

Challenges associated with using extracellular vesicles as biomarkers in neurodegenerative disease

Yvonne Couch

To cite this article: Yvonne Couch (2023) Challenges associated with using extracellular vesicles as biomarkers in neurodegenerative disease, Expert Review of Molecular Diagnostics, 23:12, 1091-1105, DOI: [10.1080/14737159.2023.2277373](https://doi.org/10.1080/14737159.2023.2277373)

To link to this article: <https://doi.org/10.1080/14737159.2023.2277373>



© 2023 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 02 Nov 2023.



Submit your article to this journal [↗](#)



Article views: 1416



View related articles [↗](#)



View Crossmark data [↗](#)

REVIEW



Challenges associated with using extracellular vesicles as biomarkers in neurodegenerative disease

Yvonne Couch

Acute Stroke Program, Radcliffe Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK

ABSTRACT

Introduction: The hunt for new biomarkers – for the diagnosis of subcategories of disease, or for the monitoring of the efficacy of novel therapeutics – is an increasingly relevant challenge in the current era of precision medicine. In neurodegenerative research, the aim is to look for simple tools which can predict cognitive or motor decline early, and to determine whether these can also be used to test the efficacy of new interventions. Extracellular vesicles (EVs) are thought to play an important role in intercellular communication and have been shown to play a vital role in a number of diseases.

Areas Covered: The aim of this review is to examine what we know about EVs in neurodegeneration and to discuss their potential to be diagnostic and prognostic biomarkers in the future. It will cover the techniques used to isolate and study EVs and what is currently known about their presence in neurodegenerative diseases. In particular, we will discuss what is required for standardization in biomarker research, and the challenges associated with using EVs within this framework.

Expert Opinion: The technical challenges associated with isolating EVs consistently, combined with the complex techniques required for their efficient analysis, might preclude ‘pure’ EV populations from being used as effective biomarkers. Whilst biomarker discovery is important for more effective diagnosis, monitoring, prediction and prognosis in neurodegenerative disease, reproducibility and ease-of-use should be the priorities.

ARTICLE HISTORY

Received 2 August 2023
Accepted 26 October 2023

KEYWORDS

Neurodegeneration;
extracellular vesicles;
dementia; biomarkers;
Parkinson's; Alzheimer's;
Huntington's

1. Introduction to biomarkers

The aim of this review is to cover the main challenges associated with the use of extracellular vesicles (EVs) as biomarkers in neurodegenerative disease. Whilst Alzheimer's may be the most prevalent neurodegenerative disease, neurodegenerative processes occur in dementia, in Parkinson's, in Huntington's and in a number of other CNS diseases. These diseases all have a common theme, the gradual loss of neuronal function and the lack of truly effective single-analyte biomarker tests. Neurodegenerative diseases such as Alzheimer's often require a combination of clinical assessments, medical history review, neuropsychological testing, imaging studies (e.g. MRI, PET scans), and sometimes cerebrospinal fluid analysis to make a diagnosis. But with the introduction of machine learning algorithms and large ‘omics studies, there is the potential to use ‘patterns’ to diagnose disease. In MS, for example, studies have shown that a specific pattern of metabolites can be used to predict disease [1]. Here, we will explore how biomarkers are discovered, what biomarkers are currently used in neurodegenerative disease, and how EVs might be used as a novel biomarker.

Before embarking on the use of EVs as a novel biomarker, it is important to sit back and contemplate the definitions of the word biomarker. According to the NIH biomarker working group, the term is defined as ‘a defined characteristic that is

measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention.’ It is important to separate biomarkers from clinical outcomes, or clinical outcome assessments (COAs), as the NIH terms them. COAs are assessments which show how the disease is affecting the person. For example, cognitive decline in dementia is a measurable characteristic but it's a COA, not a biomarker. The goal of finding a biomarker is to have something which is measurable which might predict the COA. This can then be used to gauge the efficacy of new drugs, to monitor disease progression, or to stratify patient groups into subcategories of disease. Approaching the development of biomarkers for neurodegenerative disease requires understanding of these different categories of biomarker and how they might be applied to specific diseases and trials.

Diagnostic biomarkers are the most commonly used in clinics and require the most careful consideration before implementation. False-positive results from a blood cholesterol test would result in some dietary modifications and a re-check in six months, false-positive results from a blood test for uterine cancer would result in invasive biopsies, imaging sessions and considerable stress for the patient. Diagnostic biomarkers must be considered both necessary and sufficient to be worth using. They must also be rigorously validated and be associated with an easily measurable COA. A good example of

a diagnostic biomarker in current use is troponin, a biomarker used to help diagnose heart attack. However, the assays used to analyze troponin vary widely, and the outcomes of this can affect the specific clinical pathway the patient takes [2]. In neurodegenerative disease, especially at the early stages, a false-negative diagnosis because of the apparent absence of a biomarker could result in years of lost cognition. Biomarkers for neurodegeneration need to be able to accurately pick up the disease at an early stage so that patients can be put on specific medications or on specific treatment regimens before significant decline occurs.

Monitoring biomarkers are often used in clinical trials as a way of monitoring the efficacy of the intervention. They can also be used as a way of monitoring disease more generally. Imaging techniques are often used as monitoring biomarkers and with the increased use of machine learning and artificial intelligence, the large pool of imaging data available is being used to analyze patients with both mild cognitive impairment (MCI) and Alzheimer's [3,4]. Indeed, the combination of machine learning algorithms and multimodal approaches to monitoring have allowed this type of biomarker to morph into the prognostic category, with longitudinal data now being used to determine the conversion from MCI to Alzheimer's or dementia [5].

Prognostic biomarkers must be kept separate from predictive biomarkers, which are used as a way of monitoring an intervention or predicting a particular clinical event. For example, imaging data provides a predictive biomarker of the

efficacy of thrombolytic therapy in stroke [6] but it does not, as yet, provide a great deal of prognostic value about recovery or the development of dementia. Here, what might be more useful is a risk biomarker. One that is similar to a prognostic biomarker but is found prior to the onset of disease. A stroke increases the risk of dementia, but it's not a fatal prognosis.

A final important consideration in biomarker research is the discovery-validation pathway (Figure 1). When investigating a disease for a new biomarker, it is important to consider the noise you might experience within the population you're studying. For example, if you were investigating the presence or absence of a cancer, your data is likely to be less noisy and more binary. However, the majority of neurodegenerative diseases are, by nature, a declining scale of cognitive and motor symptoms. As such, including patients with mild cognitive impairment and those with severe dementia can muddy the waters. Here, researchers must make a choice as to what is clinically useful for the question they have in mind. For example, a diagnosis of Alzheimer's from the above pool might be challenging because of the inclusion of multiple types of dementia, but a marker that is present or absent in the mild cognitive impairment group and the reverse in all the dementias might be a good indicator of cognitive decline.

This discovery phase of biomarker research is crucial to finding novel ways of diagnosing, monitoring and prognosing disease, but Pepe and colleagues feel that sample selection for this stage has traditionally been neglected [7]. They are keen

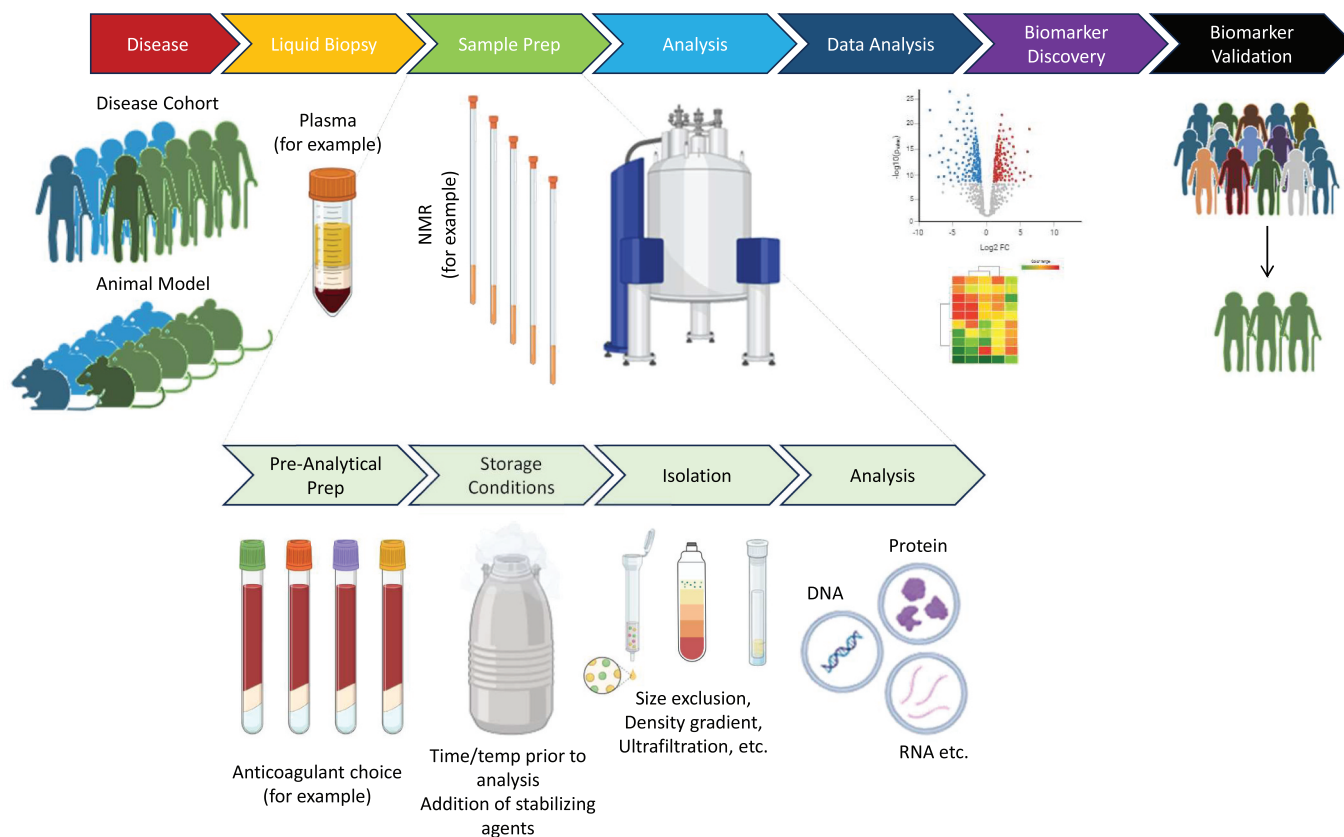


Figure 1. Example of biomarker discovery pipeline using plasma and nuclear magnetic resonance (NMR) as an example of biofluid and technique. The addition of EV isolation to the sample prep stage (inset) adds a number of caveats that need to be considered including the preparation of the samples to standardize pre-analytical variables, storage conditions (including the use of stabilization agents such as serum albumin), method of isolation and specific analytes of interest. Created using Biorender.

to include statisticians at this stage and highlight the need for sample sets *'that are large enough to reliably identify useful biomarkers,'* suggesting that one way to do this would be to open up large biobanks to discovery research. This would not only improve the statistical reliability of the research done at the discovery stage but also improve pre-analytical variables, as biobanks are often required to adhere to strict processing and storage protocols. By effectively choosing samples at the discovery stage, the chances of success at the validation phase are much higher [7] and improve the potential for discovering a novel biomarker for neurodegenerative disease.

