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A multi-centre, randomised controlled trial to compare the use of the Decellularised Dermis Allograft in addition to standard care versus standard care alone for the treatment of Venous Leg Ulceration – DAVE trial.

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ABSTRACT

Introduction: Venous leg ulceration (VLU), the most common type of chronic ulcer, can be difficult to heal and is a major cause of morbidity and reduced quality of life. Although compression bandaging is the principal treatment, it is time consuming and bandage application requires specific training. There is evidence that intervention on superficial venous incompetence can help ulcer healing and recurrence, but this is not accessible to all patients. Hence, new treatments are required to address these chronic wounds. One possible adjuvant treatment for VLU is human decellularised dermis (DCD), a type of skin graft derived from skin from deceased tissue donors. Although DCD has the potential to promote ulcer healing, there is a paucity of data for its use in patients with VLU.

Methods and analysis: This is a multi-centre, parallel group, pragmatic randomised controlled trial. One hundred and ninety-six patients with VLU will be randomly assigned to receive either the DCD allograft in addition to standard care, or standard care alone. The primary outcome is the proportion of participants with a healed index ulcer at 12-weeks post randomisation in each treatment arm. Secondary outcomes include the time to index ulcer healing and the proportion of participants with a healed index ulcer at 12-months. Changes in quality of life scores and cost-effectiveness will also be assessed. All analyses will be carried out on an intention-to-treat (ITT) basis. A mixed-effects, logistic regression on the outcome of the proportion of those with the index ulcer healed at 12-weeks, will be performed. Secondary outcomes will be assessed using various statistical models appropriate to the distribution and nature of these outcomes.

Ethics and dissemination: Ethical approval was granted by the Bloomsbury Research Ethics Committee (19/LO/1271). Findings will be published in a peer-reviewed journal and presented at national and international conferences.

Trial registration: ISRCTN21541209.

Keywords: Venous leg ulceration, decellularised dermis allograft, compression bandaging

ARTICLE SUMMARY

Strengths and Limitations of the study

- To the authors' knowledge, this is the first randomised controlled trial evaluating the use of the decellularised dermis (DCD) allograft solely in patients with venous leg ulceration (VLU).
- The trial will follow up participants for 12-months, thus providing longer-term data on ulcer healing and recurrence.
- The data obtained from this study will support the development of VLU treatment and management strategies.
- The cost-effectiveness analysis will assess the economic impact of utilizing the DCD allograft for the management of patients with VLU, whose care consumes significant financial resource.
- The trial concentrates specifically on hard to heal ulcers, solely recruiting patients who have had an ulcer for at least 6-months.

Word count: 3995

INTRODUCTION

Background and rationale

Venous leg ulceration (VLU) describes a persistent wound in the lower limbs caused by a poorly functioning venous system. Characterised by chronicity and a protracted and intensive treatment, these wounds affect approximately 1-2% of the population, with prevalence increasing to up to 4% in those over 65 years of age (1,2).

Venous leg ulceration has a devastating impact on quality of life and social function especially in the elderly (3–5). The wounds can be very painful, resulting in reduced mobility, and require regular dressing changes, which can be extremely painful and time-consuming. Together, these factors result in negative quality of life effects as severe as those seen in other life limiting chronic conditions, such as congestive cardiac failure and chronic obstructive pulmonary disease (6).

Venous leg ulceration presents a significant burden to the healthcare service (7). Up to 50% of district nurse time is spent caring for people with chronic wounds, of which 70% will be venous in origin (8,9). Furthermore, ulcers can recur many times with up to 48% recurring at 5 years, thus requiring further treatment (10,11). Combined with the social cost due to loss of work and productivity, venous leg ulceration is estimated to cost up to 2% of the annual healthcare budget which equates to approximately £2.5 billion in the UK in 2017 (12). This is predicted to increase as a result of the ageing population (13).

The management of chronic VLU is therefore an important priority and public health concern. Compression, in the form of bandaging and stockings, is the underlying principle of treatment, with the aim of reducing venous hypertension (14). However, applying compression is time consuming; bandage application requires skill and stockings are not suitable for everyone (14,15). Furthermore, the reduction in community nursing numbers has resulted in increasing difficulty for patients to access this service (16,17).

Evidence from the ESCHAR and EVRA trials show that interventions to abolish superficial venous incompetence improve ulcer healing and recurrence (8,18). Although promising, such intervention is not accessible to all patients (19). Moreover, although EVRA reported that early intervention performed

in ulcers with a duration of less than 6-months was beneficial, many patients present within leg ulceration of greater duration than this, recurrent ulceration despite eradication of venous incompetence, or may have underlying deep venous incompetence. These chronic wounds are known to be hard to heal and require considerable nursing resources (10,20). The current treatments offered are therefore insufficient for the management of VLU.

Skin grafting represents an adjuvant treatment that can promote and expedite ulcer healing (21). Grafts can be taken from the patient's own skin, from a donor or from tissue engineered skin (22). An autograft (graft from own skin) can be performed in different ways, including pinch and punch grafting, mincing and meshing (23). Despite promoting ulcer healing, drawbacks exist, including poor cosmetic outcomes and the need for a formal surgical procedure in an operating theatre (24,25). Furthermore, surgical waiting lists can be lengthy and, in the current NHS climate, bed availability is not guaranteed (26). Thus, routine autografts are not accessible to all ulcer patients. Allografts (donor skin) and xenografts (animal skin) have been successfully employed, but present similar drawbacks to autografts and the potential for immunogenicity and disease transmission (27). Tissue engineered skin is donor skin that has been processed to be made inert, and therefore is not immunogenic (28). A Cochrane review found that tissue-engineered skin in conjunction with compression increased the healing rate in venous ulceration; however, there was insufficient evidence to determine the effectiveness of any other skin graft material (29).

Human decellularised dermis (DCD) is generated from skin donations from deceased tissue donors processed to remove epidermal and dermal cells while preserving dermal structures and is supplied nationally by NHS Blood and Transplant [(NHSBT) (30,31)]. This provides an immunologically inert scaffold to support cellular repopulation and tissue re-vascularisation. The advantage of the DCD allograft is that it can be applied to the wound with local anaesthesia (via tissue staples or sutures) or without (via tissue glue), and therefore does not require admission for a procedure under general anaesthetic. The procedure can be performed in the outpatient department, avoiding inpatient admission and theatre use, making the technique more accessible to a larger group of patients.

The majority of DCD studies, including randomised controlled trials, have been performed in diabetic populations (32–35). DCD allografts have been reported as safe, to promote angiogenesis (36) and, in

randomised controlled trials, to significantly reduce ulcer healing time (by up to 50%), (37,38). Cohort study data reveals a reduction in wound surface area, improved healing and volume in venous ulceration, with evidence of angiogenesis, host cell migration and proliferation (39). This study addresses the lack of robust research evidence about the effects of DCD allografts on VLU healing.

This prospective, randomised, open (non-blinded), pragmatic trial will explore whether the DCD allograft in addition to standard care, compared to standard care alone, will improve healing rates, reduce recurrence, increase ulcer-free time and improve quality of life for those with VLU. In addition, a cost-effectiveness analysis will be performed to assess the economic impact of utilizing the DCD allograft for the management of this patient population, whose care consumes significant financial resource.

Currently, the annual cost to manage VLU is approximately £1,200 per patient (14); however, in chronic ulceration this is likely to be more. The NHS per patient costs for graft application will be approximately £400. If a positive outcome results from this trial, the reduced ulcer healing time will likely result in significantly reduced NHS costs with an improvement in quality adjusted life years (QALYs).

Objectives

The primary objective is to determine whether the use of the DCD allograft in patients with VLU, in addition to standard care, improves healing at 12-weeks compared to standard care alone. Secondary objectives include comparisons of time to ulcer healing, change in ulcer area at 12-weeks, ulcer recurrence at 12-months, quality of life (QoL) assessment at 12-weeks, 6-months and 12-months and cost-effectiveness analysis.

METHODS

Trial design

This is a prospective, randomised, open (non-blinded), pragmatic trial with a follow-up of 12 months.

Study Setting

Eligible participants will be recruited from at least 10 sites in the United Kingdom. A full list of the study sites can be found on the International Standard Randomised Controlled Trial Number (ISRCTN) registry (ISRCTN21541209) (40).

Eligibility Criteria

Inclusion criteria are: adult patients (> 18 years), able to provide informed consent with a diagnosis of VLU with documented evidence of venous incompetence on duplex ultrasound, ulcer duration for > 6-months and ulcer surface area ≥ 2 cm^2. Exclusion criteria include: a diagnosis of sickle cell disease, an ankle brachial pressure index (ABPI) < 0.8, a clinically infected ulcer, treatment with biomedical or topical growth factors within the previous 30 days, a previous history of an inability to tolerate compression therapy or a foot ulcer (i.e. below the ankle). The DCD allograft preparation entails the use of a number of components, including specific antibiotics, which are then washed away. There have been no documented allergic or hypersensitivity reactions to the DCD graft reported. Patients with known allergies to the DCD preparation components are therefore able to participate at the discretion of the clinical team.

Interventions

All eligible patients will be informed about the study and provided with a written information sheet. Consenting participants will be randomised to receive either the DCD allograft in addition to standard care or standard care alone (Figure 1). Baseline demographic data will be collected for each participant, including details of their past medical history and any concomitant medication. The EQ-5D (41) and Charing Cross Venous Ulceration Questionnaire (CCVUQ) (42) will also be completed for generic and disease-specific quality of life assessment respectively.

Participants in the standard care arm will undergo wound cleaning and debridement, plus standard compression therapy in the form of multilayer elastic compression bandaging or stockings. Participants in the DCD arm will undergo wound cleaning and debridement and DCD allograft application. Following application of the DCD allograft, a non-adhesive, non-absorbent, non-medicated primary dressing will be applied, followed by the appropriate bolster/secondary dressings (31). Compression therapy will then be applied in the form of multilayer elastic compression. Practice/district nurses will be advised not to

change the primary dressing the first 7-days post DCD allograft application. If the DCD allograft has not adhered to the wound bed at the 1-week visit, the graft can be rinsed in saline (if it appears viable) and reapplied and re-secured. Additional grafts will not be reapplied as part of the trial.

[Figure 1 about here]

As this is a pragmatic trial, the ulcer care in both arms will be as per local unit standard practice. All participants will have their ulcers irrigated, cleaned and debrided according to best local practice. Compression therapy will be according to local practice and may include multilayer elastic compression bandaging or stockings designed to deliver between 20 to 40mm/Hg pressure. Wound dressing and compression application will be performed by trained research nurses or community/district/practice nurses as per standard care. The use of negative pressure wound therapy device will be left to the discretion of the treating clinician. All participants may be offered interventional procedures in the form of endovenous ablation (in the presence of superficial venous disease) dependent on whether local recruitment site practice is to intervene upon ulcers over 6 months' duration. Once the wound has healed, the participant will be given a minimum of Class II compression hosiery (18 – 24 mmHg) to wear to prevent ulcer recurrence as per local practice. Endovenous ablation, amongst other procedures, at any point post-randomisation, will be recorded at the 12-month follow-up.

