Leeches (Hirudinea) for osteoarthritis

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Editorial group: Cochrane Musculoskeletal Group.


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ABSTRACT

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

To assess the benefits and harms of leeches compared with placebo, no intervention or any other active treatment in people with osteoarthritis.

BACKGROUND

Description of the condition

Epidemiology

Osteoarthritis (OA) is defined as a failure of one or more synovial joints (Felson 2012). It is a condition in which all the structures of the joint have undergone pathological change, often in concert. Its cardinal features are: focal loss of articular cartilage, proliferation of new bone and remodelling of the joint contour (Doherty 2010). OA preferentially targets certain large and small joints, while sparing others. The knee and hip are the principal large joints involved (Doherty 2010).

OA is the most common disease of joints in adults around the world (Felson 1988). In the United States, OA is the most frequent cause of disability among adults, and the fourth most common cause of hospitalisation (Murphy 2012). OA is the leading indication for joint replacement surgery in high-income countries; 905,000 knee and hip replacements were performed in the United States in 2009, at a cost of $42.3 billion (Murphy 2012). Between 2005 and 2010 the number of total hip arthroplasties in the United Kingdom increased by 16% (Pivec 2012).

Risk factors

OA is a complex disorder with multiple risk factors (Doherty 2010). These risk factors include heredity, trauma, advancing age and obesity (Sinusas 2012). OA is uncommon in adults aged less than 40 years and highly prevalent in those aged over 60 years (Felson 2012). OA affects 10% to 25% of people aged over 65 years (Doherty 2010). Women aged over 55 years experience OA more commonly than men of equivalent age (Shipley 2009), and this gender differential increases with advancing age (Felson 2012). Different racial groups are affected differently; hip OA, for example, is more common in Europeans than in Asians, but knee OA less common (Shipley 2009).
Clinical presentation

The characteristic symptoms of OA are joint pain, joint ‘gelling’ (i.e. stiffening and pain after any period of immobility), joint instability and loss of function. The characteristic signs are joint tenderness, crepitus (i.e. a cracking or grating feeling or sound) on movement, limitation of range of movement, joint effusion, bony swelling, and wasting of muscles (Shipley 2009). The joint pain of OA is typically activity-related. It comes on during or just after joint use (e.g. hip pain when climbing stairs), then gradually subsides. Early in the disease course, the pain of OA is episodic and self-limiting. With advancing disease, however, the pain becomes continuous, and may be troublesome even at night (Felson 2012). Inflammation is not usually a prominent clinical feature of OA (Doherty 2010).

Diagnosis

The diagnosis of OA is based on a history of joint pain worsened by movement (Sinusas 2012). Plain radiography often helps in confirming the diagnosis, but laboratory testing usually does not. The American College of Rheumatology (ACR) has developed diagnostic criteria for the presence of OA based on clinical criteria alone (for hand OA), and on clinical, laboratory and radiographic criteria (for hip and knee OA) (Brion 2010). The diagnostic criteria for knee OA are the most complex, and have recently been validated using arthroscopically-defined cartilage damage scores (Wu 2005). The ACR diagnostic criteria are summarised in Appendix 1, Appendix 2 and Appendix 3.

Management

Interventions to treat OA have been assessed in previous Cochrane reviews and the number of effective treatment options is limited, as shown below:

- **Therapeutic exercise** has a modest impact on pain reduction in OA of the knee and hip (Fransen 2008; Fransen 2009).
- **Surgical intervention** may be of benefit in some presentations of OA (Singh 2010; Verra 2013).
- **There may be benefit from transcutaneous electrostimulation (TENS), acupuncture, therapeutic ultrasound and electromagnetic field treatment** (Rutjes 2009; Manheimer 2010; Rutjes 2010; Li 2013).
- **There is no benefit from joint lavage for knee OA** (Reichenbach 2010) or from doxycycline for hip or knee OA (da Costa 2012).
- **Total joint replacement of the hip, knee, or shoulder is currently recommended for patients who have persistent severe OA pain and disability, despite maximal medical therapy** (Sinusas 2012).

Description of the intervention

‘Leech’ is derived from the Old English word læce, meaning physician (Porshinsky 2011). Leeches have been used extensively for the past half-century in plastic, reconstructive and trauma surgery (Derganc 1960; Porshinsky 2011). In the 1980s French microsurgeons began using leeches to assist with distal digital replantation involving arterial repairs only (Foucher 1981). The use of leeches for phlebotomy (i.e. bloodletting), and for specific therapeutic indications, dates back to ancient Egypt (Whitaker 2012). Leeching, or hirudotherapy, was popular in Western society until the First World War, when supplies of wild leeches could no longer be guaranteed from the usual sources in Eastern Europe. There has, however, been a steady resurgence from the 1970s onwards, associated with the commercial farming of leeches (Koeppen 2013). In Germany alone, approximately 100,000 therapeutic leech sessions take place each year, corresponding to around 350,000 leeches used annually (Koeppen 2013).