1.1. Current biomarkers in neurodegenerative disease

In addition to understanding what a standard biomarker might need to consist of, it is also important to understand how neurodegenerative diseases are currently diagnosed, what the current predictive and monitoring biomarkers are and why a novel biomarker might be needed. Neurodegenerative diseases are a challenge for clinicians as they require considerable patient input into the diagnostic process. They may require blood tests and imaging, detailed personal and family histories or even invasive spinal fluid testing. Even with a litany of tests, and care within specialized centers, it is estimated that >20% of dementia patients are misdiagnosed when their pre-mortem and postmortem tests are compared [8].

There are currently no 'gold-standard' biomarkers for neurodegeneration, unlike circulating troponin for myocardial infarction, the biomarkers for neurodegenerative disease very much depend on what is available at the point of diagnosis. Patients with access to teaching hospitals using the latest positron emission tomography (PET) ligands and the latest magnetic resonance imaging (MRI) sequences are likely to get a more accurate diagnosis than those without access to these facilities.

An excellent review on the current use of biomarkers in neurodegenerative disease highlights some of the major issues with current testing regimes [9]. Our knowledge of the etiology of neurodegenerative diseases such as Alzheimer's is still lacking, and this is often confounding our use of good diagnostic biomarkers. Whilst there is a high degree of accuracy using β -amyloid and tau measurements in cerebrospinal fluid to predict a decline from mild cognitive impairment to Alzheimer's [10], in diseases such as Parkinson's, the story is not so simple. α -synuclein can be found in the cerebrospinal fluid in the majority of cases of Parkinson's [11] but this pathology is also present in some dementias [12] and thus the diagnostic value of this marker is reduced. Similar problems occur with less invasive methods. For example, β -amyloid pathology within the brain is a good indicator of Alzheimer's, but there are a number of instances of high amyloid load with no cognitive decline [13]. As such, β -amyloid PET scans can rule out Alzheimer's (cognitive decline with no amyloid pathology means another type of dementia), but they cannot definitely say what type of dementia the patient might have.

A large number of neurodegenerative diseases are diagnosed postmortem. Techniques such as imaging or invasive

spinal fluid sampling, are only applicable once symptoms such as motor issues or cognitive impairment begin to present. They are also often expensive, time consuming or invasive, requiring radioactive tracers or specialist radiologists to analyze images. What is required in neurodegenerative research are earlier predictive biomarkers and effective monitoring biomarkers to stratify patients into trials, novel medications and preventative treatment strategies and these biomarkers need to be easily accessible and testable in a liquid biopsy from blood or urine so that routine screening can take place early. However, to date, finding such a biomarker has been challenging.

2. Introduction to extracellular vesicles

Extracellular vesicles have garnered significant interest in recent decades, especially in biomarker research. Whereas previously they were thought to be cellular debris or were referred to by colloquial names like 'placental mist,' they are now known to be an important means of intercellular communication. The plethora of nomenclature increases every year as new isolation techniques and new methods of quantifying and visualizing extracellular vesicles are developed [14]. The field has moved on from exosomes and microvesicles to small and large extracellular vesicles, highlighting an understanding of overlapping sizes and novel means of biogenesis. Witwer and Thery [14] suggest extracellular vesicle as an effective umbrella term because it 'acts as a scaffold' on which you can pin more precise definitions. In this review, the aim is to use the term extracellular vesicles even when the original authors have used alternate nomenclature.

Extracellular vesicles (EVs) describes lipid bilayer-delineated vesicles produced by the majority of known cells (Figure 2). Subcategories of EVs have previously been based on biogenesis; smaller EVs formed from the endosomal compartments being known as 'exosomes', and larger ones formed from the cell membrane as 'microvesicles' or 'microparticles'. Their biogenesis pathways were often thought to be synonymous with size, leading to defined size ranges of 30-150 nm for exosomes and 150-1 μ m for microvesicles, now more commonly called ectosomes [15]. However, a combination of improved techniques for measuring and sizing EVs, as well as a better understanding of their biogenesis has led us to the realization that these ranges and biogenesis pathways often overlap. This means that when using standard isolation techniques, samples contain a mixture of EV sizes and a mixture of EVs from different parts of the cell.

It has also resulted in the addition of a number of new subgroups of EVs. Whilst there are extensive literature reviews on the biogenesis of EVs from the multivesicular body (MVB) and the cell surface [15], newer categories of EVs such as migrasomes and oncosomes – which originate from other parts of the cell – have received less attention. Some have suggested that subcategorizing EVs into functions with terms such as migrasome and oncosome is misleading [16], and that one may as well go back to the days of subcategorizing according to organ of origin, bringing back terms such as 'prostosome' [17]. However, migrasomes in particular are phenotypically distinct from MVB-derived EVs and from cell

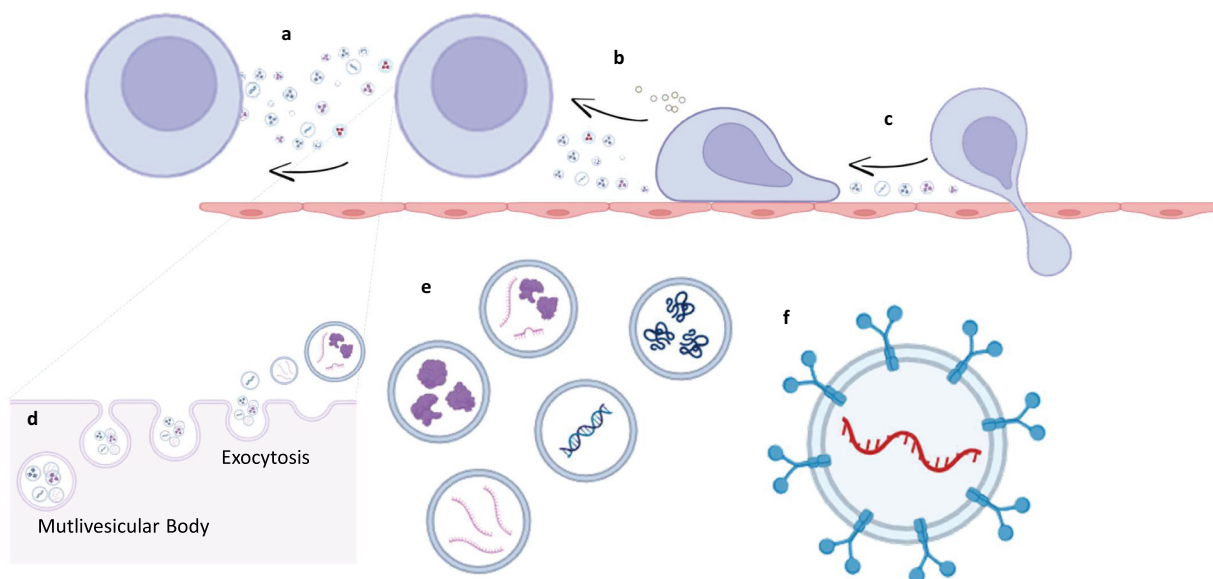


Figure 2. Examples of EV release from cells. All cells release EVs with a variety of cargo (A) and these EVs can be as small as exomeres or supermeres (B). When cells migrate they can leave behind trails of migrasomes (C). Normal EV release occurs through a variety of pathways including exocytosis of EVs from the multivesicular body (D). EVs can contain a variety of cargo including proteins, RNA and DNA species (E) and have a variety of different membrane proteins and sugars (F). Any number of these processes or components could be harnessed as part of the biomarker discovery pipeline. Created using Biorender.

surface shed vesicles [18]. They are produced during cell migration and can be anywhere between 500 nm and 3 μ m [19]. They contain their own set of internal vesicles and are taken up by other cells. Recent work suggests that the mechanisms of migrasome formation result in the clustering of adherence proteins such as integrins [18] and that perhaps they are produced as a 'trail of breadcrumbs' to induce further cell migration [20]. Oncosomes seem to be less distinct, with a size range of anywhere between 100–400 nm for 'regular' oncosomes [21] and 1–10 μ m for 'large' oncosomes [22]. The characteristic of this type of EV seems to be that they carry oncogenic cargo with the capacity to transform target cells and produce a metastatic niche at their destination [23]. Both migrasomes and oncosomes are likely to co-isolate from biofluids with the more 'classical' MVB- and surface-derived EVs, where EVs are going to come from a range of different cell types. As such, it is important to be aware of them within the field of EV biology.

At the smaller end of the spectrum lie exomeres and supermeres, particles of around 15–50 nm which are amembranous. Whilst this lack of membrane precludes them from being termed an EV, their size and shape often result in them being isolated with other EVs by commonly used techniques such as size exclusion and ultracentrifugation. Indeed, exomeres were only discovered because the researchers were using a highly specialized technique – asymmetrical field flow fractionation – and found a unique population of, what they considered to be, EVs [24]. The group went on to determine that the supernatant of the exomere isolation contained further particles which they termed supermeres (supernatant of exomeres) and which was found to be enriched with nucleic acids of various types [25]. Witwer, a veteran of the EV field, proposes caution when studying these unique

nanoparticles. An analysis run by the group on the existing data from several groups found enrichment for proteins such as LGALS3BP, a protein known to self-assemble [26]. The authors speculate on whether many of these can be considered distinct particle subtypes, or whether the size they achieve after aggregation simply results in co-isolation with particles of a similar size.

2.1. Technical challenges measuring EVs

This plethora of different EV subtypes highlights a distinct technical limitation of the current field of EV biology, isolation techniques and measurement techniques are often not precise enough to acquire data on these unique populations. There are a number of extensive and comprehensive reviews on the technical challenges of working with EVs, which should be compulsory reading for anyone starting in the field [27–29]. As a starting point, isolation techniques for EVs from biofluids vary enormously across the globe, and these different isolation techniques can result in different physicochemical outcomes [30]. In their paper on myocardial infarction, Paget and colleagues found that whilst isolation technique did not affect a core set of protein and lipid markers, their capacity to correlate with infarct size varied according to isolation technique [31]. In fact, the MISEV guidelines do not specifically state a recommended isolation technique, rather they say that '*there is no single optimal separation method, so choose based on the downstream applications and scientific question*' [15]. As such, those embarking on biomarker discovery using EVs nearly to clearly consider whether they want to have high recovery but low purity, or low recovery but high purity, and choose their isolation technique accordingly.

For example, the most commonly used technique to measure the number and size of EVs is some form of dynamic light scattering [27]. This technique provides an idea of the number and size of 'particles' in a solution but cannot provide more specific information on their composition. Biofluid samples are likely to contain not only EVs but lipoproteins of various sizes as well as aggregated proteins. An enrichment of smaller EVs (<50 nm) in these samples observed using dynamic light scattering does not necessarily mean there is an enrichment in exomeres and supermeres and further, more specialized characterization would be required to confirm this.

