REVIEW

Extracellular Vesicles for the Diagnosis of Parkinson's Disease: Systematic Review and Meta-Analysis

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ABSTRACT: Parkinson's disease (PD) biomarkers are needed by both clinicians and researchers (for diagnosis, identifying study populations, and monitoring therapeutic response). Imaging, genetic, and biochemical biomarkers have been widely studied. In recent years, extracellular vesicles (EVs) have become a promising material for biomarker development. Proteins and molecular material from any organ, including the central nervous system, can be packed into EVs and transported to the periphery into easily obtainable biological specimens like blood, urine, and saliva. We performed a systematic review and meta-analysis of articles (published before November 15, 2022) reporting biomarker assessment in EVs in PD patients and healthy controls (HCs). Biomarkers were analyzed using random effects meta-analysis and the calculated standardized mean difference (Std.MD). Several proteins and ribonucleic acids have been identified in EVs in PD patients, but only α-synuclein (aSyn) and leucine-rich repeat kinase 2 (LRRK2) were reported in sufficient studies (n = 24 and 6, respectively) to perform a meta-analysis. EV aSyn was significantly increased in neuronal L1 cell adhesion molecule (L1CAM)–positive blood EVs in PD patients compared to HCs (Std.MD = 1.84, 95% confidence interval = 0.76–2.93, $P = 0.0009$). Further analysis of the biological sample and EV isolation method indicated that L1CAM-IP (immunoprecipitation) directly from plasma was the best isolation method for assessing aSyn in PD patients. Upcoming neuroprotective clinical trials immediately need peripheral biomarkers for identifying individuals at risk of developing PD. Overall, the improved sensitivity of assays means they can identify biomarkers in blood that reflect changes in the brain. CNS-derived EVs in blood will likely play a major role in biomarker development in the coming years. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: exosomes; biomarker; Parkinson's disease; L1 cell adhesion molecule; α-synuclein; plasma

Introduction

Timely and correct diagnosis of Parkinson's disease (PD) is challenging for clinicians and researchers. By the time motor symptoms appear, there has been an irreversible loss of the majority of central dopaminergic neurons.[1] Thus far, clinical trials of potentially neuroprotective agents in early motor PD have yielded

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negative results. Early and accurate diagnosis of patients with PD is needed for the development of diagnostic tools and for epidemiological and treatment studies. Such tools will also improve clinical care by providing a larger window for neuroprotective and therapeutic interventions-the prodromal phase of PD is up to 20 years.[2]

The definite diagnosis of PD can be confirmed only by neuropathological postmortem examination, and the accuracy of the clinical motor diagnosis is low, especially in the first 5 years of the disease.[3-5] Diagnostic accuracy increases with disease duration, mainly due to the development in motor fluctuations.[6]

Research efforts have focused on developing objective tests and biomarkers to facilitate earlier and more accurate diagnosis of PD and to support clinical trials. Despite advances in imaging tools,[7,8] and in genetic[9-11] and biochemical biomarkers,[12-15] the diagnostic validity of PD has not improved. The recent advancement in using seed amplification assays (SAA) to detect pathologic α-synuclein (aSyn) seeds is currently well established in the cerebrospinal fluid (CSF)[16] and broadly under development for peripheral biospecimens, but the assay is not yet optimized to reflect disease progression.[17] Therefore, medical practitioners urgently need a biomarker that can be effectively used, enable early diagnosis, and reflect disease progression and response to interventions.

Several CSF biomarkers have been studied.[12,13] The invasiveness of CSF collection makes it difficult to use for larger population screenings to identify, for example, those at risk of developing PD in the future. Peripheral, minimally invasive, or non-invasive biological specimens like blood, urine, and saliva have also been used for the development of biomarkers, but these have yet to be validated in clinical practice.[18] In recent years, brain-derived extracellular vesicles (EVs) isolated from samples have garnered increasing attention as possible biomarkers: they are considered an attractive target with unique properties and a cargo rich in signaling molecules from the central nervous system (CNS).[19-21]

EVs are secreted by cells in both physiological and pathological conditions.[22] They are single-membrane vesicles of endosomal origin, ~30 to 200 nm in diameter, with a characteristic cup-shaped morphology observed under the microscope. They can carry proteins, deoxyribonucleic acids, ribonucleic acids (RNA), lipids, and glycoconjugates.[23] EVs contain the majority of proteins from plasma membranes, endosomes and cytosol, and some proteins from the nucleus, mitochondria, and Golgi body from the cells of origin.[24] In addition to proteins, various RNA subtypes, namely mRNAs, microRNA (miRNAs), long non-coding RNAs, and circular RNAs, have been identified in EVs, uncovering new mechanisms in the genetic exchange between cells and intercellular transcriptional and translational regulation.[25-29] These vesicles are secreted by neural, muscle, and epithelial cells. They are vital in cell-to-cell communication and can be found in biofluids like CSF, blood, saliva, and urine.[30-33] EVs can cross the blood–brain barrier (BBB) in both directions and can, therefore, act as mediators between the CNS and the periphery.[34,35] These properties render EVs a very attractive biomarker; they can carry the contents of specific cell types from different organs in biological fluids that are easily collected and analyzed. Moreover, EV surface markers allow the isolation of EVs with specific cellular origin in the periphery, making it possible to assess e.g. brain derived neuronal or glial contents transported to the periphery via EVs.[36] Overall, these properties indicate that the contents of EVs or certain EV subgroups compared to total biofluid contents can provide more relevant information on the studied tissues and diseases.

In this systematic review and meta-analysis, we aimed to identify EV biomarkers for the diagnosis of PD and assess their clinical significance. In addition, we assessed the importance of the biological specimen and the EV isolation method.

Patients and Methods

This systematic review was performed in accordance with the transparent reporting guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 (PRISMA 2020).[37]

Search Strategy

The literature search was performed using PubMed, Web of Science, and Google Scholar databases from their inception through November 15, 2022. We did not impose any restrictions on publication language. The keywords "Parkinson's disease," "exosomes," "biomarker," and "diagnosis" were used in different combinations. The search flow chart is shown in Figure 1.

Selection Process and Eligibility Criteria

All articles retrieved from the electronic databases were imported to Mendeley Reference Manager. Duplicates, reviews, posters, bulletins, news, and conference abstracts were removed manually. The titles and abstracts of the retrieved articles were screened by two authors (M.X. and A.C.), and studies on cellular and animal models or studies irrelevant to PD were excluded (n = 79). After this step, 82 studies were selected for full-text reading.

The inclusion and exclusion criteria were developed based on the research question. Inclusion criteria considered cohort and case–control studies consisting of patients diagnosed with PD. For PD, we excluded possible co-pathologies with other neurodegenerative

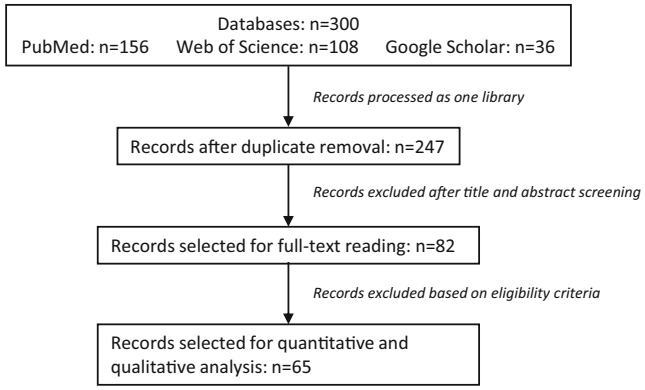

FIG. 1. Flow chart of study selection.

diseases, and for control groups of healthy individuals, we excluded those with signs of neurological or related diseases. Only studies presenting EV isolation from biological specimens and dysregulation of EV composition and contents were included.

Data Collection

Data collected from the selected studies include first author, year of publication, sample matrix, EV isolation method, EV characterization method, biomarker, biomarker analysis method, study design, number of PD patients and HCs, male-to-female ratio, age of HCs, age of PD patients during sample collection, age of PD patients at disease onset, disease duration, Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) score, Hoehn & Yahr stage, Mini-Mental Status Examination (MMSE), Montreal Cognitive Assessment (MoCa), and information on medications. Biomarker outcomes of the studies were collected as means and standard deviations (SD). When data were provided in other forms, the appropriate conversions and calculations were performed according to Hozo et al and Wan et al.[38,39] Biomarker outcomes of the studies were collected as means and SD. When data were provided only in graphs, we used Web plot digitizer to extract the values.[40]

Risk of Bias/Quality Assessment

Risk of bias assessment was performed separately by two reviewers for each study using the QUADAS-2 tool (Quality Assessment of Diagnostic Accuracy Studies).[41] QUADAS-2 assesses four domains: patient selection, index test, reference standard, and flow and timing. The risk of bias is determined in each domain by answering "yes" or "no" to each of the signaling questions. If the answer is "yes" to all signaling questions, the bias for this domain is considered low. Domains including "no" answers are generally considered as high risk for bias but should be determined individually to assess if the negative answer to the question can render the whole domain problematic.