Leeches belong to the phylum Annelida (i.e. segmented invertebrate worms), class Hirudinea. An accepted high-level leech taxonomy is given at Appendix 4. Medicinal leeches all fall under the family Hirudinidae. The important biological characteristics of leeches are listed in Appendix 5.

Three leech species, Hirudo medicinalis, Hirudo verbena and Hirudo orientalis, have been widely used in Europe as therapy. There is currently some debate as to whether these are three reproductively isolated biospecies, or whether they are in fact a complex of closely related and potentially interbreeding species (Kutschera 2012). Other leech species that have been regularly used in therapy include Macrobodella decora (the American medicinal leech), Hirudinaria granulosa (the Indian medicinal leech), Hirudo michaeli and Hirudo nipponia, (Zaidi 2011).

Leech therapy involves (Yantis 2009):

- an initial leech bite, which is usually painless;
- an attachment period lasting from 20 to 45 minutes, during which the leech typically sucks between 5 and 15 mL of blood; and
- a post-attachment period, during which the wound may continue to bleed, for some hours.

Clear fluid flows copiously from the skin of the leech as it feeds (Worth 1951). After feeding, the tripartite jaw of the leech leaves a 0.3 cm diameter three-pronged bite wound on the host’s skin, sometimes referred to as the ‘Mercedes-Benz sign’. After the post-attachment period, the wound scabs over and the resulting scar quickly shrinks in size and becomes pale; within a few months it is indiscernible (Michalsen 2007).

Effectiveness of the intervention - empirical
There is empirical evidence, based on contemporary clinical experience in numerous countries and also on published and unpublished non-randomised studies, that leeches may be effective therapy for a wide range of medical disorders. Leeches seem to be effective in four broad disease categories, as follows (Michalsen 2007):

1. **Pain syndromes.** Benefits from leech therapy have been reported in cancer pain (Kalender 2010), cervical spine syndrome ("whiplash injury"), cervicobrachialgia, chronic low back pain/lumbago, iliosacral joint pain and migraine (Hyson 2005), osteoarthritis (ankle, hip, knee, shoulder, small joint), and sports injuries (Deuser 1971).

2. **Inflammatory states.** Benefits have been reported in abscesses associated with inflammation, acute gout (Panda 2012), inflammatory diseases of internal organs (as adjuvant therapy only), paronychia (Graham 1995), parotitis and sialadenitis (i.e. chronic inflammatory and dystrophic disease of the salivary glands) (Singh 2009), rheumatic disease (rheumatoid arthritis, fibromyalgia), systemic lupus erythematosus (Cheng 2005), tendinitis, and tendovaginitis (i.e. lateral epicondylitis).

3. **Circulatory disorders.** Benefits have been reported in acute superficial phlebitis, arterial hypertension, chronic venous insufficiency, frostbite (Munshi 2005), haematoma (including acute macroglossia, sublingual haematoma and periorbital haematoma) (Porshinsky 2011; Panda 2012), haemorrhoids, ocular circulation disorders, peripheral circulation disorders (including peripheral occlusive arterial disease), priapism, spider naevi, and varicose veins (RCT evidence from Nigar 2011). Leeches also seem to be effective in treating the circulatory complications of diabetes mellitus (Abdulkader 2013).

4. **Other conditions.** Benefits have been reported in dental pathology (alveolar abscess, oral pemphigus, periodontitis) (Srivastava 2010), herpes zoster, otitis media, otitis externa, ovarian cysts (Hyson 2005), and tinnitus (Michalsen 2007). Leeches may be of benefit in some neurological disorders, such as epilepsy (Sun 2007).

**Safety of the intervention - empirical evidence**

Leeches are contraindicated in pregnancy, in infants and in patients with chronic gastrointestinal and other bleeding disorders. They should not be used in patients taking anticoagulant medication, or in patients with anaemia, haemophilia, known protein allergies, or serious organic disease with immunosuppression (including HIV infection) (Michalsen 2007). For cosmetic reasons, leeches should not be used in patients with a tendency to keloid scar formation (Michalsen 2007).

Provided the above precautions are observed, the therapeutic use of leeches appears to be safe. Systemic infections and septicaemias due to *Aeromonas* spp bacteria, which are naturally present in the digestive tract of leeches (Appendix 5), have been described only in the context of reconstructive and trauma surgery, and in patients who were severely injured or otherwise acutely ill (Whitaker 2012). The infection risk from the use of leeches in non-traumatised patients is considered to be minimal (Lent 1986), and with careful selection and preparation of the leeches it may be as low as zero per cent (de Chalain 1996).

