A 'test and treat' strategy for elevated wound protease activity for healing in venous leg ulcers (Protocol)

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[Intervention Protocol]

A 'test and treat' strategy for elevated wound protease activity for healing in venous leg ulcers

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ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To determine the effects on wound healing of a 'test and treat' strategy for diagnosing and treating high levels of wound protease activity in people with venous leg ulcers.

BACKGROUND

Description of the condition

Venous leg ulcers are a common and recurring type of complex wound (a wound which heals by secondary intention, i.e. by the growth of new tissue rather than by primary closure). Problems with the leg veins (such as damage to the valves, or blockages) reduce the efficient return of blood to the heart and increase the pressure in the leg veins (Ghauri 2010), which may result in venous leg ulcers. The precise chain of events that links the high venous pressures (chronic venous hypertension) with skin breakdown and a chronic wound is not fully understood (Coleridge Smith 1988; Valencia 2001).

Venous leg ulcers commonly occur on the gaiter region of the lower leg (from just below the ankle up to mid calf). A venous leg ulcer is defined as any break in the skin that has either been present for longer than six weeks or occurs in a person with a history of venous leg ulceration. Differential diagnosis of the type of leg ulcer (i.e. the underlying cause) is made by taking a clinical history, physical examination, laboratory tests and haemodynamic assessment (RCN 2006; SIGN 2010). The latter typically includes an assessment of arterial supply to the leg using the ankle brachial pressure index (ABPI), measured using a hand-held Doppler ultrasound scanner. Clinically significant arterial disease as a cause of ulceration is usually ruled out by an ABPI of at least 0.8 (Ashby 2014; NICE 2012a; SIGN 2010). True venous ulcers are moist, shallow and irregularly shaped and lie wholly or partly within the gaiter area of the leg. Leg ulcers can be associated with venous disease in combination with vascular disease, which impairs arterial blood supply; in these instances they are said to have a 'mixed aetiology'. Open skin ulceration due solely to limb ischaemia from vascular disease is less common.

Venous disease is a chronic condition which is characterised by

periods of ulceration (i.e., an open wound) followed by healing and then recurrence. An early cross-sectional survey reported that half of current or recent ulcers had been open for up to nine months and that 35% of people with leg ulcers had experienced four or more episodes (Callam 1987). This picture was supported by a subsequent cross-sectional study (Nelzen 1994).

More recent analysis of almost 1200 patients documented a 24-week healing rate of 76% and a recurrence at one year of 17% (Gohel 2005). Cohort data from 20,000 people have shown that initial wound area and duration accurately predict healing in venous leg ulcers (Margolis 2004). In this study, ulcers smaller than 10cm² with durations of less than 12 months at first visit had a 29% chance of not healing by the 24th week of care, whilst ulcers larger than 10cm² with duration longer than 12 months had a 78% chance of not healing by 24 weeks (Margolis 2004). A small cohort study has suggested that percentage change in area over the first four weeks of treatment may be an indicator of whether a wound will heal within 24 weeks (Kantor 2000). Older age has been identified as an independent risk factor for delayed healing (Gohel 2005) while slow healing is also a risk factor for recurrence, possibly because it reflects the extent of underlying venous insufficiency (Gohel 2005).

Accurate, current estimates of leg ulcer prevalence are hard to identify because most surveys do not differentiate between causes of leg ulceration, or do so per limb but not per patient (Moffatt 2004; Srinivasaiah 2007; Vowden 2009a). Estimates of the prevalence of open leg ulceration (any cause) range from 0.4 to 4.8 cases per 1000 (Graham 2003; Johnson 1995; Walker 2002), with the point prevalence of venous leg ulceration in Australian and European studies being between 0.1% and 0.3% (Nelzen 2008). A recent estimate suggests that venous ulceration has a point prevalence of 0.29 cases per 1000 in the United Kingdom (UK), whilst mixed arterial/venous leg ulceration has a point prevalence of 0.11 per 1000 (Hall 2014).

Venous ulcers are painful, can be malodorous and prone to infection, and may severely affect patients' mobility and quality of life. The presence of leg ulceration has been associated with pain, restriction of work and leisure activities, impaired mobility, sleep disturbance, reduced psychological well-being and social isolation (Herber 2007; Persoon 2004). In severe cases, ulceration can lead to limb amputation although this may be more common in patients with comorbid arterial insufficiency (Dumville 2009; Nelzen 1997; Valencia 2001). Recent research suggests that people with complex wounds, including those with venous leg ulcers, commonly see complete wound healing as the most important outcome to them (Madden 2014).

The financial cost of treating an unhealed leg ulcer in the UK has been estimated at around GBP 1700 per year (price year 2012) (Ashby 2014). Another evaluation estimated the average cost of treating a venous leg ulcer in the UK (based on costs for material for dressing changes) as between EUR 814 and EUR 1994 and, in Sweden as lying between EUR 1332 and EUR 2585 (price year 2002), with higher costs associated with larger and more chronic wounds (Ragnarson Tennvall 2005). Data from a German study, which estimated total costs including those classified as indirect or intangible costs, estimated mean annual costs of leg ulcers as EUR 9060 per patient (price year 2006). This figure is higher than other estimates because it includes non-health service costs to the patient and to society (Augustin 2012). In Bradford, UK, GBP 1.69 million was spent on dressings and compression bandages, and GBP 3.08 million on nursing time (estimates derived from resource use data for all wound types) during the financial year 2006 to 2007 (Vowden 2009b).

The first line treatment for venous leg ulcers is compression therapy in the form of bandages, stockings or mechanical devices (O'Meara 2012).This application of external pressure around the lower leg assists venous return and reduces venous reflux (Woo 2013). Alongside compression, wound dressings are commonly applied to open ulcers. The primary rationale for using a dressing is to protect the surface of the ulcer; however other considerations such as absorption of exudate or antimicrobial properties also play a role in treatment selection (O'Meara 2014). Other treatments for venous leg ulcers include venous surgery (removal of incompetent superficial veins) (Gohel 2007) and drugs such as pentoxifylline (Jull 2012). Other standard therapeutic approaches for complex wounds, such as optimising nutrition, and debridement (removal of dead, damaged or infected tissue), may also be offered. Despite these approaches, as discussed above, many venous leg ulcers remain hard to heal and further specialist treatments may be considered.

Description of the intervention

A 'test and treat strategy' involves the use of a diagnostic/prognostic test or assessment which precedes the potential use of a therapeutic intervention (a treatment): the use and/or timing of the treatment being dependent on the results of the test. A diagnostic test determines the current state of disease while a prognostic test indicates the likely future course of the disease process (Rector 2012).

Evaluations of test and treat strategies assess the use of combinations of testing and treating, as opposed to evaluating diagnostic test accuracy, or the effects of an treatment, separately. Test and treat approaches are therefore the best method for implementing a test where we need to consider both its diagnostic properties (i.e. sensitivity and specificity) and the healthcare outcomes from an effective test for the relevant indication (Ferrante di Ruffano 2012; Fryback 1991; Guyatt 1986; Lord 2006). As such, test and treat evaluations are pragmatic and give an indication of the real life results of implementing the strategy in terms of its impact on patient outcomes (Bossuyt 2009).

Just as with therapeutic interventions alone, the gold standard for assessing the impact of a test plus a treatment strategy is the randomised controlled trial (RCT) (Lord 2009). Guidance on assess-

ing the impact of tests in health care has been issued by various agencies including the UK National Institute for Health and Care Excellence (NICE 2012b) and the United States (US) Agency for Healthcare Research and Quality (AHRQ 2012; Rector 2012).
In this review we evaluate test and treat interventions for high protease activity in venous leg ulcers. This involves the use of a test for high protease levels in venous leg ulcers as well as subsequent targeted treatment decisions (possible use of treatments designed to reduce protease levels) which follow the test.

