Clinical Biochemistry 140 (2025) 111015

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Corrigendum

The biological diagnosis of Alzheimer's disease using blood-based biomarkers: a Canadian prospective

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A B S T R A C T

Dementia is the most common type of neurodegenerative disease, with Alzheimer's Disease (AD) constituting about two-thirds of these cases. In Canada, an estimated 674,000 individuals may have AD by 2031, nearly doubling from 2011. The total annual economic burden of dementia in Canada was about $40 billion in 2020, with an approximate average of $67,200 per person with dementia and if current trends continue, its annual burden could grow by 275 % over 30 years. AD is a double proteinopathy with its fundamental neuropathologic features defined by amyloid-beta (Aβ) plaques and neurofibrillary tangles with aggregated tau proteins. This supports the potential for mechanism-based proteomic biomarkers to be detected in biofluids. Pathophysiologic and topographical biomarkers have significantly improved the diagnosis of typical and atypical phenotypes of AD, helping clinicians recognize and differentiate AD phenotypes from other types of dementia and neurodegenerative diseases. The cerebrospinal fluid Aβ42/Aβ40 ratio measurement is a robust biomarker in detecting cerebral Aβ pathology and AD diagnosis. A number of very sensitive assays for measuring AD blood biomarkers including p-tau217, front-runner candidate for AD diagnosis, have been developed during last years. In this review we discuss the biological configuration and normal function of involved proteomics in AD including Aβ and tau protein, particularly tau phosphorylation and biochemistry of tau isoforms and their detection feasibility in plasma using novel technologies. Then, we critically review blood-based biomarkers' analytical and clinical validations, focusing more on plasma p-tau217 and their availability and prospects in Canada.

1. Introduction

Dementia is the most common clinical syndrome caused by neurodegenerative diseases (NDDs), with Alzheimer's disease (AD) constituting about two-thirds of these cases. The prevalence of AD has risen with increasing of life expectancy; currently more than 55 million people worldwide live with dementia and AD may contribute to 60–70 % of them [1–3]. It is estimated that AD prevalence will be approximately tripled by 2050 and 43 % of these patients will need high levels of care [4]. In Canada, an estimated 674,000 individuals may have AD by 2031, nearly doubling from 2011 [5]. The total annual economic burden of dementia in Canada was about $40 billion in 2020, with an approximate average of $67,200 per person with dementia. If current trends continue, the annual burden could grow by 275 % over 30 years [6]. The foremost NDDs are defined by misfolding the normal proteins (proteinopathy) and accumulating them in the central nervous system. This supports the potential for mechanism-based proteomic biomarkers to be detected in biofluids [7,8]. AD is a double proteinopathy with its fundamental neuropathologic features defined by amyloid-beta (Aβ) plaques and neurofibrillary tangles with aggregated tau proteins [9,10]. Aβ is a small peptide derived by secretase cleavage of the amyloid precursor protein (APP) [9,10]. The deposition of Aβ in the brain as extracellular neuritic ß-amyloid plaques and in the cerebral vasculature leads to neurotoxicity, dementia, and cerebral amyloid angiopathy. Tau is a phosphoprotein and a microtubule-associated protein (MAP); its significant function is the stabilization of axonal microtubules [9,10]. Abnormal hyperphosphorylation of tau (ptau) and its accumulation as intracytoplasmic aggregates occur in AD and other tauopathies such as progressive supranuclear palsy, chronic traumatic encephalopathy, corticobasal degeneration, and frontotemporal lobar degeneration [11]. A common neuropathological feature of these diseases is the presence of deposits of ptau in various morphologies, many years before the onset of clinical findings [12,13]. Therefore, understanding the role of biomarkers in the early diagnosis of AD appears imperative. Pathophysiologic and topographical biomarkers have significantly improved the diagnosis of typical and atypical phenotypes of AD, helping clinicians recognize and differentiate AD phenotypes from other types of dementia and NDDs. Pathophysiologic biomarkers, including amyloid positron emission tomography (PET), and fluid biomarkers such as cerebrospinal fluid (CSF) concentrations of Aβ, tau proteins, and neurofilament light

DOI of original article: https://doi.org/10.1016/j.clinbiochem.2025.110980.
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https://doi.org/10.1016/j.clinbiochem.2025.111015

Available online 29 September 2025

chain (NfL), along with plasma concentrations of Aβ, tau, and NfL, play a crucial role in this diagnostic enhancement. Topographic biomarkers, such as fluorodeoxyglucose (FDG)-PET, tau PET, and magnetic resonance imaging (MRI), assess the regional involvement of AD pathology [14]. The CSF Aβ42/Aβ40 ratio measurement is a robust biomarker in detecting cerebral Aβ pathology and AD diagnosis [15]. A noticeable feature of Aβ42 and Aβ40 is that their levels are altered in the preclinical stages of AD [15–17]. They can identify Aβ pathology with high accuracy in both individuals with unimpaired cognition and patients with mild cognitive impairment (MCI) [15]. A number of very sensitive assays for measuring AD blood biomarkers including p-tau217 have been developed during last years. All available assays measure phosphorylated forms of tau using specific antibodies that target the N-terminus or mid-domain of the protein with higher concentrations than the full-length or C-terminal forms of tau [18–20]. In this review we discuss the biological configuration and normal function of involved proteomics in AD including Aβ and tau protein, particularly tau phosphorylation and biochemistry of tau isoforms and their detection feasibility in plasma using novel technologies. Then, we critically review blood-based biomarkers' analytical and clinical validations, focusing more on plasma p-tau217 than on p-tau181, p-tau231, and other established biomarkers.

2. Proteinopathies in AD

The core pathologies in development and progression of AD are accumulation of Aβ as extracellular amyloid plaques and tau toxicity which presents as an intracellular neurofibrillary tangle.

2.1. Biology and structure of amyloid beta

The Aβ peptides are cleaved from a larger protein, APP, which is a fundamental membrane protein in many tissues, particularly synapses of neurons [21]. The normal structures of APP isoforms range in size from 695 to 770 amino acids including a large extracellular glycosylated N-terminus, a single membrane-helix region and a shorter cytoplasmic C-terminus domain. (Fig. 1) [21,22]. The 695 aa APP isoform is mainly expressed in the brain, while the APP751 and APP770 isoforms with a Kunitz-type serine protease inhibitory domain (KPI), are mainly expressed on platelets and peripheral cells [21,22]. Human APP is processed through two pathways, amyloidogenic and non-amyloidogenic, by three main proteases: α-, β- and γ-secretases. In the amyloidogenic pathway, Aβ is produced through sequential cleavage of APP by β- and γ- secretases, and in the non-amyloidogenic pathway, APP is cleaved by α-secretase or by β-secretase and α-secretase. The two pathways lead to different by-products with different functional characteristics and pathophysiological implications. (Fig. 1) [23–25]. The biological function of APP is not completely clear; however, experimental studies have demonstrated its role in dendritic spine remodeling, molecular pathways of neurotransmission, and synaptic homeostasis [25,26]. In addition, soluble sAPPα and sAPPβ regulate the basal synaptic transmission and Aβ monomers and can trigger or sustain essential intracellular signaling for neurotransmission [26]. The products of the proteolytic processing of APP, especially Aβ, are removed from the brain via CSF and the glia-lymphatic (glymphatic) system. In the Aβ clearance process multiple molecular pathways are involved but the blood–brain barrier (BBB) has an important role in its homeostasis [27]. Even a slight reduction in its clearance can cause its toxic accumulation in misfolded forms. Therefore, the efficient clearance of Aβ is crucial for preventing amyloidopathy [28].