After many years of demonstrably safe clinical use, *Hirudo medicinalis* received official approval as a medical device in 1984 from the USA's Food and Drug Administration (Rados 2004).

**How the intervention might work**

The principal mechanism of action in leech therapy appears to be the secretion of a complex mixture of biologically active substances from the salivary glands of the leech at the time of biting, and the injection of these active substances into the animal host (Singh 2009). The exact composition and relative mix of the secreted bioactive substances varies between leech species (Sawyer 1986), and many substances have not yet been identified. The active substances include (Singh 2009):

1. **Anaesthetic and analgesic compounds.** A specific analgesic within leech saliva is yet to be identified (Koeppen 2013).

2. **Anti-inflammatory agents.** These include fibrinolytic enzymes (Abdulkader 2013), collagenase and hyaluronidase (Lone 2011).

3. **Anticoagulant, thrombolytic and vasodilatory agents.** These include antiplatelet agents and factor Xa inhibitors (Abdulkader 2013). All leeches appear to secrete hirudin, a potent anticoagulant which was first demonstrated in leech saliva by the English physiologist, John Haycraft (Haycraft 1884); the synthetic analogues desirudin and lepirudin have recently been approved by the FDA (Abdulkader 2013).

4. **Other substances.** Bactericidal and bacteriostatic agents.

**Why it is important to do this review**

OA is a painful chronic disease, and a major influence on a patient's quality of life.

Because of its high global prevalence, especially in the elderly, OA is now a leading worldwide cause of disability (Felson 2012). This global prevalence is increasing - both due to the progressive ageing of Western societies and because of increasing obesity, a major risk factor for OA (Felson 2012). In the United States, OA prevalence will increase by 66% to 100% by 2020 (Felson 2012). Current treatment regimens for OA are sub-optimal. There is an urgent need for agents to halt or reverse OA, but no such products exist (Shipley 2009). While acetaminophen and topical analgesics appear safe in OA, the safety profile of oral NSAIDs and of opiates is poor (Reid 2012). Most drug therapies provide mild to moderate...
pain relief only. Their long-term safety and long-term efficacy are undetermined, as are their effects in diverse populations (e.g. adults aged over 65 years) (Reid 2012). There are professional concerns around the long-term safety of metal-on-metal arthroplasties (Pivec 2012).

An inexpensive, effective, low-risk, non-pharmacological intervention would constitute a significant advance in OA treatment.

**OBJECTIVES**

To assess the benefits and harms of leeches compared with placebo, no intervention or any other active treatment in people with osteoarthritis.

**METHODS**

Criteria for considering studies for this review

**Types of studies**

Randomised controlled trials (RCTs) and controlled clinical trials. Studies may be single-blind, double-blind, triple-blind or unblinded (i.e. 'open label').

**Types of participants**

Participants will be persons of any age with clinically- or radiologically-confirmed primary osteoarthritis.

**Types of interventions**

We will consider studies for inclusion where:

- any leech species is administered to a human host;
- in any dose (i.e. number of applied leeches, periodicity of exposure); and
- at any anatomical site.

Leech-derived molecular products are outside the scope of this review, and are not included.

**Control**

The control group will receive placebo (i.e. sham leech exposure), no treatment, or any other active intervention.

**Types of outcome measures**

**Major outcomes**

1. Pain (see Appendix 6 for our proposed hierarchy of data extraction).
2. Function (see Appendix 7 for our proposed hierarchy of data extraction).
3. Radiographic joint structure changes (see Appendix 8 for our proposed hierarchy of data extraction).
4. Change in Quality of Life score.
5. Number of participants experiencing any adverse event.
6. Number of participants experiencing serious adverse events, defined as events resulting in hospitalisation, persistent or significant disability, congenital abnormality or birth defect in offspring, or other life-threatening event or death (European Commission 2010).
7. Dropouts (all-cause study withdrawal, number of participants withdrawing because of adverse effects).

If pain or function outcomes are reported at several time points, we will extract the measure at the end of the trial or at a maximum of three months after termination of therapy, whichever came first.

**Minor outcomes**

1. Number of participants experiencing non-serious local adverse events (e.g. pain, itching, infection).
2. Number of participants experiencing non-serious systemic adverse events (e.g. fever, headache, confusion).

We will report the above secondary outcomes as additional tables in our review, if appropriate.

**Search methods for identification of studies**

We will conduct systematic searches for randomised controlled trials. We will not apply any language, publication year, or publication status restrictions. If trial reports are unclear, we will attempt to contact original authors for clarification and for further data. We will arrange translations of papers where necessary. If several reports describe the same trial we will choose the most complete report as the representative study in our review, and carefully check the remaining reports for additional information on study methodology and outcomes.

**Electronic searches**

We will systematically search the following databases for primary studies from inception to current date:

- the Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library), using the generic search strategy set out in Appendix 9;
• four additional bibliographic databases: MEDLINE, EMBASE, AMED, and CINAHL.