Protease activity in wounds

Proteases are enzymes which break down proteins into their constituent peptides and amino acids. The action of different proteases tends to be restricted to different proteins. The principal proteases involved in wound healing are the matrix metalloproteinases (MMPs) and the serine proteases which breakdown extracellular matrix (ECM) and connective tissue proteins such as collagen and elastin (Ladwig 2002; Nwomeh 1999).
Proteases are thought to play key roles in the normal wound healing process, being active in three of the phases of wound healing: inflammation, proliferation and remodelling (Trengove 1999). In the inflammation phase, proteases are used for the removal of damaged ECM, bacteria and foreign material (aiding autolytic debridement); in the proliferation phase proteases have a role in the degradation of capillary basement membrane for angiogenesis (growth of new capillary blood vessels) and in aiding detachment and migration of cells; and in the remodelling phase protease activity contributes to contraction and remodelling of scar ECM. It is thought that there is a burst of protease activity at the start of acute wound healing and, in normally-healing wounds, an activity peak in the first two to three days followed by a decline to very low levels after one week (Nwomeh 1998). Proteases may be present in an active or inactive state and protease activity is regulated through complex feedback mechanisms within the wound environment; only activated proteases have an impact on the wound healing process (McCarty 2013; Nwomeh 1999; Yager 2002).
In non-healing wounds it is thought that a complex inflammatory mechanism may result in proteases reaching higher levels and also persisting for longer than in healing wounds (Trengove 1999). Correlations between elevated levels of MMPs and delayed healing have been documented in pressure ulcers (Ladwig 2002) and foot ulcers in people with diabetes (Liu 2009) as well as in venous leg ulcers (Mwaura 2006; Serra 2013). However, there is limited evidence for a causal relationship between protease activity and wound healing.

Protease-modulating treatments

Novel treatments have been designed to modify the chronic wound environment by substantially reducing the activity of key proteases. The principle of such protease-modulating matrix treatments is both to absorb and bind excess proteases from wound fluids, thereby reducing levels of protease at the wound bed (Cullen 2002). The treatments do not, however, affect the expression of proteases on a cellular level (Lobmann 2006).
Interventions that reduce harmful levels of protease activity may potentially promote healing in wounds with persistently high protease activity. However evidence for this from RCTs has been limited across unselected wounds of different aetiologies, including venous leg ulcers (e.g. Andriessen 2009; Chin 2003; Kakagia 2007; Nisi 2005; Veves 2002). There is extremely limited evidence from a very small industry-sponsored study that screened wounds may respond better to protease-modulating treatment relative to all wounds (Cullen 2011).
Treatments can target specific proteases or can be broader spectrum, designed to inhibit all protease activity. Common protease-modulating treatments and their properties are described below. Products are listed by their generic names and, when possible, with examples of corresponding trade names and manufacturers. Both dressings and ointments are available; some dressings have silver ions incorporated, which are intended to reduce wound pathogens. Types of protease-modulating treatment which are listed in the British National Formulary (BNF 2014) include the following:

- Starch-based ointment: Cadesorb® (Smith & Nephew)
- Collagen matrix (bovine cartilage): Catrix® (Cranage)
- Gel, alginate and propylene glycol with extracellular matrix proteins: Xelma® (Mölnlycke)
- Collagen and oxidised regenerated cellulose matrix dressing: Promogran® (Systagenix)
- Collagen, silver and oxidised regenerated cellulose matrix dressing: Promogran® Prisma® (Systagenix)
- Cellulose acetate matrix, impregnated with polyhydrated ionogens ointment in polyethylene glycol basis dressing: Tegaderm® Matrix (3M)
- Adherent polymer matrix dressing containing nano-oligosaccharide factor (NOSF), with polyurethane foam film backing: UrgoStart® (Urgo)
- Non-adherent wound contact dressing containing NOSF: UrgoStart® Contact (Urgo).

This list is not exhaustive and other wound dressings such as Aquacel® (ConvaTec) are sometimes listed as having protease-modulating effects (Wound Care Handbook). A pragmatic approach will be adopted, and, where such dressings are used in a protease-modulating capacity we will include them in the review.

How the intervention might work

Very weak evidence suggests an inverse association between protease levels and healing in an unadjusted analysis of a mixed sample which included venous leg ulcers, foot ulcers in people with diabetes, and pressure ulcers (Cullen 2011; Serena 2011). On this basis, a test and treat process has been proposed, which involves

the testing of venous leg ulcers for levels of protease activity followed by targeted treatment of those deemed to have high levels of protease activity (and alternative care for the remaining wounds) (Systagenix 2013). This might mean that only wounds where high protease activity is present receive treatment designed to lower it. It is suggested that this strategy may reduce the time taken to heal for the wounds receiving targeted treatment, whilst avoiding unnecessary, expensive and potentially harmful use of the protease-modulating treatments in wounds where protease activity levels were not increased.

Why it is important to do this review

Venous leg ulcers are a relatively common type of complex wound that have a negative impact on people's lives and incur high costs for health services and society. Leg ulcers are painful, sometimes malodorous, prone to infection, and may severely affect patients' mobility and quality of life, and in severe cases, there is a risk of limb amputation. There are a number of treatments for venous leg ulcers, but many ulcers prove hard to heal.

There is a widespread view among experts in the field that proteases have an important role in wound healing and that a point of care test for elevated activity of commonly identified proteases has value (International Consensus 2011; Barrett 2011; Snyder 2011; Snyder 2012). Identification of wounds in which there is elevated protease activity is not considered possible on the basis of clinical examination alone; delayed wound healing is not proposed to be a universal indicator (Sibbald 2012; Snyder 2012). Limited data from an industry-sponsored study found that only 28% of 162 non-healing wounds of mixed aetiology were determined to have high protease activity (Serena 2011).

However, although a test for protease activity is now available, the impact of its use, in combination with subsequent targeted treatments with protease-modulating therapies where indicated, is unclear, and we are not aware of other reviews that address this question. A Cochrane review of the use of protease-modulating dressings in venous leg ulcers is currently underway (Westby 2014). A review of the diagnostic test accuracy of protease activity tests is also planned (Dumville 2015 [personal communication]).

In the current review we will assess the impact of testing venous leg ulcers for high levels of protease activity and treating those which record a positive test result; we will therefore be assessing the relative effectiveness of one or more tests for protease activity (together with thresholds for treatment) and the subsequent protease-modulating treatments. Our review will also compare a strategy of test and treat with non-directed usual care.

OBJECTIVES

To determine the effects on wound healing of a 'test and treat' strategy for diagnosing and treating high levels of wound protease activity in people with venous leg ulcers.

METHODS

Criteria for considering studies for this review

Types of studies

We will include published and unpublished randomised controlled trials (RCTs), including cluster RCTs, irrespective of the language of report. We will exclude quasi-randomised studies. We will only include RCTs reported only as abstracts when available data (either from the abstract itself or from the study authors) are sufficient for reasonable data extraction.

Types of participants

We will include trials recruiting adults described as having venous leg ulcers, managed in any setting. We will accept study authors' definitions of venous leg ulcers and will note the diagnostic methods and criteria used.

We will include trials which recruited people with venous leg ulcers and those with other types of complex wounds if the results for people with venous leg ulcers are presented separately or are available from the authors.

We will include participants at any stage in their treatment pathway, e.g. participants with or without hard to heal ulcers and with or without clinical infection of ulcers.

