

École polytechnique de Louvain

MRI-Based Analysis of Glymphatic System Biomarkers in Alzheimer's Disease

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Abstract

Alzheimer’s disease (AD) is the leading cause of dementia worldwide, yet the mechanisms driving the accumulation of pathological proteins such as amyloid- β remain only partially understood. In this context, growing interest has focused on the glymphatic system (GS), a perivascular pathway involved in cerebrospinal fluid circulation and brain waste clearance, whose dysfunction may contribute to neurodegeneration. This work investigated four MRI-based biomarkers proposed to reflect GS alterations: choroid plexus (CP) volume, enlarged perivascular spaces (ePVS), the DTI-ALPS index, and NODDI-derived metrics (FISO, FINTRA). The aim is to assess their association with cognitive decline and to determine whether these relationships differed according to amyloid status. Analyses were conducted on an AD cohort of 117 participants (26 cognitively normal, 64 with mild cognitive impairment, and 27 with dementia), stratified by amyloid status. Dedicated processing pipelines were implemented for each biomarker, including a hybrid GMM-based CP segmentation method, a complete DTI-ALPS pipeline, and an automated ePVS quantification approach, together with a validation approach for CP segmentation and ePVS count. Finally, associations with age, cognition, diagnostic group, and amyloid status were assessed using partial correlations and ANCOVA models adjusted for age, sex, and education. Results showed that CP volume increased with disease severity and was associated with poorer cognition independently of age. In contrast, the DTI-ALPS index decreased with age and differed between cognitively normal and impaired subjects, suggesting that it may reflect broad brain changes rather than glymphatic dysfunction alone. EPVS burden showed no significant association with the investigated variables, whereas NODDI metrics suggested that FISO may act as a global marker of extracellular alterations and FINTRA as a more region-specific marker. Together, these findings suggest that MRI-derived glymphatic biomarkers capture partly distinct aspects of AD-related brain alterations, although their indirect and non-specific nature limits their interpretation.

Contents

Acknowledgements	i
Abstract	ii
Introduction	i
1 Magnetic Resonance Imaging	2
1.1 Fundamental Principles of MRI	3
1.2 MRI Signal and Image Contrast	5
1.2.1 Relaxation Mechanisms	5
1.2.2 Contrast Mechanisms	7
1.3 Diffusion Weighted Imaging	10
1.3.1 Diffusion Tensor Imaging	12
1.3.2 Advanced Multi-Compartment Diffusion Models: NODDI	14
2 Alzheimer’s Disease and Glymphatic Dysfunction	16
2.1 Neuropathological Hallmarks of AD	16
2.2 Glial Cells and Brain Clearance Mechanisms	18
3 Neuroimaging of the Glymphatic System	21
3.1 Choroid Plexus Volume	21
3.2 Enlarged Perivascular Spaces	22
3.3 DTI-ALPS index	24
3.4 Advanced dMRI-derived metrics	26
4 Materials & Methods	27
4.1 Data Acquisition	27
4.1.1 MRI Acquisition	27
4.1.2 Cognitive Assessment	28
4.1.3 Population Description	30
4.2 Data Preprocessing	31
4.2.1 Diffusion MRI Preprocessing	31
4.2.2 Structural MRI Preprocessing	32
4.2.3 Image Registration	32
4.3 Data Processing	34
4.3.1 Diffusion Model Reconstruction	34
4.3.2 Pipelines for Biomarker Assessment	34
CP Segmentation	35
DTI-ALPS Index Computation	36
EPVS Segmentation	37
NODDI-derived Metrics	39
4.4 Experiments	39
4.4.1 Biomarker Validation Strategy	39

4.4.2	Biomarker Analysis Strategy	40
4.5	Technical Resources	42
5	Results	44
5.1	Demographics	44
5.2	Biomarker Validation	45
5.2.1	Validation of CP segmentation	45
5.2.2	Validation of Automated ePVS Quantification	46
5.3	Biomarker Analysis	47
5.3.1	CP Volume	47
5.3.2	DTI-ALPS Index	48
5.3.3	EPVS Burden	50
5.3.4	NODDI Metrics	51
5.3.5	Combined CP & DTI-ALPS Cognitive Models	56
5.4	Biomarker Associations Across Cognitive Domains	56
6	Discussion	57
6.1	Methods Validation	57
6.1.1	Choroid Plexus Segmentation	57
6.1.2	Automated ePVS Quantification	58
6.2	Individual Biomarker Analysis	59
6.2.1	CP Volume	59
6.2.2	DTI-ALPS Index	59
6.2.3	EPVS Burden	60
6.2.4	NODDI-derived Metrics	60
6.3	Cross-Biomarker Analysis	61
6.3.1	Relationships Between CP Volume & DTI-ALPS Index	61
6.3.2	Relationships with Different Cognitive Domains	62
6.4	Limitations	63
6.5	Future Perspectives	65
	Conclusion	66
A	Signal detection and image acquisition	76
B	Supplementary Figures	80
B.1	Demographics	80
B.2	CP Analyses	81
B.3	DTI-ALPS Analyses	82
B.4	EPVS Analyses	82
B.5	Noddi Analyses	84

List of Figures

1	Schematic representation of the glymphatic system	i
1.1	Spin-magnetic moment	2
1.2	Behaviour of magnetic moments under B_0	4
1.3	Radiofrequency excitation and flip angles	4
1.4	Relaxation and MR signal decay	5
1.5	Longitudinal relaxation (T_1)	6
1.6	Transverse relaxation (T_2)	6
1.7	Spin-echo sequence and refocusing mechanism	8
1.8	Image contrast in SE sequences	8
1.9	Inversion recovery	9
1.10	FLAIR imaging	9
1.11	Water diffusion in biological tissues	10
1.12	Diffusion signal vs b-value	11
1.13	Diffusion images and ADC	12
1.14	DTI diffusion ellipsoid	13
1.15	NODDI signal decomposition	14
2.1	Neuropathological features of AD	17
2.2	AD biomarker cascade and structural brain changes	18
2.3	Outline of the glymphatic system	19
2.4	Neurovascular unit and astrocytic end-feet	19
2.5	Schematic representation of glymphatic dysfunction	20
3.1	Visualisation of the choroid plexus on MRI	22
3.2	Illustration of ePVS appearance in axial and sagittal MRI views	23
3.3	Schematic representation of PVS and WM fiber orientations	25
4.1	Distribution of MRI acquisition protocols across the cohort	27
4.2	Cohort composition diagram	30
4.3	Preprocessing ElikoPy pipeline	32
4.4	Aseg registration	33
4.5	Registration failures	34
4.6	GMM candidate region	35
4.7	Automated ROI definition and orientation correction for DTI-ALPS	37
4.8	Anatomical masks used for ePVS segmentation in the sagittal plan	38
4.9	ePVS segmentation in the CSO	38
4.10	ePVS segmentation in the BG	39
5.1	Education level distributions	44
5.2	Validation of the proposed CP segmentation method	45
5.3	Example of CP segmentation comparison	45

5.4	Comparison between automated and manual ePVS scores	46
5.5	Confusion matrices for ePVS visual scores	46
5.6	Age-related trajectories of normalised CP volume	47
5.7	Associations between CP volume and cognition	48
5.8	Age-related trajectories of DTI-ALPS index	49
5.9	DTI-ALPS index across diagnostic groups	50
5.10	Partial correlations between ePVS burden and global cognition	51
5.11	ePVS count across diagnostic groups — amyloid-positive subgroup	51
5.12	Age-related trajectories of NODDI metrics in the uncinate fasciculus	53
5.13	Group differences in hippocampal FISO	54
5.14	Group differences in fornix FINTRA	54
5.15	Associations between cingulate isthmus NODDI metrics and global cognition	55
5.16	Group differences in cingulate isthmus NODDI metrics	55
6.1	Examples of CP segmentation failures	58
A.1	Principle of slice selection in MRI	77
A.2	Frequency encoding principle in MRI	78
A.3	Phase encoding principle in MRI	78
B.1	Sex and amyloid status distribution across diagnostic groups	80
B.2	Age distribution by sex and by diagnostic group	81
B.3	Adjusted CP volume across diagnostic groups — amyloid-positive subgroup	81
B.4	Association between ALPS index and global cognition	82
B.5	Age-related ePVS trajectories within amyloid subgroups.	82
B.6	Age-related ePVS trajectories within amyloid subgroups.	83
B.7	ePVS distributions across diagnostic groups	83
B.8	Regional age associations for FISO and FINTRA	84
B.9	Associations between NODDI metrics and cognition across regions	85
B.10	Associations of hippocampal FISO and fornix FINTRA with global cognition	86
B.11	Group differences in cingulate isthmus with NODDI metrics	86

