Practical considerations for biomarkers in Alzheimer's disease (AD) diagnosis

This content is intended for health care professionals only for educational and informational purposes and does not substitute for sound medical judgment or clinical decision making in the context of medical treatment

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PIPN-M3224

Slide outline

Practical Zone

Assessing atrophy with MRI

Assessing atrophy: techniques

Clinical settings[1–4]

- In clinical settings, the mainstay of assessment comprises of:
    1. **Visual inspection** – both MRI and CT – to identify gyral volume, hippocampal volume, ventricular size, white matter signal pattern, etc.
    2. **Visual rating scales** to support the identification of general and focal findings e.g., medial temporal atrophy scoring, Fazekas scoring for white matter intensities

Research settings[1–3,5]

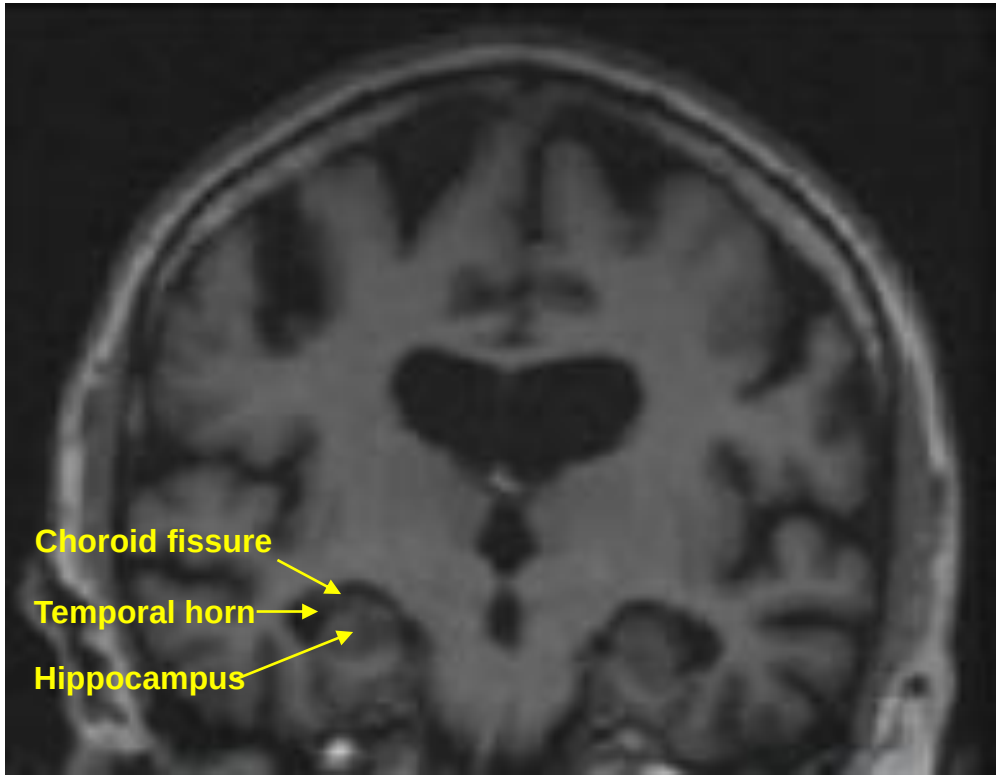
- Volumetric MRI techniques (e.g., manual and automated techniques for measuring the volume of hippocampi) are used
- Recent evidence has shown that automatically computed measurements to produce significantly higher accuracies than visual rating scales for MTA and global cortical atrophy
- **Hippocampal volumetry has not yet become a routine part of the diagnostic workup for neurodegenerative disease**

CT, computed tomography; MRI, magnetic resonance imaging; MTA, medial temporal lobe atrophy

1. Sheikh-Bahaei N, et al. J Alzheimers Dis Rep 2017;1:71–88; 2. Mortimer AM, et al. Pract Neurol 2013;13:92–103; 3. Menéndez González M, et al. Cureus 2016;8:e544; 4. Gaillard F, Jayanti S, Rasuli B, et al. Neurodegenerative MRI brain (an approach). Reference article, Radiopaedia.org (Accessed on 06 Sep 2023) https://doi.org/10.53347/rID-2836; 5. Koikkalainen JR, et al. Eur Radiol 2019;29:4937–4947;

Assessing atrophy with MRI: Scheltens scale to determine medial temporal lobe atrophy measurements (1/2)

The Scheltens scale is an example of how a coronal T1-weighted MRI scale may be used to determine medial temporal lobe atrophy



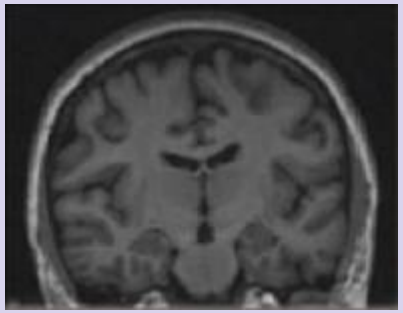
Visual assessment of medial temporal atrophy by determining the largest vertical width of choroid fissure, width of temporal horn, and height of hippocampal thickness

MRI, magnetic resonance imaging

Westman E, et al. PLoS ONE 2011;6:e22506

Assessing atrophy with MRI: Scheltens scale to determine medial temporal lobe atrophy measurements (2/2)

The Scheltens scale is provided below as an example of how a coronal T1-weighted MRI scale may be used to determine medial temporal lobe atrophy



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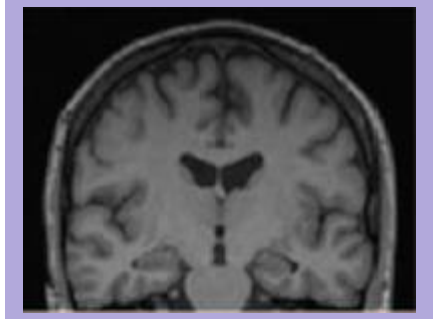
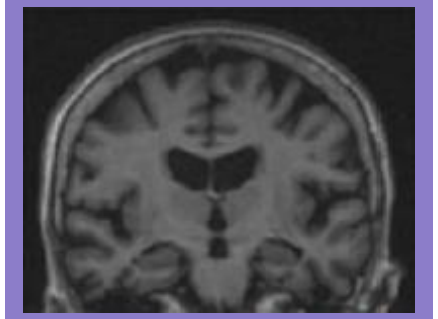


1

Choroid fissure width
- Mild ↑

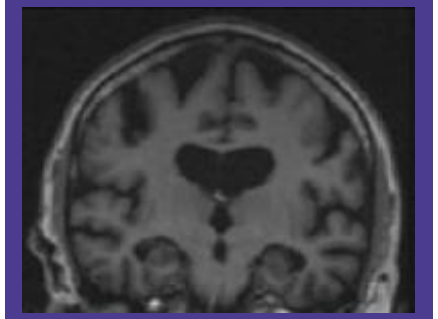


2

Choroid fissure width
- Moderate ↑

Temporal horn width
- Mild ↑

Hippocampal thickness
- Mild ↓

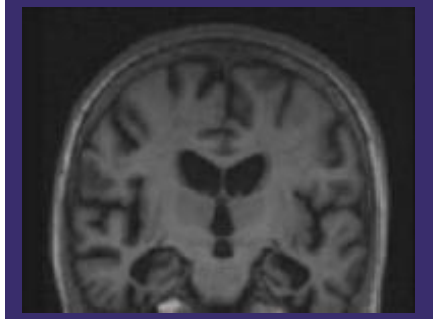


3

Choroid fissure width
- Severe ↑

Temporal horn width
- Moderate ↑

Hippocampal thickness
- Moderate ↓



4

Choroid fissure width
- Severe ↑

Temporal horn width
- Severe ↑

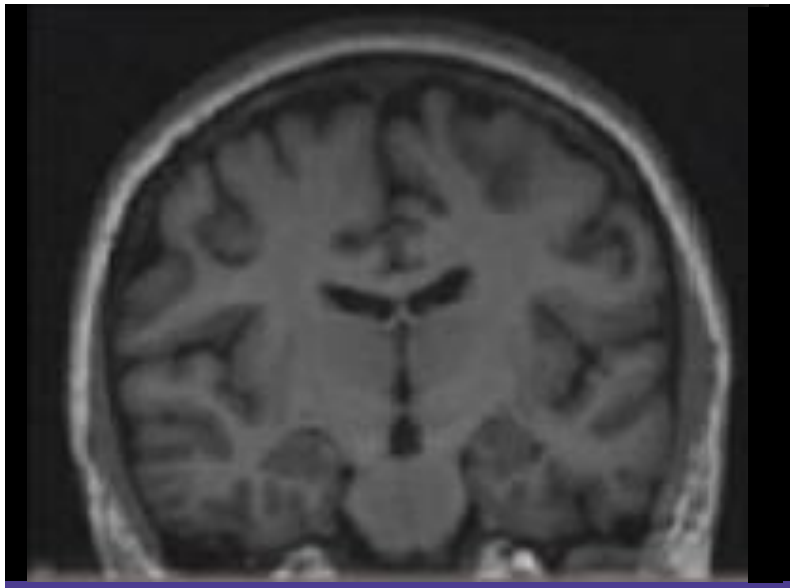
Hippocampal thickness
- Severe ↓

MRI, magnetic resonance imaging

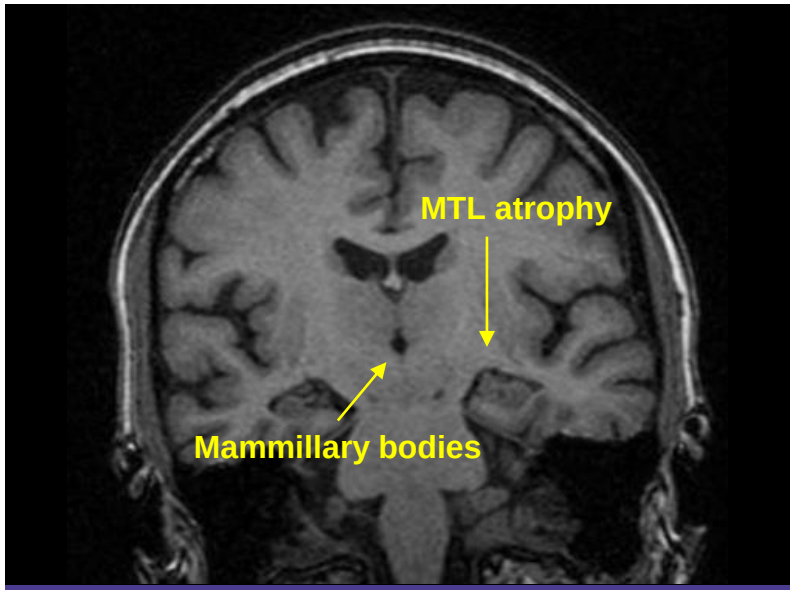
Westman E, et al. PLoS ONE 2011;6:e22506

Assessing atrophy with MRI: using mammillary bodies as a landmark for medial temporal lobe atrophy measurements

Coronal slices, identified using the mammillary bodies as a landmark, may be used to determine if medial temporal lobe atrophy is present[1]



No atrophy
(healthy control)



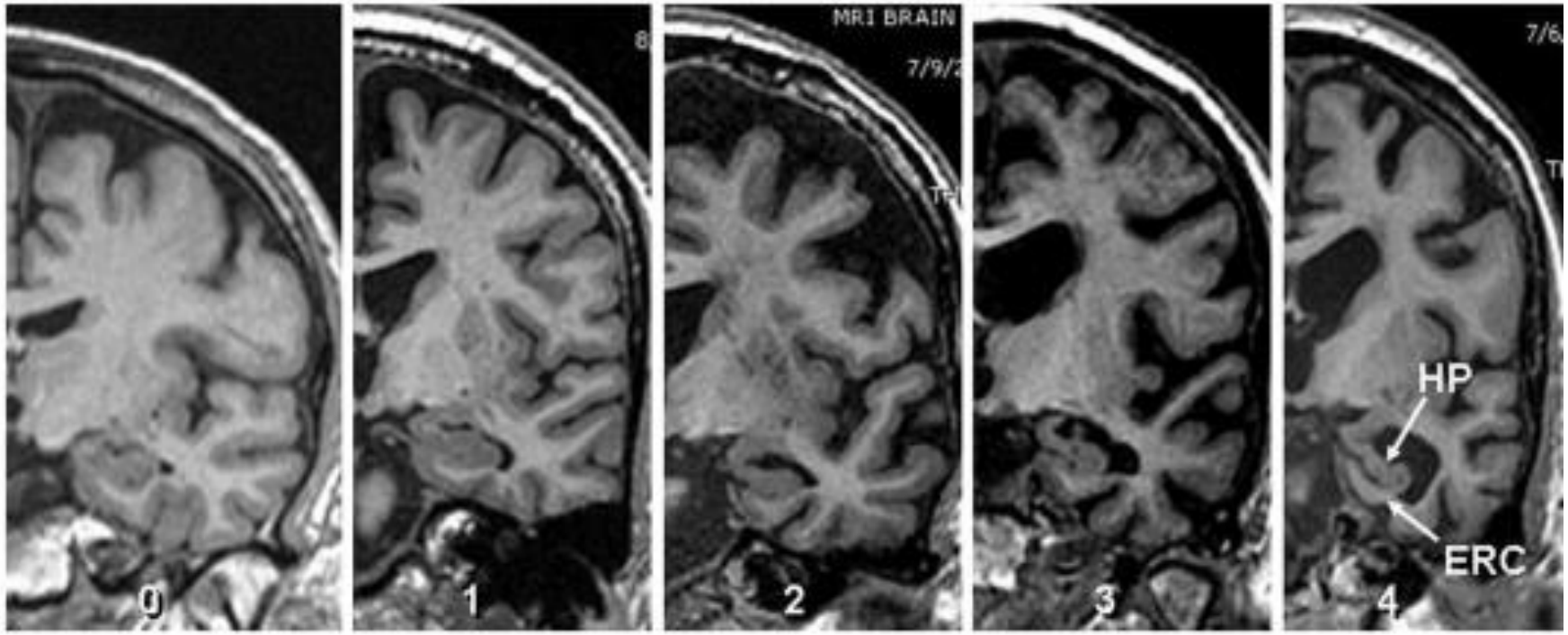
Atrophy
(patient with AD dementia)

MRI, magnetic resonance imaging; MTL, medial temporal lobe

Image on left from Westman E, et al. PLoS ONE 2011 ;6:e22506 (License: CC BY 4.0 https://creativecommons.org/licenses/by/4.0); Right hand image used with permission from Gaillard F, Alzheimer's disease. Case study, Radiopaedia.org (Accessed on 26 Jul 2023) https://doi.org/10.53347/rID-30244

