# Diagnostic utility of CSF α-synuclein species in Parkinson’s disease: protocol for a systematic review and meta-analysis

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<td>bmjopen-2016-011113</td>
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<td>Protocol</td>
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<tr>
<td>Date Submitted by the Author:</td>
<td>12-Jan-2016</td>
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<tr>
<td>Complete List of Authors:</td>
<td>Eusebi, Paolo; University of Perugia, Department of Medicine; Regional Health Authority of Umbria, Health Planning Service Giannandrea, David; Neurology Unit, USL Umbria 1 Biscetti, Leonardo; University of Perugia, Department of Medicine, Section of Neurology Abraha, Iosief; Regional Health Authority of Umbria, Health Planning Service Chiasserini, Davide; University of Perugia, Department of Medicine, Section of Neurology Orso, Massimiliano; Regional Health Authority of Umbria, Health Planning Service of Perugia Calabresi, Paolo; University of Perugia, Department of Medicine, Section of Neurology; Fondazione Santa Lucia Istituto di Ricovero e Cura a Carattere Scientifico Parnetti, Lucilla; University of Perugia, Department of Medicine, Section of Neurology</td>
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Diagnostic utility of CSF α-synuclein species in Parkinson’s disease: protocol for a systematic review and meta-analysis

Paolo Eusebi¹,², David Giannandrea³, Leonardo Biscetti¹, Iosief Abraha², Davide Chiasserini¹, Massimiliano Orso², Calabresi Paolo¹,⁴, Lucilla Parnetti¹.

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Abstract

Introduction: The diagnostic criteria currently used for Parkinson’s disease (PD) are mainly based on clinical motor symptoms. For these reasons many biomarkers are under investigation to support the diagnosis at the early stage. The neuropathological hallmark of PD is represented by Lewy bodies, which are intracytoplasmic inclusions in substantia nigra neurons. α-synuclein is the major component of the LBs and has been implicated in the pathogenesis of PD and in other "synucleinopathies" such as multi-system atrophy (MSA) and dementia with Lewy bodies (DLB). Several studies have investigated this presynaptic protein as potential biomarker of PD. The aim of our meta-analysis is to determine the ability of cerebrospinal fluid (CSF) concentrations of total α-synuclein, oligomeric α-synuclein and phosphorylated α-synuclein to discriminate PD patients from healthy subjects, non-degenerative neurological controls, patients suffering from parkinsonism and or synucleinopathies.

Methods and analysis: This systematic review protocol has been developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocol (PRISMA-P) 2015 statement and was registered on PROSPERO (CRD42016013217). We will search Cochrane Library, Web of Science, MEDLINE (via Pubmed) and EMBASE from inception using appropriate search strategies. Two independent reviewers will screen titles, abstracts and full-text articles, and will complete data abstraction. We will include studies that involved patients with Parkinson’s disease, dementia with Lewy bodies, multisystem atrophy, progressive supra-nuclear palsy, corticobasal disease, Parkinson’s disease with dementia, and vascular Parkinson’s disease with dementia, and in which at least one between total α-syn, oligomeric α-syn and phosphorylated α-syn was measured in CSF. To evaluate the risk of bias and applicability of primary diagnostic accuracy studies we will use QUADAS-2.

Ethics and dissemination: Our study will neither include any confidential data, nor interventional, so ethical approval is not required. The results of the study will be reported in international peer-reviewed journals.
Strengths and limitations of this study

This diagnostic review protocol aims to comprehensively systematically assess the evidence regarding the diagnostic utility of CSF α-syn (total concentration, oligomeric and phosphorylated form) in discriminating patients with Parkinson disease from healthy individuals.

The results of this systematic review may also help clinicians in the differential diagnosis of Parkinson’s disease.

The planned systematic review and meta-analysis will be the first summary of the evidence in the field with a rigorous methodological conduct.

However, we expect heterogeneity in the design and conduct of the primary studies and in the type of the markers used as index test; this would make difficult to reach exhaustive conclusions.

We also expect that, given the well-know inter-laboratory variation, it will be difficult to have defined and validated cut-off of α-syn markers as final outcomes.
Introduction

Together with dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), Parkinson disease (PD) is part of the synucleinopathies’ spectrum, characterized by the deposition of fibrillary aggregates of α-synuclein protein (α-syn) in the cytoplasm of selective populations of neurons (PD and DLB) and oligodendroglia (MSA) (Uversky VN. 2008).

PD is a progressive neurological disorder; it is the second most common neurodegenerative disease, immediately after Alzheimer’s disease (AD). The incidence of the disease rises abruptly with age and several data showed prevalence varying from 1% of the general population older than 60 years, to 4% of the population older than 80 years (Pringsheim T. et al. 2014). The median age of onset is 60 years and the mean duration of the disease from diagnosis to death is 15 years (Lees A.J. 2009).

Currently, the diagnosis of PD is mainly based on clinical criteria, primarily through the identification of the cardinal motor signs: bradykinesia, rest tremor and rigidity (Gelb D.J. et al. 1999). Unfortunately, when the motor signs appear, the neurodegeneration is at an advanced phase.

It has been estimated that about 70% of nigral neurons are lost when the motor symptoms are evident.