Data Analysis

The meta-analysis was performed using Review Manager.[42] We calculated the standardized mean difference (Std.MD) to estimate the average change in values of each biomarker in PD patients compared to HCs. The included studies use different methods to measure the same biomarker, and the absolute values vary significantly. An Std.MD above 1 indicates the biomarker is higher in the PD group and that below 1 indicates the biomarker is higher in the HC group. Random effects meta-analysis was performed to incorporate heterogeneity. Heterogeneity was estimated using the I^2 test. For biomarkers with more than three studies, subgroup analyses were also performed.

Results

Characteristics of Selected Studies

The first literature search of the electronic databases yielded 300 studies; 247 studies remained after duplicate removal. We then screened the studies by abstract and title and excluded 86 reviews, 61 irrelevant studies, and 18 studies on cellular and animal models; 82 studies underwent full-text review. After full-text assessment, 20 studies were excluded due to inappropriate data format or reporting, resulting in 62 studies undergoing quantitative (n = 35) or qualitative analyses (n = 27) (Fig. 1; Tables S1 and S3).[43-104]

Studies were published between 2014 and 2022, with increased publications in the last 2 years. Participants for the included studies were recruited from Asia (34 of 62 studies), Europe (19 of 62 studies), and North America (10 of 62 studies), indicating that certain populations might be not included or underrepresented.

Twenty-three were case–control studies, and 39 were cohorts. Participant numbers ranged from 7 to 290 in the PD groups and 7 to 215 in the control groups; the male-to-female ratio was reported in all but four studies. All but 13 studies properly reported the age of participants and included the age of PD diagnosis and/or disease duration. The age at sample collection was 64.74 ± 7.24 years for healthy individuals and 67.16 ± 7.90 years for PD patients. Age at disease onset was 61.26 ± 5.97 years, and disease duration was 5.58 ± 3.38 years. This is important in the case of PD diagnosis as the longer the disease, the higher the certainty that PD was correctly diagnosed. Clinical data reported included MDS-UPDRS (46 of 62 studies), MoCa (26 of 62 studies), medication (25 of 62 studies), and MMSE (24 of 62 studies). In addition, only 27 studies explained in detail the exclusion and

inclusion criteria they used to enroll study participants and properly reported pathologies other than PD.

Biological specimens from which EVs were isolated included plasma (35 of 62 studies), serum (15 of 62 studies), urine (8 of 62 studies), CSF (6 of 62 studies), and saliva (2 of 62 studies); therefore, plasma was the most commonly used biological specimen. The EV isolation methods were EV isolation kits (25 of 62 studies; ExoQuick: 13, ExoEasy: 5, MACSPlex Exosome Assays: 2, Total Exosome Isolation Kit: 2, XYCQ EV Enrichment Kit: 1, PureExo: 1, and miRCURY Exosome Isolation Serum/Plasma Kit: 1); ultracentrifugation (24 of 62 studies); immunoprecipitation (IP) (8 of 62 studies, L1 cell adhesion molecule [L1CAM]: 6, and total EV: 2); size-exclusion chromatography (SEC) (2 of 62 studies); fluorescence-activated cell sorting (FACS) (1 of 62 studies); and one study performing enzyme-linked immunosorbent assay (ELISA) directly without isolation. In nine studies, a secondary processing method was performed to select a specific subgroup of EVs with IP or FACS. In particular, L1CAM-IP followed ExoQuick isolation (5 of 62 studies), myelin-oligodendrocyte glycoprotein IP followed ExoQuick (1 of 62 studies), and L1CAM-IP followed ultracentrifugation (1 of 62 studies). Collectively, there is no indication for a preferred isolation method, although the majority of studies after 2019 use isolation kits. Interestingly, L1CAM-IP was first performed in 2014 by Shi et al and seemed to gain popularity after 2019.[81]

The majority of studies (42 of 62) used at least two different methods to characterize the isolated vesicles. The most commonly used method is immunoblotting analysis (44 of 62 studies) employing positive and negative exosome markers. Next is microscopy (37 of 62 studies), specifically electron microscopy, transmission electron microscopy, or atomic force microscopy, to visualize the vesicle shape and size, and then nanoparticle tracking analysis (33 of 62 studies), which measures the size and number of isolated vesicles. Individual studies employed dynamic light scattering, exosome antibody array, augmented colorimetric nanoplasmonic method, acetylcholinesterase activity assay, and flow cytometry. Conclusively, immunoblotting and a second method-either microscopy or a particle analysis method-seem to be the gold standards for EV characterization. As EV size varies and overlaps with the size of other types of vesicles, size and shape estimation are insufficient, and EV markers are required to confirm proper isolation. It is worth highlighting that some of the studies that analyzed RNA biomarkers did not perform any vesicle characterization but proceeded directly to RNA extraction.

The different methods of analysis used to measure the biomarkers were immunoblotting (13 of 62 studies), ELISA (13 of 62 studies), real-time quantitative polymerase chain reaction (RT qPCR) (9 of 62 studies), next-generation sequencing (NGS) (8 of 62 studies), mass spectrometry (6 of 62 studies), electrochemiluminescence (ECL) (6 of 62 studies), flow cytometry (4 of 62 studies), immunomagnetic reduction assay (3 of 62 studies), Meso Scale Discovery (MSD) (2 of 62 studies), single-molecule array (2 of 62 studies), Luminex (2 of 62 studies), and Thioflavin-T (ThT) based assay (2 of 62 studies).

The biomarkers analyzed from exosomal extraction were mainly proteins. A total of 24 studies reported data on α-synuclein, including total aSyn (24 of 24 studies), oligomeric aSyn (6 of 24 studies), and phosphorylated Ser129 aSyn (5 of 24 studies); 5 studies reported data on leucine-rich repeat kinase 2 (LRRK2), including phosphorylated Ser1292 LRRK2 (3 of 5 studies); two studies reported data on tau-protein; two studies reported data on DJ-1; and two studies reported data on clusterin. Other proteins related with neurodegenerative disorders, synaptic proteins, surface markers, and inflammatory proteins were also reported and are presented in Table S1. Only biomarkers reported in more than three studies were included in the meta-analysis.

RNA biomarkers from EVs were reported in 15 studies, including miRNAs (12 of 15 studies), long non-coding RNAs (2 of 15 studies), and mRNAs (2 of 15 studies). Four miRNAs were identified in at least three studies: miR-22, miR-24, miR-331, and miR-151. All other RNAs are presented in Table S1. Due to the incomplete vesicle characterization in some of the studies reporting miRNA biomarkers, they were not included in the meta-analysis.

Risk of Bias and Applicability Assessment

Fifty studies were classified as "low risk of bias," 12 studies were classified as "moderate risk of bias," and 2 studies were classified as "high risk of bias" and were not included in the meta-analysis. Data produced by the QUADAS tool on bias and applicability risk are presented in Table S2.

Meta-Analysis

We meta-analyzed data for aSyn, including total aSyn and oligomeric aSyn (Fig. 2), and LRRK2, including total LRRK2 and phosphorylated Ser1292 LRRK2 (Fig. 4).

Meta-analysis of total aSyn in all studies showed a nonsignificant increase in PD (Std.MD [standardized mean difference] = 1.02, 95% confidence interval [CI] = 0.23–1.82, $P = 0.01$) (Fig. 2A). However, performing the meta-analysis of total aSyn in blood EVs based on their source showed different results: total EVs isolated from blood samples did not show any change between PD patients and HCs (healthy controls,