One way to determine whether the populations of EVs in a sample are distinct is to analyze their physicochemical properties. Once again, the technical challenges of isolation of pure populations often make this task difficult. Ultracentrifugation, one of the most widely available and easy to implement means of isolating EVs, will co-isolate proteins and aggregates and as such this makes it an unsuitable method for downstream protein analysis using techniques such as mass spectrometry (MS) [27]. Size exclusion will remove free proteins by allowing them to elute later, but samples from, for example, plasma or serum may still be contaminated by lipoproteins. Whilst smaller lipid particles such as high density lipoprotein may be too small (<12 nm) very low density lipoproteins (30–80 nm) and chylomicrons (<1.2 μm [32]) are likely to co-elute with EV fractions around the same size. To circumvent this issue, specialized techniques such as acoustofluidics can be employed [32] or additional steps such as cation exchange can be added to standard size exclusion protocols [33]. At this point, it is important to consider the feasibility of the technique for the relatively high throughput nature of biomarker implementation. If the plan is to use this as a monitoring biomarker in a trial, there may be tens if not hundreds of samples to process and the slow throughput caused by the lack of purity in the original sample may not be optimal. If the plan is to use this as a starting point to identify potential biomarkers, then throughput may not be an issue until the experiment is scaled further down the line.

As mentioned above, the MISEV guidelines [15] encourage researchers to think about what their specific questions are prior to deciding on isolation and analysis techniques. This may mean that adjustments need to be made according to their own unique molecule or molecules of interest. For example, surface proteins may be more robust in terms of sample processing than miRNA or other nucleic acid species. There are a number of excellent technical guidelines, including those by ISEV, which go into extensive detail on this topic [27–29].

2.2. The EV surface

To date, the most popular technique to analyze the protein composition of EVs is MS. However, the lysis of whole EVs results in a mixture of surface and cargo proteins being analyzed. A better approach is to analyze proteins on intact EVs, but Bauzá-Martínez and colleagues note that technological limitations of super-resolution microscopy and flow cytometry still limit us to observing only a handful of proteins at any one time on intact EVs [34]. They have developed a cross-linking

approach to MS that allows intact surface proteins on whole EVs to be studied [34]. The importance of analyzing surface vs cargo was highlighted by Cvjetkovic *et al.*, who found that a number of surface proteins were in an 'inside-out' conformation when found on EVs [35]. Finding these proteins by MS should not pose any technical difficulties but moving to a high-throughput assay-based technique employing antibodies may prove challenging with the protein in an aberrant conformation, an important consideration for the translatability of biomarker discovery.

As well as considering the conformation of the proteins which may be physically present within the EV bilayer, it is also important to consider the formation of a protein corona around the EV itself. For many years this has been the bane of nanoparticle-based drug delivery, where lipid particles take on an immediate 'hard' layer of proteins, surrounded by a 'soft' protein layer [36]. These often include so-called *don't eat me* proteins which preclude the uptake of the nanomedicine [36,37]. EVs in biological fluids have a net negative charge (ζ -potential) in stable or neutral solutions [38]. This means they are likely to attract proteins with a net positive charge, these include immunoglobulins, serum proteins and histones. Toth and colleagues have shown just this, demonstrating significant amounts of opsonins and immunoglobulins accumulated on the surface of nascent EVs when exposed to EV-free plasma [39]. The presence of a corona is a significant factor when considering using EVs as biomarkers. If the protein is present on the surface of the EV as part of the corona, then it is likely to have been free-floating in the plasma and as such the added complication of isolating EVs simply to access protein which was present in the biofluid anyway, may be unnecessary. However, the converse may also be true. If the protein you are searching for is relatively rare but attracted to the surface of EVs it may be a way of concentrating it for analysis.

Finally, it is also important to consider the fact that MS as a method to analyze EVs is one dimensional. The form and function of proteins is a fundamentally three-dimensional affair, and this may be particularly important in terms of EVs. Braig and colleagues, in their 2017 paper, demonstrated that C-reactive protein is shed from monocytes on EVs [40]. They suggest that one of the benefits of this could be conformational. The surface area available for the pentameric structure is smaller, with a tighter curve, exposing novel epitopes which are more capable of binding complement [40]. If this conformational change were to happen on for a rare but disease-specific epitope, the chances of picking it up using antibody-based techniques would be significantly higher and would be an important consideration for biomarker discovery. In fact, studies of α -synuclein have found that its interaction with EVs may result in its stabilization, as well as aggregate/oligomer formation [41,42]. Similar studies have been done on amyloid- β , demonstrating that EVs promote aggregation of the protein [43]. One suggested mechanism is that glycans on the surface of EVs bind monomeric amyloid, causing aggregation by clustering lots of monomers in the same space [44,45]. These protein-EV interactions potentially 'concentrate' the signal produced by the free protein, allowing more effective detection by less sensitive techniques.

2.3. The internal EV environment

As well as surface proteins, the cargo of EVs has been a source of much research focus. The lipid bilayer acts as a protective casing for easily degraded contents such as nucleic acids. In fact, RNase is used to demonstrate the presence of nucleic acid species inside EVs in sequencing experiments [15]. The relative abundance of the nucleic acid species commonly found in EVs has been clouded somewhat by the differential isolation methods used throughout the field, by the presence or absence of RNase treatment and by a bias in sequencing techniques. Many have suggested that miRNA are the most abundant species of RNA found in EVs but there are significant amounts of tRNA fragments, as well as long non-coding RNA, vault RNA, snoRNA and even mRNA [46]. In fact, the presence or absence of RNA species within EVs is likely to be biased by the type of read performed. Historically, EV research assumed that smaller RNA species were more likely to be present and therefore ran sequencing protocols which would favor the detection of small RNAs such as miRNA [46].

Concomitant with the problems of internal vs external RNA species, the protein content of EVs has often been obscured by poor isolation techniques or simply lack of interest. The techniques for capturing surface proteins, such as biotinylation, often lyse the captured EVs and analyze the captured part, not the eluent. The analysis of whole EVs, content and surface combined, by techniques such as MS are the most likely studies to yield novel biomarkers based on content simply by dint of the quantity of material required.

2.4. The potential benefits of using extracellular vesicles as biomarkers

An excellent NIH review of biomarker potential [2] highlights that the increasing number of papers producing big data has led to a maelstrom of potential biomarkers for almost anything you can think of. The main issue this creates is that these are not being considered under the rigorous frameworks outlined above and so progress toward useful and translatable biomarkers for neurodegenerative disease in particular, is stalling. EVs are often lauded as the next big thing in biomarker discovery. The 'liquid biopsy' approach is appealing with an abundance of bodily fluids – blood, urine, breast milk, saliva – available to use, many of which are minimally invasive to acquire.

EVs provide a unique way of amplifying and conserving biosignatures. The aforementioned studies demonstrating changes in the exposure of specific epitopes may provide novel binding sites for previously hidden antigens [40]. EVs may also conserve signal. The lipid membrane prevents destruction of the nucleic acid contents by circulating enzymes, potentially allowing it to be detected where it would otherwise be degraded. Signals can then be transported long distances, meaning that those originating in the brain may end up in the plasma or even the urine [47]. This would allow for noninvasive liquid biopsies and an overall easier patient experience. This conservation of signal or exposure of epitopes could be exploited for use in proteomics. The proteomic analysis of smaller sample sets often enables the

selection of a single marker which might then be detected by an antibody in cell-depleted plasma [48], saving the need for running repeated time-consuming and analytically complex proteomics. Indeed, the above-mentioned conformational changes EVs confer on surface antigens may result in more effective recognition by antibody-based technologies. The exacerbated membrane curve of EVs has been exploited by some groups using curvature-sensing peptides, which detect EVs even in the presence of their less curved parent cells [49]. Technologies like this may allow the detection of novel biomarkers on EVs *in situ* within their parent fluid without the need for complex and time-consuming isolation techniques.

For larger data sets, such as those generated by the 'omics analysis of EVs in biofluids, the advent of machine learning is increasingly enabling us to integrate larger datasets, providing us with important predictive outcomes for data where we have clinical information as well as liquid biopsy samples available. For example, platforms using a combination of micro flow cytometry, proteomics and sequencing offer promising ways of looking at EVs in biofluids [48]. Indeed, these kinds of approaches have been used to look at the biosignatures of EVs in urine for Parkinson's disease patients [50]. These large-scale approaches may be beneficial when studying EVs in biofluids, providing us with a signature of analytes specific to the disease being studied.

2.5. The potential pitfalls of using extracellular vesicles as biomarkers

The main issues with EVs as biomarkers arise from the fundamental technical challenges associated with their isolation and use which have been highlighted throughout the review thus far. Given the potential for these markers to direct clinical care, a marker which does not correlate effectively and rigorously with a COA is not a useful marker. There are currently no commonly used 'standard' COAs for the majority of neurodegenerative diseases and as such, correlating any new biomarker with anything beyond symptomatic decline is challenging.

In addition to the previously highlighted issues with isolation techniques (section 2.1), pre-analytical variability is also likely to affect the content and surface properties of EVs. In their work on using EV-bound tissue factor as a potential cancer biomarker, Gardiner and colleagues highlight the importance of standardizing pre-analytical variables such as anticoagulant use, blood draw technique and time to isolation, noting that all of these factors can influence the amount of tissue factor present on EVs [51]. This variability has implications for clinical care with insensitive assays potentially missing a diagnosis and ultrasensitive assays resulting in a different clinical pathway [2]. If standardized protocols are not globally implemented then the development of new biomarkers will have almost no impact on clinical care because pre- and post-analytical variables will have too much of an impact on their measurement.

These analytical variables are being added to the background of an already complex disease model, and this will often hinder the aforementioned discovery-validation pathway (section 1). Studies have shown that a significant proportion of patients with all-cause dementia, for example, have



Table 1. Created in October 2023 using the PubMed search string (extracellular vesicles[Title/abstract]) and (disease[Title/abstract]) where disease was either Alzheimer's, Parkinson's, Huntington's, ALS or multiple sclerosis. ALS = amyotrophic lateral sclerosis; NSF = no significant findings; NS = reported as not significant; NR = not reported; NU = not undertaken. Where AUC and specificity/sensitivity are reported these are compared to healthy controls. Where two sets of AUC are reported, these are the discovery and validation cohorts, respectively.

