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Autologous bone marrow-derived and blood-derived biological therapies (including cellular therapies and platelet-rich plasma) for bone healing in adults (Protocol)

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Autologous bone marrow-derived and blood-derived biological therapies (including cellular therapies and platelet-rich plasma) for bone healing in adults

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To assess the effects (benefits and harms) of autologous bone marrow-derived and blood-derived biological therapies on bone healing in adults.

BACKGROUND

Description of the condition

Bone fractures (broken bones) are a common injury that affect people worldwide. A recent study found around 3% of white adults aged 50 years or over sustain a fracture each year in the USA (estimated incidence 2704 per 100,000 persons/year) (Amin 2014). Most often, bones are broken as a consequence of trauma; though sometimes they are broken as part of a treatment, such as osteotomy, where the bone is cut usually to facilitate realignment. Overall, bones tend to heal after surgical or non-surgical treatment. However, in approximately 1 in 10 cases they fail to heal normally,

resulting in either nonunion or delayed union (Einhorn 2014). The risk of nonunion, which represents a failure of the bone healing process, varies according to the bone involved. An epidemiological study of the nonunion in 18 types of bones reported an overall nonunion rate of 4.9%, with the highest nonunion rates occurring in the scaphoid (15.5%), tibia and fibula (14%) and femur (13.9%), and the lowest in the metacarpal (1.5%) and radius (2.1%) bones (Zura 2016).

Bone healing requires multiple factors including: 1) an adequate mechanical environment; 2) osteogenic progenitor cells (cells that can make bone); 3) growth factors and inflammatory mediators (signalling molecules); 4) vascular supply; and 5) an osteoconductive scaffold. This process demonstrates a remarkable potential

for regeneration. Bone healing follows most instances in which there is bone discontinuity (i.e. bone fracture or defect), usually taking several weeks or months (Loi 2016). For example, nondisplaced distal radius fractures in adults typically require six weeks of short-arm cast immobilisation to achieve bone union (Roth 2013). Humeral fractures, treated conservatively with functional bracing, take an average of 10 weeks to heal (Papasoulis 2010). The time taken for the bone to heal can represent an opportunity to enhance and optimise treatments in order to allow patients to resume their activities sooner.

Furthermore, as indicated above, fractures may take longer to heal (delayed union) or fail to heal. These are often associated with risk factors including: 1) patient-related factors, such as medical comorbidities, smoking, metabolic disease and nutritional deficiency; and 2) injury-related factors, such as complex fracture pattern, open fracture, high energy trauma, displacement, soft tissue injury and bone loss (Hak 2014). Zura 2016 narrowed down the main risk factors to “fracture severity, fracture location, disease comorbidity, and medication use”. The treatment of these challenging conditions embraces a multimodal approach where surgical treatment to achieve mechanical stabilisation is partnered with host optimisation and biological supplementation to the nonunion site (Dimitriou 2011).

Nonunion and delayed union represent a substantial clinical challenge and cause considerable comorbidity for patients, prolonging their disability and pain (Einhorn 2014; Antonova 2013). Additionally, these conditions are difficult to treat and consume substantial healthcare resources (Antonova 2013). Strategies targeted to enhancing bone healing would allow patients to resume their daily life activities and return to work, thus improving their health outcomes while reducing the direct and indirect costs.

Description of the intervention

Tissue engineering strategies, which include the combined use of cells, scaffolds and biologically-active signalling molecules, are becoming increasingly available technologies to augment bone healing (Muschler 2004). When cells or tissues are obtained and used in the same individual, they are called autologous therapies, and they present the benefit of not having the risk of being rejected by the recipient's immune system. The two categories of autologous interventions covered in this Cochrane Review are bone marrow-derived products and autologous blood-derived products. Both therapies can be applied as percutaneous injections or during surgical procedures, alone or in combination with scaffolds, and in a single or a series of applications (Patterson 2008).