Types of interventions

We will include any RCT which evaluates a test and treat strategy for elevated protease activity in venous leg ulcers. In these studies the use of a specific test and treat strategy will be the only systematic difference between treatment groups. This will include trials in which all participants in the comparison arm received the same protease-modulating treatment but where a test and treat strategy was applied in the intervention arm as well as trials comparing test and treatment combinations versus each other, versus other interventions, or versus standard care. This may include comparisons of different test thresholds for the same test.

We will include RCTs whether or not compression therapy is reported as a concurrent treatment as long as the study groups received the same compression protocols. Where possible we will assess the impact of concurrent compression therapy on the treatment effect (see Subgroup analysis and investigation of heterogeneity).

We will exclude studies in which the test result is part of the inclusion criteria, i.e. participants with a positive test result were randomised to different protease-modulating treatments or to protease-modulating versus alternative treatments, as these will be included in a concurrent review evaluating protease-modulating matrix treatments for venous leg ulcers (Westby 2014).

Types of outcome measures

We list primary and secondary outcome measures below. If a trial is otherwise eligible (correct study design, population and intervention/comparator) but does not report a listed outcome, then we will contact the study authors where possible in order to establish whether a relevant outcome was measured but not reported. Trials will be included only where we are able to obtain data on a listed outcome.

We will report outcome measures at the latest time point available for a study (assumed to be length of follow-up if not specified) and the time point specified in the methods as being of primary interest (if this is different from the latest time point available). Where appropriate, for all outcomes we will class (and categorise) outcomes from:

- < 1 week to 8 weeks as short term;
- > 8 weeks to 24 weeks as medium term; and
- > 24 weeks as long term.

We will use our judgement to decide whether statistical pooling within these time categories is appropriate.

Primary outcomes

The primary effectiveness outcome for this review is wound healing. Trialists use a range of different methods of measuring and reporting this outcome. We will regard the following as the most relevant and rigorous measures of wound healing:

- Time to complete wound healing (correctly analysed using survival, time-to-event approaches). Ideally the outcome will be adjusted for appropriate covariates, e.g. baseline ulcer area/duration.
- Proportion of wounds completely healed during follow-up (frequency of complete healing).

We will use authors' definitions of complete wound healing; these will be reported.

Where both of the outcomes above are reported, we will present all data in a summary outcome table for reference but will focus on reporting time to healing. When time is analysed as a continuous measure, but it is not clear whether all wounds healed, we will document the use of the outcome in the study, but we will not extract, summarise or use the data in any meta-analysis.

The primary safety outcome is all reported adverse events. Where reported, we will extract data on all serious adverse events and all non-serious adverse events where a clear methodology for the collection of adverse event data was provided. This methodology should make it clear whether events were reported at the participant level or, where multiple events/person were reported, that an appropriate adjustment has been made for data clustering. We will not extract individual types of adverse events other than pain or infection (see Secondary outcomes). We will note where events are reported as being treatment-related.

Secondary outcomes

We will include the following secondary outcomes:

- Health-related quality of life: we will include quality of life where it is reported using a validated scale such as the SF-36 or EQ-5D or a validated disease-specific questionnaire such as the Cardiff Wound Impact Schedule. Ideally reported data will be adjusted for the baseline score. We will not include ad hoc measures of quality of life that are unlikely to be validated and would not be common to multiple trials.
- Pain scores: we will include pain (including pain at dressing change) only where mean scores with a standard deviation are reported using a scale validated for the assessment of pain levels, such as a visual analogue scale (VAS).
- Change (and rate of change) in wound size, with adjustment for baseline size (we will contact study authors to request adjusted means when not presented). When change or rate of change in wound size is reported without adjustment for baseline size, use of the outcome in the study will be documented, but data will not be extracted, summarised or used in any meta-analysis.
- Change in wound infection status (as defined by the study authors): we will include measures of incident cases of infection and cases of existing infections being resolved. We will not extract data on microbiological assays not clearly linked to a diagnosis of infection. We will use authors' definitions of infection.
- Resource use (when presented as a mean with standard deviation) including measures of resource use such as appointments for undergoing tests and receiving test results, number of dressing changes, number of nurse visits, length of hospital stay, need for other interventions.
- Costs associated with resource use (including estimates of cost-effectiveness).

Search methods for identification of studies

Electronic searches

We will search the following electronic databases:

- The Cochrane Wounds Group Specialised Register.
- The Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*) (latest issue).
- Ovid MEDLINE (1946 to present).

- Ovid EMBASE (1974 to present).
- EBSCO Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1982 to present).

We will use the following provisional search strategy in CENTRAL:
#1 MeSH descriptor: [Leg Ulcer] explode all trees
#2 (varicose ulcer* or venous ulcer* or leg ulcer* or stasis ulcer* or crural ulcer* or ulcus cruris or ulcer cruris):ti,ab,kw (Word variations have been searched)
#3 {or #1-#2}
#4 MeSH descriptor: [Peptide Hydrolases] explode all trees
#5 (protease* or proteinase* or metalloproteinase* or peptidase* or "peptide hydrolase" or "peptide hydrolases" or "proteolytic enzymes" or "proteolytic enzyme" or esteroprotease*):ti,ab,kw (Word variations have been searched)
#6 {or #4-#5}
#7 {and #3, #6} in Trials
We will adapt this strategy to search Ovid MEDLINE, Ovid EMBASE and EBSCO CINAHL. We will combine the Ovid MEDLINE search with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials in MEDLINE: sensitivity- and precision-maximising version (2008 revision) (Lefebvre 2011). We will combine the EMBASE search with the Ovid EMBASE filter developed by the UK Cochrane Centre (Lefebvre 2011). We will combine the CINAHL searches with the trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN 2011). There will be no restrictions with respect to language, date of publication or study setting.
We will also search the following clinical trials registries:
- ClinicalTrials.gov (http://www.clinicaltrials.gov/).
- WHO International Clinical Trials Registry Platform (http://apps.who.int/trialsearch/Default.aspx).
- EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/).

A shared search strategy will be employed for the current review and the review of effectiveness of protease-modulating treatments (Westby 2014).

Searching other resources

We will try to identify other potentially-eligible trials or ancillary publications by searching the reference lists of retrieved included trials, as well as relevant systematic reviews, meta-analyses and health technology assessment reports. We will contact corresponding authors of trials and the manufacturers and distributors of protease-modulating treatments or of tests for wound protease activity including Systagenix. We will search the websites and briefing documentation of regulatory bodies including the US Food and Drug Administration and the European Medical Association.

Data collection and analysis

Selection of studies

Two review authors will independently assess the titles and abstracts of the citations retrieved by the searches for relevance. After this initial assessment, we will obtain full text copies of all studies considered to be potentially relevant. Two review authors will independently check the full papers for eligibility; disagreements will be resolved by discussion and, where required, the input of a third review author. Where the eligibility of a study is unclear we will attempt to contact study authors. We will record all reasons for exclusion of studies for which we had obtained full copies. We will complete a PRISMA flowchart to summarise this process (Liberati 2009).
Where studies have been reported in multiple publications/reports, we will obtain all associated publications. Whilst the study will be included only once in the review, we will extract data from all reports to ensure that all available relevant data are obtained.