List of Tables

4.1	Main MRI acquisition parameters	28
4.2	Combined cognitive and amyloid-status classification	30
4.3	Characteristics of the study cohort	31
5.1	Interaction effects between age and sex or amyloid status on DTI-ALPS index	49
5.2	Interaction effects between age and sex or amyloid status on ePVS counts .	50
5.3	Effect of IQR-based outlier removal in the uncinate fasciculus	52
5.4	Stratified age-related associations for NODDI metrics	52
5.5	Partial correlation between cognitive scores and imaging biomarkers.	56

List of Abbreviations

ADC	Apparent Diffusion Coefficient
AD	Alzheimer's Disease
ALPS	Analysis Along the Perivascular Space
ANCOVA	Analysis of Covariance
APP	Amyloid Precursor Protein
AQP4	Aquaporin-4
AxD	Axial Diffusivity
BG	Basal Ganglia
CNN	Convolutional Neural Network
CN	Cognitively Normal
CNS	Central Nervous System
CP	Choroid Plexus
CSF	Cerebrospinal Fluid
CSO	Centrum Semiovale
dMRI	Diffusion Magnetic Resonance Imaging
DTI	Diffusion Tensor Imaging
DTI-ALPS	Diffusion Tensor Image Analysis Along the Perivascular Space
DWI	Diffusion-Weighted Imaging
ePVS	Enlarged Perivascular Spaces
EPI	Echo-Planar Imaging
FA	Fractional Anisotropy
FCSRT	Free and Cued Selective Reminding Test
FID	Free Induction Decay
FINTRA	Intra-neurite Fraction
FISO	Isotropic (Free-Water) Fraction

FLAIR	Fluid-Attenuated Inversion Recovery
FSPGR	Fast Spoiled Gradient Echo
FW	Free Water
GMM	Gaussian Mixture Model
GS	Glymphatic System
ICV	Intracranial Volume
IR	Inversion Recovery
IQR	Interquartile Range
ISF	Interstitial Fluid
JHU	Johns Hopkins University
LV	Lateral Ventricle
MB	Multiband
MCI	Mild Cognitive Impairment
MD	Mean Diffusivity
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
MP-PCA	Marchenko–Pastur Principal Component Analysis
MPRAGE	Magnetization Prepared Rapid Gradient Echo
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NDI	Neurite Density Index
NMR	Nuclear Magnetic Resonance
NODDI	Neurite Orientation Dispersion and Density Imaging
ODI	Orientation Dispersion Index
OLS	Ordinary Least Squares
PD	Proton Density
PET	Positron Emission Tomography
PGSE	Pulsed Gradient Spin Echo
PVS	Perivascular Spaces
QC	Quality Control

RD	Radial Diffusivity
RF	Radiofrequency
ROI	Region of Interest
SE	Spin Echo
SE-EPI	Spin-Echo Echo-Planar Imaging
TE	Echo Time
TI	Inversion Time
TMT	Trail Making Test
TR	Repetition Time
WM	White Matter
WMH	White Matter Hyperintensities

Introduction

The progressive ageing of the population has led to a major increase in age-related neurodegenerative diseases, among which Alzheimer’s disease (AD) represents the leading cause of dementia worldwide [1]. AD is characterized by a progressive decline in cognitive functions, including memory, executive functions, language, and reasoning abilities, ultimately leading to loss of autonomy [2]. Although the neuropathological hallmarks of AD, namely amyloid- β ($A\beta$) plaques and tau neurofibrillary tangles, have been extensively studied, the mechanisms contributing to disease progression remain only partially understood. Why do toxic proteins such as $A\beta$ and tau progressively accumulate in some individuals ?

These questions gained renewed interest after Iliff and colleagues proposed the existence of the *glymphatic system* (GS) in 2012 [3], a perivascular transport pathway potentially involved in cerebrospinal fluid (CSF) circulation and brain waste clearance (see Figure 1). The term *glymphatic* highlights the central role of glial cells, particularly astrocytes, in supporting this lymphatic-like clearance pathway. This discovery challenged the long-standing assumption that the brain lacked a dedicated waste clearance system owing the absence of conventional lymphatic vessels. Using fluorescent tracer injections in the mouse striatum, Iliff et al. demonstrated that $A\beta$ protein can be cleared from the brain parenchyma along perivascular pathways, suggesting that glymphatic transport may contribute to amyloid clearance. Whether similar mechanisms operate at scale in the human brain, however, remains an open and actively debated question [4, 5].

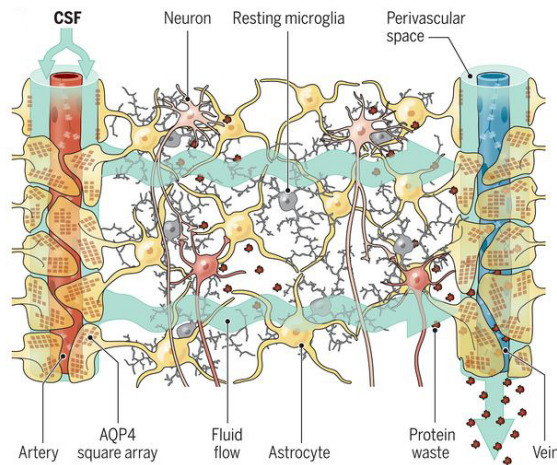


Figure 1: Schematic representation of the GS under physiological conditions. CSF flows along periarterial spaces and exchanges with interstitial fluid through aquaporin-4 (AQP4) water channels, facilitating the clearance of metabolic waste products toward perivenous spaces [4].

In this context, neuroimaging and magnetic resonance imaging (MRI) in particular, has become an essential tool for studying brain disorders by allowing non-invasive, in vivo visualization of brain structures. The present work investigates four MRI biomarkers: choroid plexus (CP) volume, enlarged perivascular spaces (ePVS), the diffusion tensor image analysis along the perivascular space (DTI-ALPS) index, and diffusion metrics derived from neurite orientation dispersion and density imaging (NODDI), in a cohort spanning different cognitive stages: cognitively normal (CN), mild cognitive impairment (MCI), and dementia, stratified according to amyloid status. This stratification enables distinction between amyloid-positive ($A\beta+$) participants, and amyloid-negative ($A\beta-$) participants, whose cognitive decline, if present, may be attributable to non-AD aetiologies.

The objective of this study is to assess the extent to which GS alterations, as captured by these biomarkers, are associated with cognitive decline, and whether such associations differ according to amyloid status.

In the future, if reliably detectable on routine MRI acquisitions, such biomarkers could complement existing molecular markers and contribute to more accessible and longitudinal monitoring of disease-related alterations in brain homeostasis. Ultimately, improved characterization of these fluid-related imaging changes may help refine early detection strategies, better track disease progression, and support the development of more personalized approaches to patient monitoring and therapeutic intervention.

The thesis is, therefore, organized as follows. First, the theoretical principles underlying MRI are introduced, with particular focus on diffusion MRI techniques and diffusion models used throughout this work, including DTI and NODDI. The clinical and neuropathological aspects of AD are then presented, together with the current understanding of the glymphatic system and its potential role in brain waste clearance and neurodegeneration. A state-of-the-art review of MRI biomarkers proposed to investigate glymphatic-related alterations is subsequently provided. The study cohort, MRI acquisition protocols, preprocessing steps, and statistical analysis strategies are then described. Dedicated processing pipelines were implemented for each biomarker, including a validation step, to ensure the robustness of measurements across this cohort. Finally, the results are presented and discussed, together with the main limitations and future perspectives of the study.

Chapter 1

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a widely used medical imaging technique that provides high-resolution images of soft tissues, particularly the brain [6]. More specifically, MRI is based on electromagnetic interactions between magnetic fields and moving electric charges, enabling detailed visualisation of internal anatomical structures [7].

In 1952, F. Bloch and E. Purcell applied these principles to atomic nuclei and received the Nobel Prize in Physics for the discovery of *nuclear magnetic resonance (NMR)*. This phenomenon arises from the intrinsic property of atomic nuclei known as *spin*. Spin describes the intrinsic angular momentum of a charged particle, represented by the vector $\vec{S}$. Because the particle carries an electric charge, this angular momentum is associated with a magnetic moment (magnetisation vector) $\vec{\mu}$ oriented along the spin axis, as illustrated in Figure 1.1 [6, 7].

