding 22.5 kHz LFCUD to usual care on wound healing outcomes of a vascular surgery wound clinic patient population. It is hypothesized that treatment with LFCUD will remove necrotic debris, reduce bacterial burden and stimulate the rate of wound healing in the vascular population. It is also hypothesized that improved healing times will also reduce complication events of infection, amputation and health care system usage in this high-risk population. Using this prospective randomized controlled study design, the following study questions will be addressed:

> 1. Does four weeks of treatment with LFCUD added to usual wound care improve wound healing outcomes compared to a similar group of patients receiving usual care?

2. Does the application of ultrasonic assisted debridement reduce bacterial load and lower clinical signs of infection in a vascular population compared to usual care?

3. Does the application of LFCUD in a high risk population of patients with PVD improve patient outcomes and complications, including, amputations, deaths, emergency room visits and admission days?

4.3   Methods

The study is comprised of a two arm prospective randomized controlled trial with single assessor blinding. The sample size calculation is presented in Appendix 3. A study flow diagram is outlined in Figure 5. The study was approved by both Western University and Ottawa Hospital Research Institute Research Ethics Boards as required (see Appendices 1 and 2). The study was registered at the U.S National Institutes of Health Registry (ClinicalTrials.gov, at identifier NCT01973361). As per local requirements for the administration of LFCUD by an ET RN, the Department of Vascular Surgery approved a Medical Directive and a Delegated Medical Act. The ET RN, who is a specialized nurse with graduate education in wound care, completed the screening process and provided patients with a letter of information that was approved by the Research Ethics Boards.

75

Figure 5 *Patient flow diagram*
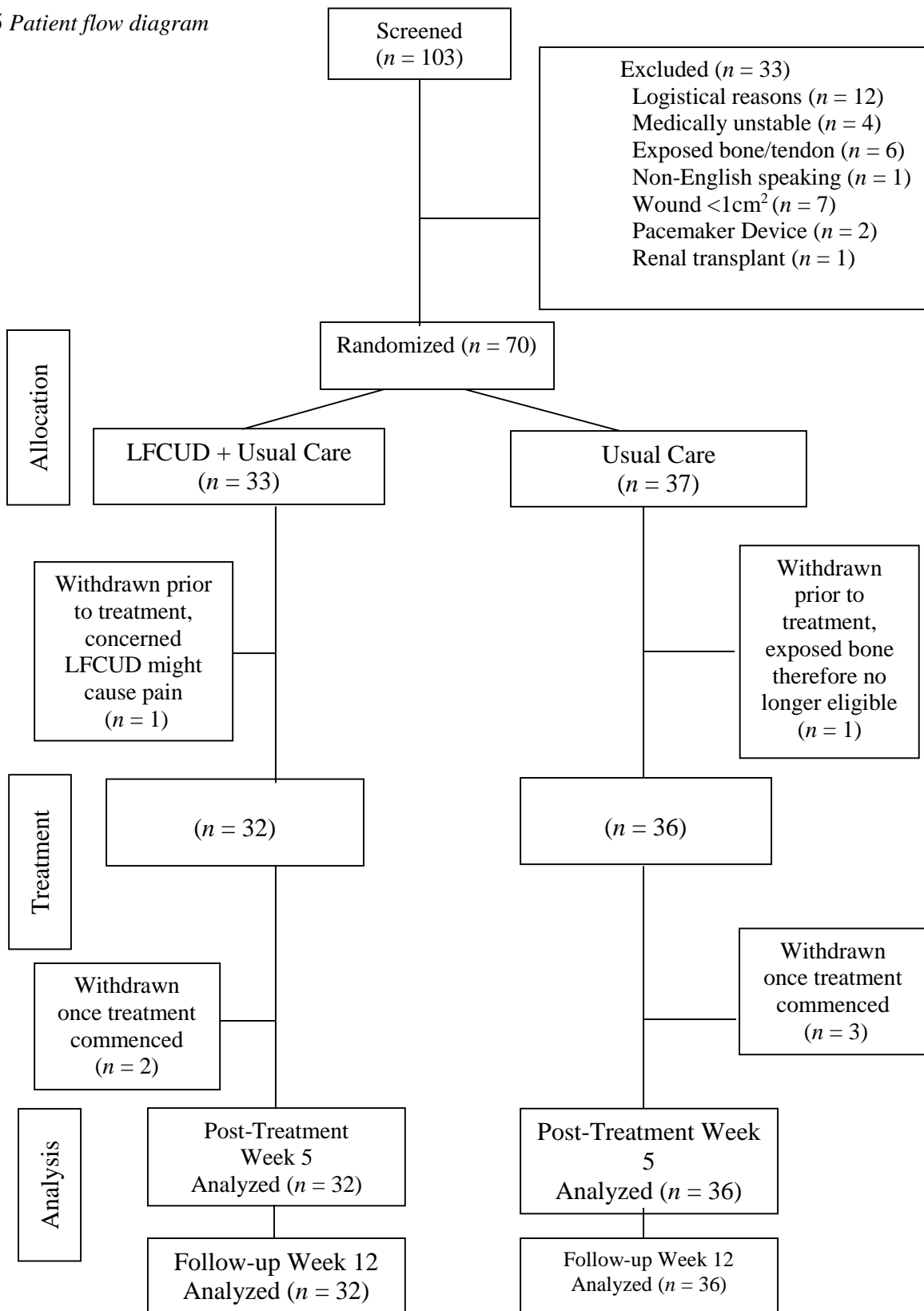
All patients with lower extremity wounds of at least 1cm^2 in size, who were referred to the vascular service of a tertiary care hospital, were approached consecutively by members of the vascular service team and asked if they would participate in the study. Patients were eligible if they were over 18 years of age, and had a full thickness wound below the knee greater than 1cm^2 in surface area. Patients were excluded if they had conditions that prevent healing or if they had a medical condition that could contraindicate ultrasound treatment or could cause undue pain or post procedural bleeding. Patients were also excluded if they were concurrently receiving alternate advanced therapy treatments, had exposed bone or tendon in the wound, or were unwilling to complete the 12 week study protocol. Patients with more than one ulcer were included and all ulcers were treated. However, only one ulcer (the largest area measured at baseline) was followed for study purposes. All patients were screened by a vascular surgeon and the Infectious Diseases (ID) physician to rule out the presence of serious or potentially life or limb threatening ischemia or infections.

4.3.1   Vascular Assessment

All patients underwent an extensive vascular assessment by one of six vascular surgeons. Typically, this included palpation of pedal pulses. If pulses were not appreciated or if there were any other vascular concerns, there was an evaluation of limb perfusion using various methods performed as part of usual care. Vascular tests used in this study included the evaluation of Ankle Brachial Pressure Index (ABPI) or Toe Brachial Index in a clinical vascular laboratory as well as more invasive tests such as computerized tomography angioplasty or digital subtraction angioplasty. As part of the screening process, the vascular surgeon confirmed that vascular status was sufficient for healing and that debridement was not contraindicated. Throughout the study, the vascular surgeons were not blinded so that patients could discuss any concerns and safety could be monitored.

4.3.2   Randomization

All eligible and consenting patients were enrolled and randomly allocated during their baseline visit. Patients were allocated to the low frequency contact ultrasound

debridement (LFCUD) group plus usual care or just usual care (UC) by a concealed computer-generated sequencing method. The computer program stratified patients who were receiving negative pressure wound therapy (NPWT) for equal distribution between groups. Typical of this population, 25 patients (36.8%) were receiving negative pressure wound therapy. The stratification process resulted in 11 (34.4%) patients receiving NPWT in the LFCUD group and 14 (38.9%) in the UC group. This concealed stratification and allocation process was performed by a university-based computer system (Empower, Inc., London, Canada), which was off-site and independent of any of the researchers.

4.3.3   Infectious Diseases Assessment

All patients were assessed for infection by the same ID physician who was blinded to treatment allocation. This physician had previous extensive training and experience in debridement procedure, tissue biopsy and infection analyses which included clinical impression. Additional training was provided regarding entering the data into the computerized database and using the infrared thermometer for taking wound temperature.

As part of this assessment, the ID physician obtained a tissue sample for analysis. Briefly, these samples were obtained by 3mm dermal punch biopsy or scalpel after cleansing with physiological sterile saline. An extensive debridement procedure was then performed to remove all necrotic tissue in the wound surface. This sharp debridement procedure involved cleansing with chlorhexidine 0.05% and completely removing all visible necrotic debris on the wound surface with sterile curette, forcep and/or scalpel. The ID physician determined at this visit whether the wound was infected or not, and antibiotics were prescribed as needed according to the Infectious Diseases Society of America's Practice Guidelines for Skin and Soft Tissue Infection[42] and Diabetic Foot Infection.[43] An accredited medical laboratory then analyzed the samples semi-quantitatively in aerobic and anaerobic conditions.

4.3.4   Initial Assessment

The study timeline is outlined in Figure 6. All patients who were enrolled in the study underwent a comprehensive assessment conducted by the ET RN to identify risk factors

for delayed healing. A patient history form was used to fully describe patient characteristics and identify all co-morbidities known to affect healing (e.g. diabetes and associated complications, any recent or serious illness and/or any recent surgeries). A blood sample was drawn to identify factors that may affect healing (e.g., infection, nutritional markers, and anemia). The ET RN applied the treatments and therefore was not blinded to treatment allocation.

4.3.5   Wound Assessments

Wounds were assessed at each visit by the Registered Practical Nurse assessor (RPN), who is a nurse familiar with wound care. This RPN was trained to photograph and trace the wound, compute planimetry measurements, determine visual analogue scores (VAS) for pain, and enter data into the computer database. This nurse assessor was blinded to treatment allocation and performed assessments prior to any treatments so that visual cues of group allocation were absent.

