BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2015-010787
Article Type:	Protocol
Date Submitted by the Author:	06-Dec-2015
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Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Oncology
Keywords:	CHEMOTHERAPY, HAEMATOLOGY, ONCOLOGY, Adult oncology < ONCOLOGY, Toxicity < THERAPEUTICS

Efficacy and Safety of Acupuncture for Chemotherapy-induced Leucopenia：Protocol for A Systematic Review

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Abstract

Introduction: Many cancer patients experience leucopenia during chemotherapy. The main treatment for chemotherapy-induced leucopenia(CIL) involves granulocyte-colony-stimulating factor (G-CSF), which has various limitations. Clinical trials indicated that acupuncture may prevent bone marrow suppression and increase leukocyte counts after chemotherapy. The objective of this review is to assess the efficacy and safety of acupuncture for treating CIL.

Methods and analysis: This systematic review will electronically search the following databases: the Cochrane Central Register of Controlled Trials(CENTRAL); the Cochrane Library; Medicine; EMBASE; China National Knowledge Infrastructure Database (CNKI); Chinese Biomedical Literature Database(CBM);Chinese Scientific Journal Database(VIP Database);Wan-Fang Database from their inception to 1 November 2015.Other sources will also be searched including potential grey literatures, conference proceedings and the reference lists of identified publications and existing systematic reviews. Two reviewers will independently search the databases, perform data extraction and assess the quality of studies. Data will be synthesized by either the fixed-effects or the random model according to a heterogeneity test. White blood cell counts(WBCs) will be assessed as the primary outcome. Adverse effects and quality of life will be evaluated as the secondary outcome. RevMan V.5.3 will be employed for data analysis. The results will be expressed as risk ratio(RR) for dichotomous data and mean difference(MD) for continuous data.

Ethics and dissemination: The protocol does not need ethical approval because individuals cannot be identified from it. The review will be reported through a peer-review publication or a relevant conference for dissemination.
Trial registration number: PROSPERO CRD42015027594

INTRODUCTION

According to the data reported recently, cancer continues to be a severe worldwide health issue and has become one of the foremost causes of disease-related death [1-3]. Chemotherapy is a main treatment used for cancer but can lead to dose-limiting toxicity, of which, leucopenia has emerged as a common adverse effect [4]. Leucopenia is defined as a decrease in the number of circulating White Blood Cells(WBCs), to counts less than 4×10^9/L [5]. It can lead to life-threatening infections [6], dose reductions and delays of chemotherapy [7], subsequently affecting success of treatment. Although some medications, such as G-CSF or CSF, are explored as prophylactic measures to reduce the depth and duration of CIL [8]. Because of their temporary effects, it requires dose-intensity administration to sustain the pharmacologic efficacy [9-10]. The consequent increasing in frequency or dose can, in turn, produce bone pain, myalgia, fever, rashes and other adverse reactions [11]. In addition, supplementing chemotherapy with G-CSF were reported to result in acute myeloid leukemia or myelodysplastic [12], even stimulating angiogenesis and promoting tumor growth [13]. Nevertheless, not all patients who experience CIL are recommended to be treated with G-CSF/CSF, patients' overall risk of febrile neutropenia are needed to be estimated in decision making [14]. Considering the limitations of medications in treating chemotherapy-induced leucopenia(CIL), other types of therapy are needed to be used as adjuncts to pharmacological interventions.

Description of the intervention

Acupuncture, which is a therapy that stimulates specific points on the body surface, has been used for prevention and treatment of diseases for thousands of years in China as well as other Eastern countries. Many clinical trials and animal studies reported recently indicated that acupuncture could alleviate CIL, while having fewer side effects and relatively lower cost [15].

How the intervention might work

Acupuncture is believed to function by stimulating acupoints to regulate the balance of Qi circulation in the theory of traditional Chinese medicine [16]. In Western medicine, the mechanism involved has not been well established [17]. Studies in animals and humans suggested that acupuncture might play a positive role on preventing leucopenia by stimulating anticancer immunity, promoting protective effects on bone marrow [18,19] or increasing the activity of serum colony-stimulating factor [20,21].

Why it is important to do this review

CSFs is recommended to be used for those patients who have high risk of febrile neutropenia (approximately 20% or higher) or ever have experienced a neutropenic complication from a previous cycle of chemotherapy regimen. That means not every patient with CIL can be treated by G-SCF/CSF, especially for those with lower risk of febrile neutropenia [14]. Considering the limited application scope of these medications and their adverse effects, it is essential to introduce other supplementary interventions in order to benefit more patients.

In recent years, there has been an increasing number of studies on acupuncture in treating CIL. Some studies reported acupuncture could decrease the occurrence rate of leucopenia or stimulate the activity of G-CSF. However, the methodological quality of these reviews were not substantial enough to drive strong recommendations. The acupoints and matching acupoints with optimal efficacy for the treatment of CIL still remain unidentified. This systematic review seeks to provide high-quality evidence with rigorous methodology.

Objectives

The review aims to systematically evaluate the efficacy and safety of acupuncture for treating CIL.

METHODS AND ANALYSIS

Inclusion criteria for study selection

Types of studies

Randomized controlled trials(RCTs) will be included, while animal studies, case reports, commentaries or quasi-RCTs will be excluded.