Data extraction and management

We will extract and summarise details of the eligible studies. Where possible we will extract data by treatment group for the prespecified interventions and outcomes in this review. Data will be extracted independently by two review authors; discrepancies will be resolved through discussion or by consultation with a third author. Where data are missing from reports, we will attempt to contact the study authors to request this information.
Where a study with more than two intervention arms is included, only data from intervention and control groups that meet the eligibility criteria will be extracted. Where the reported baseline data relate to all patients rather than to those in relevant treatment arms, the data for the whole trial will be extracted and this will be noted.
Outcome data will be collected for relevant time points as described in the Types of outcome measures section, and will be extracted on an intention-to-'test and treat' basis. However, where possible, we will also extract separate outcome data for those in the intervention arm who have positive results followed by protease-modulating treatment and those who have negative results followed by a different treatment.
Where possible we will extract the following data:
- bibliographic data including date of completion/publication
- country of origin
- unit of randomisation (participant/ulcer)
- unit of analysis
- trial design, e.g. parallel; cluster
- care setting
- number of participants randomised to each trial arm and number included in final analysis

- eligibility criteria and key baseline participant data including duration of venous insufficiency and current ulcer(s)
- details of treatment regimen received by each group including the nature, threshold and timing of test and the nature, timing and duration of subsequent treatment initiation. Details of treatment for participants with negative test results will also be reported
- details of any co-interventions
- number (%) of patients with positive and negative test results and the number of patients receiving each treatment
- primary and secondary outcome(s) (with definitions and, where applicable, time points)
- outcome data for primary and secondary outcomes (by group) including outcomes for participants randomised to the intervention(s) but with negative test results
- duration of follow-up
- number of withdrawals (by group), and number of withdrawals (by group) due to adverse events. Where possible separate data will be extracted for participants in the intervention group(s) with positive and negative test results
- publication status of trial
- source of funding for trial.

Assessment of risk of bias in included studies

Two review authors will independently assess included studies using the Cochrane Collaboration tool for assessing risk of bias (Higgins 2011a). This tool addresses six specific domains: sequence generation, allocation concealment, blinding, incomplete data, selective outcome reporting and other issues (Appendix 1). In this review we will record issues with unit of analysis, for example where a cluster trial has been undertaken but analysed at the individual level in the study report. For this review we will not assess blinding of patients and personnel as this is unlikely to be possible in a trial of the interventions included (testing and treating according to test results); all other domains will be assessed. We will assess blinding of outcome assessment and completeness of outcome data for each of the review outcomes separately. Because this is a review of a test and treat process, we will also consider differences in completeness of outcome data between patients with positive versus negative test results in the intervention group(s).
We will present our assessment of risk of bias using two 'Risk of bias' summary figures; one which is a summary of bias for each item across all studies, and a second which shows a cross-tabulation of each trial by all of the 'Risk of bias' items. We will summarise a study's risk of selection bias, detection bias, attrition bias, reporting bias and other bias. We anticipate that in many comparisons blinding of participants and personnel may not be possible.Therefore the assessment of the risk of detection bias will focus on whether blinded outcome assessment was reported. (Because wound healing can be a subjective outcome, it can be at high risk of measurement bias when outcome assessment is not blinded).
For trials using cluster randomisation, we will also examine the risk of bias considering: recruitment bias, baseline imbalance, loss of clusters, incorrect analysis and comparability with individually randomised trials (Higgins 2011b) (Appendix 2).

Measures of treatment effect

Time-to-event data (e.g. time to complete wound healing) will be reported as hazard ratios (HRs) when possible, in accordance with the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Deeks 2011). If studies reporting time-to-event data (e.g. time to healing) do not report an HR, then, when feasible, we plan to estimate this using other reported outcomes, such as numbers of events, through the application of available statistical methods (Parmar 1998; Tierney 2007). For dichotomous outcomes, we will calculate the risk ratio (RR) with 95% confidence intervals (CIs). For continuous outcome data, we will use the mean difference (MD) with 95% CIs for trials that use the same assessment scale. When trials use different assessment scales, we will use the standardised mean difference (SMD) with 95% CIs.

Unit of analysis issues

Where studies have been randomised at the participant level and outcomes measured at the wound level, for example for wound healing, we will treat the participant as the unit of analysis when the number of wounds assessed appears to be equal to the number of participants (e.g. one wound per person).
A possible unit of analysis issue that may occur is that randomisation has been carried at the participant level with the allocated treatment used on multiple wounds per participant (or perhaps only on some participants) but data are presented and analysed per wound (clustered data).
In cases where included studies contain some or all clustered data we plan to report this, noting whether data had been (incorrectly) treated as independent. We will record this as part of the risk of bias assessment. We do not plan to undertake further calculation to adjust for clustering as part of this review.

Dealing with missing data

It is common to have data missing from trial reports. Excluding participants from the analysis post randomisation or ignoring participants who are lost to follow-up compromises the randomisation and may introduce bias into the trial. If it is thought that study authors might be able to provide some missing data, we will contact them; however, it is likely that data will often be missing because of loss to follow-up. In individual studies, when data on the proportion of ulcers healed are presented, we plan to assume that randomly assigned participants not included in an analysis had an unhealed wound at the end of the follow-up period (i.e.

they will be considered in the denominator but not in the numerator).

When a trial does not specify participant group numbers before dropout, we will present only complete case data. For time-to-healing analysis using survival analysis methods, dropouts should be accounted for as censored data. Hence all participants will be contributing to the analysis. We acknowledge that such analysis assumes that dropouts are missing at random and there is no pattern of missingness. We will present data for all secondary outcomes as a complete case analysis.

For continuous variables, e.g. length of hospital stay, and for all secondary outcomes we will present available data from the study reports/study authors and do not plan to impute missing data. Where measures of variance are missing we will calculate these wherever possible (Higgins 2011a). If calculation is not possible we will contact study authors. Where these measures of variation remain unavailable and cannot be calculated, we will exclude the study from any relevant meta-analyses that we conduct.

Assessment of heterogeneity

Assessment of heterogeneity can be a complex, multi-faceted process. Firstly, we will consider clinical and methodological heterogeneity: that is the degree to which the included studies vary in terms of participant, intervention, outcome and characteristics such as length of follow-up. This assessment of clinical and methodological heterogeneity will be supplemented by information regarding statistical heterogeneity - assessed using the Chi^2 test (a significance level of $P < 0.10$ will be considered to indicate statistically significant heterogeneity) in conjunction with I^2 measure (Higgins 2003). I^2 examines the percentage of total variation across RCTs that is due to heterogeneity rather than chance (Higgins 2003). Very broadly, we will consider that I^2 values of 25%, or less, may mean a low level of heterogeneity (Higgins 2003), and values of more than 75%, or more, indicate very high heterogeneity (Deeks 2011). We will also examine the variability of the point estimates and the overlap of the confidence intervals, when I^2 values are less than 50%. Where there is evidence of high heterogeneity we will attempt to explore this further; see Data synthesis.

Assessment of reporting biases

Reporting biases arise when the dissemination of research findings is influenced by the nature and direction of results. Publication bias is one of a number of possible causes of 'small study effects', that is, a tendency for estimates of the intervention effect to be more beneficial in smaller RCTs. Funnel plots allow a visual assessment of whether small study effects may be present in a meta-analysis. A funnel plot is a simple scatter plot of the intervention effect estimates from individual RCTs against some measure of each trial's size or precision (Sterne 2011). Funnel plots are only informative when there are a substantial number of studies included in an analysis; we plan to present funnel plots for meta-analyses which include at least 10 RCTs using RevMan 5.3 (RevMan 2014).

Data synthesis

We will combine details of included studies in narrative review according to the comparison between intervention and comparator, the population and the time point of the outcome measurement. We will also use the timing of the protease activity test and the threshold for a positive result to structure the synthesis. We will consider clinical and methodological heterogeneity and undertake pooling when studies appear appropriately similar in terms of ulcer characteristics, intervention type, duration of treatment and outcome assessment.