1. Duara R, et al. Neurology 2008;71:1986–1992

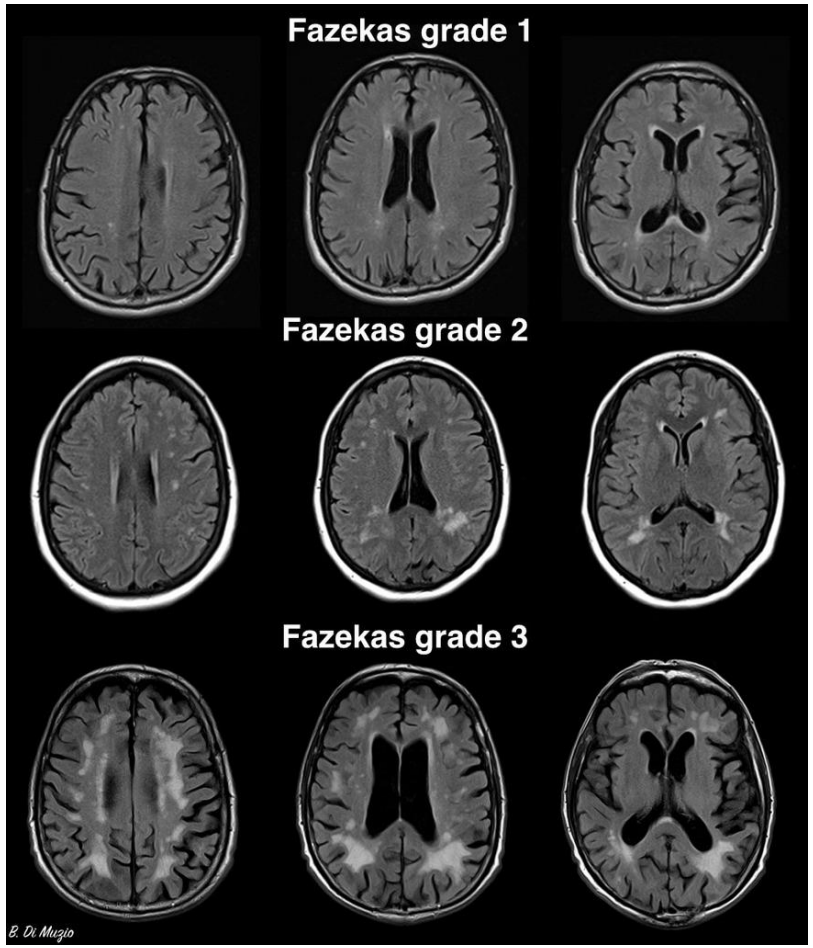
Assessing atrophy with MRI: using coronal slices for medial temporal lobe atrophy measurements



No atrophy (score = 0) → Severe atrophy (score = 4)

Images show coronal slices (1.2 to 1.5-mm thickness), perpendicular to the AC/PC line and intersecting the mammillary bodies
AC/PC, anterior/posterior commissure; ERC, entorhinal cortex; HP, hippocampus; MRI, magnetic resonance imaging

Deep white matter changes: Fazekas scale



Fazekas 0: absence

Fazekas 1: punctate foci

Fazekas 2: beginning confluency of foci

Fazekas 3: large confluent areas

Image from: Gaillard F, El-Feky M, Qureshi P, et al. Fazekas scale for white matter lesions. Reference article, Radiopaedia.org (Accessed on 06 Sep 2023) https://doi.org/10.53347/rID-28447

Advantages and limitations of structural imaging

Patterns of atrophy on structural imaging are not indicative of the specific underlying pathology; this is considered a neurodegenerative marker, and is indicative of neuronal injury[1]

Advantages[2]

- Needs to be done to exclude other pathologies
- Usually available in urban areas
- Can detect co-existing vascular changes
- Provides information on the temporal and spatial evolution of AD

Limitations

- Reflects downstream processes and not molecular pathology[2]
- Volume changes may be produced by other factors not related to progression of neuronal loss[2]
- A normal scan does not exclude AD[3]

AD, Alzheimer's disease; MRI, magnetic resonance imaging

1. Jack CR Jr, et al. Alzheimers Dement 2018;14:535–562; 2. Johnson KA, et al. Cold Spring Harb Perspect Med 2012;2:a006213; 3. Frisoni GB, et al. Nat Rev Neurol 2010;6:67–77

Utilizing CT scans in place of MRI

Benefits	Limitations
Contraindications to MRI (e.g., pacemaker)	Lower spatial resolution
Less expensive	Lower sensitivity to detect some types of lesions
More widespread availability	Inability to measure progression of lesions
Shorter time of examination	

CT scan shows enlargement of cerebral sulci and loss of gyral volume. This is most marked in the parietal regions

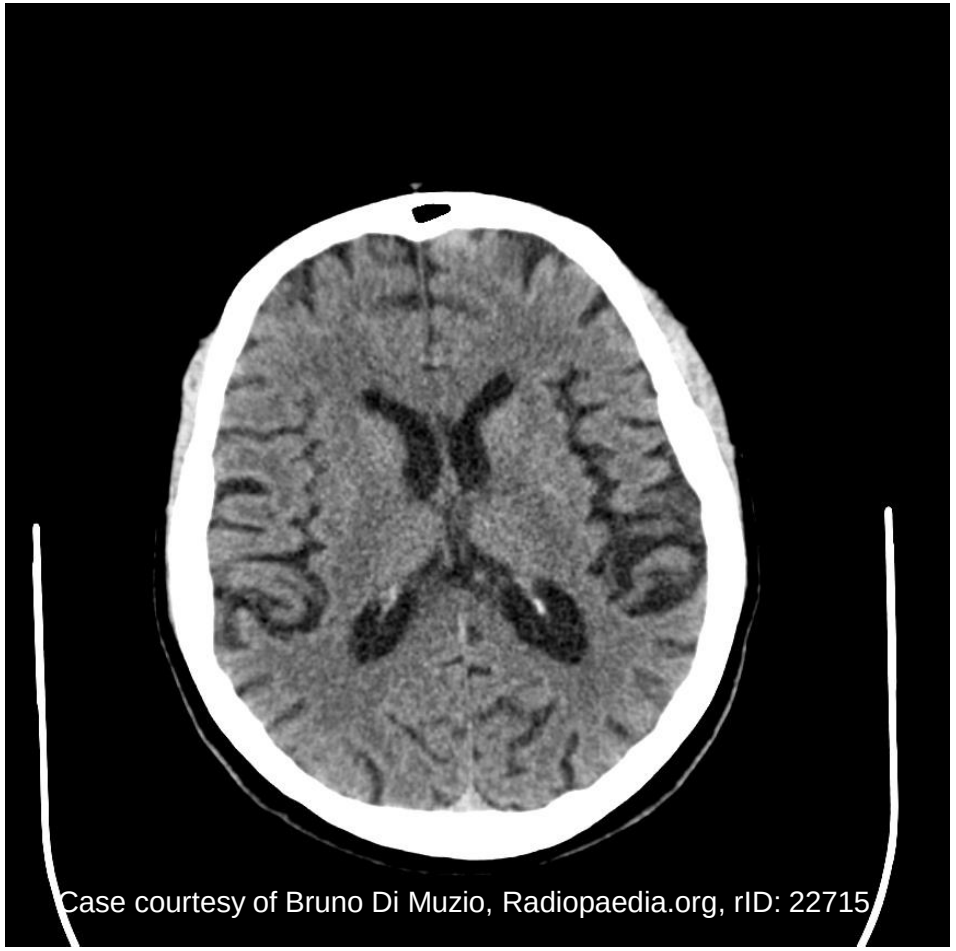


Image from Di Muzio B, Alzheimer disease. Case study, Radiopaedia.org (Accessed on 06 Sep 2023) https://doi.org/10.53347/rID-22715

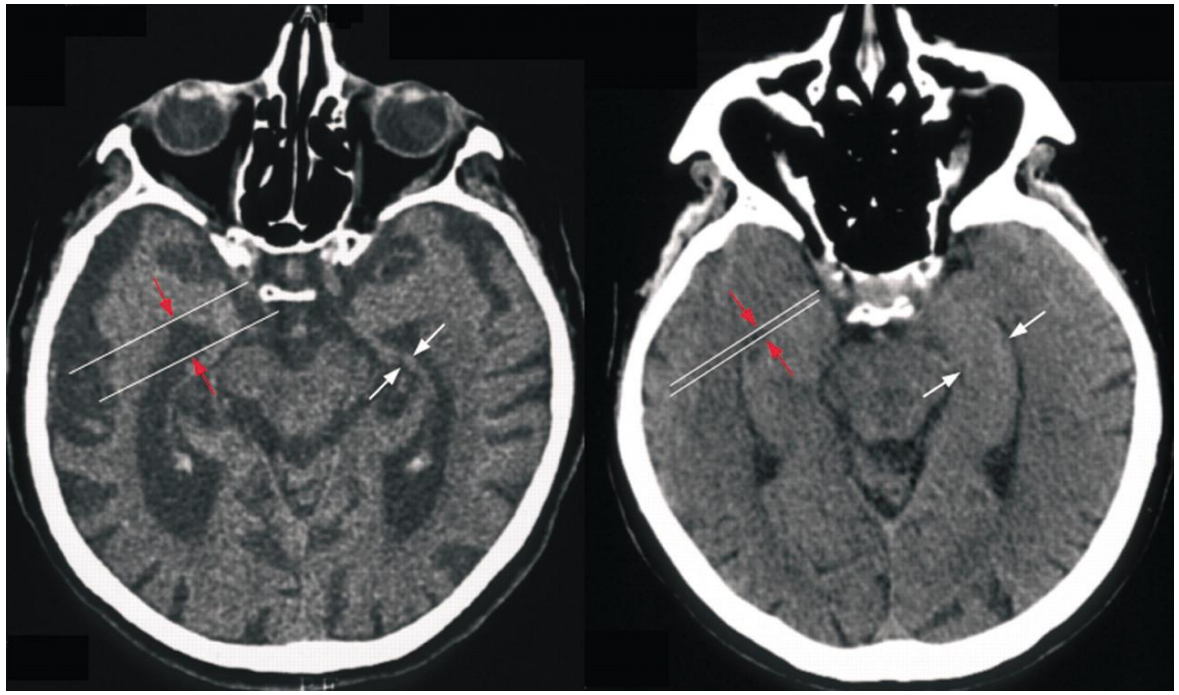
CT, computed tomography; MRI, magnetic resonance imaging

1. Pasi M, et al. Int Psychogeriatr 2011;5(Suppl 2):S6–S12;

Utilizing CT scans to measure medial temporal atrophy

High-resolution CT scans can be used to measure medial temporal atrophy, subject to the appropriate orientation of the scanner[1]

- **Minimum thickness of the medial temporal lobe:** The measurement is taken in the parahippocampal gyrus region[2] (**white arrows**)
- **Radial width of the temporal horn:** the measurement is the distance between two parallel lines drawn tangential to the tip of the temporal horns[2] (**red arrows**)



Alzheimer's disease

Normal

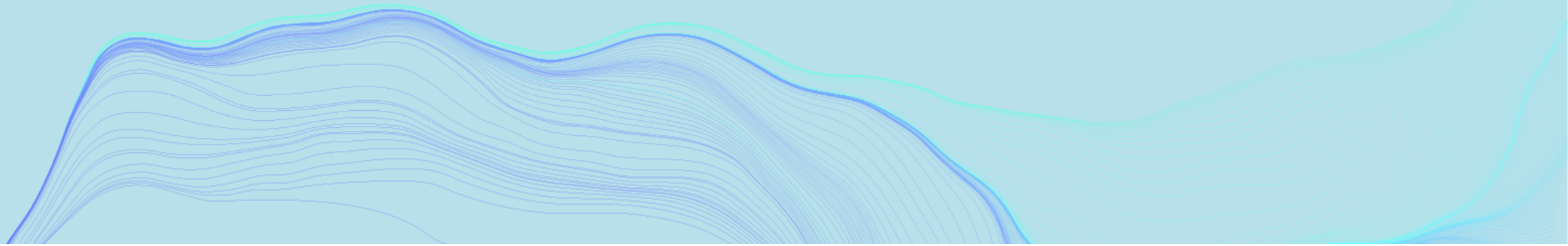
Image used with permission from Frisoni G, et al. J Neurol Neurosurg Psychiatry 2003;74:1371–1381

CT, computed tomography; MRI, magnetic resonance imaging

1. Pasi M, et al. Int Psychogeriatr 2011;5(Suppl 2):S6–S12; 2. Frisoni G, et al. J Neurol Neurosurg Psychiatry 2003;74:1371–1381

Practical Zone

Application of biomarkers to support a diagnosis



NIA-AA research framework defines Alzheimer's disease as a biomarker-driven pathophysiological construct