Types of patients

This protocol will include cancer patients with CIL, regardless of age (>18 years old), gender, race, education status, tumor type and stage. All cancer patients were confirmed to have cancer according to the criteria by WHO [22]. Participants with primary disease of hematopoietic system or psychiatric disorders will be excluded.

Types of interventions

Acupuncture is defined as the forms of stimulating acupoints by needles, including body acupuncture, scalp acupuncture, manual acupuncture, auricular acupuncture,

electro acupuncture, fire needling and plum blossom needle. Other forms of stimulation methods including acupressure without needles, moxibustion (other than a warming needle method), transcutaneous electrical nerve stimulation, laser acupuncture or drug injection therapy will be excluded.

We will investigate the treatment comparisons as follows:

1. Acupuncture versus no treatment.
2. Acupuncture versus sham acupuncture [23].
3. Acupuncture versus other treatment.
4. Acupuncture with another treatment versus the same treatment.

Neither will the studies with patients in control groups treated by acupuncture except for sham acupuncture be included, nor the studies with patients in either group undertaking radiotherapy, molecular targeted therapy or Chinese herbal medicine be included. Because different kind of chemotherapy drugs have incomparable grades of WBCs suppression. Chemotherapeutic medications must have been claimed without significant difference among experimental and control groups in order to exclude the effects of chemotherapy on trial results.

Types of outcome measures

Primary outcomes

The primary outcome will be the changes of WBC counts from baseline to endpoint. We will contact the authors of original articles for additional information about the primary outcome if it were expressed as the changes in decrease of WBCs at grade of I°-IV° (according to the criteria of toxicity grading scale for determining the severity of adverse events by WHO,2003).

Secondary outcomes

1. Incidence and type of adverse events of acupuncture.
2. Quality of life, as measured by validated instruments (e.g. Karnofsky Performance Score(KPS), the European Organization for Treatment of Cancer(EORTC) QLQ-C30(Aaronson 1993), et al).

Search methods for identification of studies

Electronic searches

We will electronically search the following database: the Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Library, Medline (via PubMed), EMBASE (via embase.com), China National Knowledge Infrastructure Database(CNKI), Chinese Biomedical Literature Database (CBM), Chinese Scientific Journal Database (VIP database), Wan-Fang Database from their inception to 1 November 2015. The following search terms will be used: acupuncture, manual acupuncture, electroacupuncture, fire needling, auricular acupuncture, ear acupuncture, dermal needle, plum blossom needle, leucopenia, leukopenia, aleucocytosis, aleukocytosis, hypoleucocytosis, hypoleukocytosis,

oligoleukocythemia, oligoleukocytosis, hypoleukia, G-CSF, Granulocyte colony-stimulating factor, GM-CSF, Granulocyte monocyte colony-stimulating factor. The search words with the same meaning as the English version will be used in Chinese databases. Restriction will be made to English or Chinese language and human subjects. The search strategy for Medline (via PubMed) is shown in table 1.

No	Search items
1	randomized controlled trial [pt]
2	controlled clinical trial [pt]
3	randomized [tiab]
4	placebo [tiab]
5	clinical trials as topic [mesh: noexp]
6	randomly [tiab]
7	trial [ti]
8	1 or 2 or 3 or 4 or 5 or 6 or 7
9	Acupuncture therapy [mh]
10	Acupuncture [tiab]
11	Acupoints [tiab]
12	Body acupuncture [tiab]
13	Scalp acupuncture [tiab]
14	manual acupuncture [tiab]
15	Auricular acupuncture [tiab]
16	ear acupuncture [tiab]
17	Electroacupuncture [tiab]
18	Fire needling [tiab]
19	dermal needle [tiab]
20	plum blossom needle [tiab]
21	9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
22	Leucopenia [mh]
23	Leukopenia [tiab]
24	Aleucocytosis [tiab]
25	Aleukocytosis [tiab]
26	Hypoleucocytosis [tiab]
27	Hypoleukocytosis [tiab]
28	oligoleukocythemia [tiab]
29	Oligoleukocytosis [tiab]
30	Hypoleukia [tiab]
31	G-CSF [tiab]
32	Granulocyte colony-stimulating factor [tiab]
33	GM-CSF [tiab]
34	Granulocyte monocyte colony-stimulating factor [tiab]
35	22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34
36	8 and 21 and 35

Table 1 Search strategy used in PubMed

Searching other sources

The reference lists of potentially eligible studies and relevant systematic reviews will be manual retrieved for additional trials. The conference proceedings in relation to this topic will be also searched to identify further studies. We will also search OpenGrey.eu for potential grey literatures. In addition, we plan to search relevant trial protocols through the WHO International Clinical Trial Registry Platform(ICTRP) and ClinicalTrials.gov for ongoing and newly completed studies.

Data collection and analysis

All reviewers have received training at the beginning in order to get familiar with the purpose and the whole process of the review. The outputs obtained from electronic searching and other sources will be uploaded to a database created by Endnote X7. Two reviewers (XS and GJ) will independently screen the titles, abstracts and keywords of all retrieved records. Studies, meeting the predefined eligibility criteria, will be included for full text screening, but their duplicates will be excluded. All excluded studies will be recorded in the" Reasons of excluded studies" table. And any disagreement will be resolved by discussion between the two reviewers (XS and GJ) for consensus or consulting the arbiter (CY) when necessary.