	Number of Samples Used				Specificity, Sensitivity or AUC or other reported measure	Sample Type	Refs.
	Cargo/Surface Marker	Increased	Decreased	Discovery	Validation		
Alzheimer's	miR9-5p, miR106-5p, miR125-5p, miR132-5p		miR29a-5p	6–11	NU	Plasma	[87]
	C1q		MAPK	9	NU	Serum	[88]
				24	100	Cerebrospinal fluid	[89]
			L1CAM, Aβ40, Aβ42, pTau, NMDAR	45	66	Plasma	[90]
	Aβ42			45	NU	Plasma	[64]
	let-7e-5p, miR-96-5p, miR-484		miR-99b-5p, miR-100-5p, miR-30e-5p, miR-378, miR-145-5p, miR-378c, miR-451a	23	NU	Plasma	[91]
	Synaptophysin		C48Pa	9–32	12	Serum	[92]
				19	NU	Cerebrospinal fluid	[93]
	Haemoglobin			20	NU	Serum	[94]
	Mitochondrial transcripts		DJ-1, progranulin, α-synuclein	15	NU	Plasma	[95]
Parkinson's	HSPA1A, NPEPPS, PTGFRN			5	NU	Plasma	[96]
				10	NU	Cerebrospinal fluid	[97]
	MMP9			31	NU	Plasma	[98]
	CD31 + CD41-, CD144, MOG, CD171		let-7-5p, miR-126-3p, miR-1290, miR-130a-3p, miR-142-3p, miR-144-3p, miR-146a-5p	9–13	NU	Plasma	[99]
	CCL11			10	NU	Plasma, serum	[100]
	IGF1R			73	NU	Serum	[101]
			miR212-3p, miR132-3p	31	NU	Plasma	[102]
			miR451a, miR21-5p	10	NU	Plasma	[103]
	Total EVs (fluorescence)			8–18	NU	Saliva	[104]
	GLT, SYN211, MJFR14			106	NU	Plasma	[105]
Parkinson's contd.	Aβ1–42, CD62P			66–73	20 (repeat)	Plasma	[65]
	NSF			3	NU	Cerebrospinal fluid	[106]
	Oligomeric α-synuclein		miR128	25	NU	Plasma	[107]
	NSF		Tau oligomers	70	NU	Serum	[108]
	Oligomeric α-synuclein			84	NU	Plasma	[90]
	AChE activity			30	NU	Plasma	[109]
				34	NU	Plasma	[110]
	α-synuclein			116	NU	Plasma	[111]
	α-synuclein			96	47	Plasma	[112]
	α-synuclein (in oligoEVs)			51	50	Serum	[112]
p-IRS-1 ^{S312}				215	75	Serum	[113]
				94	NU	Plasma	[114]

(Continued)

Table 1. (Continued).

Cargo/Surface Marker		Number of Samples Used		Specificity, Sensitivity or AUC or other reported measure	Sample Type	Refs.
Increased	Decreased	Discovery	Validation			
Oligomeric α -synuclein	STX1A, VAMP	32	NU	0.824 AUC, sensitivity = 78.1%, specificity = 75.0%	Serum	[115]
Changes in Raman spectra PCA plots						
Calbindin, SNAP23		22	NU	0.71 AUC	Serum	[116]
Oligomeric α -synuclein		28	57	0.86 AUC	Urine	[117]
		74	NU	0.941 AUC, sensitivity = 92%, specificity = 86%	Saliva	[118]
Neuron-derived EV number		15	NU	0.82 AUC	Plasma	[119]
AKR1A1, ATP5A1	AIDA, NADSYN1, ABHD14B	60	42	NR	Plasma	[120]
miR146a-5p, miR199a-3p, miR151a-3p, miR151-5p, miR199a-5p	miR4454, miR10b-5p, miR29b-3p	10	10	NS	Plasma	[121]
miR151a-5p, miR146a-5p	miR4454, miR10b-5p, miR29b-3p	20	50	NR	Plasma	[122]
miR23c	miR192-5p	12	18	0.909 AUC (quantile)	Plasma	[123]
IGLL1		10	NU	NR	Saliva	[124]
miR146a-5p, miR199a-3p, miR151a-3p, miR151-5p, miR199a-5p	miR4454, miR10b-5p, miR29b-3p	50	NU	NR	Plasma	[125]
TNF-R2, CHIT1, JAM-A	MB	9	NU	0.90–0.96 AUC	Cerebrospinal fluid	[126]
miR-342-3p	miR-1254	15	NU	NR	Serum, CNS tissue	[127]
Raman Shift Analysis						
	BLMH	20	NU	0.64–0.84 AUC	Plasma	[128]
		20	NU	NR	Cerebrospinal fluid	[129]
Huntingtin protein		14	NU	NR	Plasma	[75]
NSF		59	NU	NR	Plasma	[130]
C16:0 Sulfatide		9	NU	NR	Plasma	[131]
>50 proteins		4	NU	NR	Cerebrospinal fluid	[132]
NR		7	NU	NR	Cerebr. fluid, tears	[133]
Total number of EVs		50	NU	1.01 Hazard Ratio	Cerebrospinal fluid	[134]
Multiple Sclerosis contd.						
circNEIL3		10	NU	NR	Plasma	[135]
CD31/CD105		59	NU	NR	Plasma	[136]
miR-16-5p, miR-451a		16	NU	0.78–0.86 AUC	Cerebr. fluid, serum	[137]
Myelin basic protein		67	NU	0.916–0.988 AUC	Serum	[138]
MBLP1, CFH		86	NU	NR	Serum	[139]
Myelin basic protein		136	NU	95.2% sensitivity, 88.2% specificity	Cerebr. fluid, serum	[140]
GALC, CD107a	EBNA-1	59	NU	NR	Plasma	[141]
		11	NU	NR	Plasma	[142]

some kind of comorbidity [52,53]. A large proportion of these is cardiovascular diseases, with known effects on platelets and the vasculature [53]. These diseases are likely to be contributing significant numbers of EVs to the circulation which may not be specific to the CNS pathology. Obtaining well-characterized clinical samples that accurately represent the disease state can be challenging, particularly for rare or heterogeneous diseases. Limited sample availability can hinder the validation and large-scale studies necessary for biomarker development. In regular biomarker studies, significant numbers are normally used. For example, a Danish biomarker study for rheumatoid arthritis is aiming to run between 2015 and 2030 and collect > 5000 samples [54]. A study from Finland on predictive markers for outcomes after traumatic brain injury used > 600 samples and then validated them using a further 500 using a different platform [55]. These kinds of numbers simply aren't present in the EV literature at the moment likely due to the time constraints, expense and complexity of isolating EVs. The ongoing development of *in situ* assays that do not require isolation of EVs and novel high-throughput techniques is needed to avoid these issues.

3. Extracellular vesicles in neurodegeneration

EVs in neurodegenerative diseases are particularly appealing as they are known to cross the blood brain barrier [56]. This has led to the hunt for so-called 'brain-derived' EVs in the circulation (Table 1). There have been various markers picked up for this task, some of which have snowballed in the fashion-driven world of EV research. One example which has been surrounded by some degree of controversy is the surface marker L1CAM, a purportedly neuron-specific surface adhesion molecule. There are a number of studies showing conflicting results suggesting that L1CAM may not be an ideal brain-derived EV marker [57,58]. Despite these issues, the interest in EVs in neurodegenerative disease, and specifically our capacity to find brain-derived EVs in the circulation, is rising. Despite this, the number of trials registered to study EVs in neurodegenerative disease is surprisingly low, with the majority of research on EVs as biomarkers in neurodegeneration being done on small cohorts available in-house. A search on clinicaltrials.gov (as of October 2023) using the filters 'neurodegenerative disease' and 'extracellular vesicles' gave 12 trials currently recruiting. Of these, only one was aimed at studying biomarkers in dementia with a recruitment pool of 100, several were to study EVs in familial Parkinson's and several were irrelevant. This search highlights the relatively low numbers of official trials being done on EVs in neurodegenerative disease. Here, this review will highlight the EV-associated discoveries across a range of neurodegenerative diseases as a way of highlighting the breadth of the biomarker discovery landscape and the potential it has to be expanded.

With the recent advent of antibody therapies for dementia [59,60], the use of monitoring and prognostic biomarkers is becoming increasingly important. To date, studies on EVs as biomarkers in dementia number in the hundreds but this is rapidly increasing. A significant proportion of papers study amyloid and tau in EVs [61–64]. Here, the aim is often to determine whether the protein crosses the blood-brain barrier

as a free molecule or whether it comes associated with EVs. In terms of use as a biomarker, one study points out that detection in the circulation can be done without the enrichment of EVs [65]. Indeed, the follow-up investigations of the successful donanemab trial found that pTau and glial fibrillary acidic protein were reduced compared to placebo in patients who had received treatment with absolutely no need to co-isolate these molecules on EVs [66].

The potential for EVs to act as vectors for the transport of aberrant protein species is prevalent across the field of neurodegenerative disease. In Parkinson's disease (PD), EVs associated with oligomeric α -synuclein have been found in a variety of liquid biopsies [67], and EVs are thought to be an important way for cells to rid themselves of the misfolded protein [68] and could be used as a way of monitoring disease progression. In fact, a meta-analysis determined that isolating neuronal EVs (determined by L1CAM-positivity) and looking for α -synuclein was much more likely to be discriminatory for PD than looking for total α -synuclein in the blood or looking for α -synuclein in total EVs [67]. The authors also note that studies using plasma were much more successful than those using serum, again highlighting the importance of considering pre-analytical variables.

In amyotrophic lateral sclerosis (ALS), the picture is more complex. The predominant protein associated with the disease, TDP-43, is present in aggregated and fragmented form in the majority of ALS patients and it's the fragments which have been most commonly found associated with EVs [69]. However, reports have found that the transfer of this protein between cells often happens in an EV-independent manner [70]. A second protein often found mutated in ALS, SOD1, has been found to be associated with EVs in the brains and spinal cords of both mutant mice and human ALS patients [71]. However, where TDP-43 pathology has been associated with disease severity [72] and thus could be used in association with EVs as a potential biomarker, SOD1 mutations are varied, and correlations between mutant protein and disease progression are not as clear and as such it's association with EVs is a moot point in terms of use as a monitoring biomarker.

The transport of misfolded proteins carries across to other neurodegenerative diseases. Pink and colleagues have shown that specific molecular chaperones are responsible for the export of mutant huntingtin protein in vesicles [73]. Levels of mutant huntingtin have been shown to be associated with disease severity [74] but are most frequently measured in the cerebrospinal fluid, an invasive and often uncomfortable procedure. Huntingtin has been found to be associated with EVs in the plasma [75] and presents a novel and less invasive way of monitoring disease progression and therapeutic interventions. The presence of mutant protein on the surface of EVs also has significant effects on their physicochemical properties, specifically their viscoelasticity. One group has taken advantage of this and has shown that changes in viscoelasticity can be used to indicate the presence of mutant huntingtin [76]. The authors suggest that the presence of the mutant protein affects the way EVs adsorb to surfaces. This could be an important approach to rapidly measuring misfolded protein in neurodegenerative disease, considering the number of studies suggesting that EVs are a mechanism by which cells rid themselves of aberrant protein.

Prion protein has also been found to be associated with EVs in a number of different biofluids [77] and provides an interesting candidate for biomarker discovery in neurodegeneration. Native prion protein (PrP) is converted to an abnormal isoform (PrP^{TSE}) during the course of the disease and as such what often happens is a decrease in native protein during symptomatic periods [78]. Detection of the abnormal isoform *per se* is challenging [79], largely due to the insensitivity of current assay techniques, but studies have found that EVs appear to enrich PrP [80] and have been found to carry PrP^{TSE} [81] and this may be a way for cells to accidentally concentrate the signal, making it easier to detect using techniques like the ELISA.

Finally, in neurodegenerative diseases without an obvious misfolded protein at the root cause, using EVs as a biomarker of disease progression remains more challenging. Multiple sclerosis is one of the more challenging neurological diseases to diagnose, with a wide range of symptoms many of which overlap with other neurodegenerative diseases. Furthermore, the disease in its relapse-remitting form, has a temporal element, where patients often require imaging multiple times in order to establish a definitive diagnosis. In rodent models of the disease, animals are immunized against myelin oligodendrocyte glycoprotein (MOG), resulting in a gradual symptom onset followed by a recovery, similar to a relapse in the clinical setting. In humans, MOG is not often found in MS patients [82] and as such its use as a biomarker is minimal. It has been found associated with EVs [83] but using precipitation methods, which may have resulted in contamination with aggregated proteins and IgG. Because of the lack of an obvious protein candidate biomarker in multiple sclerosis, the majority of studies have focused on miRNAs in EVs [84]. These provide an interesting snapshot of the inflammatory processes underlying the disease, but the technical complexities associated with their isolation and measurement render them challenging biomarkers.