Primary outcome

The primary outcome is the proportion of participants with a healed index ulcer 12-weeks post randomisation.

Secondary outcomes

The secondary outcomes include:

- Time to index ulcer healing from randomisation
- The percentage change in index ulcer area at 12-weeks from randomisation
- The proportion of participants with a healed index ulcer at 12-months from randomisation
- The proportion of those whose index ulcer healed for whom an ulcer recurred at the index site within 12-months from randomisation

- Change in QoL score at 12-weeks, 6-months and 12-months from randomisation using the EQ-5D and CCVUQ
- Cost-effectiveness analysis, measured using the Incremental Cost-Effectiveness Ratio (ICER)

Sample Size and study duration

To detect an absolute difference of 25% in the proportion of participants with a healed index ulcer at 12 weeks (assuming a healing rate of 30% in the control group and 55% in the intervention group) and allowing for a 10% loss to follow up with a power of 90% and 5% level of significance, 196 patients are required (Stata/IC 15.1 for Mac, Statacorp, College Station, Texas, USA; procedure 'power twoprop', with continuity correction). The effect size was estimated from previously published literature on diabetic and venous ulceration, showing an absolute difference in the proportion of participants with a healed ulcer of 25% between intervention and control groups at 12 weeks (32,38,39). With the 12-month follow-up, this study will run for 36-months.

Interim analysis

When we have mature 12-week primary outcome data on the first 50 participants randomised, we will review the sample size with the independent TSC on the basis of recruitment rate, the overall (blinded) primary outcome of index ulcer healed proportion (expected to be (30+55/2) =~40%) and attrition rate (expected to be 10%).

We plan on having a formal interim analysis with the possibility of stopping early for futility (no prospect of a clinically meaningful treatment effect, or for overwhelming evidence of effectiveness) at this point (of n=50 with mature primary outcome data, or at around 25% of the total scheduled events observed). This single interim analysis using a Lan-DeMets alpha spending approach with Fleming O'Brien boundaries has negligible effect on the required sample size (R 3.4.1 for Windows, package gsDesign).

Recruitment

Potential participants will be identified at outpatient clinic appointments. Posters and leaflets will also be displayed in the outpatient clinics and other appropriate locations.

Potentially eligible patients will receive a verbal explanation of the study and a patient information sheet

by the attending clinical/research team.

Randomisation

Consent forms are completed on the day of treatment. Following confirmation of eligibility, consent and completion of baseline assessments, participants will then be randomly allocated to receive one of the two possible treatment options using an online computerised web system (REDCap, managed by the study data centre, University of Edinburgh). A minimization algorithm using centre, index ulcer size and duration will be used, including a random component to lessen predictability.

Blinding

As the DCD allograft is visible after application for a period of time, it is not possible to mask participants or the research/clinical teams to the treatment strategy. However the primary outcome assessments (verification of index ulcer healing visits) will be completed by an independent clinical assessor trained in the assessment of wound healing, who will have no previous involvement with, or knowledge of, the participant's index ulcer treatment and as such will be blind to the randomised treatment strategy (the DCD allograft is not expected to be visible after 4 weeks).

Follow-up periods

All participants will attend for follow-up at 1-week, 3-weeks, 6-weeks and 12-weeks, 6-months, 9-months and 12-months post-randomisation. At all follow-up visits, a clinical assessment will be undertaken and a photograph and planimetry tracing of the ulcer will be collected (unless healing has been confirmed). The EQ-5D and the CCVUQ will be collected at baseline and the 12-week, 6- and 12-month follow-ups. Healthcare resource use will also be collected at 6- and 12-months.

Fortnightly calls will be made after the 6-week follow-up to check if the ulcer has healed. If the participant reports that their ulcer has healed, they will be invited to attend a verification visit, where a photograph of the ulcer will be taken. This photograph will be sent to an independent assessor (blinded to treatment allocation) for assessment and confirmation of healing status.

If the ulcer is confirmed as healed, monthly telephone calls will be performed to check for recurrence. In the event that an ulcer is confirmed as healed, the recurrence, safety, resource use and health

questionnaire data can be collected over the telephone or by post. If the participant fails to attend their appointment, attempts will be made to collect the QoL and resource use questionnaires by telephone or post. Participants will receive up to £10 for each visit attended as a contribution towards travel expenses.

Data collection and confidentiality

Participant data will be stored in the password-protected REDCap database. Participant details will be anonymised as each participant will be allocated a participant number. Identifiable data, including contact information, will also be recorded on paper forms and will be kept in a locked filing cabinet in a locked office at each investigational site. Data will be monitored for quality and completeness and missing data will be requested from the participating sites, as per the data monitoring plan.

ANALYSIS

Statistical analysis

All analyses will be carried out on an intention-to-treat (ITT) basis. A mixed-effects, logistic regression on the outcome of the proportion of those with the index ulcer healed at 12-weeks, with site as a random effect and randomised group as the treatment effect, along with index ulcer size and duration at baseline (the minimisation factors) and any other baseline factors known or suspected to be strongly related to good or poor outcome, will form the model. Goodness of model fit will be examined using the Hosmer-Lemeshow approach. The robustness of the findings to any patterns of missing data (both assuming data are missing at random; and, if appropriate, informatively missing (missing not at random)) will be explored using appropriate sensitivity analyses.

Secondary outcomes (including the primary outcome at 12-months, time to index ulcer healing, reduction in ulcer area at 12-weeks, ulcer recurrence at 12-months, and quality of life) will be assessed using various statistical models appropriate to the distribution and nature of these outcomes, with the same modelling strategy as per the primary outcome above (e.g. missing data and appropriate model diagnostics).

The proportion healed at 12-months and the recurrence of the index ulcer at 12-months will be analysed as the primary outcome above. The time to index ulcer healing will be analysed using a survival type

model (e.g. Cox proportional hazards model), and if the assumption regarding proportional hazards fails, using a Restricted Mean Survival Time (RMST) approach. The reduction in area of the index ulcer at 12 weeks over baseline will be analysed using a linear mixed model. The quality of life data (EQ-5D and CCVUQ questionnaire) will be analysed using a repeated measures mixed linear models (with repeated measures at 6-weeks, 6-months and 12-months and a suitable specified covariance structure), with the overall treatment effect and the evolution of any treatment effect over time modelled.

Cost-effectiveness analysis

A literature review will be conducted to identify other economic studies and other trials in comparable populations. A within-trial analysis and a decision model will be constructed. In both cases, the main analyses will be performed from the perspective of the NHS and Personal Social Services. Secondary analyses will be performed from a societal perspective. The price year will be 2018-2019. Discounting will be applied according to UK Government guidelines. The study will be reported according to consolidated guidelines for economic evaluation (CHEERS) (43).

The within-trial analysis will compare the treatment strategies within the 12-month time horizon of the clinical trial on an ITT basis. Data will be collected by case note review and questionnaires completed at baseline and follow-up.

Resource use items in hospital and community care, adverse events or complications will be recorded for each patient at 6- and 12-months. Resource use will be multiplied by UK unit costs obtained from published literature, Healthcare Resource Groups, and manufacturers' list prices to calculate overall costs. Utilities and QALYs will be calculated from the EQ-5D questionnaire. The extent of missing data will be assessed and appropriate methods to handle missing data will be applied.

The decision model provides a framework to incorporate evidence from other relevant studies and to extrapolate outcomes, such as ulcer healing and recurrence, beyond the trial reporting period. The Markov model will include the key ulcer-related health states and events that may occur during the lifetime of the patient. The data to support extrapolation may be taken from the trial (e.g. fitting parametric time-to-event functions to the trial data) or may come from external sources (such as the literature review or observational data)(44,45).

In both the within trial and model analyses, the incremental cost-effectiveness ratio will be calculated and compared to current UK decision making thresholds. Sensitivity analysis will be carried out to test the robustness of results to alternative assumptions about model structure or data. The cost-effectiveness acceptability curve will be calculated using probabilistic sensitivity analysis (43).

Data monitoring, safety and quality control

An independent Trial Steering Committee (TSC) and independent Data Monitoring Committee (iDMC) have been appointed. The main role of the TSC is to provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of Good Clinical Practice (GCP) and the relevant regulations, whilst the main role of the iDMC is to safeguard the interests of trial participants and to monitor the main outcome measures including safety and efficacy. A clinical trial manager, together with the Trial Management Group (TMG), will oversee trial progress.

All treatment related adverse events (AEs; related to the skin graft or leg ulcer only) will be collected as will all serious adverse events (SAEs). The chief investigator (CI) will be notified of all SAEs within 24 hours. All SAEs will be reported to the research ethics committee (REC) if, in the opinion of the CI, the event was related to the intervention. All related AEs and SAEs will be recorded and summarised by treatment strategy. These analyses will be descriptive, with any p-values calculated to be interpreted descriptively.

DISCUSSION

Although compression therapy is the mainstay of treatment, there is a need to explore new treatments for wounds that are chronic and persistent in nature. This is the first randomised controlled trial to evaluate the use of DCD allograft for the treatment of VLU. This study will provide important data on whether the use of the DCD allograft plus standard care is associated with improved outcomes compared to standard care alone and will provide important data on its effects on quality of life and healthcare costs.

Patient and public involvement

Focus groups were held with patients accessing the vascular clinic at Imperial College Healthcare NHS Trust to obtain views on the proposed study and the acceptability of the DCD allograft. The focus group helped to inform important aspects of the trial, including the number of visits and questionnaires used

in the study. A Patient and Public Involvement (PPI) representative was included as a co-applicant and provided invaluable input in the study design. A PPI representative also sits on the TSC, providing real time input on study progress. He will also aid with dissemination of the results.

Ethics approval and consent to participate

Ethical approval was granted by the Bloomsbury Research Ethics Committee (19/LO/1271). Amendments to the protocol will be updated on the ISRCTN record. All amendments to the protocol will be submitted to the sponsor for review before applying for approval from the REC and the Health Research Authority (HRA). Standard informed consent will be taken with freedom to withdraw at any time.

Publication of data

The findings from this study will be published in a peer-reviewed journal and presented at national and international conferences.

Current study status: The current version of the protocol is v8.0. The study commenced recruitment in October 2019.

Trial sponsor

Imperial College London is the main sponsor for this study. Delegated responsibilities are assigned to the NHS trusts taking part in this study.

Funding statement: This study is supported by the J P Moulton Charitable Foundation. The DCD allograft is provided free of charge by NHSBT. The design, management, analysis and reporting of the study are entirely independent of J P Moulton Charitable Foundation and NHSBT.