Data extraction and management

Two reviewers (XS and GJ) will independently perform data extraction of selected studies and fill in the data extraction which has been developed before. The authors of trials will be contacted for further details when it is necessary. We will extract data including the following information:

1.General information of the articles including the first author, year, country, sample size.

2. Demographic characteristics including gender, age and other information including tumor type, tumor stage.

3. Intervention parameters including type of acupuncture, acupionts used, chemotherapeutic medications, other treatment, frequency and duration of treatment.

4.Outcome information including results, adverse events, costs, quality of life.

Assessment of risk of bias in included studies

The risk of bias of all studies will be assessed by two reviewers (XS and GJ) using the Cochrane Collaboration's "Risk of Bias" Tool (Higgins 2011), which includes the following seven domains: randomized sequence generation, allocation concealment, blinding of participants and personnel, binding of outcome assessment, incomplete outcome data, selective reporting and other issues. The assessment of each domain will be categorized into three levels of bias: low, unclear and high risk of bias, and

then filled in the form" risk of bias" table. Any discrepancies will be resolved by discussion between the two reviewers (XS and GJ) or consulting the third reviewer (CY).

Measures of treatment effect

Mean difference (MD) with 95% confidence intervals will be used to assess continuous outcomes, while dichotomous outcomes will be expressed as the relative risk (RR) with 95% confidence intervals.

Unit of analysis issue

Only the first phase data will be assessed in randomized cross-over trials to prevent carry-over effects. In the case of studies with multiple intervention groups, we will create pairwise comparison if the groups meet the predefined inclusion criteria. Non-randomized controlled trials will not be included for analysis.

Dealing with missing data

We will attempt to contact the original investigators to obtain the missing or insufficient data when the data prevents a study from being included.

Assessment of heterogeneity

Heterogeneity will be assessed by visually inspecting the forest plot and investigated by Chi^2(significance level: $P<0.10$) and I^2 statistics. $I^2<50\%$ will be taken as evidence of no statistical heterogeneity, while $I^2\geq50\%$ will be considered to indicate substantial heterogeneity (Higgins 2011) and the causes of heterogeneity among results of studies will be explored through subgroup analysis or sensitivity analysis.

Assessment of publication biases

We will generate funnel plots to detect publication bias or small-study effects with sufficient numbers of included studies (at least 10 studies).

Data synthesis

The RevMan V.5.3 will be employed for data analysis when a meta-analysis is allowed. The MD with 95% confidence intervals will be used to assess continuous outcomes, while the RR with 95% confidence intervals will be expressed for dichotomous data. If I^2 test is less than 50%, the RR, MD will be calculated by

fixed-effects model. If I^2 test is higher than 50%, random effects model will be used to synthesize the data and subgroup analysis or sensitivity analysis will be conducted to explore the causes including clinical or methodological reasons. We may conduct narrative synthesis if meta-analysis is not appropriate (e.g. incidence of adverse events of acupuncture).

Subgroup analysis

We will perform subgroup analysis, if there is adequate number of studies (at least 10 trials), to explore the possible causes of heterogeneity.

Sensitivity analysis

Sensitivity analysis will be used to determine whether the conclusions are robust to the decisions which were made during the review process. We will conduct a one-study-removed analysis by excluding one study in each turn and reanalyze the remained studies in order to explore the potential influential effects on composite endpoint.

Grading the quality of evidence

We will use the Grading of Recommendations Assessment, Development and Evaluation(GRADE) to assess the quality of confidence for primary outcomes. Meanwhile, the conclusions will be classified into four levels: high, moderate, low or very low.

DISCUSSION

CIL is one of the most common adverse events during chemotherapeutic period. It can bring about severe adverse reactions, which may affect the efficacy of treatment. Granulocyte colony-stimulating factors (G-CSF), for instance, have been demonstrated a significant reduction in leucopenia. While per-cycle administration (daily use), adverse events, economic burden accompanied with expensive antineoplastic agents were the main problems of G-CSF, which limited its clinical application. Studies have reported that acupuncture could prevent the decline of WBCs and could be used as adjuncts to medications in increasing the activity of G-CSF, while having fewer side effects and relatively lower cost. However, there still exists a problem of quality synthesis in the evidence currently. Therefore, we will conduct a systematic review in order to provide more convincing evidence for clinicians. This systematic review will be separated into four parts including identification, study inclusion, data extraction and data synthesis.

Some potential limitations may affect in drawing highly creditable conclusions during the review. Firstly, as the restriction in the language of included studies, it may limit the broad searching of potential findings, such as articles in Japanese, Korean or German. Secondly, the studies which are still ongoing or unpublished may prevent data synthesis. Thirdly, various types of acupuncture, diverse kinds of chemotherapy medications, mixed tumor types and stages may increase the risk of heterogeneity. Finally, difficulty in undertaking blinding measures in acupuncture therapy may cause the occurrence of bias test results.

PRISMA-P Checklists of the protocol is suppled in appendix 1.

Acknowledgements

The authors would like to thank Feng Y and Zhou AJ for their assistance to improve the paper.