4. Conclusion

The hunt for new biomarkers in neurodegenerative disease is crucial. With an increasingly aging population and the advent of new and exciting drugs to battle diseases like dementia, it is becoming increasingly important to be able to monitor and prognose patients without the use of time-consuming and expensive imaging techniques. EVs offer a promising way of protecting circulating nucleic acid species from degradation, but our current methods of isolation and characterization make them too inconsistent and time-consuming to be what could be considered effective biomarkers. Researchers have alternative options for biomarker studies beyond EVs. Large, well-characterized clinical cohorts can be effectively studied using accessible techniques like nuclear magnetic resonance (NMR), which eliminates the complexity and time-consuming nature of isolating EVs while still providing valuable and translatable insights [1].

Biomarker development requires rigorous validation across large and diverse patient cohorts to demonstrate clinical utility and predictive power. Validation studies involve independent replication, clinical correlation, and assessment of the

biomarker's performance against existing gold standards or clinical endpoints. Addressing these challenges requires continued advancements in technologies, standardization of experimental and analytical approaches, collaboration among researchers and clinicians, and robust validation frameworks. Overcoming these hurdles will improve the translation of current data into clinically useful biomarkers for neurodegenerative disease, whether those are in EVs or not remains to be seen.

5. Expert opinion

Extracellular vesicles present new and exciting challenges in biomarker discovery, an area becoming increasingly important in the age of precision medicine. The potential to diagnose diseases such as dementia and Parkinson's prior to neurodegeneration using rapid liquid biopsies gives us the opportunity for the development of preventative medicines. At the other end of the spectrum, novel drugs and interventions are being introduced to the field and the capacity to monitor disease progression without using costly imaging techniques is vital. Such strategies will require easy implementation, high throughput and high accuracy. There are important studies currently being carried out in the field of cancer biology which may be transferable to neurodegenerative disease, where researchers have studied a broad molecular signature on extracellular vesicles, rather than looking for specific biomarkers.

Technological developments continue to be exciting in this field and highlight the importance of interdisciplinary research. The tools being used to study plant and bacterial extracellular vesicles, for example curvature sensing peptides [85], need to be taken up by those of us studying human disease. Combining these with extant microfluidic technologies has the potential for high throughput analysis of extracellular vesicles in liquid biopsy samples using an on-chip reader [86], for example. Much like many technologies in their infancy, these devices are sparsely available and often not being developed in bulk, and as such tend to be expensive and inaccessible to the majority of researchers. However, if we take this approach a step further into the future, it is possible to envision a time where on-chip techniques might be linked to localized readers with output to the patient, much like continuous glucose monitoring, or directly to clinicians to flag specifics for investigation.

Extracellular vesicle researchers also need to consider the scope and heterogeneity of neurodegenerative disease. The numbers currently being used in the majority of 'biomarker' papers show the presence of one protein or nucleic acid on a small handful of samples carefully prepared using precise and time-consuming techniques. Biomarkers for any disease must be robust and as such have been demonstrated to be present in a significant number of samples. Going forward, the field needs to consider how to scale research in order to determine the reproducibility of any discovered biomarkers in larger, more heterogeneous populations.

It is important to remember the goal of biomarker research is to find an easily accessible marker for disease. Whilst extracellular vesicles represent a safe haven for many potential biomarkers, such as miRNA, in their pure form they do not always

represent the easiest way to analyze liquid biopsies (Figure 1). Researchers need to consider whether they ‘need’ to isolate EVs in order to look for their biomarker of interest, or whether it could be done more simply in whole plasma or whole urine. Novel approaches that allow us to use the characteristics of extracellular vesicles, such as their enhanced membrane curvature or the altered properties of their surface proteins, should be being investigated over long and arduous methods for isolating pure populations which may not result in translatable markers. If markers are discovered which harness these unique properties of EVs, then we need to focus on how to translate them into high-throughput, reproducible techniques that can be easily implemented. Studying ‘pure’ populations is important in terms of fundamental physiology and pathology, but their isolation and use as pools for biomarkers is currently not reproducible and biomarker studies will continue to fail at the validation stage. To prove ultimately successful as biomarkers, the near future of extracellular vesicle research lies in the development of easily accessible and affordable technologies which can take full advantage of their unique properties.

Funding

This paper was funded by Alzheimer’s Research UK (no. ARUK-RF2019B-004).

Declaration of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Acknowledgments

The author would like to acknowledge Alzheimer’s Research UK for funding their ongoing work via grant ARUK-RF2019B-004 and to thank Professor Pia Siljander, Dr Elizabeth Dellar and Dr Cherie Blenkiron for their insightful thoughts, support and ongoing discussions on the subject of EVs as biomarkers in disease

References

Papers of special note have been highlighted as either of interest (*) or of considerable interest () to readers.**

- Dickens AM, Larkin JR, Griffin JL, et al. A type 2 biomarker separates relapsing-remitting from secondary progressive multiple sclerosis. *Neurology*. 2014;83(17):1492–1499. doi: [10.1212/WNL.0000000000000905](#)
- Califf RM. Biomarker definitions and their applications. *Exp Biol Med* (Maywood). 2018;243(3):213–221. doi: [10.1177/1535370217750088](#)
- Young PNE, Estarellas M, Coomans E, et al. Imaging biomarkers in neurodegeneration: current and future practices. *Alzheimers Res Ther*. 2020;12(1):49. doi: [10.1186/s13195-020-00612-7](#)
- Yan Y, Somer E, Grau V. Classification of amyloid PET images using novel features for early diagnosis of Alzheimer’s disease and mild cognitive impairment conversion. *Nucl Med Commun*. 2019;40(3):242–248. doi: [10.1097/MNM.0000000000000953](#)
- Ottoy J, Niemantsverdriet E, Verhaeghe J, et al. Association of short-term cognitive decline and MCI-to-AD dementia conversion with CSF, MRI, amyloid- and (18)F-FDG-PET imaging. *NeuroImage Clin*. 2019;22:101771. doi: [10.1016/j.nicl.2019.101771](#)
- Schellinger PD, Fiebach JB, Hacke W. Imaging-based decision making in thrombolytic therapy for ischemic stroke: present status. *Stroke*. 2003;34(2):575–583. doi: [10.1161/01.STR.0000051504.10095.9C](#)
- Pepe MS, Li CI, Feng Z. Improving the quality of biomarker discovery research: the right samples and enough of them. *Cancer Epidemiol Biomarkers Prev*. 2015;24(6):944–950. doi: [10.1158/1055-9965.EPI-14-1227](#)
- Beach TG, Monsell SE, Phillips LE, et al. Accuracy of the clinical diagnosis of Alzheimer disease at national institute on aging alzheimer disease centers, 2005–2010. *J Neuropathol Exp Neurol*. 2012;71(4):266–273. doi: [10.1097/NEN.0b013e31824b211b](#)
- Hansson O. Biomarkers for neurodegenerative diseases. *Nat Med*. 2021;27(6):954–963.
- Mattsson N, Lonneborg A, Boccardi M, et al. Geneva task force for the roadmap of Alzheimer’s B. Clinical validity of cerebrospinal fluid Abeta42, tau, and phospho-tau as biomarkers for Alzheimer’s disease in the context of a structured 5-phase development framework. *Neurobiol Aging*. 2017;52:196–213. doi: [10.1016/j.neurobiolaging.2016.02.034](#)
- Parnetti L, Gaetani L, Eusebi P, et al. CSF and blood biomarkers for Parkinson’s disease. *Lancet Neurol*. 2019;18(6):573–586. doi: [10.1016/S1474-4422\(19\)30024-9](#)
- Shahnawaz M, Mukherjee A, Pritzkow S, et al. Discriminating alpha-synuclein strains in Parkinson’s disease and multiple system atrophy. *Nature*. 2020;578(7794):273–277. doi: [10.1038/s41586-020-1984-7](#)
- Snowdon DA, Nun S. Healthy aging and dementia: findings from the Nun study. *Ann Intern Med*. 2003;139(5 Pt 2):450–454. doi: [10.7326/0003-4819-139-5_Part_2-200309021-00014](#)
- Witwer KW, Thery C. Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *J Extracell Vesicles*. 2019;8(1):1648167. doi: [10.1080/20013078.2019.1648167](#)
- Important article highlighting the discrepancies in nomenclature in the EV field.**
- Thery C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7(1):1535750. doi: [10.1080/20013078.2018.1535750](#)
- The MISEV guidelines are regularly updated and are recommended reading for anyone thinking about starting EV research.**
- Jaiswal R, Sedger LM. Intercellular vesicular transfer by exosomes, Microparticles and oncosomes - implications for cancer biology and treatments. *Front Oncol*. 2019;9:125. doi: [10.3389/fonc.2019.00125](#)
- Ronquist G. Prostatomes are mediators of intercellular communication: from basic research to clinical implications. *J Intern Med*. 2012;271(4):400–413. doi: [10.1111/j.1365-2796.2011.02487.x](#)
- Ding T, Ji J, Zhang W, et al. The phosphatidylinositol (4,5)-bisphosphate-Rab35 axis regulates migrasome formation. *Cell Res*. 2023;33(8):617–627. doi: [10.1038/s41422-023-00811-5](#)
- Ma L, Li Y, Peng J, et al. Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration. *Cell Res*. 2015;25(1):24–38. doi: [10.1038/cr.2014.135](#)
- Di Daniele A, Antonucci Y, Campello S. Migrasomes, new vesicles as Hansel and Gretel white pebbles? *Biol Direct*. 2022;17(1):8. doi: [10.1186/s13062-022-00321-1](#)
- Di Vizio D, Kim J, Hager MH, et al. Oncosome formation in prostate cancer: association with a region of frequent chromosomal deletion in metastatic disease. *Cancer Res*. 2009;69(13):5601–5609. doi: [10.1158/0008-5472.CAN-08-3860](#)