We will include as an appendix in our systematic review the precise search strategy used for each separate electronic database.

Searching other resources
We will search national and international trial registers (www.clinicaltrials.gov, www.controlled-trials.com, www.actr.org.au and www.umin.ac.jp/ctr) to identify ongoing or unpublished studies. We will search dissertation abstracts and central databases (e.g. Networked Digital Library of Theses and Dissertations), conference abstracts (e.g. proceedings of the European League against Rheumatism, the American College of Rheumatology and the Osteoarthritis Research Society) and other ‘grey literature’ to identify potentially relevant studies. We will search the reference lists of all primary studies and relevant review articles for additional references. We will contact authors of identified trials and ask them to identify other published and unpublished studies. We will also contact suppliers of leeches and leech-derived products, and experts in this area.

Data collection and analysis

Selection of studies
All phases of screening and selection of studies to be included in the review will be done independently and in duplicate, by two review authors (AC and AM). Where there is disagreement on the potential relevance of a particular report, we will resolve this through discussion. Where doubt persists, we will retrieve the full text of the report for inspection. We will retrieve for further assessment, and for a final decision on inclusion, the full text of all those reports judged to be potentially relevant (see Criteria for considering studies for this review). Once the full texts are obtained, AC and AM will inspect the full reports and independently decide whether or not they meet the inclusion criteria. AC and SC will not be blinded to the names of the authors, source institutions, or journal of publication. Where difficulties or disputes on study eligibility arise, we will ask SC or GPF for help; if agreement is still not reached, we will add these disputed studies to those awaiting assessment, and contact the authors of the original reports for clarification.

PRISMA flow diagram
We will present a PRISMA flow diagram to illustrate the results of our searches and the process of screening and selecting studies for inclusion in the review (Moher 2009).

Data extraction and management
We will design a data extraction form to record data from five key domains of each included study, as follows:

1. **Study characteristics.** Date of study, total study duration, number of study centres and their location, study withdrawals.
2. **Participants.** N, mean age, age range, gender distribution, sociodemographic characteristics, ethnicity, OA site and severity, inclusion criteria, exclusion criteria.
3. **Interventions.** For each intervention: total participants in intervention arm, leech species and number of leeches used, leeching regimen, cost.
4. **Controls.** For each control: total participants in control arm. Where control is an active pharmacological intervention: nature, dose, route of administration, cost.
5. **Outcomes.** Outcomes specified and outcomes actually recorded and reported, time points reported.

For included studies, AC and AM will then independently extract the data, using the agreed form. AC and AM will resolve discrepancies through discussion; failing resolution, we will consult SC or GPF. We will enter data into Review Manager (RevMan) software version 5.2 (RevMan 2013) and check data for accuracy. When information regarding any data items is unclear, we will contact the authors of the original reports to seek further details.

Assessment of risk of bias in included studies
AC and AM will independently assess the methodological quality of each included study using the Cochrane Collaboration’s ‘Risk of bias’ tool (Higgins 2011).

The study features assessed will include:

1. Random sequence generation.
2. Allocation concealment.
3. Blinding of participants and personnel.
5. Incomplete outcomes data.
7. Other potential sources of bias (e.g. baseline imbalance, the use of an insensitive instrument to measure outcomes, and the use of blocked randomisation).

We will record each of these domains as ‘Low risk’, ‘High risk’ or ‘Unclear risk’, with a brief summary of our reasoning provided in table format. If we identify a domain as being at ‘Unclear risk’, we will attempt to seek clarification from trial authors. We will resolve disagreements between ourselves by discussion and failing this, by involving a third review author (SC or GPF).

Appendix 10 gives more information about our planned bias assessment scheme.

Measures of treatment effect
Dichotomous data
We will calculate risk ratios (RRs) with 95% confidence intervals (CIs) for dichotomous outcomes. Where appropriate, we will express estimated effects as NNTB (number needed to treat, to benefit). The NNTB corresponds mathematically to the inverse of the risk difference, and clinically to the number of patients to be treated to achieve one desirable event. It will be calculated using the pooled RR. We will also calculate NNTH (number needed to treat to harm).

Continuous data
For continuous variables, we will calculate a mean difference (MD) or standardised mean difference (SMD), along with 95% CIs, as follows:
- when two or more studies present their data derived from the same instrument of evaluation, and with the same units of measurement, we will pool data as an MD;
- conversely, when primary studies express the same domains through different instruments, and different units of measurement, we will use the SMD.

Unit of analysis issues
If any trials have multiple treatment groups, the 'shared' comparison group will be divided into the number of treatment groups, and comparisons between each treatment group and the split comparison group will be treated as independent comparisons. For RCTs with a crossover study design, where the only data available are summary measures of effect, along with precision estimates, we will use the generic inverse variance method to analyse the data.