Since PD has a long pre- or pauci-symptomatic phase, in which only non-motor symptoms are often present - such as REM sleep behaviour disorder (RBD), olfactory disorders, constipation, depression and forms of dysautonomia (Munhoz R.P. et al. 2015), it becomes increasingly significant to identify diagnostic tools that can differentiate individuals at risk of developing overt PD from healthy individuals.

Moreover, the differential diagnosis between PD and the atypical parkinsonisms – e.g. MSA, progressive supranuclear palsy (PSP), cortico-basal degeneration (CBD) - can be difficult, particularly at the early stages of the disease, primarily because PD symptoms overlap with the symptoms of other diseases (Shi M. et al. 2011).

When PD is only diagnosed on the basis of clinical sign (sequential neurological examinations to detect cardinal motor deficits, the disease progression, the responsiveness to levodopa treatment and to exclude atypical signs), the diagnostic accuracy for PD is about 75-90% depending on whether it is a general neurologist or an expert of movement disorders (Hughes A.J. et al. 2002).

Therefore, the research on the identification of a reliable and reproducible biomarker for the early PD diagnosis is fundamental to improve the precision in early diagnosis compared to control and to increase the accuracy of the differential diagnosis against other parkinsonism, which rarely respond to levodopa.

The cerebrospinal fluid (CSF) is in close contact with the extracellular space of the brain, therefore it is believed to mirror many of the biochemical processes of the brain.
Several studies were performed to assess the role of CSF biomarkers in PD diagnosis/prognosis, but the data are inconsistent or conflicting (Parnetti L. et al. 2013).

In the recent years several systematic reviews and meta-analyses were published (Gao L. et al. 2014, Sako W. et al. 2014, Zhou B. et al. 2015) but each of them lacks of at least one crucial aspect such as: analysis of diagnostic data, assessment of risk of bias, search strategy with multiple electronic databases, and analysis of phosphorylated α-syn.

The aim of our systematic review and meta-analysis is to evaluate the diagnostic utility of CSF α-syn (total concentration, oligomeric and phosphorylated form) to distinguish between PD and healthy subjects - primary outcome -, and between PD and patients suffering from atypical parkinsonism - secondary outcome -. 
Methods and analysis

1. Search strategy

Electronic Search

We will search through multiple sources of information to guarantee that all relevant studies are included in the review according to the eligibility criteria. In particular, we will search in: Cochrane Library, ISI Web of Science, MEDLINE (via Pubmed) and EMBASE. We will search without any language restriction. See Appendix 1 for a proposed draft strategy to be run.

Searching other resources

Interrogation of electronic databases will include also conference proceedings, ensuring that grey literature will be taken into account. We will scan reference lists of all eligible studies and reviews in the field for further possible titles and the process will be repeated until no new titles are found (Greenhalgh 2005).

This review protocol was prepared according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis Protocols (PRISMA-P) 2015 Statement (Shamseer et al 2015) and the results will be presented following the PRISMA flow diagram.

2. Eligibility criteria

Types of studies

We will consider prospective and retrospective cohort studies as well as clinical trials that have evaluated the diagnostic accuracy of CSF markers to discriminate patients with PD from healthy subjects (primary objective) or from subjects with other parkinsonism (secondary objective). Results of baseline assessment in longitudinal studies are also of interest.

Participants

Studies must include a group of participants with PD and another group of subjects that can be either a group of neurological/healthy controls and/or with patients with other parkinsonism. The diagnoses of parkinsonism will be based on internationally established operational criteria (McKeith IG et al.2005, Gilman S et al 2008, Litvan I et al.1996 and 2006, Alexander SK et al 2014, Armstrong MJ et al 2013, Bak TH et al 2008)

The diagnosis for PD will be established using the UK Parkinson’s Disease Society Brain Bank criteria (Hughes et al. 1992) or the National Institute of Neurological Disorders and Stroke (NINDS; Gelb et al., 1999).
Index tests

Studies that included the following markers will be considered in our assessment:

• CSF total α-synuclein
• CSF oligomeric α-synuclein
• CSF phosphorylated α-synuclein

All the markers will be evaluated for both the primary and secondary outcome.

There are currently no generally accepted standards for positivity threshold in such CSF biomarkers, and therefore it is not possible to pre-specify test positivity threshold.

We will use the criteria that were applied in each included primary study to classify participants as either test positive or test negative. We will compare the index tests with the reference standards specified below.

Target condition

Parkinson’s disease.

Reference standards

For the purpose of this review, we will consider the following clinical criteria as a suitable reference standard: the UK Parkinson’s Disease Society Brain Bank criteria (UKPDSBB; Hughes et al., 1992) or the National Institute of Neurological Disorders and Stroke (NINDS; Gelb et al., 1999).

Study selection

Two researchers will screen all titles and abstracts generated by the electronic database searches for relevance. Two researchers will then independently assess full manuscripts against the eligibility criteria. When necessary, a third arbitrator will resolve disagreements that the two researchers cannot resolve through discussion.

Where a study includes usable data but these are not presented in the published manuscript, we will contact the authors to request further information. If the same data set is presented in more than one paper we will include only the first published paper. We will detail the steps of the selection process in a PRISMA flow diagram.