Contributions of authors

XMW is the guarantor of the review. JYN and XS drafted the manuscript. LY and JYN developed the search strategy. XS and JG will independently screen the potential studies, perform data extraction, assess the risk of bias, enter data into RevMan and finish the data synthesis.CY will arbitrate any disagreements and ensure no errors occur during the review. GWY, MWY and GLZ all got involved in the conception, design, revision and final approval of the review. All authors read and approved the final manuscript.

Declarations of interests

None declared.

Sources of support

No sources of support provided.

Provenance and peer review

Not commissioned; externally peer reviewed.

Protocol amendments

If it is necessary to amend the protocol, we will give the detailed explanation of the date, the change and the rationale.

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Strengthens and limitations of this study

This is the first systematic review which we have retrieved on “acupuncture” and “chemotherapy-induced leucopenia(CIL)”. Our review will assess the efficacy and safety of acupuncture in chemotherapy-induced leucopenia.

Current therapy in CIL has many limitations, acupuncture may be used as adjuncts to current medications.

Different types of acupuncture and cancer stages may cause heterogeneity in this review.

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	1
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	9
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	9
Support:			
Sources	5a	Indicate sources of financial or other support for the review	9
Sponsor	5b	Provide name for the review funder and/or sponsor	9
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	9
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	2-3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	3
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	3-4
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	4-5
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	5

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	6
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	6
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	6
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	4
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	6-7
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	7-8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	7-8
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	8
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	8
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	7
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647

BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2015-010787.R1
Article Type:	Protocol
Date Submitted by the Author:	29-Jan-2016
Complete List of Authors:	Nian, Jiayun; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Oncology Department; Beijing University of Chinese Medicine, School of Graduates Sun, Xu; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Oncology Department Guo, Jiao; Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Oncology ; Beijing University of Chinese Medicine, School of Graduates Yan, Chen; Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Oncology ; Beijing University of Chinese Medicine, School of Graduates Wang, Xiaomin; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Oncology Department Yang, Guowang; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Oncology Department Yang, Lin; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Oncology Department Yu, Mingwei; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Oncology Department Zhang, Ganlin; Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Oncology Department
Primary Subject Heading:	Complementary medicine
Secondary Subject Heading:	Health economics, Oncology
Keywords:	CHEMOTHERAPY, ONCOLOGY, HAEMATOLOGY, Health & safety < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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Abstract

Introduction: Many cancer patients experience leucopenia during chemotherapy. The main treatment for chemotherapy-induced leucopenia(CIL) involves granulocyte-colony-stimulating factor (G-CSF), which has various limitations. Clinical trials indicated that acupuncture may prevent bone marrow suppression and increase leukocyte counts after chemotherapy. The objective of this review is to assess the efficacy and safety of acupuncture for treating CIL.

Methods and analysis: This systematic review will electronically search the following databases: the Cochrane Central Register of Controlled Trials(CENTRAL); the Cochrane Library; Medicine; EMBASE; China National Knowledge Infrastructure Database (CNKI); Chinese Biomedical Literature Database(CBM);Chinese Scientific Journal Database(VIP Database);Wan-Fang Database from their inception to 1 January 2016.Other sources will also be searched including potential grey literatures, conference proceedings and the reference lists of identified publications and existing systematic reviews. Two reviewers will independently search the databases, perform data extraction and assess the quality of studies. Data will be synthesized by either the fixed-effects or the random model according to a heterogeneity test. White blood cell counts(WBCs) will be assessed as the primary outcome. Adverse effects, incidence of leucopenia, quality of life and physical condition will be evaluated as the secondary outcome. RevMan V.5.3 will be employed for data analysis. The results will be expressed as risk ratio(RR) for dichotomous data and mean difference(MD) for continuous data.

Ethics and dissemination: The protocol does not need ethical approval because individuals cannot be identified from it. The review will be reported through a peer-review publication or a relevant conference for dissemination.
Trial registration number: PROSPERO CRD42015027594

INTRODUCTION

According to the data reported recently, cancer continues to be a severe worldwide health issue and has become one of the foremost causes of disease-related death [1-3]. Chemotherapy is a main treatment used for cancer but can lead to dose-limiting toxicity, of which, leucopenia has emerged as a common adverse effect [4]. Leucopenia is defined as a decrease in the number of circulating White Blood Cells(WBCs), to counts less than 4×10^9/L [5]. It can lead to life-threatening infections [6], dose reductions and delays of chemotherapy [7], subsequently affecting success of treatment. Although medications, such as G-CSF or CSF, are explored as prophylactic measures to reduce the depth and duration of CIL [8], it requires dose-intensity administration to sustain the pharmacologic efficacy [9-10]. The consequent increasing in frequency or dose can, in turn, produce bone pain, myalgia, fever, rashes and other adverse reactions [11]. In addition, supplementing chemotherapy with G-CSF were reported to result in acute myeloid leukemia or myelodysplastic syndrome [12], even stimulating angiogenesis and promoting tumor growth [13]. Nevertheless, CSFs is recommended in guideline to be used for those patients who have high risk of febrile neutropenia (approximately 20% or higher) or ever have experienced a neutropenic complication from a previous cycle of chemotherapy regimen. That means not every patient with CIL can be treated by G-SCF/CSF, especially for those with lower risk of febrile neutropenia [14]. Considering the limited application scope of these medications and their adverse effects, it is essential to introduce other supplementary interventions in order to benefit more patients.