Availability of data and materials

Not applicable

Author contributions

AHD, SO, TL, FH and LB were involved in the design of the study and securing funding. AHD, SO and FH drafted the protocol and applied for ethical approval. AHD and SO supervise the project. FH and SP coordinate the project. SO, AHD and SP drafted the manuscript. JN and RL will conduct the statistical analysis. DE will conduct the cost-effectiveness analysis. All authors have read and approved the final manuscript. AHD acts as guarantor.

Competing interests

AC and RL are affiliated to NHS Blood and Transplant (NHSBT), who are providing the DCD allografts free of charge. There are no other conflicts of interest to declare.

Abbreviations

ABPI: Ankle Brachial Pressure Index; AE: Adverse Event; CI: Chief Investigator; CCVUQ: Charing Cross Venous Ulcer Questionnaire; CRN: Clinical Research Network; CHEERS: Consolidated Health Economic Evaluation Reporting Standards; DCD: Decellularised dermis; EQ-5D: EuroQol Five-Dimension; GP: General Practitioner; GCP: Good Clinical Practice; HRA: Health Research Authority; ICER: Incremental Cost-Effectiveness Ratio; iDMC: Independent Data Monitoring Committee; ITT: Intention-to-treat; ISRCTN: International Standard Randomised Controlled Trial Number; NHSBT: National Health Service Blood and Transplant; NIHR: National Institute for Health Research; Patient and Public Involvement: PPI; REDCap: Research Electronic Data Capture; REC: Research Ethics Committee; RMST: Restricted Mean Survival Time; TMG: Trial Management Group; TSC: Trial Steering Committee; QALY: Quality-adjusted life year; QoL: Quality of Life; Venous leg ulceration: VLU.

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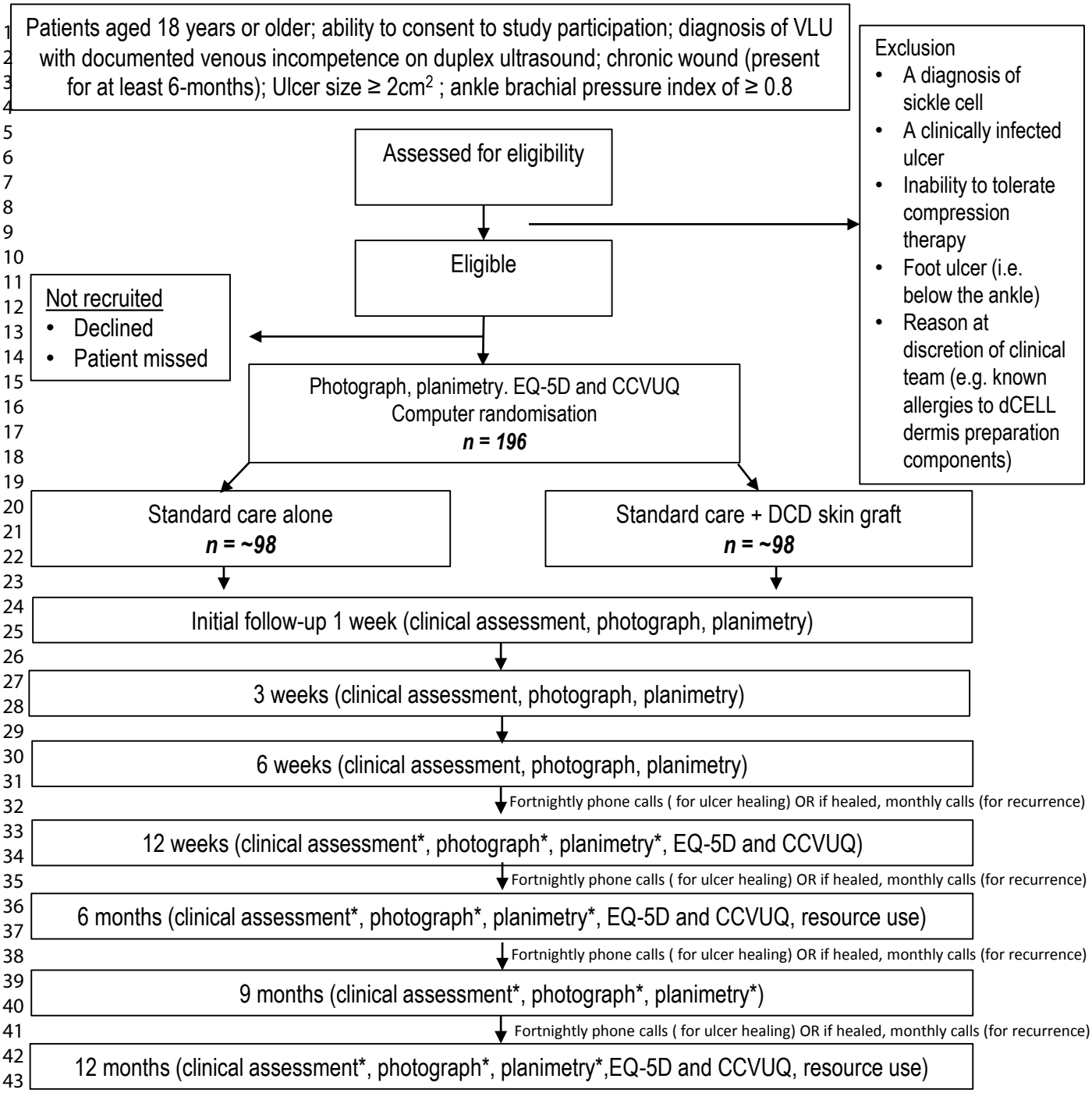
Evaluation of decellularised dermis allograft for the treatment of venous leg ulceration



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____3_____
	2b	All items from the World Health Organization Trial Registration Data Set	Included in trial registry and throughout manuscript
Protocol version	3	Date and version identifier	____15___
Funding	4	Sources and types of financial, material, and other support	_____15_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_____1-2_____
	5b	Name and contact information for the trial sponsor	_____15_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____15_____

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10, 12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10-12
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_8,12_
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	____11-12_____
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	____12____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____12-13____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	__10, 13-14_
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____12_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____14_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____10_
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____14_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	____N/A____
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	15
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10-11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided on request

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	___N/A____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

A study protocol for a multi-centre, randomised controlled trial to compare the use of the Decellularised Dermis Allograft in addition to standard care versus standard care alone for the treatment of Venous Leg Ulceration – DAVE trial

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Primary Subject Heading:	Cardiovascular medicine

Secondary Subject Heading:	Nursing
Keywords:	VASCULAR MEDICINE, WOUND MANAGEMENT, Vascular surgery < SURGERY

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Title: A study protocol for a multi-centre, randomised controlled trial to compare the use of the Decellularised Dermis Allograft in addition to standard care versus standard care alone for the treatment of Venous Leg Ulceration – DAVE trial.

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ABSTRACT

Introduction: Venous leg ulceration (VLU), the most common type of chronic ulcer, can be difficult to heal and is a major cause of morbidity and reduced quality of life. Although compression bandaging is the principal treatment, it is time consuming and bandage application requires specific training. There is evidence that intervention on superficial venous incompetence can help ulcer healing and recurrence, but this is not accessible to all patients. Hence, new treatments are required to address these chronic wounds. One possible adjuvant treatment for VLU is human decellularised dermis (DCD), a type of skin graft derived from skin from deceased tissue donors. Although DCD has the potential to promote ulcer healing, there is a paucity of data for its use in patients with VLU.

Methods and analysis: This is a multi-centre, parallel group, pragmatic randomised controlled trial. One hundred and ninety-six patients with VLU will be randomly assigned to receive either the DCD allograft in addition to standard care, or standard care alone. The primary outcome is the proportion of participants with a healed index ulcer at 12-weeks post randomisation in each treatment arm. Secondary outcomes include the time to index ulcer healing and the proportion of participants with a healed index ulcer at 12-months. Changes in quality of life scores and cost-effectiveness will also be assessed. All analyses will be carried out on an intention-to-treat (ITT) basis. A mixed-effects, logistic regression on the outcome of the proportion of those with the index ulcer healed at 12-weeks, will be performed. Secondary outcomes will be assessed using various statistical models appropriate to the distribution and nature of these outcomes.

Ethics and dissemination: Ethical approval was granted by the Bloomsbury Research Ethics Committee (19/LO/1271). Findings will be published in a peer-reviewed journal and presented at national and international conferences.

Trial registration: ISRCTN21541209.

Keywords: Venous leg ulceration, decellularised dermis allograft, compression bandaging

ARTICLE SUMMARY

Strengths and Limitations of the study

- To the authors' knowledge, this is the first randomised controlled trial evaluating the use of the decellularised dermis (DCD) allograft solely in patients with venous leg ulceration (VLU).
- The trial will follow up participants for 12-months, thus providing longer-term data on ulcer healing and recurrence.
- The cost-effectiveness analysis will assess the economic impact of utilizing the DCD allograft for the management of patients with VLU, whose care consumes significant financial resource.
- The trial concentrates specifically on hard to heal ulcers, solely recruiting patients who have had an ulcer for at least 6-months.

Word count: 4034

INTRODUCTION

Background and rationale

Venous leg ulceration (VLU) describes a persistent wound in the lower limbs caused by a poorly functioning venous system. Characterised by chronicity and a protracted and intensive treatment, these wounds affect approximately 1-2% of the population, with prevalence increasing to up to 4% in those over 65 years of age (1,2).

Venous leg ulceration has a devastating impact on quality of life and social function especially in the elderly (3–5). The wounds can be very painful, resulting in reduced mobility, and require regular dressing changes, which can be extremely painful and time-consuming. Together, these factors result in negative quality of life effects as severe as those seen in other life limiting chronic conditions, such as congestive cardiac failure and chronic obstructive pulmonary disease (6).

Venous leg ulceration presents a significant burden to the healthcare service (7). Up to 50% of district nurse time is spent caring for people with chronic wounds, of which 70% will be venous in origin (8,9). Furthermore, ulcers can recur many times with up to 48% recurring at 5 years, thus requiring further treatment (10,11). Combined with the social cost due to loss of work and productivity, venous leg ulceration is estimated to cost up to 2% of the annual healthcare budget which equates to approximately £2.5 billion in the UK in 2017 (12). This is predicted to increase as a result of the ageing population (13).

The management of chronic VLU is therefore an important priority and public health concern. Compression, in the form of bandaging and stockings, is the underlying principle of treatment, with the aim of reducing venous hypertension (14). However, applying compression is time consuming; bandage application requires skill and stockings are not suitable for everyone (14,15). Furthermore, the reduction in community nursing numbers has resulted in increasing difficulty for patients to access this service (16,17).