Figure 6 *Study visit diagram*

Recruitment	Consecutive Referral from Vascular Surgeon
	Screening Vascular Assessment
	Baseline Assessment Week 0 Vascular surgeon evaluation Demographic information and blood test ID physician evaluation: Sharp wound debridement and assessment of wound infection† Wound healing (wound size, pain score, and revPWAT score)††, Randomization (Stratified for NPWT)

Treatment Phase	**1st Treatment (Week 1)** **LFCUD + UC** Wound healing (wound size, pre- and post-treatment pain score)††	**1st Treatment (Week 1)** **UC** Wound healing (wound size, pre- and post-treatment pain score)††
	2nd Treatment (Week 2) **LFCUD + UC** Wound healing (wound size, pre- and post-treatment pain score)††	**2nd Treatment (Week 2)** **UC** Wound healing (wound size, pre- and post-treatment pain score)††
	3rd Treatment (Week 3) **LFCUD + UC** Wound healing (wound size, pre- and post-treatment pain score)††	**3rd Treatment (Week 3)** **UC** Wound healing (wound size, pre- and post-treatment pain score)††
	4th Treatment (Week 4) **LFCUD + UC** Wound healing (wound size, pre- and post-treatment pain score)††	**4th Treatment (Week 4)** **UC** Wound healing (wound size, pre- and post-treatment pain score)††

Follow-up	**Post-treatment Week 5 (1 week after last treatment)** Wound healing (wound size, pain score, and revPWAT score)†† ID physician evaluation: Sharp wound debridement and assessment of wound infection†
	Post-treatment Week 12 Wound measurement, pain score and wound photography††

†Wound infection assessed via semi-quantitative analysis of tissue sample culture and visual wound inspection
††Wound measurement, photography and pre-treatment pain scores obtained and documented by blinded nurse assessor. Abbreviations: LFCUD = Low Frequency Contact Ultrasound Debridement; UC = Usual Care; revPWAT = Revised Photographic Wound Assessment Tool [45]

4.3.6   Usual Care (UC)

All patients in the control group, known as usual care (UC), continued to receive routine wound care on the same visit frequency as the treatment group. In the vascular wound clinic, usual wound care includes removing/observing the dressing, cleansing the wound with chlorhexidine 0.05%, performing a conservative sharp debridement of any necrotic debris from the wound base, pairing the periwound callus, and replacing with a dressing. For the study, patients in both groups received a consistent silver alginate dressing (Silvercel®, Acelity, San Antonio, TX)

4.3.7   Negative Pressure Wound Therapy (NPWT)

For patients with cavity wounds that extend adjacent to deep structures or with post-operative cavity defects, usual care includes the use of NPWT as the wound dressing. For these patients, one of two NPWT devices was used (VAC®, Acelity, Antonio, TX, or Renasys®, Smith & Nephew, London, UK). NPWT was set at intermittent suction to support granulation response unless wound structural support was needed or the seal was problematic in which case continuous suction was selected. To reduce the influence of this active therapy on study outcomes, patients were evenly distributed between groups by computer stratification during the process of random allocation.

4.3.8   Low Frequency Contact Ultrasound Debridement (LFCUD)

In addition to routine wound care, all patients assigned to the treatment group received high intensity, low frequency (22.5 kHz) contact continuous ultrasonic debridement (Sonic One®, kindly supplied by, Misonix, Farmingdale, NY. [See Appendix 4]). The hand-piece and probe were sterilized in the central processing department by autoclave as per manufacturer's instructions. Treatment continued until light bleeding occurred and all necrotic tissue was removed. The treatment was applied by placing a sterile probe in direct contact with the wound bed. The Sonic One® LFCUD device produces a 22.5 kHz ultrasonic frequency at amplitude settings of one to five through a piezoelectric crystal in the hand piece which, in turn, transfers the acoustic energy into the tissue via direct contact with the saline medium. The saline irrigation rate was set at the lowest setting. The probe type was selected based on patient pain sensation, wound shape and tissue

adherence. The gold (standard) probe was the usual selection while the green (gentle) probe was used for patients with any described discomfort or preference, the blue (tunnel) probe was used for wounds with undermined areas, and the magenta (aggressive) probe was used for very adherent necrotic debris.

4.3.9   Treatments

All treatments were administered under medically aseptic conditions in the combined vascular surgery in-patient and wound clinic setting. The probe type was recorded at all sessions, and all hand-held probe attachment components were autoclaved at the hospital instrument processing centre prior to every treatment and returned in sterile packaging for the next use. Personal protective equipment, including a face visor, was used for the aerosol generating procedure[22] as per local infection control practices. Local anesthesia was available by injection prior to the LFCUD procedure. Each patient was informed at the initial visit and in the study letter of information that local anaesthesia was available on request if they felt uncomfortable. Additionally, patients were reminded that local anaesthesia was available if they appeared uncomfortable at any point during their visit.

Treatment was applied until necrotic debris was removed and light bleeding achieved at each of the four weekly treatments by the ET RN who had received training and certification on use of the device from the company representatives (Misonix, Farmingdale, NY).

4.3.9.1   Wound Size

Wound surface area was recorded by the RPN who traced the wound perimeter three times onto a multi-layer acetate designed for single patient use (Visitrak, Smith & Nephew, London, UK). All tracings at the treatment visits were taken after cleansing the wound and before debridement at treatment visits (Week 1 to Week 4)so that visual cues to treatment allocation were not present. Tracings were obtained after the ID physician debridements at Week 0 and Week 5 so that necrotic debris did not obscure the wound edges. Tracings were digitized using the previously validated Visitrak planimetry system[44] (Smith & Nephew, London, UK), and the mean of three tracings was calculated to determine the area in centimetres squared (cm^2) with minimal error of measurement.

4.3.9.2   Wound Closure

Wound closure was determined by the RPN by a wound measurement of $0cm^2$ and absence of exudate, which was subsequently confirmed by the ET RN.

4.3.9.3   Wound Appearance

Using a Canon Rebel 300D EOS, 8 megapixel resolution, 60mm macro lens digital camera with a ring flash, automatic focus photographic images were taken to assess wound appearance. In order to assess the wounds using the rev Photographic Wound Assessment Tool (revPWAT), the patient was positioned in a similar fashion in a room that has the same examination lighting. The wound dressing was removed and then a ruler was placed against the skin near the wound and labelled with the subject ID number and the date the photo was taken. Digital images were captured after wound cleansing but before any debridement (to prevent visual cues of group allocation) at the beginning of treatment sessions (Weeks 1-4). The digital image was assigned a de-identified number that was not linked to the patient or the session. In this way, the single assessor who evaluated all photographs, which were mixed and assessed in large groups, did not know who had received LFCUD or the sequence of visit when the photo was taken.

Each photo was assessed using by using a validated scoring tool called the revPWAT,.[45] which is a pen and paper tool that is used to systematically assess eight different characteristics of the wound base, edges and peri-ulcer skin using a photograph of the wound and peri-ulcer skin. Each of the eight domains of the revPWAT is ranked on a four point scale with zero representing a closed wound and 32 signaling the highest possible score.

4.3.9.4   Pain

Pain was measured using the validated Visual Analogue Scale (VAS).[46] This involved asking the patient to identify on a 100mm ruler with slide indicator, the level of wound pain experienced with 0mm = no pain, and 100mm representing the worst pain imaginable. This question was asked by the blinded RPN assessor at the beginning of every visit. The VAS pain score was re-evaluated immediately after treatment by the ET

RN who did all the debridement procedures. Each assessor recorded their results independently into the computer database.

4.3.9.5   Complications

The number and type of hospital admissions, amputations and deaths were recorded and reported to the relevant ethics boards.

4.3.10 Statistical Analysis

Data were analyzed using SPSS version 23.0 (SPSS, IBM Corporation, Armonk, NY). Baseline characteristics were compared between groups by X^2 Test for categorical data and Student's t-Test for continuous variables. The wound healing outcome data of change in wound surface area (cm^2) and total revPWAT scores were calculated using a covariate analysis (ANCOVA) to adjust for baseline. A two-sided p- value of <0.05 was considered statistically significant and all patients were analyzed in the group to which they were allocated. All missing data, which included patients who withdrew once treatment started, were imputed with the last outcome carried forward.

4.4 Results

One hundred-and-three patients were recruited for the study from December 2013 until May 2015; however 33 were screened out during the initial assessment. Of the 33 patients who were screened out, 12 declined to participate for multiple reasons (e.g., parking costs and frequency of visits); seven had wounds that were smaller than $1cm^2$ in area; six had an exposed bone or tendon visible in the wound; four were medically unstable; two had pacemaker devices in situ; one did not speak English; and one had a previous renal transplant (see Figure 5).

A total of 70 patients were randomly assigned to either the experimental group (LFCUD) or the control group (UC). Of the 70 patients, two patients withdrew at the initial visit (after the randomization had occurred), one patient chose not to continue in the study (due to concerns about the potential treatment pain), and the other patient originally assigned to the UC group had exposed bone after initial sharp debridement and therefore was no longer eligible. In total, 68 patients received one of the two treatment

interventions with 32 patients allocated to the LFCUD group and 36 patients to the UC group.

Of the 68 patients followed in the study, five patients (three in UC group and two in LFCUD group) withdrew later in the treatment phase. However, they had attended most treatment visits and were equally distributed between groups. These patients withdrew for: practical reasons such as parking costs and frequency of visits ($n = 2$, UC group); medical issues including medical decline to palliative status ($n = 1$, LFCUD group); infection requiring toe amputation ($n = 1$, UC group); and change of treatment plan initiated by homecare nurse ($n = 1$, LFCUD group). None withdrew as result of the treatments. In addition to those who withdrew, there were ten patients who missed one treatment visit (including eight patients receiving UC treatment and two patients receiving LFCUD) due to practicality of visit reasons. Including both withdrawals and missed visits, a total of 16 patients (23.5%) did not return for evaluation at the 12 week follow-up visit. All data were included and missing information was imputed with the last outcome carried forward to provide an intention-to-treat analysis for conservative estimation of treatment effect.

Patient Characteristics

For information on all patients in the study, see Table 8. Of the patients in the study, most were male and the majority had evidence of significant vascular disease (having undergone either a previous angioplasty or bypass procedure or a major or distal amputation). Patients in the LFCUD group had a longer mean duration of diabetes, longer wound duration, lower hemoglobin and fewer bypass graft procedures, but these differences were not statistically significant. Of the 47 patients with diabetes, the disease was advanced with mean duration of 20.5 years. Twenty-three patients in the LFCUD group had diabetes (48.9%), as did 24 patients in the UC group (51.1%). There were more patients in the LFCUD group who had undergone previous trans-metatarsal or digital amputation procedures, and this was statistically significant ($X^2_{(1)} = 5.88$, $p = 0.015$). Mean ankle brachial pressure index (ABPI) was significantly lower in the LFCUD group (0.83, $p = 0.033$), but this was calculated from an incomplete sample (n

25) since many patients had other forms of vascular testing. Additionally, a greater proportion of the patients in the UC group had a wound infection when they were enrolled (33%) than those in the LFCUD group (19%), but this difference was not significant between groups ($X^2_{(1)} = 1.85$, $p = 0.174$).