2.2. The biological characteristics of tau protein

Tau is a multifaceted MAP predominantly expressed in neurons, which plays an important role in stabilizing microtubules, promoting neuronal function and support neuronal plasticity [29,30]. It has six isoforms ranging from 352 to 441 amino acids in length in the adult

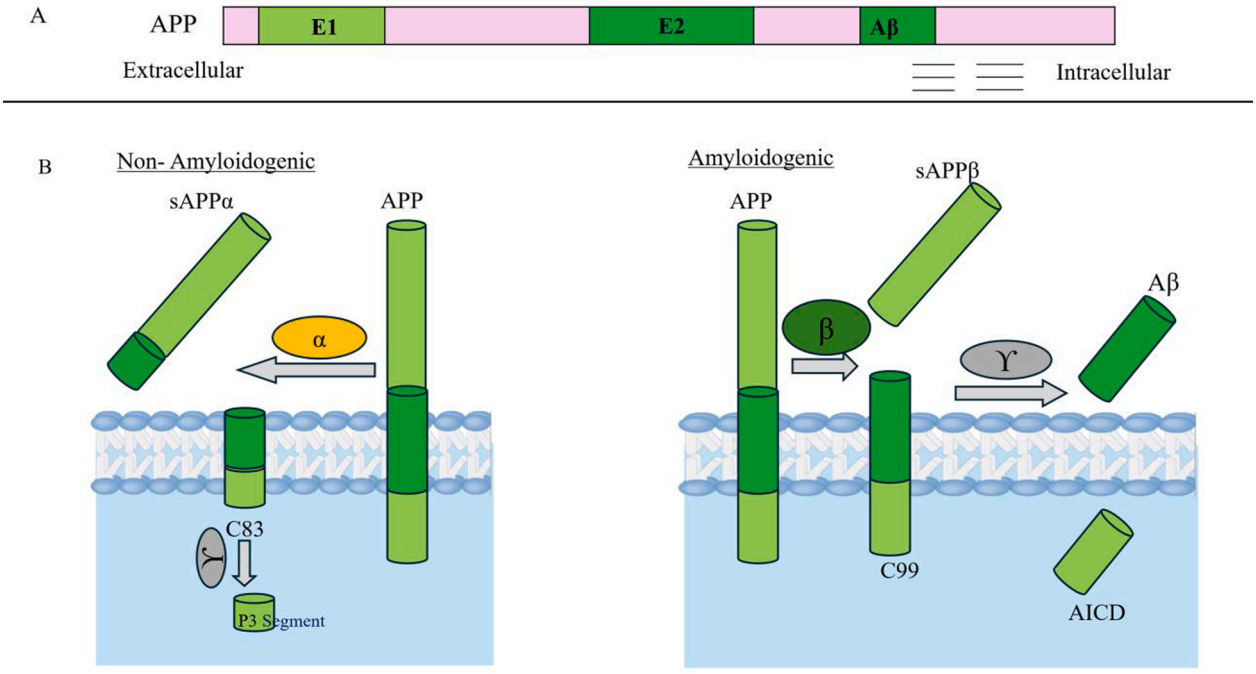

Fig. 1. APP proteolysis. A. APP normal structure: It is a type 1 transmembrane protein consisting of 695–770 amino acids. APP proteolytic pathways are amyloidogenic or non-amyloidogenic. Most APP is processed through the amyloidogenic pathway, which produce Aβ. In this pathway, APP molecules cleaved by β-secretase, releasing an ectodomain (sAPPβ), and C99 within the membrane. The first amino acid of C99 is the first amino acid of Aβ. C99 is subsequently cleaved by the γ-secretase complex to release Aβ. This cleavage predominantly produces Aβ1–40, and the more amyloidogenic Aβ1–42 at a ratio of 10:1 and APP intracellular domain (AICD). In non-amyloidogenic pathway, the first enzymatic cleavage of APP is mediated by α-secretase which occurs within the Aβ domain, thereby preventing the generation and release of the Aβ peptide. In this pathway first two fragments are released, the larger ectodomain (sAPPα) and the smaller carboxy-terminal fragment (C83). Furthermore, C83 can also undergo an additional cleavage mediated by γ-secretase to generate P3.

human brain, encoded by the microtubule-associated protein tau (*MAPT*) gene on chromosome 17q21 [31–33]. The tau protein sequence is divided into an amino-terminal region, a mid-region, a microtubule-binding region, and a carboxy terminus (Fig. 2). The six tau isoforms differ in containing three (3R) or four (4R) microtubule-binding repeats of 31–32 amino acids and one (1N), two (2N), or zero (0N) amino-terminal insertions of 29 amino acids each. Consequently, different splicing of tau pre-mRNA results in the expression of three 3R tau isoforms (0N3R, 1N3R, 2N3R) and three 4R tau isoforms (0N4R, 1N4R, 2N4R). The 2N4R isoform is the largest, with a total length of 441 amino acids, while the 0N3R isoform is the smallest, with a length of 352 amino acids [31,33–35]. These normal taus are natively unfolded and express a very low tendency towards misfolding, aggregation, and accumulation in intracellular and extracellular situations. Despite the essential role of phosphorylation in modifying tau under normal conditions, excessive phosphorylation can lead to the formation of self-aggregated and hyperphosphorylated tau (p-tau), which serves as a hallmark of several tauopathies [36–40]. In addition, several posttranslational modifications, including truncation, acetylation, ubiquitination, and sumoylation, can lead to tau aggregation. However, the most extensively studied posttranslational modification in AD is the abnormal phosphorylation of tau. There are approximately 85 potential phosphorylation sites in the longest isoform of tau (2N4R) and about 45 of these phosphorylation sites have been observed experimentally [41–43].

2.3. The normal neurofilament light chain (NfL)

NfL is a subunit of neurofilaments, which are cylindrical proteins and predominantly exist in large-caliber myelinated axons. They support axonal stability and enable radial axonal growth. (Fig. 3) [44,45]. Physiologically, low concentrations of NfL are released from axons into the CSF and consequently appear at lower levels in the blood. Aging, axonal damage, and neurodegeneration accelerate the release of NfL. Therefore, its levels increase in CSF and blood to the degree of axonal damage in inflammatory and degenerative neurological disorders, traumatic injuries, and cerebrovascular diseases.

2.4. Glial fibrillary acidic protein (GFAP)

GFAP is a type III intermediate filament protein with 432 amino acids encoded by a gene on chromosome 17q21.1-q25. Although GFAP is also expressed in the Schwann cells, mature glial cells in the gut, hepatic stellate cells, and other non-neural cells, it is specifically present in mature astrocytes in the central nervous system (CNS), and ten specific isoforms of it have been identified in the CNS. (Fig. 4) Astrocytes are an integral part of the BBB, at the center of normal synaptic function and axonal metabolic maintenance. GFAP is a general marker for reactive astrogliosis, and it is an FDA-approved blood biomarker for assessing neurodegeneration and gliosis in routine clinical practice [46–48].

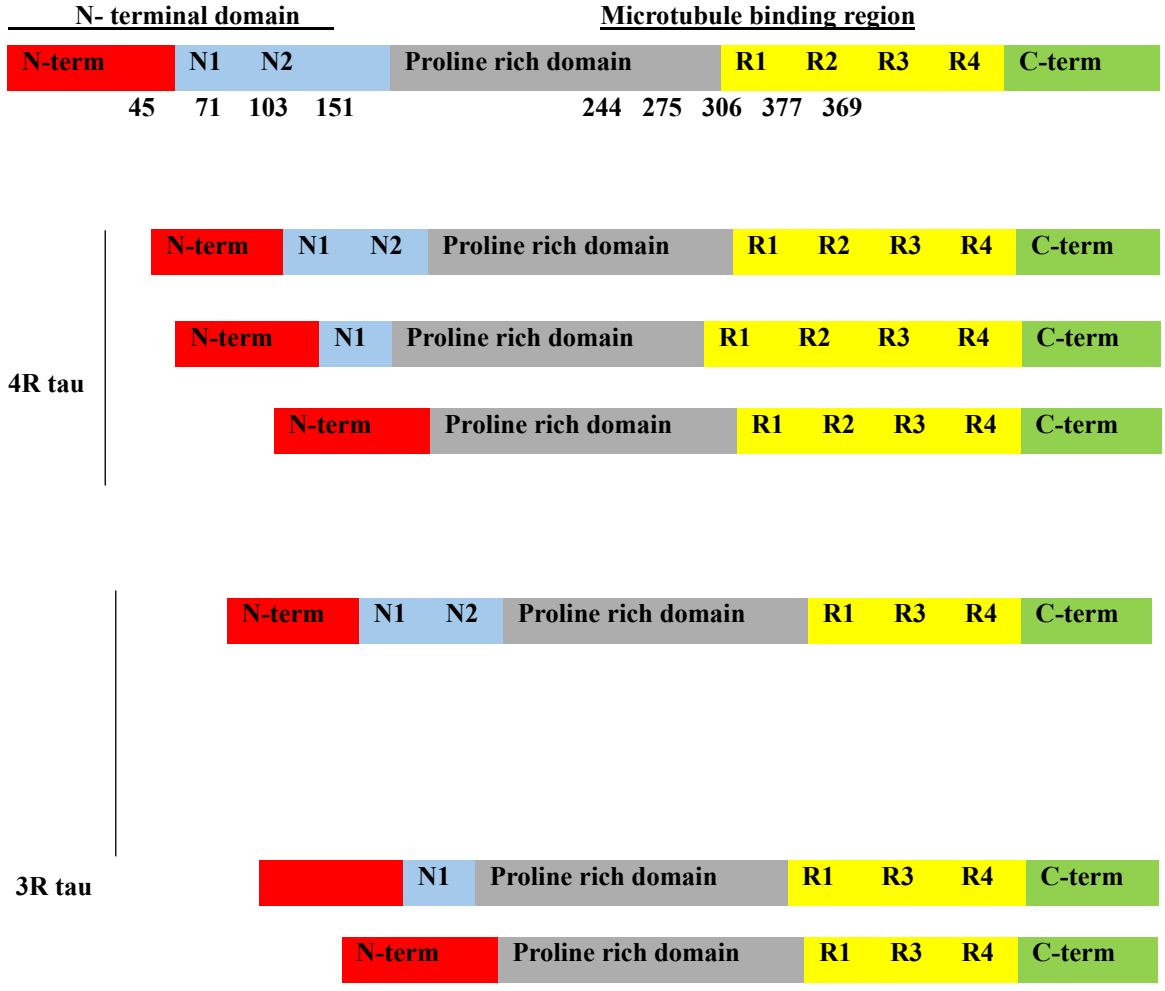

Fig. 2. Tau protein structure that includes a predominantly acidic N-terminal region, a proline-rich central region, and a relatively neutral C-terminal region. The tau isoforms vary in containing either three (3R) or four (4R) microtubule-binding repeats (R) as well as one (1N), two (2N), or zero (0N) amino-terminal insertions.