3. Data extraction

We will extract the following data on study characteristics:

Bibliographic details of primary paper: author, title of study, year and journal;
Demographics: number of subjects; age; gender;

Study design: (prospective or retrospective; cross-sectional studies or randomised controlled trials)

Clinical information: PD staging (Hoehn & Yahr stage); duration of disease; illness severity (UPDRS-III);

Inclusion and exclusion criteria for individual studies;

The type of index test: CSF total α-synuclein, CSF oligomeric α-synuclein CSF phosphorylated α-synuclein;

Measurement used for the index test: e.g., ELISA commercial, ELISA in-house, Luminex, others;

Details of the reference standard: criteria for the clinical diagnosis of PD;

Diagnostic data: number of true positives, false positives, false negatives, and true negatives;

Funding source and conflict of interest.

4. Assessment of methodological quality and risk of bias

We will assess methodological quality of each study using the QUADAS-2 tool (Whiting 2011). The tool is made up of four domains: Patient selection; Index test; Reference standard; flow and timing. Each domain is assessed in terms of risk of bias, with the first three domains also considered in terms of applicability. The components of each of these domains and a rubric that details how judgments concerning risk of bias are made are detailed in Appendix 2.

We will perform a pilot QUADAS-2 assessment on two papers. If agreement is poor, we will refine the signalling questions. We will not use QUADAS-2 data to provide with a summary quality score. We will produce a narrative summary describing numbers of studies that we considered contained high/low/unclear risk of bias as well as concerns regarding applicability.

5. Data synthesis

Statistical analysis

We will first report the calculation of summary standardized mean differences with their 95% confidence intervals. We will calculate Standardized Mean Differences and their 95% confidence intervals and combine them in a single measure using random effects models in case of significant heterogeneity. Heterogeneity will be assessed by means of Q-statistics and presented as I^2.

Where we are able to extract enough information, we will apply the diagnostic test accuracy framework for the analysis of a single test and extract the data from a study into a 2x2 table, showing the binary test results cross-classified with the binary reference standard.

Abstracted data will be tabulated as true positives (TP), false negatives (FN), false positives (FP), and true negatives (TN) and entered into STATA SE to calculate the sensitivities, specificities and
their 95% confidence intervals. We will also present individual study results graphically, by plotting estimates of sensitivities and specificities in both a forest plot and a receiver operating characteristic (ROC) space.

After the acquisition of an adequate set of data, we will meta-analyse them using the hierarchical summary ROC curve (HSROC) method proposed by Rutter and Gatsonis (Rutter 2001). We will conduct these analyses using STATA SE software.

We will explore the implications of any credible summary accuracy estimates emerging by considering the numbers of false positives and false negatives in populations with different prevalence of PD, and by presenting the results as natural frequencies and using alternative metrics such as likelihood ratios and predictive values.

**Investigations of heterogeneity**

Several factors could be relevant in clinical practice as they relate to the interpretation of the test result. Knowledge of potential sources of heterogeneity that can be referenced within the clinical setting is crucial to understand. This includes patient factors such as age, illness severity and genetic risk as well as different assay methods for the CSF biomarkers. All these factors may have an influence on the accuracy of the test itself as it is applied in practice.

The framework for the investigation of possible sources of heterogeneity includes the following factors:

*Index test:* exclusion of blood contaminated samples; type of assay for CSF biomarkers measurements (ELISA commercial, ELISA in-house, Luminex, others).

*Target population:* age; gender; UPDRS-III; Hoehn and Yahr stage; disease duration.

To investigate the effects of the sources of heterogeneity, we will perform a descriptive analysis by visual examination of the forest plot of SMD, sensitivity and specificity and the ROC plot. If the number of included studies is sufficient, subgroup analyses will be performed as well as meta-regressions.

**Sensitivity analyses**

In order to investigate the influence of study quality on overall diagnostic accuracy of the CSF biomarkers, we will perform additional analyses omitting studies at high risk of bias.

**Assessment of reporting bias**

We will investigate reporting bias using both funnel plot when analysing the SMD outcome or
Deek’s plot for evaluating diagnostic data.

**Interpretation of results**

We will produce a Summary of Findings Table according to GRADE for diagnosis. Implications for practice and future research will be discussed.

**Ethics and dissemination**

Our study will neither include any confidential data, nor interventional, so ethical approval is not required. The results of the study will be reported in international peer-reviewed journals.

**Footnotes**

**Contributors** PE, DG, LB, IA and LP conceived the idea, planned and designed the study protocol. PE and DG wrote the first draft; MO and IA designed the search strategy; PE planned the data extraction and statistical analysis; DC and PC provided critical insights. PE, DG, LB, IA, MO, DC, PC and LP have approved and contributed to the final written manuscript.

**Funding:** This study was supported by internal resources from Section of Neurology, Department of Medicine, University of Perugia.

**Competing interests:** none declared.
References


accuracy studies. Annals of Internal Medicine, 155(8), 529-536.