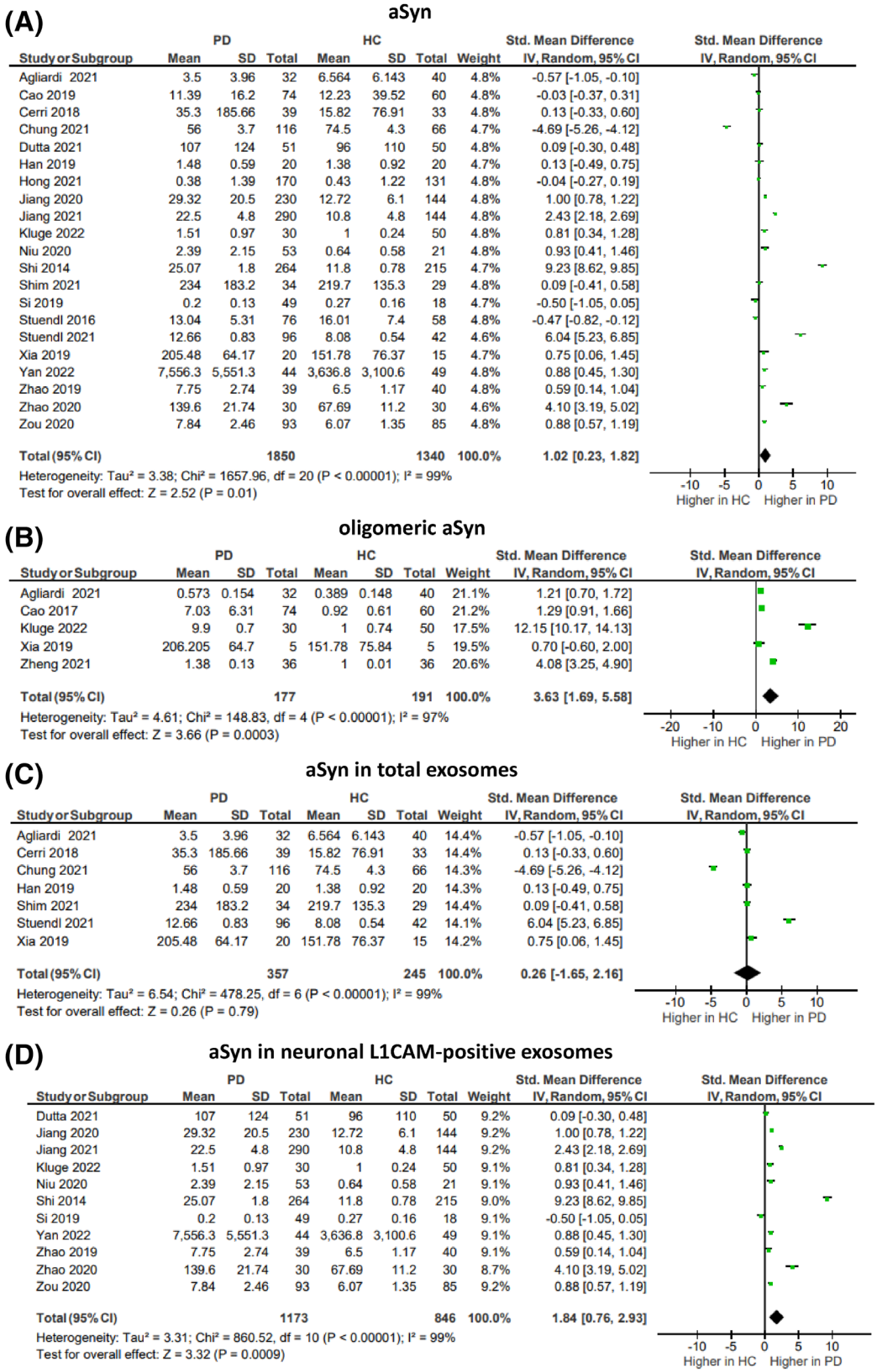

FIG. 2. Forest plots of aSyn (α-synuclein) and oligomeric aSyn in EVs (extracellular vesicles) and subgroup forest plots of aSyn in total and neuronal-derived EVs. Forest plots showing the standardized mean difference of (**A**) aSyn and (**B**) oligomeric aSyn and subgroup forest plots showing the standardized mean differences of aSyn in (**C**) total and (**D**) L1CAM (L1 cell adhesion molecule)–positive neuronal-derived EVs. Standardized mean difference and their corresponding 95% confidence intervals are indicated by green squares. Standardized differences and 95% confidence intervals for each plot are represented by diamonds. [Color figure can be viewed at wileyonlinelibrary.com]

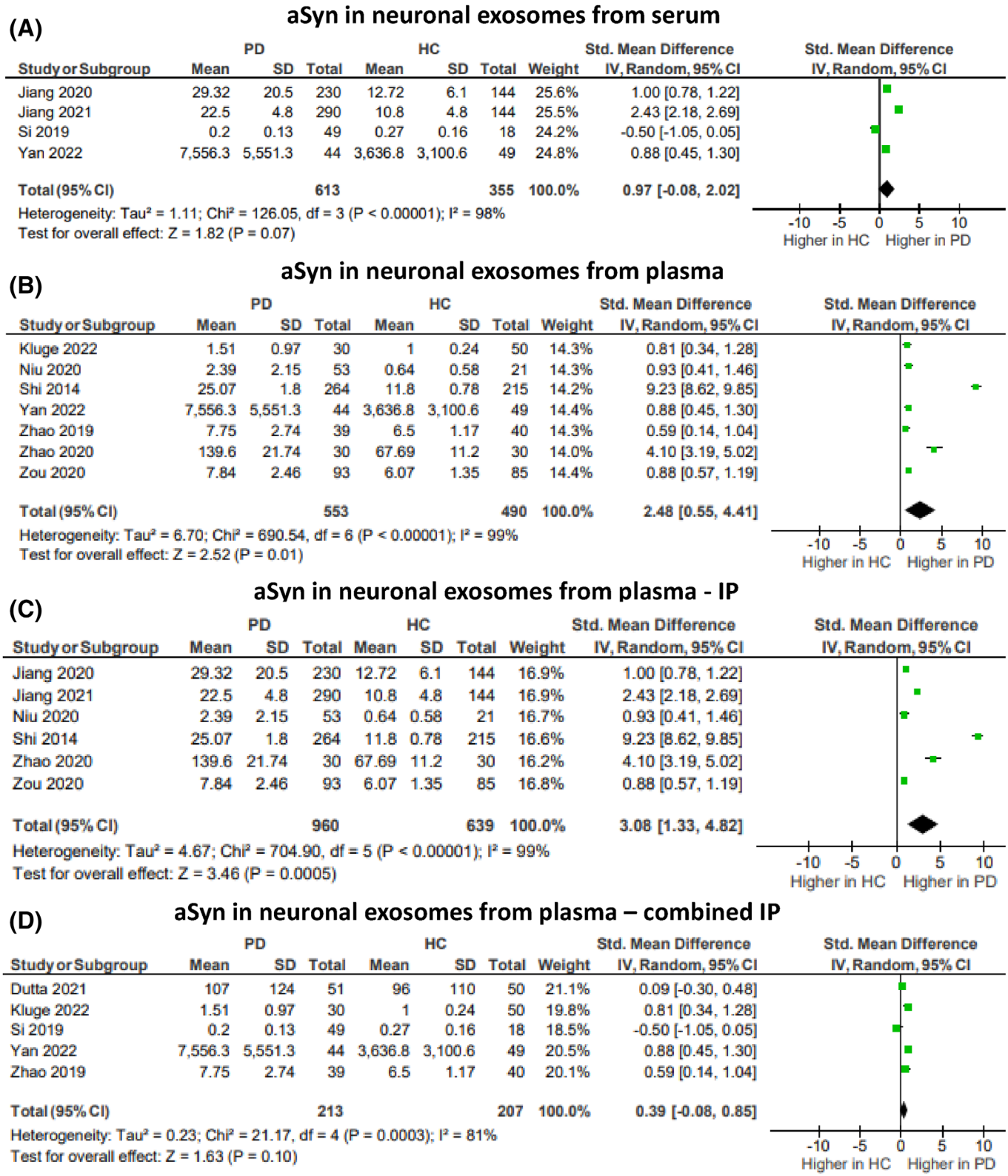

FIG. 3. Forest plots of aSyn in EVs (extracellular vesicles) from different sources and isolation methods. Forest plots showing the standardized mean differences in aSyn isolated from (**A**) serum and (**B**) plasma and further classification of plasma EVs isolated using (**C**) IP (immunoprecipitation) only and (**D**) IP combined with another method. Standardized mean differences and their corresponding 95% confidence intervals are indicated by green squares. Standardized differences and 95% confidence intervals for each plot are represented by diamonds. [Color figure can be viewed at wileyonlinelibrary.com]