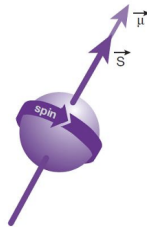


Figure 1.1: Relationship between nuclear spin and magnetic moment [6].

During the 1970s, major advances transformed NMR into an imaging modality. In particular, P. Lauterbur introduced the use of magnetic field gradients to spatially encode NMR signals and published the first magnetic resonance images in 1973 [8]. Shortly after, P. Mansfield developed rapid acquisition and reconstruction methods that considerably accelerated image formation. These pioneering contributions laid the foundation for modern clinical MRI systems, which progressively emerged during the 1980s [9]. Lauterbur and Mansfield were later awarded the Nobel Prize in Physiology or Medicine in 2003 for their major contributions to the development of MRI.

1.1 Fundamental Principles of MRI

MRI primarily relies on the signal generated by hydrogen nuclei, which are abundant in the human body due to its high water content [7]. The hydrogen nucleus consists of a single proton, whose electric charge and intrinsic spin give rise to a magnetic moment. As a result, each hydrogen nucleus behaves as a microscopic magnetic dipole.

The spin of the hydrogen nucleus is characterised by a quantum number $I = \frac{1}{2}$ and has a magnitude. These microscopic magnetic moments only become relevant for MRI when placed in an external magnetic field. In the absence of such field, the magnetic moments of hydrogen nuclei are randomly oriented, resulting in zero net magnetisation [6].

When a subject is placed inside an MRI scanner, a strong static magnetic field B_0 is applied, altering the random orientation of the magnetic moments. Under the influence of this field, hydrogen nuclei preferentially align either parallel or antiparallel to B_0 . Since the parallel configuration corresponds to a lower-energy state, a slightly larger proportion of spins occupies this orientation. This imbalance, described by the Boltzmann distribution, produces a net longitudinal magnetisation $\vec{M}_0$, whose magnitude increases with the strength of the applied magnetic field [6, 7]. As a result, a macroscopic magnetisation appears along the longitudinal direction (M_z), as illustrated in Figure 1.2a [6].

Although the magnetic moments preferentially align with the external magnetic field, they do not remain static. Under the action of the magnetic torque

$$\vec{\Gamma} = \vec{\mu} \times \vec{B}_0, \quad (1.1)$$

the magnetic moments undergo a rotational motion around the direction of B_0 , referred to as *precession*. The angular frequency associated with this motion is called the *Larmor frequency* and is defined as

$$\omega_0 = \gamma B_0 = 2\pi f_0, \quad (1.2)$$

where γ is the gyromagnetic ratio, B_0 the external magnetic field strength, and f_0 the corresponding precession frequency.

This rotational motion follows a cone-like trajectory around the magnetic field axis, as illustrated in Figure 1.2b. Consequently, a nonzero longitudinal magnetisation component is observed, whereas the transverse magnetisation components cancel out because of the random distribution of proton phases, leading to $M_{xy} = 0$.

At equilibrium, the net magnetisation remains aligned with the longitudinal direction and cannot be directly measured because its magnitude is several orders of magnitude smaller than that of the external magnetic field B_0 . To generate a measurable MRI signal, this magnetisation must be perturbed from its equilibrium state [6, 7]. This is achieved by applying a radiofrequency (RF) pulse, which generates an oscillating magnetic field B_1 perpendicular to B_0 . For efficient excitation to occur, the RF pulse frequency must match the Larmor frequency of the nuclei ($\omega_{\text{RF}} = \omega_0$). Under this resonance condition, the nuclei can efficiently absorb energy from the RF field. The interaction between nuclei and the RF field in the presence of a static magnetic field is known as magnetic resonance (MR), resulting in the absorption and subsequent re-emission of energy. In MRI, this phenomenon is exploited to generate a measurable signal from hydrogen nuclei [6, 7].

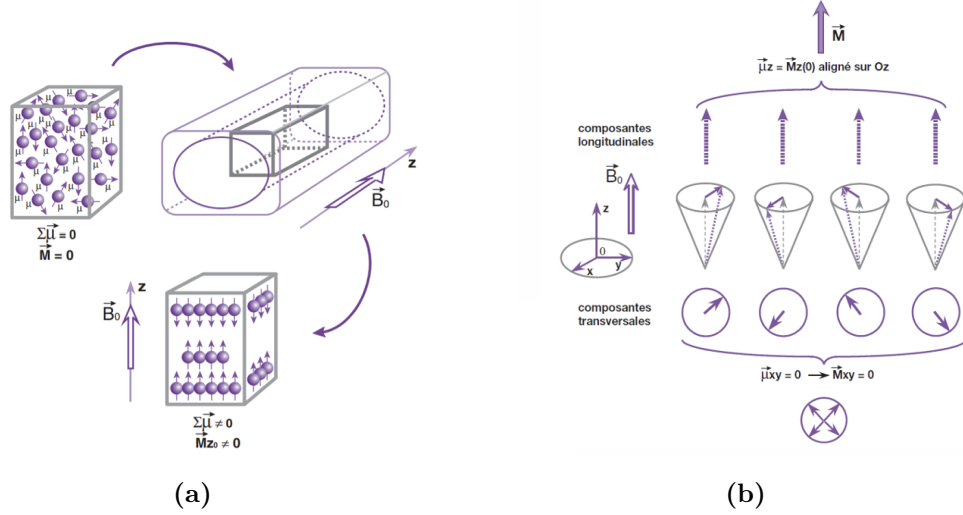


Figure 1.2: Behaviour of hydrogen nuclear magnetic moments under a static magnetic field B_0 . (a) B_0 induces a preferential alignment of the spins along the z -axis, resulting in a nonzero longitudinal magnetisation $\vec{M}_0$. (b) The magnetic moments undergo a precessional motion around B_0 . Decomposition of the resulting magnetisation into longitudinal ($\vec{M}_z$ aligned with B_0) and transverse components ($\vec{M}_{xy}$) that cancel out due to the random distribution of the proton phases, leading to $\vec{M}_{xy} = 0$ [6].

The application of an RF pulse produces two main effects on the spin system. First, it rotates the net magnetisation away from the longitudinal axis by an angle θ , called the *flip angle*, defined as

$$\theta = \gamma \int_0^{T_{\text{RF}}} B_1(t) dt. \quad (1.3)$$

Second, the RF excitation induces phase coherence between spins. Before excitation, the transverse components of individual spins are randomly distributed and therefore cancel each other out. When a resonant RF pulse is applied, the spins become synchronized and precess in phase, generating a non-zero transverse magnetisation M_{xy} [6, 7].

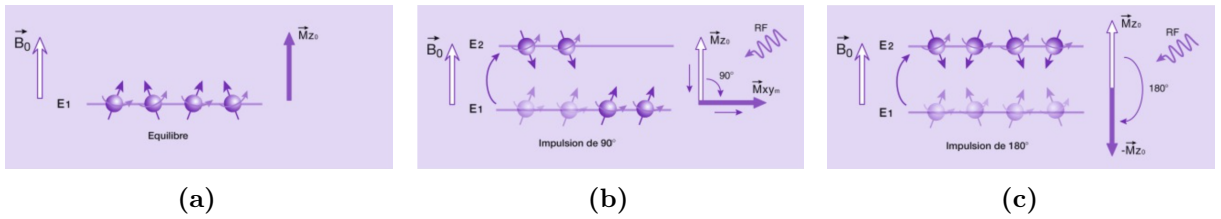


Figure 1.3: Effect of RF pulses on net magnetisation. (a) At thermal equilibrium, the net magnetisation $\vec{M}_0$ is aligned with the static magnetic field B_0 along the longitudinal axis. (b) Application of a 90° RF pulse rotates the magnetisation into the transverse plane, creating a measurable transverse component $\vec{M}_{xy}$. (c) A 180° RF pulse inverts the magnetisation, flipping it from $+\vec{M}_z$ to $-\vec{M}_z$ [6].

At thermal equilibrium, the net magnetisation is aligned with B_0 along the longitudinal axis, as shown in Figure 1.3a. A 90° RF pulse rotates the magnetisation into the transverse plane, as illustrated in Figure 1.3b, maximizing the transverse magnetisation and therefore the detectable MRI signal. In contrast, a 180° pulse inverts the longitudinal magnetisation, as shown in Figure 1.3c [6, 7].

1.2 MRI Signal and Image Contrast

After the RF pulse is switched off, the transverse magnetisation continues to precess around B_0 , while decreasing (Figure 1.4a). This induces an oscillating voltage in the receiver coils (Figure 1.4b), which constitutes the detected MR signal [6, 7].

