BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026232
Article Type:	Protocol
Date Submitted by the Author:	23-Aug-2018

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Keywords:	vascular calcification, bone mineral density, fracture, abdominal aorta, vascular disease

Abdominal aortic calcification, bone mineral density and fractures: a systematic review and meta-analysis protocol

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Word count: 2004

Abstract

Introduction: Abdominal aortic calcification (AAC) is associated with low bone mass and increased fracture risk. Two previous meta-analyses have investigated the association between AAC and fracture. However, these meta-analyses undertook limited searches and did not explore potential sources of between-study heterogeneity. Our aim is to undertake a sensitive and comprehensive assessment of the relationship between AAC, bone mineral density (BMD) as well as prevalent and incident fractures.

Methods: We will search MEDLINE, EMBASE, Web of Science core collection, and Google scholar (top 200 articles sorted by relevance) from their inception until 1st June 2018. Reference lists of included studies and previous systematic reviews will be hand searched for additional eligible studies. Retrospective and prospective cohort studies (cross-sectional, case-control and longitudinal) reporting the association between AAC, BMD and fracture at any site will be included. At least two investigators will independently: (A) evaluate study eligibility and extract data, with a third investigator to adjudicate when discrepancies occur, (B) assess study quality by the Newcastle-Ottawa Scale for each cohort/study. The meta-analysis will be reported in adherence to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) criteria. AAC will be grouped as either: (1) AAC present or absent, (2) AAC categorised as "low" (referent – lowest reported group) vs. "high" (all other groups) or (3) dose-response when AAC was assessed in three or more groups. Where primary event data was reported in individual studies, pooled risk differences and risk ratios with 95%CI will be calculated, from which, a summary estimate will be determined using DerSimonian-Laird random effects models. For the AAC and BMD pooled analyses estimates will be expressed as standardised mean difference with 95%CI. We will examine the likelihood of publication bias and where possible, investigate potential reasons for between-study heterogeneity using subgroup analyses and meta-regression.

Prospero registration number: CRD42018088019

Key words: vascular calcification, bone mineral density, fracture, abdominal aorta, vascular disease

Article summary: strengths and limitations

- Previous meta-analyses have not utilised comprehensive search strategies and have found moderate to high amounts of heterogeneity, which for the most part has been unexplained. The planned comprehensive meta-analysis is warranted and will help address uncertainties regarding the measurement of AAC for the prediction of fracture outcomes. Additionally, this study will use meta-regression to identify sources of heterogeneity and identify subgroups or subpopulations where AAC is more or less predictive of poorer outcomes.
- To our knowledge there has been no systematic review and meta-analysis that has investigated the association between AAC and BMD, which is along the hypothesised causal pathway to fracture.
- The main limitation of this review is that causality cannot be established due to the observational nature of the studies.
- A further limitation is the differences in imaging modality, measurement and reporting of AAC.

Introduction

Vascular and bone diseases are both chronic age-related disease that share many common dietary and lifestyle risk factors and cause considerable morbidity and mortality [1]. Atherosclerotic lesions in the abdominal aorta generally begin around the major vessel bifurcations and branching arteries such as the inferior mesenteric artery and the lumbar arteries that supply blood and nutrients to the lumbar vertebrae. [2] Occlusion of these vessels may causes ischemia in the lumbar spine and may result in disc degeneration and asymptomatic vertebral fractures. [3] Additionally the underlying processes regulating arterial calcification share many similarities to bone physiology [4] and calcified atherosclerotic plaques release both local and systemic osteochondrogenic factors that may affect regional and systemic bone homeostasis. [5] Conversely circulating levels of factors regulating bone homeostasis may also regulate vascular calcifications [6] with a number of studies demonstrating osteoporosis and bone mineral density being a risk factor for CVD disease. [7 8]

Assessment of lateral spine images are often undertaken to detect prevalent vertebral fractures and have been shown to improve fracture prediction. [9-11] These images can also be used to assess the degree of abdominal aortic calcification (AAC). To date there are conflicting findings as to whether AAC is associated with bone mineral density and fractures and whether or not these associations are due to ageing, shared fracture risk factors or are a non-traditional independent fracture risk factor. Recent meta-analyses published in 2016 [12] and 2017 [13], looking at observational studies, showed that people with any or high AAC were at greater risk of fractures than those with no or low AAC. However, the previous studies did not systematically review and search the literature (searches found 91 and 105 articles respectively) and the meta-analyses missed many of the known studies. Furthermore studies identified moderate-high heterogeneity without properly exploring or explaining the cause. As such uncertainty exists as to the importance of identifying AAC for predicting incident

fractures. We will therefore undertake a meta-analysis of studies reporting on AAC, bone mineral density (BMD) at any site and prevalent and incident fractures at any site.

Objectives

1. To determine the association between AAC with BMD at any site.
2. To determine the association between AAC with prevalent fractures (cross-sectional) by reported prevalent fracture sites.
3. To determine the association between AAC with incident fractures by reported incident fracture sites.
4. To assess the impact of potential effect modifiers on previous published findings.

Methods and Analysis

The systematic review and meta-analysis has been registered with PROSPERO (CRD42018088019) and reported in adherence to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting criteria [14].

Patient and public involvement statement

There is no patient or public involved in this systematic review/meta-analysis.