86

Table 8 *Demographics of Patients in LFCUD and UC Groups*

	Total Sample	LFCUD	UC	p - value
	($n = 68$)	($n =32$)	($n = 36$)	
Age (Years)	65.71 ±10.49	67.22 ± 11.49	64.36 ±9.50	$p = 0.265$
Male	76.5 (52)	75.0 (24)	77.8 (28)	$p = 0.788$
BMI	26.43 ± 5.61	25.1 ± 4.84	27.61 ± 6.05	$p = 0.065$
Initial Wound Area (cm^2)	14.64 ± 20.25	13.55 ± 23.35	15.64 ± 17.31	$p = 0.675$
Wound Duration (Months) ($n= 76$)	14.75 ± 27.32	17.06 ±36.85	12.57 ± 13.57	$p = 0.675$
Wound infection at baseline visit	25.5 (18)	18.8 (6)	33.3 (12)	$p = 0.174$
Wound Location				
Toe/ Toe Amputation Site	16.2(11)	15.6 (5)	16.7 (6)	$p = 0.907$
Mid-Foot/Plantar	27.9 (19)	31.3 (10)	25.0 (9)	$p = 0.566$
Heel	20.6 (14)	18.8 (6)	22.2 (8)	$p = 0.724$
Malleolar	7.4 (5)	9.4 (3)	5.6 (2)	$p = 0.660$
Leg	27.9 (19)	25.0 (8)	30.6 (11)	$p = 0.610$
Diabetes	69.1(47)	71.9(23)	66.7 (24)	$p = 0.643$
Duration Diabetes (Years) ($n= 45$)	20.56 ±12.30	22.23 ± 13.72	18.96 ±10.85	$p = 0.379$
Anti-coagulant Medication	64.7 (44)	65.6 (21)	63.9 (23)	$p = 0.881$
Antibiotic Medication	64.7 (44)	62.5 (20)	66.7 (24)	$p = 0.720$
Hemoglobin ($n = 77$)	114.21 ± 18.99	112.2 ± 17.33	116.1 ±20.47	$p = 0.409$
HbA1C	7.61 ±1.45	7.59 ± 1.24	7.63 ± 1.65	$p = 0.933$
Albumin ($n = 70$)	31.4 ±5.68	30.9 ± 6.67	31.84 ± 4.65	$p = 0.541$
NPWT	36.8 (25)	34.4 (11)	38.9 (14)	$p = 0.700$
ABPI ($n = 25$)	0.92 ± 2.34	0.83 ± 0.19	1.03 ± 0.25	$p = 0.033^*$
Arterial Insufficiency	**$n = 60$**	**$n = 29$**	**$n = 31$**	
Pedal Pulse Present [†]	18.3 (11)	13.8 (4)	22.6 (7)	$p = 0.416$
Angioplasty	55.0(33)	55.2 (16)	54.8 (17)	$p = 0.979$
Bypass Graft	33.3 (20)	27.6 (8)	38.7 (12)	$p = 0.361$
Prior Amputation:				
Major: (Transtibial/ Transfemoral)	11.7 (7)	6.9 (2)	16.1 (5)	$p = 0.426$
Distal: (Pedal/Digital)	30.0 (18)	44.8 (13)	16.1 (5)	$p = 0.015^{**}$

Unless otherwise stated values expressed as mean ± Standard Deviation with range in parentheses, or percentage (n). Abbreviations: LFCUD = Low Frequency (22.5 kHz) Contact Ultrasound Debridement; UC = Usual Care; BMI = Body Mass Index; HbA1C = Glycated Hemoglobin; NPWT = Receiving Negative Pressure Wound Therapy to wound.

[†] Pedal Pulse Palpable = Dorsalis Pedis and/or Posterior Tibial pedal pulse palpable in affected limb.

*Statistically significant difference but partial sample only: $t_{(23)=}$ -2.270, $p = 0.033$, $n = 25$.

**Statistically significant difference: $X^2{}_{(1)} = 5.88$, $p = 0.015$
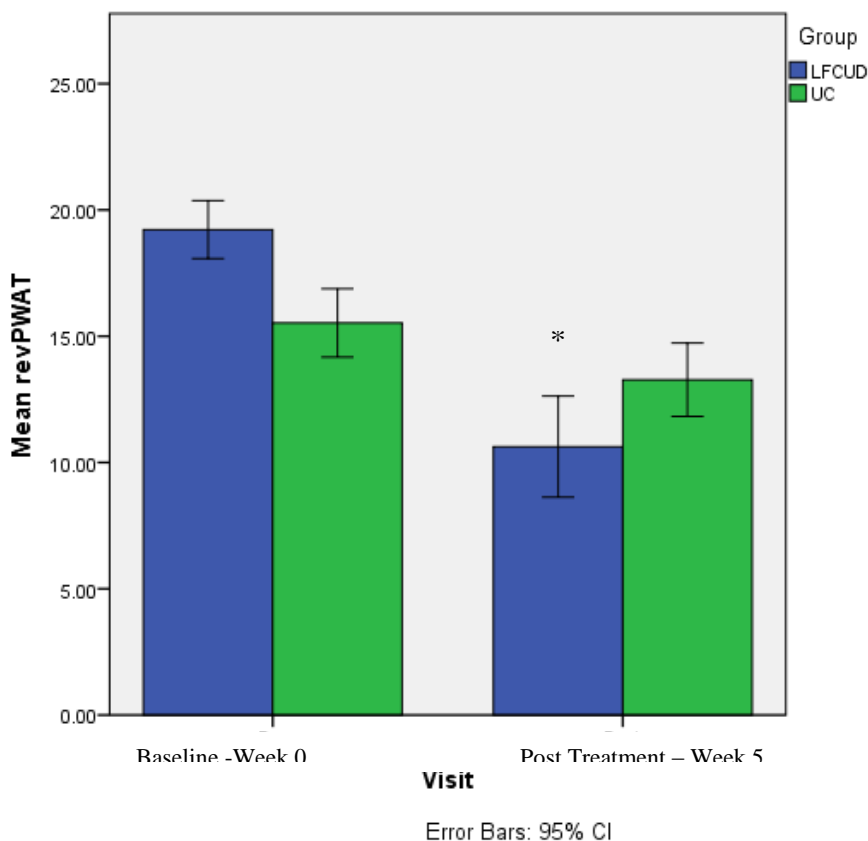
4.4.1   Ultrasound Debridement Treatments

LFCUD was consistently applied in continuous mode at amplitude five with physiological saline flow at 20% until surface debris was removed and light bleeding response was obtained. The average length of LFCUD treatment was two minutes and 59 seconds (with a range from 19 seconds to six minutes). Most patients were treated with the green (gentle) probe (44.0 % and the gold (regular) probe (40.8%). Two patients received a total of six treatment episodes with the blue (tunnel shape) probe due to wound shape (4.8%), and one patient received the magenta (aggressive) probe at two visits (1.6%) to treat adherent slough.

4.4.2   Wound Appearance (revPWAT score)

The LFCUD group demonstrated a significantly greater improvement in wound appearance (M = 7.34, 5.81 – 8.88, 95% CI) than the UC group (M = 2.98, 1.36 – 4.60, 95% CI). Put another way, there was improved wound tissue appearance in the LFCUD group by 4.36 points (2.07 – 6.66, 95% CI). This significant difference between groups was found after controlling for baseline revPWAT score as a covariate ($p = $ <0.01, Figure 7).

Figure 7 *Change in wound appearance (week 0 – week 5)*


Error Bars: 95% CI

Abbreviations: LFCUD = Low Frequency Contact Ultrasound Debridement; UC = Usual Care; revPWAT = revised Photographic Wound Assessment Tool. * Change in wound appearance was significantly greater in the LFCUD group post-treatment (week 5) after controlling for baseline revPWAT score (p = <0.01)

4.4.3   Wound Surface Area (WSA)

There was progressive trend in decreasing wound surface area (WSA) over the four weekly LFCUD treatments (see Figure 8), which was significant (p = <0.01) but this trend was not found to be significant for patients in the UC group (p = 0.935). However, the mean difference in percentage WSA reduction between groups was not statistically significant (p = 0.485) as calculated using the WSA baseline as a covariate in the analysis of covariance (ANCOVA) test. This mean %WSA reduction post-treatments (Week 5) was greater (31.63%, 3.54 – 59.70, 95% CI) in the LFCUD group than in the UC group (18.06%, -8.42 – 44.54, 95% CI), but not significant. Two patients in the LFCUD group had closed wounds by Week 5, and two others had been transferred to plastic surgery for

skin grafting. None of the UC group had closed wounds at Week 5 or were ready for skin grafting.

Figure 8 *Percentage change in wound size from baseline*
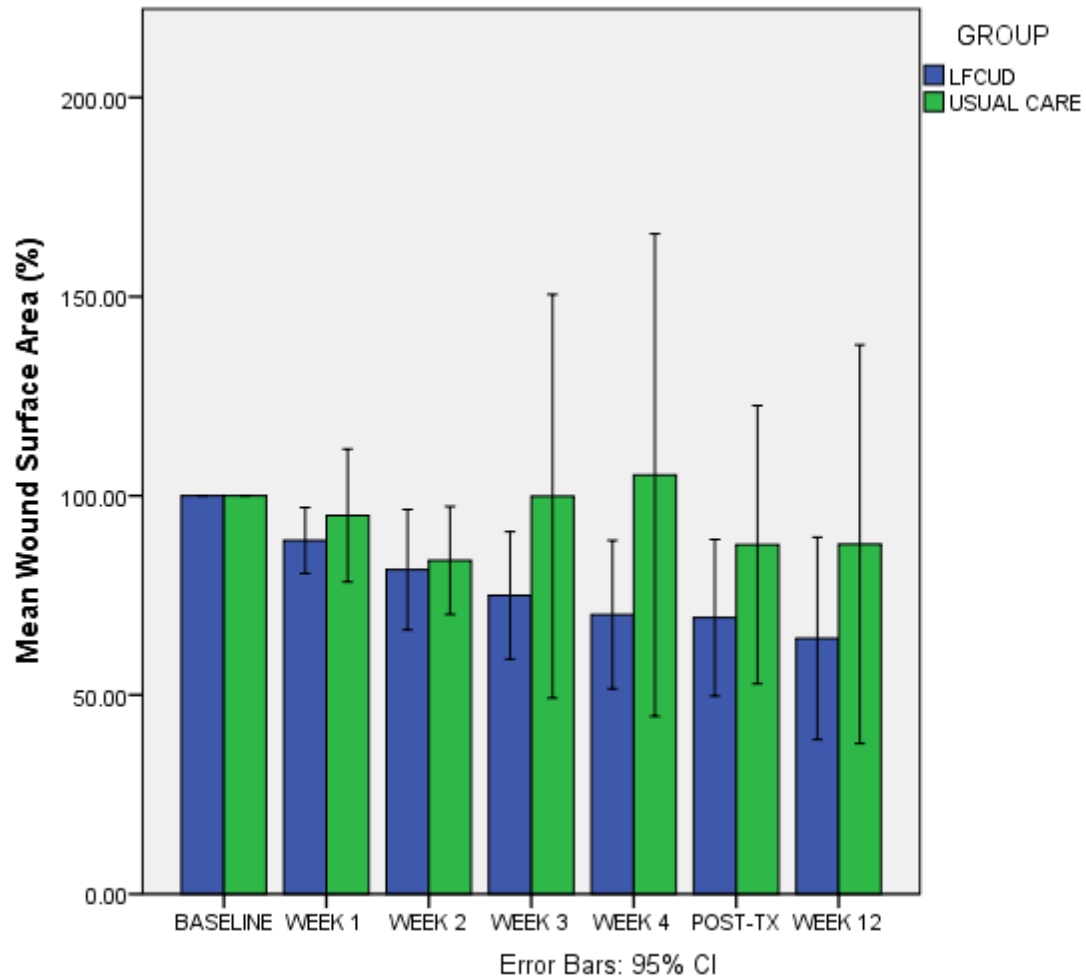
Abbreviation: LFCUD = Low Frequency Contact Ultrasound Debridement, UC = Usual Care.
(Baseline wound surface area (WSA) = 100%). Linear trend in WSA reduction was significant (p = <0.01) for LFCUD group during treatment period (from week 0 – 5) but not significant for UC p = (0.935) as adjusted not assuming equal variances