Appendix 1 – Search strategy

**Pubmed**
(synuclein>Title/Abstract) OR alpha-synuclein>Title/Abstract) OR SNCA>Title/Abstract) OR "Synucleins"[Mesh])
AND
(spinal fluid>Title/Abstract) OR cerebro spinal fluid>Title/Abstract) OR cerebrospinal fluid>Title/Abstract) OR cerebral spinal fluid>Title/Abstract) OR csf>Title/Abstract) OR "Cerebrospinal Fluid"[Mesh])
AND
(parkinson*>Title/Abstract) OR pd>Title/Abstract) OR parkinson disease OR "Parkinson Disease"[Mesh])

**Embase**
synuclein:ab,ti OR 'synuclein'/exp OR 'alpha-synuclein':ab,ti 'alpha-synuclein'/exp OR snca:ab,ti OR 'snca'/exp
AND
('spinal fluid':ab,ti OR 'spinal fluid'/exp OR 'cerebro spinal fluid':ab,ti OR 'cerebro spinal fluid'/exp OR 'cerebrospinal fluid':ab,ti OR 'cerebrospinal fluid'/exp OR csf:ab,ti OR csf/exp)
AND
(parkinson*:ab,ti OR 'Parkinson'/exp OR pd:ab,ti)
## Appendix 2 - The QUADAS-2 tool.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Patient selection</th>
<th>Index test</th>
<th>Reference standard</th>
<th>Flow and timing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Describe methods of patient selection. Describe included patients (prior testing, presentation, intended use of index test and setting).</td>
<td>Describe the index test and how it was conducted and interpreted.</td>
<td>Describe the reference standard and how it was conducted and interpreted.</td>
<td>Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram). Describe the time interval and any interventions between index test(s) and reference standard.</td>
</tr>
</tbody>
</table>
| **Signalling questions (yes/no/unclear)** | - Was a consecutive or random sample of patients enrolled?  
- Did the study avoid inappropriate exclusions?  
- Was a case-control design avoided? | - Were the index test results interpreted without knowledge of the results of the reference standard?  
- Were the methods for CSF collection and handling appropriately described?  
- Do the he study forecasts the exclusion of blood-contaminated samples on the basis of an accurate cut – off? | - Is the reference standard likely to correctly classify the target condition?  
- Were the reference standard results interpreted without knowledge of the results of the index test? | - Was there an appropriate interval between index test(s) and reference standard?  
- Did all patients receive a reference standard?  
- Did all patients receive the same reference standard?  
- Were all patients included in the analysis? |
| **Concerns regarding applicability: High/low/ unclear** | Are there concerns that the included patients do not match the review question? | Are there concerns that the index test, its conduct, or interpretation differs from the review question? | Are there concerns that the target condition as defined by the reference standard does not match the review question? | |
### PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

<table>
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<tr>
<th>Section and topic</th>
<th>Item No</th>
<th>Checklist item</th>
<th>Page</th>
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<tr>
<td>ADMINISTRATIVE INFORMATION</td>
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<tr>
<td>Title:</td>
<td>1a</td>
<td>Identify the report as a protocol of a systematic review</td>
<td></td>
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<tr>
<td>Update</td>
<td>1b</td>
<td>If the protocol is for an update of a previous systematic review, identify as such.</td>
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<td>Registration</td>
<td>2</td>
<td>If registered, provide the name of the registry (such as PROSPERO) and registration number</td>
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<tr>
<td>Authors:</td>
<td>3a</td>
<td>Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author</td>
<td></td>
</tr>
<tr>
<td>Contact</td>
<td>3b</td>
<td>Describe contributions of protocol authors and identify the guarantor of the review</td>
<td></td>
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<tr>
<td>Amendments</td>
<td>4</td>
<td>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</td>
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<tr>
<td>Support:</td>
<td>5a</td>
<td>Indicate sources of financial or other support for the review</td>
<td></td>
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<td>Sources</td>
<td>5b</td>
<td>Provide name for the review funder and/or sponsor</td>
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<tr>
<td>Sponsor</td>
<td>5c</td>
<td>Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol</td>
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| INTRODUCTION       |         |                                                                               |      |
| Rationale         | 6       | Describe the rationale for the review in the context of what is already known |      |
| Objectives        | 7       | Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO) |      |

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**METHODS**

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<tr>
<th>Eligibility criteria</th>
<th>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.</th>
<th>Pages 6,7 – Eligibility criteria</th>
</tr>
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<tbody>
<tr>
<td>Information sources</td>
<td>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.</td>
<td>Page 6 – Search strategy</td>
</tr>
<tr>
<td>Search strategy</td>
<td>Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated.</td>
<td>Appendix 1 – Search strategy</td>
</tr>
<tr>
<td>Study records:</td>
<td><strong>Data management</strong> Describe the mechanism(s) that will be used to manage records and data throughout the review.</td>
<td>Pages 7,8 – Data extraction</td>
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<td></td>
<td><strong>Selection process</strong> 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).</td>
<td>Page 2 – Abstract (Methods and Analysis)</td>
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<td></td>
<td><strong>Data collection process</strong> 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.</td>
<td>Page 7 – Study selection</td>
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<td></td>
<td><strong>Data items</strong> List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications.</td>
<td>Pages 7,8 – Data extraction</td>
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<td></td>
<td><strong>Outcomes and prioritization</strong> List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale.</td>
<td>Pages 8,9 – Statistical analysis</td>
</tr>
<tr>
<td></td>
<td><strong>Risk of bias in individual studies</strong> 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.</td>
<td>Pages 8 - Assessment of methodological quality and risk of bias</td>
</tr>
<tr>
<td></td>
<td><strong>Data synthesis</strong> Describe criteria under which study data will be quantitatively synthesised.</td>
<td>Pages 8,9 - Statistical analysis</td>
</tr>
<tr>
<td></td>
<td><strong>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 I², Kendall’s τ)</strong></td>
<td>Pages 8,9 - Statistical analysis</td>
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<tr>
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<td><strong>Describe any proposed additional analyses (such as sensitivity or</strong></td>
<td>Pages 9,10 - Statistical analysis</td>
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<td>15d</td>
<td>If quantitative synthesis is not appropriate, describe the type of summary planned.</td>
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<tr>
<td>Meta-bias(es)</td>
<td>16</td>
<td>Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)</td>
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<tr>
<td>Confidence in cumulative evidence</td>
<td>17</td>
<td>Describe how the strength of the body of evidence will be assessed (such as GRADE)</td>
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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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Abstract