Eligibility criteria for studies included in this review

Criteria for considering studies for review

a) Observational studies in humans. These include cohort (both retrospective and prospective cohort studies), case control and cross-sectional studies that report eligible exposure(s) and outcome(s)

b) Abdominal aortic calcification assessed by any methodology.

c) Report any bone mineral density measure or prevalent or incident fracture outcome.

Exclusion criteria

a) Reviews of existing literature.

Exposure

AAC identified from either radiography, DXA machine or CT. AAC will be presented as;

a) AAC present or absent or

b) AAC categorized as low (referent – lowest reported group) vs. moderate to high (all other reported groups).

c) AAC dose-response when AAC was assessed in three or more groups categorised as low (lowest reported category), moderate (middle reported category[ies]) and high (highest reported category).

Outcomes

1. Bone mineral density (by site).
2. Prevalent fractures (by fracture site).
3. Incident fractures (by fracture site).

Cohort characteristics for meta-regression (where available)

- Cohort age (cohort mean)
- Gender (% female)
- Years since menopause (cohort mean)
- Hormone replacement therapy (%)
- Modality of assessing AAC (DXA, standard radiograph or CT).
- Cut points choses for comparison (low vs high, tertiles etc.)
- Diabetes (% of cohort)
- Current smoker (% of cohort)
- History of smoking (% of cohort)
- Body mass index (cohort mean)
- Chronic kidney disease (% of cohort)

- History of CVD (% of cohort)
- Location of study (Europe, Asia-Pacific, North America), i.e. are association consistent across ethnicities and nation wealth
- Prevalence of CVD medication use (% of cohort)
- History of fracture (% of cohort)

Study Design

Search strategies

A comprehensive literature search within MEDLINE, Web of Science core collection and EMBASE databases will be conducted to source all possibly relevant studies for review, without language restriction. Google scholar will be searched for the top 200 articles sorted by relevance. The search terms will be combined with the boolean "AND" to find all potentially relevant studies. Conference proceedings and abstracts will also be evaluated. A hand search of reference lists of eligible studies and previous meta-analyses will also be undertaken. Non-English papers will be translated and evaluated for eligibility. If more than one publication of a study is retrieved, articles with the most up to date and complete information will be included, although additional unique data from all sources will be considered and included when relevant. Examples of the search strategy are shown in **Table 1**.

Process for selecting studies

Two or more independent authors [A.J.R., K.L., M.S. and J.R.L.] will assess retrieved citations to assess studies for eligibility. Briefly the process for selecting studies for inclusion in the review and meta-analysis will be as follows: merge all identified records using EndNote; remove duplicate records of the same report; retrieve full text of the potentially relevant reports; link together multiple reports of the same study (using the first or largest report as the primary record and subsequent reports to supplement other data); examine full-

text reports for compliance with eligibility criteria; correspond with investigators, where appropriate, to clarify study eligibility and request missing data; make final decisions on study inclusion. Discrepancies about inclusion will be resolved via iteration and consensus or a third reviewer if consensus cannot be reached between the two reviewers. Excluded studies identified that may plausibly be expected to be an included will be reported in supplementary data with a detailed explanation for the reason of exclusion.

Risk of bias and quality assessment

The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale (NOS). An example of this scale is provided in Supplementary Material 1A, 1B, 1C, 1D. In addition publication bias will be assessed by visual inspection of a funnel plots and the Egger's and Begg's regression tests. Summary estimates of the confidence placed on the evidence will be evaluated using the Grading of Recommendations Assessment Development and Evaluation (GRADE) of evidence about prognosis. GRADE for evidence about prognosis starts with high quality evidence that can then be rated down. These criteria are based on; a) 5 domains diminishing confidence (-1 for risk of bias, inconsistency, imprecision, indirectness, and publication bias) and b) 2 situations increasing confidence (+1 or +2 for large-very large effect size and a +1 for a dose-response gradient [increasing pooled relative risks for fractures with increasing severity of AAC]).[15]

Statistical analysis and data synthesis

Analysis of outcome variables will be presented according to either: (1) AAC present or absent (2) AAC categorised as "low" (referent – lowest reported group) vs. "high" (all other groups) or (3) dose response when AAC was assessed in three or more groups. For the dose-response analysis the lowest reported group (low AAC group) will be compared to the middle group(s) vs the highest reported AAC group (high AAC). Where data on more than three groups of AAC were presented the middle groups were combined as "moderate AAC". This approach was selected due to many studies reporting on variable number of AAC groups with

the majority of studies using different cut-points for these groupings. Data on the severity of AAC quantification presented as a continuous measure or in three or more groupings of AAC will be used to determine the impact of increased abdominal aortic calcium load on outcomes. Where primary event data was reported in individual studies, pooled risk differences and risk ratios (RR) with 95% confidence intervals will be calculated, from which, a summary estimate was determined using DerSimonian-Laird random effects models. For the AAC and BMD pooled analyses estimates will be expressed as standardised mean difference (SMD) with 95% CI. Values will be considered significant if the 95%CI of the point estimate does not cross unity. Between-study heterogeneity will also investigated by using subgroup analyses and the I^2 statistic by study ID which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis.[16 17] We will evaluate for heterogeneity using the I^2 statistic and considered the I^2 thresholds of <25%, 25-49%, 50-75% and >75% to represent low, moderate, high and very-high heterogeneity.