Immediately after excitation, the signal amplitude progressively decreases as the spins lose coherence. The transverse magnetisation decreases, producing the so-called free induction decay (FID). At the same time, the longitudinal magnetisation gradually recovers towards its equilibrium state. These signal variations arise from relaxation mechanisms occurring at the microscopic level. Since relaxation rates depend on tissue molecular composition and dynamics, they represent the primary source of contrast in MRI [6, 7].

A more detailed mathematical description of MRI signal formation, including the Bloch equations governing magnetisation dynamics and the k-space formalism used for image reconstruction, is provided in Appendix A.

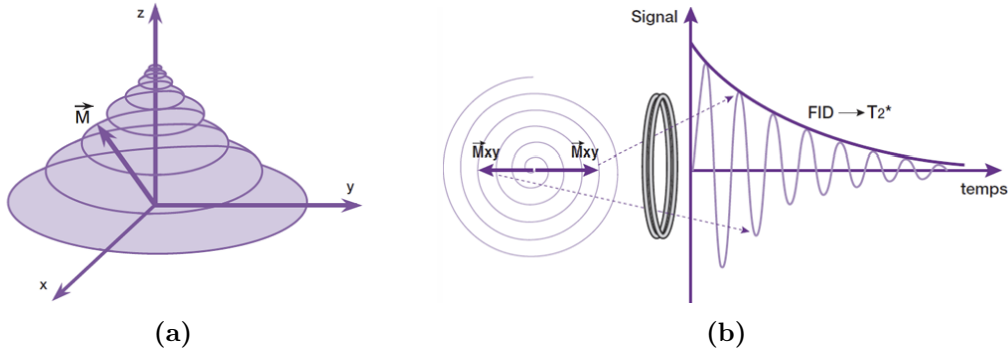


Figure 1.4: Relaxation of the transverse magnetisation and resulting MR signal. (a) Precessing of net magnetisation around B_0 . (b) While precessing, $\vec{M}_{xy}$ induces a signal in the receiver coil, producing the FID; the signal decays through the loss of phase coherence, characterised by the effective transverse relaxation time T_2^* [6].

1.2.1 Relaxation Mechanisms

Longitudinal Relaxation (T_1)

Longitudinal relaxation corresponds to the recovery of magnetisation along the direction of the static magnetic field B_0 . This process occurs through energy exchange between excited spins and their surrounding molecular environment, commonly referred to as the *lattice*. For this reason, T_1 relaxation is also known as *spin-lattice relaxation* [6, 7]. The longitudinal recovery follows an exponential behaviour as illustrated in Figure 1.5.

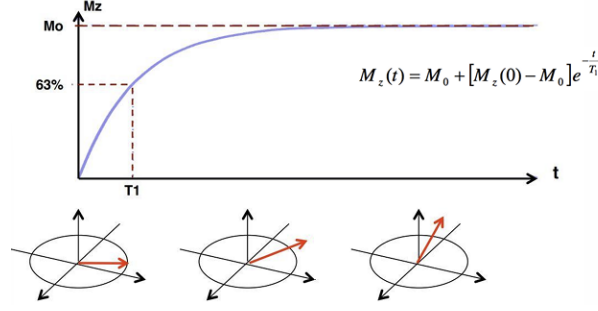


Figure 1.5: Longitudinal magnetisation recovery (T_1 relaxation). After excitation, the longitudinal component recovers exponentially towards its equilibrium value with a characteristic time constant T_1 . Here, $M_z(t)$ denotes the longitudinal magnetisation at time t , while M_0 corresponds to the equilibrium magnetisation. At $t = T_1$, approximately 63% of the equilibrium value has been recovered. The lower schematics illustrate the progressive realignment of the net magnetisation vector with the main magnetic field B_0 [7].

Transverse Relaxation (T_2 and T_2^*)

Transverse relaxation describes the progressive loss of magnetisation in the plane perpendicular to B_0 . The transverse signal decreases exponentially according to the Figure 1.6a. Also referred to as *spin-spin relaxation*, this process arises from microscopic interactions between neighbouring spins that gradually disrupt their phase coherence (Figure 1.6b).

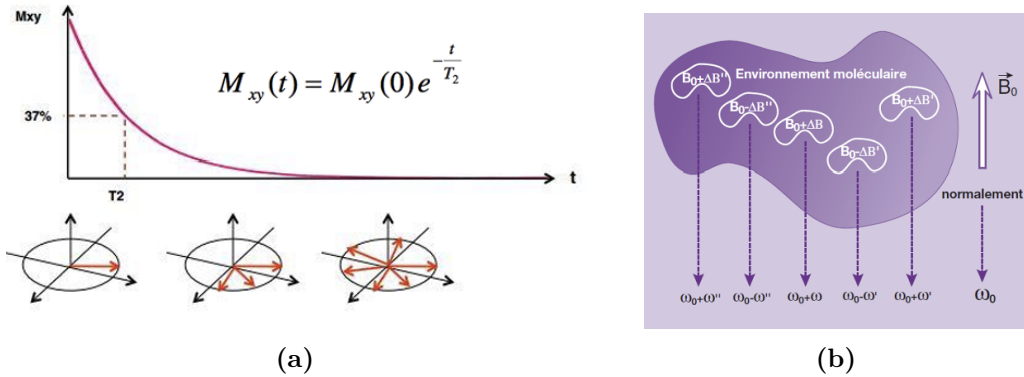


Figure 1.6: Transverse magnetisation decay (T_2 relaxation). (a) After RF excitation, the transverse magnetisation M_{xy} decreases exponentially over time due to loss of phase coherence between spins, characterised by the time constant T_2 . At $t = T_2$, approximately 37% of the initial transverse magnetisation remains. The lower schematics illustrate the progressive dephasing of spins in the transverse plane. (b) Microscopic origin of dephasing: local magnetic field variations induced by the molecular environment cause spins to precess at slightly different frequencies, leading to a progressive loss of phase coherence [6, 7].

Unlike T_1 relaxation, transverse relaxation does not involve energy exchange with the surrounding environment. Instead, intrinsic T_2 relaxation originates from microscopic irreversible magnetic field variations caused by spin-spin interactions. These interactions induce slight differences in spin precession frequencies, progressively reducing phase coherence and leading to transverse signal decay. In biological tissues, T_2 is generally shorter than T_1 . Rapid molecular motion, as observed in liquids, tends to average out local magnetic field variations and prolong T_2 , whereas restricted motion in solids or macromolecular environments maintains these variations and shortens transverse relaxation

times.

In practice, additional dephasing also arises from magnetic field inhomogeneities and magnetic susceptibility variations within tissues. These static or slowly varying magnetic field variations produce reversible dephasing effects, further accelerating the decay of the transverse magnetisation. Consequently, the experimentally observed FID reflects an effective transverse relaxation, denoted T_2^* , rather than the intrinsic T_2 process alone.

The relationship between these contributions is given by

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_{\text{inh}}}, \quad (1.4)$$

where T_{inh} represents dephasing caused by magnetic field inhomogeneities. Because T_2^* includes both irreversible spin–spin interactions and reversible magnetic field inhomogeneity effects, it is always shorter than the intrinsic T_2 relaxation time.

1.2.2 Contrast Mechanisms

In MRI, signal acquisition is controlled by predefined combinations of RF pulses and magnetic field gradients called *pulse sequences*. These sequences determine how the magnetisation evolves over time and how the MR signal is measured. By adjusting the timing and amplitude of RF pulses and gradients, different tissue properties can be emphasized, allowing the generation of various image contrasts and types of information in the final image. Two key timing parameters govern this process. The echo time (TE), defined as the time between excitation and echo formation, determines when the signal is measured, while the repetition time (TR), corresponding to the interval between successive excitation pulses, controls the recovery of longitudinal magnetisation and thus contributes to the contrast and the type of information contained in the final image.

Among the various pulse sequences, the *Spin Echo (SE)* sequence is one of the most fundamental and widely used. It allows reliable measurement of intrinsic tissue properties, in particular T_2 relaxation, while reducing the effects of magnetic field inhomogeneities.

Spin Echo Sequence

The SE sequence begins with a 90° RF pulse that tips the magnetisation into the transverse plane, followed after a delay of $TE/2$ by a 180° refocusing pulse, as shown in Figure 1.7a.

This refocusing pulse reverses the dephasing caused by reversible static or slowly varying magnetic field inhomogeneities, which are responsible for the additional T_2^* signal decay. As a result, the spins rephase and form a measurable signal, called the *spin echo*, at time TE , allowing the measured contrast to predominantly reflect the intrinsic T_2 relaxation properties of the tissue rather than T_2^* effects.