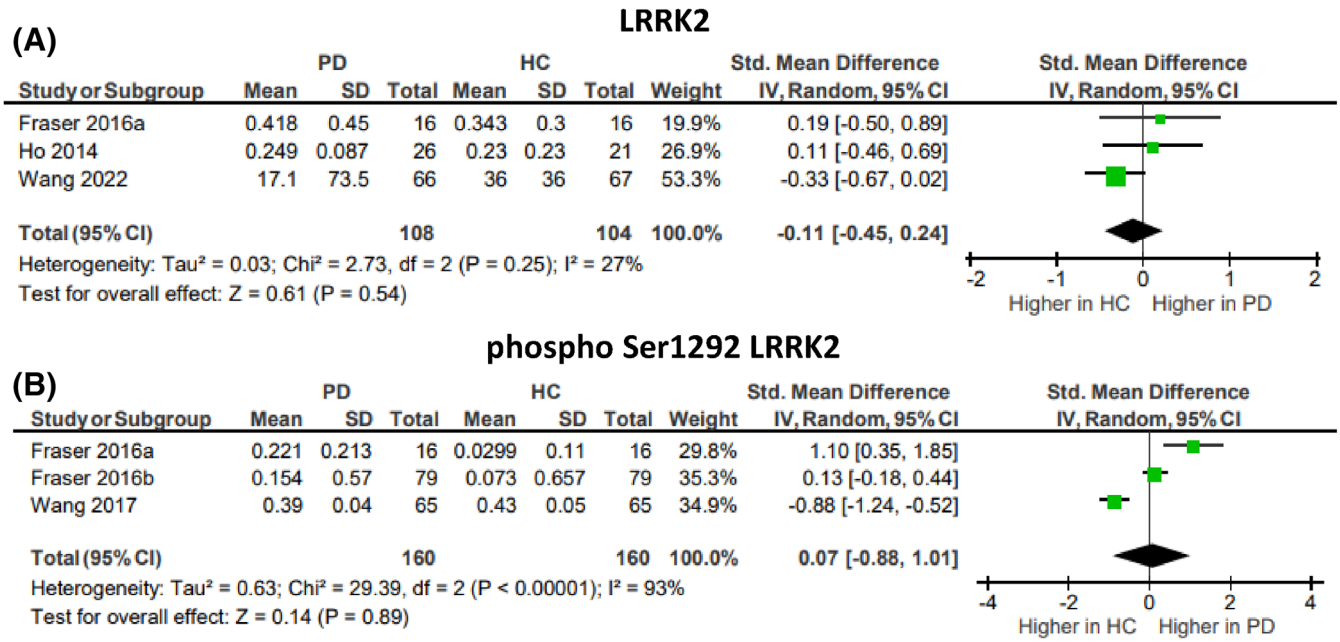

FIG. 4. Forest plots of LRRK2 (leucine-rich repeat kinase 2) and phospho Ser1292 LRRK2. Forest plots showing the standardized mean differences in (**A**) LRRK2 and (**B**) phospho Ser1292 LRRK2. Standardized mean differences and their corresponding 95% confidence intervals are indicated by green squares. Standardized differences and 95% confidence intervals for each plot are represented by diamonds. [Color figure can be viewed at wileyonlinelibrary.com]

Std.MD = 0.26, 0.95% CI = −1.65–2.16, $P = 0.79$) (Fig. 2C), whereas neuronal L1CAM-positive EVs showed a significant increase in the PD group (Std. MD = 1.84, 95% CI = 0.76–2.93, $P = 0.0009$) (Fig. 2D). Interestingly, further filtering based on the biological specimen indicated significance only for the neuronal EVs isolated from plasma (Std.MD = 2.48, 95% CI = 0.57–4.41, $P = 0.01$) (Fig. 3B) and not from serum (Std.MD = 0.97, 95% CI = −0.08–2.02, $P = 0.07$) (Fig. 3A). Surprisingly, further analysis of the EV isolation method indicated the observed effects are mostly attributed to studies that performed direct IP from the plasma sample (Std.MD = 3.08, 95% CI = 1.33–4.82, $P = 0.00001$) (Fig. 3C) rather than combining IP following another isolation method (Std. MD = 0.39, 95% CI = −0.08–0.85, $P = 0.0003$) (Fig. 3D). Collectively, aSyn quantified in neuronal EVs isolated from blood plasma with L1CAM-IP seems to be the best method for differentiating PD and HC.

Meta-analysis of oligomeric aSyn levels showed significantly higher levels in PD patients compared to HCs (Std.MD = 3.36, 95% CI = 1.69–5.58, $P = 0.0003$) (Fig. 2B). Due to the small number of studies measuring oligomeric aSyn and the combination of biological specimens and methods used, subgroup analysis was not performed.

Meta-analysis of LRRK2 indicated no change in total LRRK2 among PD patients and HCs in urinal EVs (Std.MD = −0.11, 95% CI = −0.45–0.24, $P = 0.54$) (Fig. 4A) and no change in phospho Ser1292 LRRK2 (Std.MD = 0.07, 95% CI = −0.88–1.01, $P = 0.89$) (Fig. 4B). For this meta-analysis, PD patients and HCs with and without LRRK2 mutation were pooled as only two of three studies reported data on these groups separately. In these two studies, LRRK2 levels differed among mutant and nonmutant carriers in both groups with higher levels of LRRK2 and phospho Ser1292 LRRK2 detected in urinal EVs of the mutant carriers. More studies will be needed to assess the diagnostic value of EV LRRK2 and phospho Ser1292 LRRK2 levels for LRRK2 mutant carriers.

Discussion

The development of a biomarker that will enable the early and accurate diagnosis of PD and support novel therapeutic approaches is an urgent need. Here we present reports on the collection and extraction of EV biomarkers from CSF, plasma, serum, urine, and saliva and present the optimal biomarker differentiating PD from healthy individuals. Our meta-analysis indicated that total aSyn in neuronal L1CAM-positive EVs isolated from blood plasma using IP is a promising biomarker. In addition, interesting results were found for oligomeric aSyn isolated from EVs irrespective of their origin and isolation method. Finally, although some studies assess LRRK2 levels in urinal EVs, the meta-analysis failed to identify a significant change between PD and HC (Fig. 5).

To date, there is only one additional study presenting a similar meta-analysis,[105] but we used different

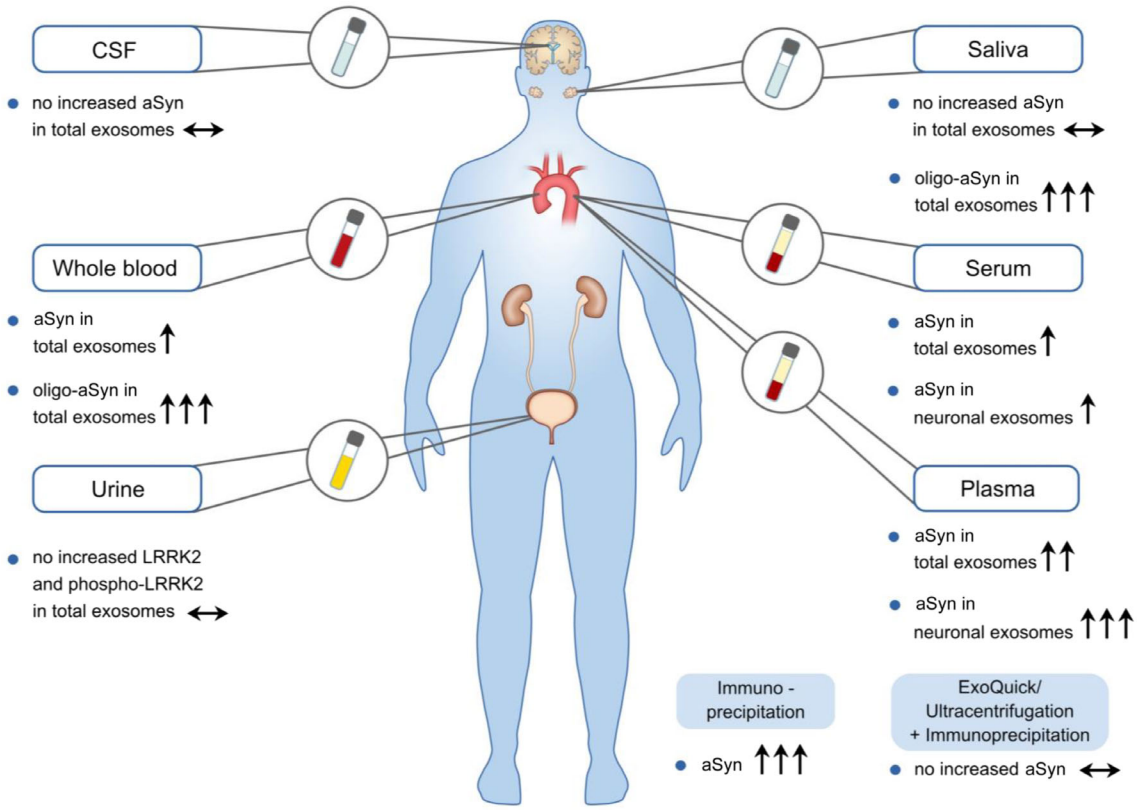

FIG. 5. Overview of the protein-level changes in the EVs (extracellular vesicles) isolated from different biological specimens in PD (Parkinson's disease) patients compared to HCs (healthy controls). Oligomeric aSyn (oligo-aSyn) is increased in total EVs isolated from blood and saliva. Total aSyn levels are found increased in EVs isolated from blood samples, with higher levels observed in neuronal EVs isolated from plasma using direct immunoprecipitation. [Color figure can be viewed at wileyonlinelibrary.com]

exclusion criteria. Most studies on biomarker discovery focus on aSyn, which clearly plays a major role in PD pathogenesis.[106] Studies on aSyn levels in CSF indicate that aSyn is significantly lower in PD patients compared to controls but presented moderate specificity.[107-110] Conversely, studies on aSyn levels in blood samples show high variability.[111-113] This can be attributed to the fact that aSyn in the blood can originate from the aSyn-rich red blood cells or any peripheral tissue.[114,115] aSyn has also been assessed in saliva and urine, but the diagnostic value of these biological specimens for aSyn remains uncertain.[116-118]