4.4.4   Infection

Both the experimental LFCUD group and the UC group had a reduced number of wound infections after the treatment phase. From the baseline to the post-treatment visit, the wound infection rate in the LFCUD group decreased from 18.8% to 12.5%. Similarly, the

wound infection rate in the UC group reduced from 33.3% to 11.1%. That said, this change was not significant between groups ($p = 0.317$, Wilcoxian Signed Ranks Test). During the four weekly treatment visits for LFCUD or UC, six patients developed a new infection.

Patients reported a decrease in pain after LFCUD at every visit, which was statistically significant. The difference in mean pain scores during the four treatment visits ranged between 9.3mm (3.5 – 15.1, $p = 0.003$, 95% CI) at Week 3, and 16.6mm (9.0 – 24.2, $p =$ <0.001, 95% CI) at Week 2. However the UC group had a significant difference only at Week 1 of 6.11mm (0.152 – 12.1, $p = 0.045$, 95% CI). Patients receiving LFCUD commonly reported a sensation of vibration rather than pain and frequently noted that they were surprised since they had anticipated pain at the first application. Two patients requested a local anaesthetic at initial treatments, which was administered by the ID physician or vascular physician prior to treatment. Both of these patients did not require a local anaesthetic for other treatments since wounds improved and they became more comfortable.

4.4.5   Adverse Events

Adverse events were rare, and none were related to a treatment. In total there were 12 adverse events, including six in the LFCUD group and six in the UC group. In the LFCUD group, there were: two new infections; one arterial occlusion requiring admission to hospital for angioplasty; one dressing reaction; one burn injury from a house fire; and one death which occurred several weeks after treatment had concluded. In the UC group, four patients developed new wound infections; one patient developed an infection requiring toe amputation; and one patient developed a medication-related rash.

In total, six patients (two in the LFCUD group and four in the UC group) were diagnosed with new infections during the study. This included one emergency room admission and toe amputation related to a new wound infection in a UC group patient. As per the REB requirements, all complications were reported to the Data Safety Monitoring Board, and were later deemed unrelated to the device by both the blinded ID physician and the unblinded vascular surgeons.

Patient experience during ultrasound debridement was generally favourable. Interestingly, there was minimal bleeding with ultrasound debridement despite most patients receiving anticoagulant therapy. Many patients in the LFCUD group reported they believed the treatment was improving their wound, and one patient reported restored sensation to his forefoot that had been absent for an extended time. It is possible that local angiogenesis was increased as a result of the ultrasound, providing improved perfusion and nerve function. No patients perceived wound deterioration from the treatment.

4.4.5.1   Follow Up: 12 Week WSA

Of the 47 patients who attended the 12 week follow up visit, the mean WSA was smaller in the LFCUD group at 4.83cm^2 (-10.673 – 1.85, 95% CI) than in the UC group, where the mean WSA was 9.25cm^2 (-10.56 – 1.74, 95% CI). However, this difference in WSA was not statistically significant ($t_{(46)}$ = -1.42, p= 0.163). Put another way, 18.4% of the LFCUD group attained a wound size of less than 6cm^2 compared to 36% of the UC group. Of the patients assessed at 12 weeks, seven (24.1%) in the LFCUD group had closed wounds compared to three (9.1%) in the UC group.

The results of this study demonstrate that four weekly LFCUD treatments resulted in a significantly improved wound appearance as well as a WSA reduction trend in a vascular population with challenging wounds. Importantly, LFCUD was well tolerated, did not induce additional pain, and did not bring about any treatment-related adverse events. LFCUD was feasible to apply by the nurse in a vascular wound clinic. LFCUD successfully facilitated the removal of necrotic debris without the need for a surgical team or operating room.

Although the LFCUD group had a greater change in mean WSA than the UC group, this finding was not statistically significant. However, we did find a significant trend in WSA reduction that was not noted in the UC group. In our pilot study we were pleased with achieving a 39.4% mean WSA reduction after four weekly treatments since a vascular population may have healing challenges. In the current study, although we achieved a similar 31.6% reduction mean WSA with LFCUD, some variability of wound

progression in the UC group may have reduced the ability to detect a significant mean difference in WSA. The finding that the mean WSA for UC (18.06%) was similar to our anticipated rate of healing for non-healers, and was similar to the sample size calculation estimated at 19.4% supports this view. However, since our findings did not achieve statistical significance, we cannot draw conclusions on the difference in WSA from this trial.

A previous study showed that weekly LFCUD treatments (25kHz) applied to diabetic foot ulcers with osteomyelitis accelerated wound healing when compared to UC group at two and three month time-points.[47] When compared to our study, it is possible that a single wound type with an extended period of time yielded these effects since those patients received over twice as many treatments. As such, it could be that the four treatments administered in our study were insufficient in number to show a significant effect. Additional treatment time could improve wound contraction. That said, treatment was given until light bleeding was achieved, and extended treatment times could deepen the wound.

Based on prior studies, we believed our schedule would be feasible and reasonable to attain a treatment effect. For example, one study[51] found seven out of 19 patients healed chronic leg ulcers with a LFCUD treatment every two to three weeks and a total average of five or six treatments. Another study used the same schedule, permitting some wounds to be ready for skin grafting.[38] The ideal timing between LFCUD treatments has yet to be determined. Even though it is known that an increase of conventional sharp debridement sessions promotes hastened wound contraction,[13] there is no consensus on the best schedule with that method to achieve the greatest effect. It is unlikely that it would be feasible in our population with advancing vascular disease to increase the number of treatments per week or the number of weeks the treatment is applied. Given that treatment schedules were varied in previous LFCUD trials, we designed our visits to fit usual and reasonable clinic scheduling and to reflect the range of similar studies.

Our findings show that applying LFCUD results in a significant improvement of wound appearance (Figure 7). Furthermore, our wound assessment occurred one week after

treatment and therefore the improved wound appearance effect was lasting. This change of wound appearance was largely due to less necrotic tissue and improved granulation tissue appearance. Since this is the first study to evaluate the effect of LFCUD on wound appearance using the revPWAT validated wound assessment tool, we cannot determine if this effect is consistent with other populations. However, Herberger and colleagues reported a subjective improvement in wound appearance, positing LFCUD treatment is as equally efficient as surgical wound debridement.[52] For a complicated vascular population, it is possible that wound depth and severity influences the speed of wound contraction. It is also recognized that, as unhealthy tissue is removed, some increase in wound size is expected and viewed as positive progress.[48] Additionally, since wound depth can be difficult to measure consistently, evaluating the wound appearance may be the best early measure of progress for more extensive wounds. The improvement of wound appearance among LFCUD patients was not surprising as this finding reflects the results of previous in vitro and animal studies.[28, 49]

This is the first time that LFCUD has been shown to produce a significant improvement in wound healing outcomes for patients with PVD. Our results of early wound improvement are consistent with a previous study which examined diabetic foot ulcers with osteomyelitis.[47] However, results in that study are difficult to interpret over extended time because of possible variations in antibiotic treatments common to that population.

Our data do not confirm that LFCUD has an effect to diminish the recurrence of infection or biofilms. However it is widely accepted that biofilms form more readily on inert or dead material,[54] and since wound appearance was improved the environment was rendered less conducive for bacterial growth after treatments. It is also possible that the LFCUD administration time was too short or too infrequent to note a significant effect.

Additionally, as we have previously explored in Chapter 2 of this document, signs of bacterial burden and infection are difficult to detect in this population. Furthermore, patients were receiving a variety of antibiotic therapies and were at various stages within that therapy. Given these circumstances, it could be that it was not possible to isolate the

effect of LFCUD within the "noise" of medical therapies. Further research is warranted as emerging and more precise methods of wound infection diagnosis become available.

Surprisingly, we found pain was significantly reduced in the LFCUD group after each debridement experience. In our experience, sharp debridement procedures are frequently painful which is unpleasant for the patient, and often require local anaesthetic. However, patients receiving LFCUD commonly reported a sensation of vibration rather than pain and frequently noted that they were surprised since they had anticipated pain at the first application. This is encouraging since anxiety and stress affect healing.[50] Our findings again reflect those of previous researchers, who have found patients with chronic leg ulcers treated with LFCUD reported little pain,[51] and that LFCUD was less painful than surgical debridement.[52] We were pleased to note that LFCUD induced little discomfort, and did not worsen the baseline pain status. This finding confirms our previous results from the pilot study, and suggests that the LFCUD approach may be less painful than current usual practice.

The patient group in our study was different than previous populations as we targeted patients with vascular disease, who are often excluded from wound trials. For various reasons, our population had problems with tissue perfusion, which is typical of a vascular surgery clinic population. With that, our sample is representative of patients that typically require acute care vascular services for complex and hard-to-heal wounds. It is possible that the significantly higher number of patients who had experienced previous toe amputations in the LFCUD group may represent a subset of patients with specific vascular pathology and that these wounds may reflect a worsening distal vessel disease. While this was expected to cast a conservative effect on our results, we were extremely pleased to find that no wounds became worse, and that we achieved a 31.6% reduction in four weeks, which is considered impressive for a vascular population. It is not surprising that there was one death and one toe amputation given the degree of illness in this population.