Subgroup analysis and investigation of heterogeneity

We will perform meta-regression of cohort characteristics to identify factors potentially explaining heterogeneity as well as performing subgroup analyses. P values of <0.01 will be considered statistically significant for subgroup analyses. Pre-planned subgroup analyses to explore statistical heterogeneity will include stratification by:

1. Subgroups based on clinical heterogeneity e.g. disease populations (general population, diabetics, chronic kidney disease, other) and age groups (<60 years, 60-69 years and ≥70 years).
2. Methodological heterogeneity e.g. AAC assessment methods (Radiography, Dual X-ray absorptiometry or CT), fracture reporting and validation.
3. Statistical heterogeneity e.g. cohort characteristics (mean ages of the cohorts)

Further analyses

Where data on the severity of AAC quantification is presented as a continuous measure or in tertile/categories these data will be used to determine the impact of increased abdominal aortic calcium load on prognosis. *Where AAC is not scored using the AAC24 scale equivalent values will be relative to estimated vertebral heights from similar aged populations. Where AAC is assessed by CT the categorical low vs moderate and high AAC will be used.

Sensitivity analysis

We will carry out sensitivity analyses for:

1. Large studies alone to establish how much they dominate the results (n > 500 participants).
2. Methodology - we will assess the methodological quality of studies using the Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses (Supplementary Material 1). For the purpose of this sensitivity analysis we will use 3 categories of quality (Good, Fair, or Poor).
3. Studies conducted in individuals without a history of a prior fracture (as this is the biggest risk factor for a new fracture).
4. Studies conducted in high income vs. low income countries.
5. Studies that included non-osteoporotic fractures (fractures of the toes, fingers, face and skull fractures)

Concluding statement

Previous meta-analyses on this topic have a number of important limitations. By undertaking the pre-planned comprehensive review and meta-analysis, we will gain better understanding of the relationship between abdominal aortic calcification (AAC), bone mineral density and

increased fracture risk. The review will provide impetus for further research, diagnosis and treatment of this novel fracture risk factor. This review will also evaluate the quality of the published evidence and our confidence in the estimates for the meta-analysis, while identifying important knowledge gaps, potential sources of between-study heterogeneity and issues with imaging, assessing or reporting of AAC in published studies.

Ethics and dissemination: The systematic review and meta-analysis does not require ethical approval. The study will be submitted to a peer reviewed journal and disseminated via research presentations.

Data statement

Technical appendix and dataset will be available on request from the corresponding author (J.R.L).

Conflicts of interest

The authors declare that there is no conflict of interests in this study protocol.

Funding and acknowledgements

The salaries of J.R.L. and D.S. are supported by a National Health and Medical Research Council of Australia (NHMRC) Career Development Fellowship (ID: 1107474 and 1123014, respectively). Funding agencies had no input into any aspect of the design and management of this study.

Author contribution

All authors contributed to the study concept and design. A.R. and K.L. led the writing of the manuscript and is the primary designer of the protocol under the guidance of J.R.L, J.T.S and P.S and all-authors conceived the conceptual ideas presented in the revised protocol critically. All authors read and approved the revised version and final supported versions. J.R.L has the primary responsibility for the final content.

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Table 1. Example search strategies

Keyword	MEDLINE	Embase
Population = Adults	No search strategy	No search strategy
Intervention/Test = aortic calcification	exp Vascular Calcification/ or exp Calcinosis/ or exp Vascular Diseases/ or arterial calcification.mp or exp Arteriosclerosis/ or exp Arterial Occlusive Diseases/ or exp Aortic Diseases/ or aortic.mp or vascular calcifications.mp. or exp Vascular Calcification/ or calcified atherosclerosis.mp or calcification.mp or calcified atherosclerotic plaque.mp or arterial calcium.mp or aortic calcification.mp or aorta calcification.mp and aort$.mp and calc$.mp	vascular calcification.mp. or exp blood vessel calcification/ or artery calcification.mp. or exp artery calcification/ or exp coronary artery disease/ or exp arteriosclerosis/ or calcified atherosclerosis.mp or arterial calcium.mp or calcified atherosclerotic plaque.mp or calcification.mp or aortic calcification.mp or aorta calcification.mp or vascular calcifications.mp or arteriosclerosis.mp or extracoronary.mp and aort$.mp and calc$.mp
Methodology = observational	No search strategy	No search strategy
Comparator = None	No search strategy	No search strategy
Outcome =	bone mineral density.mp or exp Bone Density/ or Fracture.mp or Fractures.mp	bone mineral density.mp or exp bone density/ or fracture.mp or fractures.mp or exp fracture/
Additional specific filters	Human	Human

*The reference lists of recent literature reviews and guidelines will be hand-searched for possibly relevant studies.

SUPPLEMENTARY MATERIAL 1A

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE - CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?

a) yes, with independent validation *

b) yes, e.g. record linkage or based on self reports

c) no description

2) Representativeness of the cases

a) consecutive or obviously representative series of cases *

b) potential for selection biases or not stated

3) Selection of Controls

a) community controls *

b) hospital controls

c) no description

4) Definition of Controls

a) no history of disease (endpoint) *

b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

a) study controls for fracture risk factors (age) *

b) study controls for any additional factor * (other fracture risk factors)