Introduction: The diagnostic criteria currently used for Parkinson’s disease (PD) are mainly based on clinical motor symptoms. For these reasons many biomarkers are under investigation to support the diagnosis at the early stage. The neuropathological hallmark of PD is represented by Lewy bodies (LBs), which are intracytoplasmic inclusions in substantia nigra neurons. α-synuclein (α-syn) is the major component of the LBs and has been implicated in the pathogenesis of PD and in other "synucleinopathies" such as multi-system atrophy (MSA) and dementia with Lewy bodies (DLB). Several studies have investigated this presynaptic protein as potential biomarker of PD. The aim of our meta-analysis is to determine the ability of cerebrospinal fluid (CSF) concentrations of total α-syn, oligomeric α-syn and phosphorylated α-syn to discriminate PD patients from healthy subjects, non-degenerative neurological controls, patients suffering from parkinsonism and or synucleinopathies.

Methods and analysis: This systematic review protocol has been developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocol (PRISMA-P) 2015 statement and was registered on PROSPERO (CRD42016013217). We will search Cochrane Library, Web of Science, MEDLINE (via Pubmed) and EMBASE from inception using appropriate search strategies. Two independent reviewers will screen titles, abstracts and full-text articles, and will complete data abstraction. We will include studies that involved patients with PD, DLB, MSA, progressive supra-nuclear palsy (PSP), corticobasal disease (CBD) and vascular PD (VPD), and in which at least one between total α-syn, oligomeric α-syn and phosphorylated α-syn was measured in CSF. To evaluate the risk of bias and applicability of primary diagnostic accuracy studies we will use QUADAS-2.

Ethics and dissemination: Our study will neither include any confidential data, nor interventional, so ethical approval is not required. The results of the study will be reported in international peer-reviewed journals.
Strengths and limitations of this study

This diagnostic review protocol aims to comprehensively systematically assess the evidence regarding the diagnostic utility of CSF α-syn (total concentration, oligomeric and phosphorylated form) in discriminating patients with Parkinson disease from healthy individuals.

The results of this systematic review may also help clinicians in the differential diagnosis of Parkinson’s disease.

The planned systematic review and meta-analysis will be the first summary of the evidence in the field with a rigorous methodological conduct.

However, we expect heterogeneity in the design and conduct of the primary studies and in the type of the markers used as index test; this would make difficult to reach exhaustive conclusions.

We also expect that, given the well-know inter-laboratory variation, it will be difficult to have defined and validated cut-off of α-syn markers as final outcomes.
Introduction

Together with dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), Parkinson disease (PD) is part of the synucleinopathies’ spectrum, characterized by the deposition of fibrillary aggregates of α-synuclein protein (α-syn) in the cytoplasm of selective populations of neurons (PD and DLB) and oligodendroglia (MSA) (1).

PD is a progressive neurological disorder; it is the second most common neurodegenerative disease, immediately after Alzheimer’s disease (AD). The incidence of the disease rises abruptly with age and several data showed prevalence varying from 1% of the general population older than 60 years, to 4% of the population older than 80 years (2). The median age of onset is 60 years and the mean duration of the disease from diagnosis to death is 15 years (3). Currently, the diagnosis of PD is mainly based on clinical criteria, primarily through the identification of the cardinal motor signs: bradykinesia, rest tremor and rigidity (4). Unfortunately, when the motor signs appear, the neurodegeneration is at an advanced phase. It has been estimated that about 70% of nigral neurons are lost when the motor symptoms are evident.

Since PD has a long pre- or pauci-symptomatic phase, in which only non-motor symptoms are often present - such as REM sleep behaviour disorder, olfactory disorders, constipation, depression and forms of dysautonomia (5), it becomes increasingly significant to identify diagnostic tools that can differentiate individuals at risk of developing overt PD from healthy individuals.

Moreover, the differential diagnosis between PD and the atypical parkinsonisms – e.g. MSA, DLB, progressive supranuclear palsy (PSP), cortico-basal degeneration (CBD) and vascular PD (VPD) - can be difficult, particularly at the early stages of the disease, primarily because PD symptoms overlap with the symptoms of other diseases (6).

When PD is only diagnosed on the basis of clinical sign (sequential neurological examinations to detect cardinal motor deficits, the disease progression, the responsiveness to levodopa treatment and to exclude atypical signs), the diagnostic accuracy for PD is about 75-90% depending on whether it is a general neurologist or an expert of movement disorders (7).

Therefore, the research on the identification of a reliable and reproducible biomarker for the early PD diagnosis is fundamental to improve the precision in early diagnosis compared to control and to increase the accuracy of the differential diagnosis against other parkinsonian syndromes, which rarely respond to levodopa.

The cerebrospinal fluid (CSF) is in close contact with the extracellular space of the brain, therefore it is believed to mirror many of the biochemical processes of the brain.