Bone marrow-derived products

Whole bone marrow contains multiple active components including plasma, red blood cells, platelets, nucleated cells including

white blood cells, hematopoietic stem cells, connective tissue progenitor cells, growth factors and cytokines. Bone marrow samples are obtained through percutaneous or open harvest, and the sample is processed by centrifugation to obtain bone marrow concentrate (BMC). This is believed to increase the concentration of components of bone marrow aspirate that are believed to have a beneficial effect in bone healing. In a further attempt to increase the number of progenitor cells, since they only make up to approximately 0.01% of nucleated cells in the bone marrow, harvested cells can be cultured in-vitro and expanded to obtain bone marrow mesenchymal stromal/stem cells (BM-MSC) at higher concentrations (Hoch 2014).

Autologous blood-derived products

Whole blood and relevant fractions of blood obtained by separating its components have been used to enhance bone healing. Platelet-rich plasma (PRP) therapies are the most often employed, which consist of a concentration of platelets in a small volume of plasma, and can be easily isolated from freshly-drawn peripheral whole blood (Amini 2012; Piuze 2017a). The PRP can be obtained at the bedside both by centrifugation or filtering of extracted whole blood mixed with an anti-coagulant. Depending on the preparation protocol employed, different types of PRP can be obtained, which all contain a supra-physiological concentration (above whole blood baseline value) of platelets: leukocyte-poor PRP (LP-PRP), leukocyte-rich PRP (LR-PRP), or leukocyte- and platelet-rich fibrin (LR-PRF) or Choukroun's PRF. These types of PRP vary on the presence of cell content (mostly leukocytes) and the fibrin architecture (Dohan Ehrenfest 2009).

How the intervention might work

Osteogenic stem cells and progenitor cell populations involved in bone healing are present in the bone (marrow, endosteum and periosteum) and local soft tissues (muscle and fat) (Marcucio 2015). In the clinical setting where bone fails to heal, there may be an underlying deficiency of cellular activity contributing to the delayed healing or nonunion. Therefore harvesting cells from a different healthy source, such as bone marrow from an alternative anatomical site, and transplanting these, whether as they are or in concentrated form, at the fracture or nonunion site is a plausible way of enhancing bone healing. The rationale supporting the transplantation of osteogenic stem and progenitor cells (in bone marrow and related concentrated products) to the site of bone healing is based on the understanding that these cells are required to form bone (Connolly 1998). These bone marrow-derived cell-based therapies could potentially enhance bone healing by different mechanisms: 1) repopulating of osteogenic stem and progenitor cells at the nonunion site; 2) modulation of the nonunion environment, either by secretion of signalling molecules or through cell-to-cell interactions; and (3) by homing of cells to the nonunion site

(Patterson 2008). Furthermore, these stem and progenitor cells can be cultured in the attempt to increase the number of cells delivered (mesenchymal stromal/stem cells (MSC)), with the intention of increasing their effect on bone healing.

Regulation and legislation even in the use of autologous cell-based therapies is complex. Research continues to assess the feasibility and safety of many autologous cellular therapies, and many challenges must be overcome in order for them to become safely, effectively and routinely used in the clinical setting (Halme 2006). Growth factors are mediators of the inflammatory process and, during different phases of tissue healing, they constitute a key element in promoting tissue regeneration (Roffi 2017). Platelets, which are a component of whole blood, are an important source of these growth factors and play a crucial role in the pathway signalling required for successful bone healing. A more detailed rationale in support of the use of autologous whole blood and derived fractional products, such as PRP, as a therapy in bone healing is that multiple bioactive molecules (e.g. transforming growth factor beta (TGF- β 1), platelet-derived growth factor BB (PDGF-BB), vascular endothelial growth factor A (VEGF-A) and insulin-like growth factor 1 (IGF-1)) released by platelets, when activated, can ultimately promote bone healing by mechanisms of cellular recruitment (chemotaxis), cell proliferation or growth, morphogenesis and immunomodulation (Piuze 2017a; Roffi 2017). To date, different formulations of PRP have been shown to be a safe treatment with only minor adverse events, including transient mild pain and local swelling (Imam 2017; Piuze 2017a).

