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

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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. The major component of LBs, α-synuclein (α-syn), has been implicated in the pathogenesis of PD and in other 'synucleinopathies' such as multisystem atrophy (MSA) and dementia with LBs (DLBs). Several studies have investigated this presynaptic protein as a 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 patients with PD from healthy participants, non-degenerative neurological controls and 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 supranuclear palsy, corticobasal disease and vascular PD, 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 not include confidential data, and no intervention will be involved, 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 cerebrospinal fluid α-synuclein (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 markers used as index test; this would make it difficult to reach exhaustive conclusions.
- We also expect that, given the well-know interlaboratory 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 (DLBs) and multiple system atrophy (MSA), Parkinson disease (PD) is part of the synucleinopathies' spectrum, characterised by the deposition of fibrillar aggregates of α-synuclein protein (α-syn) in the cytoplasm of selective populations of neurons (PD and DLBs) and oligodendroglia (MSA).[1]

PD is a progressive neurological disorder; it is the second most common neurodegenerative disease, immediately after Alzheimer's disease. 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 presymptomatic or paucisymptomatic phase, in which only non-motor symptoms are often present—such as rapid eye movement (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—for example, MSA, DLB, progressive supranuclear palsy, corticobasal degeneration and vascular PD—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 diagnosed only on the basis of clinical signs (sequential neurological examinations to detect cardinal motor deficits, the disease progression, the responsiveness to levodopa treatment and to exclude atypical signs), the diagnostic accuracy is about 75–90%, depending on whether it is diagnosed by a general neurologist or an expert of movement disorders.[7]

Therefore, the research on the identification of a reliable and reproducible biomarker for 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.

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 have been performed to assess the role of CSF biomarkers in PD diagnosis/prognosis, but the data are either 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 a CSF biomarker for these neurodegenerative disorders, often referred to as α-synucleinopathies.[9]

Moreover, among synucleinopathies, CSF α-syn levels might also be different; this could reflect a differential brain localisation of α-syn in these pathologies (glial cells in MSA and neurons in PD), the different extension of LB spreading (more localised in PD than in DLB), as well as interactions between α-syn misfolding and other co-occurring neuropathological 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 have been published,[14–16] but each lacks 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 participants—primary outcome; and between PD and patients suffering from atypical parkinsonism—secondary outcome.

METHODS AND ANALYSIS

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 online supplementary appendix 1 for the proposed draft strategy to be run.

Searching other resources

Interrogation of electronic databases will also include conference proceedings, ensuring that the 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.

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 participants (primary objective) or from participants 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 participants that can be either a group of neurological/healthy controls and/or 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 those of 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-syn
- CSF oligomeric α-syn
- CSF phosphorylated α-syn

All markers will be evaluated for 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 prepecify a 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 being of a suitable reference standard: the UK Parkinson's Disease Society Brain Bank criteria (UKPDSBB;[25]) or those of 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.

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 participants; 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-syn, CSF oligomeric α-syn, CSF phosphorylated α-syn;

Measurement used for the index test: for example, 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 (TP), false positives (FP), false negatives (FN) and true negatives (TN);

Funding source and conflict of interest.

Assessment of methodological quality and risk of bias

We will assess methodological quality of each study using the QUADAS-2 tool.[26] This 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 judgements concerning risk of bias are made are detailed in online supplementary 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 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.

Data synthesis

Statistical analysis

We will first report the calculation of standardised mean differences using Hedges' g. Standardised mean differences and their 95% CIs 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 2×2 table, showing the binary test results cross-classified with the binary reference standard.

Abstracted data will be tabulated as TP, FN, FP and TN and entered into STATA SE to calculate the sensitivities, specificities and their 95% CIs. 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 the data, 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 possess. 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 standardised mean differences, sensitivity and specificity, and the ROC plot. If the number of included studies is sufficient, subgroup analyses as well as meta-regressions will be performed.

Sensitivity analyses

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 not include any confidential data, and will not be interventional, so ethical approval is not required. The results of the study will be reported in international peer-reviewed journals.

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Contributors PE, DG, LB, IA and LP conceived the idea, and 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 approved and contributed to the final written manuscript.

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