Acupuncture, which is a therapy that stimulates specific points on the body surface, has been used for prevention and treatment of diseases for thousands of years in China as well as other Eastern countries. Many clinical trials and animal studies reported recently indicated that acupuncture could alleviate CIL, while having fewer side effects and relatively lower cost when compared to G-CSF/CSF [15]. Acupuncture is believed to function by stimulating acupoints to regulate the balance of Qi circulation in the theory of traditional Chinese medicine [16]. In Western medicine, the mechanism involved has not been well established [17]. Studies in animals and humans suggested that acupuncture might play a positive role on preventing leucopenia by stimulating anticancer immunity, promoting protective effects on bone marrow [18,19] or increasing the activity of serum colony-stimulating factor [20,21].

In recent years, there has been an increasing number of studies on acupuncture in treating CIL. Some studies reported acupuncture could decrease the occurrence rate of leucopenia or stimulate the activity of G-CSF. A exploratory meta-analysis of

acupuncture for CIL which was published in 2007 found that acupuncture was associated with an increase in leukocytes ($p < .0001$)[22]. However, the methodological quality of these reviews were not substantial enough to drive strong recommendations. The acupoints and matching acupoints with optimal efficacy for the treatment of CIL still remain unidentified. The review aims to systematically synthesize the primary research which explores the efficacy and safety of acupuncture for treating CIL.

METHODS AND ANALYSIS

Inclusion criteria for study selection

Types of studies

Randomized controlled trials(RCTs) will be included, while animal studies, case reports, commentaries or quasi-RCTs will be excluded.

Types of patients

It will be included studies on cancer patients with CIL, regardless of age (> 18 years old), gender, race, education status, tumor type and stage and it will be excluded studies whose participants had primary disease of hematopoietic system or psychiatric disorders.

Types of interventions

Experimental interventions

Acupuncture is used in experimental groups, which is defined as the forms of stimulating acupoints by needles, including body acupuncture, scalp acupuncture, manual acupuncture, auricular acupuncture, electro acupuncture, fire needling and plum blossom needle. Other forms of stimulation methods including acupressure without needles, moxibustion (other than a warming needle method), transcutaneous electrical nerve stimulation, laser acupuncture or drug injection therapy will be excluded.

Comparator interventions

All studies in which patients in the control group were treated with acupuncture will be excluded except studies that carried out sham acupuncture to the control group. It will also be excluded all studies in which patients received radiotherapy, molecular targeted therapy or Chinese herbal medicine.

We will investigate the treatment comparisons as follows:

1. Acupuncture versus no treatment.
2. Acupuncture versus sham acupuncture[23].
3. Acupuncture versus other treatment.

4. Acupuncture with another treatment versus the same treatment without acupuncture.

Types of outcome measures

Primary outcomes

The primary outcome will be the changes of WBC counts from baseline to endpoint. We will contact the authors of original articles for additional information about the primary outcome if it were expressed as the changes in decrease of WBCs at grade of I°-IV° (according to the criteria of toxicity grading scale for determining the severity of adverse events by WHO,2003).

Secondary outcomes

1. Decrease in incidence of leucopenia.(Number needed to treat ,NNT)
2. Incidence and type of adverse events of acupuncture(e.g. acupuncture-related infection, fainting during acupuncture treatment, sticking of needle)
3. Quality of life or physical condition, as measured by validated instruments (e.g. Karnofsky Performance Score(KPS), the European Organization for Treatment of Cancer(EORTC) QLQ-C30(Aaronson 1993), et al).

Search methods for identification of studies

Electronic searches

We will electronically search the following database: the Cochrane Central Register of Controlled Trials (CENTRAL), the Cochrane Library, Medline (via PubMed), EMBASE (via embase.com), China National Knowledge Infrastructure Database(CNKI), Chinese Biomedical Literature Database (CBM), Chinese Scientific Journal Database (VIP database), Wan-Fang Database from their inception to 1 January 2016. The following search terms will be used: acupuncture, manual acupuncture, electroacupuncture, fire needling, auricular acupuncture, ear acupuncture, dermal needle, plum blossom needle, leucopenia, leukopenia, aleucocytosis, aleukocytosis, hypoleucocytosis, hypoleukocytosis, oligoleukocythemia, oligoleukocytosis, hypoleukia, G-CSF, Granulocyte colony-stimulating factor, GM-CSF, Granulocyte monocyte colony-stimulating factor. The search words with the same meaning as the English version will be used in Chinese databases. Restriction will be made to English or Chinese language and human subjects. The search strategy for Medline (via PubMed) is shown in table 1. Search strategies used for EMBASE, CENTRAL, CNKI, CBM, VIP and Wan-Fang Database are suppled in appendix1-4.