Why it is important to do this review

Despite widespread marketing, cellular (also referred as 'stem cell-based') interventions remain unproven and their efficacy for musculoskeletal applications is yet to be established (Chahla 2016; Piuze 2017c; Srivastava 2016). Nevertheless, commercial 'stem-cell' clinics worldwide offer cell therapies for a wide array of conditions, of which musculoskeletal are the most frequent (Turner 2016). Many of these clinics are currently delivering autologous cells, such as bone marrow-derived or blood-derived products, and market such interventions directly to consumers (Piuze 2017b; Rachul 2017; Ramkumar 2017).

So called 'stem cell' therapies, which oftentimes are bone marrow-derived cellular therapies, have raised substantial hope and enthusiasm regarding their therapeutic potential. However, to date, their treatment effect remains uncertain. A systematic review, with a narrower scope to this Cochrane Review, reported on a substantial number of human clinical studies that investigated on the use of BMC; it suggested benefit with this therapy (Imam 2017). However, there were limitations in terms of the review methodology and the low quality and heterogeneity of the included studies (Piuze 2018).

While positive preclinical findings on the biological potential of PRP favouring bone healing have been reported, the overall avail-

able literature in clinical trials has been reported to have major limitations in terms of low quality and heterogeneity, which have limited the reliability of estimates of any treatment effect (Chahla 2017; Griffin 2012; Roffi 2017). Griffin 2012 concluded in a previous Cochrane systematic review on the use of PRP for long bone healing in adults that, although potential clinical benefit could not be ruled out, the available evidence was insufficient to support the routine use of this intervention in clinical practice.

Our review seeks to evaluate the available up to date evidence on the use of autologous bone marrow-derived and blood-derived products for enhancing bone healing in adults and, where possible, provide a reliable estimate of their treatment effects.

OBJECTIVES

To assess the effects (benefits and harms) of autologous bone marrow-derived and blood-derived biological therapies on bone healing in adults.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomised controlled trials and quasi-randomised controlled trials (method of allocating participants to a treatment that is not strictly random, e.g. by hospital number) that assess the effects of autologous bone marrow-derived and blood-derived biological therapies on bone healing in adults.

Types of participants

We will include trials of adult participants (i.e. over the age of 16 years or with closed physes (growth plates)) with a bone discontinuity involving the appendicular skeleton that includes any one of the following:

- acute complete fracture;
- stress fracture;
- delayed union;
- non-union (or pseudoarthrosis);
- osteotomy (which is the surgical cutting of a bone or removal of a piece of bone);
- critical size defect (bone defect that cannot heal by itself);
- arthrodesis (this is the surgical immobilisation of a joint by a fusion of the adjacent bones);
- docking sites during distraction osteogenesis (this procedure is used in bone lengthening; it involves surgical

cutting of a bone and slowly separating the broken ends, allowing the bone healing process to fill in the gap).

We will exclude trials focused exclusively on bone discontinuities that do not involve the appendicular skeleton, such as fractures of the spine, and on children. However, we will consider including trials of mixed populations provided the separate data are available for bones within our scope or the percentage of non-eligible participants is small (under 10%) and comparable among treatment groups.

Types of interventions

We will include the following autologous bone marrow-derived and blood-derived biological therapies:

- autologous bone marrow-derived products:
 - whole bone marrow
 - bone marrow concentrate (BMC)
 - bone marrow mesenchymal stromal/stem cells (BM-MSD);
- autologous blood-derived products:
 - whole blood
 - platelet-rich plasma (PRP)
 - ◊ leukocyte-poor PRP (LP-PRP)
 - ◊ leukocyte-rich PRP (LR-PRP)
 - ◊ leukocyte- and platelet-rich fibrin (LR-PRF) or Choukroun's PRF.

Our comparator will be placebo (e.g. sham therapy such as sham bone marrow harvest and giving a placebo infusion of saline) or a no treatment control (none of the above interventions). We will include trials where autologous bone marrow-derived or blood-derived intervention was the only treatment but we anticipate that it would be delivered in addition to a standard-of-care treatment applied to all trial participants. The standard-of-care treatment could be operative or non-operative.