Evidence from the ESCHAR and EVRA trials show that interventions to abolish superficial venous incompetence improve ulcer healing and recurrence (8,18). Although promising, such intervention is not accessible to all patients (19). Moreover, although EVRA reported that early intervention performed in ulcers with a duration of less than 6-months was beneficial, many patients present within leg

ulceration of greater duration than this, recurrent ulceration despite eradication of venous incompetence, or may have underlying deep venous incompetence. These chronic wounds are known to be hard to heal and require considerable nursing resources (10,20). The current treatments offered are therefore insufficient for the management of VLU.

Skin grafting represents an adjuvant treatment that can promote and expedite ulcer healing (21). Grafts can be taken from the patient's own skin, from a donor or from tissue engineered skin (22). An autograft (graft from own skin) can be performed in different ways, including pinch and punch grafting, mincing and meshing (23). Despite promoting ulcer healing, drawbacks exist, including poor cosmetic outcomes and the need for a formal surgical procedure in an operating theatre in some instances (24,25). Furthermore, surgical waiting lists can be lengthy and, in the current NHS climate, bed availability is not guaranteed (26). Thus, routine autografts are not accessible to all ulcer patients. Allografts (donor skin) and xenografts (animal skin) have been successfully employed, but present similar drawbacks to autografts and the potential for immunogenicity and disease transmission (27). Tissue engineered skin is donor skin that has been processed to be made inert, and therefore is not immunogenic (28). A Cochrane review found that tissue-engineered skin in conjunction with compression increased the healing rate in venous ulceration; however, there was insufficient evidence to determine the effectiveness of any other skin graft material (29).

Human decellularised dermis (DCD) is generated from skin donations from deceased tissue donors processed to remove epidermal and dermal cells while preserving dermal structures and is supplied nationally by NHS Blood and Transplant [(NHSBT) (30,31)]. This provides an immunologically inert scaffold to support cellular repopulation and tissue re-vascularisation. Although allografts can only serve as temporary cover, the advantage of the DCD allograft is that it can be applied to the wound with local anaesthesia (via tissue staples or sutures) or without (via tissue glue), and therefore does not require admission for a procedure under general anaesthetic. The procedure can be performed in the outpatient department, avoiding inpatient admission and theatre use, making the technique more accessible to a larger group of patients.

The majority of DCD studies, including randomised controlled trials, have been performed in diabetic populations (32–35). DCD allografts have been reported as safe, to promote angiogenesis (36) and, in

randomised controlled trials, to significantly reduce ulcer healing time (by up to 50%), (37,38). Cohort study data reveals a reduction in wound surface area, improved healing and volume in venous ulceration, with evidence of angiogenesis, host cell migration and proliferation (39). This study addresses the lack of robust research evidence about the effects of DCD allografts on VLU healing.

This prospective, randomised, open (non-blinded), pragmatic trial will explore whether the DCD allograft in addition to standard care, compared to standard care alone, will improve healing rates, reduce recurrence, increase ulcer-free time and improve quality of life for those with VLU. In addition, a cost-effectiveness analysis will be performed to assess the economic impact of utilizing the DCD allograft for the management of this patient population, whose care consumes significant financial resource.

Currently, the annual cost to conservatively manage VLU is approximately £1,200 per patient (14); however, in chronic ulceration this is likely to be more. The NHS per patient costs for graft application will be approximately £400. If a positive outcome results from this trial, the reduced ulcer healing time will likely result in significantly reduced NHS costs with an improvement in quality adjusted life years (QALYs).

Objectives

The primary objective is to determine whether the use of the DCD allograft in patients with VLU, in addition to standard care, improves healing at 12-weeks compared to standard care alone. Secondary objectives include comparisons of time to ulcer healing, change in ulcer area at 12-weeks, ulcer recurrence at 12-months, quality of life (QoL) assessment at 12-weeks, 6-months and 12-months and cost-effectiveness analysis.

METHODS AND ANALYSIS

Trial design

This is a prospective, randomised, open (non-blinded), pragmatic trial with a follow-up of 12 months.

Study Setting

Eligible participants will be recruited from at least 10 sites in the United Kingdom. A full list of the study sites can be found on the International Standard Randomised Controlled Trial Number (ISRCTN) registry (ISRCTN21541209) (40).

Eligibility Criteria

Inclusion criteria are: adult patients (> 18 years), able to provide informed consent with a diagnosis of VLU with documented evidence of venous incompetence on duplex ultrasound, ulcer duration for > 6-months and ulcer surface area ≥ 2 cm^2. Exclusion criteria include: a diagnosis of sickle cell disease, an ankle brachial pressure index (ABPI) < 0.8, a clinically infected ulcer, treatment with biomedical or topical growth factors within the previous 30 days, a previous history of an inability to tolerate compression therapy or a foot ulcer (i.e. below the ankle). The DCD allograft preparation entails the use of a number of components, including specific antibiotics, which are then washed away. There have been no documented allergic or hypersensitivity reactions to the DCD graft reported. Patients with known allergies to the DCD preparation components are therefore able to participate at the discretion of the clinical team.

Interventions

All eligible patients will be informed about the study and provided with a written information sheet. Consenting participants will be randomised to receive either the DCD allograft in addition to standard care or standard care alone (Figure 1). Baseline demographic data will be collected for each participant, including details of their past medical history and any concomitant medication. The EQ-5D (41) and Charing Cross Venous Ulceration Questionnaire (CCVUQ) (42) will also be completed for generic and disease-specific quality of life assessment respectively.

Participants in the standard care arm will undergo wound cleaning and debridement, plus standard compression therapy in the form of multilayer elastic compression bandaging or stockings. Participants in the DCD arm will undergo wound cleaning and debridement and DCD allograft application. The DCD graft will be applied by trained registered healthcare professionals (physicians or nurses). Training on the application of the DCD graft will be provided by NHSBT. The DCD will be applied to the debrided index ulcer wound bed. Recommendations will be made that the DCD should be secured with surgical

glue, staples and/or sutures to optimise graft adhesion. The DCD graft should be fenestrated liberally with a scalpel or scissors to allow wound exudate to pass through to reduce risk of seroma/haematoma developing under DCD. Following application of the DCD allograft, a non-adhesive, non-absorbent, non-medicated primary dressing will be applied, followed by the appropriate bolster/secondary dressings (31). Compression therapy will then be applied in the form of multilayer elastic compression. Practice/district nurses will be advised not to change the primary dressing the first 7-days post DCD allograft application. If the DCD allograft has not adhered to the wound bed at the 1-week visit, the graft can be rinsed in saline (if it appears viable) and reapplied and re-secured. Additional grafts will not be reapplied as part of the trial.

[Figure 1 about here]

As this is a pragmatic trial, the ulcer care in both arms will be as per local unit standard practice. All participants will have their ulcers irrigated, cleaned and debrided according to best local practice. Compression therapy will be according to local practice and may include multilayer elastic compression bandaging or stockings designed to deliver between 20 to 40mm/Hg pressure. Wound dressing and compression application will be performed by trained research nurses or community/district/practice nurses as per standard care. In the event of a missed visit, local study teams will liaise with/ask the participant to liaise with the district/community/practice nurse to arrange dressing change and compression application. The use of negative pressure wound therapy device will be left to the discretion of the treating clinician. All participants may be offered interventional procedures in the form of endovenous ablation (in the presence of superficial venous disease) dependent on whether local recruitment site practice is to intervene upon ulcers over 6 months' duration. Once the wound has healed, the participant will be given a minimum of Class II compression hosiery (18 – 24 mmHg) to wear to prevent ulcer recurrence as per local practice. Endovenous ablation, amongst other procedures, at any point post-randomisation, will be recorded at the 12-month follow-up.

Primary outcome

The primary outcome is the proportion of participants with a healed index ulcer 12-weeks post randomisation.

Secondary outcomes

The secondary outcomes include:

- Time to index ulcer healing from randomisation
- The percentage change in index ulcer area at 12-weeks from randomisation
- The proportion of participants with a healed index ulcer at 12-months from randomisation
- The proportion of those whose index ulcer healed for whom an ulcer recurred at the index site within 12-months from randomisation
- Change in QoL score at 12-weeks, 6-months and 12-months from randomisation using the EQ-5D and CCVUQ
- Cost-effectiveness analysis, measured using the Incremental Cost-Effectiveness Ratio (ICER)

Sample Size and study duration

To detect an absolute difference of 25% in the proportion of participants with a healed index ulcer at 12 weeks (assuming a healing rate of 30% in the control group and 55% in the intervention group) and allowing for a 10% loss to follow up with a power of 90% and 5% level of significance, 196 patients are required (Stata/IC 15.1 for Mac, Statacorp, College Station, Texas, USA; procedure 'power twoprop', with continuity correction). The effect size was estimated from previously published literature on diabetic and venous ulceration, showing an absolute difference in the proportion of participants with a healed ulcer of 25% between intervention and control groups at 12 weeks (32,38,39). With the 12-month follow-up, this study will run for 36-months.

Interim analysis

When we have mature 12-week primary outcome data on the first 50 participants randomised, we will review the sample size with the independent TSC on the basis of recruitment rate, the overall (blinded) primary outcome of index ulcer healed proportion (expected to be (30+55/2) =~40%) and attrition rate (expected to be 10%).

We plan on having a formal interim analysis with the possibility of stopping early for futility (no prospect of a clinically meaningful treatment effect, or for overwhelming evidence of effectiveness) at this point

(of n=50 with mature primary outcome data, or at around 25% of the total scheduled events observed). This single interim analysis using a Lan-DeMets alpha spending approach with Fleming O'Brien boundaries has negligible effect on the required sample size (R 3.4.1 for Windows, package gsDesign).

Recruitment

Potential participants will be identified at outpatient clinic appointments. Posters and leaflets will also be displayed in the outpatient clinics and other appropriate locations.

Potentially eligible patients will receive a verbal explanation of the study and a patient information sheet by the attending clinical/research team.

Randomisation

Consent forms are completed on the day of treatment. Following confirmation of eligibility, consent and completion of baseline assessments, participants will then be randomly allocated to receive one of the two possible treatment options using an online computerised web system (REDCap, managed by the study data centre, University of Edinburgh). A minimization algorithm using centre, index ulcer size and duration will be used, including a random component to lessen predictability.

Blinding

As the DCD allograft is visible after application for a period of time, it is not possible to mask participants or the research/clinical teams to the treatment strategy. However the primary outcome assessments (verification of index ulcer healing visits) will be completed by an independent clinical assessor trained in the assessment of wound healing, who will have no previous involvement with, or knowledge of, the participant's index ulcer treatment and as such will be blind to the randomised treatment strategy (the DCD allograft is not expected to be visible after 4 weeks).