Exposure

1) Ascertainment of exposure

a) secure record (verified fracture) *

b) structured interview where blind to case/control status *

c) interview not blinded to case/control status

d) written self report or medical record only

e) no description

2) Same method of ascertainment for cases and controls

a) yes *

b) no

3) Non-Response rate

a) same rate for both groups *

b) non respondents described

c) rate different and no designation

SUPPLEMENTARY MATERIAL 1B

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE - COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average general population of that age in the community *

b) somewhat representative of the average general population of that age in the community *

c) selected group of users eg nurses, volunteers

d) no description of the derivation of the cohort

2) Selection of the non exposed cohort

a) drawn from the same community as the exposed cohort *

b) drawn from a different source

c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure

a) secure record (verified fracture) *

b) structured interview *

c) written self report

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes *

b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for age*

b) study controls for any additional fracture risk factors*

Outcome

1) Assessment of outcome

a) independent blind assessment *

b) record linkage *

c) self report

d) no description

2) Was follow-up long enough for outcomes to occur

a) yes (fracture - 1 year) *

b) no

3) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for *

b) subjects lost to follow up unlikely to introduce bias - small number lost - <20% lost to follow up, or description provided of those lost) *

c) follow up rate < 80% (select an adequate %) and no description of those lost

d) no statement

SUPPLEMENTARY MATERIAL 1C

CODING MANUAL FOR COHORT STUDIES

Selection

1) Representativeness of the Exposed Cohort

Item is assessing the representativeness of exposed individuals in the community, not the representativeness of the sample of women from some general population. For example, subjects derived from groups likely to contain middle class, better educated, health oriented women are likely to be representative of postmenopausal estrogen users while they are not representative of all women (e.g. members of a health maintenance organisation (HMO) will be a representative sample of estrogen users. While the HMO may have an under-representation of ethnic groups, the poor, and poorly educated, these excluded groups are not the predominant users of estrogen).

Allocation of stars as per rating sheet

2) Selection of the Non-Exposed Cohort

Allocation of stars as per rating sheet

3) Ascertainment of Exposure

Allocation of stars as per rating sheet

4) Demonstration That Outcome of Interest Was Not Present at Start of Study

In the case of mortality studies, outcome of interest is still the presence of a disease/ incident, rather than death. That is to say that a statement of no history of disease or incident earns a star.

Comparability

1) Comparability of Cohorts on the Basis of the Design or Analysis

A maximum of 2 stars can be allotted in this category

Either exposed and non-exposed individuals must be matched in the design and/or confounders must be adjusted for in the analysis. Statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. Note: If the relative risk for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.

There may be multiple ratings for this item for different categories of exposure (e.g. ever vs. never, current vs. previous or never)

Age = , Other controlled factors =

Outcome

1) Assessment of Outcome

For some outcomes (e.g. fractured hip), reference to the medical record is sufficient to satisfy the requirement for confirmation of the fracture. This would not be adequate for vertebral fracture outcomes where reference to x-rays would be required.

a) Independent or blind assessment stated in the paper, or confirmation of the outcome by reference to secure records (x-rays, medical records, etc.)
b) Record linkage (e.g. identified through ICD codes on database records)
c) Self-report (i.e. no reference to original medical records or x-rays to confirm the outcome)
d) No description.

2) Was Follow-Up Long Enough for Outcomes to Occur

An acceptable length of time should be decided before quality assessment begins (e.g. 5 yrs. for exposure to breast implants)

3) Adequacy of Follow-Up of Cohorts

This item assesses the follow-up of the exposed and non-exposed cohorts to ensure that losses are not related to either the exposure or the outcome.

Allocation of stars as per rating sheet.

SUPPLEMENTARY MATERIAL 1D

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE – CROSS-SECTIONAL STUDIES

Selection (Maximum 5 stars)

1) Representativeness of the exposed cohort

a) Truly representative of the average general population of that age in the community * (all subjects or random sampling)

b) Somewhat representative of the average general population of that age in the community *(non-random sampling)

c) Selected group of users eg nurses, volunteers

d) No description of the derivation of the cohort

2) Sample size

a) Justified*

b) Non-justified

3) Non-respondents

a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory*

b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory

c) No description of the response rate or the characteristics of the responders and the non-responders

4) Ascertainment of the exposure (risk factor):

a) Validated measurement tool **

b) Non-validated measurement tool, but the tool is available or described *

c) No description of the measurement tool

Comparability (Maximum 2 stars)

1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled

a) Study controls for age *

b) Study controls for any additional fracture risk factors *

Outcome (Maximum 3 stars)

1) Assessment of outcome

a) Independent blind assessment **

b) Record linkage **

c) Self-report *

d) No description

2) Statistical test

a) The statistical test used to describe the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value) *

b) The statistical test is not appropriate, not described or incomplete

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	
ADMINISTRATIVE INFORMATION			**Page number**
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	NA
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2,6
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	12
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	NA
Support:			
Sources	5a	Indicate sources of financial or other support for the review	12
Sponsor	5b	Provide name for the review funder and/or sponsor	12
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	12
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
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	6-8
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	8-9
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	16

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8
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)	8
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	9
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	9
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
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	9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9-10
	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)	9-10
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10-11
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	NA
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	9

*** 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

Abdominal aortic calcification, bone mineral density and fractures: a systematic review and meta-analysis protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026232.R1
Article Type:	Protocol
Date Submitted by the Author:	19-Dec-2018
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Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Cardiovascular medicine, Radiology and imaging
Keywords:	vascular calcification, bone mineral density, fracture, abdominal aorta, vascular disease

Abdominal aortic calcification, bone mineral density and fractures: a systematic review and meta-analysis protocol

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Word count: 1979

Abstract

Introduction: Abdominal aortic calcification (AAC) is associated with low bone mass and increased fracture risk. Two previous meta-analyses have investigated the association between AAC and fracture. However, these meta-analyses only identified articles until December 2016, undertook limited searches and did not explore potential sources of between-study heterogeneity. We aim to undertake a sensitive and comprehensive assessment of the relationship between AAC, bone mineral density (BMD) as well as prevalent and incident fractures.