Co-interventions, such as autologous bone grafting or bone graft substitutes, could be used provided they were administered as standard or irrespective of the treatment allocation.

We will compare the following:

- whole bone marrow versus placebo or no intervention;
- BMC versus placebo or no intervention;
- BM-MSD versus placebo or no intervention;
- whole blood versus placebo or no intervention;
- PRP versus placebo or no intervention.

Types of outcome measures

Bone healing is usually defined as the radiographic assessment of callus with bone bridging including periosteal, endosteal, and inter-cortical patterns of at least three of four cortices on orthogonal radiographs or computerised tomography scan, or clinical assessment of absence of pain or tenderness on weightbearing or on

palpation of the fracture site. For the purposes of this Cochrane Review, we will accept the trial authors' statements on and definitions of bone healing.

Primary outcomes

- Physical function reported as measurements using validated patient-reported outcome instruments (e.g. Disability Rating Index (DRI), Hip Disability and Osteoarthritis Outcomes Survey (HOOS), Foot and Ankle Disability Index (FADI), Disabilities of the Arm, Shoulder, and Hand Score (DASH), Knee Injury and Osteoarthritis Outcome Score (KOOS));
- time-to-union (defined as period of time elapsed since trial entry (randomisation) until achieving bone healing). This will be collected in conjunction with the incidence of union at a set follow-up (preferably long-term);
- serious adverse events: defined as the number of individuals suffering at least one serious adverse event, such as bone infection (osteomyelitis), surgical skin infection or deep wound infection, deep vein thrombosis, pulmonary embolism, readmission or death. If data for the total of participants with serious adverse events are not available, we will consider the individual incidence of each serious adverse event.

Secondary outcomes

- Non-union or delayed union: defined as the failure to achieve bone healing or the need for additional treatment to achieve bone healing;
- pain reported by validated pain scores (e.g. visual analogue scale (VAS));
- health-related quality-of-life reported as measurements using validated patient-reported outcome instruments (e.g. Veterans RAND 12, EuroQol-5D, SF-6, other);
- minor adverse events, defined as the number of individuals suffering at least one of the following adverse events: pain at injection site, soreness, infection at harvesting site, allergic reactions, and other mild adverse events.

Timing of outcome assessment

We plan to group assessments of the numbers of people with bone healing into three categories; short (up to three months), medium (between three and 12 months) and long-term follow-up (one year or more). These time points are necessarily a compromise to encompass data from studies that include different bones with different typical healing times.

Cost and resource use

We will assess each trial report for cost and resource data, such as length of hospital stay and number of clinic attendances.

Search methods for identification of studies

Electronic searches

We will search for all published, unpublished and ongoing relevant RCTs, without restrictions on language or publication status and in consultation with the Information Specialist of the Cochrane Bone, Joint and Muscle Trauma Group.

Systematic searches will be conducted of the following databases from their inception:

- the Cochrane Bone Joint and Muscle Trauma Group Specialised Register;
- Cochrane Central Register of Controlled Trials (CENTRAL);
- MEDLINE (Ovid Online) (1946 onwards);
- Embase (Ovid Online) (1974 onwards);
- [ClinicalTrials.gov](#);
- [ISRCTN registry](#);
- [World Health Organization International Clinical Trials Registry Platform](#).

The subject strategies for the above databases will be modelled on the search strategy designed for MEDLINE ([Appendix 1](#)). The subject specific MEDLINE search will be combined with the sensitivity-maximizing version of the Cochrane Highly Sensitive Search Strategy for identifying randomised trials ([Lefebvre 2011](#)).

Searching other resources

We will search reference lists of articles retrieved from the electronic searches. We will contact experts in the field for any additional or unpublished articles. We will also search abstracts of the [Orthopaedic Trauma Association \(OTA\)](#) annual meetings and the [Bone and Joint Journal \(BJJ\)](#) [Orthopaedic Proceedings](#).