Follow-up periods

All participants will attend for follow-up at 1-week, 3-weeks, 6-weeks and 12-weeks, 6-months, 9-months and 12-months post-randomisation. At all follow-up visits, a clinical assessment will be undertaken and a photograph and planimetry tracing of the ulcer will be collected (unless healing has

been confirmed). The EQ-5D and the CCVUQ will be collected at baseline and the 12-week, 6- and 12-month follow-ups. Healthcare resource use (procedures, hospital, GP and community nurse visits, physiotherapy and other interventions), days lost from work and normal activities, carer time and out-of-pocket expenses related to leg ulcer care will also be collected from case notes and patient diaries during the initial procedure and at 6- and 12-months.

Fortnightly calls will be made after the 6-week follow-up to check if the ulcer has healed. If the participant reports that their ulcer has healed, they will be invited to attend a verification visit, where a photograph of the ulcer will be taken. This photograph will be sent to an independent assessor (blinded to treatment allocation) for assessment and confirmation of healing status. Ulcer healing is defined as complete re-epithelialisation of the index ulcer in the absence of a scab (eschar) with no dressing required confirmed by blinded photo assessment of healing.

If the ulcer is confirmed as healed, monthly telephone calls will be performed to check for recurrence. In the event that an ulcer is confirmed as healed, the recurrence, safety, resource use and health questionnaire data can be collected over the telephone or by post. If the participant fails to attend their appointment, attempts will be made to collect the QoL and patient resource use diaries by telephone or post. Participants will receive up to £10 for each visit attended as a contribution towards travel expenses.

Data collection and confidentiality

Participant data will be stored in the password-protected REDCap database. Participant details will be anonymised as each participant will be allocated a participant number. Identifiable data, including contact information, will also be recorded on paper forms and will be kept in a locked filing cabinet in a locked office at each investigational site. Data will be monitored for quality and completeness and missing data will be requested from the participating sites, as per the data monitoring plan.

Statistical analysis

All analyses will be carried out on an intention-to-treat (ITT) basis. A mixed-effects, logistic regression on the outcome of the proportion of those with the index ulcer healed at 12-weeks, with site as a random effect and randomised group as the treatment effect, along with index ulcer size and duration at baseline

(the minimisation factors) and any other baseline factors known or suspected to be strongly related to good or poor outcome, will form the model. Goodness of model fit will be examined using the Hosmer-Lemeshow approach. The robustness of the findings to any patterns of missing data (both assuming data are missing at random; and, if appropriate, informatively missing (missing not at random)) will be explored using appropriate sensitivity analyses.

Secondary outcomes (including the primary outcome at 12-months, time to index ulcer healing, reduction in ulcer area at 12-weeks, ulcer recurrence at 12-months, and quality of life) will be assessed using various statistical models appropriate to the distribution and nature of these outcomes, with the same modelling strategy as per the primary outcome above (e.g. missing data and appropriate model diagnostics).

The proportion healed at 12-months and the recurrence of the index ulcer at 12-months will be analysed as the primary outcome above. The time to index ulcer healing will be analysed using a survival type model (e.g. Cox proportional hazards model), and if the assumption regarding proportional hazards fails, using a Restricted Mean Survival Time (RMST) approach. The reduction in area of the index ulcer at 12 weeks over baseline will be analysed using a linear mixed model. The quality of life data (EQ-5D and CCVUQ questionnaire) will be analysed using a repeated measures mixed linear models (with repeated measures at 6-weeks, 6-months and 12-months and a suitable specified covariance structure), with the overall treatment effect and the evolution of any treatment effect over time modelled.

Cost-effectiveness analysis

A literature review will be conducted to identify other economic studies and other trials in comparable populations. A within-trial analysis and a decision model will be constructed. In both cases, the main analyses will be performed from the perspective of the NHS and Personal Social Services. Secondary analyses will be performed from a societal perspective. The price year will be 2018-2019. Discounting will be applied according to UK Government guidelines. The study will be reported according to consolidated guidelines for economic evaluation (CHEERS) (43).

The within-trial analysis will compare the treatment strategies within the 12-month time horizon of the clinical trial on an ITT basis. Data will be collected by case note review and questionnaires completed at baseline and follow-up.

Resource use items in hospital and community care, adverse events or complications will be recorded for each patient at 6- and 12-months. Resource use will be multiplied by UK unit costs obtained from published literature, Healthcare Resource Groups, and manufacturers' list prices to calculate overall costs. Utilities and QALYs will be calculated from the EQ-5D questionnaire. The extent of missing data will be assessed and appropriate methods to handle missing data will be applied.

The decision model provides a framework to incorporate evidence from other relevant studies and to extrapolate outcomes, such as ulcer healing and recurrence, beyond the trial reporting period. The Markov model will include the key ulcer-related health states and events that may occur during the lifetime of the patient. The data to support extrapolation may be taken from the trial (e.g. fitting parametric time-to-event functions to the trial data) or may come from external sources (such as the literature review or observational data)(44,45).

In both the within trial and model analyses, the incremental cost-effectiveness ratio will be calculated and compared to current UK decision making thresholds. Sensitivity analysis will be carried out to test the robustness of results to alternative assumptions about model structure or data. The cost-effectiveness acceptability curve will be calculated using probabilistic sensitivity analysis (43).

Data monitoring, safety and quality control

An independent Trial Steering Committee (TSC) and independent Data Monitoring Committee (iDMC) have been appointed. The main role of the TSC is to provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of Good Clinical Practice (GCP) and the relevant regulations, whilst the main role of the iDMC is to safeguard the interests of trial participants and to monitor the main outcome measures including safety and efficacy. A clinical trial manager, together with the Trial Management Group (TMG), will oversee trial progress.

All treatment related adverse events (AEs; related to the skin graft or leg ulcer only) will be collected as will all serious adverse events (SAEs). The chief investigator (CI) will be notified of all SAEs within 24 hours. All SAEs will be reported to the research ethics committee (REC) if, in the opinion of the CI, the event was related to the intervention. All related AEs and SAEs will be recorded and summarised by treatment strategy. These analyses will be descriptive, with any p-values calculated to be interpreted descriptively.

DISCUSSION

Although compression therapy is the mainstay of treatment, there is a need to explore new treatments for wounds that are chronic and persistent in nature. This is the first randomised controlled trial to evaluate the use of DCD allograft for the treatment of VLU. This study will provide important data on whether the use of the DCD allograft plus standard care is associated with improved outcomes compared to standard care alone and will provide important data on its effects on quality of life and healthcare costs.

Patient and public involvement

Focus groups were held with patients accessing the vascular clinic at Imperial College Healthcare NHS Trust to obtain views on the proposed study and the acceptability of the DCD allograft. The focus group helped to inform important aspects of the trial, including the number of visits and questionnaires used in the study. A Patient and Public Involvement (PPI) representative was included as a co-applicant and provided invaluable input in the study design. A PPI representative also sits on the TSC, providing real time input on study progress. He will also aid with dissemination of the results.

Ethics and dissemination: Ethical approval was granted by the Bloomsbury Research Ethics Committee (19/LO/1271). Amendments to the protocol will be updated on the ISRCTN record. All amendments to the protocol will be submitted to the sponsor for review before applying for approval from the REC and the Health Research Authority (HRA). Standard informed consent will be taken with freedom to withdraw at any time. The findings from this study will be published in a peer-reviewed journal, presented at national and international conferences and to participants (via emails and letters at the end of the study).

Current study status: The current version of the protocol is v9.0. The study commenced recruitment in October 2019.

Trial sponsor

Imperial College London is the main sponsor for this study. Delegated responsibilities are assigned to the NHS trusts taking part in this study.

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Availability of data and materials

Data will be made available on reasonable request.

Author contributions

AHD, SO, TL, FH and LB were involved in the design of the study and securing funding. MG, KP, NC, AB and KD were involved in the design of the study. AHD, SO and FH drafted the protocol and applied for ethical approval. AHD and SO supervise the project. FH and SP coordinate the project. SO, AHD and SP drafted the manuscript. JN and RLe will conduct the statistical analysis. DE will conduct the cost-effectiveness analysis. AC and RLo advise on any DCD-related issues. All authors have read and approved the final manuscript. AHD acts as guarantor.

Competing interests

AC and RL are affiliated to NHS Blood and Transplant (NHSBT), who are providing the DCD allografts free of charge. There are no other conflicts of interest to declare.

Abbreviations

ABPI: Ankle Brachial Pressure Index; AE: Adverse Event; CI: Chief Investigator; CCVUQ: Charing Cross Venous Ulcer Questionnaire; CRN: Clinical Research Network; CHEERS: Consolidated Health Economic Evaluation Reporting Standards; DCD: Decellularised dermis; EQ-5D: EuroQol Five-Dimension; GP: General Practitioner; GCP: Good Clinical Practice; HRA: Health Research Authority; ICER: Incremental Cost-Effectiveness Ratio; iDMC: Independent Data Monitoring Committee; ITT: Intention-to-treat; ISRCTN: International Standard Randomised Controlled Trial Number; NHSBT: National Health Service Blood and Transplant; NIHR: National Institute for Health Research; Patient and Public Involvement: PPI; REDCap: Research Electronic Data Capture; REC: Research Ethics Committee; RMST: Restricted Mean Survival Time; TMG: Trial Management Group; TSC: Trial Steering Committee; QALY: Quality-adjusted life year; QoL: Quality of Life; Venous leg ulceration: VLU.