Methods: We will search MEDLINE, EMBASE, Web of Science core collection, and Google scholar (top 200 articles sorted by relevance) from their inception until 1st June 2018. Reference lists of included studies and previous systematic reviews will be hand searched for additional eligible studies. Retrospective and prospective cohort studies (cross-sectional, case-control and longitudinal) reporting the association between AAC, BMD and fracture at any site will be included. At least two investigators will independently: (A) evaluate study eligibility and extract data, with a third investigator to adjudicate when discrepancies occur, (B) assess study quality by the Newcastle-Ottawa Scale for each cohort/study. The meta-analysis will be reported in adherence to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) criteria. AAC will be grouped as either: (1) AAC present or absent, (2) AAC categorised as "low" (referent – lowest reported group) vs. "high" (all other groups) or (3) dose-response when AAC was assessed in ≥3 groups. Where primary event data was reported in individual studies, pooled risk differences and risk ratios with 95%CI will be calculated, from which, a summary estimate will be determined using DerSimonian-Laird random effects models. For the AAC and BMD pooled analyses estimates will be expressed as standardised mean difference with 95%CI. We will examine the likelihood of publication bias and where

possible, investigate potential reasons for between-study heterogeneity using subgroup analyses and meta-regression.

Prospero registration number:CRD42018088019

Key words: vascular calcification, bone mineral density, fracture, abdominal aorta, vascular disease

Article summary: strengths and limitations

- Previous meta-analyses have only searched the literature until 2016, have found limited numbers of studies and identified moderate to high amounts of between-study heterogeneity, which for the most part has been unexplained. The planned comprehensive meta-analysis is warranted and will help address uncertainties regarding the measurement of AAC for the prediction of fracture outcomes and understand the role of AAC alongside, but independent of BMD in fracture risk prediction. Additionally, this study will use meta-regression to identify sources of heterogeneity and identify subgroups or subpopulations where AAC is more or less predictive of poorer outcomes.
- To our knowledge there has been no systematic review and meta-analysis that has investigated the association between AAC and BMD, which is along the hypothesised causal pathway to fracture.
- The main limitation of this review is that causality cannot be established due to the observational nature of the studies.
- A further limitation is the differences in imaging modality, measurement and reporting of AAC across studies but we attempted to overcome this by exploring these aspects in pre-specified sub-analyses.

Introduction

Vascular and bone diseases are both chronic age-related disease that share many common dietary and lifestyle risk factors and cause considerable morbidity and mortality [1]. Atherosclerotic lesions in the abdominal aorta generally begin around the major vessel bifurcations and branching arteries such as the inferior mesenteric artery and the lumbar arteries that supply blood and nutrients to the lumbar vertebrae. [2] Occlusion of these vessels may causes ischemia in the lumbar spine and may result in disc degeneration and asymptomatic vertebral fractures. [3] Additionally the underlying processes regulating arterial calcification share many similarities to bone physiology [4] and calcified atherosclerotic plaques release both local and systemic osteochondrogenic factors that may affect regional and systemic bone homeostasis. [5] Conversely circulating levels of factors regulating bone homeostasis may also regulate vascular calcifications [6] with a number of studies demonstrating osteoporosis and bone mineral density being a risk factor for cardiovascular disease (CVD). [7 8]

Assessment of lateral spine images are often undertaken to detect prevalent vertebral fractures and have been shown to improve fracture prediction. [9-11] These images can also be used to assess the degree of abdominal aortic calcification (AAC). To date there are conflicting findings as to whether AAC is associated with bone mineral density and fractures and whether or not these associations are due to ageing, shared fracture risk factors or are a non-traditional independent fracture risk factor. Recent meta-analyses published in 2016 [12] and 2017 [13], looking at observational studies, showed that people with any or high AAC were at greater risk of fractures than those with no or low AAC. However, the previous studies by Chen et al.[12] and Wei et al.[13], only identified a limited number of articles due to the search strategies employed (searches found 91 and 105 articles respectively) and the meta-analyses missed many of the known studies in the area (by way of example - both studies missed Wang et al. [14]). For example, our recent search identified 1561 potentially eligible reports. Furthermore, studies

identified moderate-high unexplained heterogeneity that needs to be explored further. As such uncertainty exists as to the importance of identifying AAC for incident fracture risk, particularly with respect to AAC cut points, types of fracture and potential explanations for the observed between-study heterogeneity.We will therefore undertake a meta-analysis of studies reporting on AAC, bone mineral density (BMD) at any site and prevalent and incident fractures at any site.

Objectives

1. To determine the association between AAC with BMD at any site.
2. To determine the association between AAC with prevalent fractures (cross-sectional) by reported prevalent fracture sites.
3. To determine the association between AAC with incident fractures by reported incident fracture sites.
4. To assess the impact of potential effect modifiers, including aspects of clinical, methodological and statistical heterogeneity on previous published findings.

Methods and Analysis

The systematic review and meta-analysis has been registered with PROSPERO (CRD42018088019) and reported in adherence to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting criteria [15].

Patient and public involvement statement

There is no patient or public involved in this systematic review/meta-analysis.