Dealing with missing data
Where data are missing, we will contact trial authors directly to obtain this missing information. For all outcomes, in all studies, we will carry out analyses, as far as possible, on an intention-to-treat basis, i.e. we will attempt to include all participants randomised to each group in the analyses, and we will analyse all participants in the group to which they were allocated, regardless of whether or not they received the allocated intervention.

For continuous data that are missing, we will estimate standard deviations from other available data (e.g. standard errors), or else impute them using methods suggested by Higgins (Higgins 2011). We will make no assumptions about loss to follow up for continuous data, and will base our analyses on those participants completing the trial. We will perform a sensitivity analysis by calculating the treatment effect of including and excluding the imputed data, to see whether this alters the outcome of the analysis. We will investigate the effect of study withdrawals and exclusions by conducting worst- versus best-case scenario analyses.

If there is discrepancy between the number randomised and the number analysed in each treatment group, we will calculate and report the percentage lost to follow up in each group. If study withdrawals exceed 10% for any trial, we will assign the worst outcome to those lost to follow up for dichotomous outcomes, and assess the impact of this sensitivity analysis against the results for those completing the study. Where it is not possible to obtain missing data, this will be recorded in the data extraction form and reported in the ‘Risk of bias’ table. For included studies, we will note levels of attrition. We will explore the impact of including studies with high levels of missing data in the overall assessment of treatment effect, by using sensitivity analyses. The potential impact of missing data will be explained in the Discussion section of our systematic review.

Assessment of heterogeneity
We will assess heterogeneity between pooled trials using the Chi² test; in conjunction with the I² statistic, which describes the percentage of the variability in effect estimates that is due to heterogeneity rather than to sampling error, or to chance (Higgins 2011). All P values will be two-sided. We will consider a P value of < 0.10 as statistically significant.

If enough trials are identified, we will explore sources of between-trial heterogeneity using subgroup analyses. We will display results graphically using forest plots, with a summary statistic presented if there is no major statistical heterogeneity (i.e. no overlap of confidence intervals in the forest plots). As recommended in the Cochrane Handbook (Higgins 2011), we will interpret different magnitudes of I² to indicate between-trial heterogeneity broadly as follows, and while additionally taking into account the size of the individual trials:
- 0% to 40%: heterogeneity might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- ≥ 75%: may represent considerable heterogeneity.

Assessment of reporting biases
If ten or more trials contribute data to a meta-analysis, we will assess publication bias by preparing a funnel plot. We will then perform a visual assessment of funnel plot asymmetry. We will carry out exploratory analyses to investigate any suggestion of visual asymmetry in the funnel plots. Our searches for trials listed in clinical trial registers and trial protocols should help to avoid publication bias and to assess outcome selection bias. Where necessary, we will contact study authors in an attempt to either establish a full dataset or else obtain reasons for the non-reporting of certain outcomes.
Data synthesis

We will perform statistical analysis using RevMan 5.2 (RevMan 2013). We will pool treatment effect estimates across trials using a standard inverse variance random-effects model, which will account for anticipated between-study variance (Higgins 2011). We will express continuous outcomes as effect sizes in standard deviation units, with the differences in mean values at the end of follow-up across treatment groups being divided by the pooled standard deviation. If differences in mean values at the end of treatment are unavailable, we will use differences in mean changes. If some of the required data are unavailable, we will use approximations as proposed by Reichenbach 2007.

We will make the following judgments in respect of effect sizes between the experimental and the control groups (Jüni 2006):
- an effect size of -0.20 we will consider a small difference;
- an effect size of -0.50 we will consider a moderate difference; and
- an effect size of -0.80 we will consider a large difference.

We will convert effect sizes of pain intensity and function to odds ratios (Higgins 2011) as the first step in deriving numbers needed for both the intervention and placebo, and also the NNTH for both intervention and placebo.

We will define treatment response as a 50% improvement in scores (Clegg 2006). With a median standardised pain intensity at baseline of 2.4 standard deviation units, as observed in large OA trials (Nüesch 2009), we anticipate that a median of 31% participants in the placebo group will achieve an improvement in pain scores of 50% or more. We will use this percentage as the control group response rate to derive from ORs the response rate in the experimental group.

We will derive NNTHs for treatment response on pain by calculating the inverse of the difference between the experimental and the control group response rates. Based on the median standardised Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) function score at baseline of 2.7 standard deviation units, and the median standardised decrease in function score of 0.50 standard deviation units (Nüesch 2009), we anticipate that 26% of participants in the placebo group will achieve a reduction in function of 50% or more. Again, we will use this percentage in the control group response rate to derive from ORs the response rate in the experimental group, and then use these values to calculate NNTHs for treatment response on function.