The presence of aSyn in EVs was first reported by Lee and colleagues[119] followed by the characterization of those vesicles as EVs and the importance of this finding in aSyn spread and pathology.[120,121] Using specific neuronal markers like L1CAM, it became clear that a portion of aSyn can be released from the CNS via the BBB to the blood and other peripheral biofluids.[81]

Assessing those EVs is like opening a window to the PD brain and allows us to differentiate brain-derived aSyn from aSyn from other tissues that may end up in the blood. Indeed, neuronal EVs showed the best results in differentiating PD, whereas total EVs isolated from blood showed no difference between PD and HC. This indicates that we should measure neuronal-derived aSyn in the blood as opposed to total aSyn that also originates from other peripheral tissues. This suggests that the increased levels of aSyn in blood EVs are from neuronal EVs. The levels of aSyn in total blood EVs are not as specific as the neuronal-only aSyn, highlighting the importance of measuring specific types of EVs and not the entire blood pool. Interestingly, this is not the case for oligomeric aSyn, which is significantly increased in PD in total EVs isolated from saliva and total as well as neuronal EVs isolated from plasma or serum. This indicates oligomeric aSyn is a more robust biomarker as it is the most toxic form and is also implicated in disease propagation.[121,122]

The majority of studies assess EV aSyn in plasma and serum, whereas only a few assessed urine, CSF, and saliva EVs. We could compare only plasma and serum as EV sources. More studies are needed to assess the diagnostic potential of EVs from other fluids. We show that plasma EVs have a higher and significant increase in aSyn in PD compared to HC versus a smaller nonsignificant change that was found in serum EVs. The main difference between plasma and serum is clot formation. Different coagulants are used to prevent clotting in the case of plasma. Studies comparing EV

isolation from plasma and serum showed that although there is no significant change in the number of EVs isolated from these two samples, there are many differences in EV contents, and the optimal source should be assessed based on the research questions.[123-126] Although it is not clear why the EVs derived from plasma and serum differ, plasma EVs seem to be richer in protein contents and present more neuronal surface markers.[124,126]

In addition to the EV source, the isolation method can introduce further variability. Each technique has advantages and disadvantages that are relevant to the research question. Classic ultracentrifugation has been the first isolation method used for EVs and is being gradually replaced by EV isolation kits that are based on precipitation principles. Although ultracentrifugation seems to be the most inexpensive and easiest method with a very high yield, it also requires more careful handling and purification steps to ensure that EV preparation is free of other vesicles and impurities in the form of aggregated protein and nucleic acids.[127] Exosome isolation kits, on the contrary, are more expensive and easier to use due to standardized procedures but have the disadvantage of impurities either from the sample itself or from the reagents employed by the kit for precipitation and isolation.[127,128] Precipitation of EVs in such kits is performed either chemically or by affinity IP with the use of certain antibodies; precipitation-based kits seem to work well for RNA-seq studies, but affinity IP should be preferred in protein studies because chemical precipitation is mostly accountable for impurities such as sodium dodecyl sulfate, high-salt concentrations, and binding reagents that can hinder protein quality.[128] Similar to antibody-based total EV IP isolation employed by different kits, IP with specific antibodies to isolate the EVs of interest is a method gaining ground. The major advantages of this method are EV purity and high specificity and efficiency, and the disadvantages are the high costs and the use of antibodies that need careful optimization and add high variability.[128,129] Despite presented results with L1CAM-IP, it is worth highlighting that L1CAM is specific for not only neuronal EVs but also EVs of different origin,[130] underlining that the use of antibodies for the isolation of EVs with specific origin needs to be better characterized and standardized among laboratories. Fewer studies are using SEC or FACS. Overall, SEC appears to be an inexpensive and effective method but requires a large volume of samples and produces a large volume of samples with highly diluted EVs, and the EVs passing through the column are subjected to high friction that can lead to bursting or loss of surface proteins, limiting the possibility of certain applications. Another limitation for SEC is sample purity, as lipoproteins with similar size to that of EVs can be isolated in parallel. SEC coupled with other approaches like ultracentrifugation, ultrafiltration, and chemical IP can minimize this issue and increase sample purity.[131] FACS, on the contrary, can be a very specific and efficient method for EV isolation but is not yet standardized.[132] One would think that combining two methods would give better results, but along with the advantages of each method, the disadvantages might also add up. Our meta-analysis indicated that for aSyn-level assessment, it is preferred to perform L1CAM-IP.

It is better to perform IP on a straight sample rather than perform IP after another isolation method. This could be due to impurities introduced in the sample that can interfere with the antibody binding or clustering of several EVs that decrease specificity.

Heterogeneity calculated using I^2 was very high in our meta-analysis, indicating high variability among studies. We tried to eliminate heterogeneity by performing subgroup analyses, but the EV source and isolation method do not seem to eliminate variability. To assess the source of heterogeneity, we performed a regression analysis using relevant parameters like sex, age, disease duration, and disease severity in the form of MDS-UPDRS score during sample collection. We did not find a correlation between these parameters and the mean difference of aSyn (data not shown). This indicates that all these variables, in addition to individual cohort recruitment and patient selection and interlab variabilities in the experimental procedures, can be the source of heterogeneity. Only a few studies reported on participant inclusion and exclusion criteria for participant recruitment. Some studies accepted certain pathologies for the non-PD group and chose to record and monitor them, whereas others excluded individuals with many complications like hypertension, cardiovascular and cerebrovascular diseases, diabetes, inflammatory diseases, autoimmune diseases, kidney disease, thyroid problems, blood deficiencies, and anemia. These additional variabilities between studies may increase heterogeneity. In terms of experimental procedures, variability can be introduced at every step, starting with the use of different coagulants for plasma preparation to the biomarker measurement method. We did not assess the biomarker analysis methods as the studies included in the meta-analysis quantify aSyn using antibody-based methods (ELISA, ECL, and MSD). Thus, the variability is expected to be high and analogous to the sensitivity and specificity of the different antibodies used in the different assays. To eliminate this, or at least point out a definite variability source, more studies are necessary. Moreover, such meta-analyses aid the establishment of standard procedures for biomarker measurement and help create reference standards that aid biomarker development.

Measuring aSyn levels has been a promising biomarker, but other methods of analysis might be proven more useful and require further studies to validate their

potential. For example, the ratio of oligomeric aSyn levels to the total levels provides a good measure of disease while eliminating errors of systemic errors in analysis methods.[102] In addition, measuring the amount of aSyn-positive EVs in blood showed good sensitivity and specificity as well as correlation with disease measures.[69,72] Finally, the potential of the aSyn species packed in EVs to form aggregates and propagate disease was assessed in two studies with ThT-based measures in a simple setup[96] or a more systematic way by performing SAA.[68]

Although several biomarkers are reported in the literature, there are not sufficient studies to perform a meta-analysis for most of them. In short, aSyn remains interesting for PD diagnosis and also in EV studies that showed a higher aSyn concentration in neuronal blood EVs isolated using IP from plasma. Very few studies performed exploratory analysis, only six studies assessed EV protein contents using mass spectrometry, and only eight studies assessed RNA contents using NGS. Identifying novel targets is important for diagnosis and mechanistic studies as the EV cargo reflects the content of the cell of origin and can shed light on overseen mechanisms.