Eligibility criteria for studies included in this review

Criteria for considering studies for review

a) Observational studies in humans. These include cohort (both retrospective and prospective cohort studies), case control and cross-sectional studies that report eligible exposure(s) and outcome(s)

b) Abdominal aortic calcification assessed by any methodology.

c) Report any bone mineral density measure or prevalent or incident fracture outcome.

Exclusion criteria

a) Reviews of existing literature.

Exposure

AAC identified from either radiography, DXA machine or CT. AAC will be presented as;

a) AAC present or absent or

b) AAC categorized as low (referent – lowest reported group) vs. moderate to high (all other reported groups).

c) AAC dose-response when AAC was assessed in three or more groups categorised as low (lowest reported category), moderate (middle reported category[ies]) and high (highest reported category).

Outcomes

1. Bone mineral density (by site).

2. Prevalent fractures (by fracture site).

3. Incident fractures (by fracture site).

Cohort characteristics for meta-regression (where available)

- Cohort age (cohort mean)
- Gender (% female)
- Years since menopause (cohort mean)
- Hormone replacement therapy (%)

- Modality of assessing AAC (DXA, standard radiograph or CT).
- Cut points choses for comparison (low vs high, tertiles etc.)
- Diabetes (% of cohort)
- Current smoker (% of cohort)
- History of smoking (% of cohort)
- Body mass index (cohort mean)
- Chronic kidney disease (% of cohort)
- History of CVD (% of cohort)
- Location of study (Europe, Asia-Pacific, North America), i.e. are association consistent across ethnicities and nation wealth
- Prevalence of CVD medication use (% of cohort)
- History of fracture (% of cohort)

Study Design

Search strategies

A comprehensive literature search within MEDLINE, Web of Science core collection and EMBASE databases will be conducted to source all possibly relevant studies for review, without language restriction. Google scholar will be searched for the top 200 articles sorted by relevance. The search terms will be combined with the boolean "AND" to find all potentially relevant studies. Conference proceedings and abstracts will also be evaluated. A hand search of reference lists of eligible studies and previous meta-analyses will also be undertaken. Non-English papers will be translated and evaluated for eligibility. If more than one publication of a study is retrieved, articles with the most up to date and complete information will be included, although additional unique data from all sources will be considered and included when relevant. Examples of the search strategy are shown in **Table 1**.

Process for selecting studies

Two or more independent authors [A.J.R., K.L., M.S. and J.R.L.] will assess retrieved citations to assess studies for eligibility. Briefly the process for selecting studies for inclusion in the review and meta-analysis will be as follows: merge all identified records using EndNote; remove duplicate records of the same report; retrieve full text of the potentially relevant reports; link together multiple reports of the same study (using the first or largest report as the primary record and subsequent reports to supplement other data); examine full-text reports for compliance with eligibility criteria; correspond with investigators, where appropriate, to clarify study eligibility and request missing data; make final decisions on study inclusion. Discrepancies about inclusion will be resolved via iteration and consensus or a third reviewer if consensus cannot be reached between the two reviewers. Excluded studies identified that may plausibly be expected to be an included will be reported in supplementary data with a detailed explanation for the reason of exclusion.

Risk of bias and quality assessment

The risk of bias for observational studies will be assessed using the Newcastle-Ottawa Scale (NOS). An example of this scale is provided in Supplementary Material 1A, 1B, 1C, 1D. In addition publication bias will be assessed by visual inspection of a funnel plots and the Egger's and Begg's regression tests. Summary estimates of the confidence placed on the evidence will be evaluated using the Grading of Recommendations Assessment Development and Evaluation (GRADE) of evidence about prognosis. GRADE for evidence about prognosis starts with high quality evidence that can then be rated down. These criteria are based on; a) 5 domains diminishing confidence (-1 for risk of bias, inconsistency, imprecision, indirectness, and publication bias) and b) 2 situations increasing confidence (+1 or +2 for large-very large effect size and a +1 for a dose-response gradient [increasing pooled relative risks for fractures with increasing severity of AAC]).[16]

Statistical analysis and data synthesis

Analysis of outcome variables will be presented according to either: (1) AAC present or absent (2) AAC categorised as "low" (referent – lowest reported group) vs. "high" (all other groups) or (3) dose response when AAC was assessed in three or more groups. For the dose-response analysis the lowest reported group (low AAC group) will be compared to the middle group(s) vs the highest reported AAC group (high AAC). Where data on more than three groups of AAC were presented the middle groups were combined as "moderate AAC". This approach was selected due to many studies reporting on variable number of AAC groups with the majority of studies using different cut-points for these groupings. Data on the severity of AAC quantification presented as a continuous measure or in three or more groupings of AAC will be used to determine the impact of increased abdominal aortic calcium load on outcomes. Where primary event data was reported in individual studies, pooled risk differences and risk ratios (RR) with 95% confidence intervals will be calculated, from which, a summary estimate was determined using DerSimonian-Laird random effects models. For the AAC and BMD pooled analyses estimates will be expressed as standardised mean difference (SMD) with 95% CI. Values will be considered significant if the 95%CI of the point estimate does not cross unity. Between-study heterogeneity will also investigated by using subgroup analyses and the I^2 statistic by study ID which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis.[17 18] We will evaluate for heterogeneity using the I^2 statistic and considered the I^2 thresholds of <25%, 25-49%, 50-75% and >75% to represent low, moderate, high and very-high heterogeneity.