A major benefit of LFCUD is the ease of application by a non-physician. Debridement requires specific knowledge and skills, and carries inherent risks which prohibit the

availability of the procedure in many areas of practice. Furthermore, there are gaps in education delivery, and policy, with few definitive protocols available.[21] In a supported environment, the availability of nurse-applied LFCUD allowed for improved access to care. This benefit was also described for the nurse-applied LFCUD treatment of a peri-stomal wound, which allowed for an earlier skin graft and decreased hospital stay.[53]

4.4.6   Limitations

There were several limitations in this study. Patients were recruited with a variety of wound etiologies related to vascular disease. While it may have been preferable to restrict the sample to a particular wound etiology, it was considered unlikely that sufficient participants could be recruited within the catchment area to permit analysis. We were pleased that our sample was representative of a typical vascular surgery department population, which was clinically relevant.

We were unable to blind the participants. It is possible that the participants may have adjusted their response to pain questioning based on attempting to support the study, or in anticipation of a wound treatment that induces bleeding. The two nurses asked about pain to encourage open dialogue about the pain experience.

Additionally, the ET RN could not be blinded in order to provide the LFCUD treatments, which is a common problem for wound trials. That said, every effort to reduce bias was implemented, including the blinding of the RPN for wound measurements and the ID physician for consistent antibiotic treatment across groups. While the revPWAT scores were calculated by the ET RN, the analysis was performed using unidentifiable photos. The photos were not identifiable by time or by the patient, and—when analyzed—it was not possible to recall sequential scores in relation to visit number. Additionally, as recruitment continued, the ET RN could no longer recall the group allocation of many patients which had to be verified at each clinic visit.

The use of silver alginate dressings or NPWT may have affected healing outcomes. The antimicrobial properties of silver may have prevented the usual re-growth of bacteria in the wounds and so reduced our ability to detect the effects on bacteria or biofilm

reduction. However, since systemic antimicrobial delivery may be further diminished by PVD and silver is frequently considered as usual care for this population, we considered it unethical to withdraw all local antimicrobial therapy during the trial. Therefore we decided to provide every patient with the same dressing protocol.

We chose to administer four weekly treatments, which may have been insufficient to detect a significant effect. During the treatment phase of the fewer than 15% of patients failed to attend their visits, and this was within our sample size calculation limitations. However, even at 12 weeks, the follow-up was not so well attended, which speaks to the difficulties of gathering evidence of long term treatment effects in a population with challenging health needs. We acknowledge that the long term follow-up of patients with multiple health problems can be problematic since there is a high frequency of medical appointments with different specialists, and the frequency of travel becomes tiring with advancing disease states. Also, the follow-up period was not long enough to determine whether wound closure would be more likely with LFCUD treatments.

It is likely that overall there were fewer incidences of wound deterioration than might be expected due to the intensive visit schedule with examination by an expert team. However, since treatment and control groups were evaluated by the same team, it is expected that this would have a moderating effect on the results. Also, since LFCUD was not combined with other advanced therapies that may be commonly used in an expert clinic, the full clinical potential of the treatment may not have been realized.

4.5   Conclusion

We found that four weekly LFCUD treatments added to usual care significantly improved wound appearance in a vascular population (as noted by revPWAT score) and was well tolerated without adverse events. Our results were inconclusive whether LFCUD enhances WSA contraction or improves time to complete wound healing. However, since there was a significant trend in WSA reduction with LFCUD, this aspect warrants future research since it is possible that a consistent healing trajectory may yield more successful wound closure.

We could not determine if four weekly LFCUD treatments significantly reduced bacterial load or lowered clinical signs of infection in a vascular population. We were unable to determine if LFCUD could reduce the number of whole patient outcomes at 12 weeks such as amputations, deaths, ER visits or admission days since there were few in this study. Importantly, LFCUD did not cause wounds to deteriorate, and did not increase the number of adverse events.

4.5.1   Clinical Implications and Future Research

Our study found LFCUD is a feasible and well-tolerated method of debridement for a vulnerable population with vascular disease, who are often excluded from research and have few treatment options. The improvement of wound appearance is a clinically important finding since challenging patients may then become eligible for wound closure by skin graft. Additionally, LFCUD may improve access to debridement procedures since it was found to be a well-tolerated, was without adverse events, and was feasible to offer without extensive surgical personnel or resources.

Importantly, we believe that high risk populations with vascular diseases should be included in future LFCUD trials. Future research to determine if better healing outcomes or reduced infection may be attained for specific wound types with increased applications in combination with other therapies and over an extended time. Our study suggests that it is a safe and efficient method of wound preparation which is well-tolerated and feasible to apply by the ET RN in a tertiary care vascular wound clinic.

References

1. Rooke TW, Hirsch AT, Misra S, Sidawy AN, Beckman JA, Findeiss LK, Golzarian J, Gornik HL, Halperin JL, Jaff MR, et al. 2011 ACCF/AHA focused update of the guideline for the management of patients with peripheral artery disease (updating the 2005 guideline): A report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines: Developed in collaboration with the Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society for Vascular Medicine, and Society for Vascular Surgery. Catheter Cardiovasc Interv 2012 Mar 1;79(4):501-31.

2. Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, Krook SH, Hunninghake DB, Comerota AJ, Walsh ME, et al. Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA 2001 Sep 19;286(11):1317-24.

3. Bonham P, Flemister BG, Goldberg M, Crawford PE, Johnson JJ, Varnado MF. What's new in lower-extremity arterial disease? WOCN's 2008 Clinical Practice Guideline. Journal of Wound Ostomy and Continence Nursing 2009;36(1):37-44.

4. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: Epidemiology, pathophysiology, and management. JAMA : The Journal of the American Medical Association 2002;287(19):2570-81.

5. Association. CD. An economic tsunami: The cost of diabetes in Canada. 2009.

6. Canadian Diabetes Association and Diabete Quebec. Diabetes: Canada at the tipping point. Charting a new path. 2011;5:1-58.

7. Apelqvist J, Larsson J. What is the most effective way to reduce incidence of amputation in the diabetic foot? Diabetes Metab Res Rev 2000 Sep-Oct;16 Suppl 1:S75-83.

8. Sussman C, Bates-Jensen BM. Wound healing and physiology: Acute and chronic. In: C. Sussman, B. M. Bates-Jensen, editors. Philadelphia, PA: Lippincott, Williams & Williams; 2007. ID: 3751; M1: Book, Section.

9. Zammit MC, Fiorentino L, Cassar K, Azzopardi LM, LaFerla G. Factors affecting gentamicin penetration in lower extremity ischemic tissues with ulcers. International Journal of Lower Extremity Wounds 2011;10(3):130-7.

10. Attinger CE, Evans KK. Operative management of diabetic foot wounds and infections: Maximizing length and optimizing biomechanics. In: A. N. Sidawy, editor. Philadelphia: Lippincott, Williams and Wilkins; 2006. ID: 823; M1: Book, Section.

11. Cardinal M, Eisenbud DE, Armstrong DG, Zelen C, Driver V, Attinger C, Phillips T, Harding K. Serial surgical debridement: A retrospective study on clinical outcomes in chronic lower extremity wounds. Wound Repair & Regeneration 2009 05;17(3):306-11.

12. Williams D, Enoch S, Miller D, Harris K, Price P, Harding KG. Effect of sharp debridement using curette on recalcitrant nonhealing venous leg ulcers: A concurrently controlled, prospective cohort study. Wound Repair Regen 2005 Mar-Apr;13(2):131-7.

13. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic ulcer study group. J Am Coll Surg 1996 Jul;183(1):61-4.

14. Steed DL. Debridement. Am J Surg 2004 May;187(5):71S-4S.

15. Kirshen C, Woo K, Ayello EA, Sibbald RG. Debridement: A vital component of wound bed preparation. Adv Skin Wound Care 2006;19(9):506-17.

16. Sibbald R, Goodman L, Woo KY, Krasner DL, Smart H, Tariq G, Ayello EA, Burrell RE, Keast DH, Mayer D, et al. Special considerations in wound bed preparation 2011: An update. Advances in Skin and Wound Care 2011;24(9):415-36.

17. Wolcott RD, Rumbaugh KP, James G, Schultz G, Phillips P, Yang Q, Watters C, Stewart PS, Dowd SE. Biofilm maturity studies indicate sharp debridement opens a time-dependent therapeutic window. Journal of Wound Care 2010;19(8):320-8.

18. James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J, Costerton JW, Stewart PS. Biofilms in chronic wounds. Wound Repair Regen 2008 Jan-Feb;16(1):37-44.

19. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001;14(2):244-69.

20. Shannon RJ. A cost-utility evaluation of best practice implementation of leg and foot ulcer care in the Ontario community. Wound Care Canada 2007;5:S53-6.

21. Rodd-Nielsen E, Harris CL. Conservative sharp wound debridement: An overview of Canadian education, practice, risk, and policy. J Wound Ostomy Continence Nurs 2013 Nov-Dec;40(6):594-601.

22. Kloth LC, Niezgoda JA. Ultrasound for wound debridement and healing. In: J. M. Muccullogh, L. C. Kloth, editors. Philadelphia, PA: F.A Davis; 2010. ID: 2814; M1: Book, Section.

23. Webster DF, Pond JB, Dyson M, Harvey W. The role of cavitation in the in vitro stimulation of protein synthesis in human fibroblasts by ultrasound. Ultrasound Med Biol 1978;4(4):343-51.

24. Dyson M, Luke DA. Induction of mast cell degranulation in skin by ultrasound. IEEE Trans Ultrason Ferroelectr Freq Control 1986;33(2):194-201.

25. Young SR, Dyson M. The effect of therapeutic ultrasound on angiogenesis. Ultrasound Med Biol 1990;16(3):261-9.

26. Harvey W, Dyson M, Pond JB, Grahame R. The 'in vitro' stimulation of protein synthesis in human fibroblasts by therapeutic levels of ultrasound. Excerpta Medica, 363 1975:10-21.

27. Sugita Y, Mizuno S, Nakayama N, Iwaki T, Murakami E, Wang Z, Endoh R, Furuhata H. Nitric oxide generation directly responds to ultrasound exposure. Ultrasound Med Biol 2008 Mar;34(3):487-93.