Data Availability Statement

Data sharing not applicable-no new data generated

References

1. Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. Ann Neurol 2010;67:715–725.
2. Kalia LV, Lang AE. Parkinson's disease. Lancet 2015;386:896–912.
3. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease. Neurology 2016;86:566–576.
4. Braak H, del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197–211.
5. Adler CH, Beach TG, Hentz JG, Shill HA, Caviness JN, Driver-Dunckley E, et al. Low clinical diagnostic accuracy of early vs advanced Parkinson disease: clinicopathologic study. Neurology 2014;83:406–412.
6. Joutsa J, Gardberg M, Röyttä M, Kaasinen V. Diagnostic accuracy of parkinsonism syndromes by general neurologists. Parkinsonism Relat Disord 2014;20:840–844.
7. Pavese N, Brooks DJ. Imaging neurodegeneration in Parkinson's disease. Biochim Biophys Acta 2009;1792:722–729.
8. Mitchell T, Lehéricy S, Chiu SY, Strafella AP, Stoessl AJ, Vaillancourt DE. Emerging neuroimaging biomarkers across disease stage in Parkinson disease: a review. JAMA Neurol 2021;78:1262–1272.
9. Singleton AB, Farrer MJ, Bonifati V. The genetics of Parkinson's disease: progress and therapeutic implications. Mov Disord 2013;28:14–23.
10. Berg D, Schweitzer K, Leitner P, Zimprich A, Lichtner P, Belcredi P, et al. Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease. Brain 2005;128:3000–3011.
11. Johnson PH, Weinreb NJ, Cloyd JC, Tuite PJ, Kartha Rv. GBA1 mutations: prospects for exosomal biomarkers in α-synuclein pathologies. Mol Genet Metab 2020;129:35–46.
12. Mollenhauer B, Caspell-Garcia CJ, Coffey CS, Taylor P, Shaw LM, Trojanowski JQ, et al. Longitudinal CSF biomarkers in patients with early Parkinson disease and healthy controls. Neurology 2017;89:1959–1969.
13. Parnetti L, Gaetani L, Eusebi P, Paciotti S, Hansson O, El-Agnaf O, et al. CSF and blood biomarkers for Parkinson's disease. Lancet Neurol 2019;18:573–586.
14. Ge F, Ding J, Liu Y, Lin H, Chang T. Cerebrospinal fluid NFL in the differential diagnosis of parkinsonian disorders: a meta-analysis. Neurosci Lett 2018;685:35–41.
15. Paciotti S, Bellomo G, Gatticchi L, Parnetti L. Are we ready for detecting α-synuclein prone to aggregation in patients? The case of "Protein-Misfolding Cyclic Amplification" and "Real-Time Quaking-Induced Conversion" as diagnostic tools. Front Neurol 2018;9:415.
16. Siderowf A, Concha-Marambio L, Lafontant D-E, Farris CM, Ma Y, Urenia PA, et al. Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study. Lancet Neurol 2023;22:407–417.
17. Bellomo G, Paciotti S, Gatticchi L, Rizzo D, Paoletti FP, Fragai M, et al. Seed amplification assays for diagnosing synucleinopathies: the issue of influencing factors. Front Biosci 2021;26:1075–1088.
18. Delenclos M, Jones DR, Mclean PJ, Uitti RJ, Clinic M, Clinic M, et al. Biomarkers in Parkinson's disease: advances and strategies. Parkinsonism Relat Disord 2017;22:1–10.
19. Upadhya R, Shetty AK. Extracellular vesicles for the diagnosis and treatment of Parkinson's disease. Aging Dis 2021;12:1438–1450.
20. Blommer J, Pitcher T, Mustapic M, Eren E, Yao PJ, Vreones MP, et al. Extracellular vesicle biomarkers for cognitive impairment in Parkinson's disease. Brain 2022;146:195–208.
21. Leggio L, Paternò G, Vivarelli S, Falzone GG, Giachino C, Marchetti B, et al. Extracellular vesicles as novel diagnostic and prognostic biomarkers for Parkinson's disease. Aging Dis 2021;12:1494–1515.
22. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science 1979;2020:367.
23. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002;2:569–579.
24. Théry C, Boussac M, Véron P, Ricciardi-Castagnoli P, Raposo G, Garin J, et al. Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Immun 2001;166:7309–7318.
25. Han Z, Chen H, Guo Z, Shen J, Luo W, Xie F, et al. Circular RNAs and their role in exosomes. Front Oncol 2022;12:1807.
26. D'Anca M, Fenoglio C, Serpente M, Arosio B, Cesari M, Scarpini EA, et al. Exosome determinants of physiological aging and age-related neurodegenerative diseases. Front Aging Neurosci 2019;11:232.
27. Wang J, Yue B-L, Huang Y-Z, Lan X-Y, Liu W-J, Chen H, et al. Exosomal RNAs: novel potential biomarkers for diseases— a review. Int J Mol Sci 2022;23:2461.
28. Brás IC, Khani MH, Riedel D, Parfentev I, Gerhardt E, van Riesen C, et al. Ectosomes and exosomes modulate neuronal spontaneous activity. J Proteomics 2022;269:104721.
29. Brás IC, Khani MH, Vasili E, Möbius W, Riedel D, Parfentev I, et al. Molecular mechanisms mediating the transfer of disease-associated proteins and effects on neuronal activity. J Parkinsons Dis 2022;12:1–26.
30. Bobrie A, Colombo M, Raposo G, Théry C. Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 2011;12:1659–1668.
31. Ogawa Y, Kanai-Azuma M, Akimoto Y, Kawakami H, Yanoshita R. Exosome-like vesicles with dipeptidyl peptidase IV in human saliva. Biol Pharm Bull 2008;31:1059–1062.

32. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A 2004;101:13368–13373.
33. Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C. Exosomal-like vesicles are present in human blood plasma. Int Immunol 2005;17:879–887.
34. Banks WA, Sharma P, Bullock KM, Hansen KM, Ludwig N, Whiteside TL. Transport of extracellular vesicles across the blood-brain barrier: brain pharmacokinetics and effects of inflammation. Int J Mol Sci 2020;21:1–21.
35. Matsumoto J, Stewart T, Sheng L, Li N, Bullock K, Song N, et al. Transmission of α-synuclein- containing erythrocyte-derived extracellular vesicles across the blood-brain barrier via adsorptive mediated transcytosis: another mechanism for initiation and progression of Parkinson's disease? Acta Neuropathol Commun 2017;5:71.
36. Younas N, Fernandez Flores LC, Hopfner F, Höglinger GU, Zerr I. A new paradigm for diagnosis of neurodegenerative diseases: peripheral exosomes of brain origin. Transl Neurodegener 2022; 11:1–15.
37. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ 2021;372:n160.
38. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol 2005;5:13.
39. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol 2014;14:135.
40. Rohatgi A. WebPlotDigitizer; 2022. https://automeris.io/WebPlotDigitizer. Accessed November 15, 2022.
41. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011; 155:529.
42. Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane handbook for systematic reviews of interventions. Cochrane Database Syst Rev 2019;10:ED000142.
43. Barbagallo C, Mostile G, Baglieri G, Giunta F, Luca A, Raciti L, et al. Specific signatures of serum miRNAs as potential biomarkers to discriminate clinically similar neurodegenerative and vascular-related diseases. Cell Mol Neurobiol 2020;40:531–546.
44. Cao X-Y, Lu J-M, Zhao Z-Q, Li M-C, Lu T, An X-S, et al. MicroRNA biomarkers of Parkinson's disease in serum exosome-like microvesicles. Neurosci Lett 2017;644:94–99.
45. Cao Z, Wu Y, Liu G, Jiang Y, Wang X, Wang Z, et al. α-Synuclein in salivary extracellular vesicles as a potential biomarker of Parkinson's disease. Neurosci Lett 2019;696:114–120.
46. Cerri S, Ghezzi C, Sampieri M, Siani F, Avenali M, Dornini G, et al. The exosomal/total α- synuclein ratio in plasma is associated with glucocerebrosidase activity and correlates with measures of disease severity in PD patients. Front Cell Neurosci 2018;12:125.
47. Chan L, Chung CC, Chen JH, Yu RC, Hong CT. Cytokine profile in plasma extracellular vesicles of Parkinson's disease and the association with cognitive function. Cell 2021;10:1–9.
48. Chou S-Y, Chan L, Chung C-C, Chiu J-Y, Hsieh Y-C, Hong C-T. Altered insulin receptor substrate 1 phosphorylation in blood neuron-derived extracellular vesicles from patients with Parkinson's disease. Front Cell Dev Biol 2020;8:564641.
49. Chung CC, Chan L, Chen JH, Bamodu OA, Hong CT. Neurofilament light chain level in plasma extracellular vesicles and Parkinson's disease. Ther Adv Neurol Disord 2020;13:1–9.
50. Chung CC, Huang PH, Chan L, Chen JH, Chien LN, Hong CT. Plasma exosomal brain-derived neurotrophic factor correlated with the postural instability and gait disturbance–related motor symptoms in patients with Parkinson's disease. Diagnostics 2020; 10:684.
51. Chung CC, Chan L, Chen JH, Bamodu OA, Chiu HW, Hong CT. Plasma extracellular vesicles tau and β-amyloid as biomarkers of cognitive dysfunction of Parkinson's disease. FASEB J 2021;35: 1–10.
52. Chung C-C, Chan L, Chen J-H, Hung Y-C, Hong C-T. Plasma extracellular vesicle α-synuclein level in patients with Parkinson's disease. Biomolecules 2021;11:744.
53. dos Santos MCT, Barreto-Sanz MA, Correia BRS, Bell R, Widnall C, Perez LT, et al. miRNA-based signatures in cerebrospinal fluid as potential diagnostic tools for early stage Parkinson's disease. Oncotarget 2018;9:17455–17465.
54. Dutta S, Hornung S, Kruayatidee A, Maina KN, del Rosario I, Paul KC, et al. Alpha-synuclein in blood exosomes immunoprecipitated using neuronal and oligodendroglial markers distinguishes Parkinson's disease from multiple system atrophy. Acta Neuropathol 2021;142:495–511.
55. Fraser KB, Moehle MS, Alcalay RN, West AB. Urinary LRRK2 phosphorylation predicts parkinsonian phenotypes in G2019S LRRK2 carriers. Neurology 2016;86:994–999.
56. Fraser KB, Rawlins AB, Clark RG, Alcalay RN, Standaert DG, Liu N, et al. Ser(P)-1292 LRRK2 in urinary exosomes is elevated in idiopathic Parkinson's disease. Mov Disord 2016;31:1543–1550.
57. Grossi I, Radeghieri A, Paolini L, Porrini V, Pilotto A, Padovani A, et al. MicroRNA-34a-5p expression in the plasma and in its extracellular vesicle fractions in subjects with Parkinson's disease: an exploratory study. Int J Mol Med 2021;47:533–546.
58. Gualerzi A, Picciolini S, Carlomagno C, Terenzi F, Ramat S, Sorbi S, et al. Raman profiling of circulating extracellular vesicles for the stratification of Parkinson's patients. Nanomedicine 2019; 22:102097.
59. Gui Y, Liu H, Zhang L, Lv W, Hu X. Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease. Oncotarget 2015;6:37043–37053.
60. Han C, Xiong N, Guo X, Huang J, Ma K, Liu L, et al. Exosomes from patients with Parkinson's disease are pathological in mice. J Mol Med 2019;97:1329–1344.
61. He M, Zhang H-N, Tang Z-C, Gao S-G. Diagnostic and therapeutic potential of Exosomal MicroRNAs for neurodegenerative diseases. Neural Plast 2021;2021:8884642.
62. Ho DH, Yi S, Seo H, Son I, Seol W. Increased DJ-1 in urine exosome of Korean males with Parkinson's disease. Biomed Res Int 2014;2014:1–8.
63. Hong Z, Tian C, Stewart T, Aro P, Soltys D, Bercow M, et al. Development of a sensitive diagnostic assay for Parkinson disease quantifying α-synuclein–containing extracellular vesicles. Neurology 2021;96:e2332–e2345.
64. Jiang R, Rong C, Ke R, Meng S, Yan X, Ke H, et al. Differential proteomic analysis of serum exosomes reveals alterations in progression of Parkinson disease. Medicine 2019;98:e17478.
65. Jiang C, Hopfner F, Katsikoudi A, Hein R, Catli C, Evetts S, et al. Serum neuronal exosomes predict and differentiate Parkinson's disease from atypical parkinsonism. J Neurol Neurosurg Psychiatry 2020;91:720–729.
66. Jiang C, Hopfner F, Berg D, Hu MT, Pilotto A, Borroni B, et al. Validation of α-synuclein in L1CAM- immunocaptured exosomes as a biomarker for the stratification of parkinsonian syndromes. Mov Disord 2021;36:2663–2669.
67. Kitamura Y, Kojima M, Kurosawa T, Sasaki R, Ichihara S, Hiraku Y, et al. Proteomic profiling of Exosomal proteins for blood-based biomarkers in Parkinson's disease. Neuroscience 2018;392:121–128.
68. Kluge A, Bunk J, Schaeffer E, Drobny A, Xiang W, Knacke H, et al. Detection of neuron-derived pathological alpha-synuclein in blood. Brain 2022;145:3058–3071.
69. Lamontagne-Proulx J, St-Amour I, Labib R, Pilon J, Denis HL, Cloutier N, et al. Portrait of blood- derived extracellular vesicles in patients with Parkinson's disease. Neurobiol Dis 2019;124: 163–175.
70. Leng B, Sun H, Zhao J, Liu Y, Shen T, Liu W, et al. Plasma exosomal prion protein levels are correlated with cognitive decline in PD patients. Neurosci Lett 2020;723:134866.