The underlying mechanism is illustrated in Figure 1.7b: after an initial dephasing period, spins accumulate phase differences due to local magnetic field variations. The application of a 180° pulse inverts the relative spin phases, causing faster spins to lag behind and slower spins to catch up, ultimately leading to spin rephasing and echo formation.

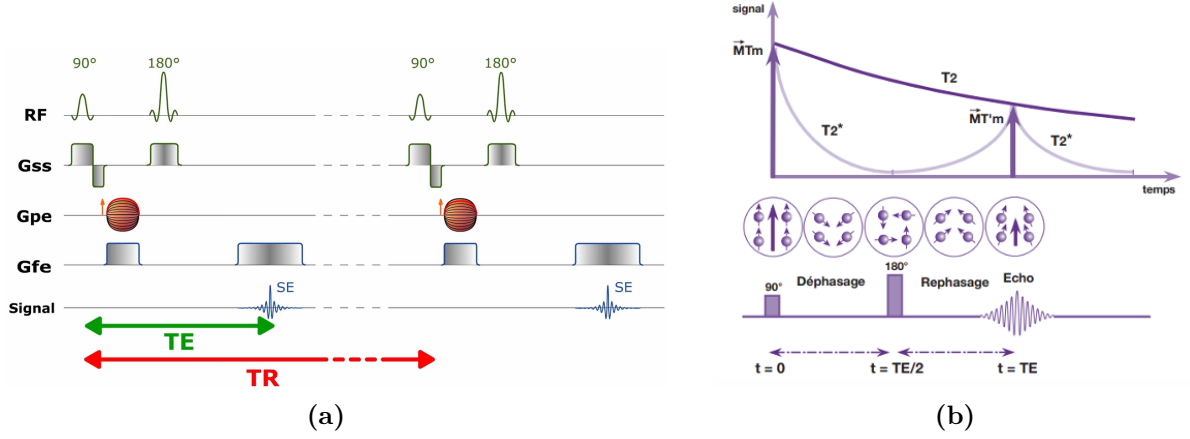


Figure 1.7: Spin-echo imaging principle. (a) SE pulse sequence diagram showing RF pulses and gradient fields used for spatial encoding of the signal [10]. (b) Spin-echo refocusing mechanism illustrating the reduction of T_2^* effects and the measurement of intrinsic T_2 relaxation [7].

Image contrast in SE sequences is primarily controlled by two key acquisition parameters: TR and TE , effects are represented on Figure 1.8. By adjusting these parameters, images emphasise different tissue properties, namely T_1 -weighting, T_2 -weighting, or proton density (PD) weighting:

- **T_1 -weighted images:** short TR and short TE
- **T_2 -weighted images:** long TR and long TE
- **PD-weighted images:** long TR and short TE

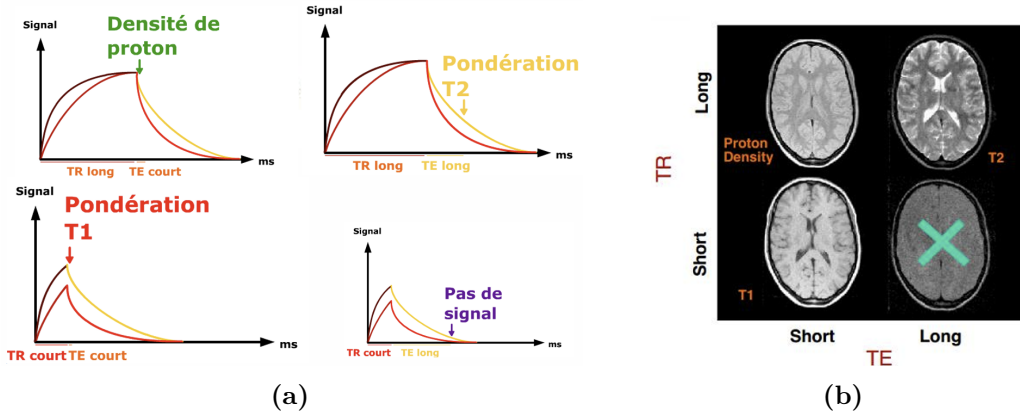


Figure 1.8: Image contrast SE sequences as a function of TR and TE . (a) Signal evolution curves illustrating how different combinations of TR and TE produce T_1 -, T_2 -, and PD-weighted contrasts. (b) Corresponding MRI brain images showing the effect of these weightings on tissue contrast.

Inversion Recovery and FLAIR Imaging

Inversion-recovery (IR) sequences are based on a magnetisation preparation step consisting of a 180° RF pulse that inverts the longitudinal magnetisation (M_z), followed by a recovery period during which the magnetisation returns towards equilibrium through T_1

relaxation. A subsequent 90° RF pulse converts the remaining longitudinal magnetisation into transverse magnetisation, generating the measurable MR signal. The delay between the inversion pulse and the excitation pulse, known as the inversion time (TI), determines the degree of longitudinal recovery and therefore the image contrast. By selecting a TI corresponding to the null point of a specific tissue, its signal can be suppressed, as no transverse magnetisation is generated when $M_z = 0$, as illustrated in Figure 1.9 [11].

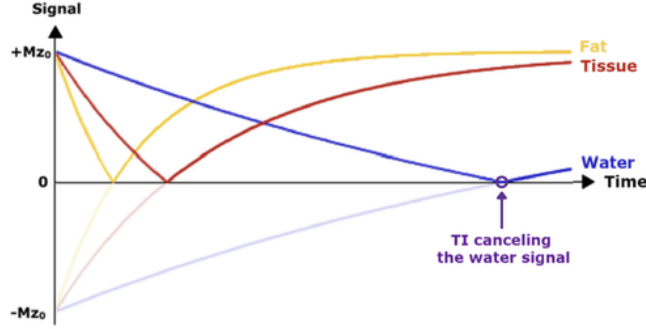


Figure 1.9: Longitudinal magnetisation recovery after inversion. An appropriate TI allows selective suppression of water signal, in this case [11].

Fluid-Attenuated Inversion Recovery (FLAIR) is an inversion-recovery MRI sequence designed to suppress CSF signal. By selecting an TI corresponding to the null point of CSF longitudinal magnetisation, CSF appears dark while surrounding brain tissues retain signal, improving contrast between parenchyma and fluid (Figure 1.10a) [11, 12].

FLAIR can be acquired with different contrast weightings. T_2 -weighted FLAIR uses long TR and TE values to emphasise T_2 contrast while suppressing CSF, whereas T_1 -weighted FLAIR uses shorter timing parameters to produce predominantly T_1 -weighted contrast with CSF suppression [12]. The Figure 1.10b shows an example of T_2 -weighted FLAIR image.

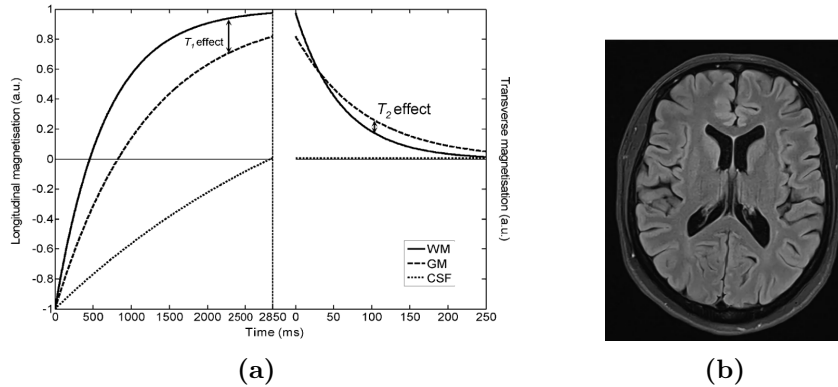


Figure 1.10: FLAIR contrast mechanism. An inversion pulse with appropriate TI suppresses CSF signal while preserving T_2 contrast. (a) Evolution of longitudinal and transverse magnetisation for different tissues [13]. (b) Example of a T_2 -weighted FLAIR image, with TR 9000, TE 105, TI 2500. From [12].

In this work, T_2 -Weighted FLAIR images are used. Those images are particularly sensitive to lesions with high water content, such as oedema or white matter abnormalities, and are widely used in neuroimaging due to its ability to highlight pathological changes adjacent to CSF spaces [12, 13].