In terms of meta-analytical approach, in the presence of clinical heterogeneity (review author judgement) and/or evidence of statistical heterogeneity we will use the random-effects model. We will only use a fixed-effect approach when clinical heterogeneity is thought to be minimal and statistical heterogeneity is estimated as non-statistically significant for the Chi^2 value and 0% for the I^2 assessment (Kontopantelis 2012). We will adopt this approach as it is recognised that statistical assessments can miss potentially important between-study heterogeneity in small samples, hence the preference for the more conservative random-effects model (Kontopantelis 2013). Where clinical heterogeneity is thought to be acceptable or of interest we may meta-analyse even when statistical heterogeneity is high but we will attempt to interpret the causes behind this heterogeneity and will consider using meta-regression for that purpose, if possible (Thompson 1999; Thompson 2002).

We will present data using forest plots where possible. For dichotomous outcomes we will present the summary estimate as an RR with 95% CI. Where continuous outcomes are measured in the same way across studies, we plan to present a pooled MD with 95% CI; we plan to pool SMD estimates where studies measure the same outcome using different methods. For time-to-event data, we plan to plot (and, if appropriate, pool) estimates of HRs and 95% CIs as presented in the study reports using the generic inverse variance method in RevMan 5.3 (RevMan 2014). Where time to healing is analysed as a continuous measure but it is not clear if all wounds healed, we will document the use of the outcome in the study but will not summarise or use the data in any meta-analysis.

'Summary of findings' tables

We will present the main results of the review in 'Summary of findings' tables. These tables present key information concerning the quality of the evidence, the magnitude of the effects of the interventions examined and the sum of available data for the main outcomes (Schünemann 2011a). The 'Summary of findings' tables also include an overall grading of the evidence related to each of the main outcomes using the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) approach. The

GRADE approach defines the quality of a body of evidence as the extent to which one can be confident that an estimate of effect or association is close to the true quantity of specific interest. The quality of a body of evidence involves consideration of within-trial risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias (Schünemann 2011b). We plan to present the following outcomes in the 'Summary of findings' tables for each comparison:

- Time to complete ulcer healing when analysed using appropriate survival analysis methods.
- Proportion of ulcers completely healed during the trial period.
- Study-defined serious and non-serious adverse events.

Subgroup analysis and investigation of heterogeneity

When possible we will perform subgroup analyses according to whether the intervention was delivered in conjunction with compression therapy or not. RCTs in which it is unclear whether concurrent compression therapy was used will be excluded from these analyses.

When possible we will conduct subgroup analyses based on the type of test for protease activity employed and/or the threshold used to define a positive test result. For example laboratory-based assays could be compared to point-of-care tests.

When possible, we will explore the influence of risk of bias on effect size. We will assess the influence of removing from meta-analyses studies classed as having high and unclear risk of bias. We will explore subgroups of studies that are assessed as having low risk of bias in all key domains, namely selection bias, detection bias and attrition bias.

Elements of this methods section are based on the standard Cochrane Wounds Group Protocol Template.

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REFERENCES

Additional references

AHRQ 2012
Agency for Healthcare Research and Quality (AHRQ), Chang SM, Matchar DB (Eds). *Methods Guide for Medical Test Reviews*. Vol. **AHRQ Publication No. 12-EHC017**, Rockville, MD: Agency for Healthcare Research and Quality (AHRQ), 2012.

Andriessen 2009
Andriessen A, Polignano R, Abel M. Monitoring the microcirculation to evaluate dressing performance in patients with venous leg ulcers. *Journal of Wound Care* 2009;**18**(4):148–50.

Ashby 2014
Ashby RL, Gabe R, Ali S, Adderly U. Clinical and cost-effectiveness of compression hosiery versus compression bandages in treatment of venous leg ulcers (Venous leg Ulcer Study IV, VenUS IV): a randomised controlled trial. *Lancet* 2014;**383**:871–9.

Augustin 2012
Augustin M, Brocatti LK, Rustenbach SJ, Schafer I, Herberger K. Cost-of-illness of leg ulcers in the community. *International Wound Journal* 2012;**11**(3):283–92.

Barrett 2011
Barrett S, Davis L, Edwards J, Fletcher J, Harding K, Jack, L, et al. Protease diagnostic in wound care: round-table meeting. *Wounds UK* 2011;**4**(4):supplement.

BNF 2014
British National Formulary, British Medical Association, British Royal Pharmaceutical Society of Great Britain. *British National Formulary*. London: British Medical Association, 2014.

Bossuyt 2009
Bossuyt PMM, McCaffery K. Additional patient outcomes and pathways in evaluations of testing. *Medical Decision Making* 2009;**29**(5):E30–8.

Callam 1987
Callam MJ, Harper DR, Dale JJ, Ruckley CV. Chronic ulcer of the leg: clinical history. *BMJ* 1987;**294**(6584): 1389–91.

Chin 2003
Chin GA, Thigpin TG, Perrin KJ, Moldawer LL, Schulz GS. Treatment of chronic ulcers in diabetic patients with a topical metalloproteinase inhibitor, doxycycline. *Wounds* 2003;**15**:315–23.

Coleridge Smith 1988
Coleridge Smith PD, Thomas P, Scurr JH, Dormandy JA. Causes of venous ulceration: a new hypothesis. *BMJ* 1988;

296(6638):1726–7.

Cullen 2002
Cullen B, Smith R, McCulloch E, Silcock D, Morrisson L. Mechanism of action of PROMOGRAN, a protease modulating matrix, for the treatment of diabetic foot ulcers. *Wound Repair and Regeneration* 2002;**10**(1):16–25.

Cullen 2011
Cullen B, Gibson M, Nesbit L. Targeted use of protease modulating dressings improves clinical outcomes. Wounds UK, Harrogate. 2011:Poster.

Deeks 2011
Deeks JJ, Higgins JPT, Altman DG (editors). Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. The Cochrane Collaboration.

Dumville 2009
Dumville JC, Worthy G, Soares MO, Bland JM. VenUS II: a randomised controlled trial of larval therapy in the management of leg ulcers. *Health Technology Assessment* 2009;**13**(55):1–206.

Dumville 2015 [personal communication]
Dumville JC, Westby M, Norman G. A point of care test for elevated protease activity in venous leg ulcers [Protocol]. Personal communication to G Norman March 2015.

Ferrante di Ruffano 2012
Ferrante di Ruffano L, Hyde CJ, McCaffery KJ, Bossuyt PMM, Deeks JJ. Assessing the value of diagnostic tests: a framework for designing and evaluating trials. *BMJ* 2012; **344**:e686. [DOI: http://dx.doi.org/10.1136/bmj.e686]

Fryback 1991
Fryback DG, Thornbury JR. The efficacy of diagnostic imaging. *Medical Decision Making* 1991;**11**(2):88–94.

Ghauri 2010
Ghauri ASK, Nyamekye IK. Leg ulceration: the importance of treating the underlying pathophysiology. *Phlebology / Venous Forum of the Royal Society of Medicine* 2010;**25**(S1): 42–51.

Gohel 2005
Gohel MS, Taylor M, Earnshaw JJ, Heather BP, Poskitt KR, Whyman MR. Risk factors for delayed healing and recurrence of chronic venous leg ulcers - an analysis of 1324 legs. *European Journal of Vascular and Endovascular Surgery* 2005;**29**(1):74–7.

Gohel 2007
Gohel MS, Barwell JR, Taylor M, Chant T, Foy C, Earnshaw JJ, et al. Long term results of compression therapy alone versus compression plus surgery in chronic venous ulceration (ESCHAR): randomised controlled trial. *BMJ* 2007;**335**(7610):83–7.