Data collection and analysis

Any review author who is a trial investigator of a trial within the scope of this review will not be party to decisions made on their trial. However, we will approach them for additional information and data, where necessary as per protocol.

Selection of studies

We will use Covidence software for study selection, including the initial removal of duplicate records ([Covidence 2017](#)). Two review authors (NSP, JIO) will independently scan the abstract, title, or both, of the remaining records to identify potentially eligible studies, for which we will obtain full-text versions. They will independently map records to studies, and classify studies as either included studies, excluded studies, studies awaiting classification, or ongoing studies in accordance with the review inclusion criteria.

We will resolve any discrepancies through consensus or recourse to a third review author (JVAf). If necessary, we will contact study authors for clarification to inform study selection decisions.

We will document reasons for exclusion of studies that may have reasonably been expected to be included in the review in a 'Characteristics of excluded studies' table. We will present an adapted PRISMA flow diagram showing the process of study selection ([Liberati 2009](#)).

Data extraction and management

We will develop a dedicated data extraction form and pilot this using a sample of included studies. The data collected will include information on study design, study population, interventions and outcomes measurement, and results. Two review authors (NSP, JIO) will independently extract data and study characteristics information. We will resolve any disagreements by discussion, or, if required, by consulting a third review author (JVAf).

We will collect the following information on each study, which will be presented in the 'Characteristics of included studies' table. We will state where key information is not available.

- study design;
- study dates;
- study settings and country;
- participant inclusion and exclusion criteria;
- participant details, baseline demographics. These will include data on gender, age, type and locality of fracture, mechanism of injury, open versus closed status, the duration of the delayed or nonunion, size of critical defect, and comorbidities of the participants;
- the number of participants, overall and by study arm;
- details of relevant experimental and comparator interventions such as dose, route, frequency and duration;
- details about co-interventions such as bone grafting and surgery;
- definitions of relevant outcomes, and method and timing of outcome measurement, as well as any relevant subgroups;
- study funding sources;
- declarations of interest by primary investigators.

In addition for each group of interventions we will extract the following:

- for bone marrow-derived products: initial volume of bone marrow, anticoagulant used, collection site and technique, processing machine, number of spins (with rotations per minute (RPM) or gravitational forces when reported, and time), method of platelet activation, initial and final nucleated cell count, percentage increase in cell-count, colony-forming-count (CFU), qualitative characterisation (e.g. cluster of differentiation (CD) surface-markers), culture expansion technique, final volume, delivery method;
- for autologous whole blood and fractional products (PRP): initial whole blood volume, anticoagulant used, processing

machine, number of spins (with RPM and time), platelet activation, final platelet count, fold increase in platelet count, growth factor analysis, final volume, delivery method.

We will attempt to contact authors of included studies to obtain key missing data.

Duplicate and companion publications

In the event of duplicate publications, companion documents or multiple reports of a primary study, we will maximise yield of information by mapping all publications to unique studies and collating all available data. We will use the most complete data set aggregated across all known publications. In case of doubt, we will give priority to the publication reporting the longest follow-up associated with our primary or secondary outcomes.

Assessment of risk of bias in included studies

Two review authors (NSP, JIO) will independently assess the risk of bias of each included study. We will resolve disagreements by consensus, or by consulting a third review author (JVAf).

We will assess risk of bias using Cochrane's 'Risk of bias' assessment tool ([Higgins 2011a](#)). We will assess the following domains:

- random sequence generation (selection bias);
- allocation concealment (selection bias);
- blinding of participants and personnel (performance bias);
- blinding of outcome assessment (detection bias);
- incomplete outcome data (attrition bias);
- selective reporting (reporting bias);
- other sources of bias: selection bias from major imbalances in key baseline characteristics (such as age, sex and smoking behaviour).

We will judge risk of bias domains as 'low risk', 'high risk' or 'unclear risk' and will evaluate individual bias items as described in the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2011a](#)). We will present a 'Risk of bias' summary figure to illustrate these findings. Where information on risk of bias relates to unpublished data or correspondence with trial authors, we will note this in the 'Risk of bias' tables.

For performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment) and attrition bias (incomplete outcome data), we will evaluate the risk of bias separately for subjective and objective outcomes. We will consider the following outcomes as subjective outcomes: physical function, pain and health-related quality of life.

We will assess the risk of detection bias separately for the primary outcome of bone healing, which is variably defined in the literature. We anticipate that studies will define healing clinically and radiographically. We anticipate that bias might be introduced by inter- and intraobserver error in the reading of radiographs. Thus we will assess this risk of detection bias and ascribe a low risk

where two or more specialist radiologists or orthopaedic surgeons read the radiographs and reached agreement. Any study employing other methodologies, such as multiple independent observers, will be ascribed a high risk of bias.

We will further summarise the risk of bias across domains for each outcome in each included study, as well as across studies and domains for each outcome in each comparison.

Measures of treatment effect

We will express dichotomous data as risk ratios (RRs) with 95% confidence intervals (CIs). We will express continuous data as mean differences (MDs) with 95% CIs unless we pool studies that used different measures to assess the same outcome, in which case we will express data as standardised mean differences with 95% CIs. Where meta-analysis is possible and studies have reported mean time-to-union, we will consider calculating standardised mean differences (SMDs) with 95% CIs for this outcome because we anticipate widely differing mean time-to-union values in different studies, including different bones and types of discontinuity. In general, for continuous data, we will use results based on change scores where final values are not available.

Unit of analysis issues

Where possible, we will analyse the extracted data by participant. However, it is possible that trials that include individuals with several fractures might report outcomes such as 'non-union or delayed union', by the individually-treated fracture sites or involved bones. This might pose unit of analysis issues as described in the *Cochrane Handbook for Systematic Reviews of Interventions* Section 9.3.7 ([Higgins 2011b](#)), given that multiple site or bone data from the same individual cannot be considered independent. Should this unit of analysis issue arise and appropriate adjustments have not been made, we will conduct sensitivity analyses to explore the potential effects of the incorrect analysis, including where pooled with data from other trials, where practical.

We expect that most studies will report functional outcome scores at a number of follow-up times; for example, at six and 12 weeks. Dependent on the nature of reporting, we will make separate analyses at each of the commonly-reported occasions; representing perhaps, short-, medium- and long-term follow-up. We expect that all studies will report simple parallel group designs. However, should we identify cross-over trials, cluster-randomised trials, or trials with more than two intervention groups for inclusion in the review, we will handle these in accordance with guidance provided in the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2011b](#)), using generic inverse variance methods to combine data where appropriate.

Dealing with missing data

We will contact trial investigators for missing data. If feasible, we will perform intention-to-treat (ITT) analyses; otherwise we will perform available-case analyses for the main analyses. We will investigate attrition rates, such as losses to follow-up and withdrawals, and will critically appraise the effects of missing data.

Where standard deviations are not specifically reported, we will attempt to determine these from standard errors, confidence intervals or exact P values, if available.

When the results of the primary meta-analysis suggest a statistically significant treatment effect for the primary outcomes, we will conduct sensitivity meta-analyses using plausible assumptions to impute events in participants with missing outcome data in each study, as proposed by [Guyatt 2017](#). We will give details of assumptions used in the sensitivity analyses in our review. We will use the robustness of the results to the sensitivity analyses to assess the risk of bias associated with missing data in our GRADE assessment ([Guyatt 2017](#)).

Assessment of heterogeneity

We will identify heterogeneity (inconsistency) through visual inspection of the forest plots to assess the amount of overlap of CIs, and the I^2 statistic which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis ([Higgins 2002](#); [Higgins 2003](#)). We will interpret the I^2 statistic as follows:

- 0% to 40%: may not be important;
- 30% to 60%: may indicate moderate heterogeneity;
- 50% to 90%: may indicate substantial heterogeneity;
- 75% to 100%: considerable heterogeneity.

When we find heterogeneity, we will attempt to determine possible reasons for it by examining individual study and subgroup characteristics.