1.3 Diffusion Weighted Imaging

Water molecules in the liquid state continuously undergo random thermal motion, known as Brownian motion [14]. This diffusion phenomenon is an intrinsic physical property of water and is therefore also present in biological tissues. While diffusion is isotropic in homogeneous media, it can become constrained by cellular structures such as membranes and fibres, making it dependent on tissue microstructure [14]. Diffusion-weighted imaging (DWI) exploits this property to probe water displacement and infer tissue organisation. In the brain, diffusion is anisotropic in white matter due to the preferential alignment of axonal fibres, whereas it is more isotropic in grey matter, whose microstructure consists of neuronal cell bodies, dendrites, and axons without a dominant orientation [14, 15].

Diffusion Encoding and Signal Modelling

Diffusion sensitization is most commonly achieved using the Pulsed Gradient Spin Echo (PGSE) sequence, which combines a spin-echo acquisition with two diffusion-encoding gradients of equal amplitude and opposite polarity applied before and after the refocusing pulse [7, 16]. These gradients encode water displacement by introducing phase shifts that depend on molecular position. If molecules remain stationary between the two gradients, the phase shifts cancel out and no signal attenuation occurs. In contrast, diffusion-induced displacement prevents complete rephasing, resulting in signal loss. Greater molecular motion therefore produces stronger attenuation, as illustrated in Figure 1.11 [7].

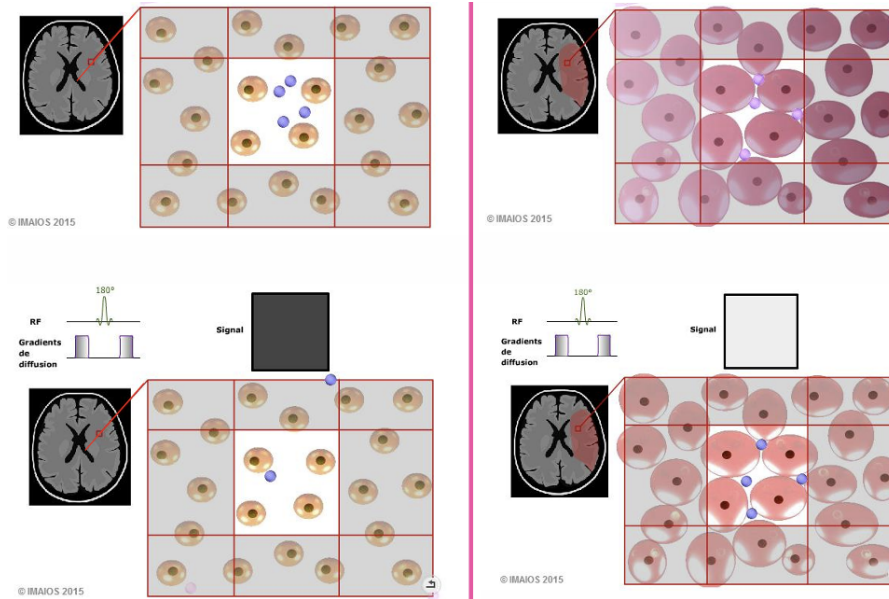


Figure 1.11: Water diffusion in biological tissues. Unrestricted diffusion leads to stronger signal attenuation, whereas restricted diffusion reduces signal loss [7].

The sensitivity of diffusion weighting is determined by the *b-value*, which depends on the strength and timing of the diffusion gradients:

$$b = (\gamma G \delta)^2 \left(\Delta - \frac{\delta}{3} \right), \quad (1.5)$$

where G is the gradient amplitude, δ its duration, and Δ the interval between gradients. Diffusion-weighted acquisitions typically include a reference image acquired without diffusion weighting ($b = 0$) together with images acquired at higher b-values. Increasing the b-value enhances sensitivity to diffusion but also reduces signal-to-noise ratio due to stronger attenuation [17].

For a freely diffusing medium, signal attenuation follows the Stejskal–Tanner equation [17]:

$$S(b) = S_0 e^{-bD}, \quad (1.6)$$

where $S(b)$ is the diffusion-weighted signal, S_0 the signal acquired at $b = 0$, and D the diffusion coefficient. This relationship predicts an exponential decrease in signal intensity with increasing diffusion weighting, as illustrated in Figure 1.12. Larger diffusion coefficients correspond to greater molecular mobility and therefore faster signal decay.

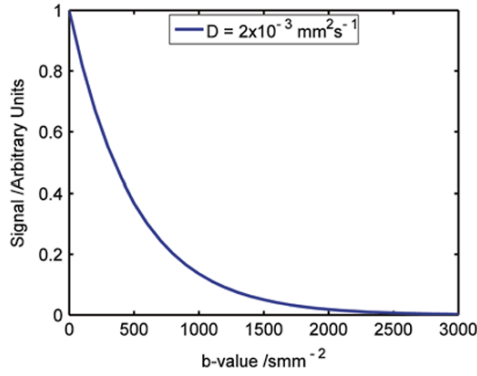


Figure 1.12: Diffusion-weighted MRI signal as a function of b-values for a given D value [17].

In practice, MRI measures signal attenuation rather than diffusion directly. In biological tissues, diffusion is hindered by microstructural barriers, meaning that the measured coefficient reflects both water mobility and tissue organisation. This quantity is therefore referred to as the *apparent diffusion coefficient* (ADC) [17].

Replacing D with ADC in Equation 1.6 gives:

$$S(b) = S_0 e^{-bADC}, \quad (1.7)$$

from which ADC can be estimated:

$$ADC = -\frac{1}{b} \ln \left(\frac{S(b)}{S_0} \right). \quad (1.8)$$

ADC maps provide a voxel-wise measure of diffusion magnitude: low values indicate restricted diffusion, whereas higher values reflect greater molecular mobility [17, 18]. Figure 1.13 illustrates the progressive signal attenuation observed with increasing b-values together with the corresponding ADC map derived from these measurements.

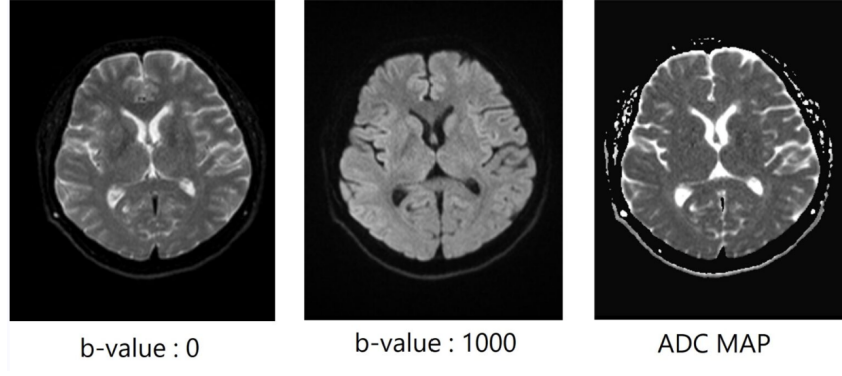


Figure 1.13: Example of diffusion-weighted images acquired with different b -values and the derived ADC map [18].

Because ADC is a scalar quantity, it does not capture directional dependence. In anisotropic tissues such as white matter, diffusion must therefore be sampled along multiple gradient orientations. Each acquisition provides information about diffusion along a specific direction, allowing estimation of the diffusion tensor [17].

At least six non-collinear directions are required to estimate the tensor, although 30–60 directions are commonly acquired to improve robustness. Gradient orientations are defined by b -vectors, while diffusion weighting remains controlled by b -values [19].

For a given direction $\mathbf{g}$, diffusion is described by:

$$ADC(\mathbf{g}) = \mathbf{g}^T \mathbf{D} \mathbf{g}, \quad (1.9)$$

where $\mathbf{D}$ is the symmetric diffusion tensor:

$$\mathbf{D} = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{pmatrix}. \quad (1.10)$$

1.3.1 Diffusion Tensor Imaging

This framework forms the basis of Diffusion Tensor Imaging (DTI), in which diffusion properties are reconstructed by fitting a tensor model to multiple diffusion-weighted measurements acquired along different gradient directions.

Once estimated, the tensor can be diagonalised to obtain three eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) and corresponding eigenvectors, which represent the magnitude and direction of diffusion along the principal axes [19, 17]. These quantities can be represented geometrically as a diffusion ellipsoid, whose principal axes correspond to the eigenvectors and whose lengths are proportional to the eigenvalues, as illustrated in Figure 1.14. From the eigenvalues of the diffusion tensor, several scalar diffusion metrics can be derived:

- **Mean diffusivity (MD):** MD reflects the overall magnitude of water diffusion within a voxel.