With regard to adverse events, large placebo-controlled OA trials have observed the following risks (Nüesch 2009):
- a median risk of 150 participants with any adverse events per 1000 patient-years;
- a median risk of 150 participants with any adverse events per 1000 patient-years; and
- 17 drop-outs due to adverse events per 1000 patient-years.

We will use these estimates to calculate NNTHs for safety outcomes.

Subgroup analysis and investigation of heterogeneity

If sufficient data are available, we will perform subgroup analyses to explore the effects of:
- different exposure regimens (single exposure to leeches versus multiple exposures); and
- leeches for OA at different anatomical sites (knee, hip, shoulder, etc).

Sensitivity analysis

Where possible, we will perform sensitivity analyses to explore the effects of various aspects of trial and review methodology, including the effects of missing data and of whether or not allocation was concealed.

If sufficient data are available, we will also perform sensitivity analyses to determine the impact of excluding studies of lower methodological quality, for example:
- trials at high or unclear risk of bias;
- unpublished studies (since these may not have been subjected to the peer review process and may have intrinsic biases);
- industry-sponsored studies; and
- trials that have not assessed adherence.

'Summary of findings' table

We will generate a 'Summary of findings' (SoF) table using outcome data for the following seven outcomes:
1. Pain.
2. Function.
3. Radiographic joint structure.
4. Quality of Life.
5. Any adverse event.
6. Serious adverse events.
7. Dropouts.

Within the SoF table we will use the GRADE approach (Higgins 2011) to assess and summarise the strength of the evidence for each outcome. We will rate evidence as 'High', 'Moderate', 'Low' or 'Very low' quality.

Domains that may decrease the quality of the evidence are:
1. Limitations in the study design.
2. Indirectness of evidence.
3. Unexplained heterogeneity or inconsistency of results.
4. Imprecision of results (i.e. wide confidence intervals).
5. High probability of publication bias.

We will downgrade the quality of the evidence by one level (by up to a maximum of three levels for all factors), for each domain in which poor quality is encountered (Higgins 2011).

Conversely, we will upgrade the quality of the evidence where there is a large magnitude of effect, where all plausible confounding
would reduce a demonstrated effect (or else suggest a spurious effect when results show no effect), or where there is an obvious dose-response gradient (Higgins 2011).

ACKNOWLEDGEMENTS

We thank Tamara Rader for her assistance in developing the search strategy.

REFERENCES

Additional references

Abdualkader 2013

Bielecki 2011

Brion 2010

Cheng 2005

Clegg 2006

da Costa 2012

de Chalain 1996

Derganc 1960

Deuser 1971

Doherty 2010

Eroglu 2001

European Commission 2010

Felson 1988

Felson 2012

Foucher 1981

Fransen 2008

Fransen 2009
Leeches (Hirudinea) for osteoarthritis (Protocol)

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Leeches (Hirudinea) for osteoarthritis (Protocol)

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Reid 2012

RevMan 2013 [Computer program]

Rutjes 2009

Rutjes 2010

Sawyer 1986

Shipley 2009

Singh 2009

Singh 2010

Sinusas 2012

Srivastava 2010

Sun 2007

Valauri 1991

Verra 2013

Wade 1990

West 1994

Whitaker 2012

Worth 1951

Wu 2005

Yantis 2009

Zaidi 2011

* Indicates the major publication for the study
APPENDICES

Appendix 1. American College of Rheumatology diagnostic criteria for hand OA\textsuperscript{a,b}

Hand pain, aching or stiffness with three out of the following four features:

1. Hard tissue enlargement of two or more of ten selected hand joints.\textsuperscript{c}

2. Fewer than three swollen metacarpophalangeal joints.

3. Hard tissue enlargement of two or more distal interphalangeal (DIP) joints

4. Deformity of one or more of ten selected hand joints.\textsuperscript{c}

\textsuperscript{a} Adapted from Brion 2010.
\textsuperscript{b} Hand OA is readily diagnosed by clinical criteria alone; there are no recommended laboratory and/or radiographic diagnostic criteria.
\textsuperscript{c} Selected joints are the second and third DIP joints, the second and third proximal interphalangeal joints and the first carpometacarpal joint of both hands.

Appendix 2. American College of Rheumatology diagnostic criteria for hip OA\textsuperscript{a,b}

Hip pain for most days in the previous month, with two of the following three features:

1. Femoral and/or acetabular osteophytes on radiograph.

2. Erythrocyte sedimentation rate (ESR) of \( \geq 20 \) mm/h.

3. Joint space narrowing on radiograph.

\textsuperscript{a} Adapted from Brion 2010.
\textsuperscript{b} Clinical and laboratory criteria alone yield poor results; only clinical with laboratory and radiographic criteria are recommended.