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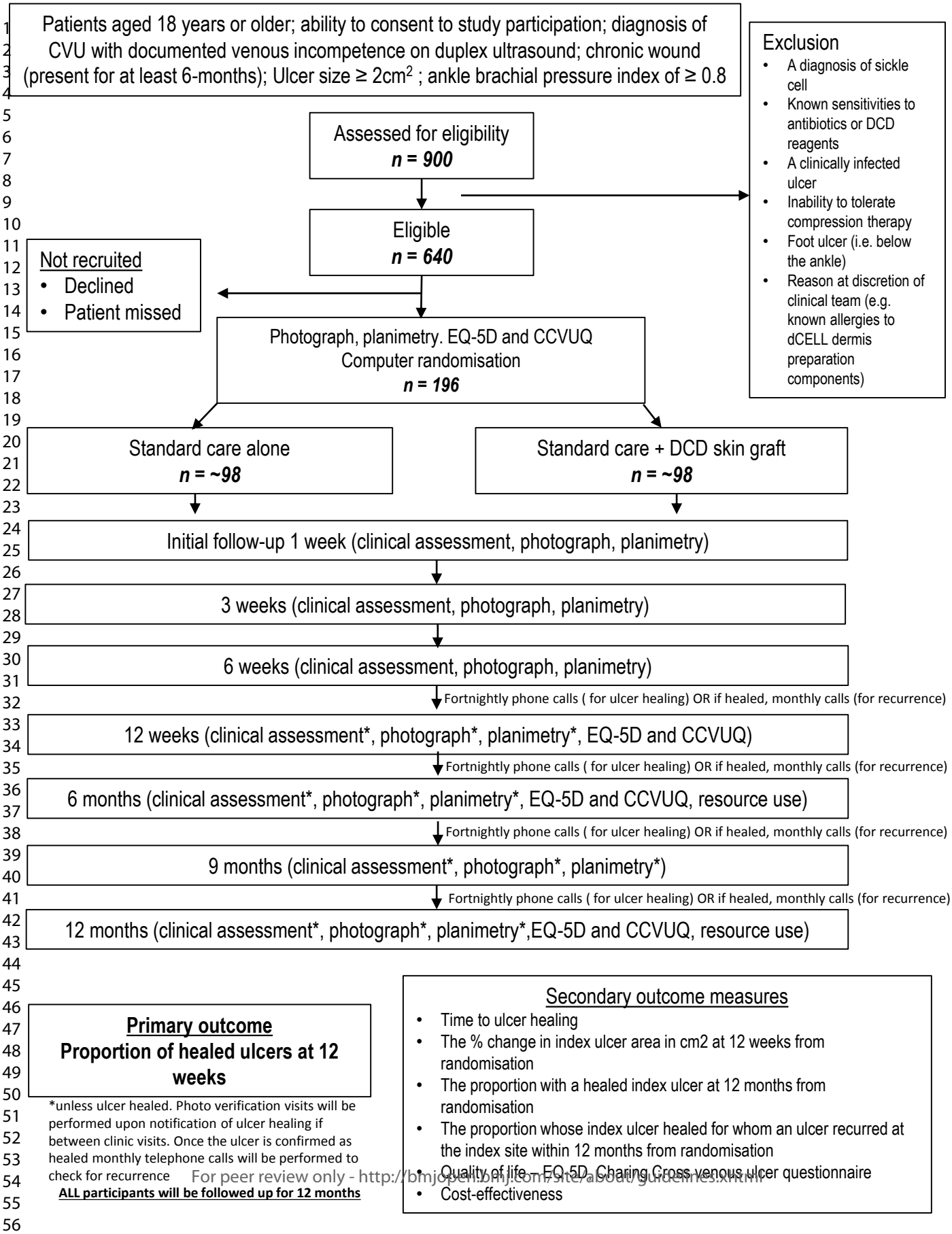
Figure legend

Figure 1: Flow diagram of the study protocol

Figure 1: Flow diagram of the study protocol



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____3_____
	2b	All items from the World Health Organization Trial Registration Data Set	Included in trial registry and throughout manuscript
Protocol version	3	Date and version identifier	____15___
Funding	4	Sources and types of financial, material, and other support	_____16_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_____1-2_____
	5b	Name and contact information for the trial sponsor	_____15_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____15_____

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10, 12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10-12
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_8,12_
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	____11-12_____
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	____12____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____12-13____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	__10, 13-14_
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____12_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____14_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____10_
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____14_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	____N/A____
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	15
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10-11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	16
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided on request

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	___N/A____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

A study protocol for a multi-centre, randomised controlled trial to compare the use of the Decellularised Dermis Allograft in addition to standard care versus standard care alone for the treatment of Venous Leg Ulceration – DAVE trial

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Primary Subject Heading:	Cardiovascular medicine

Secondary Subject Heading:	Nursing
Keywords:	VASCULAR MEDICINE, WOUND MANAGEMENT, Vascular surgery < SURGERY

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Title: A study protocol for a multi-centre, randomised controlled trial to compare the use of the Decellularised Dermis Allograft in addition to standard care versus standard care alone for the treatment of Venous Leg Ulceration – DAVE trial.

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ABSTRACT

Introduction: Venous leg ulceration (VLU), the most common type of chronic ulcer, can be difficult to heal and is a major cause of morbidity and reduced quality of life. Although compression bandaging is the principal treatment, it is time consuming and bandage application requires specific training. There is evidence that intervention on superficial venous incompetence can help ulcer healing and recurrence, but this is not accessible to all patients. Hence, new treatments are required to address these chronic wounds. One possible adjuvant treatment for VLU is human decellularised dermis (DCD), a type of skin graft derived from skin from deceased tissue donors. Although DCD has the potential to promote ulcer healing, there is a paucity of data for its use in patients with VLU.

Methods and analysis: This is a multi-centre, parallel group, pragmatic randomised controlled trial. One hundred and ninety-six patients with VLU will be randomly assigned to receive either the DCD allograft in addition to standard care, or standard care alone. The primary outcome is the proportion of participants with a healed index ulcer at 12-weeks post randomisation in each treatment arm. Secondary outcomes include the time to index ulcer healing and the proportion of participants with a healed index ulcer at 12-months. Changes in quality of life scores and cost-effectiveness will also be assessed. All analyses will be carried out on an intention-to-treat (ITT) basis. A mixed-effects, logistic regression on the outcome of the proportion of those with the index ulcer healed at 12-weeks, will be performed. Secondary outcomes will be assessed using various statistical models appropriate to the distribution and nature of these outcomes.

Ethics and dissemination: Ethical approval was granted by the Bloomsbury Research Ethics Committee (19/LO/1271). Findings will be published in a peer-reviewed journal and presented at national and international conferences.

Trial registration: ISRCTN21541209.

Keywords: Venous leg ulceration, decellularised dermis allograft, compression bandaging

ARTICLE SUMMARY

Strengths and Limitations of the study

- This is the first randomised controlled trial evaluating the use of the decellularised dermis (DCD) allograft solely in patients with venous leg ulceration (VLU).
- The cost-effectiveness analysis will assess the economic impact of utilising the DCD allograft for the management of patients with VLU.
- This is a pragmatic study hence compression and debridement technique will be up to local guidelines/standard care.
- This study only evaluates applications in patients with chronic venous ulceration.
- This study does not address long-term recurrence rates beyond 1 year.

Word count: 4073

INTRODUCTION

Background and rationale

Venous leg ulceration (VLU) describes a persistent wound in the lower limbs caused by a poorly functioning venous system. Characterised by chronicity and a protracted and intensive treatment, these wounds affect approximately 1-2% of the population, with prevalence increasing to up to 4% in those over 65 years of age (1,2).

Venous leg ulceration has a devastating impact on quality of life and social function especially in the elderly (3–5). The wounds can be very painful, resulting in reduced mobility, and require regular dressing changes, which can be extremely painful and time-consuming. Together, these factors result in negative quality of life effects as severe as those seen in other life-limiting chronic conditions, such as congestive cardiac failure and chronic obstructive pulmonary disease (6).

Venous leg ulceration presents a significant burden to the healthcare service (7). Up to 50% of district nurse time is spent caring for people with chronic wounds, of which 70% will be venous in origin (8,9). Furthermore, ulcers can recur many times with up to 48% recurring at 5 years, thus requiring further treatment (10,11). Combined with the social cost due to loss of work and productivity, venous leg ulceration is estimated to cost up to 2% of the annual healthcare budget, which equates to approximately £2.5 billion in the UK in 2017 (12). This is predicted to increase as a result of the ageing population (13).

The management of chronic VLU is therefore an important priority and public health concern. Compression, in the form of bandaging and stockings, is the underlying principle of treatment, with the aim of reducing venous hypertension (14). However, applying compression is time consuming; bandage application requires skill and stockings are not suitable for everyone (14,15). Furthermore, the reduction in community nursing numbers has resulted in increasing difficulty for patients to access this service (16,17).

Evidence from the ESCHAR and EVRA trials show that interventions to abolish superficial venous incompetence improve ulcer healing and recurrence (8,18). Although promising, such intervention is not accessible to all patients (19). Moreover, although EVRA reported that early intervention performed

in ulcers with a duration of less than 6-months was beneficial, many patients present within leg ulceration of greater duration than this, recurrent ulceration despite eradication of venous incompetence, or may have underlying deep venous incompetence. These chronic wounds are known to be hard to heal and require considerable nursing resources (10,20). The current treatments offered are therefore insufficient for the management of VLU.

Skin grafting represents an adjuvant treatment that can promote and expedite ulcer healing (21). Grafts can be taken from the patient's own skin, from a donor or from tissue engineered skin (22). An autograft (graft from own skin) can be performed in different ways, including pinch and punch grafting, mincing and meshing (23). Despite promoting ulcer healing, drawbacks exist, including poor cosmetic outcomes and the need for a formal surgical procedure in an operating theatre in some instances (24,25). Furthermore, surgical waiting lists can be lengthy and, in the current NHS climate, bed availability is not guaranteed (26). Thus, routine autografts are not accessible to all ulcer patients. Allografts (donor skin) and xenografts (animal skin) have been successfully employed, but present similar drawbacks to autografts and the potential for immunogenicity and disease transmission (27). Tissue engineered skin is donor skin that has been processed to be made inert, and therefore is not immunogenic (28). A Cochrane review found that tissue-engineered skin in conjunction with compression increased the healing rate in venous ulceration; however, there was insufficient evidence to determine the effectiveness of any other skin graft material (29).

Human decellularised dermis (DCD) is generated from skin donations from deceased tissue donors processed to remove epidermal and dermal cells while preserving dermal structures and is supplied nationally by NHS Blood and Transplant [(NHSBT) (30,31)]. This provides an immunologically inert scaffold to support cellular repopulation and tissue re-vascularisation. Although allografts can only serve as temporary cover, the advantage of the DCD allograft is that it can be applied to the wound with local anaesthesia (via tissue staples or sutures) or without (via tissue glue), and therefore does not require admission for a procedure under general anaesthetic. The procedure can be performed in the outpatient department, avoiding inpatient admission and theatre use, making the technique more accessible to a larger group of patients.

The majority of DCD studies, including randomised controlled trials, have been performed in diabetic

populations (32–35). DCD allografts have been reported as safe, to promote angiogenesis (36) and, in randomised controlled trials, to significantly reduce ulcer healing time (by up to 50%), (37,38). Cohort study data reveals a reduction in wound surface area, improved healing in venous ulceration, with evidence of angiogenesis, host cell migration and proliferation (39). This study addresses the lack of robust research evidence about the effects of DCD allografts on VLU healing.

This prospective, randomised, open (non-blinded), pragmatic trial will explore whether the DCD allograft in addition to standard care, compared to standard care alone, will improve healing rates, reduce recurrence, increase ulcer-free time and improve quality of life for those with VLU. In addition, a cost-effectiveness analysis will be performed to assess the economic impact of utilizing the DCD allograft for the management of this patient population, whose care consumes significant financial resource.

Currently, the annual cost to conservatively manage VLU is approximately £1,200 per patient (14); however, in chronic ulceration this is likely to be more. The NHS per patient costs for graft application will be approximately £400. If a positive outcome results from this trial, the reduced ulcer healing time will likely result in significantly reduced NHS costs with an improvement in quality adjusted life years (QALYs).