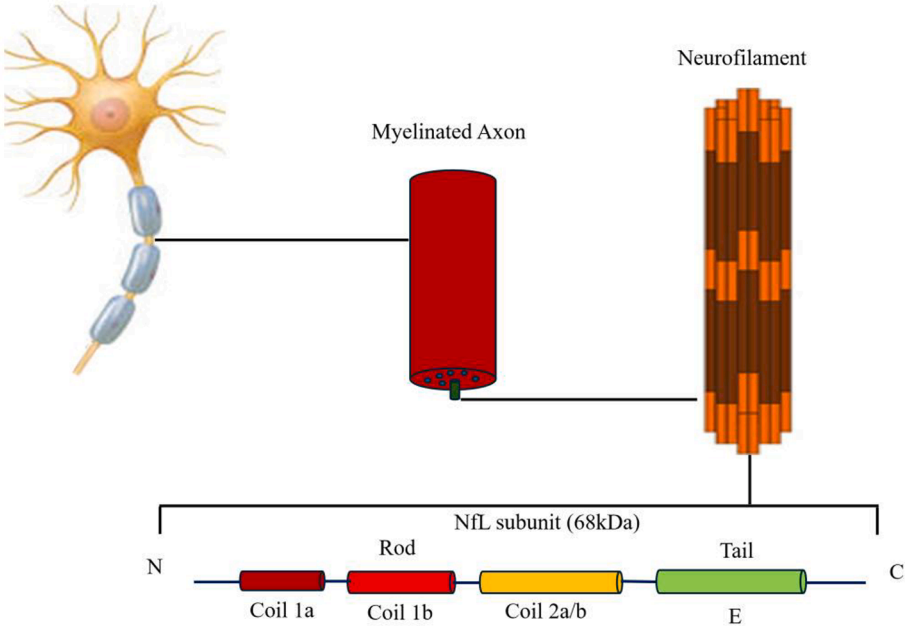

Fig. 3. Nfl is a cylindrical protein and a subunit of Nfs that exist in large-caliber myelinated axons.

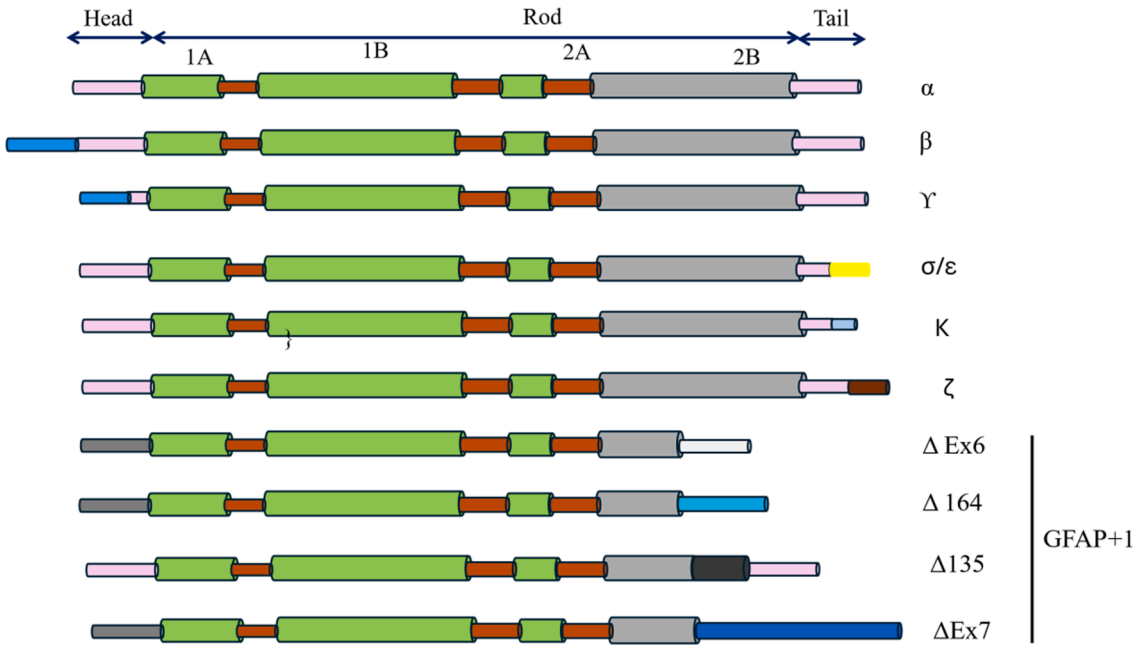

Fig. 4. GFAP is a type III intermediate filament protein with 432 amino acid and ten specific isoforms of it have been identified in the CNS.

3. AD diagnosis

3.1. Clinical diagnosis of AD

The clinical features of AD are related to its pathological processes. AD neuropathological cascades start years before the apparent beginning of the clinical symptoms of the disease and spread in a consistent pattern with subsequent neuroanatomical and neurophysiological changes. Therefore, clinical findings of AD are heterogenous but exist in a continuum from cognitively unimpaired through MCI to severe dementia [49–51]. The picture is even rendered more heterogeneous with the inclusion of atypical AD. Although an amnestic syndrome with insidious onset and gradual progression is the typical clinical phenotype of AD (about 85 %), an important proportion of cases with AD pathology present with non-amnestic cognitive disorders. Logopenic variant of primary progressive aphasia (lvPPA), posterior cortical atrophy (PCA), corticobasal syndrome (CBS), behavioral or dysexecutive variants of frontal AD, non-fluent primary progressive aphasia (nfPPA), and semantic variant primary progressive aphasia (svPPA) are atypical clinical presentations of AD pathology (Fig. 5). MCI is a prodromal stage of AD and patients with amnestic MCI progress to probable AD at a rate of ~10–15 % per year whilst 11–33 % of them develop it within the first 2 years. However, 50 % of patients with MCI will return to normal in the follow up evaluations. Thus, the accurate diagnosis and the prediction of developing of AD in its prodromal phase particularly within 1 to 2 years of MCI onset, can help treat early and could minimize progression of the

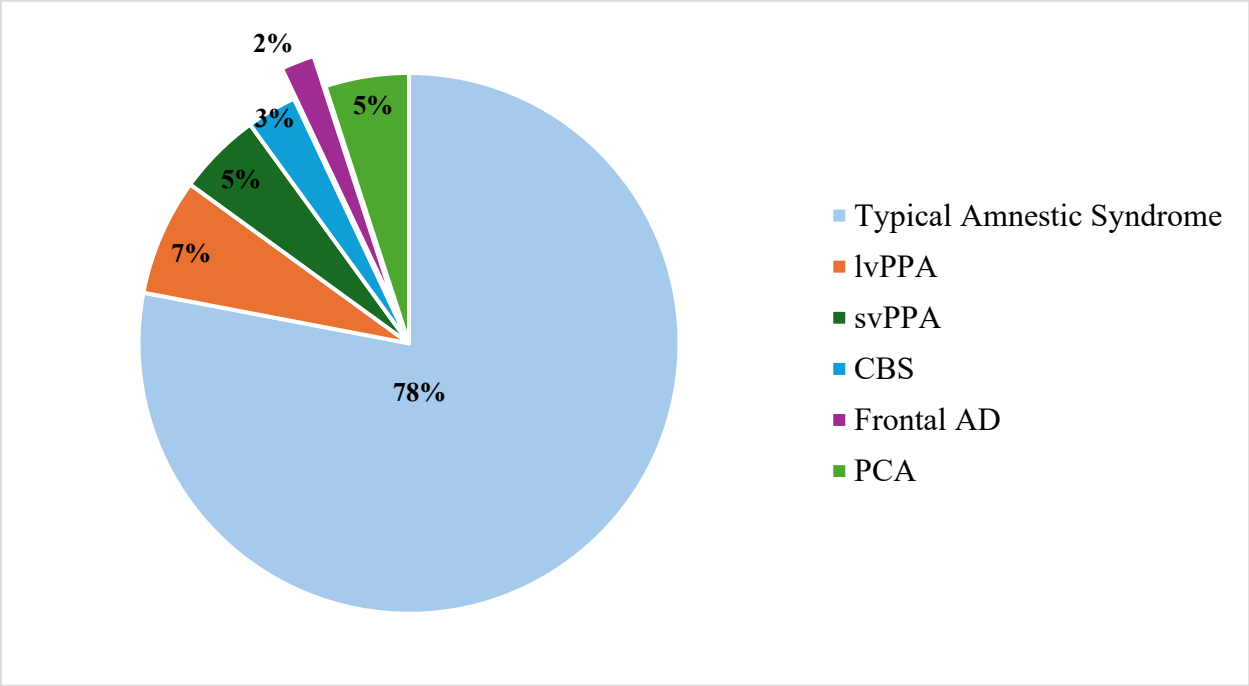

Fig. 5. Prevalence of typical and atypical clinical phenotypes of AD.