Appendix 3. American College of Rheumatology diagnostic criteria for knee OA\textsuperscript{a}

Clinical criteria. Knee pain for most days of the previous month, with three of the following six features:

1. Age \( \geq 50 \) years.

2. Morning stiffness of < 30 min duration.

3. Crepitus on active joint motion.

4. Bony enlargement on examination.
5. Bony tenderness on examination.

6. No palpable warmth.

Clinical and laboratory criteria. Knee pain for most days of the previous month, with five of the following nine features:

1. Age > 50 years.

2. Morning stiffness of < 30 min duration.

3. Crepitus on active joint motion.

4. Bony enlargement on examination.

5. Bony tenderness on examination.

6. No palpable warmth.

7. ESR < 40 mm/h.

8. Rheumatoid factor titre < 1:40.

9. Synovial fluid suggestive of OA.

Clinical, laboratory and radiographic criteria. Knee pain for most days of the previous month, with osteophytes on the radiograph and one of the following three features:

1. Age > 50 years.

2. Morning stiffness of < 30 min duration.

3. Crepitus on active joint motion.

Adapted from Brion 2010.

Synovial fluid suggestive of OA has a clear colour, a viscous consistency, and a white cell count of < 2000/mm³.

Appendix 4. Currently-agreed leech taxonomy

<table>
<thead>
<tr>
<th>Class</th>
<th>Hirudinea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subclass</td>
<td>Euhirudinea</td>
</tr>
<tr>
<td>Order</td>
<td>Rhyncobdellida</td>
</tr>
<tr>
<td>Suborder</td>
<td>Erpobdelliformes</td>
</tr>
</tbody>
</table>
Appendix 5. Biological characteristics of leeches

1. Leeches are ‘sanguivorous’ or blood-sucking aquatic worms, found in both freshwater and marine environments (Porshinsky 2011). They are distributed all over the world, except the polar zones, deserts and altitudes exceeding 3700 m (Lone 2011).

2. The freshwater leeches of Europe reach their greatest abundance in the numerous shallow lakes and ponds of north-central temperate countries, particularly Poland, Romania and Ukraine (Sawyer 1986). In Poland alone, 47 different species of leech have been recorded (Bielecki 2011).

3. Since it is an invertebrate segmented worm without an exoskeleton, the leech has the ability to greatly expand or contract its body (Wade 1990).

4. Leeches are usually hermaphrodite; however they require a second leech to reproduce, and the two leeches mutually impregnate each other (Mory 2000). They are oviparous, with 6 to 15 eggs per clutch. The eggs are deposited near the edges of freshwater ponds and are hatched by the heat of the sun (Hyson 2005).

5. After hatching, young leeches first live off the yolk mass in the cocoon, then start to feed on benthic and planktonic organisms; they then proceed to suck blood from amphibians and other invertebrates. Some species then progress to vertebrate prey, including mammals (Michalsen 2007). Adult leeches can be very discriminating in their feeding behaviour, preferring blood from certain vertebrate species, while ignoring others (Mory 2000).

6. Most leech species prefer standing water to running water (Sawyer 1986). Hungry leeches tend to rest in submerged vegetation at the water’s edge, from where they swim out with great speed and accuracy towards large objects that produce waves (Lent 1988). The act of feeding on humans is stimulated by the proximity of mammalian-range temperature and by the sodium and arginine that the leech detects in the potential host’s blood (Lent 1986).

7. *Hirudo medicinalis*, the medicinal leech, is the species most commonly used in clinical practice; this is because it inflicts the deepest bite in its class, and has the most prolonged post-bite extravasation (Porshinsky 2011). *H. medicinalis* consists of 102 annuli, each annulus usually comprising five segments; it can grow to approximately 12 cm in length, with its resting length being approximately one-third of its maximal length (Valauri 1991).

8. *H. medicinalis* crawls using a large posterior sucker. Anteriorly there is a small sucker that assists in locomotion and is utilised for non-blood feeding. It has three blade-like jaws posteriorly, armed with teeth and arranged in a triradiate configuration; the jaws attach to and incise the skin of its human host (Valauri 1991). Leech saliva is secreted from the cutting edge of the jaws, and contains the powerful anticoagulant hirudin (Haycraft 1884).

9. Like *H. medicinalis*, most leech species bite through the host’s skin, making a Y-shaped incision (Lone 2011). Some species, however, secrete enzymes onto the host’s skin surface instead; the enzymes help digest an opening through the skin and the leech inserts a tubular proboscis into this opening, through which it sucks blood (Sawyer 1986).

10. The bite of a leech is similar in its intensity to a mosquito bite; it is only very slightly painful, due to an anaesthetic contained in leech saliva (Singh 2009).