Assessment of reporting biases

We will attempt to obtain study protocols to assess for selective outcome reporting.

If we include 10 studies or more investigating a particular outcome, we will use funnel plots to assess small study effects. Several explanations can be offered for the asymmetry of a funnel plot, including true heterogeneity of effect with respect to trial size, poor methodological design (and hence bias of small trials) and publication bias. We will, therefore, interpret results carefully.

Data synthesis

We will pool data only if we judge participants, interventions, comparisons and outcomes to be sufficiently similar to ensure an answer that is clinically meaningful. Where appropriate, we will pool results of comparable studies using both fixed-effect and random-effects models. We will decide the choice of the model to report by careful consideration of the extent of heterogeneity

and whether it can be explained, in addition to other factors, such as the number and size of included studies. We will use 95% CIs throughout. We will consider not pooling data where there is considerable heterogeneity (I^2 statistic value of greater than 75%) that cannot be explained by the diversity of methodological or clinical features among trials. Where it is inappropriate to pool data, we will still present trial data in the analyses or tables for illustrative purposes and report these in the text.

‘Summary of findings’ tables

We will present the overall quality of the evidence for each outcome according to the GRADE approach, which takes into account five criteria that are not only related to internal validity (risk of bias, inconsistency, imprecision, publication bias), but also to external validity, such as directness of results ([Guyatt 2008](#)). For each comparison, two review authors (NSP, JIO) will independently rate the quality of evidence for each outcome as either ‘high’, ‘moderate’, ‘low’, or ‘very low’ using GRADEpro GDT software ([GRADEpro 2015](#)). We will resolve any discrepancies by consensus, or, if needed, through arbitration by a third review author (JVAf). For each comparison, we plan to present a summary of the evidence for the main outcomes in a ‘Summary of findings’ table, which provides key information about: the best estimate of the magnitude of the effect in relative terms and absolute differences for each relevant comparison of alternative management strategies; numbers of participants and studies addressing each important outcome; and the rating of the overall confidence in effect estimates for each outcome ([Guyatt 2011a](#); [Guyatt 2011b](#); [Schünemann 2011](#)). Where meta-analysis is not possible, we will present results in a narrative ‘Summary of findings’ table. We will use the controlled vocabulary suggested by [Glenton 2010](#) to summarise the findings of the ‘Summary of findings’ table in the Plain Language Summary and Abstract.

We will produce ‘Summary of findings’ tables for the comparisons listed in the ‘Types of interventions’ section.

We will include the following critical outcomes in the ‘Summary of findings’ tables:

- physical function (preferably long term follow-up);
- time-to-union (including consideration of number healed at long-term follow-up);
- serious adverse events (no follow-up restriction);
- non-union or delayed union (generally with need for additional treatment indicated) (no follow-up restriction);
- health-related quality-of-life (preferably long-term follow-up);
- minor adverse events (short-term follow-up).

Subgroup analysis and investigation of heterogeneity

Where there is sufficient evidence available, we plan to conduct the following subgroup analyses for the primary outcomes of this review:

- type of condition: acute fracture versus delayed or non-union;
- bone defects by size and chronicity;
- anatomical site of the fracture or bone defect: upper limb versus lower limb as a pragmatic proxy for weight bearing versus non-weight bearing bones;
- acute versus elective surgery (osteotomy, arthrodesis and docking sites during distraction osteogenesis will be analysed as a distinct subgroup if possible since they are usually scheduled (elective) orthopaedic procedures);
- type of co-intervention: no other treatment versus non-surgical treatment versus surgical treatment.

We will investigate whether the results of subgroups are significantly different by inspecting the overlap of CIs and performing the test for subgroup differences available in Review Manager 5 (RevMan 2014).

Sensitivity analysis

We will undertake sensitivity analyses to assess whether the results of the review are robust to the decisions made during the review process. We plan to examine the effects on the review findings of:

- excluding trials at high or unclear risk of bias, either overall or selection bias reflecting inadequate or lack of allocation concealment;
- excluding trials reported in abstracts only;

- excluding trials not reporting clinical or radiographic confirmation of bone healing;
- excluding mixed population trials;
- excluding studies with unbalanced co-interventions;
- adjusting for missing data (see the 'Dealing with missing data' section);
- different interpretations of data where there are potential or known unit of analysis issues; and
- using fixed-effect versus random-effects models for pooling.