22. Minciaccchi VR, You S, Spinelli C, et al. Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles. *Oncotarget*. 2015;6(13):11327–11341. doi: [10.18632/oncotarget.3598](#)
23. Meehan B, Rak J, Di Vizio D. Oncosomes – large and small: what are they, where they came from? *J Extracell Vesicles*. 2016;5(1):33109. doi: [10.3402/jev.v5.33109](#)
24. Zhang H, Freitas D, Kim HS, et al. Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. *Nat Cell Biol*. 2018;20(3):332–343. doi: [10.1038/s41556-018-0040-4](#)
25. Zhang Q, Jeppesen DK, Higginbotham JN, et al. Supermeres are functional extracellular nanoparticles replete with disease biomarkers and therapeutic targets. *Nat Cell Biol*. 2021;23(12):1240–1254. doi: [10.1038/s41556-021-00805-8](#)
26. Tosar JP, Cayota A, Witwer K. Exomeres and supermeres: monolithic or diverse? *J Extracell Biol*. 2022;1(6). doi: [10.1002/jex2.45](#)
27. Ramirez MI, Amorim MG, Gadelha C, et al. Technical challenges of working with extracellular vesicles. *Nanoscale*. 2018;10(3):881–906. doi: [10.1039/C7NR08360B](#)
28. Reithmair M, Lindemann A, Mussack V, et al. Isolation and characterization of urinary extracellular vesicles for MicroRNA biomarker signature development with reference to MISEV compliance. *Methods Mol Biol*. 2022;2504:113–133.
29. Witwer KW, Goberdhan DC, O'Driscoll L, et al. Updating MISEV: evolving the minimal requirements for studies of extracellular vesicles. *J Extracell Vesicles*. 2021;10(14):e12182. doi: [10.1002/jev2.12182](#)
30. Veerman RE, Teeuwen L, Czarnewski P, et al. Molecular evaluation of five different isolation methods for extracellular vesicles reveals different clinical applicability and subcellular origin. *J Extracell Vesicles*. 2021;10(9):e12128. doi: [10.1002/jev2.12128](#)
31. Paget D, Checa A, Zöhrer B, et al. Comparative and integrated analysis of plasma extracellular vesicle isolation methods in healthy volunteers and patients following myocardial infarction. *J Ex Biol*. 2022;1(11):e66. doi: [10.1002/jex2.66](#)
32. Wu M, Chen C, Wang Z, et al. Separating extracellular vesicles and lipoproteins via acoustofluidics. *Lab Chip*. 2019;19(7):1174–1182. doi: [10.1039/C8LC01134F](#)
33. Ter-Ovanesyan D, Gilboa T, Budnik B, et al. Improved isolation of extracellular vesicles by removal of both free proteins and lipoproteins. *Elife*. 2023;12. doi: [10.7554/eLife.86394](#)
34. Bauza-Martinez J, Armony G, Pronker MF, et al. Characterization of protein complexes in extracellular vesicles by intact extracellular vesicle crosslinking mass spectrometry (iEVXL). *J Extracell Vesicles*. 2022;11(8):e12245. doi: [10.1002/jev2.12245](#)
- **This paper uses a novel technique, iEVXL, which uses protein chemistry to characterise EV surface topology.**
35. Cvjetkovic A, Jang SC, Konecna B, et al. Detailed analysis of protein topology of extracellular vesicles—evidence of unconventional membrane protein orientation. *Sci Rep*. 2016;6(1):36338. doi: [10.1038/srep36338](#)
36. Heidarzadeh M, Zarebkohan A, Rahbarghazi R, et al. Protein corona and exosomes: new challenges and prospects. *Cell Commun Signal*. 2023;21(1):64. doi: [10.1186/s12964-023-01089-1](#)
37. Kamerkar S, LeBleu VS, Sugimoto H, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature*. 2017;546(7659):498–503. doi: [10.1038/nature22341](#)
38. Midekessa G, Godakumara K, Ord J, et al. Zeta potential of extracellular vesicles: toward understanding the attributes that determine colloidal stability. *ACS Omega*. 2020;5(27):16701–16710. doi: [10.1021/acsomega.0c01582](#)
39. Toth EA, Turiak L, Visnovitz T, et al. Formation of a protein corona on the surface of extracellular vesicles in blood plasma. *J Extracell Vesicles*. 2021;10(11):e12140. doi: [10.1002/jev2.12140](#)
40. Braig D, Nero TL, Koch HG, et al. Transitional changes in the CRP structure lead to the exposure of proinflammatory binding sites. *Nat Commun*. 2017;8(1):14188. doi: [10.1038/ncomms14188](#)
- **An important paper showing that EVs may change the conformation, and a such function or exposure, of proteins on their surface.**
41. Stuenkel A, Kunadt M, Kruse N, et al. Induction of alpha-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. *Brain*. 2016;139(Pt 2):481–494. doi: [10.1093/brain/awv346](#)
42. Ruf WP, Meirelles JL, Danzer KM. Spreading of alpha-synuclein between different cell types. *Behav Brain Res*. 2023;436:114059. doi: [10.1016/j.bbr.2022.114059](#)
43. Rajendran L, Honsho M, Zahn TR, et al. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc Natl Acad Sci U S A*. 2006;103(30):11172–11177. doi: [10.1073/pnas.0603838103](#)
44. Yamamoto N, Matsubara T, Sato T, et al. Age-dependent high-density clustering of GM1 ganglioside at presynaptic neuritic terminals promotes amyloid beta-protein fibrillogenesis. *Biochim Biophys Acta*. 2008;1778(12):2717–2726. doi: [10.1016/j.bbame.2008.07.028](#)
45. Yuyama K, Igarashi Y. Exosomes as carriers of Alzheimer's amyloid-ss. *Front Neurosci*. 2017;11:229. doi: [10.3389/fnins.2017.00229](#)
46. Dellar ER, Hill C, Melling GE, et al. Unpacking extracellular vesicles: RNA cargo loading and function. *J Ex Biol*. 2022;1(5):e40. doi: [10.1002/jex2.40](#)
47. Sun R, Wang H, Shi Y, et al. A pilot study of urinary exosomes in Alzheimer's disease. *Neurodegen Dis*. 2020;19(5–6):184–191. doi: [10.1159/000505851](#)
48. Hoshino A, Kim HS, Bojmar L, et al. Extracellular Vesicle and Particle Biomarkers Define Multiple Human Cancers. *Cell*. 2020;182(4):1044–1061 e1018. doi: [10.1016/j.cell.2020.07.009](#)
49. Kawano K, Yokoyama F, Kawamoto J, et al. Development of a simple and rapid method for in situ vesicle detection in cultured media. *J Mol Biol*. 2020;432(22):5876–5888. doi: [10.1016/j.jmb.2020.09.009](#)
- **This study used curvature sensing peptides to find EVs in solution without the need for isolation techniques.**
50. Hadisurya M, Li L, Kuwaranancharoen K, et al. Quantitative proteomics and phosphoproteomics of urinary extracellular vesicles define putative diagnostic biosignatures for Parkinson's disease. *Commun Med (Lond)*. 2023;3(1):64. doi: [10.1038/s43856-023-00294-w](#)
51. Gardiner C, Harrison P, Belting M, et al. Extracellular vesicles, tissue factor, cancer and thrombosis—discussion themes of the ISEV 2014 educational day. *J Extracell Vesicles*. 2015;4(1):26901. doi: [10.3402/jev.v4.26901](#)
52. Varkey BP, Joseph J, Haokip HR, et al. The prevalence of comorbidities and associated factors among patients with dementia in the Indian setting: meta-analysis of observational studies. *Indian J Psychol Med*. 2023;45(4):338–344. doi: [10.1177/02537176221130252](#)
53. Xiao X, Xiang S, Xu Q, et al. Comorbidity among inpatients with dementia: a preliminary cross-sectional study in West China. *Aging Clin Exp Res*. 2023;35(3):659–667. doi: [10.1007/s40520-023-02349-3](#)
54. Kringelbach TM, Glinborg B, Hogdall EV, et al. Identification of new biomarkers to promote personalised treatment of patients with inflammatory rheumatic disease: protocol for an open cohort study. *BMJ Open*. 2018;8(2):e019325. doi: [10.1136/bmjopen-2017-019325](#)
55. Thomas I, Dickens AM, Posti JP, et al. Serum metabolome associated with severity of acute traumatic brain injury. *Nat Commun*. 2022;13(1):2545. doi: [10.1038/s41467-022-30227-5](#)
56. Alvarez-Erviti L, Seow Y, Yin H, et al. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol*. 2011;29(4):341–345. doi: [10.1038/nbt.1807](#)
57. Gomes DE, Witwer KW. L1CAM-associated extracellular vesicles: a systematic review of nomenclature, sources, separation, and characterization. *J Extracell Biol*. 2022;1(3). doi: [10.1002/jex2.35](#)
58. Norman M, Ter-Ovanesyan D, Trieu W, et al. L1CAM is not associated with extracellular vesicles in human cerebrospinal fluid or plasma. *Nat Methods*. 2021;18(6):631–634. doi: [10.1038/s41592-021-01174-8](#)
59. Wessels AM, Dennehy EB, Dowsett SA, et al. Meaningful clinical changes in alzheimer disease measured with the iADRS and illustrated using the donanemab TRAILBLAZER-ALZ study findings. *Neurol Clin Pract*. 2023;13(2):e200127. doi: [10.1212/CPJ.000000000000200127](#)