28. Thawer HA, Houghton PE. Effects of ultrasound delivered through a mist of saline to wounds in mice with diabetes mellitus. J Wound Care 2004 May;13(5):171-6.

29. Roper J, Harrison A, Bass MD. Induction of adhesion-dependent signals using low-intensity ultrasound. J Vis Exp 2012 May 8;(63):e4024. doi(63):e4024.

30. Dyson M, Pond JB. The effects of ultrasound on circulation. Physiotherapy 1973;59(9):284-7.

31. Schoenbach SF, Song IC. Ultrasonic debridement: A new approach in the treatment of burn wounds. Plastic & Reconstructive Surgery 1980 Jul;66(1):34-7.

32. Qian Z, Sagers RD, Pitt WG. The effect of ultrasonic frequency upon enhanced killing of P. aeruginosa biofilms. Ann Biomed Eng 1997 Jan-Feb;25(1):69-76.

33. Carmen JC, Nelson JL, Beckstead BL, Runyan CM, Robison RA, Schaalje GB, Pitt WG. Ultrasonic-enhanced gentamicin transport through colony biofilms of pseudomonas aeruginosa and escherichia coli. J Infect Chemother 2004 Aug;10(4):193-9.

34. Rediske AM, Hymas WC, Wilkinson R, Pitt WG. Ultrasonic enhancement of antibiotic action on several species of bacteria. J Gen Appl Microbiol 1998 Aug;44(4):283-8.

35. Romanenko IM. The characteristics of the ultrastructural changes in yeast-like fungi of the genus candida exposed to therapeutic-range ultrasound and nystatin. Zh Mikrobiol Epidemiol Immunobiol 1992;(9-10)(9-10):13-8.

36. Phillips PL, Yang Q, Sampson E, Schultz G. Effects of antimicrobial agents on an in vitro biofilm model of skin wounds. Advances in Skin and Wound Care 2010;1:299-304.

37. Cheng LH, Stewart J, Thompson M, Adlam DM. Ultrasonic debridement of contaminated facial wounds. Br J Oral Maxillofac Surg 2002 Apr;40(2):149-50.

38. Breuing KH, Bayer L, Neuwalder J, Orgill DP. Early experience using low-frequency ultrasound in chronic wounds. Ann Plast Surg 2005 Aug;55(2):183-7.

39. Carmo M, Mazzaccaro D, Barbetta I, Settembrini AM, Roveri S, Fumagalli M, Tassinari L, Settembrini G. Use of ultrasound debridement as an adjunctive tool for treating infected prosthetic vascular grafts in the lower extremities. Ann Vasc Surg 2015;29(3):607-15.

40. Tewarie L, Moza AK, Zayat R, Autschbach R, Goetzenich A, Menon AK. Ultrasound-assisted treatment of sternocutaneous fistula in post-sternotomy cardiac surgery patients. Eur J Cardiothorac Surg 2015 May;47(5):e180,7; discussion e187.

41. Drisko CH. Root instrumentation. power-driven versus manual scalers, which one? Dent Clin North Am 1998 Apr;42(2):229-44.

42. Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJC, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. Clinical Infectious Diseases 2005;41:S395-519.

43. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJG, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. 2012 infectious diseases society of america clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clinical Infectious Diseases 2012;54(12):132-73.

44. Sugama J, Matsui Y, Sanada H, Konya C, Okuwa M, Kitagawa A. A study of the efficiency and convenience of an advanced portable wound measurement system (VISITRAK). J Clin Nurs 2007 Jul;16(7):1265-9.

45. Thompson N, Gordey L, Bowles H, Parslow N, Houghton P. Reliability and validity of the revised photographic wound assessment tool on digital images taken of various types of chronic wounds. Adv Skin Wound Care 2013 Aug;26(8):360-73.

46. Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 1983 Sep;17(1):45-56.

47. Amini S, ShojaeeFard A, Annabestani Z, Hammami MR, Shaiganmehr Z, Larijani B, Mohseni S, Afshani HR, Rad MA, Mohajeri-Tehrani MR. Low-frequency ultrasound debridement in patients with diabetic foot ulcers and osteomyelitis. Wounds 2013 Jul;25(7):193-8.

48. Adam K, Mahmoud S, I SM, Widatalla A, Shawer MAG, Ahmed M. Extended leg infection of diabetic foot ulcers: Risk factors and outcome. J Wound Care 2011 09;20(9):440-4.

49. Dyson M, Williams AR. Ultrasound interaction in biology and medicine. Ultrasound Med Biol 1982;8(1):119.

50. Kielcot-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. The Lancet 1995;346:1194-6.

51. Tan J, Abisi S, Smith A, Burnand KG. A painless method of ultrasonically assisted debridement of chronic leg ulcers: A pilot study. European Journal of Vascular & Endovascular Surgery 2007 Feb;33(2):234-8.

52. Herberger K, Franzke N, Blome C, Kirsten N, Augustin M. Efficacy, tolerability and patient benefit of ultrasound-assisted wound treatment versus surgical debridement: A randomized clinical study. Dermatology 2011;222(3):244-9.

53. Shannon MK, Williams A, Bloomer M. Low-frequency ultrasound debridement (sonaca-185) in acute wound management: A case study. Wound Practice and Research 2012;20(4):200-5.

5   Thesis Discussion

This research was one of the first of a series of works responding to the needs of this high risk population. This doctoral research, comprised of three distinct clinical studies, has sought to address the needs of a particular population with challenging health needs. With a view to improving wound care for patients with vascular disease, we have contributed to understandings about how to identify and address signs of wound infection and how to treat wound infection using an ultrasound system of debridement.

Chapter 2 presented our study on the validity of clinical signs of wound infection in patients with vascular disease. Since patients with vascular disease have a high incidence of limb threatening infection, the study aimed to develop an assessment tool that would aid wound care clinicians in detecting infection. The study, again, involved 78 patients with vascular compromise and a wound located on the lower extremity. The presence or absence of 13 signs of infection was noted by a single nurse with advanced wound care training. The sensitivity and specificity of clinical signs of infection either individually or in combination were compared to the actual infection using as positive tissue biopsy and/or diagnosis by an infectious diseases physician.

Our study, reported in Chapter 2, found certain clinical signs to be specific (>0.9) but not very sensitive. Combining three or four clinical signs together improved the specificity, but did not change the sensitivity. Our results suggest that when clinical signs such as heat, increasing wound size, wound breakdown, odour, increased serous exudate and induration are present, the clinician can be confident an infection is present. However, with such low values for sensitivity, the absence of these clinical signs of infection does not mean infection is not there. These results demonstrate that clinical tools that have been used previously to detect infection in other patient populations with chronic wounds (e.g. diabetic foot ulcers) cannot be applied to this high risk population with vascular compromise. The poor sensitivity assonated with clinical signs of infection speaks to the urgent need for an objective point of care test for this population to promote better outcomes.

Chapters 3 and 4 focused on evaluating the feasibility and effectiveness of a novel treatment intervention that uses low frequency ultrasound energy (LFCUD) to remove excess debris commonly found in wounds with vascular compromise. This therapy physically removes dead or foreign material from the wound, and is purported to prevent infection by removing a site for bacterial invasion.

Chapter 3 detailed an initial single arm uncontrolled study involving ten patients with PVD. The purpose of this pilot study was to assess the feasibility of doing a larger randomized controlled trial where assessor blinding was involved. Specifically, the effect of LFCUD on healing outcomes and complication rates (infection, amputation, and hospital admissions) was evaluated. We also assessed procedural related pain, and the feasibility of a weekly treatment schedule for outpatients traveling to a clinic for patients with lower extremity wounds associated with PVD. To prepare for this pilot study using LFCUD, necessary approvals were secured, a medical directive to allow the nurse clinician to deliver LFCUD was obtained, and training by the manufacturer (Misonix) was completed (Plate 1). A treatment procedure that adhered to strict medical asepsis, equipment sterilization, and used protective equipment was developed and refined.

In this small pilot study—discussed in Chapter 3—it was concluded that LFCUD was feasible and safe to apply by a nurse specialist in a supported out-patient centre. LFCUD was well tolerated by patients and a weekly treatment schedule was deemed feasible for patients attending an outpatient clinic. Wound size reduction after four weekly treatments was clinically significant (39.4% ± 29.3). These data were then used to calculate a sample size needed to detect statistically significant differences between LFCUD and UC (see Appendix 3). The study found a sample size of 32 patients per group or 64 patients in total would be required to determine a treatment effect.

In Chapter 4, we described an assessor-blinded randomized controlled trial conducted to compare a group of vascular patients receiving LFCUD and usual care (UC) with a group of vascular patients receiving UC. Seventy patients with lower extremity wounds and vascular disease were enrolled over a 13-month period with 68 patients participating in the trial and 63 patients completing all four weeks of treatment. All subjects underwent

an initial assessment by a vascular surgeon and an ID physician to confirm healing potential and assess/treat wound infections. A consistent UC wound program was developed that included conservative sharp debridement. Patients were then randomly assigned to either continue UC or also to receive UC and four weekly treatments of LFCUD delivered directly to the wound bed in order to remove any debris and produce fresh bleeding. We measured pain, wound size and appearance along with any complications at Week 0 (baseline), at Week 5 (one week after treatment), and Week 12 (seven weeks after treatment). Again, a total of 63 patients completed four weeks of treatment and attended 94.5% of scheduled appointments. Missing data for 68 patients were imputed as last outcome carried forward. Drop-outs were equal between groups and unrelated to treatment.

As reported in Chapter 4, results from this controlled clinical trial showed LFCUD significantly improves wound appearance and is well tolerated with minimal bleeding or pain. While a progressive reduction in wound size was seen in the LFCUD group, the mean percentage wound surface area was not statistically significant. It is possible that the LFCUD treatment schedule was not sufficient enough to produce a large enough change in the treatment group. We found LFCUD treatment improves wound appearance, is well tolerated, and results in minimal bleeding.

Moving forward, future studies should evaluate a LFCUD treatment with longer treatment application times (our average treatment time was less than three minutes), with more frequent appointments (two or more times a week), and/or over longer periods of time (more than four weeks). By doing a pilot study prior to the controlled clinical trial, a sample size could be calculated to ensure the number of subjects in the trial is sufficient to detect a difference in the primary outcome (wound size reduction).

This research involved a group of patients with advancing vascular disease. Based on the low number of complications, the absence of treatment-related events, and the few withdrawals, more research evaluating new wound treatments should be conducted on this high risk population.