Graham 2003
Graham ID, Harrison MB, Nelson EA, Lorimer K, Fisher A. Prevalence of lower-limb ulceration: a systematic review of prevalence studies. *Advances in Skin and Wound Care* 2003;**16**(6):305–16.

Guyatt 1986
Guyatt GH, Tugwell PX, Feeny DH, Haynes RB, Drummond M. A framework for clinical evaluation of diagnostic technologies. *Canadian Medical Association Journal* 1986;**134**:587–94.

Hahn 2005
Hahn S, Puffer S, Torgerson DJ, Watson J. Methodological bias in cluster randomised trials. BMC Medical Research Methodology 2005; Vol. 5, issue 10. [DOI: 10.1186/1471-2288-5-10]

Hall 2014
Hall J, Buckley HL, Lamb KA, Stubbs N, Saramago P, Dumville JC, et al. Point prevalence of complex wounds in a defined United Kingdom population. *Wound Repair and Regeneration* 2014;**22**(6):694–700.

Herber 2007
Herber OR, Schnepp W, Rieger MA. A systematic review on the impact of leg ulceration on patients' quality of life. *Health and Quality of Life Outcomes* 2007;**5**:44. [DOI: 10.1186/1477-7525-5-44]

Higgins 2003
Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327** (7414):557–60.

Higgins 2011a
Higgins JPT, Altman DG, Sterne JAC (editors). Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from: www.cochrane-handbook.org. The Cochrane Collaboration.

Higgins 2011b
Higgins JPT, Deeks JJ, Altman DG (editors). Chapter 16: Special topics in statistics. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. The Cochrane Collaboration.

International Consensus 2011
Harding KG (ed), International Consensus Expert Working Group. *International consensus. The role of proteases in wound diagnostics. An expert working group review*. London: Wounds International, 2011.

Johnson 1995
Johnson M. The prevalence of leg ulcers in older people: implications for community nursing. *Public Health Nursing* 1995;**12**(4):269–75.

Jull 2012
Jull AB, Arroll B, Parag V, Waters. Pentoxifylline for treating venous leg ulcers. *Cochrane Database of Systematic Reviews* 2012, Issue 12. [DOI: 10.1002/14651858.CD001733.pub3]

Kakagia 2007
Kakagia DD, Kazakos KJ, Xarchas KC, Karanikas M, Georgiadis GS, Tripsiannis G, et al. Synergistic action of protease-modulating matrix and autologous growth factors in healing of diabetic foot ulcers. A prospective randomized trial.. *Journal of Diabetes and its Complications* 2007;**21**(6): 387–91.

Kantor 2000
Kantor J, Margolis DJ. A multicentre study of percentage change in venous leg ulcer area as a prognostic index of healing at 24 weeks. *British Journal of Dermatology* 2000; **142**(5):960–4.

Kontopantelis 2012
Kontopantelis E, Reeves D. Performance of statistical methods for meta-analysis when true study effects are non-normally distributed: a simulation study. *Statistical Methods in Medical Research* 2012;**21**(4):409–26.

Kontopantelis 2013
Kontopantelis E, Springate DA, Reeves D. A re-analysis of the Cochrane Library data: the dangers of unobserved heterogeneity in meta-analyses. *PLoS One* 2013;**26**:e69930.

Ladwig 2002
Ladwig GP, Robson MC, Liu R, Kuhn MA, Muir DF, Schultz GS. Ratios of activated matrix metalloproteinase-9 to tissue inhibitor of matrix metalloproteinase-1 in wound fluids are inversely correlated with healing of pressure ulcers. *Wound Repair and Regeneration* 2002;**10**(1):26–37.

Lefebvre 2011
Lefebvre C, Manheimer E, Glanville J. Chapter 6: Searching for studies. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. The Cochrane Collaboration.

Liberati 2009
Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Medicine* 2009;**6**:e1000100.

Liu 2009
Liu Y, Min D, Bolton T Nube V, Twigg SM, Yue DK, Mclennan SV. Increased matrix metalloproteinase-9 predicts poor wound healing in diabetic foot ulcers. *Diabetes Care* 2009;**32**(1):117–9.

Lobmann 2006
Lobmann R, Zemlin C, Motzkau M, Rescke K, Lehnert H. Expression of matrix metalloproteinases and growth factors in diabetic foot wounds treated with a protease absorbent dressing. *Journal of Diabetes and its Complications* 2006;**20** (5):329–35.

Lord 2006
Lord SJ, Irwig L, Simes RJ. When is measuring sensitivity and specificity sufficient to evaluate a diagnostic test, and when do we need randomized trials?. *Annals of Internal Medicine* 2006;**144**:850–5.

Lord 2009
Lord SJ, Irwig L, Bossuyt PMM. Using the principles of randomized controlled trial design to guide test evaluation. *Medical Decision Making* 2009;**29**(5):E1–12.

Madden 2014
Madden M. Is research in chronic wound care focusing on the outcomes that matter most to patients?. BSA Annual Conference. 2014; Vol. Changing Society.

Margolis 2004
Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. The accuracy of venous leg ulcer prognostic models in a wound care system. *Wound Repair and Regeneration* 2004;**12**(2): 163–8.

McCarty 2013
McCarty SM, Percival SL. Proteases and delayed wound healing. *Advances in Wound Care* 2013;**2**(8):438–47.

Moffatt 2004
Moffatt CJ, Franks PJ, Doherty DC, Martin R, Blewett R, Ross F. Prevalence of leg ulceration in a London population. *QJM: Monthly Journal of the Association of Physicians* 2004; **97**(7):431–7.

Mwaura 2006
Mwaura B, Mahendran B, Hynes N, Defreitas D, Avalos G, Adegbola T, et al. The impact of differential expression of extracellular matrix metalloproteinase inducer, matrix metalloproteinase-2, tissue inhibitor of matrix metalloproteinase-2 and PDGF-AA on the chronicity of venous leg ulcers. *European Journal of Vascular and Endovascular Surgery* 2006;**31**(3):306–10.

Nelzen 1994
Nelzen O, Berqvist D, Lindhagen A. Venous and non-venous leg ulcers: clinical history and appearance in a population study. *British Journal of Surgery* 1994;**81**:182–7.

Nelzen 1997
Nelzen O, Bergqvist D, Lindhagen A. Long-term prognosis for patients with chronic leg ulcers: a prospective cohort study. *European Journal of Vascular and Endovascular Surgery* 1997;**15**:500–8.

Nelzen 2008
Nelzen O. Prevalence of venous leg ulcer: the importance of the data collection method. *Phlebolymphology* 2008;**15**(4): 143–50.

NICE 2012a
National Institute for Health and Care Excellence. Clinical knowledge summaries. Leg ulcer - venous. http://cks.nice.org.uk/leg-ulcer-venous#!topic summary. Last revised September 2012. Accessed November 2014.

NICE 2012b
National Institute for Health and Clinical Excellence (NICE). *The Guidelines Manual*. London: National Institute for Health and Clinical Excellence. Available from www.nice.org.uk, 2012.

Nisi 2005
Nisi G, Brandi C, Grimaldi L, Calabro M, D'Aniello C. Use of a protease-modulating matrix in the treatment of pressure sores. *Chirugia Italiana* 2005;**57**(4):465–8.

Nwomeh 1998
Nwomeh BC, Liang HX, Diegelmann RF, Cohen IK, Yager DR. Dynamics of the matrix metalloproteinases MMP-1 and MMP-8 in acute open human dermal wounds. *Wound Repair and Regeneration* 1998;**6**(2):127–34.

Nwomeh 1999
Nwomeh BC, Liang HX, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. *Journal of Surgical Research* 1999;**81**(2): 189–95.