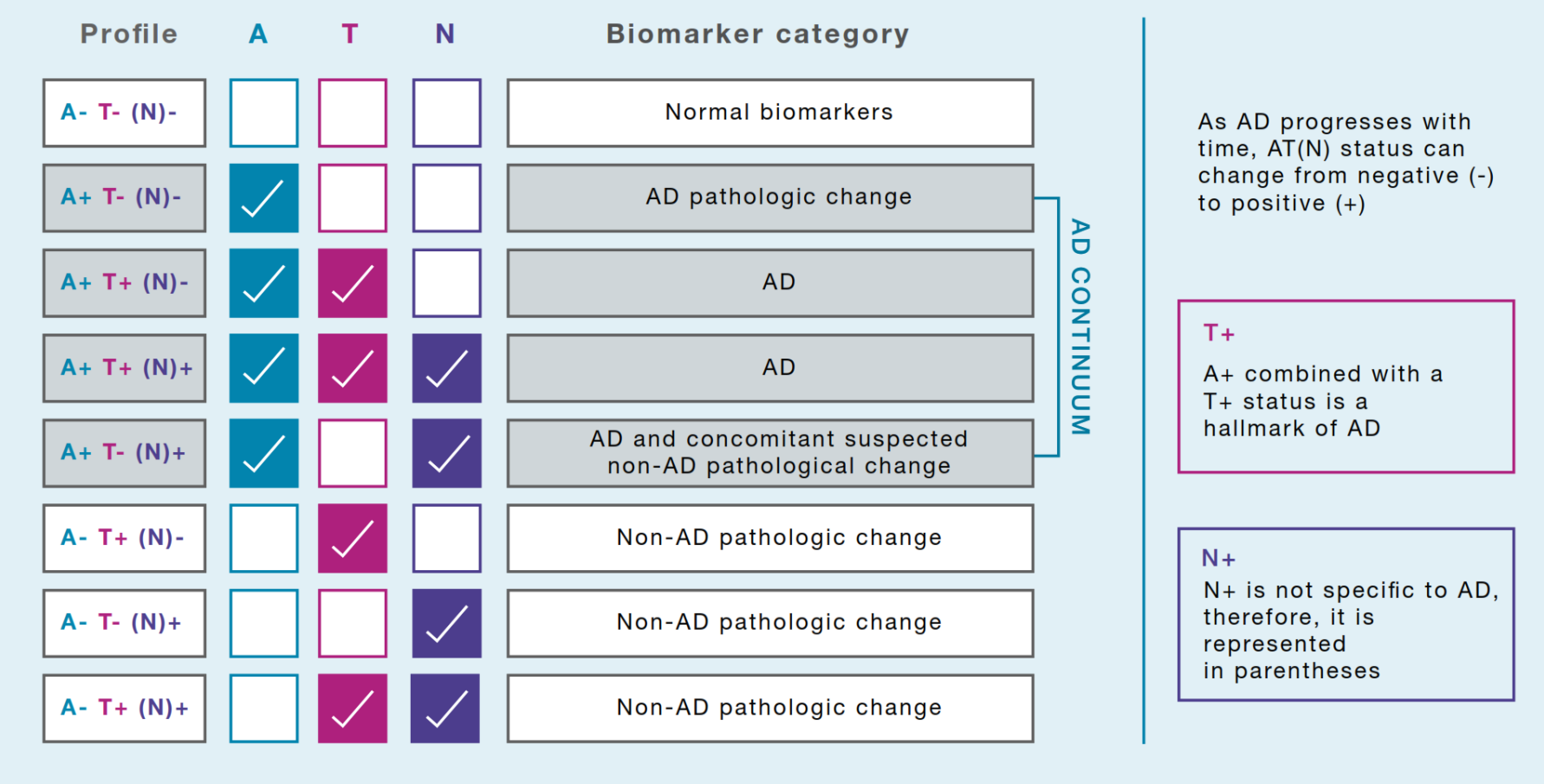
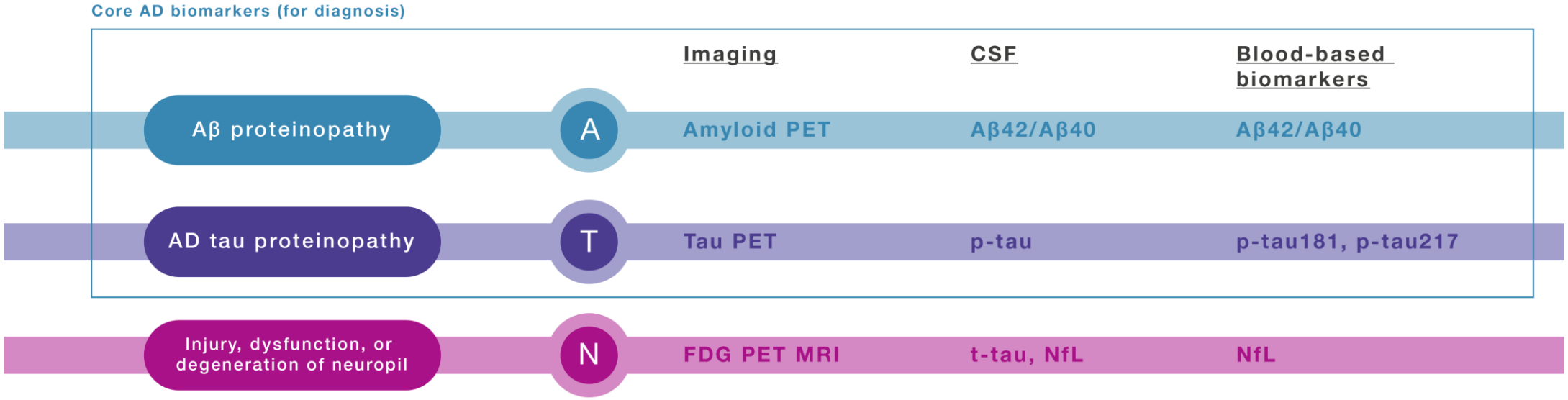


Table reprinted with permission from Jack CR Jr, et al. Alzheimers Dement 2018;14:535–562 (CC-BY 4.0: http://creativecommons.org/licenses/by/4.0)
AD, Alzheimer's disease; NIA-AA, National Institute on Aging—Alzheimer's Association
Jack CR Jr, et al. Alzheimers Dement 2018;14:535–562

Clinically available and emerging biomarker diagnostic modalities



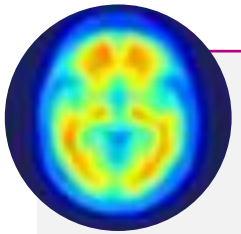
Emerging/other biomarkers	• GFAP • Neuroinflammation (Astrocytic activation) • *Neurogranin* • *Synaptic dysfunction* • *MTBR tau243* • *Insoluble aggregated tau*	• GFAP • Neuroinflammation (Astrocytic activation)

Note: not an exhaustive list of emerging biomarkers

Aβ, amyloid beta; cSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; FDG, fludeoxyglucose F18; MTBR, microtubule-binding region; NfL, neurofilament light chain; PET, positron emission tomography; p-tau, phosphorylated tau; WMH, white matter hyperintensities

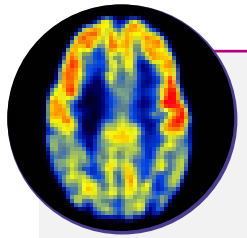
1. Jack CR Jr, et al. Alzheimers Dement 2018;14:535–562; 2. NIA-AA Revised clinical criteria for Alzheimer's disease. 2023 Available from: https://aaic.alz.org/downloads2023/NIA-AA-Revised-Clinical-Criteria-Figures-and-Tables-AAIC-2023.pdf

Using PET in AD



Amyloid and tau PET[1]

- Molecular biomarkers providing in-vivo evidence for Alzheimer pathology
- Visualizes extracellular amyloid plaques composed of Aβ
- Visualizes intracellular neurofibrillary tangles composed of p-tau



FDG PET

- Visualizes brain glucose metabolism, reflecting neuronal injury/dysfunction[2]
- Is not specific to any neurodegenerative disorder, but identifies regional neurodegeneration[3,4]

Aβ, amyloid beta; AD, Alzheimer's disease; FDG PET, fluorodeoxyglucose positron emission tomography; p-tau, phosphorylated tau; PET, positron emission tomography

1. Jack CR Jr, et al. Alzheimers Dement 2018;14:535–562; 2. Jospeh-Mathurin N, et al. Alzheimers Dement (Amst) 2018;10:669–677; 3. Levin F, et al. Alzheimers Res Ther 2021;13:49; 4. Massa F, et al. Eur J Nucl Med Mol Imaging 2022;49:1263–1274

Current limitations of PET imaging for AD diagnosis

- Single tracer assessed (amyloid, tau, FDG) in each PET imaging modality[1–3]
- Clinical test results are qualitative (under the current labels)[4]
- May be less sensitive than other available biomarkers (i.e., CSF biomarkers) to detect abnormalities in earlier stages of AD[5]
- Infrastructure requirements; in 2017, the availability of PET scanners was limited and location-dependent. In order, for a patient to access an amyloid tracer, a PET scanner must be within a 200-mile radius of a cyclotron[6]
- Requires trained and experienced raters[7,8]
- Cost/reimbursement may be a barrier to access[9]

AD, Alzheimer's disease; CSF, cerebrospinal fluid; FDG, fluorodeoxyglucose; PET, positron emission tomography

1. Villemagne VL, et al. Nat Rev Neurol. 2018;14(4):225–236; 2. TAUVID™ Prescribing Information. May 2020; Available from https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/212123s000lbl.pdf; 3. Bohnen NI, et al. J Nucl Med. 2012;53(1):59–71; 4. Chiotis K, et al. Neurobiol Aging. 2017;52:214-227; 5. Schindler SE, et al. Nat Aging. 2023;3(5):460-462; 6. Liu JL, et al. Assessing the preparedness of the U.S. health care system infrastructure for an Alzheimer's treatment. Santa Monica, CA. RAND Corporation. 2017. Available from: https://www.rand.org/pubs/research_reports/RR2272.html ; 7. Kolanko MA, et al. Pract Neurol. 2020;20(6):451–462; 8. Johnson KA, et al. J Nucl Med. 2013;9(1):e-1–16; 9. Hampel H, et al. Nat Aging. 2022;2(8):692-703

Practical Zone

Utilizing amyloid PET biomarkers

Introduction to amyloid PET tracers

Amyloid PET tracers that bind to aggregated Aβ peptides in amyloid plaques provide a means to directly assess relative brain amyloid pathology[1]

Currently available tracers include:

- ^{18}F-Florbetapir [AMYViD®]
- ^{18}F-Florbetaben [Neuraceq®]
- ^{18}F-Flutemetamol [VIZAMYL®]
- ^{11}C-Pittsburgh compound B
- ^{18}F-NAV4694

These tracers have received approval from the US FDA as well as the EMA for clinical use[2]

Aβ, amyloid beta; EMA, European Medicines Agency; FDA, Food and Drug Administration; PET, positron emission tomography

1. Clark CM, et al. JAMA 2011;305:275–283; 2. Villemagne VL, et al. Nat Rev Neurol 2018;14:225–236

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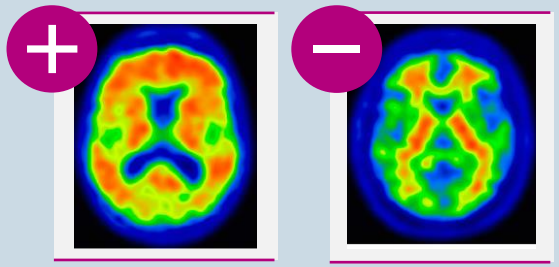
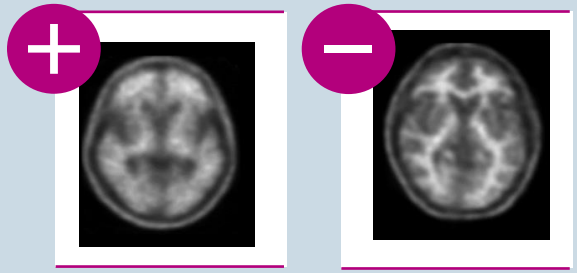
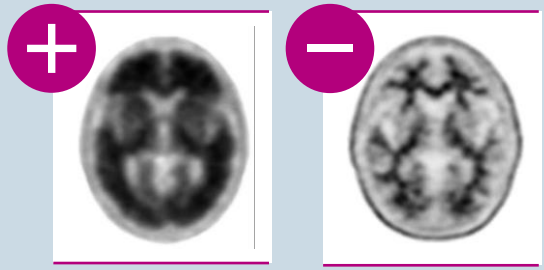
- ^{18}F-Flutemetamol [VIZAMYL®]
- ^{11}C-Pittsburgh compound B
- ^{18}F-NAV4694

These are currently used only in research and not clinically; however, the short half-life of ^{11}C-PiB and need for a PET cyclotron program has restricted use of this tracer[2–4]

Aβ, amyloid beta; PET, positron emission tomography

1. Clark CM, et al. JAMA 2011;305:275–283; 2. Villemagne VL, et al. Nat Rev Neurol 2018;14:225–236; 3. Kobylecki C, et al. J Nucl Med 2015;56:386–391; 4. Buccino P, et al. EJNMMI Radiopharm Chem 2019;4:14

Clinical characteristics of available amyloid PET tracers

		^{18}F-Flutemetamol	^{18}F-Florbetaben	^{18}F-Florbetapir
Trade name		Vizamyl™	Neuraceq™	Amyvid™
Interpretation		Color scale (red positive uptake)	Gray scale (white positive uptake)	Gray scale (black positive uptake)
				
Concordance with post-mortem neuropathology[1–3]	Sensitivity (95% CI)	93% (86, 93)	96% (90, 100)	92% (78, 98)
	Specificity (95% CI)	84% (60, 92)	77% (47, 80)	100% (80, 100)
Meta-analyses of clinical studies with different reference standards[4*]	Sensitivity (95% CI)	95% (85, 98)	89% (55, 98)	90% (75, 96)
	Specificity (95% CI)	87% (75, 94)	89% (81, 94)	81% (24, 98)

*different reference standards used in studies included in the meta-analyses

Images from left to right: This research was originally published in JNMT. Mantel E, Williams J. J Nucl Med Technol 2019;47:203–209. © SNMMI; Middle: Images used with permission from Jovalekic A, et al. Eur J Nucl Med Mol Imaging. 2023 Jun 10. doi: 10.1007/s00259-023-06279-0. Epub ahead of print (CC-BY 4.0: http://creativecommons.org/licenses/by/4.0/); Right: This research was originally published in JNMT. Trembath L, et al. J Nucl Med Technol 2015;43:175–184. © SNMMI

1.Vizamyl™ Prescribing Information. December 2019; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/203137s013lbl.pdf; 2. Neuraceq™ Prescribing Information. March 2014; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/204677s000lbl.pdf; 3. Amyvid™ Prescribing Information. December 2019; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/202008s036lbl.pdf ; 4. Morris E, et al. Eur J Nucl Med Mol Imaging. 2016; 43: 374–385.