disease [51,52]. This is especially true in atypical AD characterized by presentations of non-amnestic features of AD including predominant impairment in visual, language, executive, behavioral, and motor functions. These syndromes present as a young onset dementia, before age 65, and over half of these patients are misdiagnosed. It is probable that the early precise diagnosis of non-amnestic AD can reduce disease progression and morbidity and improve quality of life, thus understanding the role of biomarkers in the early diagnosis of AD appears imperative [53]. Indeed, this clinical complexity causes approximately 25–30 % misdiagnosis of patients with clinical symptoms of AD when assessed at specialized dementia clinics. This number increases to 50–70 % when symptomatic AD is evaluated in primary care when a screening cognitive test is usually not performed. This high rate of misdiagnosis is due to a lack of accurate, easily accessible, and cost-effective diagnostic tools [54–56]. Hence, the clinical diagnosis of AD requires a comprehensive assessment, including a detailed clinical and neurological evaluation, cognitive testing, laboratory investigations, and neuroimaging to rule out other causes and to determine the onset, progression, and clinical phenotype of the disease. Standardized cognitive tests such as the Mini-Mental State Examination (MMSE) [57], Montreal Cognitive Assessment (MoCA) [58], or Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) [59] are used to evaluate impairments in memory, language, executive function, visuospatial skills, and attention in various clinical stages of AD. Neuroimaging techniques, including structural MRI and computed tomography (CT) scans, are utilized to detect brain atrophy, cerebrovascular disease, brain tumor and other potential reversible causes of dementia such as normal pressure hydrocephalus [60–62]. In addition, functional imaging with FDG-PET may reveal hypometabolism in temporoparietal regions, and amyloid and tau PET imaging show evidence for core AD pathology. Furthermore, the AD clinical diagnosis is guided by established criteria such as the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision *(*DSM-5-TR), the National Institute on Aging–Alzheimer's Association (NIA-AA), and the International Working Group (IWG) for AD. In DSM-5-TR AD is classified under the mild and major neurocognitive disorders. The essential criterion for this classification includes a decline in memory and learning as well as at least one other cognitive domain such as complex attention, executive function, language, perceptual-motor, and social cognition. Additionally, the cognitive function impairment must not be explained by other medical or psychiatric conditions and must interfere with the basic or instrumental activities of daily living. The clinical diagnosis AD has evolved significantly over the past four decades, shifting from a symptom-based approach to a biomarker-driven framework. In 1984, the McKhann criteria for clinical diagnosis of AD emphasized insidious onset of memory decline accompanied by impairment of another cognitive function domain, and exclusion of other medical or psychiatric causes [63] and the condition cannot be diagnosed by laboratory tests. The criteria stressed that laboratory tests are important to exclude other possible causes of dementia, however neuropsychological tests can provide more diagnostic confirmatory evidence and help to monitor the disease progression and therapeutic response [63]. Accordingly these criteria proposed the probable, possible, and definite clinical AD (Table 1) [63]. By 2011, the Alzheimer's Association (NIA-AA) updated the McKhann criteria with incorporating multiple cognitive domains such as memory, language, visuospatial skills, and praxis and added genetic factors and supportive biomarkers, including MRI and PET imaging, to detect brain atrophy and amyloid deposition [64]. The clinical, neuropsychiatric, and biomarker assessments can improve the clinical diagnosis of AD by categorizing it as possible, probable, or probable/possible [64]. The 2018 NIA–AA revision introduced a conceptual shift and research framework defining AD biologically by amyloid and tau pathology [64,65]. They changed their 2011 framework of AD (possible, probable probable/possible), to a new framework including a research platform with β amyloid deposition, pathologic tau, and neurodegeneration (termed ATN). This reform provides opportunities for moving from clinical pathological diagnosis of AD to clinical/biological and biological definition of AD [65]. Most recently, the 2024 update by Jack et al. continues this trend by identifying AD as a biological process that emphasizes core biomarkers of amyloid and tau pathology, as well as neurodegeneration and inflammation markers like GFAP as well as MRI and α-synuclein for non-AD dementias such as vascular dementia, Lewy body dementia and Parkinson's with dementia (Table 1). This progressive evolution highlights a paradigm shift from clinical observation toward biological definitions of AD, with growing emphasis on early and even preclinical detection of AD [66]. This revised guideline

Table 1
Evolution of the Diagnostic Criteria for Alzheimer Disease.

1984 (McKhann et al., Neurology)	2011 (McKhann et al., Alz & Dem, NIA-AA)	2018 (Jack et al., Alz & Dem, NIA-AA)	2024 (Jack et al., Alz & Dem)
Insidious onset memory decline plus another cognitive domain	2+ cognitive domains (memory, language, behaviour, visuospatial, praxis) & functional decline	Alzheimer Disease refers to the pathology (amyloid & tau), not defined by symptoms	Biological process of AD pathology (symptoms not necessary?! Very controversial!)
Functional impairment from usual activities	Genetics (causative mutations)		
No other medical or psychiatric Disorder	CSF biomarkers supportive	A (amyloid) – low CSF Ab42 or positive amyloid PET	Core 1 biomarkers – amyloid pathology (CSF Ab42, Ab to tau ratio, plasma p-tau-217, amyloid PET)
Imaging to exclude structural lesions	MRI atrophy or abnormal FDG-PET (evidence of degeneration)	T (tau) – CSF p-tau, or positive tau PET	Core 2 biomarkers – tau pathology (CSF p-tau, tau PET)
	PET biomarkers (amyloid, tau)	N (neuronal injury) – MRI (atrophy), FDG-PET, CSF t-tau, NfL	Non-specific biomarkers – neurodegeneration/ inflammation (NfL, GFAP)
	Possible mixed pathologies, also added preclinical & MCI stages	Biomarker Staging of AD based on biomarkers based on ATN vs. cognitive staging (CDR)	Non-AD dementia (vascular – MRI, a-synuclein)

provide a framework for diagnosing AD across clinical stages: preclinical AD, MCI due to AD, and AD dementia. This model incorporates clinical assessment and biomarker evidence such as CSF Aβ and tau levels or amyloid/tau PET imaging emphasizing a biologically defined diagnosis, requiring both clinical symptoms and biomarker evidence of AD pathology [66].

3.2. Biological diagnosis of AD

Given that numerous new therapeutic agents for AD, thought to be most effective in the early stages of the disease (MCI and mild AD), are undergoing clinical trials, and that two of them, donanemab and lecanemab, have been approved by the U.S. FDA and are very likely to receive approval from Health Canada in the coming months, the biological diagnosis of AD has become imperative [67,68]. Biological determination of disease has long been standard in medical areas such as oncology. It is also recognized as a common framework for understanding the pathology and clinical diagnosis of all NDDs, particularly AD during the last two decades [69].

The discovery of biofluid biomarkers over the last decade has significantly improved our knowledge of the dynamic pathological changes underlying AD. It has allowed in vivo detection of AD pathologies and improved the diagnosis of typical and atypical phenotypes of AD, helping clinicians recognize and differentiate AD phenotypes from other types of dementia and NDDs [70]. In addition, development of biofluid biomarkers has introduced the potential for diagnosis of presymptomatic and early stages of AD [71]. The diagnostic landscape of AD has undergone a significant transformation toward a biomarker-based approach, as outlined in the Revised Criteria for Diagnosis and Staging of AD by the Alzheimer's Association in 2024 [66]. These criteria support diagnosing AD based solely on biomarkers, without the need for clinical symptoms (Table 1). In this regard, the AD biomarkers were classified into three major categories: core biomarkers of AD neuropathological features [72], non-specific biomarkers that are involved in pathogenesis of both AD and other NDDs, and biomarkers of non-AD co-pathologies [66]. Core AD biomarkers include core 1 (Aβ 42, p-tau217, p-tau181, p-tau231 and amyloid PET) and core 2 [microtubule-binding region (MTBR-tau243), other phosphorylated tau forms (e.g., p-tau205), non-phosphorylated mid-region tau fragments biomarkers and tau PET]. Core 1 biomarkers can identify early stage and asymptomatic AD, but core 2 biomarkers cannot individually be used as AD diagnostic tests. In other words, an abnormal Aβ 42 or p-tau217 or amyloid PET is nearly always necessary for neocortical AD tauopathy and an A–T2+biomarker profile is not compatible with AD diagnosis [73–75]. Moreover, according to the 2024 US FDA Level 1 guidance, only alterations in surrogate biomarker levels can be accepted as efficacy evidence for the licensing of treatments for asymptomatic preclinical AD individuals. Therefore, biomarker measurements can be used both to diagnose (Revised Alzheimer's Association criteria) and as evidence of treatment response (FDA guideline) in asymptomatic individuals [69].