O'Meara 2012
O'Meara S, Cullum N, Nelson EA, Dumville JC. Compression for venous leg ulcers. *Cochrane Database of Systematic Reviews* 2012, Issue 11. [DOI: 10.1002/14651858.CD000265.pub3]

O'Meara 2014
O'Meara S, Al-Kurdi D, Ologun Y, Ovington LG, Martyn St-James M, Richardson R. Antibiotics and antiseptics for venous leg ulcers. *Cochrane Database of Systematic Reviews* 2014, Issue 1. [DOI: 10.1002/14651858.CD003557.pub5]

Parmar 1998
Parmar MKB, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Statistics in Medicine* 1998; **17**:2815–34.

Persoon 2004
Persoon A, Heinen MM, van der Vleuten CJM, de Rooji MJ, van de Kerkhof PCM, van Achterberg T. Leg ulcers: a review of their impact on daily life. *Journal of Clinical Nursing* 2004;**13**(3):341–54.

Ragnarson Tennvall 2005
Ragnarson Tennvall G, Hjelmgren J. Annual costs of treatment for venous leg ulcers in Sweden and the United Kingdom. *Wound Repair and Regeneration* 2005;**13**(1): 13–18.

RCN 2006
Royal College of Nursing. The nursing management of patients with venous leg ulcers: recommendations. http://www.rcn.org.uk/__data/assets/pdf_file/0003/107940/003020.pdf 2006. Accessed November 2014.

Rector 2012
Rector TS, Taylor BC, Wilt TJ. Chapter 12: Systematic review of prognostic tests. *Methods Guide for Medical Test Reviews (AHRQ Publication No. 12-EHC017)*. Rockville, MD. Available at www .effectivehealthcare .ahrq.gov/reports/final.cfm: Agency for Healthcare Research and Quality (AHRQ), 2012.

RevMan 2014
The Nordic Cochrane Centre. Review Manager (RevMan). 5.3. Copenhagen: The Cochrane Collaboration, 2014.

Schünemann 2011a
Schünemann HJ, Oxman AD, Higgins JPT, Deeks JJ, Glasziou P, Guyatt GH. Chapter 12: Interpreting results and drawing conclusions. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration.

Schünemann 2011b
Schünemann HJ, Oxman AD, Higgins JPT, Vist GE, Glasziou P, Guyatt GH. Chapter 11: Presenting results and 'Summary of findings' tables. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration.

Serena 2011
Serena T, Cullen B, Bayliff S, Gibson M, DeMarco D, Galbraith J, et al. Protease activity levels associated with healing status of chronic wounds. Wounds UK (Harrogate). 2011:Poster.

Serra 2013
Serra R, Buffone G, Falcone D, Molinari V, Scaramuzzino M, Gallelle L, et al. Chronic venous leg ulcers are associated with high levels of metalloproteinases-9 and neutrophil gelatinase-associated lipocalin. *Wound Repair and Regeneration* 2013;**21**(3):395–401.

Sibbald 2012
Sibbald RG, Snyder RJ, Botros M, Burrows C, Coutts P, D'Souza L, et al. Role of a point-of-care protease activity diagnostic test in Canadian clinical practice: a Canadian expert consensus. *Advances in Skin and Wound Care* 2012; **25**(6):267–75.

SIGN 2010
Scottish Intercollegiate Guidelines Network (SIGN). Management of chronic venous leg ulcers. Edinburgh: Scottish Intercollegiate Guidelines Network, Healthcare Improvement Scotland, 2010; Vol. 120.

SIGN 2011
Scottish Intercollegiate Guidelines Network (SIGN). Search filters. http://www.sign.ac.uk/methodology/filters.html#random (accessed December 2011).

Snyder 2011
Snyder RJ, Driver V, Fife CE, Lantis J, Peirce B, Serena T, et al. Using a diagnostic tool to identify elevated protease activity levels in chronic and stalled wounds: a consensus panel discussion. *Ostomy Wound Management* 2011;**57**(12): 36–46.

Snyder 2012
Snyder RJ, Cullen B, Nisbet LT. An audit to assess the perspectives of US wound care specialists regarding the importance of proteases in wound healing and wound assessment. *International Wound Journal* 2012;**10**(6): 653–60.

Srinivasaiah 2007
Srinivasaiah N, Dugdall H, Barrett S, Drew PJ. A point prevalence survey of wounds in north-east England. *Journal of Wound Care* 2007;**16**(10):413–9.

Sterne 2011
Sterne JAC, Egger M, Moher D. Chapter 10: Addressing reporting biases. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. Version 5.1.0 [updated March 2011]. The Cochrane Collaboration.

Systagenix 2013
Systagenix. WOUNDCHEK™ Protease Status. http://www.systagenix.co.uk/our-products/lets-test/woundchekandtrade-protease-status-218. Accessed November 2014 2013.

Thompson 1999
Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. *Statistics in Medicine* 1999;**18**:2693–708.

Thompson 2002
Thompson SG, Higgins JPT. How should meta-regression analyses be undertaken and interpreted?. *Statistics in Medicine* 2002;**21**:1559–74.

Tierney 2007
Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007;**7**(8):16.

Trengove 1999
Trengove NJ, Stacey MC, MacAuley S, Bennett N, Gibson J, Burslem F, et al. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Repair and Regeneration* 1999;**7**(6):442–52.

Valencia 2001
Valencia IC, Falabella A, Kirsner RS, Eaglestein WH. Chronic venous insufficiency and venous leg ulceration. *Journal of the American Academy of Dermatology* 2001;**44**: 401–21.

Veves 2002
Veves A, Sheehan P, Hau T, Pham DPM. Promogram Diabetic Foot Ulcer Study. A randomized controlled trial of Promogram (a collagen/oxidised regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. *Archives of Surgery* 2002;**137**(7):822–7.

Vowden 2009a
Vowden KR, Vowden P. The prevalence, management and outcome for patients with lower limb ulceration identified in a wound care survey within one English health care district. *Journal of Tissue Viability* 2009;**18**(1):13–19.

Vowden 2009b
Vowden K, Vowden P. The resource costs of wound care in Bradford and Airedale primary care trust in the UK. *Journal of Wound Care* 2009;**18**(3):93–102.

Walker 2002
Walker N, Rodgers A, Birchall N, Norton R, MacMahon S. The occurrence of leg ulcers in Auckland: results of a population-based survey. *New Zealand Medical Journal* 2002;**115**(1151):159–62.

Westby 2014
Westby MJ, Dumville JC, Stubbs N. Protease-modulating matrix treatments for venous leg ulcers. *Cochrane Database of Systematic Reviews* 2014, Issue [in preparation].

Woo 2013
Woo KY, Alavi A, Evans R, Despatis M, Allen J. New advances in compression therapy for venous leg ulcers. *Surgical Technology International* 2013;**23**:61–8.

Wound Care Handbook
Journal of Wound Care. Wound Care Handbook. http://www.woundcarehandbook.com/ Accessed February 2015.

Yager 2002
Yager DR, Nwomeh BC. The proteolytic environment of chronic wounds. *Wound Repair and Regeneration* 2002;**7**(6): 433–41.

* *Indicates the major publication for the study*

APPENDICES

Appendix 1. Assessment of risk of bias

1. Was the allocation sequence randomly generated?

Low risk of bias

The investigators describe a random component in the sequence generation process such as: referring to a random number table; using a computer random-number generator; coin tossing; shuffling cards or envelopes; throwing dice; drawing of lots.

High risk of bias

The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach, for example: sequence generated by odd or even date of birth; sequence generated by some rule based on date (or day) of admission; sequence generated by some rule based on hospital or clinic record number.