71. Leng B, Sun H, Li M, Zhao J, Liu X, Yao R, et al. Blood neuroexosomal excitatory amino acid transporter-2 is associated with cognitive decline in Parkinson's disease with RBD. Front Aging Neurosci 2022;14:952368.

72. Lucien F, Benarroch EE, Mullan A, Ali F, Boeve BF, Mielke MM, et al. Poly (ADP-Ribose) and α– synuclein extracellular vesicles in patients with Parkinson disease: a possible biomarker of disease severity. PLoS One 2022;17:e0264446.

73. Manna I, Quattrone A, De Benedittis S, Vescio B, Iaccino E, Quattrone A. Exosomal miRNA as peripheral biomarkers in Parkinson's disease and progressive supranuclear palsy: a pilot study. Parkinsonism Relat Disord 2021;93:77–84.

74. Nie C, Sun Y, Zhen H, Guo M, Ye J, Liu Z, et al. Differential expression of plasma Exo-miRNA in neurodegenerative diseases by next-generation sequencing. Front Neurosci 2020;14:438.

75. Niu M, Li Y, Li G, Zhou L, Luo N, Yao M, et al. A longitudinal study on α-synuclein in plasma neuronal exosomes as a biomarker for Parkinson's disease development and progression. Eur J Neurol 2020;27:967–974.

76. Ohmichi T, Mitsuhashi M, Tatebe H, Kasai T, El-Agnaf OMA, Tokuda T, et al. Quantification of brain-derived extracellular vesicles in plasma as a biomarker to diagnose Parkinson's and related diseases. Parkinsonism Relat Disord 2019;61:82–87.

77. Petropoulou-Vathi L, Simitsi A, Valkimadi PE, Kedariti M, Dimitrakopoulos L, Koros C, et al. Distinct profiles of LRRK2 activation and Rab GTPase phosphorylation in clinical samples from different PD cohorts. NPJ Parkinsons Dis 2022;8:73.

78. Calvani R, Picca A, Landi G, Marini F, Biancolillo A, Coelho-Junior HJ, et al. A novel multi-marker discovery approach identifies new serum biomarkers for Parkinson's disease in older people: an EXosomes in PArkiNson disease (EXPAND) ancillary study. Geroscience 2020;42:1323–1334.

79. Rani K, Mukherjee R, Singh E, Kumar S, Sharma V, Vishwakarma P, et al. Neuronal exosomes in saliva of Parkinson's disease patients: a pilot study. Parkinsonism Relat Disord 2019;67:21–23.

80. Ruf WP, Freischmidt A, Grozdanov V, Roth V, Brockmann SJ, Mollenhauer B, et al. Protein binding partners of dysregulated mirnas in Parkinson's disease serum. Cell 2021;10:1–13.

81. Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, Ginghina C, et al. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. Acta Neuropathol 2014;128:639–650.

82. Shi M, Kovac A, Korff A, Cook TJ, Ginghina C, Bullock KM, et al. CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. Alzheimers Dement 2016;12:1125–1131.

83. Shim KH, Go HG, Bae H, Jeong D-E, Kim D, Youn YC, et al. Decreased Exosomal acetylcholinesterase activity in the plasma of patients with Parkinson's disease. Front Aging Neurosci 2021;13:665400.

84. Si X, Tian J, Chen Y, Yan Y, Pu J, Zhang B. Central nervous system-derived Exosomal alpha- synuclein in serum may be a biomarker in Parkinson's disease. Neuroscience 2019;413:308–316.

85. Sproviero D, Gagliardi S, Zucca S, Arigoni M, Giannini M, Garofalo M, et al. Different miRNA profiles in plasma derived small and large extracellular vesicles from patients with neurodegenerative diseases. Int J Mol Sci 2021;22:2737.

86. Stuendl A, Kunadt M, Kruse N, Bartels C, Moebius W, Danzer KM, et al. Induction of α-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. Brain 2016;139:481–494.

87. Stuendl A, Kraus T, Chatterjee M, Zapke B, Sadowski B, Moebius W, et al. <scp>α- synuclein</scp> in plasma-derived extracellular vesicles is a potential biomarker of Parkinson's disease. Mov Disord 2021;36:2508–2518.

88. Tong G, Zhang P, Hu W, Zhang K, Chen X. Diagnostic test to identify Parkinson's disease from the blood sera of Chinese population: a cross-sectional study. Parkinsons Dis 2022;2022:8683877.

89. Vacchi E, Burrello J, Burrello A, Bolis S, Monticone S, Barile L, et al. Profiling inflammatory extracellular vesicles in plasma and cerebrospinal fluid: an optimized diagnostic model for Parkinson's disease. Biomedicine 2021;9:230.

90. Vacchi E, Burrello J, Di Silvestre D, Burrello A, Bolis S, Mauri P, et al. Immune profiling of plasma- derived extracellular vesicles identifies Parkinson disease. Neurol Neuroimmunol Neuroinflamm 2020;7:e866.

91. Wang L, Zhang L. Circulating Exosomal miRNA as diagnostic biomarkers of neurodegenerative diseases. Front Mol Neurosci 2020;13:53.

92. Wang S, Liu Z, Ye T, Mabrouk OS, Maltbie T, Aasly J, et al. Elevated LRRK2 autophosphorylation in brain-derived and peripheral exosomes in LRRK2 mutation carriers. Acta Neuropathol Commun 2017;5:86.