4. Biomarkers in AD

4.1. Positron emission tomography (PET)

For showing the pattern and density of AD neuropathology in the brain, PET, a molecular imaging technique with different radiotracers has been utilized (amyloid and tau PET) [76,77]. Amyloid PET exhibits remarkable sensitivity in detecting AD [78–80] and can prognosticate future cognitive impairment over long follow-up periods [17,81] as a result of the significant delay between amyloid deposition and clinical findings [82]. Tau PET is the second neuropathological biomarker, though its clinical implementation is still limited. In research settings, it can accurately differentiate AD cases from controls and illustrate the spatial and temporal correlation with clinical presentations of AD [83–86]. Moreover, tau PET has been shown to be positive in 3R and 4R tauopathies, although its ability to differentiate from control individuals is less clear compared to AD [87,88]. Multiple studies have demonstrated that tau PET signals in the neocortex are highly prognostic for clinical progression in cognitively unimpaired individuals [89]. While PET has been used as a reference diagnostic tool for both amyloid and tau pathologies in research studies, recent evidence suggests that tau PET can verify both amyloid and tau pathologies as a single diagnostic test [90].

4.2. CSF biomarkers

Accumulation of Aβ in growing neuritic plaques in AD causes the selective reduction of Aβ42 from the CSF, while the Aβ40 is nearly unchanged. Therefore, the CSF Aβ42/Aβ40 ratio has stronger diagnostic accuracy for AD compared to CSF Aβ42 alone [91–93] and its measurement is a robust biomarker in detecting cerebral Aβ pathology. In addition, CSF p-tau, particularly p-tau181, increases alongside the reduction of Aβ [94,95]. The ratios between T-tau/Aβ42 and p-tau181/Aβ42 have also been evaluated in some studies as an alternative to the Aβ42/Aβ40 ratio and these ratios show high concordance with amyloid PET [96,97], and Aβ42/Aβ40 ratio [98]. While the accuracy of clinical criteria for AD diagnosis compared to neuropathological findings is not optimal [55,99] CSF biomarkers are used increasingly in the clinical practice to rule out AD as an underlying cause of cognitive impairment [65,100]. These CSF biomarkers also have been incorporated in research studies and clinical trials as an AD diagnostic tool for improvement of enrollment criteria [101]. Despite the widespread use of CSF Aβ and p-tau181 in clinical practice, remarkable variability in measured concentrations was reported between laboratories and across sample batches [102,103], hindering the establishment of uniform cutoff values

to utilize globally for defining abnormality. Indeed, the most commonly used technique to measure CSF AD biomarkers, the enzyme-linked immunosorbent assay (ELISA), has been demonstrated to cause inter-laboratory variation up to 15–25 % [104]. Three possible sources of variability are: pre-analytical (differences in the collection, handling, and storage of CSF) [105,106], analytical (variations in technician skill, and lot-to-lot variability of reference materials, kits and their components) [103,107] and biological/patient-related (confounding factors such as age) [103,106]. This variability has been addressed using standard protocols for CSF collection and storage [106] and fully automated platforms [108]. To minimize the variability in measurement and provide a standard protocol for CSF biomarker analysis, a workgroup led by the Alzheimer's Association developed a standardized pre-analytical protocol for CSF collection, handling, and analysis for routine clinical use (Fig. 6) [106]. These protocols also can facilitate implementing unified cut-point levels among different laboratories which use the same platform and advance the use of CSF biomarkers in AD diagnosis [106]. In the first publication Bittner and colleagues reported a full validation and analytical performance of a fully automated electrochemiluminescence immunoassay (Cobas Elecsys®) for CSF Aβ42, with a repeatability coefficient of variation (CV) 1.0–1.6 % and intermediate CVs of 1.9–4.0 % (compared to ~15 % for ELISA methods) [109–111]. Novel assays on the Elecys instrument for CSF P-tau and T-tau with good performance in the Alzheimer's Association quality control (QC) program have also been launched [112]. Similar automated platforms for AD biomarkers have since been launched, including those from Euroimmun [113], and Fujirebio (LUMIPULSE®) [114,115], and have shown superior performance in the QC program [104]. The certified reference materials for Aβ42 have now been fully implemented and will allow for full standardization of all commercially available CSF Aβ42 methods [116]. Health Canada has approved the use of CSF biomarkers for the diagnosis of AD, focusing on measuring Aβ and tau proteins since April 14th, 2023. The Elecsys and Fujirebio (LUMIPULSE) are available in a few labs over the country.

4.3. Plasma biomarkers

4.3.1. The importance of blood-based biomarkers in diagnosis of AD

Although PET and CSF biomarkers for diagnosis of AD have already been approved by the FDA, invasiveness, accessibility, cost, and availability limit the use of CSF and PET biomarkers in clinical practices. The development of ultrasensitive technologies such as the Single-molecule Array (Simoa) facilitated the early detection of biomarkers in the plasma with high sensitivity. These emerging technologies and novel neurodegenerative biomarkers in the simple blood-based test offer a unique advantage to use them in clinical testing and drug development trials for the diagnosis and management of AD and other NDDs [117].

5. The role of medical laboratory diagnostics in biological diagnosis of AD

Medical laboratory diagnostics play a key role in the development and implementation of new laboratory tests for the biological diagnosis of AD in clinical practice settings [118]. These laboratories are responsible for the analytical validation, including defining limit of blank (LoB), limit of detection (LoD), limit of quantification (LoQ), clinical reportable range, intra-laboratory precision, sample stability and interference, verifying precision, reproducibility, accuracy, sensitivity, specificity, standardized measurement, quality control, and interpretation of the measurement results [119]. They also contribute in clinical validation to demonstrate how accurately these biomarkers correlate with established and gold diagnostic standards such amyloid and tau PET imaging and neuropathology confirmation. Clinical validation aims to demonstrate an association between the biomarker and the endpoint of interest and to show the usefulness of the biomarker [119]. It relies on external validation and by using retrospective clinical trial data or prospective clinical trials [120]. Additionally, medical laboratories help establish analytical performance specifications by applying principles such as biological variation to define acceptable levels of assay imprecision and variability [121,122]. With the emergence of AD biomarkers, laboratories are helpful in early diagnosis and disease staging in clinical settings. In addition, by quality control studies, and adherence to different regulatory guidelines, medical laboratory diagnostics confirm the reliability of biomarker assays to improve diagnosis, and guiding treatment decisions.

6. Biological variation of plasma biomarkers

Biological variation (BV) is a fundamental principle in clinical chemistry, and understanding the BV of plasma biomarkers is crucial for their safe implementation in diagnostic laboratory medicine and for minimizing the risk of miscalculation [123]. As BV refers to the variability detected in clinical laboratory measurements generated by individual physiology, BV studies must follow strict guideline-defined

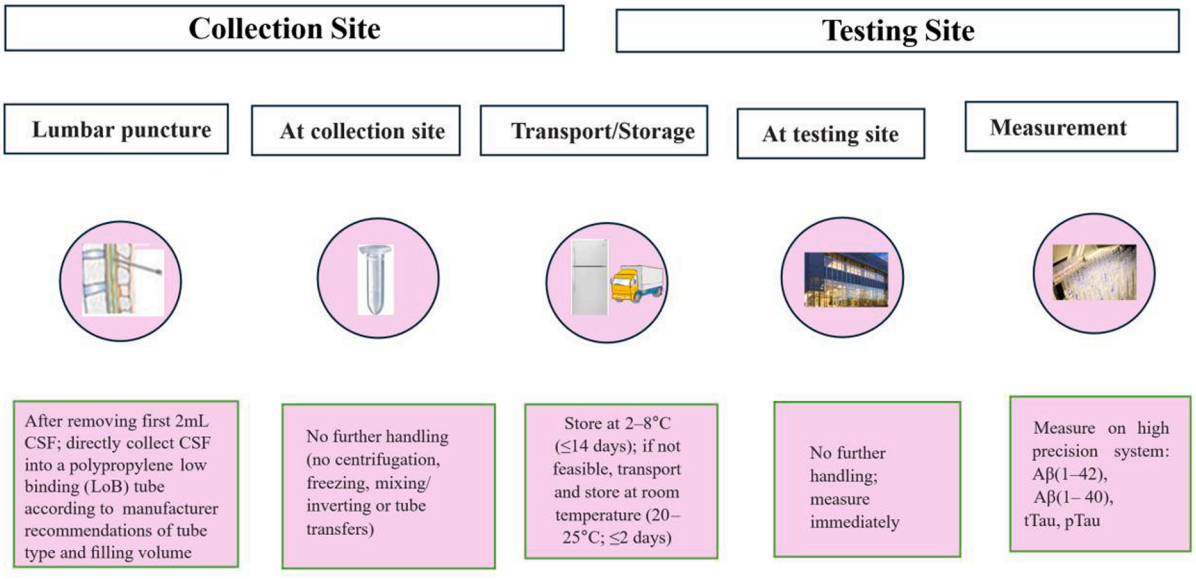

Fig. 6. Recommended pre-analytical protocol for collection, storage, sample handling, and measurement of fresh CSF.