Variability in amyloid PET imaging tracers[a]

	Vizamyl™ (^{18}F-flutemetamol)[1]	Neuraceq™ (^{18}F-florbetaben)[2]	Amyvid™ (^{18}F-florbetapir)[3]
Dosage form	Sterile injectable	Sterile injectable	Sterile injectable
Dose	185 MBq/10ml	300 MBq/10ml	370 MBq/10ml
Dosage route	Single IV bolus (<40 secs); followed by a 0.9% sterile sodium chloride IV flush	Slow, single IV bolus (6 sec/mL); followed by a 0.9% sterile sodium chloride IV flush	Single IV bolus; followed by a 0.9% sterile sodium chloride IV flush
Single scan time	10–20 mins	15–20 mins	10 mins
Optimal time from injection	60-120 mins	45–130 mins	30–50 mins
Cortical vs white matter uptake	Similar uptake[4]	Similar uptake[4]	Similar uptake[4]
Radiation absorbed from dose	5.92 mSv	5.8 mSv	7 mSv
Image display	Display images in axial, sagittal, and coronal planes using color scale	Transaxial orientation using gray scale or inverse gray scale	Transaxial orientation using black-white scale

FDA-approved PET tracers are not all read with the same specifications—training from respective manufacturers is available and recommended

[a]The PET tracers are trademarked by: Amyvid™, Eli Lilly; Neuraceq™, Piramal Imaging; Vizamyl™, General Electric Company
FDA, Food and Drug Administration; IV, intravenous; MBq, megabecquerel; mSv, millisievert; PET, positron emission tomography

1. Vizamyl™ Prescribing Information. December 2019; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/203137s013lbl.pdf; 2. Neuraceq™ Prescribing Information. March 2014; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/204677s000lbl.pdf; 3. Amyvid™ Prescribing Information. December 2019; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/202008s036lbl.pdf ; 4. Rowe CC, Villemagne VL. *J Nucl Med Technol*. 2013;41(1):11–8

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Amyloid PET recommendations and guidelines (1/2)

The Amyloid Imaging Task Force (Society for Nuclear Medicine and Molecular Imaging and the Alzheimer's Association) and the European Federation of Neurological Societies and have similar recommendations for clinical use of amyloid PET[1,2]

Appropriate use of amyloid PET[1,2]

To qualify for an amyloid PET scan, patients should have all three of the following criteria:

1. When there is a cognitive complaint with objectively confirmed impairment
2. Where AD is a possible diagnosis but is uncertain after a comprehensive evaluation by a dementia expert
3. When knowledge of the presence or absence of Aβ pathology is expected to increase diagnostic certainty and alter management

Appropriate indications:

- In patients with persistent or progressive unexplained MCI
- In patients who satisfy core clinical criteria for possible AD because of unclear clinical presentation, either an atypical clinical course or an etiologically mixed presentation
- In patients with progressive dementia and atypically early age of onset (usually defined as age ≤65 years)

Aβ, amyloid beta; AD, Alzheimer's disease; MCI, mild cognitive impairment; PET, positron emission tomography

1. Sheikh-Bahaei N, et al. J Alzheimers Dis Rep 2017;1:71–88; 2. Johnson KA, et al. Alzheimers Dement 2013;9:e1–e16

Amyloid PET recommendations and guidelines (2/2)

The Amyloid Imaging Task Force (Society for Nuclear Medicine and Molecular Imaging and the Alzheimer's Association) and the European Federation of Neurological Societies and have similar recommendations for clinical use of amyloid PET[1,2]

Inappropriate use of amyloid PET[1,2]

- In patients with core clinical criteria for probable AD with typical age of onset[2]
- To determine dementia severity[1,2]
- On the sole basis of a positive family history of dementia or presence of apolipoprotein E ε4[1,2]
- Patients with a cognitive complaint that is unconfirmed on clinical examination[2]
- *In lieu* of genotyping for suspected autosomal mutation carriers[2]
- In asymptomatic individuals[1,2]
- Non-medical use (such as legal, insurance coverage, or employment screening)[2]

AD, Alzheimer's disease; PET, positron emission tomography

1. Sheikh-Bahaei N, et al. J Alzheimers Dis Rep 2017;1:71–88; 2. Johnson KA, et al. Alzheimers Dement 2013;9:e1–e16

Amyloid PET's impact on medical management

IDEAS was launched in 2016 by the Alzheimer's Association to test the impact of a brain amyloid PET scan on medical management of patients with MCI or dementia of uncertain etiology[1]

- A total of 946 dementia specialists at 595 US sites enrolled 16008 patients between February 2016 and September 2017; patients were followed up through January 2018[2]
  - Results in 11409 patients (60.5% with MCI) showed that the use of **amyloid PET was associated with changes in clinical management within 90 days in 60.2% of patients with MCI and 63.5% of patients with AD dementia**[2]
  - Etiologic diagnosis changed from AD to non-AD in 25.1% of patients and from non-AD to AD in 10.5% of patients[2]
- In clinically ambiguous cases of cognitive impairment from 2 academic institutions (n=112) enrolled in IDEAS, **lower cognitive test scores were predictive of positive amyloid PET** scan[3]
- Of the 30 patients with a negative amyloid PET scan, 90% had a diagnosis of non-AD etiology suggesting **negative amyloid PET can rule out AD diagnosis**[3]

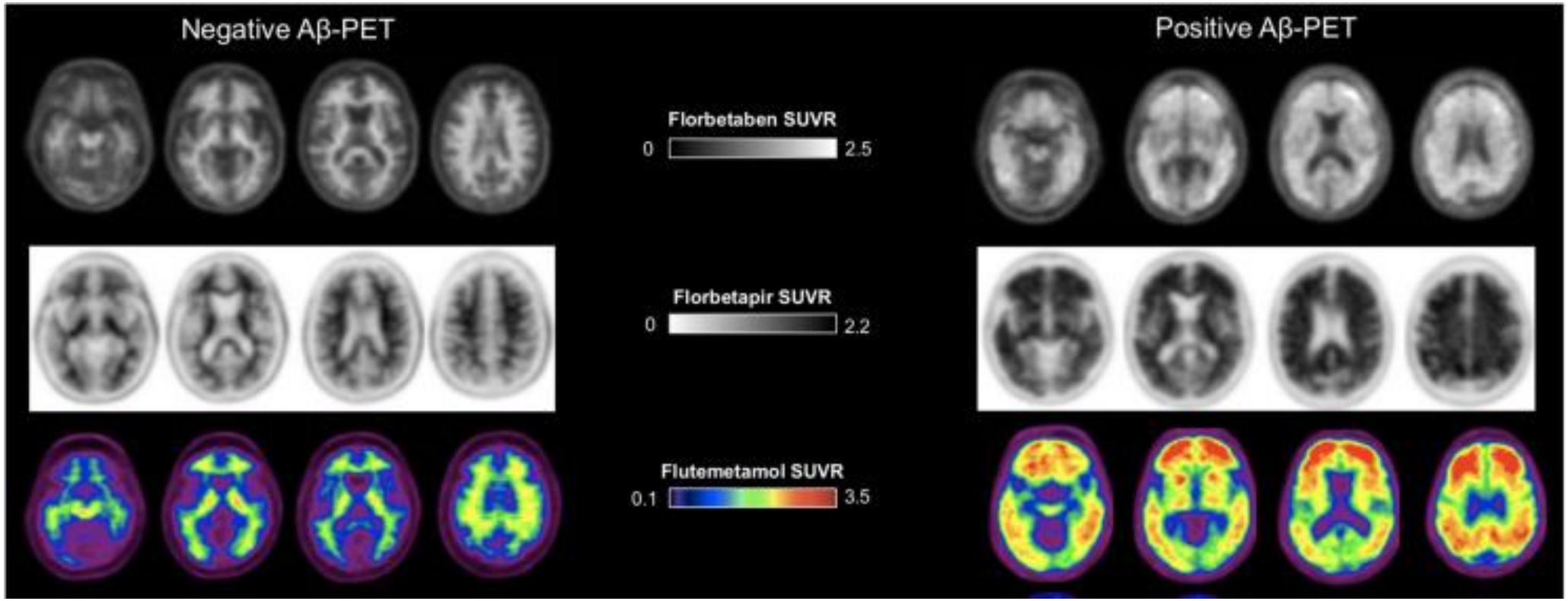
AD, Alzheimer's disease; IDEAS, Imaging Dementia—Evidence for Amyloid Scanning; MCI, mild cognitive impairment; PET, positron emission tomography

1. IDEAS study reaches recruitment goal, demonstrates value of PET scans. 2018. Available from: https://www.ideas-study.org/Original-Study; 2. Rabinovici GD, et al. JAMA 2019;321:1286–1294; 3. Pletnikova A, et al. Alzheimer Dis Assoc Disord. 2023 Aug 11. doi: 10.1097/WAD.0000000000000575. Epub ahead of print.

Visual read of negative and positive amyloid PET scans by tracer

Negative scan:
binding is restricted to white matter, showing a preserved gray matter-to-white matter contrast

Positive scan:
cortical gray matter binding is equal to or greater than binding in the white matter, with loss of gray matter-to-white matter contrast



This research was originally published in *JNM*. Chapleau M, et al. J Nucl Med. 2022;63(Suppl 1):13S-19S. © SNMMI.

Aβ, amyloid beta, PET, positron emission tomography

Chapleau M, et al. J Nucl Med. 2022;63(Suppl 1):13S-19S

Amyloid PET acquisition

- The current ADNI[1] protocol has been adopted by many studies for standardization and uses the following acquisition methods for ^{18}F-florbetapir and ^{18}F-florbetaben:
  - **Florbetapir:** 370 MBq (10.0 mCi) ± 10%, 20 min (4×5 min frames) acquisition at 50–70 min post injection
  - **Florbetaben:** 300 MBq (8.1 mCi) ± 10%, 20 min (4×5 min frames) acquisition at 90–110 min post injection
- The BIOFINDER[2] study used the following acquisition method for **^{18}F-flutemetamol**: approximately 180.9 MBq (range 171.5–187.6 MBq),[3] obtaining 10–20 min PET images starting approximately 60–120 minutes after intravenous injection[3–5]

ADNI, Alzheimer's Disease Neuroimaging Initiative; MBq, megabecquerel; mCi, millicurie; PET, positron emission tomography

1. ADNI. PET analysis. Available from: http://adni.loni.usc.edu/methods/pet-analysis-method/pet-analysis/ (Accessed June 19, 2023); 2. The Swedish BIOFINDER Study. Available from: http://biofinder.se/ (Accessed June 20, 2023); 3. Nelissen N, et al. J Nucl Med 2009;50:1251–1259; 4. VIZAMYL® (^{18}F-flutemetamol) package insert. 2013. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/203137s005lbl.pdf (Accessed December 17, 2021);
5. The Swedish BioFINDER 2 Study. Available from: https://clinicaltrials.gov/ct2/show/record/NCT03174938 (Accessed June 13, 2023)

Amyloid PET quantification (1/2)

- Routine clinical use of amyloid PET tracers involves visual assessment and binary categorization of scans, based on tracer-specific manufacturers' guidelines;[1,2] alternatively, a quantitative method, such as the standardised uptake value ratio (SUVR) can be implemented through CE/FDA-approved software[2]
  - SUVR is the ratio of radiotracer uptake in the cortex relative to reference region, such as the cerebellum and the pons (a site that does not accumulate amyloid in AD dementia)[1]
- Quantitative analysis and visual interpretation have generally been found to have similar sensitivity and specificity[1]
  - However, quantitative analysis may be more sensitive to low levels of amyloid[3]

Currently available quantitative measures: SUVR; the Centiloid (CL) scale; and reference-based z-scores. Both CL and z-scores are calculated based on SUVR[2]

AD, Alzheimer's disease; PET, positron emission tomography; SUVR, standardized uptake value ratio

1. Morris E, et al. Eur J Nucl Med Mol Imaging 2016;43:374–385; 2. Pemberton H, et al. Eur J Nucl Med Mol Imaging. 2022;49(10):3508–3528; 3. Suppiah S, et al. Diagnostics (Basel) 2019;9:65

Amyloid PET quantification (2/2)

Considerations for visual assessment[1]

- Readers require training[2]
- Interpretation depends on the observer's experience, which can influence diagnostic accuracy[1]
- Lacks precision (a binary scale; no cutoff value)[1]
- Possible partial volume effects[3]
- Comorbidities such as normal pressure hydrocephalus or other neurodegenerative disorders can complicate visual assessment[3]

Considerations for SUVR[2]

- Several factors may affect the outcome, e.g.:
  - Scan time after injection
  - Image reconstruction and processing
  - Partial volume correction
  - Region of interest delineation method
  - Reference region
  - Standard of truth
- As a result, optimal SUVR diagnostic thresholds can differ between sites

PET, positron emission tomography; SUVR, standardized uptake value ratio

1. Morris E, et al. Eur J Nucl Med Mol Imaging 2016;43:374–385; 2. Bullich S, et al. Neuroimage Clin 2017;15:325–332; 3. Pemberton H, et al. Eur J Nucl Med Mol Imaging. 2022;49(10):3508–3528

Standardization of PET imaging across clinics: the Centiloid (CL) method

The CL method is an approach to the quantification of amyloid radiotracer uptake, currently undergoing validation and **limited to research use**

The aims of widespread use of the CL approach are to:

1. Allow direct comparison of results across labs even when different analysis methods or radiotracers are employed
2. Provide a clear definition of cutoffs for the earliest signs of amyloid positivity in the brain
3. Provide further definition of the range of amyloid positivity characteristics of AD
4. Provide a more consistent representation of longitudinal change in standard units rather than as percent change
5. Allow for direct comparison of the characteristics of different radiotracers

AD, Alzheimer's disease; CL, Centiloid; PET, positron emission tomography

Klunk WE, et al. Alzheimers Dement 2015;11:1–31

Current evidence for the validation of the Centiloid method

Method

- CL is ranked on a 0 to 100 scale defined by young controls aged <45 years and patients with typical AD[1]
- The CL value is derived from the SUVR using a predetermined equation that varies depending on the tracer used[1]
- Data are available for the following radiotracers:
  - ^{18}F-NAV4694[2]
  - ^{11}C-PiB[1,2]
  - ^{18}F-florbetaben[3]
  - ^{18}F-flutemetamol[4]
- **However, using different methods or radiotracers requires lab-specific analyses for CL calculations[1]**

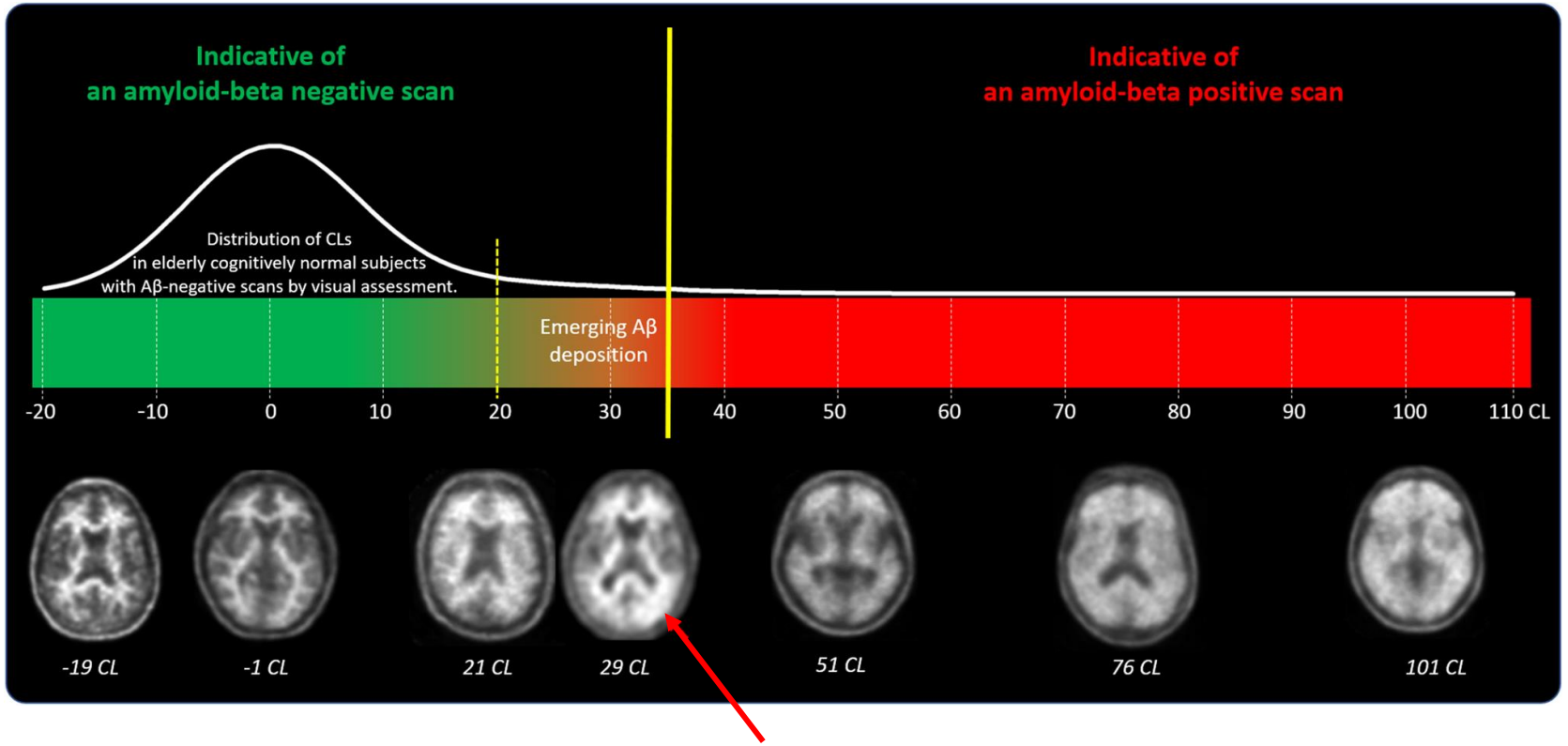
Evidence

- Pathology-based analysis has identified a score of >24.4 CL as a positive amyloid result using ^{11}C-PiB in 29/30 cases[5]
  - This threshold is reported to identity clinically relevant intermediate-to-high AD neuropathological changes
- For ^{18}F-florbetapir, a threshold of 24.1 CLs was found to equate to the SUVR value of 1.10[6]
  - This discriminates cases neuropathologically verified with none-to-sparse Aβ vs moderate-to-frequent neuritic plaques in an autopsy-confirmed cohort