93. Wang S, Zhang X, Guo Y, Rong H, Liu T. The long noncoding RNA HOTAIR promotes parkinson's disease by upregulating LRRK2 expression. Oncotarget 2017;8:24449–24456.

94. Wang S, Kojima K, Mobley JA, West AB. Proteomic analysis of urinary extracellular vesicles reveal biomarkers for neurologic disease. EBioMedicine 2019;45:351–361.

95. Wang S, Unnithan S, Bryant N, Chang A, Rosenthal LS, Pantelyat A, et al. Elevated urinary Rab10 phosphorylation in idiopathic Parkinson disease. Mov Disord 2022;37:1454–1464.

96. Xia Y, Zhang G, Han C, Ma K, Guo X, Wan F, et al. Microglia as modulators of exosomal alpha- synuclein transmission. Cell Death Dis 2019;10:174.

97. Yan YQ, Pu JL, Zheng R, Fang Y, Gu LY, Guo T, et al. Different patterns of exosomal α-synuclein between Parkinson's disease and probable rapid eye movement sleep behavior disorder. Eur J Neurol 2022;29:3590–3599.

98. Yao YF, Qu MW, Li GC, Zhang FB, Rui HC. Circulating exosomal miRNAs as diagnostic biomarkers in Parkinson's disease. Eur Rev Med Pharmacol Sci 2018;22:5278–5283.

99. Yeh S h, Lin CH, Yang YJ, Lin LW, Tseng CW, Yang KD. Higher senescence associated secretory phenotype and lower defense mediator in urinary extracellular vesicles of elders with and without Parkinson disease. Sci Rep 2021;11:1–12.

100. Zhao Z-HH, Chen Z-TT, Zhou R-LL, Zhang X, Ye Q-YY, Wang Y-ZZ. Increased DJ-1 and α-synuclein in plasma neural-derived exosomes as potential markers for Parkinson's disease. Front Aging Neurosci 2019;10:438.

101. Zhao A, Li Y, Niu M, Li G, Luo N, Zhou L, et al. SNCA hypomethylation in rapid eye movement sleep behavior disorder is a potential biomarker for Parkinson's disease. J Parkinsons Dis 2020;10:1023–1031.

102. Zheng H, Xie Z, Zhang X, Mao J, Wang M, Wei S, et al. Investigation of α-synuclein species in plasma exosomes and the oligomeric and phosphorylated α-synuclein as potential peripheral biomarker of Parkinson's disease. Neuroscience 2021;469:79–90.

103. Zou J, Guo Y, Wei L, Yu F, Yu B, Xu A. Long noncoding RNA POU3F3 and α-synuclein in plasma L1CAM exosomes combined with β-glucocerebrosidase activity: potential predictors of Parkinson's disease. Neurotherapeutics 2020;17:1104–1119.

104. Agliardi C, Meloni M, Guerini FR, Zanzottera M, Bolognesi E, Baglio F, et al. Oligomeric alpha- syn and SNARE complex proteins in peripheral extracellular vesicles of neural origin are biomarkers for Parkinson's disease. Neurobiol Dis 2021;148:105185.

105. Nila IS, Sumsuzzman DM, Khan ZA, Jung JH, Kazema AS, Kim SJ, et al. Identification of exosomal biomarkers and its optimal isolation and detection method for the diagnosis of Parkinson's disease: a systematic review and meta-analysis. Ageing Res Rev 2022;82:101764.

106. Spillantini MG. Parkinson's disease, dementia with Lewy bodies and multiple system atrophy are alpha-synucleinopathies. Parkinsonism Relat Disord 1999;5:157–162.

107. Shi M, Bradner J, Hancock AM, Chung KA, Quinn JF, Peskind ER, et al. Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression. Ann Neurol 2011;69:570–580.

108. Tokuda T, Salem SA, Allsop D, Mizuno T, Nakagawa M, Qureshi MM, et al. Decreased α- synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. Biochem Biophys Res Commun 2006;349:162–166.

109. Mollenhauer B, Cullen V, Kahn I, Krastins B, Outeiro TF, Pepivani I, et al. Direct quantification of CSF α-synuclein by ELISA and first cross-sectional study in patients with neurodegeneration. Exp Neurol 2008;213:315–325.

110. Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, Galasko D, et al. DJ-1 and α-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. Brain 2010;133:713–726.

111. Foulds PG, Diggle P, Mitchell JD, Parker A, Hasegawa M, Masuda-Suzukake M, et al. A longitudinal study on a-synuclein in blood plasma as a biomarker for Parkinson's disease. Sci Rep 2013;3:2540.

112. El-Agnaf OMA, Salem SA, Paleologou KE, Curran MD, Gibson MJ, Court JA, et al. Detection of oligomeric forms of α-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. FASEB J 2006;20:419–425.

113. Shi M, Zabetian CP, Hancock AM, Ginghina C, Hong Z, Yearout D, et al. Significance and confounders of peripheral DJ-1 and alpha-synuclein in Parkinson's disease. Neurosci Lett 2010;480:78–82.

114. Malek N, Swallow D, Grosset KA, Anichtchik O, Spillantini M, Grosset DG. Alpha-synuclein in peripheral tissues and body fluids as a biomarker for Parkinson's disease-a systematic review. Acta Neurol Scand 2014;130:59–72.

115. Barbour R, Kling K, Anderson JP, Banducci K, Cole T, Diep L, et al. Red blood cells are the major source of alpha-synuclein in blood. Neurodegener Dis 2008;5:55–59.

116. Giri B, Seamon M, Banerjee A, Chauhan S, Purohit S, Morgan J, et al. Emerging urinary alpha- synuclein and miRNA biomarkers in Parkinson's disease. Metab Brain Dis 2021;37:1687–1696.

117. Nam D, Lee JY, Lee M, Kim J, Seol W, Son I, et al. Detection and assessment of α-synuclein oligomers in the urine of Parkinson's disease patients. J Parkinsons Dis 2020;10:981–991.

118. Bougea A, Koros C, Stefanis L. Salivary alpha-synuclein as a biomarker for Parkinson's disease: a systematic review. J Neural Transm 2019;126:1373–1382.

119. Lee H-J, Patel S, Lee S-J. Intravesicular localization and exocytosis of α-synuclein and its aggregates. J Neurosci 2005;25:6016–6024.

120. Emmanouilidou E, Melachroinou K, Roumeliotis T, Garbis SD, Ntzouni M, Margaritis LH, et al. Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. J Neurosci 2010;30:6838–6851.

121. Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, Zhu L, et al. Exosomal cell-to- cell transmission of alpha synuclein oligomers. Mol Neurodegener 2012;7:42.

122. Danzer KM, Krebs SK, Wolff M, Birk G, Hengerer B. Seeding induced by alpha-synuclein oligomers provides evidence for spreading of alpha-synuclein pathology. J Neurochem 2009;111:192–203.

123. Palviainen M, Saraswat M, Varga Z, Kitka D, Neuvonen M, Puhka M, et al. Extracellular vesicles from human plasma and serum are carriers of extravesicular cargo-implications for biomarker discovery. PLoS One 2020;15:e0236439.

124. Cao F, Gao Y, Chu Q, Wu Q, Zhao L, Lan T, et al. Proteomics comparison of exosomes from serum and plasma between ultracentrifugation and polymer-based precipitation kit methods. Electrophoresis 2019;40:3092–3098.

125. Zhang X, Takeuchi T, Takeda A, Mochizuki H, Nagai Y. Comparison of serum and plasma as a source of blood extracellular vesicles: increased levels of platelet-derived particles in serum extracellular vesicle fractions alter content profiles from plasma extracellular vesicle fractions. PLoS One 2022;17:e0270634.

126. Muraoka S, Hirano M, Isoyama J, Nagayama S, Tomonaga T, Adachi J. Comprehensive proteomic profiling of plasma and serum phosphatidylserine-positive extracellular vesicles reveals tissue-specific proteins. IScience 2022;25:104012.

127. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. Theranostics 2017;7:789–804.

128. Patel GK, Khan MA, Zubair H, Srivastava SK, Khushman M, Singh S, et al. Comparative analysis of exosome isolation methods using culture supernatant for optimum yield, purity and downstream applications. Sci Rep 2019;9:5335.

129. Greening DW, Xu R, Ji H, Tauro BJ, Simpson RJ. A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods. Methods Mol Biol 2015;1295:179–209.

130. Gomes DE, Witwer KW. L1CAM-associated extracellular vesicles: a systematic review of nomenclature, sources, separation, and characterization. J Ex Bio 2022;1:e35.

131. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option? Int J Mol Sci 2020;21:1–19.

132. Contreras-Naranjo JC, Wu HJ, Ugaz VM. Microfluidics for exosome isolation and analysis: enabling liquid biopsy for personalized medicine. Lab Chip 2017;17:3558–3577.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.