protocols including serial and tightly controlled sampling from healthy individuals at a regular interval, and duplicate analyte quantifications [121,124]. Key BV components include within-subject biological variation (CVI), and between-subject variation (CVG) that along with known assay-dependent analytical variation (CVA) including the reference change value (RCV), analytical performance specifications (APS), and the index of individuality (II) can provide useful information for clinical implementation of plasma biomarkers [125,126]. In addition, validated BV data are important for setting appropriate APS for plasma biomarkers, particularly by ensuring that CVA is equal or less than half of CVI. In a recent study conducted by Brum and Blennow et al. to determine BV, associated APS and RCVs for plasma Aβ42, Aβ40, Aβ42/Aβ40, p-tau181, p-tau217, p-tau231, GFAP, and NfL in 20 healthy adults between 40 and 60 years from the European Biological Variation Study (EuBIVAS) [127,128], they estimated CVI, CVG, CVA, and RCV for these plasma biomarkers. While Aβ42/Aβ40 had the lowest CVI (≈3%) and p-tau181 the highest (≈16 %), other biomarkers had a range from 6 % to 10 %. They also demonstrated that CVAs of plasma biomarkers were slightly higher than the targets for most biomarkers, however the plasma Aβ42 and Aβ40 (not Aβ42/Aβ40) and p-tau181 were within the acceptable range, and plasma p-tau217 was very close to the desirable range (observed CVA = 5.7 % and desired CVA ≤ 5.2 %). In addition, they suggested that CVI and CVG can differ significantly for the measured AD plasma biomarkers, which may influence their utility depending on the implementation setting. However, BV estimates are not primary criteria for determining APS for assays but rather are a complementary tool to improve analytical goals based on the clinical context of each analyte [122,129]. Additional prospective studies are required to evaluate the BV of plasma biomarkers, especially p-tau217, in AD diagnosis.

7. Performance of plasma p-tau217 assays

7.1. Plasma p-tau217 as a front runner AD biomarker

p-tau217 is the leading candidate for AD diagnosis due to its strong correlation with core AD pathology determined by both CSF biomarkers and PET or post-mortem examination. In addition, plasma p-tau217 is a highly efficacious biomarker in the early detection of Aβ pathology, making it a feasible test for AD screening in clinical practice. In several studies, p-tau217 demonstrates high performance in discriminating AD from non-AD neurodegenerative disorders and detecting AD pathology in MCI patients [130,131]. In a head-to-head comparison study of different plasma and CSF tau immunoassays, Ashton et al. found that p-tau217 shows the strongest association with both cross-sectional and longitudinal changes in Aβ pathology, likely due to a more pronounced continuing increase in symptomatic cases [132]. Additionally, p-tau217 indicates the direction of the disease course, with its increasing levels aligned with declining cognitive function and worsening brain atrophy related to elevated Aβ pathology [133].

7.2. Validation of plasma p-tau

Despite the promising potential of plasma p-tau217 as a diagnostic biomarker for AD, its widespread evaluation has been hindered by the limited availability of commercial assays. A few studies analytically and clinically have validated these immunoassays as laboratory diagnostic tests for AD diagnosis and differentiating from non-AD NDDs in clinical practice (Table 3) [119]. However, plasma p-tau assays such as the Lilly immunoassay using the MSD ECL platform and the Janssen SIMOA immunoassay using the Quanterix HD-X platform have been developed for measuring plasma p-tau217 (Table 2). Although these are 'home-brewed' using proprietary antibodies and have not been made widely available for clinical testing since their initial development for research, Groot et al., in a study consisting of two cohorts, compared these two immunoassays head-to-head [134]. The performance of two assays was evaluated by comparing receiver operating characteristic (ROC) analyses using a cohort of 27 controls and 25 individuals with MCI, as well as a cohort of 147 individuals with MCI who were followed for an average of 4.92 years. Both assays showed similar performance in detecting amyloid-β status in CSF, differentiating MCI from controls, and predicting future conversion from MCI to AD dementia. Additionally,

Table 2
Comparison of the accuracy different platforms for measurement of plasma p-tau217.

	Platform	Clinical stages	Threshold (pg/L)	AUC	Specificity	Sensitivity	PPV	NPV
Ashton, N. J. [142]	ALZpath	Preclinical to MCI	Binary: >0.42	0.92–0.96	74.5–85.1	85.0–98.2	48–85	77.6–97.7
			<0.40 (negative), >0.63 (positive)	0.93–0.97	94.1–98.6	86.7–98.2	87.5–98.5	88.5–98.4
Palmqvist, S. [143]	Eli Lilly	AD vs. non-AD	–	0.96–0.99	86 – 92	93	–	–
Kivisäkk, P. [144]	MSD S-PLEX *	AD vs. control	–	0.98	–	–	–	–
Janelidze, S. [145]	WashU	MCI-AD	–	0.947	90.6	94.4	–	–
	Lilly	Non-progressors A−		0.886	84.4	85.9		
	Janss	Non progressors A+		0.858	87.5	87.5		
Suárez-Calvet M et al., [146]	Janssen			0.96				
	Lilly			0.94				
Groot, C. [134]	Janssen	Controls, MCI, MCI to AD	–	0.91	–	–	–	–
Arranz, J. [137]	Lumipule	Controls, MCI, MCI to AD		0.92–0.97				
			0.130		97.8 %	65.5 %	96.9 %	73.1 %
			0.186		95.0 %	82.1 %	94.4 %	83.5 %
			0.247		90.6 %	89.7 %	90.9 %	89.4 %
			0.388		69.8 %	95.2 %	76.7 %	93.3 %
Zhong X [147]	Lumipulse	Normal, MCI, AD		0.94–0.97				
Pilotto A [148]	Lumipulse	MCI, Mild AD	0.291	0.952	–	–	–	–
	ALZpath		0.542	0.955				
Figdore DJ [149]	ALZPath	MCI, AD	0.91	–	84	84	–	–
	Lumipulse		0.93		86	88	–	–
Frykman H. [119]	ALZpath		0.34	0.94	67.4	95.8	83.1	90.6
			0.40	0.94	76.7 %	87.5 %	86.3 %	78.6 %
			0.63	0.94	95.3 %	79.2 %	96.6 %	73.2 %
	Lumipulse	Neuropathology confirmed cases	0.13	0.90	46.5 %	96.6 %	71.3 %	90.9 %
			0.18	0.90	67.4 %	88.1 %	78.9 %	80.5 %
			0.37	0.90	93.0 %	67.8 %	93.0 %	67.8 %

* Developed by Meso Scale Discovery using a sandwich immunoassay format using monoclonal antibodies and electrochemiluminescence.

Table 3
Comparison of analytical performance of two Plasma p-tau217 Immunoassays.

Analytical performance	ALZpath p-tau 217	Lumipulse p-tau 217
Clinical reportable range	0.032–10.00 ng/L	0.06–10.00 ng/L
Limit of blank (LoB)	0.0035 ng/L	0.040 ng/L
Limit of detection (LoD)	0.0074 ng/L	0.052 ng/L
Limit of quantification (LoQ)	0.032 ng/L	0.060 ng/L
Intra-laboratory precision	≤10 % above LoQ	≤13 % above LoQ
Inter-laboratory precision	≤11 % above LoQ	NA
Sample Stability	≤7 days at 2–8 °C ≤4 weeks −20 °C ≤4 weeks −80 °C ≤5 freeze/thaw cycles	≤7 days at 2–8 °C ≤1 weeks −20 °C ≤4 weeks −80 °C ≤3 freeze/thaw cycles
Interference	No interference detected to the maximum concentration for bilirubin (unconjugated and conjugated), hemoglobin, intralipid, biotin, and heterophilic antibodies	No interference detected to the maximum concentrations tested for bilirubin (unconjugated and conjugated), hemoglobin, and intralipid. Interference was detected for heterophilic antibodies