60. van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in Early Alzheimer's Disease. *N Engl J Med.* 2023;388(1):9–21. doi: [10.1056/NEJMoa2212948](https://doi.org/10.1056/NEJMoa2212948)
61. Perez M, Avila J, Hernandez F. Propagation of tau via extracellular vesicles. *Front Neurosci.* 2019;13:698. doi: [10.3389/fnins.2019.00698](https://doi.org/10.3389/fnins.2019.00698)
62. Lee S, Mankhong S, Kang JH. Extracellular vesicle as a source of Alzheimer's biomarkers: opportunities and challenges. *Int J Mol Sci.* 2019;20(7):1728. doi: [10.3390/ijms20071728](https://doi.org/10.3390/ijms20071728)
63. Andras IE, Garcia-Contreras M, Yanick C, et al. Extracellular vesicle-mediated amyloid transfer to neural progenitor cells: implications for RAGE and HIV infection. *Mol Brain.* 2020;13(1):21. doi: [10.1186/s13041-020-0562-0](https://doi.org/10.1186/s13041-020-0562-0)
64. Li TR, Yao YX, Jiang XY, et al. Beta-amyloid in blood neuronal-derived extracellular vesicles is elevated in cognitively normal adults at risk of Alzheimer's disease and predicts cerebral amyloidosis. *Alzheimers Res Ther.* 2022;14(1):66. doi: [10.1186/s13195-022-01010-x](https://doi.org/10.1186/s13195-022-01010-x)
65. Wang Z, Zheng Y, Cai H, et al. Abeta1-42-containing platelet-derived extracellular vesicle is associated with cognitive decline in Parkinson's disease. *Front Aging Neurosci.* 2023;15:1170663. doi: [10.3389/fnagi.2023.1170663](https://doi.org/10.3389/fnagi.2023.1170663)
66. Pontecorvo MJ, Lu M, Burnham SC, et al. Association of donanemab treatment with exploratory plasma biomarkers in early symptomatic alzheimer disease: a secondary analysis of the TRAILBLAZER-ALZ randomized clinical trial. *JAMA Neurol.* 2022;79(12):1250–1259. doi: [10.1001/jamaneurol.2022.3392](https://doi.org/10.1001/jamaneurol.2022.3392)
67. Xylaki M, Chopra A, Weber S, et al. Extracellular vesicles for the diagnosis of Parkinson's disease: systematic review and meta-analysis. *Mov Disord.* 2023;38(9):1585–1597. doi: [10.1002/mds.29497](https://doi.org/10.1002/mds.29497)
68. Danzer KM, Kranich LR, Ruf WP, et al. Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Mol Neurodegener.* 2012;7(1):42. doi: [10.1186/1750-1326-7-42](https://doi.org/10.1186/1750-1326-7-42)
69. Casarotto E, Sproviero D, Corridori E, et al. Neurodegenerative Disease-Associated TDP-43 Fragments Are Extracellularly Secreted with CASA Complex Proteins. *Cells.* 2022;11(3):516. doi: [10.3390/cells11030516](https://doi.org/10.3390/cells11030516)
70. Sackmann C, Sackmann V, Hallbeck M. TDP-43 is efficiently transferred between neuron-like cells in a manner enhanced by preservation of its N-Terminus but independent of extracellular vesicles. *Front Neurosci.* 2020;14:540. doi: [10.3389/fnins.2020.00540](https://doi.org/10.3389/fnins.2020.00540)
71. Silverman JM, Christy D, Shyu CC, et al. CNS-derived extracellular vesicles from superoxide dismutase 1 (SOD1)(G93A) ALS mice originate from astrocytes and neurons and carry misfolded SOD1. *J Biol Chem.* 2019;294(10):3744–3759. doi: [10.1074/jbc.RA118.004825](https://doi.org/10.1074/jbc.RA118.004825)
72. Brettschneider J, Arai K, Del Tredici K, et al. TDP-43 pathology and neuronal loss in amyotrophic lateral sclerosis spinal cord. *Acta Neuropathol.* 2014;128(3):423–437. doi: [10.1007/s00401-014-1299-6](https://doi.org/10.1007/s00401-014-1299-6)
73. Pink D, Donnelier J, Lewis JD, et al. Cysteine string protein controls two routes of export for misfolded huntingtin. *Front Neurosci.* 2021;15:762439. doi: [10.3389/fnins.2021.762439](https://doi.org/10.3389/fnins.2021.762439)
74. Wild EJ, Boggio R, Langbehn D, et al. Quantification of mutant huntingtin protein in cerebrospinal fluid from Huntington's disease patients. *J Clin Investig.* 2015;125(5):1979–1986. doi: [10.1172/JCI80743](https://doi.org/10.1172/JCI80743)
75. Ananbeh H, Novak J, Juhas S, et al. Huntingtin co-isolates with small extracellular vesicles from blood Plasma of TgHD and KI-HD pig models of Huntington's disease and human blood Plasma. *Int J Mol Sci.* 2022;23(10):5598. doi: [10.3390/ijms23105598](https://doi.org/10.3390/ijms23105598)
76. Mazouzi Y, Sallem F, Farina F, et al. Biosensing Extracellular Vesicle Subpopulations in Neurodegenerative Disease Conditions. *ACS Sens.* 2022;7(6):1657–1665. doi: [10.1021/acssensors.1c02658](https://doi.org/10.1021/acssensors.1c02658)
77. Khadka A, Spiers JG, Cheng L, et al. Extracellular vesicles with diagnostic and therapeutic potential for prion diseases. *Cell Tissue Res.* 2023;392(1):247–267. doi: [10.1007/s00441-022-03621-0](https://doi.org/10.1007/s00441-022-03621-0)
78. Minikel EV, Kuhn E, Cocco AR, et al. Domain-specific quantification of prion protein in cerebrospinal fluid by targeted mass spectrometry. *Mol & Cell Proteomics.* 2019;18(12):2388–2400. doi: [10.1074/mcp.RA119.001702](https://doi.org/10.1074/mcp.RA119.001702)
79. Erana H, Charco JM, Gonzalez-Miranda E, et al. Detection of pathognomonic biomarker PrP(Sc) and the Contribution of cell free-amplification techniques to the diagnosis of prion diseases. *Biomolecules.* 2020;10(3):469. doi: [10.3390/biom10030469](https://doi.org/10.3390/biom10030469)
80. Vella LJ, Greenwood DL, Cappai R, et al. Enrichment of prion protein in exosomes derived from ovine cerebral spinal fluid. *Vet Immunol Immunopathol.* 2008;124(3–4):385–393. doi: [10.1016/j.vetimm.2008.04.002](https://doi.org/10.1016/j.vetimm.2008.04.002)
81. Vella LJ, Sharples RA, Lawson VA, et al. Packaging of prions into exosomes is associated with a novel pathway of PrP processing. *J Pathol.* 2007;211(5):582–590. doi: [10.1002/path.2145](https://doi.org/10.1002/path.2145)
82. Spadaro M, Gerdes LA, Krumbholz M, et al. Autoantibodies to MOG in a distinct subgroup of adult multiple sclerosis. *Neurol(r) Neuroimmunol Neuroinflammation.* 2016;3(5):e257. doi: [10.1212/NXI.0000000000000257](https://doi.org/10.1212/NXI.0000000000000257)
83. Galazka G, Mycko MP, Selmaj I, et al. Multiple sclerosis: serum-derived exosomes express myelin proteins. *Mult Scler.* 2018;24(4):449–458. doi: [10.1177/1352458517696597](https://doi.org/10.1177/1352458517696597)
84. D'Anca M, Fenoglio C, Buccellato FR, et al. Extracellular vesicles in multiple sclerosis: role in the pathogenesis and potential usefulness as biomarkers and therapeutic tools. *Cells.* 2021;10(7):1733. doi: [10.3390/cells10071733](https://doi.org/10.3390/cells10071733)
85. Yan L, Liang J, Yin H. Chemical biology probes for extracellular vesicles facilitate studies of Neuroinflammation. *ACS Chem Neurosci.* 2016;7(4):418–419. doi: [10.1021/acschemneuro.5b00310](https://doi.org/10.1021/acschemneuro.5b00310)
86. Liu J, Chen Y, Pei F, et al. Extracellular Vesicles in Liquid Biopsies: Potential for Disease Diagnosis. *Biomed Res Int.* 2021;2021:6611244. doi: [10.1155/2021/6611244](https://doi.org/10.1155/2021/6611244)
87. Kumar A, Su Y, Sharma M, et al. MicroRNA expression in extracellular vesicles as a novel blood-based biomarker for Alzheimer's disease. *Alzheimers Dement.* 2023. doi: [10.1002/alz.13055](https://doi.org/10.1002/alz.13055)
88. Ferreira MJC, Soares Martins T, Alves SR, et al. Bioinformatic analysis of the SPs and NFTs proteomes unravel putative biomarker candidates for Alzheimer's disease. *Proteomics.* 2023;23(15):e2200515. doi: [10.1002/pmic.202200515](https://doi.org/10.1002/pmic.202200515)
89. Chatterjee M, Ozdemir S, Kunadt M, et al. C1q is increased in cerebrospinal fluid-derived extracellular vesicles in Alzheimer's disease: a multi-cohort proteomics and immuno-assay validation study. *Alzheimers Dement.* 2023. doi: [10.1002/alz.13066](https://doi.org/10.1002/alz.13066)
90. Tian C, Stewart T, Hong Z, et al. Blood extracellular vesicles carrying synaptic function- and brain-related proteins as potential biomarkers for Alzheimer's disease. *Alzheimer's & Dementia.* 2022;19(3):909–923. doi: [10.1002/alz.12723](https://doi.org/10.1002/alz.12723)
91. Durur DY, Tastan B, Ugur Tufekci K, et al. Alteration of miRNAs in small neuron-derived extracellular vesicles of Alzheimer's disease patients and the effect of extracellular vesicles on microglial immune responses. *J Mol Neurosci.* 2022;72(6):1182–1194. doi: [10.1007/s12031-022-02012-y](https://doi.org/10.1007/s12031-022-02012-y)
92. Soares Martins T, Marcalo R, da Cruz ESCB, et al. Novel exosome biomarker candidates for Alzheimer's disease unravelled through mass spectrometry analysis. *Mol Neurobiol.* 2022;59(5):2838–2854. doi: [10.1007/s12035-022-02762-1](https://doi.org/10.1007/s12035-022-02762-1)
93. Utz J, Berner J, Munoz LE, et al. Cerebrospinal fluid of patients with Alzheimer's disease contains increased percentages of synaptophysin-bearing microvesicles. *Front Aging Neurosci.* 2021;13:682115. doi: [10.3389/fnagi.2021.682115](https://doi.org/10.3389/fnagi.2021.682115)
94. Arioz BI, Tufekci KU, Olcum M, et al. Proteome profiling of neuron-derived exosomes in Alzheimer's disease reveals hemoglobin as a potential biomarker. *Neurosci Lett.* 2021;755:135914. doi: [10.1016/j.neulet.2021.135914](https://doi.org/10.1016/j.neulet.2021.135914)
95. Ben Khedher MR, Haddad M, Laurin D, et al. Apolipoprotein E4-driven effects on inflammatory and neurotrophic factors in peripheral extracellular vesicles from cognitively impaired, no dementia participants who converted to Alzheimer's disease. *Alzheimers Dement (N Y).* 2021;7(1):e12124. doi: [10.1002/trc2.12124](https://doi.org/10.1002/trc2.12124)
96. Kim KM, Meng Q, Perezde Acha O, et al. Mitochondrial RNA in Alzheimer's Disease Circulating Extracellular Vesicles. *Front Cell Dev Biol.* 2020;8:581882. doi: [10.3389/fcell.2020.581882](https://doi.org/10.3389/fcell.2020.581882)
97. Muraoka S, Jedrychowski MP, Yanamandra K, et al. Proteomic profiling of extracellular vesicles derived from cerebrospinal fluid of Alzheimer's disease patients: a pilot study. *Cells.* 2020;9(9):1959. doi: [10.3390/cells9091959](https://doi.org/10.3390/cells9091959)