Appendix 1*University Original Research Ethics Board Approval Notice*

Western Research **Research Ethics**

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Pamela Houghton
File Number:103762
Review Level: Full Board
Approved Local Adult Participants:0
Approved Local Minor Participants:0
Protocol Title:A proposal for randomized controlled trial to investigate if application of ultrasonic-assisted debridement may improve healing and infection outcomes compared to usual care in the person with vasculopathy and recalcitrant wounds of the lower extremity.
Department & Institution: Health Sciences\Health & Rehabilitation Sciences,Western University
Sponsor:
Ethics Approval Date: June 10, 2013
Ethics Expiry Date: September 26, 2014

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Protocol		2013/04/17
Western University Protocol		
Instruments	Data collection forms-Received for Information	2013/05/29

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.
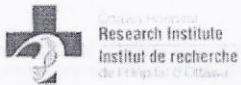
The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health &

Erika Basile	Grace Kelly	Shantel Walcott
(ebasile@uwo.ca)	(grace.kelly@uwo.ca)	(swalcot@uwo.ca)

This is an official document. Please retain the original in your files.

Western University, Research, Support Services Bldg., Rm. 5150
London, ON, Canada N6A 3K7 t. 519.661.3036 f. 519.850.2466 www.uwo.ca/research/services/ethics

Appendix 2 *Clinical Site Original Research Ethics Board Approval Notice*

Research Institute
Institut de recherche
de l'Hôpital d'Ottawa

uOttawa

UNIVERSITY OF OTTAWA
HEART INSTITUTE
INSTITUT DE CARDIOLOGIE
DE L'UNIVERSITÉ D'OTTAWA

Ottawa Health Science Network Research Ethics Board/Réseau des sciences de la santé d'Ottawa Conseil d'éthique de la recherche
Civic Box 411 725 Parkdale Avenue, Ottawa, Ontario K1Y 4E9 613-798-5555 ext. 14902 Fax: 613-761-4311
http://www.ohri.ca/ohsn-reb

Wednesday, August 28, 2013

Ms. Christine Murphy
The Ottawa Hospital, Civic Campus
Department of Surgery
Division of Vascular Surgery
1053 Carling Ave., Room JM46
Ottawa, ON K1Y 4E9

Dear Ms. Murphy:

Re: Protocol # 20130152-01H   A Proposal for Randomized Controlled Trial to Investigate if Application of Ultrasonic-Assisted Debridement may Improve Healing and Infection Outcomes Compared to Usual Care in the Person with Vasculopathy and Recalcitrant Wounds of the Lower Extremity

Protocol approval valid until -   Monday, March 17, 2014

Thank you for your email dated August 22, 2013. This protocol was reviewed by the full Board of the Ottawa Health Science Network Research Ethics Board (OHSN-REB) at the meeting held on March 18, 2013. You have met the requirements of the OHSN-REB and your protocol has been granted approval by the OHSN-REB. No changes, amendments or addenda may be made to the protocol or the consent form without the OHSN-REB's review and approval.

Your request for a French exemption has been approved and the study may proceed in English only.

PLEASE NOTE: THE APPROVAL OF THIS PROTOCOL IS CONDITIONAL UPON A FULLY-SIGNED STUDY CONTRACT/AGREEMENT BETWEEN THE OTTAWA HOSPITAL RESEARCH INSTITUTE, THE PRINCIPAL INVESTIGATOR AND THE SPONSOR (OR AS OTHERWISE REQUIRED). YOU CANNOT START THE STUDY, OR BEGIN TO RECRUIT RESEARCH PARTICIPANTS INTO THE STUDY UNTIL THE STUDY CONTRACT/AGREEMENT HAS BEEN SIGNED BY ALL PARTIES, AND HAS BEEN RECEIVED BY THE OTTAWA HOSPITAL RESEARCH INSTITUTE'S CONTRACTS OFFICE. FOR FURTHER DETAILS, PLEASE CONTACT ALISON IRWIN, CONTRACTS ADMINISTRATOR AT AIRWIN@OHRI.CA OR AT 613-798-5555 EXT. 19690.

Approval is for the following:
- Electronic Application
- Proposal dated June 17, 2013
- Sonic One Ultrasonic Wound Care System Brochure, uploaded March 3, 2013
- English Information Sheet and consent Form, dated August 22, 2013

The REB no longer requires a 'valid until' date at the bottom of all approved informed consent forms. The consent forms currently approved for use by the REB are listed above.

.../2

Appendix 3 *Sample Size Calculation*

The sample size for the randomized controlled trial was initially calculated using percentage area reduction at four weeks as the primary outcome measure. The minimally important difference between healers and non-healers was considered as 20% contraction based on a conservative estimate from available literature of normally perfused patients.[112] Variability within the sample was considered at 10%. Once the pilot data were analyzed, the minimally important difference of this population estimate remained at 20% since it was found that the mean WSA reduction which was 39.4%, for which a 20% difference wound render an 19.4% estimation of for non-healers. Because it is known that only 25% of arterial insufficient wounds heal at six months,[113] 19.4% was considered to be an optimistic target for non-healing vascular wounds, and so these estimations would yield a conservative sample size calculation.[113]

Therefore sample size = $\dfrac{2(1.96 + 0.84)^2\,\sigma^2}{(\delta - M)^2}$ = $\dfrac{2(1.96 + 0.84)^2\,10^2}{(20 \text{ -}10)^2}$ = 31.36 /group
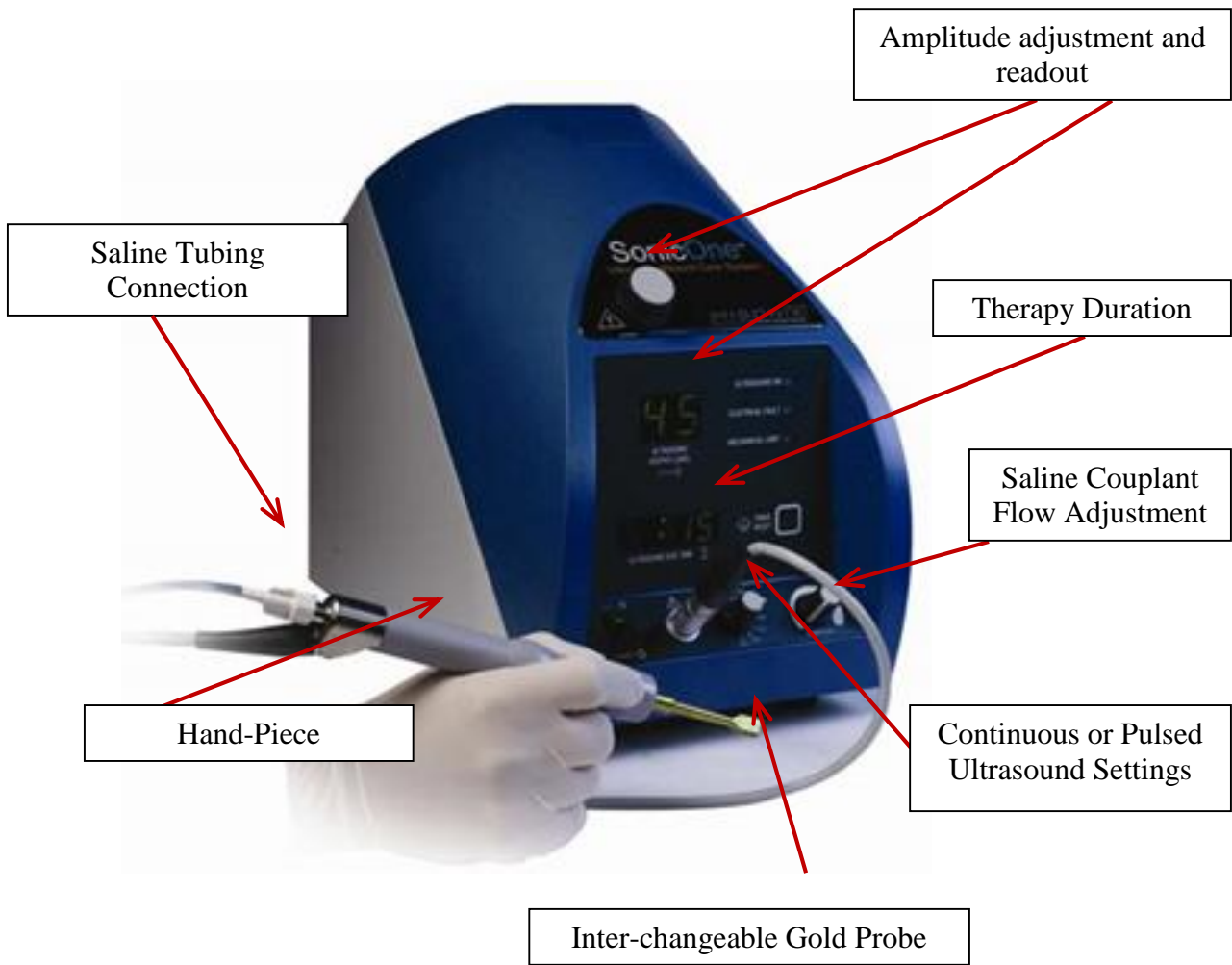
Using this formula, the sample size was calculated to be 32 patients per group (as calculated using a 95% confidence interval and continuous data). This calculation was based on a two-sided analysis (within a superiority design for a conservative assessment), which was used to identify a significant treatment effect considering $p = 0.05$ and $\beta = 0.2$. Assuming a 15% drop out rate, this calculation determined that recruitment of 36 subjects per group would be reasonable to maintain the target of 32 patients in each of the two groups (64 in total) to determine a significant effect.

1. Cardinal M, Eisenbud DE, Phillips T, Harding K. Early healing rates and wound area measurements are reliable predictors of later complete wound closure. *Wound Repair Regen*. 2008;16(1):19-22.

2. Marston WA, Davies SW, Armstrong B, et al. Natural history of limbs with arterial insufficiency and chronic ulceration treated without revascularization. *J Vasc Surg*. 2006;44(1):108-114.