Table 1 Search strategy used in PubMed

No	Search items
1	Acupuncture therapy [mh]
2	Acupuncture [tiab]
3	Acupoints [tiab]
4	Body acupuncture [tiab]
5	Scalp acupuncture [tiab]
6	manual acupuncture [tiab]
7	Auricular acupuncture [tiab]
8	ear acupuncture [tiab]
9	Electroacupuncture [tiab]
10	Fire needling [tiab]
11	dermal needle [tiab]
12	plum blossom needle [tiab]
13	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12
14	Leucopenia [mh]
15	Leukopenia [tiab]
16	Aleucocytosis [tiab]
17	Aleukocytosis [tiab]
18	Hypoleucocytosis [tiab]
19	Hypoleukocytosis [tiab]
20	oligoleukocythemia [tiab]
21	Oligoleukocytosis [tiab]
22	Hypoleukia [tiab]
23	G-CSF [tiab]
24	Granulocyte colony-stimulating factor [tiab]
25	GM-CSF [tiab]
26	Granulocyte monocyte colony-stimulating factor [tiab]
27	14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26
28	randomized controlled trial [pt]
29	controlled clinical trial [pt]
30	randomized [tiab]
31	placebo [tiab]
32	clinical trials as topic [mesh: noexp]
33	randomly [tiab]
34	trial [ti]
35	28 or 29 or 30 or 31 or 32 or 33 or 34
36	13 and 27 and 35

Searching other sources

The reference lists of potentially eligible studies and relevant systematic reviews will be manual retrieved for additional trials. The conference proceedings in relation to this topic will be also searched to identify further studies. We will also search OpenGrey.eu for potential grey literatures. In addition, we plan to search relevant trial protocols through the WHO International Clinical Trial Registry Platform(ICTRP) and ClinicalTrials.gov for ongoing and newly completed studies.

Data collection and analysis

All reviewers have received training at the beginning in order to get familiar with the purpose and the whole process of the review. The studies obtained from electronic searching and other sources will be uploaded to a database created by Endnote X7. Two reviewers (XS and GJ) will independently screen the titles, abstracts and keywords of all retrieved records. Studies, meeting the predefined eligibility criteria, will be included for full text screening, but their duplicates will be excluded. All excluded studies will be recorded in the" Reasons of excluded studies" table. And any disagreement will be resolved by discussion between the two reviewers (XS and GJ) for consensus or consulting the arbiter (CY) when necessary.

Data extraction and management

Two reviewers (XS and GJ) will independently perform data extraction of selected studies and fill in the data extraction which has been developed before. The authors of trials will be contacted for further details when it is necessary. We will extract data including the following information:

1.General information of the articles including the first author, year, country, sample size.

2. Demographic characteristics including gender, age and other information including tumor type, tumor stage.

3. Intervention parameters including type of acupuncture, acupionts used, chemotherapeutic medications, other treatment, frequency and duration of treatment.

4.Outcome information including results, adverse events, costs, quality of life.

Data extraction sheet is suppled in appendix 5.

Assessment of risk of bias in included studies

The risk of bias of all studies will be assessed by two reviewers (XS and GJ) using the Cochrane Collaboration's "Risk of Bias" Tool (Higgins 2011), which includes the following seven domains: randomized sequence generation, allocation concealment, blinding of participants and personnel, binding of outcome assessment, incomplete outcome data, selective reporting and other issues. The assessment of each domain will be categorized into three levels of bias: low, unclear and high risk of bias, and

then filled in the form" risk of bias" table. Any discrepancies will be resolved by discussion between the two reviewers (XS and GJ) or consulting the third reviewer (CY).

Measures of treatment effect

Mean difference (MD) with 95% confidence intervals will be used to assess continuous outcomes, while dichotomous outcomes will be expressed as the relative risk (RR) with 95% confidence intervals.

Unit of analysis issue

Only the first phase data will be assessed in randomized cross-over trials to prevent carry-over effects. In the case of studies with multiple intervention groups, we will create pairwise comparison if the groups meet the predefined inclusion criteria. Non-randomized controlled trials will not be included for analysis.

Dealing with missing data

We will attempt to contact the original investigators by email or by phone to obtain the missing or insufficient data when the data prevents a study from being included. If we are unable to obtain missing data, analyses will be based on patient populations in which outcomes were reported. Where studies report statistic based on intention-to-treat(ITT) ,we will perform available case analyses.

Assessment of heterogeneity

Heterogeneity will be assessed by visually inspecting the forest plot and investigated by Chi^2(significance level: $P<0.10$) and I^2 statistics. $I^2<50\%$ will be taken as evidence of no statistical heterogeneity, while $I^2\geq50\%$ will be considered to indicate substantial heterogeneity (Higgins 2011) and the causes of heterogeneity among results of studies will be explored through subgroup analysis or sensitivity analysis.

Assessment of publication biases

We will generate funnel plots to detect publication bias or small-study effects with sufficient numbers of included studies (at least 10 studies).

Data synthesis

The RevMan V.5.3 will be employed for data analysis when a meta-analysis is allowed. The MD with 95% confidence intervals will be used to assess continuous outcomes, while the RR with 95% confidence intervals will be expressed for dichotomous data. If I^2 test is less than 50%, the RR, MD will be calculated by fixed-effects model. If I^2 test is higher than 50%, random effects model will be used to synthesize the data and subgroup analysis or sensitivity analysis will be conducted to explore the causes including clinical or methodological reasons. We may conduct narrative synthesis if meta-analysis is not appropriate (e.g. incidence of adverse events of acupuncture).

Subgroup analysis

We will perform subgroup analysis to explore the possible causes of heterogeneity if there is adequate number of studies (at least 10 trials).The effect of different types of acupuncture therapies and CSF(G-CSF/GM-CSF) will be included for analysis. We will also remove studies with low and/or medium quality to examine the robustness of the results.