Objectives

The primary objective is to determine whether the use of the DCD allograft in patients with VLU, in addition to standard care, improves healing at 12-weeks compared to standard care alone. Secondary objectives include comparisons of time to ulcer healing, change in ulcer area at 12-weeks, ulcer recurrence at 12-months, quality of life (QoL) assessment at 12-weeks, 6-months and 12-months and cost-effectiveness analysis.

METHODS AND ANALYSIS

Trial design

This is a prospective, randomised, open (non-blinded), pragmatic trial with a follow-up of 12 months.

Study Setting

Eligible participants will be recruited from at least 10 sites in the United Kingdom. A full list of the study sites can be found on the International Standard Randomised Controlled Trial Number (ISRCTN) registry (ISRCTN21541209) (40).

Eligibility Criteria

Inclusion criteria are: adult patients (> 18 years), able to provide informed consent with a diagnosis of VLU with documented evidence of venous incompetence on duplex ultrasound, ulcer duration for > 6-months and ulcer surface area ≥ 2 cm^2. Where there is more than one ulcer present, the largest ulcer will be chosen as the index ulcer for the purposes of the trial. Exclusion criteria include: a diagnosis of sickle cell disease, an ankle brachial pressure index (ABPI) < 0.8, a clinically infected ulcer, treatment with biomedical or topical growth factors within the previous 30 days, a previous history of an inability to tolerate compression therapy or a foot ulcer (i.e. below the ankle). The DCD allograft preparation entails the use of a number of components, including specific antibiotics, which are then washed away. There have been no documented allergic or hypersensitivity reactions to the DCD graft reported. Patients with known allergies to the DCD preparation components are therefore able to participate at the discretion of the clinical team.

Interventions

All eligible patients will be informed about the study and provided with a written information sheet. Consenting participants will be randomised to receive either the DCD allograft in addition to standard care or standard care alone (Figure 1). Baseline demographic data will be collected for each participant, including details of their past medical history and any concomitant medication. The EQ-5D (41) and Charing Cross Venous Ulceration Questionnaire (CCVUQ) (42) will also be completed for generic and disease-specific quality of life assessment respectively.

Participants in the standard care arm will undergo wound cleaning and debridement, plus standard compression therapy in the form of multilayer elastic compression bandaging or stockings. Participants in the DCD arm will undergo wound cleaning and debridement and DCD allograft application. The DCD graft will be applied by trained registered healthcare professionals (physicians or nurses). Training on the application of the DCD graft will be provided by NHSBT. The DCD will be applied to the debrided index ulcer wound bed. Recommendations will be made that the DCD should

be secured with surgical glue, staples and/or sutures to optimise graft adhesion. The DCD graft should be fenestrated liberally with a scalpel or scissors to allow wound exudate to pass through to reduce risk of seroma/haematoma developing under DCD. Following application of the DCD allograft, a non-adhesive, non-absorbent, non-medicated primary dressing will be applied, followed by the appropriate bolster/secondary dressings (31). Compression therapy will then be applied according to local practice and may include multilayer elastic compression bandaging or stockings delivering 20 to 40mm/Hg pressure. Practice/district nurses will be advised not to change the primary dressing the first 7-days post DCD allograft application. If the DCD allograft has not adhered to the wound bed at the 1-week visit, the graft can be rinsed in saline (if it appears viable) and reapplied and re-secured. Additional grafts will not be reapplied as part of the trial.

[Figure 1 about here]

As this is a pragmatic trial, the ulcer care in both arms will be as per local unit standard practice. All participants will have their ulcers irrigated, cleaned and debrided according to best local practice. Compression therapy will be according to local practice and may include multilayer elastic compression bandaging or stockings designed to deliver between 20 to 40mm/Hg pressure. Wound dressing and compression application will be performed by trained research nurses or community/district/practice nurses as per standard care. In the event of a missed visit, local study teams will liaise with/ask the participant to liaise with the district/community/practice nurse to arrange dressing change and compression application. The use of negative pressure wound therapy device will be left to the discretion of the treating clinician. All participants may be offered interventional procedures in the form of endovenous ablation (in the presence of superficial venous disease) dependent on whether local recruitment site practice is to intervene upon ulcers over 6 months' duration. Once the wound has healed, the participant will be given a minimum of Class II compression hosiery (18 – 24 mmHg) to wear to prevent ulcer recurrence as per local practice. Endovenous ablation, amongst other procedures, at any point post-randomisation, will be recorded at the 12-month follow-up.

Primary outcome

The primary outcome is the proportion of participants with a healed index ulcer assessed with ulcer

photography at 12 weeks after randomisation. **Secondary outcomes**

The secondary outcomes include:

- Time to index ulcer healing from randomisation
- The percentage change in index ulcer area at 12-weeks from randomisation
- The proportion of participants with a healed index ulcer at 12-months from randomisation
- The proportion of those whose index ulcer healed for whom an ulcer recurred at the index site within 12-months from randomisation
- Change in QoL score at 12-weeks, 6-months and 12-months from randomisation using the EQ-5D and CCVUQ
- Cost-effectiveness analysis, measured using the Incremental Cost-Effectiveness Ratio (ICER)

Sample Size and study duration

To detect an absolute difference of 25% in the proportion of participants with a healed index ulcer at 12 weeks (assuming a healing rate of 30% in the control group and 55% in the intervention group) and allowing for a 10% loss to follow up with a power of 90% and 5% level of significance, 196 patients are required (Stata/IC 15.1 for Mac, Statacorp, College Station, Texas, USA; procedure 'power twoprop', with continuity correction). The effect size was estimated from previously published literature on diabetic and venous ulceration, showing an absolute difference in the proportion of participants with a healed ulcer of 25% between intervention and control groups at 12 weeks (32,38,39). With the 12-month follow-up, this study will run for 36-months.

Interim analysis

When we have mature 12-week primary outcome data on the first 50 participants randomised, we will review the sample size with the independent TSC on the basis of recruitment rate, the overall (blinded) primary outcome of index ulcer healed proportion (expected to be (30+55/2) =~40%) and attrition rate (expected to be 10%).

We plan on having a formal interim analysis with the possibility of stopping early for futility (no prospect of a clinically meaningful treatment effect, or for overwhelming evidence of effectiveness) at this point

(of n=50 with mature primary outcome data, or at around 25% of the total scheduled events observed). This single interim analysis using a Lan-DeMets alpha spending approach with Fleming O'Brien boundaries has negligible effect on the required sample size (R 3.4.1 for Windows, package gsDesign).

Recruitment

Potential participants will be identified at outpatient clinic appointments. Posters and leaflets will also be displayed in the outpatient clinics and other appropriate locations.

Potentially eligible patients will receive a verbal explanation of the study and a patient information sheet by the attending clinical/research team.

Randomisation

Consent forms are completed on the day of treatment. Following confirmation of eligibility, consent and completion of baseline assessments, participants will then be randomly allocated to receive one of the two possible treatment options using an online computerised web system (REDCap, managed by the study data centre, University of Edinburgh). A minimization algorithm using centre, index ulcer size and duration will be used, including a random component to lessen predictability.

Blinding

As the DCD allograft is visible after application for a period of time, it is not possible to mask participants or the research/clinical teams to the treatment strategy. However the primary outcome assessments (verification of index ulcer healing visits) will be completed by an independent clinical assessor trained in the assessment of wound healing, who will have no previous involvement with, or knowledge of, the participant's index ulcer treatment and as such will be blind to the randomised treatment strategy (the DCD allograft is not expected to be visible after 4 weeks).

Follow-up periods

All participants will attend for follow-up at 1-week, 3-weeks, 6-weeks and 12-weeks, 6-months, 9-months and 12-months post-randomisation. At all follow-up visits, a clinical assessment will be undertaken and a photograph and planimetry tracing of the ulcer will be collected (unless healing has

been confirmed). The EQ-5D and the CCVUQ will be collected at baseline and the 12-week, 6- and 12-month follow-ups. Healthcare resource use (procedures, hospital, GP and community nurse visits, physiotherapy and other interventions), days lost from work and normal activities, carer time and out-of-pocket expenses related to leg ulcer care will also be collected from case notes and patient diaries during the initial procedure and at 6- and 12-months.

Fortnightly calls will be made after the 6-week follow-up to check if the ulcer has healed. If the participant reports that their ulcer has healed, they will be invited to attend a verification visit, where a photograph of the ulcer will be taken. This photograph will be sent to an independent assessor (blinded to treatment allocation) for assessment and confirmation of healing status. Ulcer healing is defined as complete re-epithelialisation of the index ulcer in the absence of a scab (eschar) with no dressing required confirmed by blinded photo assessment of healing.

If the ulcer is confirmed as healed, monthly telephone calls will be performed to check for recurrence. In the event that an ulcer is confirmed as healed, the recurrence, safety, resource use and health questionnaire data can be collected over the telephone or by post. If the participant fails to attend their appointment, attempts will be made to collect the QoL and patient resource use diaries by telephone or post. Participants will receive up to £10 for each visit attended as a contribution towards travel expenses.

Data collection and confidentiality

Participant data will be stored in the password-protected REDCap database. Participant details will be anonymised as each participant will be allocated a participant number. Identifiable data, including contact information, will also be recorded on paper forms and will be kept in a locked filing cabinet in a locked office at each investigational site. Data will be monitored for quality and completeness and missing data will be requested from the participating sites, as per the data monitoring plan.

Statistical analysis

All analyses will be carried out on an intention-to-treat (ITT) basis. A mixed-effects, logistic regression on the outcome of the proportion of those with the index ulcer healed at 12-weeks, with site as a random effect and randomised group as the treatment effect, along with index ulcer size and duration at baseline

(the minimisation factors) and any other baseline factors known or suspected to be strongly related to good or poor outcome, will form the model. Goodness of model fit will be examined using the Hosmer-Lemeshow approach. The robustness of the findings to any patterns of missing data (both assuming data are missing at random; and, if appropriate, informatively missing (missing not at random)) will be explored using appropriate sensitivity analyses.

Secondary outcomes (including the primary outcome at 12-months, time to index ulcer healing, reduction in ulcer area at 12-weeks, ulcer recurrence at 12-months, and quality of life) will be assessed using various statistical models appropriate to the distribution and nature of these outcomes, with the same modelling strategy as per the primary outcome above (e.g. missing data and appropriate model diagnostics).

The proportion healed at 12-months and the recurrence of the index ulcer at 12-months will be analysed as the primary outcome above. The time to index ulcer healing will be analysed using a survival type model (e.g. Cox proportional hazards model), and if the assumption regarding proportional hazards fails, using a Restricted Mean Survival Time (RMST) approach. The reduction in area of the index ulcer at 12 weeks over baseline will be analysed using a linear mixed model. The quality of life data (EQ-5D and CCVUQ questionnaire) will be analysed using a repeated measures mixed linear models (with repeated measures at 12-weeks, 6-months and 12-months and a suitable specified covariance structure), with the overall treatment effect and the evolution of any treatment effect over time modelled.