Unclear

Insufficient information about the sequence generation process provided to permit a judgement of low or high risk of bias.

2. Was the treatment allocation adequately concealed?

Low risk of bias

Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: central allocation (including telephone, web-based and pharmacy-controlled randomisation); sequentially-numbered drug containers of identical appearance; sequentially-numbered, opaque, sealed envelopes.

High risk of bias

Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on: using an open random allocation schedule (e.g. a list of random numbers); assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unconcealed procedure.

Unclear

Insufficient information provided to permit a judgement of low or high risk of bias. This is usually the case if the method of concealment is not described or not described in sufficient detail to allow a definite judgement, for example if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed.

3. Blinding - was knowledge of the allocated interventions adequately prevented during the study?

Low risk of bias

Any one of the following.

- No blinding, but the review authors judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding.
- Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken.
- Either participants or some key study personnel were not blinded, but outcome assessment was blinded and the non-blinding of others was unlikely to introduce bias.

High risk of bias

Any one of the following.

- No blinding or incomplete blinding, and the outcome or outcome measurement is likely to be influenced by lack of blinding.
- Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken and the outcome or outcome measurement is likely to be influenced by lack of blinding.
- Either participants or some key study personnel were not blinded, and the non-blinding was likely to introduce bias.

Unclear

Either of the following.

- Insufficient information provided to permit a judgement of low or high risk of bias.
- The study did not address this outcome.

4. Were incomplete outcome data adequately addressed?

Low risk of bias

Any one of the following.

- No missing outcome data.

- Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias).
- Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups.
- For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk was not enough to have a clinically relevant impact on the intervention effect estimate.
- For continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes was not enough to have a clinically relevant impact on observed effect size.
- Missing data have been imputed using appropriate methods.

High risk of bias

Any one of the following.

- Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups.
- For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk was enough to induce clinically relevant bias in the intervention effect estimate.
- For continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes was enough to induce clinically relevant bias in observed effect size.
- 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomisation.
- Potentially inappropriate application of simple imputation.

Unclear

Either of the following.

- Insufficient reporting of attrition/exclusions to permit a judgement of low or high risk of bias (e.g. number randomised not stated, no reasons for missing data provided).
- The study did not address this outcome.

5. Are reports of the study free of suggestion of selective outcome reporting?

Low risk of bias

Either of the following.

- The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way.
- The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).

High risk of bias

Any one of the following.

- Not all of the study's pre-specified primary outcomes have been reported.
- One or more primary outcomes are reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified.
- One or more reported primary outcomes of the study were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect).
- One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis.
- The study report fails to include results for a key outcome that would be expected to have been reported for such a study.

Unclear

Insufficient information provided to permit judgement of low or high risk of bias. It is likely that the majority of studies will fall into this category.

6. Other sources of potential bias

Low risk of bias

The study appears to be free of other sources of bias.

High risk of bias

There is at least one important additional risk of bias. For example, the study:

- had a potential source of bias related to the specific study design used; or
- has been claimed to have been fraudulent; or
- had some other problem.

Unclear

There may be a risk of bias, but there is either:

- insufficient information to assess whether an important risk of bias exists; or
- insufficient rationale or evidence that an identified problem will introduce bias.

Appendix 2. Risk of bias in cluster randomised trials

In cluster randomised trials, particular biases to consider include: (i) recruitment bias; (ii) baseline imbalance; (iii) loss of clusters; (iv) incorrect analysis; and (v) comparability with individually randomised trials.

(i) Recruitment bias can occur when individuals are recruited to the trial after the clusters have been randomised, as the knowledge of whether each cluster is an 'intervention' or 'control' cluster could affect the types of participants recruited.

(ii) Cluster randomised trials often randomise all clusters at once, so lack of concealment of an allocation sequence should not usually be an issue. However, because small numbers of clusters are randomised, there is a possibility of chance baseline imbalance between the randomised groups, in terms of either the clusters or the individuals. Although not a form of bias as such, the risk of baseline differences can be reduced by using stratified or pair-matched randomisation of clusters. Reporting of the baseline comparability of clusters, or statistical adjustment for baseline characteristics, can help to reduce concern about the effects of baseline imbalance.

(iii) Occasionally complete clusters are lost from a trial, and have to be omitted from the analysis. Just as for missing outcome data in individually randomised trials, this may lead to bias. In addition, missing outcomes for individuals within clusters may also lead to a risk of bias in cluster randomised trials.

(iv) Many cluster randomised trials are analysed by incorrect statistical methods, not taking the clustering into account. Such analyses create a 'unit of analysis error' and produce over-precise results (the standard error of the estimated intervention effect is too small) and P values that are too small. They do not lead to biased estimates of effect. However, if they remain uncorrected, they will receive too much weight in a meta-analysis.

(v) In a meta-analysis including both cluster and individually randomised trials, or including cluster randomised trials with different types of clusters, possible differences between the intervention effects being estimated need to be considered. For example, in a vaccine trial of infectious diseases, a vaccine applied to all individuals in a community would be expected to be more effective than if the vaccine was applied to only half of the people. Another example is provided by a discussion of a Cochrane review of hip protectors (Hahn 2005). The cluster trials showed large positive effect whereas individually randomised trials did not show any clear benefit. One possibility is that there was a 'herd effect' in the cluster randomised trials (which were often performed in nursing homes, where compliance with using the protectors may have been enhanced). In general, such 'contamination' would lead to underestimates of effect. Thus, if an intervention effect is still demonstrated despite contamination in those trials that were not cluster randomised, a confident conclusion about the presence of an effect can be drawn. However, the size of the effect is likely to be underestimated. Contamination and 'herd effects' may be different for different types of cluster.

CONTRIBUTIONS OF AUTHORS

Gill Norman: developed and coordinated the protocol; wrote and edited the protocol; approved the final version of the protocol prior to submission and is a guarantor of the protocol.

Maggie Westby: developed the protocol; edited and performed part of writing the protocol; advised on the protocol and approved the final version of the protocol prior to submission.

Nikki Stubbs: developed the protocol; edited and performed part of writing the protocol; advised on the protocol and approved the final version of the protocol prior to submission.

Jo Dumville: conceived the review question; developed and coordinated the protocol; secured funding; edited and performed part of writing the protocol; advised on the protocol; approved the final version of the protocol prior to submission and is a guarantor of the protocol.

Nicky Cullum: developed the protocol; edited and performed part of writing the protocol; advised on the protocol and approved the final version of the protocol prior to submission.

Contributions of editorial base:

E Andrea Nelson, Editor: advised on methodology, interpretation and protocol content. Approved the final protocol prior to submission.
Sally Bell-Syer: coordinated the editorial process. Advised on methodology, interpretation and content. Edited the protocol.
Rocio Rodriguez-Lopez: designed the search strategy and edited the search methods section.

DECLARATIONS OF INTEREST

Nicky Cullum was supplied by Kinetic Concepts Inc (KCI) (free of charge) three VAC therapy units and starter packs for use in a pilot RCT of negative pressure wound therapy for pressure ulcers. They also provided product training, support and access to the KCI 24hr advice service for clinical and technical queries. However KCI had no input into the design, conduct, analysis or reporting of that research or this review which concerns a different technology and a different patient group.

Jo Dumville: nothing to declare

Gill Norman: nothing to declare

Nikki Stubbs has received funding from pharmaceutical companies to support training and education events in the National health Service. She has received payments for non-product related educational sessions. These have always been unrelated to the subject matter of this review and have never been in support or in pursuit of the promotion of products.

Maggie Westby previously undertook work on methods for test and treat reviews while employed by NICE and KCE on UK and Belgian clinical guidelines. She has no financial declarations of interest.

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