Several studies were performed to assess the role of CSF biomarkers in PD diagnosis/prognosis, but the data are inconsistent or conflicting (8). Since pathological changes of α-syn characterise PD,
DLB and MSA, efforts have been made to understand the value of α-syn as CSF biomarker for these neurodegenerative disorders, often referred to as α-synucleinopathies (9).

Moreover, also among synucleinopathies, CSF α-syn levels could be different; this could reflect a differential brain localization of α-syn in these pathologies (glial cells in MSA and neurons in PD), the different extension of LB spreading (more localized in PD than in DLB), as well as interactions between α-syn misfolding and other co-occurring neuro-pathological processes. Several reports have investigated the role of CSF α-syn in the differential diagnosis among parkinsonisms (10-13).

In recent years several systematic reviews and meta-analyses were published (14-16) but each of them lacks of at least one crucial aspect such as: analysis of diagnostic data, assessment of risk of bias, search strategy with multiple electronic databases, and analysis of phosphorylated α-syn.

The aim of our systematic review and meta-analysis is to evaluate the diagnostic utility of CSF α-syn (total concentration, oligomeric and phosphorylated form) to distinguish between PD and healthy subjects - primary outcome -, and between PD and patients suffering from atypical parkinsonism - secondary outcome -.
Methods and analysis

1. Search strategy

Electronic Search
We will search through multiple sources of information to guarantee that all relevant studies are included in the review according to the eligibility criteria. In particular, we will search in: Cochrane Library, ISI Web of Science, MEDLINE (via Pubmed) and EMBASE. We will search without any language restriction. See Appendix 1 for a proposed draft strategy to be run.

Searching other resources
Interrogation of electronic databases will include also conference proceedings, ensuring that grey literature will be taken into account. We will scan reference lists of all eligible studies and reviews in the field for further possible titles and the process will be repeated until no new titles are found (Greenhalgh 2005).

This review protocol was prepared according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis Protocols (PRISMA-P) 2015 Statement (17) and the results will be presented following the PRISMA flow diagram.

2. Eligibility criteria

Types of studies
We will consider prospective and retrospective cohort studies as well as clinical trials that have evaluated the diagnostic accuracy of CSF markers to discriminate patients with PD from healthy subjects (primary objective) or from subjects with other parkinsonism (secondary objective). Results of baseline assessment in longitudinal studies are also of interest.

Participants
Studies must include a group of participants with PD and another group of subjects that can be either a group of neurological/healthy controls and/or with patients with other forms of parkinsonism. The diagnoses of parkinsonism will be based on internationally established operational criteria (18-24).
The diagnosis for PD will be established using the UK Parkinson’s Disease Society Brain Bank criteria (25) or the National Institute of Neurological Disorders and Stroke (NINDS; (4)).
**Index tests**

Studies that included the following markers will be considered in our assessment:

- CSF total α-synuclein
- CSF oligomeric α-synuclein
- CSF phosphorylated α-synuclein

All the markers will be evaluated for both the primary and secondary outcome.

There are currently no generally accepted standards for positivity threshold in such CSF biomarkers, and therefore it is not possible to pre-specify test positivity threshold.

We will use the criteria that were applied in each included primary study to classify participants as either test positive or test negative. We will compare the index tests with the reference standards specified below.

**Target condition**

Parkinson’s disease.

**Reference standards**

For the purpose of this review, we will consider the following clinical criteria as a suitable reference standard: the UK Parkinson’s Disease Society Brain Bank criteria (UKPDSBB; (25)) or the National Institute of Neurological Disorders and Stroke (NINDS; (4)).

**Study selection**

Two researchers will screen all titles and abstracts generated by the electronic database searches for relevance. Two researchers will then independently assess full manuscripts against the eligibility criteria. When necessary, a third arbitrator will resolve disagreements that the two researchers cannot resolve through discussion.

Where a study includes usable data but these are not presented in the published manuscript, we will contact the authors to request further information. If the same data set is presented in more than one paper we will include only the first published paper. We will detail the steps of the selection process in a PRISMA flow diagram.

**3. Data extraction**

We will extract the following data on study characteristics:

*Bibliographic details of primary paper: author, title of study, year and journal;*
Demographics: number of subjects; age; gender;

Study design: (prospective or retrospective; cross-sectional studies or randomised controlled trials)

Clinical information: PD staging (Hoehn & Yahr stage); duration of disease; illness severity (UPDRS-III);

Inclusion and exclusion criteria for individual studies;

The type of index test: CSF total α-synuclein, CSF oligomeric α-synuclein CSF phosphorylated α-synuclein;

Measurement used for the index test: e.g., ELISA commercial, ELISA in-house, Luminex, others;

Details of the reference standard: criteria for the clinical diagnosis of PD;

Diagnostic data: number of true positives, false positives, false negatives, and true negatives;

Funding source and conflict of interest.

4. Assessment of methodological quality and risk of bias

We will assess methodological quality of each study using the QUADAS-2 tool (26). The tool is made up of four domains: Patient selection; Index test; Reference standard; flow and timing. Each domain is assessed in terms of risk of bias, with the first three domains also considered in terms of applicability. The components of each of these domains and a rubric that details how judgments concerning risk of bias are made are detailed in Appendix 2.