Cost-effectiveness analysis

A literature review will be conducted to identify other economic studies and other trials in comparable populations. A within-trial analysis and a decision model will be constructed. In both cases, the main analyses will be performed from the perspective of the NHS and Personal Social Services. Secondary analyses will be performed from a societal perspective. The price year will be 2018-2019. Discounting will be applied according to UK Government guidelines. The study will be reported according to consolidated guidelines for economic evaluation (CHEERS) (43).

The within-trial analysis will compare the treatment strategies within the 12-month time horizon of the clinical trial on an ITT basis. Data will be collected by case note review and questionnaires completed at baseline and follow-up.

Resource use items in hospital and community care, adverse events or complications will be recorded for each patient at 6- and 12-months. Resource use will be multiplied by UK unit costs obtained from published literature, Healthcare Resource Groups, and manufacturers' list prices to calculate overall costs. Utilities and QALYs will be calculated from the EQ-5D questionnaire. The extent of missing data will be assessed and appropriate methods to handle missing data will be applied.

The decision model provides a framework to incorporate evidence from other relevant studies and to extrapolate outcomes, such as ulcer healing and recurrence, beyond the trial reporting period. The Markov model will include the key ulcer-related health states and events that may occur during the lifetime of the patient. The data to support extrapolation may be taken from the trial (e.g. fitting parametric time-to-event functions to the trial data) or may come from external sources (such as the literature review or observational data)(44,45).

In both the within trial and model analyses, the incremental cost-effectiveness ratio will be calculated and compared to current UK decision making thresholds. Sensitivity analysis will be carried out to test the robustness of results to alternative assumptions about model structure or data. The cost-effectiveness acceptability curve will be calculated using probabilistic sensitivity analysis (43).

Data monitoring, safety and quality control

An independent Trial Steering Committee (TSC) and independent Data Monitoring Committee (iDMC) have been appointed. The main role of the TSC is to provide overall supervision of the trial and ensure that it is being conducted in accordance with the principles of Good Clinical Practice (GCP) and the relevant regulations, whilst the main role of the iDMC is to safeguard the interests of trial participants and to monitor the main outcome measures including safety and efficacy. A clinical trial manager, together with the Trial Management Group (TMG), will oversee trial progress.

All treatment related adverse events (AEs; related to the skin graft or leg ulcer only) will be collected as will all serious adverse events (SAEs). The chief investigator (CI) will be notified of all SAEs within 24 hours. All SAEs will be reported to the research ethics committee (REC) if, in the opinion of the CI, the event was related to the intervention. All related AEs and SAEs will be recorded and summarised by treatment strategy. These analyses will be descriptive, with any p-values calculated to be interpreted descriptively.

DISCUSSION

Although compression therapy is the mainstay of treatment, there is a need to explore new treatments for wounds that are chronic and persistent in nature. This is the first randomised controlled trial to evaluate the use of DCD allograft for the treatment of VLU. This study will provide important data on whether the use of the DCD allograft plus standard care is associated with improved outcomes compared to standard care alone and will provide important data on its effects on quality of life and healthcare costs.

Patient and public involvement

Focus groups were held with patients accessing the vascular clinic at Imperial College Healthcare NHS Trust to obtain views on the proposed study and the acceptability of the DCD allograft. The focus group helped to inform important aspects of the trial, including the number of visits and questionnaires used in the study. A Patient and Public Involvement (PPI) representative was included as a co-applicant and provided invaluable input in the study design. A PPI representative also sits on the TSC, providing real time input on study progress. He will also aid with dissemination of the results.

Ethics and dissemination: Ethical approval was granted by the Bloomsbury Research Ethics Committee (19/LO/1271). Amendments to the protocol will be updated on the ISRCTN record. All amendments to the protocol will be submitted to the sponsor for review before applying for approval from the REC and the Health Research Authority (HRA). Standard informed consent will be taken with freedom to withdraw at any time. The findings from this study will be published in a peer-reviewed journal, presented at national and international conferences and to participants (via emails and letters at the end of the study).

Current study status: The current version of the protocol is v9.0. The study commenced recruitment in October 2019.

Trial sponsor

Imperial College London is the main sponsor for this study. Delegated responsibilities are assigned to the NHS trusts taking part in this study.

Funding statement: This study is supported by the J P Moulton Charitable Foundation (grant number: N/A). The DCD allograft is provided free of charge by NHSBT. The design, management, analysis and reporting of the study are entirely independent of J P Moulton Charitable Foundation and NHSBT.

Availability of data and materials

Data will be made available on reasonable request.

Author contributions

AHD, SO, TL, FH and LB were involved in the design of the study and securing funding. MG, KP, NC, AB and KD were involved in the design of the study. AHD, SO and FH drafted the protocol and applied for ethical approval. AHD and SO supervise the project. FH and SP coordinate the project. SO, AHD and SP drafted the manuscript. JN and RLe will conduct the statistical analysis. DE will conduct the cost-effectiveness analysis. AC and RLo advise on any DCD-related issues. All authors have read and approved the final manuscript. AHD acts as guarantor.

Competing interests

AC and RL are affiliated to NHS Blood and Transplant (NHSBT), who are providing the DCD allografts free of charge. There are no other conflicts of interest to declare.

Abbreviations

ABPI: Ankle Brachial Pressure Index; AE: Adverse Event; CI: Chief Investigator; CCVUQ: Charing Cross Venous Ulcer Questionnaire; CRN: Clinical Research Network; CHEERS: Consolidated Health Economic Evaluation Reporting Standards; DCD: Decellularised dermis; EQ-5D: EuroQol Five-Dimension; GP: General Practitioner; GCP: Good Clinical Practice; HRA: Health Research Authority; ICER: Incremental Cost-Effectiveness Ratio; iDMC: Independent Data Monitoring Committee; ITT: Intention-to-treat; ISRCTN: International Standard Randomised Controlled Trial Number; NHSBT: National Health Service Blood and Transplant; NIHR: National Institute for Health Research; Patient and Public Involvement: PPI; REDCap: Research Electronic Data Capture; REC: Research Ethics Committee; RMST: Restricted Mean Survival Time; TMG: Trial Management Group; TSC: Trial Steering Committee; QALY: Quality-adjusted life year; QoL: Quality of Life; Venous leg ulceration: VLU.

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Figure legend

Figure 1: Flow diagram of the study protocol

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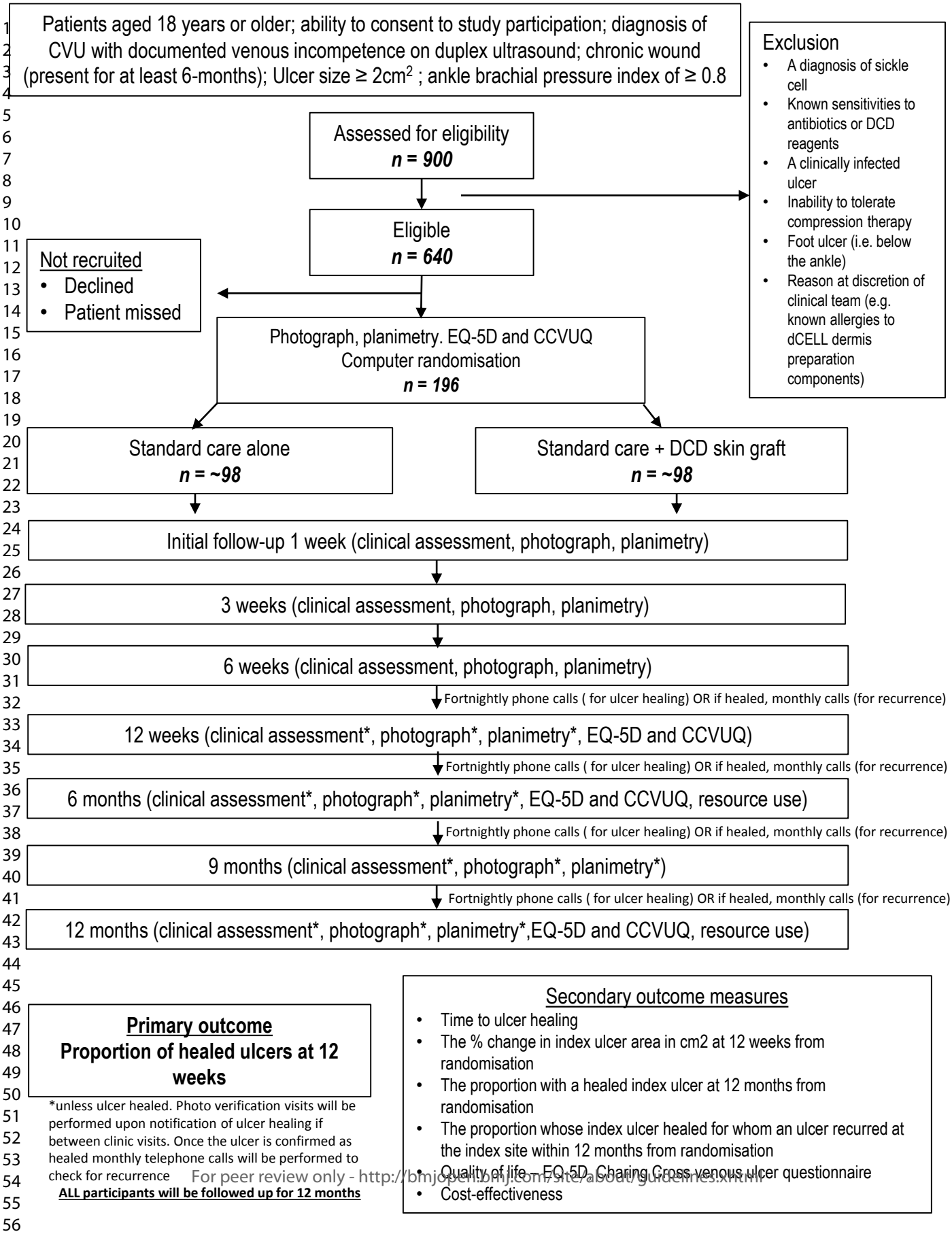
Figure 1: Flow diagram of the study protocol



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____3_____
	2b	All items from the World Health Organization Trial Registration Data Set	Included in trial registry and throughout manuscript
Protocol version	3	Date and version identifier	____15___
Funding	4	Sources and types of financial, material, and other support	_____16_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_____1-2_____
	5b	Name and contact information for the trial sponsor	_____15_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____15_____

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
	6b	Explanation for choice of comparators	5-7
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10, 12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10-12
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_8,12_
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	____11-12_____
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	____12____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____12-13____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	__10, 13-14_
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____12_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____14_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	____10_
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____14_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	____N/A____
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	15
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10-11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	16
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided on request

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	___N/A____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.