Subgroup analysis and investigation of heterogeneity

We will perform meta-regression of cohort characteristics to identify factors potentially explaining heterogeneity as well as performing subgroup analyses. P values of <0.01 will be

considered statistically significant for subgroup analyses. Pre-planned subgroup analyses to explore statistical heterogeneity will include stratification by:

1. Subgroups based on clinical heterogeneity e.g. disease populations (general population, diabetics, chronic kidney disease, other) and age groups (<60 years, 60-69 years and ≥70 years).
2. Methodological heterogeneity e.g. AAC assessment methods (Radiography, Dual X-ray absorptiometry or CT), thresholds to define high or severe AAC, fracture reporting and validation.
3. Statistical heterogeneity e.g. cohort characteristics (mean ages of the cohorts)

Further analyses

Where data on the severity of AAC quantification is presented as a continuous measure or in tertile/categories these data will be used to determine the impact of increased abdominal aortic calcium load on prognosis. *Where AAC is not scored using the AAC24 scale equivalent values will be relative to estimated vertebral heights from similar aged populations. Where AAC is assessed by CT the categorical low vs moderate and high AAC will be used.

Sensitivity analysis

We will carry out sensitivity analyses for:

1. Large studies alone to establish how much they dominate the results (n > 500 participants).
2. Methodology - we will assess the methodological quality of studies using the Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses (Supplementary Material 1). For the purpose of this sensitivity analysis we will use 3 categories of quality (Good, Fair, or Poor).

3. Studies conducted in individuals without a history of a prior fracture (as this is the biggest risk factor for a new fracture).
4. Studies conducted in high income vs. low income countries.
5. Studies that included non-osteoporotic fractures (fractures of the toes, fingers, face and skull fractures)
6. Study design bias comparing outcomes in cross-sectional and prospective studies (given that prospective studies may also include prevalent fractures and BMD measurements at baseline that can be analysed cross-sectionally).

Concluding statement

Previous meta-analyses on this topic have a number of important limitations. By undertaking the pre-planned comprehensive review and meta-analysis, we will gain better understanding of the relationship between abdominal aortic calcification (AAC), bone mineral density and increased fracture risk. The review will provide impetus for further research, diagnosis and treatment of this novel fracture risk factor. This review will also evaluate the quality of the published evidence and our confidence in the estimates for the meta-analysis, while identifying important knowledge gaps, potential sources of between-study heterogeneity and issues with imaging, assessing or reporting of AAC in published studies.

Ethics and dissemination: The systematic review and meta-analysis does not require ethical approval. The study will be submitted to a peer reviewed journal and disseminated via research presentations.

Data statement

Technical appendix and dataset will be available on request from the corresponding author (J.R.L).

Conflicts of interest

The authors declare that there is no conflict of interests in this study protocol.

Funding and acknowledgements

The salaries of J.R.L. and D.S. are supported by a National Health and Medical Research Council of Australia (NHMRC) Career Development Fellowship (ID: 1107474 and 1123014, respectively). Funding agencies had no input into any aspect of the design and management of this study.

Author contribution

A.R, K.L, P.S, D.S, P.E, M.S, G.W, W.H.L, J.T.S, D.P.K, R.L.P and J.R.L contributed to the study concept and design. A.R and K.L led the writing of the manuscript and is the primary designer of the protocol under the guidance of J.R.L, J.T.S and P.S and all-authors conceived the conceptual ideas presented in the revised protocol critically. All authors read and approved the revised version and final supported versions. J.R.L has the primary responsibility for the final content.

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Table 1. Example search strategies

Keyword	MEDLINE	Embase
Population = Adults	No search strategy	No search strategy
Intervention/Test = aortic calcification	exp Vascular Calcification/ or exp Calcinosis/ or exp Vascular Diseases/ or arterial calcification.mp or exp Arteriosclerosis/ or exp Arterial Occlusive Diseases/ or exp Aortic Diseases/ or aortic.mp or vascular calcifications.mp. or exp Vascular Calcification/ or calcified atherosclerosis.mp or calcification.mp or calcified atherosclerotic plaque.mp or arterial calcium.mp or aortic calcification.mp or aorta calcification.mp and aort$.mp and calc$.mp	vascular calcification.mp. or exp blood vessel calcification/ or artery calcification.mp. or exp artery calcification/ or exp coronary artery disease/ or exp arteriosclerosis/ or calcified atherosclerosis.mp or arterial calcium.mp or calcified atherosclerotic plaque.mp or calcification.mp or aortic calcification.mp or aorta calcification.mp or vascular calcifications.mp or arteriosclerosis.mp or extracoronary.mp and aort$.mp and calc$.mp
Methodology = observational	No search strategy	No search strategy
Comparator = None	No search strategy	No search strategy
Outcome =	bone mineral density.mp or exp Bone Density/ or Fracture.mp or Fractures.mp	bone mineral density.mp or exp bone density/ or fracture.mp or fractures.mp or exp fracture/
Additional specific filters	Human	Human

*The reference lists of recent literature reviews and guidelines will be hand-searched for possibly relevant studies.