Aβ, amyloid beta; AD, Alzheimer's disease; CL, Centiloid; PiB, Pittsburgh compound B; SUVR, standardized uptake value ratio

1. Klunk WE, et al. Alzheimers Dement 2015;11:1–31; 2. Rowe CC, et al. J Nucl Med 2016;57:1233–1237; 3. Rowe CC, et al. Eur J Nucl Med Mol Imaging 2017;44:2053–2059; 4. Battle MR, et al. EJNMMI Res 2018;8:107;
5. La Joie R, et al. Alzheimers Dement 2019;15:205–216; 6. Navitsky M, et al. Alzheimers Dement 2018;14:1565–1571

Quantitative assessment using the Centiloid method to supplement visual assessment



Subtle focal and/or unilateral amyloid accumulation

- **Centiloid (CL) values <20** represent elderly cognitively normal subjects with negative Aβ scans by visual assessment
- **Centiloid values in the range between 20 and 35 CL** are more likely to be ambiguous, can be either negative or positive by visual assessment, and correspond to subjects with emerging Aβ deposition

Image used with permission from Jovalekic A, et al. Eur J Nucl Med Mol Imaging. 2023 Jun 10. doi: 10.1007/s00259-023-06279-0. Epub ahead of print (CC-BY 4.0: http://creativecommons.org/licenses/by/4.0/).

Aβ, amyloid beta; CL, Centiloid

Advantages and limitations of the Centiloid method

Advantages

- CL scaling has low test-retest variability and may be considered a robust analysis method[1]
- This method has shown high correlations between ^{11}C-PiB and the ^{18}F tracers, ranging from 0.89 (^{18}F-florbetapir) to 0.99 (^{18}F-NAV4694) and 0.96 (^{18}F-florbetaben)[2–4]
- Data on the GAAIN website may be used to convert a global SUVR determined by a local method[2]

Limitations

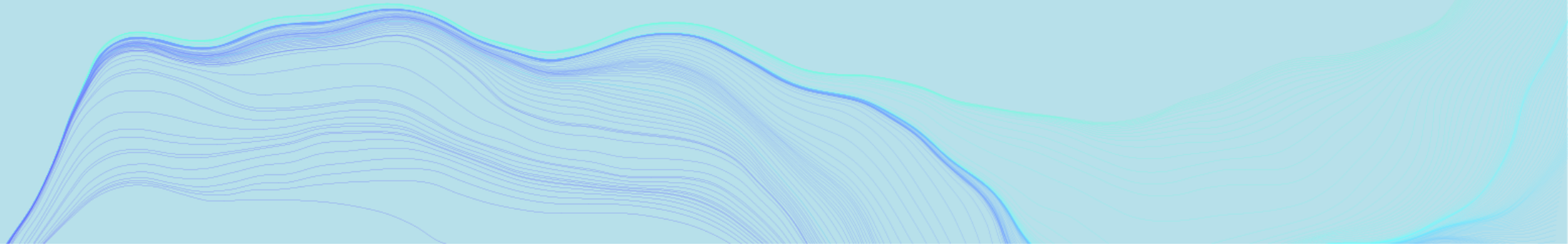
- Differences between PET systems and reconstruction methods have an effect on the use of the conversion equation across sites[2]
  - Further work is needed to determine if this could be an issue for the CL method, and whether equipment-specific equations are needed
- The CL method is being validated and is currently only used as a research tool

CL, Centiloid; GAAIN, Global Alzheimer's Association Interactive Network;
PET, positron emission tomography; PiB, Pittsburgh compound B; SUVR, standardized uptake value ratio

1. Battle MR, et al. EJNMMI Res 2018;8:107; 2. Rowe CC, et al. Eur J Nucl Med Mol Imaging 2017;44:2053–2059; 3. Rowe CC, et al. J Nucl Med 2016;57:1233–1237; 4. Navitsky M, et al. Alzheimers Dement 2018;14:1565–1571

Practical Zone

Utilizing tau PET biomarkers



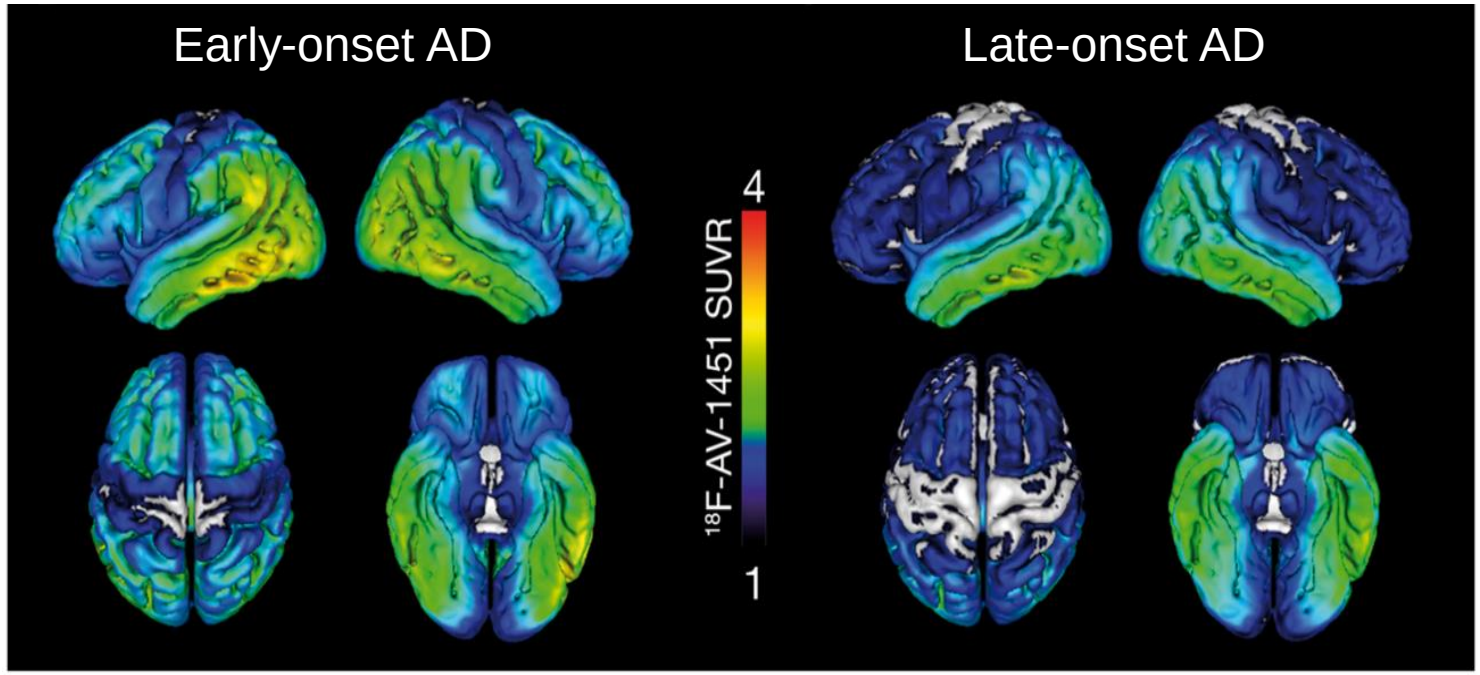
Increasing evidence demonstrates the clinical utility of tau PET

- Tau PET provides additional evidence to support AD diagnosis when combined with amyloid PET and clinical assessments[1–3]
- Tau PET correlates better with early cognitive changes in preclinical AD than amyloid PET and cortical thickness measures[4]
- The global intensity of tau PET signal, but not amyloid PET, predicts the rate of subsequent atrophy, independent of baseline cortical thickness[5]
- Tau PET positivity has been demonstrated as superior to CSF p-tau 181 and amyloid PET in predicting cognitive decline across the AD continuum within 3 years[6]
- Increased tau radiotracer, but not amyloid radiotracer, binding in specific brain regions is strongly associated with decreased cognitive performance across multiple domains[7]

AD, Alzheimer's disease; PET, positron emission tomography

1. Dubois B, et al. Lancet Neurol 2021;20:484–496; 2. Jack CR Jr, et al. Alzheimers Dement 2018;14:535–562; 3. Soleimani-Meigooni DN, et al. Brain 2020;143:3477–3494; 4. Ossenkoppele R, et al. Neurology 2019;92:e601–e612; 5. La Joie R, et al. Sci Transl Med 2020;12:eaau5732; 6. Bucci M, et al. Mol Psychiatry 2021;26:5888–5898; 7. Bejanin A, et al. Brain 2017:140;3286–3300

Tau PET imaging enhance the understanding of the pathological process of AD



Patients with early-onset AD are more prone to tau aggregation in widespread neocortical regions, whereas patients with late-onset AD showed peak tau radiotracer uptake in the medial temporal lobe[1]

AD, Alzheimer's disease; PET, positron emission tomography; SUVR, standardized uptake value ratio

Image used with permission from Schöll M, et al. Brain 2017;140:2286–2294

1. Schöll M, et al. Brain 2017;140:2286–2294

Amyloid PET vs tau PET in patients with typical and atypical AD

Unlike amyloid PET, tau PET corresponds to the clinical presentation of AD

Aβ pathology has been found to have absent-to-weak correlations with clinical features, while the severity and regional distribution of tau has been found associated with age at onset, clinical severity, phenotype, atrophy patterns, and *APOE ε4*

- **Amyloid PET** binding showed a widespread cortical distribution with subtle differences across atypical AD phenotypes and was unrelated to demographic/clinical variables or *APOE ε4*
- **Tau PET** binding was commonly elevated in temporoparietal regions, but showed marked phenotype-associated differences across different areas across atypical AD phenotypes

Aβ, amyloid beta; AD, Alzheimer's disease; APOE ε4; apolipoprotein ε4; PET, positron emission tomography
La Joie R, et al. Neurology 2021;96:e650–e661

Amyloid and tau deposition across dementia types

- Patterns of tau and amyloid deposition and accumulation can vary by dementia etiology
- As tau deposits are heterogenous (owing to different isoform confirmations and ultrastructures), the type of tauopathy can also vary by dementia etiology[1]
- Tauopathies can be classified by the number of repeats of the tau microtubule-binding domain (ie 3R, 4R, or 3R/4R)
  - Current tau PET tracers are selective for 3R/4R isoforms, which are found in AD in helical filaments, but are not present in other dementia etiologies (eg PSP and CBD contain 4R isoforms in straight filaments)[1,2]

Dementia etiology	Amyloid deposition	Tau deposition
Alzheimer's disease[3]	Yes	Yes
Hippocampal sclerosis[4]*	No	No
Dementia with Lewy bodies[3]	Yes	Yes
Frontotemporal dementia[3]	No	Yes or No
Progressive supranuclear palsy[5]	No	Yes
Corticobasal degeneration[6]	No	Yes

*Patients with hippocampal sclerosis present with hippocampal atrophy and may be differentiated from AD by biomarker testing

3R, 3-repeat tau protein; 4R, 4-repeat tau protein; AD, Alzheimer's disease; CBD, corticobasal degeneration; PET, positron emission tomography; PSP, progressive supranuclear palsy

1. Lois C, et al. Brain Imaging Behav 2019;13:333–344; 2. Soleimani-Meigooni DN, et al. Brain 2020;143:3477–3494; 3. Xia C, Dickerson BC. PET Clin 2017;12:351–359; 4. Jack Jr CR, et al. Alzheimers Dement 2018;14:535–562; 5. Ishiki A, et al. Eur J Neurol 2017;24:130–136; 6. Hassan A, et al. Expert Rev Neurother 2011;11:1569–1578

Consensus guidelines for ^{18}F-flortaucipir tau PET imaging

Patient selection

- The cause of cognitive impairment remains uncertain after clinical evaluation by an expert
- The disease history and routine examination cannot confirm the definitive diagnosis of AD
- There is a need to differentiate AD from other neurodegenerative tauopathies
- There is a need to determine the severity of tau deposition in AD

Not recommended

- To evaluate non-AD tauopathies
- Early stage tauopathy, owing to limited sensitivity
- Additional evidence is needed to support use of longitudinal assessment by tau PET

Consensus guidelines were developed by an international multidisciplinary taskforce
AD, Alzheimer's disease; PET, positron emission tomography
Tian M, et al. Eur J Nucl Med Mol Imaging 2022;49:895–904