11. If undisturbed while feeding, a leech will ingest a blood meal of up to nine times its own body weight; however this typically represents only between 2 mL to 20 mL of blood (Porshinsky 2011). Within about 10 to 30 minutes, having extracted this small amount of blood, the leech becomes satiated and detaches spontaneously from the host. The leech will not then take a further blood meal, sometimes for as long as a year, unless purged by incision of its posterior crop (West 1994).
12. Leeches themselves are colonised by endosymbiotic bacteria, mostly *Aeromonas* spp; these bacteria aid in the digestion of blood within the leech digestive system (Eroglu 2001).

**Appendix 6. Hierarchy for assessing pain**

If data on more than one pain scale are provided for a trial, we will extract data according to the following hierarchy:

1. Pain overall.
2. Pain on walking.
3. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale.
4. Pain on activities other than walking.
5. WOMAC global scale.
6. Lequesne osteoarthritis index global score.
7. Other algofunctional score.
10. Other outcome measure of pain.
11. No continuous pain outcome reported.

**Appendix 7. Hierarchy for assessing function**

If data on more than one function scale are provided for a trial, we will extract data according to the following hierarchy:

1. Global disability score.
2. Walking disability.
3. WOMAC disability subscore.
4. Composite disability scores other than WOMAC.
5. Disability other than walking.
6. WOMAC global scale.
7. Lequesne osteoarthritis index global score.
8. Other algofunctional scale.

**Appendix 8. Hierarchy for assessing radiographic joint structure changes**

We will use the following hierarchy.

1. Minimum joint space width.
2. Median joint space width.
3. Semi-quantitative measurement.

**Appendix 9. Generic search strategy for leech studies**

#1 MeSH descriptor leeches explode all trees
#2 MeSH descriptor Antigens, Leech explode all trees
#3 MeSH descriptor Antibodies, Leech explode all trees
#4 MeSH descriptor Parasitology explode all trees
#5 (leech* OR hirudin* OR hirudo* OR hirudotherap* OR phlebotom* OR bloodlett* OR sanguivorous)
#6 #1 OR #2 OR #3 OR #4 OR #5
#7 MeSH descriptor osteoarthritis explode all trees
#8 (osteoarthriti* OR osteoarthro* OR gonarthriti* OR gonarthro* OR coxarthriti* OR coxarthro* OR arthros* OR arthrot* OR ((knee* OR hip* OR joint*) near/3 (pain* OR ach* OR discomfort*)) OR ((knee* OR hip* OR joint*) near/3 stiff*)) in Clinical Trials
#9 (#7 OR #8)
Appendix 10. Strategy to assess risk of bias

We will assess each important study outcome according to the following areas of potential bias, and record our findings as 'low risk', 'high risk' or 'unclear risk'.

1. Random sequence generation
   - Was the allocation sequence adequately generated: e.g. coin toss, random number tables, computer generated, other?

2. Allocation concealment
   - Was allocation adequately concealed in a way that would not allow both the investigators and the participants to know or influence the intervention group before an eligible participant is entered into the study: e.g. central randomisation, or sequentially numbered, opaque, sealed envelopes?

3. Blinding of participants and personnel
   - Were participants blinded to the leech interventions they were receiving?
   - Were investigators blinded to the leech interventions they were administering?

We will avoid categorising studies as 'double-blinded' or 'triple-blinded' and instead will state explicitly which study groups (participants, investigators, assessors) were blinded, if any.

4. Blinding of outcome assessment
   - Were assessors blinded to the effects they were assessing?

We will avoid categorising studies as 'double-blinded' or 'triple-blinded' and instead will state explicitly which study groups (participants, investigators, assessors) were blinded, if any.

5. Incomplete outcomes data
   - Were incomplete outcome data adequately addressed?
   - If any withdrawals occurred, were these withdrawals described and reported by treatment group?
   - Were clear explanations recorded for withdrawals and dropouts in treatment groups?

Incomplete outcomes data essentially include attrition, exclusions and missing data. An example of an adequate method to address incomplete outcome data is the use of intention-to-treat analysis (ITT).

6. Selective reporting
   - Are reports of the study free from any suggestion of selective outcome reporting?

If reports are free from this suggestion, this will be interpreted as representative of no evidence that statistically non-significant results have been selectively withheld from publication (e.g. through selective under-reporting of data, or through selective reporting of a subset of the data).

7. Other bias
   - Was the study apparently free of other defects that could put it at a high risk of bias (e.g. baseline imbalance, or the use of an insensitive instrument to measure outcomes)? Did the investigators use blocked randomisation and if so, was this another potential source of bias?

Contributions of Authors

AC conceived the study and wrote the protocol. SC, GPF and AM contributed to the protocol development.
DECLARATIONS OF INTEREST

There are no financial conflicts of interest.

SOURCES OF SUPPORT

Internal sources

• No sources of support supplied

External sources

• Director of Educational and Training Services (Army), Educational grant ELC 198187, UK.
  Support to AC