SUPPLEMENTARY MATERIAL 1A

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE - CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?

a) yes, with independent validation *

b) yes, e.g. record linkage or based on self reports

c) no description

2) Representativeness of the cases

a) consecutive or obviously representative series of cases *

b) potential for selection biases or not stated

3) Selection of Controls

a) community controls *

b) hospital controls

c) no description

4) Definition of Controls

a) no history of disease (endpoint) *

b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

a) study controls for fracture risk factors (age) *

b) study controls for any additional factor * (other fracture risk factors)

Exposure

1) Ascertainment of exposure

a) secure record (verified fracture) *

b) structured interview where blind to case/control status *

c) interview not blinded to case/control status

d) written self report or medical record only

e) no description

2) Same method of ascertainment for cases and controls

a) yes *

b) no

3) Non-Response rate

a) same rate for both groups *

b) non respondents described

c) rate different and no designation

SUPPLEMENTARY MATERIAL 1B

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE - COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average general population of that age in the community *

b) somewhat representative of the average general population of that age in the community *

c) selected group of users eg nurses, volunteers

d) no description of the derivation of the cohort

2) Selection of the non exposed cohort

a) drawn from the same community as the exposed cohort *

b) drawn from a different source

c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure

a) secure record (verified fracture) *

b) structured interview *

c) written self report

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes *

b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for age*

b) study controls for any additional fracture risk factors*

Outcome

1) Assessment of outcome

a) independent blind assessment *

b) record linkage *

c) self report

d) no description

2) Was follow-up long enough for outcomes to occur

a) yes (fracture - 1 year) *

b) no

3) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for *

b) subjects lost to follow up unlikely to introduce bias - small number lost - <20% lost to follow up, or description provided of those lost) *

c) follow up rate < 80% (select an adequate %) and no description of those lost

d) no statement

SUPPLEMENTARY MATERIAL 1C

CODING MANUAL FOR COHORT STUDIES

Selection

1) Representativeness of the Exposed Cohort

Item is assessing the representativeness of exposed individuals in the community, not the representativeness of the sample of women from some general population. For example, subjects derived from groups likely to contain middle class, better educated, health oriented women are likely to be representative of postmenopausal estrogen users while they are not representative of all women (e.g. members of a health maintenance organisation (HMO) will be a representative sample of estrogen users. While the HMO may have an under-representation of ethnic groups, the poor, and poorly educated, these excluded groups are not the predominant users of estrogen).

Allocation of stars as per rating sheet

2) Selection of the Non-Exposed Cohort

Allocation of stars as per rating sheet

3) Ascertainment of Exposure

Allocation of stars as per rating sheet

4) Demonstration That Outcome of Interest Was Not Present at Start of Study

In the case of mortality studies, outcome of interest is still the presence of a disease/ incident, rather than death. That is to say that a statement of no history of disease or incident earns a star.

Comparability

1) Comparability of Cohorts on the Basis of the Design or Analysis

A maximum of 2 stars can be allotted in this category

Either exposed and non-exposed individuals must be matched in the design and/or confounders must be adjusted for in the analysis. Statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. Note: If the relative risk for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.

There may be multiple ratings for this item for different categories of exposure (e.g. ever vs. never, current vs. previous or never)

Age = , Other controlled factors =

Outcome

1) Assessment of Outcome

For some outcomes (e.g. fractured hip), reference to the medical record is sufficient to satisfy the requirement for confirmation of the fracture. This would not be adequate for vertebral fracture outcomes where reference to x-rays would be required.

a) Independent or blind assessment stated in the paper, or confirmation of the outcome by reference to secure records (x-rays, medical records, etc.)
b) Record linkage (e.g. identified through ICD codes on database records)
c) Self-report (i.e. no reference to original medical records or x-rays to confirm the outcome)
d) No description.

2) Was Follow-Up Long Enough for Outcomes to Occur

An acceptable length of time should be decided before quality assessment begins (e.g. 5 yrs. for exposure to breast implants)

3) Adequacy of Follow-Up of Cohorts

This item assesses the follow-up of the exposed and non-exposed cohorts to ensure that losses are not related to either the exposure or the outcome.

Allocation of stars as per rating sheet.

SUPPLEMENTARY MATERIAL 1D

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE – CROSS-SECTIONAL STUDIES

Selection (Maximum 5 stars)

1) Representativeness of the exposed cohort

a) Truly representative of the average general population of that age in the community * (all subjects or random sampling)

b) Somewhat representative of the average general population of that age in the community *(non-random sampling)

c) Selected group of users eg nurses, volunteers

d) No description of the derivation of the cohort

2) Sample size

a) Justified*

b) Non-justified

3) Non-respondents

a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory*

b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory

c) No description of the response rate or the characteristics of the responders and the non-responders

4) Ascertainment of the exposure (risk factor):

a) Validated measurement tool **

b) Non-validated measurement tool, but the tool is available or described *

c) No description of the measurement tool

Comparability (Maximum 2 stars)

1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled

a) Study controls for age *

b) Study controls for any additional fracture risk factors *

Outcome (Maximum 3 stars)

1) Assessment of outcome

a) Independent blind assessment **

b) Record linkage **

c) Self-report *

d) No description

2) Statistical test

a) The statistical test used to describe the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value) *

b) The statistical test is not appropriate, not described or incomplete

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	
ADMINISTRATIVE INFORMATION			**Page number**
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	NA
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2,6
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	12
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	NA
Support:			
Sources	5a	Indicate sources of financial or other support for the review	12
Sponsor	5b	Provide name for the review funder and/or sponsor	12
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	12
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
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	6-8
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	8-9
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	16

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8
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)	8
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	9
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	9
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
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	9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9-10
	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)	9-10
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10-11
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	NA
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	9

*** 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.