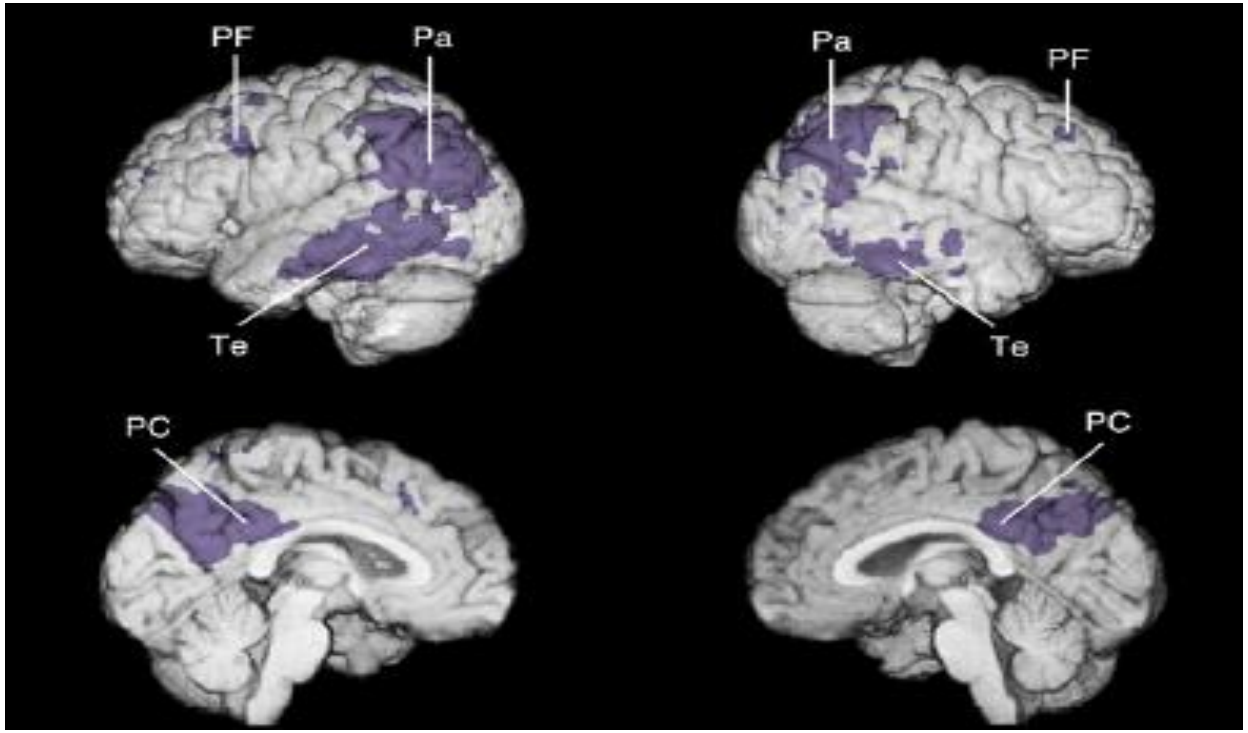
Practical Zone

Clinical utility of FDG PET

Reading FDG PET in AD

Key areas of reduced glucose metabolism in AD[1,2]

1. Parietal (lateral and precuneus)
2. Lateral temporal
3. Posterior cingulate
4. Prefrontal



AD, Alzheimer's disease; FDG PET, fluorodeoxyglucose positron emission tomography; Pa, parietal; PC, posterior cingulate; PF, prefrontal; Te, temporal
Image used with permission from Reiman EM, et al. N Engl J Med 1996;334:752–758

1. Reiman EM, et al. N Engl J Med 1996;334:752–758; 2. Marcus C, et al. Clin Nucl Med 2014;39:e413–e426

Limitations of utilizing FDG PET in AD

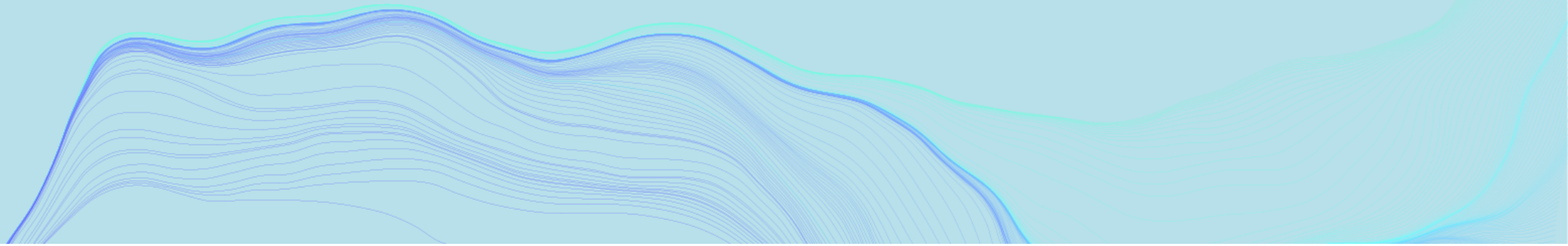
- Hypometabolism reflects clinical deficits, so findings are mild or subtle in MCI[1]
- Changes are less clear in the more elderly (>75 years of age)[1]
- Cannot exclude other causes of neurodegeneration
  - Sensitivity 77%; specificity 80% for AD vs DLB[1]

AD, Alzheimer's disease; DLB, dementia with Lewy bodies; FDG PET, fluorodeoxyglucose positron emission tomography; MCI, mild cognitive impairment

1. Massa F, et al. Eur J Nucl Med Mol Imaging 2022;49:1263–1274

Practical Zone

Clinical utility of CSF biomarkers



Automated CSF biomarker assays

ELECSYS®

- Elecsys® t-tau and p-tau CSF assays demonstrate good analytical performance with clinically relevant measuring ranges that could be incorporated into a clinical setting[1]
- An extended panel of Elecsys® CSF assays called the NeuroToolKit is being studied in relation to AD progression[2,3]
  - Data suggest that the NeuroToolKit immunoassays correlate with amyloid PET positivity[2,4]

LUMIPULSE®

- Lumipulse® G, a fully automated system, has demonstrated capability in measuring routine CSF biomarkers of AD in one instrument[5]
- Lumipulse® G correlates well with previously established methods and shows good inter-laboratory reproducibility[5]

Three FDA-approved CSF tests: Elecsys® p-tau181/Aβ42, t-tau/Aβ42; and Lumipulse® Aβ42/Aβ40 based on ~90% concordance with amyloid PET visual read[6–8]

AAIC, Alzheimer's Association International Conference; Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; PET, positron emission tomography; p-tau, phosphorylated-tau; t-tau, total-tau

1. Lifke V, et al. Clin Biochem 2019;72:30–38; 2 Molinuevo JL, et al. Journal of Neurol, Neurosurg Psychiatry 2022;93:A47-A48 ; 3. Mila-Aloma M, et al. Alzheimer's Dement. 2020;16:1358–1371; 4. Doecke JD, et al. Alzheimer's Research & Therapy (2020) 12:36; 5. Clarin M, et al. Poster presented at AAIC 2019 (Abstract P1-266); 6. FDA Permits Marketing for New Test to Improve Diagnosis of Alzheimer's Disease. Available at: https://www.fda.gov/news-events/press-announcements/fda-permits-marketing-new-test-improve-diagnosis-alzheimers-disease (Accessed June 2023); 7. Roche Gets FDA Clearance for CSF Beta-Amyloid and Phospho-Tau Alzheimer Disease Assays. Available from: https://www.neurologylive.com/view/roche-gets-fda-clearance-csf-beta-amyloid-phospho-tau-alzheimer-disease-assays (Accessed June 2023); 8. Roche Alzheimer's disease Cerebrospinal Fluid (CSF) assays receive FDA clearance, supporting more accurate and timely diagnosis. Available at: https://diagnostics.roche.com/us/en/news-listing/2022/roche-alzheimers-disease-cerebrospinal-fluid-assays-receive-fda-clearance.html (Accessed June 2023)

Clinical performance of CSF assays in the evaluation of brain amyloid positivity

	CSF Assays		
Assay	**Lumipulse G® CSF Aβ42/Aβ40**	**Elecsys® CSF p-tau181/Aβ42**	**Elecsys® CSF t-tau/Aβ42**
Assay type	Immunoassay[1,2]	Immunoassay[3]	Immunoassay[4]
Analyte	Aβ42/40	p-tau181/Aβ42	t-tau/Aβ42
Cohort size	274	646	646
Prevalence (% Amyloid PET positive)	69%	53.7%	53.7%
Sensitivity	86.3%	88.2%	85.0%
Specificity	92.9%	92.6%	94.0%
PPV	96.5%	93.3%	94.9%
NPV	75.0%	87.1%	84.4%
Regulatory Status	US IVD	US IVD	US IVD

Aβ, amyloid beta; IVD, in vitro diagnostic; NPV, negative predictive value; PET, positron emission tomography; PPV, positive predictive value; p-tau, phospho-tau; t-tau, total tau

1. FDA DEN200072: https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN200072.pdf (Accessed July 2023); 2. Esquivel RN, et al. Poster presented at AAIC 2021 July 26–July 30, 2021, Denver, CO •https://us.fujirebio.com/l/652113/2022-10-14/66vfc/652113/1665756691TIys9vB4/Clinical_validation_of_the_Lumipulse_amyloid_ratio_in_ADNI_CSF_samples.pdf (Accessed July 2023); 3. FDA K221842: https://www.accessdata.fda.gov/cdrh_docs/pdf22/K221842.pdf (Accessed July 2023); 4. FDA K231348: https://www.accessdata.fda.gov/cdrh_docs/reviews/K231348.pdf (Accessed July 2023);

Appropriate use of lumbar puncture and CSF biomarkers

Recommendations of the Alzheimer's Association

- **MCI that is persistent, progressing, and unexplained**
- Patients with symptoms that **suggest possible AD**
- Meeting **core clinical criteria for probable AD** with typical age of onset
- MCI or dementia with an **onset at an early age (<65 years)**
- Patients whose **dominant symptom is a change in behavior** (eg Capgras syndrome, paranoid delusions, unexplained delirium, combative symptoms, and depression) and where **AD diagnosis is being considered**
- Patients with subjective cognitive decline (cognitively unimpaired based on objective testing) who are considered to be **at increased risk for AD dementia**

AD, Alzheimer's disease; CSF, cerebrospinal fluid; MCI, mild cognitive impairment

Shaw LM, et al. Alzheimers Dement 2018;14:1505–1521

Inappropriate use of lumbar puncture and CSF biomarkers (1/2)

Recommendations of the Alzheimer's Association

- **Cognitively unimpaired** and **within normal range functioning** for age as established by objective testing; **no conditions suggesting high risk** and no subjective cognitive decline or expressed concern about developing AD
- **Cognitively unimpaired** patient based on objective testing, but considered by patient, family informant, and/or clinician to be **at risk for AD based on family history**
- **Patients with subjective cognitive decline** (cognitively unimpaired based on objective testing) who are not considered to be at increased risk for AD

AD, Alzheimer's disease; APOE ε4, apolipoprotein E ε4; CSF, cerebrospinal fluid

Shaw LM, et al. Alzheimers Dement 2018;14:1505–1521

Inappropriate use of lumbar puncture and CSF biomarkers (2/2)

Recommendations of the Alzheimer's Association

- Symptoms of **REM sleep behavior disorder**
- Use **to determine disease severity** in patients having already received a diagnosis of AD
- Individuals who are ***APOE* ε4 carriers with no cognitive impairment**
- Use of lumbar puncture ***in lieu* of genotyping for suspected autosomal dominant mutation carriers**
- Autosomal dominant mutation carriers, with or without symptoms

AD, Alzheimer's disease; APOE ε4, apolipoprotein E ε4; CSF, cerebrospinal fluid; REM, rapid eye movement

Shaw LM, et al. Alzheimers Dement 2018;14:1505–1521

Comparison of PET Imaging vs CSF Biomarkers

	PET imaging[1]	CSF measures[1]
Contraindications	None	Treatment with anticoagulants, spinal defects
Most common side effects	Injection site irritation and pain, flushing, increased blood pressure, and headache (<5% of patients)[2]	Post-lumbar puncture headache, which occurs in 1–10% of investigations in memory clinic settings. More serious side effects of lumbar puncture may occur, such as infection or brain herniation
Variability of the measure across centers and methods	Low	Currently considerable, but commercialized fully automated assays may have helped solving this
Individual variability of values in healthy individuals	Low	Quite high, but can be corrected for by measuring changes over time or by using ratio-based approaches
Information about tau biomarker status with the same scan or sample	Not available	Potentially available with phosphorylated tau amounts
Information on extent of amyloid pathology	Available; distribution of amyloid pathology might indicate the amyloidosis stage	Not available
Information on location of amyloid pathology	Available	Not available

CSF, cerebrospinal fluid; PET, positron emission tomography

1. Chételat G, et al. *Lancet Neurol*. 2020;19(11):951-962; 2. Amyvid™ Prescribing Information. December 2019; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/202008s036lbl.pdf (Accessed Aug 2023)

Practical Zone

Practical considerations when conducting a lumbar puncture procedure

Collecting CSF by lumbar puncture

- A lumbar puncture is a safe and standard medical procedure performed by trained HCPs:[1,2]
  - For diagnosis of neurologic conditions, such as AD
  - To investigate or exclude meningitis or subarachnoid hemorrhage
  - For measurement of CSF pressure
- It can be safely performed in outpatient settings, including in memory clinics[1]

AD, Alzheimer's disease; CSF, cerebrospinal fluid; HCP, healthcare professional

1. Duits FH, et al. Alzheimers Dement 2016;12:154–163; 2. Wright BL, et al. J Neurol 2012;259:1530–1545

Contraindications for lumbar puncture

- Space-occupying lesions with mass effect
- Posterior fossa mass

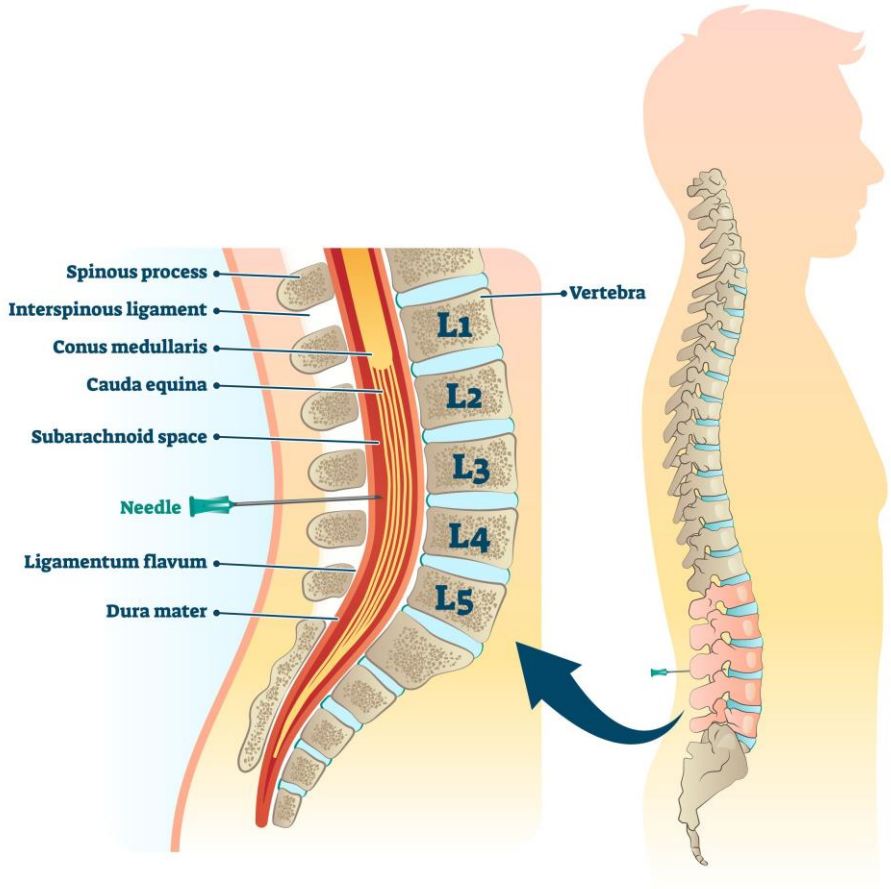
- Anticoagulant medication, coagulopathy, or uncorrected bleeding diathesis