Sensitivity analysis

Sensitivity analysis will be used to determine whether the conclusions are robust to the decisions which were made during the review process. We will conduct a one-study-removed analysis by excluding one study in each turn and reanalyze the remained studies in order to explore the potential influential effects on composite endpoint.

Grading the quality of evidence

We will use the Grading of Recommendations Assessment, Development and Evaluation(GRADE) to assess the quality of confidence for primary outcomes[24]. Meanwhile, the conclusions will be classified into four levels: high, moderate, low or very low.

DISCUSSION

CIL is one of the most common adverse events during chemotherapeutic period. It can bring about severe adverse reactions, which may affect the efficacy of treatment. Granulocyte colony-stimulating factors (G-CSF), for instance, have been demonstrated a significant reduction in leucopenia. While per-cycle administration (daily use), adverse events, economic burden accompanied with expensive antineoplastic agents were the main problems of G-CSF, which limited its clinical

application. Studies have reported that acupuncture could prevent the decline of WBCs and could be used as adjuncts to medications in increasing the activity of G-CSF, while having fewer side effects and relatively lower cost. However, there still exists a problem of quality synthesis in the evidence currently. Therefore, we will conduct a systematic review in order to provide more convincing evidence for clinicians. This systematic review will be separated into four parts including identification, study inclusion, data extraction and data synthesis.

Some potential limitations may affect in drawing highly creditable conclusions during the review. Firstly, as the restriction in the language of included studies, it may limit the broad searching of potential findings, such as articles in Japanese, Korean or German. Secondly, the studies which are still ongoing or unpublished may prevent data synthesis. Thirdly, various types of acupuncture, diverse kinds of chemotherapy medications, mixed tumor types and stages may increase the risk of heterogeneity. Finally, difficulty in undertaking blinding measures in acupuncture therapy may cause the occurrence of bias test results.

PRISMA-P Checklists of the protocol is suppled in appendix 6.

Acknowledgements

The authors would like to thank Feng Y and Zhou AJ for their assistance to improve the paper.

Contributions of authors

XMW is the guarantor of the review. JYN and XS drafted the manuscript. LY and JYN developed the search strategy. XS and JG will independently screen the potential studies, perform data extraction, assess the risk of bias, enter data into RevMan and finish the data synthesis.CY will arbitrate any disagreements and ensure no errors occur during the review. GWY, MWY and GLZ all got involved in the conception, design, revision and final approval of the review. All authors read and approved the final manuscript.

Declarations of interests

None declared.

Sources of support

No sources of support provided.

Provenance and peer review

Not commissioned; externally peer reviewed.

Protocol amendments

If it is necessary to amend the protocol, we will give the detailed explanation of the date, the change and the rationale.

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APPENDICES

Appendix1. Search strategy used for EMBASE(via OvidSp)

1. exp Acupuncture/
2. Acupuncture .tw.
3. Acupoints .tw.
4. Body acupuncture .tw.
5. Scalp acupuncture .tw.
6. manual acupuncture .tw.
7. Auricular acupuncture .tw.
8. ear acupuncture .tw.
9. Electroacupuncture .tw.
10. Fire needling .tw.
11. dermal needle .tw.
12. plum blossom needle .tw.
13. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12
14. exp Leukopenia/
15. Leucopenia .tw.
16. Aleucocytosis .tw.
17. Aleukocytosis .tw.
18. Hypoleucocytosis .tw.
19. Hypoleukocytosis .tw.
20. Oligoleukocythemia .tw.
21. Oligoleukocytosis .tw.
22. Hypoleukia .tw.
23. G-CSF .tw.
24. Granulocyte colony-stimulating factor .tw.
25. GM-CSF .tw.
26. Granulocyte monocyte colony-stimulating factor .tw.
27. 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26
28. randomized controlled trial.pt.
29. controlled clinical trial.pt.
30. randomized.ab.
31. placebo.ab.
32. clinical trials as topic.sh.
33. randomly.ab.
34. trial.ti.
35. 28 or 29 or 30 or 31 or 32 or 33 or 34
36. exp animals/ not humans.sh.
37. 35 not 36
38. 13 and 27 and 37

Appendix2. Search strategy used for CENTRAL(Wiley Online Library)

1. MeSH descriptor Acupuncture explode all tree
2. Acupuncture :ti,ab,kw
3. Acupoints :ti,ab,kw
4. Body acupuncture :ti,ab,kw
5. Scalp acupuncture :ti,ab,kw
6. manual acupuncture :ti,ab,kw
7. Auricular acupuncture :ti,ab,kw
8. ear acupuncture :ti,ab,kw
9. Electroacupuncture :ti,ab,kw
10. Fire needling :ti,ab,kw
11. dermal needle :ti,ab,kw
12. plum blossom needle :ti,ab,kw
13. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12
14. MeSH descriptor Leukopenia explode all tree
15. Leucopenia :ti,ab,kw
16. Aleucocytosis :ti,ab,kw
17. Aleukocytosis :ti,ab,kw
18. Hypoleucocytosis :ti,ab,kw
19. Hypoleukocytosis :ti,ab,kw
20. Oligoleukocythemia :ti,ab,kw
21. Oligoleukocytosis :ti,ab,kw
22. Hypoleukia :ti,ab,kw
23. G-CSF :ti,ab,kw
24. Granulocyte colony-stimulating factor :ti,ab,kw
25. GM-CSF :ti,ab,kw
26. Granulocyte monocyte colony-stimulating factor :ti,ab,kw
27. 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26
28. 13 and 27

Appendix3. Search strategy used for CNKI \Wan-Fang\VIP Database

Strategy in Chinese phonetic alphabet(i.e., Pinyin):

SU=terms which refer to title, abstract or key words.