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad (1.11)$$

- **Axial diffusivity (AxD):** AxD corresponds to diffusion along the principal fibre direction.

$$AxD = \lambda_1 \quad (1.12)$$

- **Radial diffusivity (RD):** RD characterises diffusion perpendicular to the main fibre orientation.

$$RD = \frac{\lambda_2 + \lambda_3}{2} \quad (1.13)$$

- **Fractional anisotropy (FA):** FA quantifies the degree of diffusion anisotropy and is widely used as an indicator of white matter integrity.

$$FA = \sqrt{\frac{1}{2} \frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad (1.14)$$

Together, these metrics provide complementary information about tissue microstructure and organisation, particularly in highly structured environments such as white matter tracts [17].

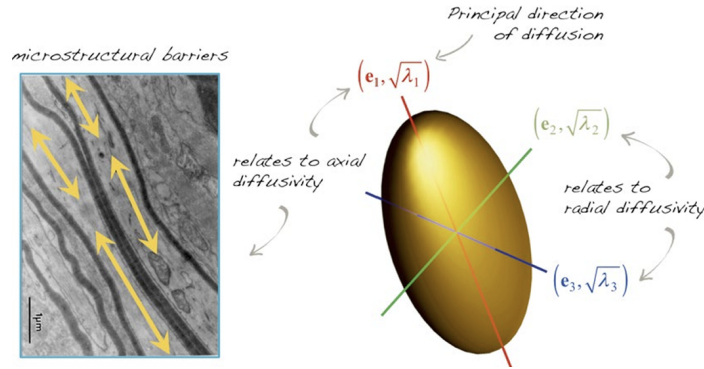


Figure 1.14: DTI representation of anisotropic diffusion in white matter. (Left) Microstructural barriers such as axonal membranes restrict water diffusion perpendicular to fibre orientation while allowing relatively free diffusion along the axonal direction. (Right) Corresponding diffusion tensor represented as an ellipsoid, where the principal eigenvector indicates the main direction of diffusion and eigenvalues reflect axial and radial diffusivities [17].

Despite its widespread use, DTI relies on the assumption that diffusion within each voxel can be described by a single Gaussian process and a single dominant fibre orientation. This assumption constitutes a major limitation in regions with complex fibre architecture. In voxels containing multiple fibre populations, the tensor model cannot represent more than one dominant direction, leading to inaccurate or averaged estimates of diffusion orientation, an issue known as the *crossing-fibres problem* [19, 20].

More generally, DTI metrics represent an average of diffusion within a voxel, which is much larger than individual cellular structures. Therefore, changes in these metrics cannot be linked to a single biological process and remain non-specific [16, 21]. In addition, DTI is sensitive to non-biological factors such as subject motion and acquisition conditions, which can further affect the reliability of the estimated parameters [16, 21].

1.3.2 Advanced Multi-Compartment Diffusion Models: NODDI

The limitations of the DTI model have motivated the development of advanced diffusion frameworks designed to better capture tissue complexity and improve microstructural specificity. Among these, neurite orientation dispersion and density imaging (NODDI), introduced by Zhang et al., provides a biologically interpretable representation of brain microstructure by modelling the diffusion signal as the contribution of multiple tissue compartments [22].

Unlike DTI, NODDI decomposes the diffusion signal into three compartments: intra-neurite, extra-neurite, and free water. Their relative contributions satisfy $f_{intra} + f_{extra} + f_{fw} = 1$, where f_{intra} , f_{extra} , and f_{fw} denote the corresponding volume fractions.

Rather than directly parameterising the model using f_{intra} , NODDI defines the intra-cellular volume fraction ν_{ic} , which represents the proportion of intra-neurite signal within the tissue compartment A_{tissue} (the structure of the model is illustrated in Figure 1.15):

$$\nu_{ic} = \frac{f_{intra}}{1 - f_{fw}}. \quad (1.15)$$

The diffusion-weighted signal is modelled as:

$$S = S_0 [(1 - f_{fw}) (\nu_{ic} A_i + (1 - \nu_{ic}) A_e) + f_{fw} A_{fw}], \quad (1.16)$$

where A_i , A_e , and A_{fw} represent the normalised signal contributions of the intra-neurite, extra-neurite, and free water compartments, respectively. Within this framework, intra-neurite diffusion is modelled as highly restricted and anisotropic, extra-neurite diffusion as hindered by surrounding tissue, and free water diffusion as isotropic.

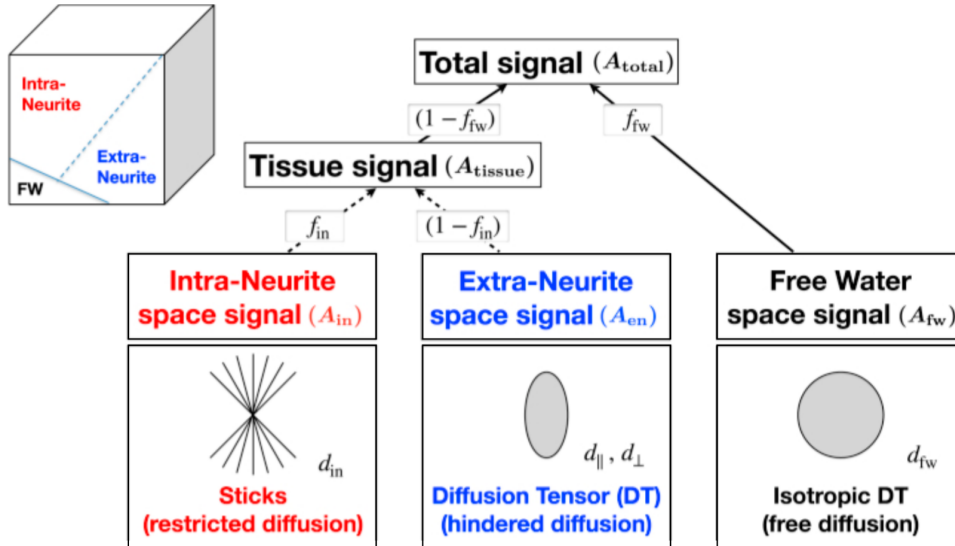


Figure 1.15: Hierarchical representation of the NODDI signal model. The diffusion signal is decomposed into tissue and free water components, with the tissue compartment further divided into intra-neurite and extra-neurite contributions [23].

To account for orientation variability within a voxel, NODDI assumes that neurite orientations follow a Watson distribution:

$$P(\mathbf{n}) = \frac{1}{Z(\kappa)} \exp \left(\kappa (\boldsymbol{\mu} \cdot \mathbf{n})^2 \right), \quad (1.17)$$

where $\boldsymbol{\mu}$ represents the principal neurite orientation and κ controls orientation concentration.

From this model, biologically meaningful biomarkers can be derived. The neurite density index (NDI) corresponds to ν_{ic} , reflecting neurite density within tissue, while the orientation dispersion index (ODI), derived from κ , quantifies the angular variability of neurite orientations [22].

To stabilise parameter estimation, NODDI assumes fixed diffusivities across compartments. Although these constraints improve robustness, they rely on empirical assumptions and may reduce sensitivity to certain pathological alterations [24]. Moreover, NODDI metrics are not uniquely specific, as distinct microstructural changes may produce similar parameter variations.

Despite these limitations, NODDI provides a more informative characterization of tissue organisation than DTI by separately modelling neurite density, orientation dispersion, and free water contributions while remaining compatible with clinically feasible acquisitions [22].

Chapter 2

Alzheimer's Disease and Glymphatic Dysfunction

With ageing, the brain undergoes progressive structural and functional changes, including neuronal loss and brain atrophy, which are associated with mild cognitive decline, notably affecting memory, processing speed, and executive functions [25]. In pathological ageing, these processes are accelerated and clinical manifestations additionally include deficits in memory, language, executive functions, concentration, and communication abilities [26]. This cognitive decline occurs along a continuum. Mild cognitive impairment (MCI) corresponds to a transitional clinical condition characterised by measurable cognitive deficits while largely preserving independent daily functioning. When cognitive impairment becomes severe enough to interfere with daily activities and compromise an individual's independence, the condition is referred to as dementia [26]. Dementia represents a major global public health challenge. According to the World Health Organisation's 2021 blueprint for dementia research, approximately 57 million people worldwide are affected [27]. Although dementia can result from various underlying conditions, Alzheimer's disease (AD) is the most common cause, accounting for approximately 60% to 70% of cases [26].