both methods showed similar correlations with baseline and annual changes in MMSE scores, and there was strong concordance between the two immunoassays in both cohorts, with strong diagnostic and prognostic results [134]. In another study, Kivisäkk et al. (Table 2) measured plasma p-tau217 in samples from 131 confirmed AD cases (by CSF biomarkers) and 70 controls using a novel p-tau217 S-PLEX® assay developed by Meso Scale Discovery (MSD; Rockville, MD). The assay demonstrated excellent performance in detecting plasma p-tau217, with a lower LoQ of 1.84 pg/mL and intra/inter-plate CVs of 5.5 % (0.3–15.0 %) and 5.7 % respectively. The plasma p-tau217 assay distinguished AD cases from controls with an AUC of 0.98 (95 % CI 0.96–1.0) and showed 3.9-fold higher levels of p-tau217 in AD cases [135]. More recently, the ALZpath p-tau217 assay, a SIMOA immunoassay for use on the Quanterix HD-X platform, has been made commercially available for research use. A recently published paper highlighted that the efficacy of the plasma ALZpath p-tau217 immunoassay in accurately diagnosing biological AD is comparable to that of CSF biomarkers and PET. This study included three cohorts with a total of 786 participants, both with and without cognitive impairment, classified by amyloid and tau status using PET or CSF biomarkers. The ALZpath p-tau217 immunoassay demonstrated high accuracy in identifying elevated Aβ (AUC, 0.92–0.96; 95 % CI, 0.89–0.99) and tau pathology (AUC, 0.93–0.97; 95 % CI, 0.84–0.99) across all cohorts, which was comparable to CSF biomarkers in determining abnormal PET signals. Additionally, using a three-range reference for detecting abnormal Aβ pathology provided reproducible results and reduced the need for confirmatory testing by approximately 80 %. Moreover, this study identified reproducible cut-offs across three cohorts to detect longitudinal alterations of plasma p-tau217, particularly at the preclinical stage. It also found that plasma p-tau217 levels showed an annual increase only in Aβ-positive individuals, with the highest increase observed in those with tau positivity [136]. Furthermore, the Fujirebio Lumipulse G instrument is a fully automated platform that has been used to measure CSF AD biomarkers in clinical laboratories. Fujirebio recently launched assays to measure p-tau217 in plasma. In another study, Arranz et al. analyzed 290 plasma samples from consecutive participants, including 66 cognitively unimpaired individuals, 130 with MCI, and 94 with dementia, all referred to a specialized memory clinic and undergoing lumbar puncture as part of routine clinical practice. Participants were categorized as amyloid positive or negative according to their CSF Aβ1–42/Aβ1–40 ratio, and their plasma p-tau217, p-tau181, Aβ1–42, and Aβ1–40 levels were measured using the fully automated LUMIPULSE platform. The study found that plasma p-tau217 and p-tau181 concentrations were higher in amyloid-positive individuals than in amyloid-negative ones. P-tau181 and the Aβ1–42/Aβ1–40 ratio showed a moderate correlation between plasma and CSF. The AUC for discriminating participants with amyloid pathology was 0.94 (95 % CI 0.92–0.97) for p-tau217, and 0.88 (95 % CI 0.84–0.92) for both p-tau181 and the Aβ1–42/Aβ1–40 ratio. Plasma p-tau217 had the highest fold change and demonstrated high predictive capability in discriminating amyloid pathology and its overall accuracy by using a two-threshold approach was robust in MCI and dementia groups, exceeding 90 % [137]. In another recent study, Pilotto et al. compared Lumipulse and ALZpath SIMOA head-to-head for detecting plasma p-tau217 in AD. They measured plasma p-tau217 in 392 participants, including 162 with AD, 70 with other NDDs with CSF biomarkers, and 160 healthy controls, using both assays. The ability of both techniques to differentiate AD from non-AD NDDs and controls, as shown by ROC analyses, demonstrated high stability, with similar correlations to CSF p-tau181 levels. While Lumipulse showed an AUC of 0.952 (95 % CI 0.927–0.978), ALZpath also exhibited similar diagnostic accuracy, with an AUC of 0.955 (95 % CI 0.928–0.982) for distinguishing AD from non-AD and healthy controls [138]. In another recent study by Figdore et al. cut-offs for the ALZpath and Lumipulse p-tau217 immunoassays were assessed in clinically diagnosed MCI and mild AD cases determined by amyloid PET status. The study, similar to previous studies, suggested a two-cutpoint approach for clinical selection of AD cases to minimize potential false-positive results and improve diagnostic accuracy. Using a one-cutpoint approach, neither assay achieved sensitivity and specificity ≥90 %. However, with a two-cutpoint approach, 92 % sensitivity and 96 % specificity were achieved, and 39 % of results were classified as indeterminate for the ALZpath assay and 22 % for the Fujirebio [139]. We assessed the analytical performance of plasma p-tau 217 using 360 samples of amyloid PET-negative healthy subjects aged 55–95, with an approximately equal distribution of males and females [140]. Plasma samples were analyzed using the ALZpath p-tau217 assay on a single Quanterix HD-X SIMOA Analyzer platform. Our validation procedures were conducted in accordance with CAP and CLIA requirements, following CLSI guidelines [141]. The ALZpath p-tau217 assay demonstrates a clinical reportable range of 0.032–10.00 ng/L, LoB of 0.0035 ng/L, LoD of 0.0074 ng/L, and LoQ of 0.032 ng/L, an interlaboratory CV 7.5–12 % and inter-laboratory CV 9.6–10.4 %. Stability is maintained at 2–8 °C for ≤1 week, at room temperature for ≤72 h, at −20 °C and −80 °C for ≤4 weeks, with freeze/thaw cycles tolerated up to 5times (Table 3). Additionally, the Lumipulse assay shows a clinical reportable range of 0.06–10.00 ng/L, a LoB of 0.040 ng/L, a LoD of 0.052 ng/L, and a LoQ of 0.060 ng/L. Stability is maintained at 2–8 °C for ≤1 week, at −20 °C for ≤one week, and at −80 °C for ≤4 weeks, with freeze/thaw cycles tolerated up to 3 times (Tables 2 and 3).

Further, to evaluate the clinical utilization of ALZpath p-tau217 immunoassay by SIMOA and Lumipulse plasma p-tau217 as a laboratory-developed test, we assessed the diagnostic performance of both assays in comparison to FDA-approved CSF testing for amyloid Aß42/40 ratio and p-tau181, as well as postmortem neuropathological evaluation of cases who had paired plasma samples at diagnosis of dementia. We conducted a clinical validation study using 115 samples of autopsy-confirmed cases as a gold standard. The ALZpath plasma p-tau217 assay outperformed the Fujirebio assay for predicting autopsy-confirmed AD cases, with a significantly higher AUC of 0.94 vs. 0.90 (p-value 0.023) for Lumipulse (Fig. 7). We also set dual decision points for the two p-tau217 tests based on the final diagnoses for the neuropathology cohort. For ALZpath p-tau217, the positive predictive value (PPV) at the threshold of 0.63 ng/L was 96.6 % (Tables 2 and 4) and negative predictive value (NPV) at the level of 0.34 ng/L was 90.6 %. For

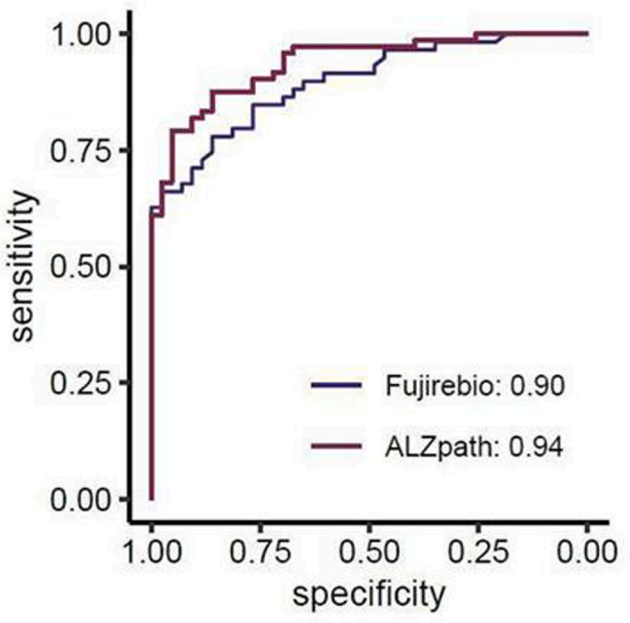

Fig. 7. The accuracy of plasma p-tau 217. Plasma p-tau217 ROC for two commercial immunoassays (ALZpath and Lumipulse) using pathology – confirmed samples.

Table 4
Comparison of clinical performance of two plasma p-tau217.