We will perform a pilot QUADAS-2 assessment on two papers. If agreement is poor, we will refine the signalling questions. We will not use QUADAS-2 data to provide with a summary quality score. We will produce a narrative summary describing numbers of studies that we considered contained high/low/unclear risk of bias as well as concerns regarding applicability.

5. Data synthesis

Statistical analysis

We will first report the calculation of standardized mean differences using Hedges’ g. Standardized mean differences and their 95% confidence intervals will be combined in a single measure using random effects models in case of significant heterogeneity. Heterogeneity will be assessed by means of Q-statistics and presented as I^2.

Where we are able to extract enough information, we will apply the diagnostic test accuracy framework for the analysis of a single test and extract the data from a study into a 2x2 table, showing the binary test results cross-classified with the binary reference standard.

Abstracted data will be tabulated as true positives (TP), false negatives (FN), false positives (FP), and true negatives (TN) and entered into STATA SE to calculate the sensitivities, specificities and
their 95% confidence intervals. We will also present individual study results graphically, by plotting estimates of sensitivities and specificities in both a forest plot and a receiver operating characteristic (ROC) space.

After the acquisition of an adequate set of data, we will meta-analyse them using the bivariate method(27) . We will conduct these analyses using STATA SE software.

We will explore the implications of any credible summary accuracy estimates emerging by considering the numbers of false positives and false negatives in populations with different prevalence of PD, and by presenting the results as natural frequencies and using alternative metrics such as likelihood ratios and predictive values.

**Investigations of heterogeneity**

Several factors could be relevant in clinical practice as they relate to the interpretation of the test result. Knowledge of potential sources of heterogeneity that can be referenced within the clinical setting is crucial to understand. This includes patient factors such as age, illness severity and genetic risk as well as different assay methods for the CSF biomarkers. All these factors may have an influence on the accuracy of the test itself as it is applied in practice.

The framework for the investigation of possible sources of heterogeneity includes the following factors:

- **Index test:** exclusion of blood contaminated samples; type of assay for CSF biomarkers measurements (ELISA commercial, ELISA in-house, Luminex, others);
- **Target population:** age; gender; UPDRS-III; Hoehn and Yahr stage; disease duration.

To investigate the effects of the sources of heterogeneity, we will perform a descriptive analysis by visual examination of the forest plot of standardized mean differences, sensitivity and specificity and the ROC plot. If the number of included studies is sufficient, subgroup analyses will be performed as well as meta-regressions.

**Sensitivity analyses**

In order to investigate the influence of study quality on overall diagnostic accuracy of the CSF biomarkers, we will perform additional analyses omitting studies at high risk of bias.

**Assessment of reporting bias**

We will investigate reporting bias using both funnel plot when analysing the SMD outcome or Deek’s plot for evaluating diagnostic data.
Interpretation of results

We will produce a Summary of Findings Table according to GRADE for diagnosis. Implications for practice and future research will be discussed.

Ethics and dissemination

Our study will neither include any confidential data, nor interventional, so ethical approval is not required. The results of the study will be reported in international peer-reviewed journals.

Footnotes

Contributors PE, DG, LB, IA and LP conceived the idea, planned and designed the study protocol. PE and DG wrote the first draft; MO and IA designed the search strategy; PE planned the data extraction and statistical analysis; DC and PC provided critical insights. PE, DG, LB, IA, MO, DC, PC and LP have approved and contributed to the final written manuscript.

Funding: This study was supported by internal resources from Section of Neurology, Department of Medicine, University of Perugia.

Competing interests: none declared.
References

Appendix 1 – Search strategy

**Pubmed**

(synuclein[Title/Abstract] OR alpha-synuclein[Title/Abstract] OR SNCA[Title/Abstract] OR "Synucleins"[Mesh])

AND


AND

(parkinson*[Title/Abstract] OR pd[Title/Abstract] OR parkinson disease OR "Parkinson Disease"[Mesh])

**Embase**

synuclein:ab,ti OR 'synuclein'/exp OR 'alpha-synuclein':ab,ti 'alpha-synuclein'/exp OR snca:ab,ti OR 'snca'/exp

AND

('spinal fluid':ab,ti OR 'spinal fluid'/exp OR 'cerebro spinal fluid':ab,ti OR 'cerebro spinal fluid'/exp OR 'cerebrospinal fluid':ab,ti OR 'cerebrospinal fluid'/exp OR csf:ab,ti OR csf/exp)

AND

(parkinson*:ab,ti OR 'Parkinson'/exp OR pd:ab,ti)
Appendix 2 - The QUADAS-2 tool.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Patient selection</th>
<th>Index test</th>
<th>Reference standard</th>
<th>Flow and timing</th>
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</thead>
<tbody>
<tr>
<td>Description</td>
<td>Describe methods of patient selection. Describe included patients (prior testing, presentation, intended use of index test and setting).</td>
<td>Describe the index test and how it was conducted and interpreted.</td>
<td>Describe the reference standard and how it was conducted and interpreted.</td>
<td>Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram). Describe the time interval and any interventions between index test(s) and reference standard.</td>
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</tbody>
</table>
| Signalling questions (yes/no/unclear) | - Was a consecutive or random sample of patients enrolled?  
- Did the study avoid inappropriate exclusions?  
- Was a case-control design avoided? | - Were the index test results interpreted without knowledge of the results of the reference standard?  
- Were the methods for CSF collection and handling appropriately described?  
- Do the he study forecasts the exclusion of blood-contaminated samples on the basis of an accurate cut-off? | - Is the reference standard likely to correctly classify the target condition?  
- Were the reference standard results interpreted without knowledge of the results of the index test? | - Was there an appropriate interval between index test(s) and reference standard?  
- Did all patients receive a reference standard?  
- Did all patients receive the same reference standard?  
- Were all patients included in the analysis? |
| Concerns regarding applicability: High/low/ unclear | Are there concerns that the included patients do not match the review question?  
Are there concerns that the index test, its conduct, or interpretation differs from the review question? | Are there concerns that the target condition as defined by the reference standard does not match the review question? | Are there concerns that the target condition as defined by the reference standard does not match the review question? | Are there concerns that the target condition as defined by the reference standard does not match the review question? |
PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