- Congenital spine abnormalities

- Skin infection at puncture site

Engelborghs S, et al. Alzheimers Dement Diagn Assess Dis Monit 2017;8:111–126

Lumbar puncture procedure (1/2)

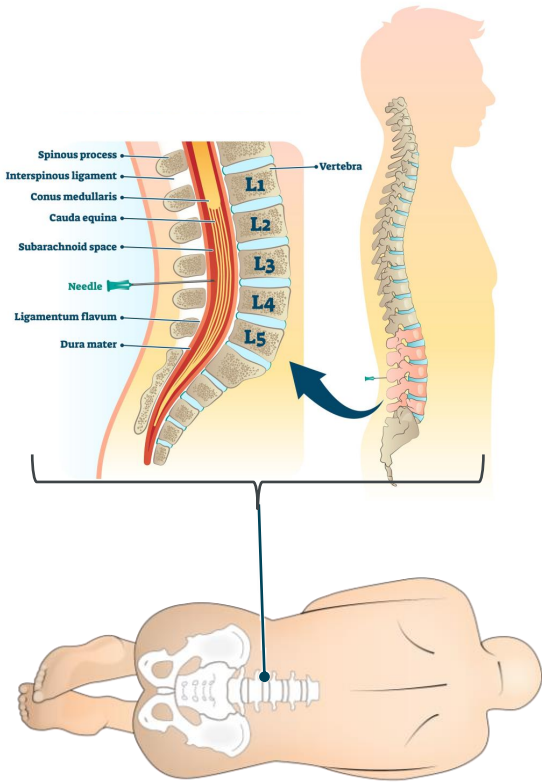


CSF is collected by inserting a needle in the subarachnoid space located below the end of spinal cord (below conus medullaris), between the third and fourth, or fourth and fifth lumbar vertebral spinous processes[1,2]

CSF, cerebrospinal fluid

1. Engelborghs S, et al. Alzheimers Dement Diagn Assess Dis Monit 2017;8:111–126; 2. Wright BL, et al. J Neurol 2012;259:1530–1545

Lumbar puncture procedure (2/2)



1. Obtain informed consent

2. Position patient

3. Identify landmarks
Supracristal line intersects L4 spinous processes

4. Preparation
Wash hands and put on sterile gloves, unwrap and check all equipment. Disinfect site and apply sterile drapes

5. Lumbar puncture
Introduce an atraumatic spinal needle through marked site. Gently advance needle through ligaments until you feel a 'give'. Withdraw stylet from needle; CSF should begin to drip out

6. Anesthesia
Inject lidocaine under skin at marked site to raise a small wheal

7. CSF collection
Serially collect enough CSF for testing, labeling polypropylene tubes in order of collection. Replace stylet, remove needle, and apply sterile dressing

8. Aftercare
Advise patient to mobilize early if able to, but not to undertake any heavy lifting, etc. after the procedure. If unable, lie flat until able to mobilize

CSF, cerebrospinal fluid; HCP, healthcare professional; LP, lumbar puncture

Bottom image taken from: Engelborghs S, et al. Alzheimers Dement Diagn Assess Dis Monit 2017;8:111–126 (License: CC BY 4.0 https://creativecommons.org/licenses/by/4.0/)
Wright BL, et al. J Neurol 2012;259:1530–1545

Post-lumbar puncture headache

Post-lumbar headache is classified as a headache following a lumbar puncture that worsens after sitting or standing and improves after lying down. It can be accompanied by:[1–4]

- Neck stiffness
- Tinnitus
- Hypacusia (hyperacusis)
- Photophobia
- Nausea
- Hearing loss

- Develops within 5 days of lumbar puncture
- Usually resolves spontaneously within 2 weeks OR after sealing of the leak with an epidural blood patch (sometimes required)

The exact pathophysiology of post-lumbar puncture headache is unknown, but it is thought to occur as a result of CSF leakage after needle removal and a fall in CSF pressure[5,6]

CSF, cerebrospinal fluid

1. Nath S, et al. Lancet 2018;391:1197–1204; 2. Headache Classification Committee of the International Headache Society. Cephalalgia 2018;38:1–211; 3. Michel O, Brusis T. Ann Otol Rhinol Laryngol 1992;101:390–394; 4. Wright BL, et al. J Neurol 2012;259:1530–1545; 5. Serpell MG, Rawal N. BMJ 2000;321:973–974; 6. Grant R, et al. J Neurol Neurosurg Psychiatry 1991;54:440–442

Considerations for lumbar puncture

Post-lumbar puncture headache:

- Occurs in 4–11% of patients, depending on needle type used[1]
- Generally mild and resolves without treatment but can be treated with mild analgesics if required.[2] In rare instances, if the headache persists, an epidural blood patch procedure may be needed for relief[2]
- Risk is lower in elderly patients and can be mitigated by using atraumatic needles[2]

Reducing the incidence of side effects associated with lumbar puncture

- **Smaller needle diameters**:
  - Reduced incidence of headache[1,2]
- **Atraumatic needles**
  - Associated with fewer side effects[1]
  - Result in fewer headaches[1]
  - Reduce the requirement for epidural blood patch[1]

Some mild side effects can be increased if patients fear complications

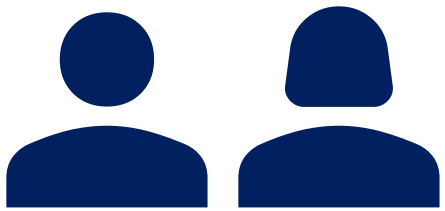
Tips to alleviate fear of complications:

1. Providing patients with information before and during the lumbar puncture
2. Including information on possible complications and steps to take if they occur after the procedure

CSF, cerebrospinal fluid

1. Nath S, et al. Lancet 2018;391:1197–1204; 2. Ahmed SV et al. Postgrad Med J 2006;82:713–716; 3. Michel O, Brusis T. Ann Otol Rhinol Laryngol 1992;101:390–394

Patient concerns around lumbar puncture (1/2)



The procedure is risky, and there are too many potential complications

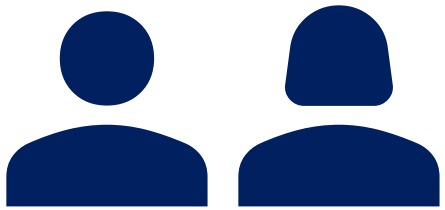
It will be painful

- The risk of complications is low[1]
- Lumbar puncture is a safe and common procedure, regularly used as a diagnostic test[1,2]

- Local anesthetic can be given at the site of the lumbar puncture[2,3]
- The risk of side effects is low, and they are usually mild[1]

1. Duits FH, et al. Alzheimers Dement 2016;12:154–163; 2. Wright BL, et al. J Neurol 2012;259:1530–1545; 3. Engelborghs S, et al. Alzheimers Dement Diagn Assess Dis Monit 2017;8:111–126

Patient concerns around lumbar puncture (2/2)

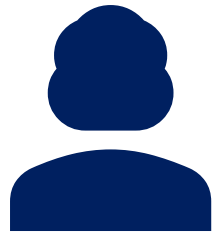


There are too many side effects

- Doctors use special techniques to lower the risk of side effects[1,2]
- Serious complications are extremely rare. Headache is the most common side effect, occurring in ~10% of patients[3]
- Headache is mild in most cases, usually resolves without treatment, and is helped by lying down[2]

What will the procedure tell me?

- To determine the cause of memory complaints, doctors will test the CSF for abnormal concentrations of Aβ and tau
- This gives us information about the concentrations of these proteins in the brain and can help your doctor confirm or rule out a diagnosis of AD[4]

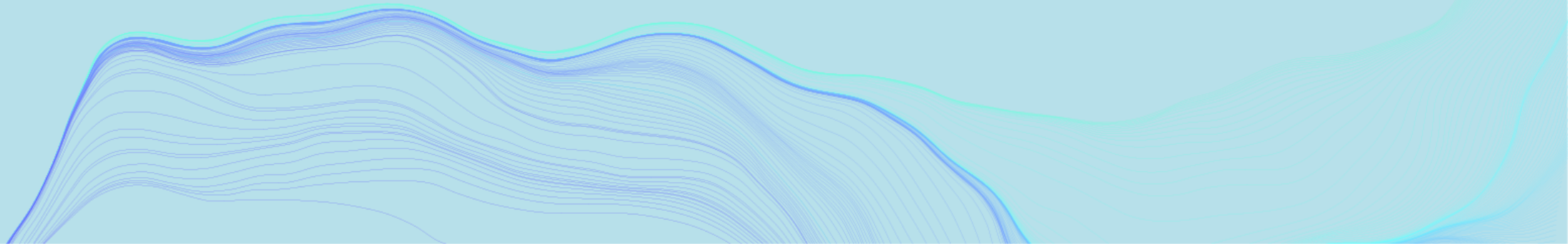


Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid

1. Hansson O, et al. Alzheimers Dement 2018;14:1313–1333; 2. Ahmed SV et al. Postgrad Med J 2006;82:713–716; 3. Duits FH, et al. Alzheimers Dement 2016;12:154–163; 4. Frisoni GB, et al. Neurobiol Aging 2017;52:119–131

Practical Zone

Example protocol for CSF collection, handling, and storage



CSF testing considerations

Ordering CSF biomarkers requires careful adherence to pre-analytical sample handling procedures. Aβ42 is highly influenced by pre-analytical factors, such as CSF collection and sampling handling

AD CSF collection has unique requirements recommended by the Alzheimer's Association International guidelines and listed in the test manufacturer package insert

Connect with the laboratory for package insert of the test and lab-specific requirements for specimen collection, storage and transport, and sourcing of low-bind tubes

- Polypropylene test tubes are recommended for CSF storage as they are 'low binding', ensuring minimal loss of Aβ through adherence to the tube surface

Aβ, amyloid beta AD, Alzheimer's disease; CSF, cerebrospinal fluid

1. Hansson O, et al. Alzheimers Dement 2021;1–8; 2. Hansson O, et al. Alzheimers Dement 2018;14:1313–1333

Impact of pre-analytical variables on CSF biomarker concentrations

Consistent CSF collection, handling, and storage conditions are recommended to ensure the validity and reliability of CSF biomarker results.[1] This is essential for establishing biomarker cutoff values, diagnosis and treatment of AD, and clinical trial enrollment[2,3]

Time taken from sample collection to analysis

CSF COLLECTION

There is limited evidence for differences between CSF aspiration or gravitational collection on biomarker results[1]

CSF STORAGE / TREATMENT

Most important considerations:[1,4]

- Tube material (polypropylene)
- Surface/volume ratio
- Storage: length of storage and sample state ie frozen or non-frozen (ambient temp)

Recommendations for CSF storage / treatment

- ✓ Polypropylene test tubes are recommended for CSF storage as they are ‘low binding’, ensuring minimal loss of Aβ through adherence to the tube surface[1]
- ✓ CSF samples can be transported at ambient temperatures within 3 days of collection and can then be stored at lower temperatures for ≤2 weeks before being analysed[4]
- ✓ CSF concentrations of Aβ42, t-tau, and p-tau have been found to be impacted by multiple freeze-thaw cycles; most studies found no impact following one freeze-thaw cycle[1]

Aβ, amyloid beta; CSF, cerebrospinal fluid; p-tau, phosphorylated tau; t-tau, total tau

1. Hansson O, et al. Alzheimers Dement 2018;14:1313–1333; 2. Hansson O, et al. Alzheimers Dement 2018;14:1470-1481; 3. Teunissen CE, et al. Neurology 2009;73:1914–1922; 4. Janelidze S, et al. Alz Res Therapy 2019;11:63

Pre-analytical protocol for CSF collection, storage, and sample handling (1/3)

A workgroup, led by the Alzheimer's Association, has developed a simplified, standardized pre-analytical protocol intended for routine clinical testing involving Aβ1–42

General recommendations

- ✓ Fasting is not required for CSF collection
- ✓ The first 1–2 mL of collected CSF should not be used for AD biomarker diagnostics
- ✓ After the first 1–2 mL has been discarded, CSF should be collected using the drip method, directly into a low-binding tube and should come from the first 20 mL. After that tube is full, additional CSF can be collected if needed for other tests
- ✓ Using a low binding tube is recommended; the same tube should be used for collection, transport and measurement of CSF. The low binding tube must be validated, and established biomarker cutoffs only apply with a certain filling; fill volume should be specified

Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; LP, lumbar puncture

1. Hansson O, et al. Alzheimers Dement 2021;1–8

Pre-analytical protocol for CSF collection, storage, and sample handling (2/3)

Fresh vs frozen CSF samples

Fresh CSF

- ✓ Fresh CSF should not be mixed or centrifuged (unless visible blood contamination)
- ✓ The validated tubes with a predetermined fill volume of CSF should be transported (with cold blocks) and stored at 2–8°C for up to 14 days before analyses
- ✓ Samples can be stored at room temperature for up to 48 hours before analyses, but transport with cold blocks is still recommended