	ALZPath p-tau217	Lumipulse p-tau217
AUC	0.94	0.90
Youden Index	0.63 ng/L	0.28 ng/L
NPV	90.6 % (0.34 ng/L)	90.9 % (0.13 ng/L)
PPV	96.6 % (0. 0.63 ng/L	93.0 % (0.37 ng/L)
Sensitivity	95.8 % (0.34 ng/L)	96.6 % (0.13 ng/L)
Specificity	95.3 % (0.63 ng/L)	93.0 % (37 ng/L)
Negative	<0.34 ng/L	<0.13 ng/L
Intermediate	0.34–0.63 ng/L	0.13–0.37 ng/L
Positive	>0.63 ng/L	>0.37 ng/L

the Fujirebio p-tau217 assay, the PPV at the higher threshold of 0. 37 ng/L was 93 % and NPV at the lower level of 0.13 ng/L was 90.9 %. Moreover, we defined a specificity of 95.3 % at the 0.63 ng/L concentration and a sensitivity of 95.8 % at the 0.34 ng/L threshold for ALZpath, with 20.1 % falling within a grey zone (concentrations between 0.34 ng/L and 0.63 ng/L). For Lumipulse, we observed a sensitivity of 96.6 % at the 0.13 ng/L threshold and a specificity of 93.0 % at the higher cut-off of 37 ng/L, with a broader intermediate zone of 32.2 %, which is higher than that of ALZpath (Table 4) [119].

8. The past, current and future of biofluid biomarkers for AD in Canada

AD neuropathological cascades are a longitudinal process that begin years before the apparent clinical symptoms. The clinical findings of AD manifest along a continuum and their precise diagnosis during the prodromal phase is difficult. Therefore, any tools that are used for early AD diagnosis must be very sensitive, practical and ideally non-invasive for use by health care providers [150]. In addition, numerous new therapeutic agents for AD are undergoing clinical trials that are thought to be most effective in the early stages of the disease (MCI and mild AD), and two of them, antibody-based drugs, donanemab and lecanemab, have received approval from the U.S. Food and Drug Administration (FDA). They also have been approved in Japan and Europe, and it is expected to be approved by Health Canada and many other countries in the coming months [151]. In preparation for potential new disease-modifying treatments for AD it is of interest to know about methods of diagnosis of AD, including imaging and other markers. PET imaging has been used for early AD diagnosis and has served as the most commonly used biomarker in many parts of the world for many years [152]. In Canada, according to clinical expert input, in Ontario, CSF biomarkers are more commonly used than PET imaging [153]. The first diagnostic radiotracer for early diagnosis of AD was approved in Canada in 2017 [154]. Furthermore, there are 57 available PET-CT scanners for use in Canada, and they are mostly used for oncology (80 %). These scanners are distributed mostly in urban centres; Prince Edward Island and the territories of Canada do not have PET-CT scanners, and New Brunswick does not pay for amyloid PET imaging for AD patients [154]. CSF biomarkers are promising diagnostic tool for AD, and the first testing program for CSF biomarkers in Canada was launched in 2017 [155]. Further, the CSF testing for AD diagnosis has been approved by Health Canada since 2023 [155]. Blood-based biomarkers offer less invasive, affordable and scalable alternatives to PET or CSF biomarkers for screening, diagnosis, and monitoring a variety of neurodegenerative and neuroinflammatory disorders [44,94,156]. Among them plasma p-tau181, Aβ42/40 ratio, NfL, and GFAP have been validated in Canada to develop both discrete and continuous reference intervals (RIs) and provide their age-specific cut-offs in the Canadian population [157]. In this regard, Cooper et al. obtained 900 plasma samples from both male and female participants aged 3–79 years old from the Statistics Canada Biobank, which stores specimens from the Canadian Health Measures Survey. They measured Aβ42/40, p-tau-181, NfL and GFAP on the Quanterix Simoa HD-X analyzer using the Neurology 4-plex E and p-tau-181 assays and produced discrete RIs according to Clinical Laboratory Standards Institute guidelines (EP28-A3c) (Table 5). This study focused on defining population-based age-specific RIs which are essential for improvement of implementation of biomarkers. Although these indices are also important for reporting and interpreting cut-off values to improve interpretation of laboratory tests in a variety of contexts, these reported RIs can only be used for interpretation of measurements on the Simao HD-X platform [157]. Hence, alongside this study, we evaluated the analytical performance and reference values of plasma NfL using the Lumipulse instrument following CLSI guidelines as a diagnostic laboratory test. In addition, we analytically and clinically validated plasma p-tau181 on the Quanterix Simoa HD-X analyzer, as well as plasma p-tau217, as explained in the previous sections. Furthermore, while plasma p-tau217 is a front-runner biomarker for AD diagnosis, to establish its diagnostic value, conducting prospective studies in the real-world memory clinics is vital to define a precise diagnostic cutoff value and reference standard. This could promote the adoption of plasma p-tau217 as a primary care screening test, improving accessibility and cost-effectiveness for initial diagnosis. Additionally, clinical validation

Table 5
The RI of plasma biomarkers in Canadian Population [157].

Biomarker	Age (years)	RI (ng/L)
Aβ42/40	3–<55	0.053–0.098
	55–<80	0.040–0.090
p-tau-181	3–<12	1.4–5.6
	12–<60y	0.8–3.1
	60–<80y	0.9–4.0
NfL	3–<40y	2.6–11.3
	40–<60y	4.6–17.7
	60–<80y	8.1–47.1
GFAP	3–<10	47.0–226
	10–<60y	21.2 to 91.9
	60–<80	40.7–228

studies focused on plasma p-tau217 are crucial for enhancing diagnostic accuracy and ensuring consistent test results, and seeking a specific threshold level, potentially revolutionizing AD diagnosis and care. Moreover, AD biomarkers are known to be influenced by risk factors of AD including age, sex, ethnicity, apolipoprotein E epsilon 4 (APOE-ε4) and other covariates such as renal disease, cardiovascular disease, body mass index and hypertension [158]. Therefore, to confidently interpret plasma p-tau217 in the biological diagnosis of AD, particularly in its preclinical stage, we need to understand how AD risk factors are associated with plasma p-tau217 in the general population. Concerning these issues, a series of prospective and community-based studies are required to address the variability of plasma p-tau217 and to specify a certain diagnostic cut-off. These types of studies also can enhance the accuracy of biological diagnosis of AD and characterize plasma p-tau217 levels across various stages of AD, thereby defining its relevance in different disease stages. In addition to improve the interpretation of plasma biomarkers, obtaining reliable BV data can facilitate the identification of the APS required for every biomarker. In this regard, implementation of plasma biomarkers requires comprehensive data about their BV. While CSF biomarkers have been approved by Health Canada, blood-based biomarkers for AD have not still been approved. Additionally, since 1989, four Canadian Consensus Conferences on the Diagnosis and Treatment of Dementia (CCCDTD) have provided evidence-based dementia guidelines for Canadian clinicians and researchers. The latest one, the 5th CCCDTD convened in October 2019, recommended that because the significance of the presence of amyloid and tau proteins in people without cognitive deterioration is not clear, amyloid and tau imaging in such individuals should only be used for research [159]. For people with cognitive decline, the recommendation was that amyloid and tau PET imaging should only be ordered by dementia experts [159]. It was also recommended that asymptomatic people should not be routinely screened for MCI or dementia (including people with relevant risk factors), either with clinical or cognitive testing or with imaging [159]. Although blood-based biomarkers can be used to diagnose (revised AA criteria) and as evidence of treatment response (FDA guideline) in asymptomatic individuals and AD patients, their practical implementation in clinical settings in Canada also faces other challenges. These include:

1. Lack of regulatory approval: currently, no blood-based biomarkers for AD diagnosing have been approved by Health Canada. While the blood-based biomarkers are relatively new, there is strong evidence from Canadian laboratories to support them to be recognized as reliable diagnostic tools in clinical settings [119].
2. Standardization of testing: while we demonstrated that both plasma p-tau217 ALZPath and Lumipulse had acceptable analytical performance [119], blood biomarker tests for AD are not yet globally standardized across the world as the variations in testing protocols, assay methods, and processing may lead to inconsistent results.
3. Cost of testing: although commercial tests for blood-based biomarkers for AD diagnosis as a laboratory diagnostic tests [119] are now available in Canada, unfortunately they are not covered by provincial health plans. This limits their accessibility and widespread use in clinical practice.
4. Patient and clinician awareness: many clinicians in Canada may not be fully aware of the latest advancements in blood biomarker testing for AD, especially outside of major research and academic hospitals. Educating healthcare providers about the benefits and limitations of blood biomarkers is crucial to fostering acceptance.

In conclusion, measurement of AD biomarkers will be a key factor in determining eligibility for disease-modifying therapy in clinical practice. Plasma p-tau 217 is a robust biomarker for diagnosis and monitoring of AD. It has also demonstrated high efficacy in identifying Aβ pathology in the early stages of the disease, making it a feasible test for AD screening in clinical practice. Several assays have been made commercially available for clinical use. Numerous studies, including ours, have evaluated these plasma p-tau immunoassays as laboratory-developed tests, reinforcing the clinical utility of plasma p-tau217 in AD diagnosis and highlighting differences in the performance of current plasma p-tau217 immunoassays.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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