(SU="zhenjiu" or SU="zhenci" or SU="zhen" or SU="tizhen" or SU="toupizhen" or SU="erzhen" or SU="dianzhen" or SU="huozhen" or SU="sanlengzhen" or SU="meihuazhen") and (SU="baixibaojianshao" or SU="baixibaojiangdi" or SU="lixibaojiluocijiyinzi" or SU="chongzurenlixibaojiluocijiyinzi") and (SU="suijiduizhaoshiyan" or SU="linchuanguancha" or SU="suiji" or SU="linchuangshiyan" or SU="shiyan")

SU=字段(题目、摘要 or 关键词)

(SU="针灸" or SU="针刺" or SU="针" or SU="体针" or SU="头皮针" or SU="耳针" or SU="电针" or SU="火针" or SU="三棱针" or SU="梅花针") and (SU="白细胞减少" or SU="白细胞降低" or SU="粒细胞集落刺激因子" or SU="重组人粒细胞集落刺激因子") and (SU="随机对照试验" or SU="临床观察" or SU="随机" or SU="临床试验" or SU="试验")

Appendix4. Search strategy used for CBM database

Strategy in Chinese phonetic alphabet(i.e., Pinyin):

SU= terms which refer to title or abstract.

(SU="zhenjiu" or SU="zhenci" or SU="zhen" or SU="tizhen" or SU="toupizhen" or SU="erzhen" or SU="dianzhen" or SU="huozhen" or SU="sanlengzhen" or SU="meihuazhen") and (SU="baixibaojianshao" or SU="baixibaojiangdi" or SU="lixibaojiluocijiyinzi" or SU="chongzurenlixibaojiluocijiyinzi") and (SU="suijiduizhaoshiyan" or SU="linchuanguancha" or SU="suiji" or SU="linchuangshiyan" or SU="shiyan")

SU=字段(题目 or 摘要)

(SU="针灸" or SU="针刺" or SU="针" or SU="体针" or SU="头皮针" or SU="耳针" or SU="电针" or SU="火针" or SU="三棱针" or SU="梅花针") and (SU="白细胞减少" or SU="白细胞降低" or SU="粒细胞集落刺激因子" or SU="重组人粒细胞集落刺激因子") and

(SU="随机对照试验" or SU="临床观察" or SU="随机" or SU="临床试验" or SU="试验")

Appendix5. Extraction sheet of selected studies

Quality of life E/C										
Adverse Events E/C										
NNT E/C										
◀ WBCs E/C										
Other treatments										
Chemotherapy										

Publication Year	Country	Sample Size E/C	Gender E/C		Age E/C	Tumor Stage E/C		Cancer	Acupuncture Type	Acupuncture Points	Intensity	Frequency

First Author										

E=Experimental Group

C=Control Group

Gender E/C: number of male patients of female patients

i.e. E: 10\12(male and female, respective) C:12\14(male and female, respective)

Age E/C: age of patients in experimental of control groups(mean age\median age)

Tumor Stage E/C: number of patients in every stage(stage I\II\III\IV)

i.e. E: 10\12\13\14 C:12\14\16\16\17

Caner: number of every kind of cancer patients

i.e. breast:10, lung:12, liver:13

Acupuncture Type: i.e. ear acupuncture

Intensity: i.e. 20 minutes

Frequency: once a day for 2 weeks

Chemotherapy: i.e. FOLFOX

WBCs E/C: difference value between baseline and endpoint(M±SD)

Otherwise: fill the sheet with baseline and endpoint value(M±SD)

Adverse Events E/C: will describe the adverse events and the frequency

i.e. E: acupuncture-related infection:20 C: acupuncture-related infection:33

Quality of life E/C: difference value between baseline and endpoint(M±SD)

All QoL measurement tool used in the studies will be synthesized(NO. of studies, if>2)

i.e. E:QLQ-C30 18±9 C:QLQ-C30 10±3

Physical condition: difference value between baseline and endpoint(M±SD)

All tools ,which are used to measure physical condition and have been used in the studies ,will be synthesized(NO. of studies, if>2)

i.e. E:KPS 18±9 C:KPS 10±3

Appendix 6

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols)

2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	1
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	9
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	10
Support:			
Sources	5a	Indicate sources of financial or other support for the review	9
Sponsor	5b	Provide name for the review funder and/or sponsor	9

Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	9
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	2-3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	3
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	3-4
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	4-6
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	5
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	6
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	6
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	6
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	4

Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	6-7
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	7-8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	7-8
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	8
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	8
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	7
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*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	1
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	9
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	10

Support:			
Sources	5a	Indicate sources of financial or other support for the review	9
Sponsor	5b	Provide name for the review funder and/or sponsor	9
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