We will report any sensitivity analyses in the text and, if numerous, by producing summary tables.

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* Indicates the major publication for the study

APPENDICES

Appendix I. Ovid MEDLINE search strategy

1. exp Fractures, Bone/
2. Fracture Healing/
3. exp Bone Remodeling/
4. Osteotomy/
5. Pseudarthrosis/
6. Osteogenesis, Distraction/
7. (bone adj4 (union or non-union or nonunion or delay* or discontinuity or heal* or repair* or regenerat* or defect*)).tw.
8. (fracture* or osteotom* or pseudarthros* or pseudo arthros* or pseudoarthros* or critical size defect* or docking site*).tw.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. Platelet-Rich Plasma/
11. Blood Transfusion, Autologous/
12. Blood Cells/ or Blood Platelets/ or exp Bone Marrow Cells/ or Mesenchymal Stromal Cells/
13. Hematopoietic Stem Cells/ or Peripheral Blood Stem Cells/
14. Hematopoietic Stem Cell Transplantation/ or Peripheral Blood Stem Cell Transplantation/ or Bone Marrow Transplantation/
15. Blood Proteins/
16. Bone Marrow/
17. exp "Cell- and Tissue-Based Therapy"/
18. (PRP or PRF or LP-PRP or LR-PRF or buffy layer).tw.
19. (platelet* adj2 (rich or gel or concentrat* or autologous)).tw.
20. "bone marrow".tw.
21. ((stem or stromal or mesenchym*) adj1 cell*).tw.
22. (blood adj2 (cell* or marrow or protein* or transfusion* or autologous or platelet* or peripher* or transplant*)).tw.
23. 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22
24. 9 and 23
25. Randomized controlled trial.pt.
26. Controlled clinical trial.pt.
27. randomi?ed.ti,ab.
28. placebo.ab.
29. Clinical trials as topic/
30. randomly.ab.
31. trial.ti. (199179)
32. 25 or 26 or 27 or 28 or 29 or 30 or 31
33. exp Animals/ not Humans/
34. 32 not 33
35. 24 and 34

Lines 25 to 34 = modified version of Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity- and precision-maximizing version (2008 revision); Ovid format

CONTRIBUTIONS OF AUTHORS

NSP: wrote the [Background](#) section of the protocol, contributed to all the remaining sections of the protocol, and coordinated writing of this protocol. NSP is the guarantor of the review.

JIO: wrote the [Background](#) section of the protocol.

VV: wrote the [Methods](#) section of the protocol.

JVAF: wrote the [Methods](#) sections of the protocol and co-coordinated the writing of this protocol.

XLG: contributed to the definitions and provided feedback on the written protocol.

Contributions of the editorial base

Helen Handoll (Co-ordinating Editor): edited the protocol; advised on methodology and protocol content; and approved the final version for publication.

Lindsey Elstub and Joanne Elliott (Managing Editors): coordinated the editorial process; advised on content; and edited the protocol.

Joanne Elliott (Information Specialist): designed the search strategy and edited the search methods section.

Zipporah Iheozor-Ejiofor (Methodologist): advised on methodology.

Maria Clarke (Information Specialist): checked the search methods section.

DECLARATIONS OF INTEREST

NSP: none known

JIO: none known

VV: none known

JVAF: none known

XLG: was an investigator on a study investigating the clinical effectiveness of PRP in fractures of the proximal femur (IS-RCTN49197425), listed as Griffin 2010 in [Griffin 2012](#). He will remain independent of decisions made on this study in this review.

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External sources

- No sources of support supplied

NOTES

The [Methods](#) section of this protocol was adapted from a published protocol by one of the authors ([Franco 2017](#)), and the published review of another author ([Griffin 2012](#)).