Frozen CSF

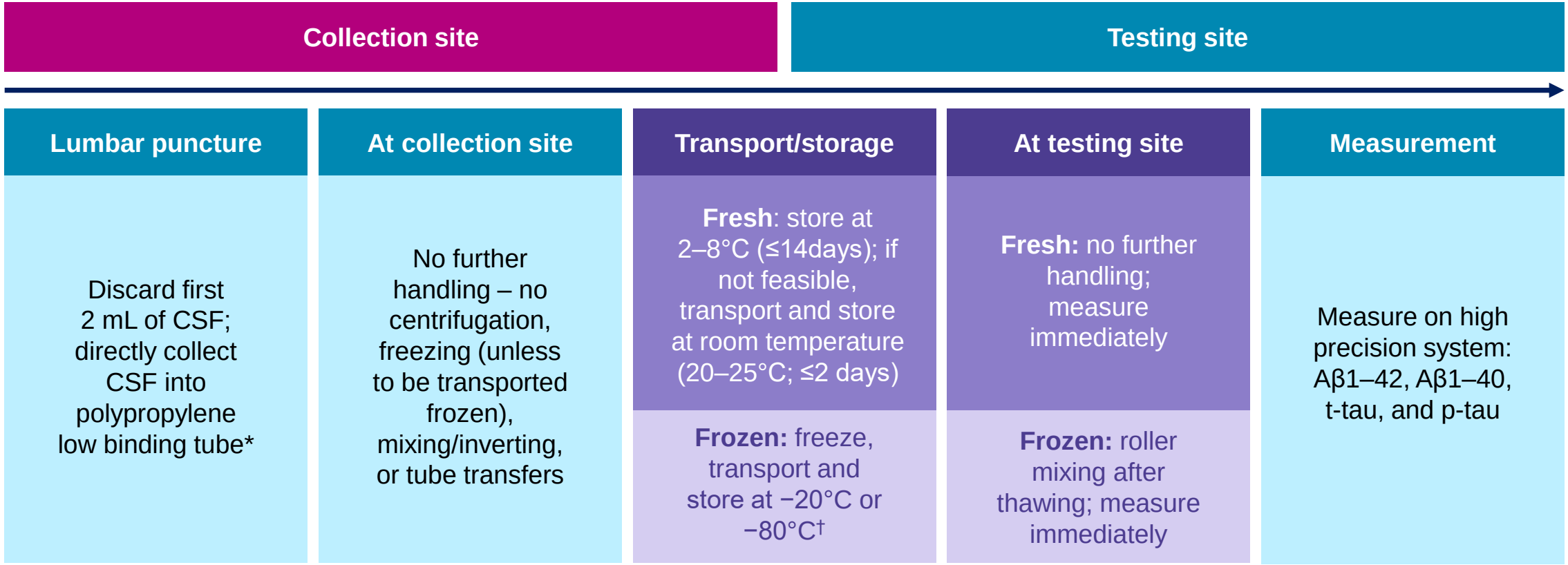
- ✓ The validated tube with a predefined volume of CSF can be frozen at −20 or −80°C during storage and transport for up to 2 weeks
- ✓ For freezing at −80°C, tubes validated for this temperature must be used

CSF, cerebrospinal fluid

Hansson O, et al. Alzheimers Dement 2021;17:1575–1582

Pre-analytical protocol for CSF collection, storage, and sample handling (3/3)

Protocol for CSF sampling



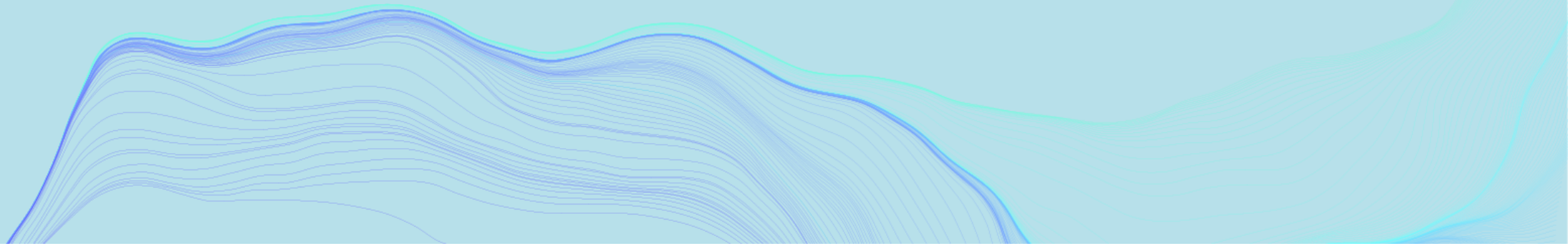
NB. Some manufacturers provide their own tubes / fact sheets for clinicians to use; the protocol allows for differences between analyzers

*Follow manufacturer recommendations of tube type and filling volume; †Follow tube and assay manufacturer's instructions for use
Aβ, amyloid beta; CSF, cerebrospinal fluid; p-tau, phosphorylated tau; t-tau, total tau

Hansson O, et al. Alzheimers Dement 2021;17:1575–1582

Practical Zone

Potential clinical utility of blood-based biomarkers



Potential utility of blood-based biomarkers in clinical practice

	Triage	Confirmatory
	Rule-in test for amyloid pathology in symptomatic individuals followed by amyloid PET or CSF testing in those with a positive test (high risk or investigated for cognitive issues) **Rule-out test** for amyloid pathology in symptomatic individuals (low risk of AD)	**Alternative** for amyloid PET or CSF as a test to determine brain amyloid pathology
Test characteristics	**For ruling in: BBBM requires high specificity, high PPV, low false positive rate** **For ruling out: BBBM requires high sensitivity, high NPV, low false-negative rate**	**Highly accurate performance with diagnostic accuracy and performance close to amyloid PET and CSF tests**

AD, Alzheimer's disease; BBBM, blood-based biomarkers; CSF, cerebrospinal fluid; LP, lumbar puncture; NPV, negative predictive value; PET, positron emission tomography; PPV, positive predictive value

Table used with permission from Hampel H, et al. Neuron 2023

Hampel H, et al. Neuron. 2023 Jun 5:S0896-6273(23)00390-2. doi: 10.1016/j.neuron.2023.05.017. Epub ahead of print.

Alzheimer's Association recommendations on the current use of blood-based biomarkers in clinical practice

Specialized memory clinics

Blood-based biomarkers with established thresholds should only be used in **symptomatic patients at specialist clinics** and the results should be confirmed with CSF or PET, whenever possible

Additional data are needed before the use of blood-based biomarkers as stand-alone diagnostic markers*

Primary care

Additional data are needed for use of blood-based biomarkers e.g., studies in diverse populations, and to assess optimal combinations of biomarkers in this setting*

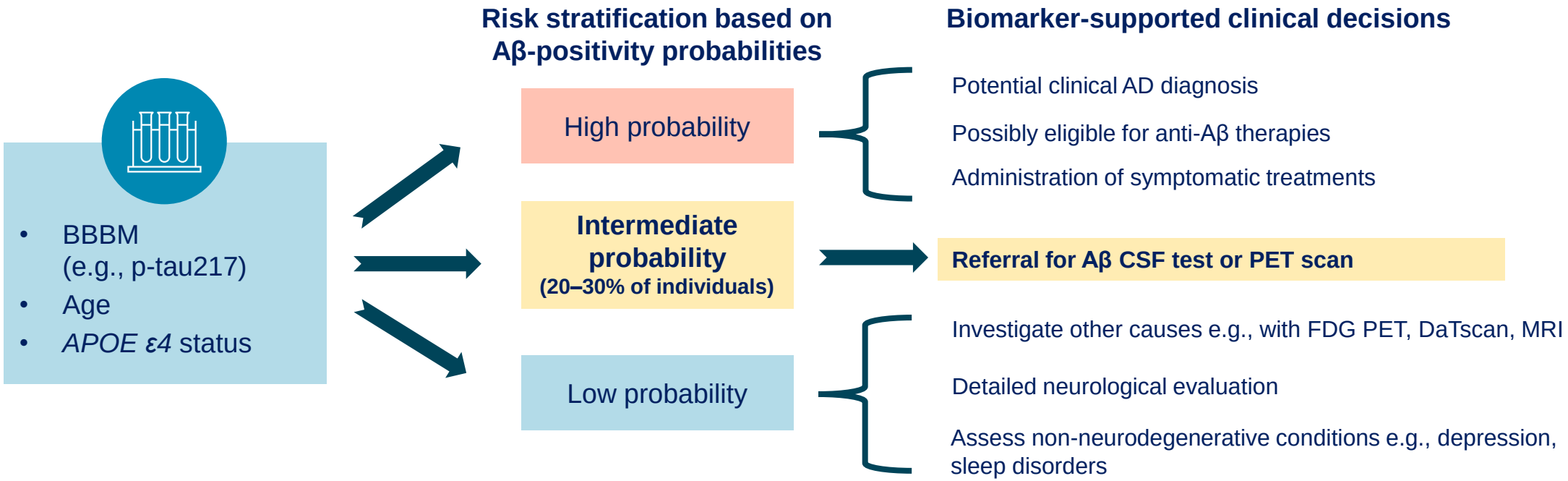
Owing to the lower cost and improved accessibility compared with CSF and PET measurements, blood-based biomarkers have the potential to revolutionize the diagnostic and prognostic workup of AD globally

***Note: Recommendations reflect the evidence base at the time of the publication and will likely evolve as new supporting data emerges**

CSF, cerebrospinal fluid; PET, position emission tomography

Hansson O, et al. Alzheimers Dement 2022;18:2269–2686

Risk stratification with BBBMs may reduce the burden of CSF or PET testing for patients and memory clinics



In a study evaluating a BBBM-based risk prediction model as a first-line screening tool in detecting amyloid PET status, applying a lenient, intermediate or stringent threshold* reduced the number of individuals requiring confirmatory testing with CSF or PET biomarkers by **85.9%**, **72.7%** and **61.2%**, respectively.

*Threshold sensitivity and specificity: lenient 90%, intermediate 95%, stringent 97.5%. The more stringent thresholds improved the accuracy of the risk prediction model in predicting AB-PET status, but also increased the size of the intermediate group requiring further testing (88.2%, 90.5% and 92.0% overall classification accuracy for the lenient, intermediate and stringent thresholds, respectively).

Aβ, amyloid beta; AD, Alzheimer's disease; BBBM, blood-based biomarker; CSF, cerebrospinal fluid; DaT, dopamine active transporter; FDG, fluorodeoxyglucose; MRI, magnetic resonance imaging; PET, positron emission tomography

Brum, WS, et al. Nat Aging 2023 E-pub ahead of print. doi: 10.1038/s43587-023-00471-5

Variables that may affect AD blood-based biomarkers

As blood-based biomarkers begin to enter clinical use, it is important to understand the factors that may affect their levels – both for interpretation of results and establishing reference ranges[1]

Participant demographics[1,2]

- Plasma biomarkers **Aβ42, Aβ40, NfL, p-tau,* and t-tau** have been shown to correlate with **age**
- **Aβ42/40** was shown to negatively correlate with **age** in participants with MCI or dementia
- **Women** who were cognitively unimpaired had higher levels of **t-tau** and lower levels of **Aβ42/40** than **men**

Medical conditions[1,2]

- Plasma biomarkers **Aβ42, Aβ40, Aβ42/40, NfL, p-tau,* and t-tau** have been shown to correlate with **CKD**
- **Myocardial infarction, stroke, hypertension, atrial fibrillation, and diabetes** have been found to influence biomarker levels

BMI[2]

- BMI **≥40** was associated with higher levels of **Aβ42, Aβ40, and t-tau** compared with participants in the BMI reference group (**18.5–24.9**)
- Participants with a BMI of **25–29.9 and 30–38.9** had lower levels of **NfL** compared with participants with a BMI of **18.5–24.9**

*P-tau181 and p-tau217

Aβ, amyloid beta, AD, Alzheimer's disease; BMI, body mass index; CKD, chronic kidney disease; MCI, mild cognitive impairment; NfL, neurofilament light chain; p-tau, phosphorylated-tau; t-tau, total tau

1. Mielke M, et al. Nat Med 2022;28:1398–1405; 2. Syrjanen JA, et al. Alzheimers Dement 2022;18:1128–1140.

Current clinical status of blood-based biomarkers
September 2023

Clinically available as a laboratory-developed test (LDT) under Clinical Laboratory Improvement Amendments (CLIA) or cleared as *in vitro* diagnostic devices (IVD) by the US FDA

Assay	Analyte	Assay type	CLIA (LDT)	IVD status
C2N: PrecivityAD™,[1]	Aβ42/40+ APOE (+age)	**IP-LC-MS/MS**	✓	Granted Breakthrough Device designation by FDA[2]
C2N: PrecivityAD2™,[3]	Aβ42/40 + p-tau217/nptau-217 ratio	**IP-LC-MS/MS**	✓	
Sysmex: HISCL™ β-Amyloid 1–42 / 1–40 Assay Kits[4,5]	Aβ42/40	**Immunoassay**		PMDA, Japan*
Quest: AD-Detect[6,7]	Aβ42/40	**IP-LC-MS/MS**	✓	
Quanterix: LucentAD[8]	p-tau181	**Simoa immunoassay**	✓	Granted Breakthrough Device designation by FDA[9]
LabCorp: Aβ42/40 test[10]	Aβ42/40	**Chemiluminescence enzyme immunoassay (CLEIA)**	✓	
LabCorp: p-tau181 test[11]	p-tau181	**Electrochemiluminescence Immunoassay (ECLIA)**	✓	

*not yet available in the US

Aβ, amyloid beta; AD, Alzheimer's disease; APOE, apolipoprotein E; FDA, Food and Drug Administration; IP, immunoprecipitation; LC, liquid chromatography; MS, mass spectrometry; PMDA, Pharmaceuticals and Medical Devices Agency

1. Hu Y, et al. JAMA Network Open 2022;5:e228392; 2. Press release. Available from: https://c2n.com/news-releases/2019/01/29/2019-1-24-c2n-diagnostics-receives-breakthrough-device-designation-from-us-fda-for-blood-test-to-screen-for-alzheimers-disease-risk (Accessed September 11, 2023); 3. PrecivityAD2™. The PrecivityAD2™ Blood Test Specifications; 4. Yamashita K, et al. Alzheimers Res Ther. 2022;14:86; 5. Press release. Available from: https://www.sysmex.co.jp/en/news/2023/230622.html (Accessed September 11, 2023); 6. Weber D, et al. Presented at AAIC 2022; 7. Quest AD-Detect Test details. Available from: https://testdirectory.questdiagnostics.com/test/test-detail/11786/quest-ad-detect-beta-amyloid-4240-ratio-plasma?cc=MASTER; 8. Lucent Diagnostics. A guide for providers. Available from: https://www.lucentdiagnostics.com/wp-content/uploads/2023/06/LucentAD_ProviderGuide_6302023.pdf (Accessed September 5, 2023); 9. Quanterix Press Release. Available from: https://www.quanterix.com/press-releases/quanterix-granted-breakthrough-device-designation-from-u-s-fda-for-blood-based-ptau-181-assay-for-alzheimers-disease/ (accessed September 2023); 10. Labcorp. Beta amyloid 42/40 ratio, plasma. Available from https://www.labcorp.com/tests/505725/beta-amyloid-42-40-ratio-plasma (Accessed September 5, 2023); 11. Labcorp. Phosphorylated tau 181 (ptau-181), plasma. Available from https://www.labcorp.com/tests/483745/phosphorylated-tau-181-ptau-181-plasma (Accessed September 5, 2023).

Eisai hhc human health care

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