Appendix 4 *Sonic One ® Device Illustration*

Generator and hand-piece with gold probe

Amplitude adjustment and readout

Saline Tubing Connection

Therapy Duration

Saline Couplant Flow Adjustment

Hand-Piece

Continuous or Pulsed Ultrasound Settings

Inter-changeable Gold Probe

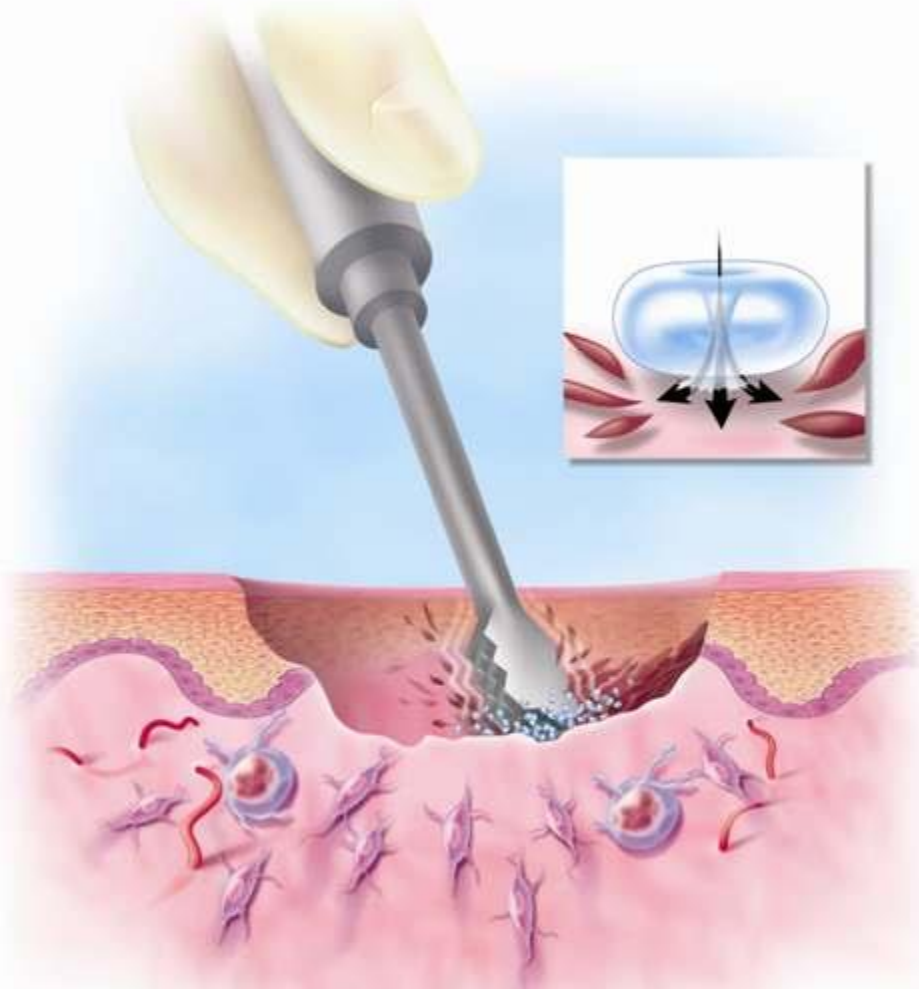Appendix 5 *Illustration of cavitation of micro-bubble (implosion) causing debridement of wound surface*

Image courtesy of Misonix, Inc., Farmingdale, NY
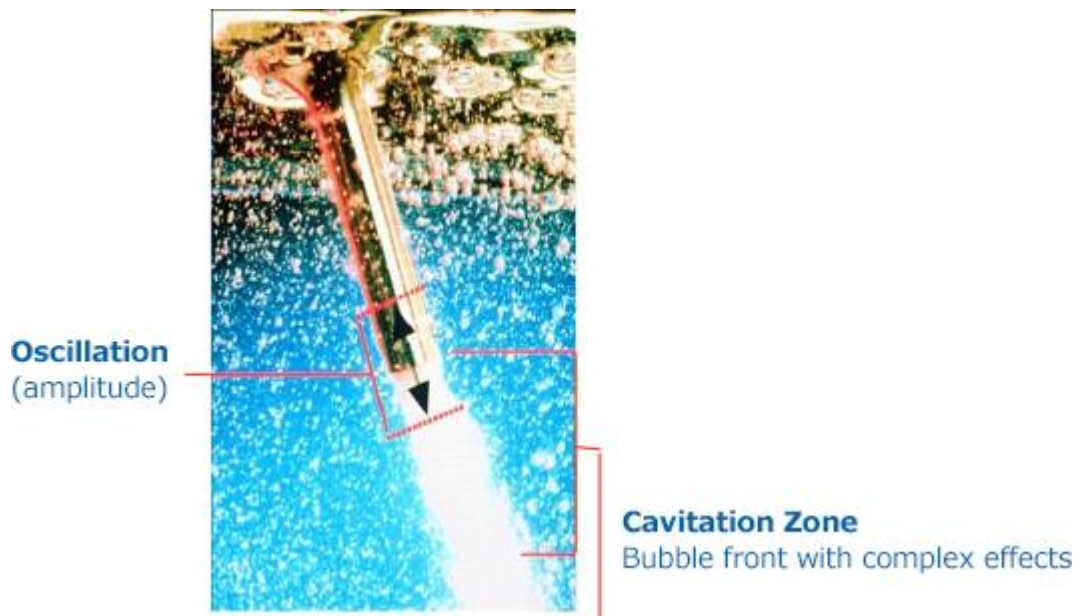
Appendix 6 Image: *Amplitude and cavitation (micro-bubbles)*

Oscillation (amplitude)

Cavitation Zone Bubble front with complex effects

Image courtesy of Misonix, Inc., Farmingdale, NY

Appendix 7 *Illustration of different probes (from product brochure)*

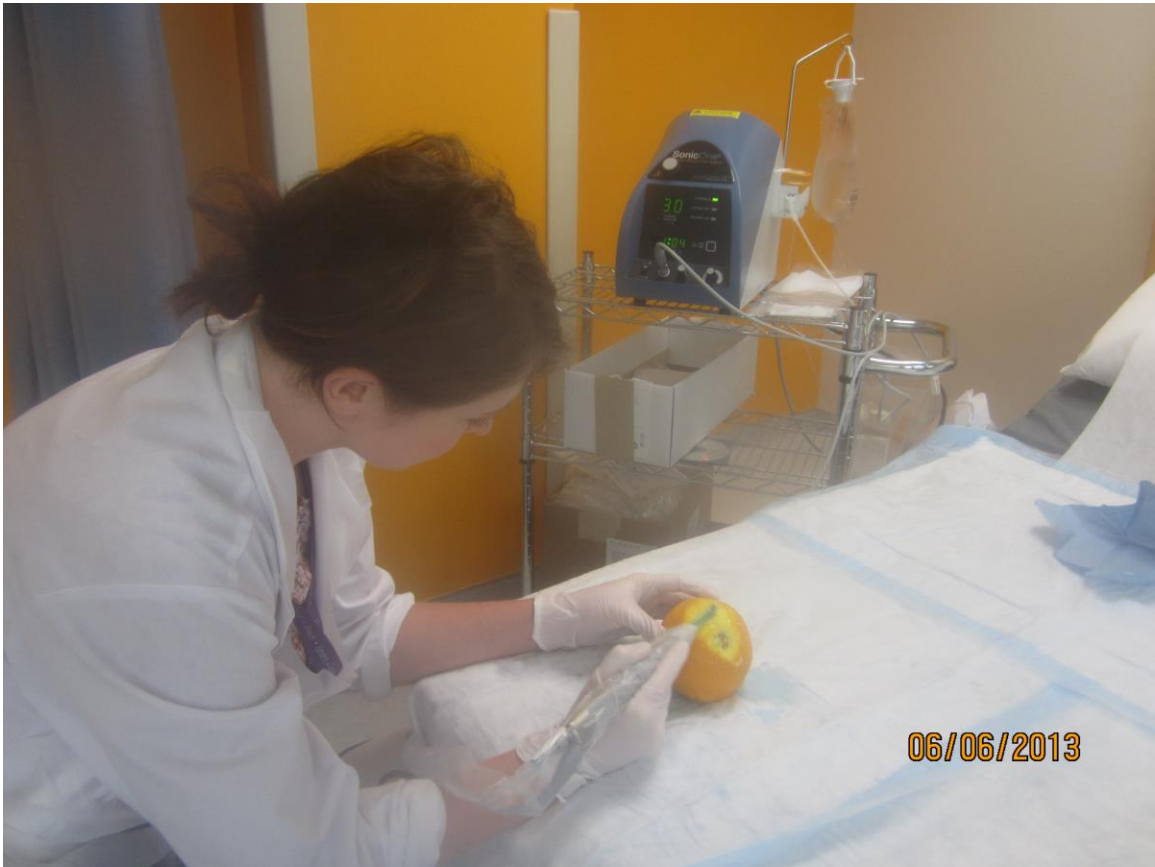Plate 1 *Photo: Training with the Sonic One ®.*

Plate 2 *Photo: Patient receiving LFCUD*

Plate 3 Photo: *Pilot patient pre-LFCUD treatment: (Week 0). Open digital amputation site*

Plate 4 **Photo:** *Same patient post-LFCUD treatment: (Week 5)*

(Ready for skin graft).

Curriculum Vitae

Name:	Christine Anne Murphy
Post-secondary Education and Degrees:	Western University London, Ontario, Canada 2011 – 2015 PhD (student) Western University London, Ontario, Canada 2009 – 2010 MClScWH Thames Valley University London, United Kingdom 2005 – 2009 BSc (hons) Algonquin College Ottawa, Ontario, Canada 1983 – 1986 Diploma (Nursing)
Honours and Awards:	The Ottawa Hospital Nursing Research Bursary 2013 Hollister Graduate Scholarship Award 2012 Cathy Harley Award for Graduate Studies 2010
Related Work Experience	Teaching Assistant The University of Western Ontario 2011-2014 Enterostomal Therapy Nurse The Ottawa Hospital 2006 - 2015 Registered Nurse The Ottawa Hospital 1986 - 2015

Publications:

Murphy, C.A.; Laforet, K.; DaRosa, P.; Tabamo, F.; M.G. Woodbury. (2012). Reliability and predictive validity of Inlow's 60-second diabetic foot screen tool. Advances in Skin and Wound Care, 25(6) 261–266.

Teague, L.M.; Bruton, K.; Coutts, P.; Goodman, L.; Murphy, C.A.; Nesbeth, H.; O' Sullivan, D.; Thompson, R.; Aknsenchuk, K.; D'Souza, E.; English, K.; Nazarali, R.; Yang, A. (2013).Registered Nurses" Association of Ontario (RNAO) Best Practice Guideline: Assessment and Management of Foot Ulcers for People with Diabetes: Update 2013.RNAO: Toronto.

Murphy, C.A.(2012). The Enterostomal Therapist in the Emergency Room. The Link.23 (1)30-31.