98. Gu D, Liu F, Meng M, et al. Elevated matrix metalloproteinase-9 levels in neuronal extracellular vesicles in Alzheimer's disease. *Ann Clin Transl Neurol.* 2020;7(9):1681–1691. doi: [10.1002/acn3.51155](#)
99. Aharon A, Spector P, Ahmad RS, et al. Extracellular vesicles of Alzheimer's disease patients as a biomarker for disease progression. *Mol Neurobiol.* 2020;57(10):4156–4169. doi: [10.1007/s12035-020-02013-1](#)
100. Ellegaard Nielsen J, Sofie Pedersen K, Vestergaard K, et al. Novel blood-derived extracellular vesicle-based biomarkers in Alzheimer's disease identified by proximity extension assay. *Biomedicines.* 2020;8(7):199. doi: [10.3390/biomedicines8070199](#)
101. Eren E, Hunt JFV, Shardell M, et al. Extracellular vesicle biomarkers of Alzheimer's disease associated with sub-clinical cognitive decline in late middle age. *Alzheimers Dement.* 2020;16(9):1293–1304. doi: [10.1002/alz.12130](#)
102. Cha DJ, Mengel D, Mustapic M, et al. miR-212 and miR-132 are downregulated in neurally derived Plasma exosomes of Alzheimer's patients. *Front Neurosci.* 2019;13:1208. doi: [10.3389/fnins.2019.01208](#)
103. Gamez-Valero A, Campdelacreu J, Vilas D, et al. Exploratory study on microRNA profiles from plasma-derived extracellular vesicles in Alzheimer's disease and dementia with Lewy bodies. *Transl Neurodegener.* 2019;8(1):31. doi: [10.1186/s40035-019-0169-5](#)
104. Rastogi S, Rani K, Rai S, et al. Fluorescence-tagged salivary small extracellular vesicles as a nanotool in early diagnosis of Parkinson's disease. *BMC Med.* 2023;21(1):335. doi: [10.1186/s12916-023-03031-1](#)
105. Wang P, Lan G, Xu B, et al. alpha-Synuclein-carrying astrocytic extracellular vesicles in Parkinson pathogenesis and diagnosis. *Transl Neurodegener.* 2023;12(1):40. doi: [10.1186/s40035-023-00372-y](#)
106. Dutta S, Hornung S, Taha HB, et al. Development of a novel electrochemiluminescence ELISA for Quantification of alpha-synuclein phosphorylated at Ser(129) in biological samples. *ACS Chem Neurosci.* 2023;14(7):1238–1248. doi: [10.1021/acscchemneuro.2c00676](#)
107. Bhattacharyya P, Biswas A, Biswas SC. Brain-enriched miR-128: reduced in exosomes from Parkinson's patient plasma, improves synaptic integrity, and prevents 6-OHDA mediated neuronal apoptosis. *Front Cell Neurosci.* 2022;16:1037903. doi: [10.3389/fncel.2022.1037903](#)
108. Meloni M, Agliardi C, Guerini FR, et al. Oligomeric alpha-synuclein and tau aggregates in NDEVs differentiate Parkinson's disease from atypical parkinsonisms. *Neurobiol Dis.* 2023;176:105947. doi: [10.1016/j.nbd.2022.105947](#)
109. Kluge A, Bunk J, Schaeffer E, et al. Detection of neuron-derived pathological alpha-synuclein in blood. *Brain.* 2022;145(9):3058–3071. doi: [10.1093/brain/awac115](#)
110. Shim KH, Go HG, Bae H, et al. Decreased exosomal acetylcholinesterase activity in the Plasma of patients with Parkinson's disease. *Front Aging Neurosci.* 2021;13:665400. doi: [10.3389/fnagi.2021.665400](#)
111. Chung CC, Chan L, Chen JH, et al. Plasma Extracellular Vesicle α -Synuclein Level in Patients with Parkinson's Disease. *Biomolecules.* 2021;11(5):744. doi: [10.3390/biom11050744](#)
112. Stuehl A, Kraus T, Chatterjee M, et al. Alpha-synuclein in Plasma-derived extracellular vesicles is a potential biomarker of Parkinson's disease. *Mov Disord.* 2021;36(11):2508–2518. doi: [10.1002/mds.28639](#)
113. Jiang C, Hopfner F, Berg D, et al. Validation of alpha-synuclein in L1CAM-Immunocaptured exosomes as a biomarker for the stratification of parkinsonian syndromes. *Mov Disord.* 2021;36(11):2663–2669. doi: [10.1002/mds.28591](#)
114. Chou SY, Chan L, Chung CC, et al. 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. doi: [10.3389/fcell.2020.564641](#)
115. Agliardi C, Meloni M, Guerini FR, 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. doi: [10.1016/j.nbd.2020.105185](#)
116. Gualerzi A, Picciolini S, Carlomagno C, et al. Raman profiling of circulating extracellular vesicles for the stratification of Parkinson's patients. *Nanomedicine.* 2019;22:102097. doi: [10.1016/j.nano.2019.102097](#)
117. Wang S, Kojima K, Mobley JA, et al. Proteomic analysis of urinary extracellular vesicles reveal biomarkers for neurologic disease. *EBioMedicine.* 2019;45:351–361. doi: [10.1016/j.ebiom.2019.06.021](#)
118. Cao Z, Wu Y, Liu G, et al. alpha-Synuclein in salivary extracellular vesicles as a potential biomarker of Parkinson's disease. *Neurosci Lett.* 2019;696:114–120. doi: [10.1016/j.neulet.2018.12.030](#)
119. Ohmichi T, Mitsuhashi M, Tatebe H, et al. Quantification of brain-derived extracellular vesicles in plasma as a biomarker to diagnose Parkinson's and related diseases. *Parkinsonism Related Disord.* 2019;61:82–87. doi: [10.1016/j.parkreldis.2018.11.021](#)
120. Lamontagne-Proulx J, St-Amour I, Labib R, et al. Portrait of blood-derived extracellular vesicles in patients with Parkinson's disease. *Neurobiol Dis.* 2019;124:163–175. doi: [10.1016/j.nbd.2018.11.002](#)
121. Banack SA, Dunlop RA, Cox PA. An miRNA fingerprint using neural-enriched extracellular vesicles from blood plasma: towards a biomarker for amyotrophic lateral sclerosis/motor neuron disease. *Open Biol.* 2020;10(6):200116. doi: [10.1098/rsob.200116](#)
122. Dunlop RA, Banack SA, Cox PA. L1CAM immunocapture generates a unique extracellular vesicle population with a reproducible miRNA fingerprint. *RNA Biol.* 2023;20(1):140–148. doi: [10.1080/15476286.2023.2198805](#)
123. Kim JA, Park C, Sung JJ, et al. Small RNA sequencing of circulating small extracellular vesicles microRNAs in patients with amyotrophic lateral sclerosis. *Sci Rep.* 2023;13(1):5528. doi: [10.1038/s41598-023-32717-y](#)
124. Sjoqvist S, Otake K. Saliva and Saliva extracellular vesicles for biomarker candidate Identification—assay development and pilot study in amyotrophic lateral sclerosis. *Int J Mol Sci.* 2023;24(6):5237. doi: [10.3390/ijms24065237](#)
125. Banack SA, Dunlop RA, Stommel EW, et al. miRNA extracted from extracellular vesicles is a robust biomarker of amyotrophic lateral sclerosis. *J Neurolog Sci.* 2022;442:120396. doi: [10.1016/j.jns.2022.120396](#)
126. Sjoqvist S, Otake K. A pilot study using proximity extension assay of cerebrospinal fluid and its extracellular vesicles identifies novel amyotrophic lateral sclerosis biomarker candidates. *Biochem Biophys Res Commun.* 2022;613:166–173. doi: [10.1016/j.bbrc.2022.04.127](#)
127. Lo TW, Figueroa-Romero C, Hur J, et al. Extracellular vesicles in Serum and central nervous system tissues contain microRNA signatures in sporadic amyotrophic lateral sclerosis. *Front Mol Neurosci.* 2021;14:739016. doi: [10.3389/fnmol.2021.739016](#)
128. Morasso CF, Sproviero D, Mimmi MC, et al. Raman spectroscopy reveals biochemical differences in plasma derived extracellular vesicles from sporadic amyotrophic lateral sclerosis patients. *Nanomedicine.* 2020;29:102249. doi: [10.1016/j.nano.2020.102249](#)
129. Thompson AG, Gray E, Mager I, et al. CSF extracellular vesicle proteomics demonstrates altered protein homeostasis in amyotrophic lateral sclerosis. *Clin Proteomics.* 2020;17(1):31. doi: [10.1186/s12014-020-09294-7](#)
130. Denis HL, Lamontagne-Proulx J, St-Amour I, et al. Platelet-derived extracellular vesicles in Huntington's disease. *J Neurol.* 2018;265(11):2704–2712. doi: [10.1007/s00415-018-9022-5](#)
131. Moyano AL, Li G, Boullerne AI, et al. Sulfatides in extracellular vesicles isolated from plasma of multiple sclerosis patients. *J Neurosci Res.* 2016;94(12):1579–1587. doi: [10.1002/jnr.23899](#)
132. Welton JL, Loveless S, Stone T, et al. Cerebrospinal fluid extracellular vesicle enrichment for protein biomarker discovery in neurological disease; multiple sclerosis. *J Extracell Vesicles.* 2017;6(1):1369805. doi: [10.1080/20013078.2017.1369805](#)
133. Pieragostino D, Lanuti P, Cicalini I, et al. Proteomics characterization of extracellular vesicles sorted by flow cytometry reveals a disease-specific molecular cross-talk from cerebrospinal fluid and tears in multiple sclerosis. *J Proteomics.* 2019;204:103403. doi: [10.1016/j.jpro.2019.103403](#)
134. Dalla Costa G, Croese T, Pisa M, et al. CSF extracellular vesicles and risk of disease activity after a first demyelinating event. *Mult Scler.* 2021;27(10):1606–1610. doi: [10.1177/1352458520987542](#)

135. Iparraguirre L, Alberro A, Hansen TB, et al. Profiling of Plasma Extracellular Vesicle Transcriptome Reveals That circRNAs Are Prevalent and Differ between Multiple Sclerosis Patients and Healthy Controls. *Biomedicines*. 2021;9(12):1850. doi: [10.3390/biomedicines9121850](https://doi.org/10.3390/biomedicines9121850)
136. Mazzucco M, Mannheim W, Shetty SV, et al. CNS endothelial derived extracellular vesicles are biomarkers of active disease in multiple sclerosis. *Fluids Barriers CNS*. 2022;19(1):13. doi: [10.1186/s12987-021-00299-4](https://doi.org/10.1186/s12987-021-00299-4)
137. Cuomo-Haymour N, Bergamini G, Russo G, et al. Differential expression of Serum extracellular vesicle miRNAs in multiple sclerosis: disease-stage specificity and relevance to pathophysiology. *Int J Mol Sci*. 2022;23(3):1664. doi: [10.3390/ijms23031664](https://doi.org/10.3390/ijms23031664)
138. Agliardi C, Guerini FR, Zanzottera M, et al. Myelin Basic Protein in Oligodendrocyte-Derived Extracellular Vesicles as a Diagnostic and Prognostic Biomarker in Multiple Sclerosis: A Pilot Study. *Int J Mol Sci*. 2023;24(1):894. doi: [10.3390/ijms24010894](https://doi.org/10.3390/ijms24010894)
139. Torres Iglesias G, Fernandez-Fournier M, Botella L, et al. Brain and immune system-derived extracellular vesicles mediate regulation of complement system, extracellular matrix remodeling, brain repair and antigen tolerance in Multiple sclerosis. *Brain Behav Immun*. 2023;113:44–55. doi: [10.1016/j.bbi.2023.06.025](https://doi.org/10.1016/j.bbi.2023.06.025)
140. Torres Iglesias G, Fernandez-Fournier M, Lopez-Molina M, et al. Dual role of peripheral B cells in multiple sclerosis: emerging remote players in demyelination and novel diagnostic biomarkers. *Front Immunol*. 2023;14:1224217. doi: [10.3389/fimmu.2023.1224217](https://doi.org/10.3389/fimmu.2023.1224217)
141. Fernandez-Fournier M, Lopez-Molina M, Torres Iglesias G, et al. Antibody Content against Epstein–Barr Virus in Blood Extracellular Vesicles Correlates with Disease Activity and Brain Volume in Patients with Relapsing–Remitting Multiple Sclerosis. *Int J Mol Sci*. 2023;24(18):14192. doi: [10.3390/ijms241814192](https://doi.org/10.3390/ijms241814192)
142. Brahmer A, Geiss C, Lygeraki A, et al. Assessment of technical and clinical utility of a bead-based flow cytometry platform for multiparametric phenotyping of CNS-derived extracellular vesicles. *Cell Commun Signal*. 2023;21(1):276. doi: [10.1186/s12964-023-01308-9](https://doi.org/10.1186/s12964-023-01308-9)