<table>
<thead>
<tr>
<th>Section and topic</th>
<th>Item No</th>
<th>Checklist item</th>
<th>Page</th>
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<tbody>
<tr>
<td>ADMINISTRATIVE INFORMATION</td>
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<tr>
<td>Title:</td>
<td>1a</td>
<td>Identify the report as a protocol of a systematic review</td>
<td>Page 1 - Title</td>
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<tr>
<td>Update</td>
<td>1b</td>
<td>If the protocol is for an update of a previous systematic review, identify as such</td>
<td>The protocol is not an update of a previous systematic review.</td>
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<tr>
<td>Registration</td>
<td>2</td>
<td>If registered, provide the name of the registry (such as PROSPERO) and registration number</td>
<td>Page 2 - Abstract</td>
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<tr>
<td>Authors:</td>
<td>3a</td>
<td>Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author</td>
<td>Page 1 – Authors, affiliations, mailing addresses of the corresponding author.</td>
</tr>
<tr>
<td>Contributions</td>
<td>3b</td>
<td>Describe contributions of protocol authors and identify the guarantor of the review</td>
<td>Page 10 - Contributors</td>
</tr>
<tr>
<td>Amendments</td>
<td>4</td>
<td>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</td>
<td>The protocol is not an update of a previous systematic review.</td>
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<tr>
<td>Support:</td>
<td>5a</td>
<td>Indicate sources of financial or other support for the review</td>
<td>Page 10 - Funding</td>
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<tr>
<td>Sponsor</td>
<td>5b</td>
<td>Provide name for the review funder and/or sponsor</td>
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<tr>
<td>Role of sponsor or funder</td>
<td>5c</td>
<td>Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol</td>
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<tr>
<td>INTRODUCTION</td>
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<tr>
<td>Rationale</td>
<td>6</td>
<td>Describe the rationale for the review in the context of what is already known</td>
<td>Pages 4,5 - Introduction</td>
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<tr>
<td>Objectives</td>
<td>7</td>
<td>Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)</td>
<td>The aim of our systematic review and meta-analysis is to evaluate the diagnostic utility of CSF α-syn (total concentration, oligomeric and phosphorylated form) to distinguish between PD and healthy subjects - primary outcome -, and between PD and patients suffering from</td>
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<td>METHODS</td>
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<tr>
<td><strong>Eligibility criteria</strong></td>
<td>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. Pages 6, 7 – Eligibility criteria</td>
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<tr>
<td><strong>Information sources</strong></td>
<td>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. Page 6 – Search strategy</td>
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<tr>
<td><strong>Search strategy</strong></td>
<td>Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated. Appendix 1 – Search strategy</td>
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<td><strong>Study records:</strong></td>
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<td>Data management</td>
<td>Describe the mechanism(s) that will be used to manage records and data throughout the review. Pages 7, 8 – Data extraction</td>
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<tr>
<td>Selection process</td>
<td>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). Page 2 – Abstract (Methods and Analysis) Page 7 – Study selection</td>
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<td>Data collection process</td>
<td>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. Pages 7, 8 – Data extraction</td>
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<tr>
<td><strong>Data items</strong></td>
<td>List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications. Page 7, 8 – Data extraction Page 9 – Investigation of heterogeneity and sensitivity analyses</td>
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<tr>
<td><strong>Outcomes and prioritization</strong></td>
<td>List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale. Pages 8, 9 – Statistical analysis</td>
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<tr>
<td><strong>Risk of bias in individual studies</strong></td>
<td>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. Page 8 - Assessment of methodological quality and risk of bias Page 9 - Sensitivity analyses</td>
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<tr>
<td><strong>Data synthesis</strong></td>
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<td>15a</td>
<td>Describe criteria under which study data will be quantitatively synthesised. Pages 8, 9 - Statistical analysis</td>
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<tr>
<td>15b</td>
<td>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 $\tau$). Pages 8, 9 - Statistical analysis</td>
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<tr>
<td>15c</td>
<td>Describe any proposed additional analyses (such as sensitivity or ...) Pages 9, 10 - Statistical analysis</td>
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For peer review only

15d If quantitative synthesis is not appropriate, describe the type of summary planned  

Pages 9,10 - Statistical analysis

<table>
<thead>
<tr>
<th>Meta-bias(es)</th>
<th>Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)</th>
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<tbody>
<tr>
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<td>Pages 9,10 - Assessment of reporting bias</td>
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</table>

**Confidence in cumulative evidence**

17 Describe how the strength of the body of evidence will be assessed (such as GRADE)  

Page 10 – Interpretation of results

*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.*