AD is characterised by progressive neuronal dysfunction, leading to synaptic loss and eventually neuronal death. In the early stages, pathological changes primarily affect memory-related regions such as the entorhinal cortex and hippocampus. As the disease progresses, these alterations spread to associative cortical areas, resulting in impairments in language, reasoning, and social behaviour, and ultimately leading to widespread brain dysfunction [25].

2.1 Neuropathological Hallmarks of AD

Beyond its clinical manifestations, AD is defined by specific neuropathological alterations that drive its progression. Since its first description by Alois Alzheimer in the early 20th century [28], the disease has been characterised by two main histopathological hallmarks: extracellular amyloid- β plaques and intracellular neurofibrillary tangles, illustrated in Figure 2.1 [29].

Amyloid- β ($A\beta$) is a peptide derived from the proteolytic cleavage of the amyloid precursor protein (APP). Under physiological conditions, $A\beta$ protein is continuously generated and cleared, and may play roles in synaptic regulation and neuronal activity. In AD, an

imbalance between production and clearance leads to its aggregation into extracellular plaques [29].

Tau is a microtubule-associated protein that stabilises the neuronal cytoskeleton and supports axonal transport. In AD, tau becomes abnormally phosphorylated, detaches from microtubules, and aggregates into intracellular neurofibrillary tangles, disrupting neuronal transport and function [25].

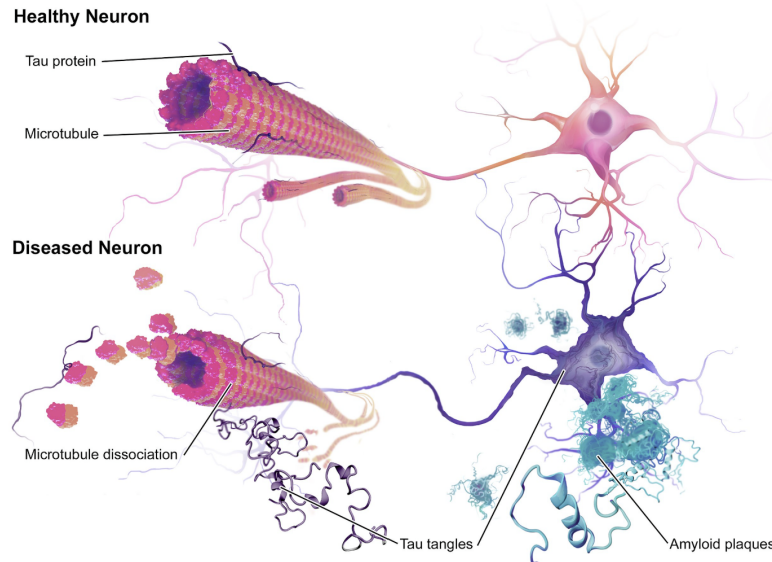


Figure 2.1: Main neuropathological features of AD: extracellular amyloid- β plaques and intracellular tau neurofibrillary tangles [30].

These pathological processes begin years before clinical symptoms. $A\beta$ protein accumulation can start decades in advance, defining a prolonged preclinical phase [31, 2]. As pathology progresses, compensatory mechanisms initially preserve cognitive function, but their failure leads to MCI and eventually dementia [26]. Cognitive decline across these stages can be objectively assessed using standardised neuropsychological tests, which allow the evaluation of specific cognitive domains and the identification of early impairments. A detailed description of the cognitive assessment battery is provided in Chapter: Materials&Methods, Section 4.1.2.

The temporal evolution of these biomarkers and cognitive changes has been described by Jack et al. (2013) [32], who proposed a model in which disease progression follows a non-linear, sigmoid-like trajectory.

As illustrated in Figure 2.2a, biomarker changes follow a sequential pattern: $A\beta$ accumulation occurs early, followed by tau pathology and neurodegeneration, while cognitive impairment emerges later and progresses more rapidly. Notably, $A\beta$ burden alone is not directly associated with clinical progression, highlighting the complex relationship between pathology and cognition [32]. This model remains a key framework for understanding disease progression. In parallel, neurodegeneration leads to progressive brain atrophy, characterised by hippocampal volume loss, cortical thinning in temporal and orbitofrontal regions, and ventricular enlargement (Figure 2.2b). These structural changes are closely associated with disease progression and cognitive decline.

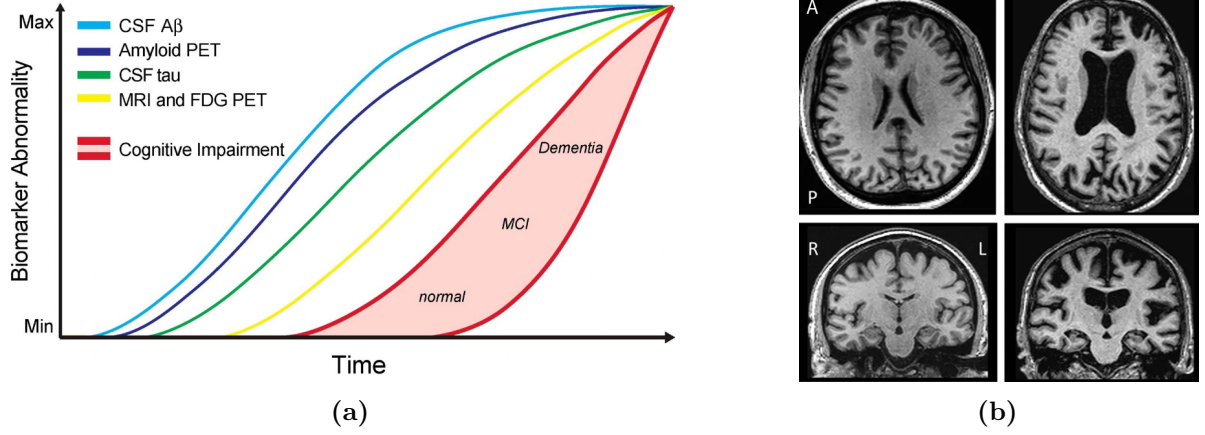


Figure 2.2: (a) Schematic representation of the hypothetical temporal evolution of AD biomarkers, from early amyloid- β accumulation through tau pathology and neurodegeneration to later cognitive decline [33]. (b) MRI images illustrating brain atrophy and ventricular enlargement associated with AD progression. Orientation markers: R = right, L = left, and P = posterior [34].

2.2 Glial Cells and Brain Clearance Mechanisms

In peripheral tissues, excess fluid and interstitial solutes are cleared through the lymphatic system. In contrast, the brain lacks conventional lymphatic vessels despite its high metabolic demand, raising the question of how metabolic waste is removed from the central nervous system (CNS) [35]. This view changed following studies by Iliff, Nedergaard and colleagues in 2012, who described a brain-wide clearance pathway termed the *glymphatic system* (GS), reflecting the combined roles of glial cells and lymphatic-like functions [3, 36]. The GS mediates exchange between CSF and interstitial fluid (ISF), contributing to waste clearance, nutrient distribution, and ionic homeostasis. In particular, it supports the removal of metabolic by-products such as amyloid- β and tau proteins [37, 38].

CSF transport follows organised pathways along the cerebral vasculature through perivascular spaces (PVS), also known as Virchow–Robin spaces, which are fluid-filled compartments surrounding blood vessels and continuous with the subarachnoid space [38]. Within the GS, CSF enters deep periarterial spaces from the subarachnoid space, driven by arterial pulsatility, vasomotion, respiration, and CSF pressure gradients [39]. CSF then moves into the brain parenchyma via aquaporin-4 (AQP4) water channels expressed in astrocytic end-feet, where it exchanges with ISF in the interstitial space (Figure 2.3). This process generates convective ISF flow towards perivenous spaces surrounding deep veins, supporting solute transport and waste clearance. ISF is subsequently collected in perivenous spaces and drains towards the cervical lymphatic system [39].

Perivascular spaces are anatomically defined by the interface between vascular structures and astrocytic end-feet, forming a pathway that supports fluid exchange between CSF and ISF, as illustrated in Figure 2.4. More broadly, this process relies on the coordinated activity of glial cells forming an integrated neurovascular unit that contributes to brain homeostasis: astrocytes regulate fluid exchange, oligodendrocytes support axonal function, and microglia provide immune surveillance [40]. CSF is primarily produced by the choroid plexus (CP) and enters the brain along periarterial pathways, driven by arterial pulsatility, respiration, and pressure gradients [38, 5]. Importantly, glymphatic activity is dynamic and modulated by physiological factors such as vascular pulsatility, neural activity, and

sleep, during which clearance efficiency is enhanced [33].

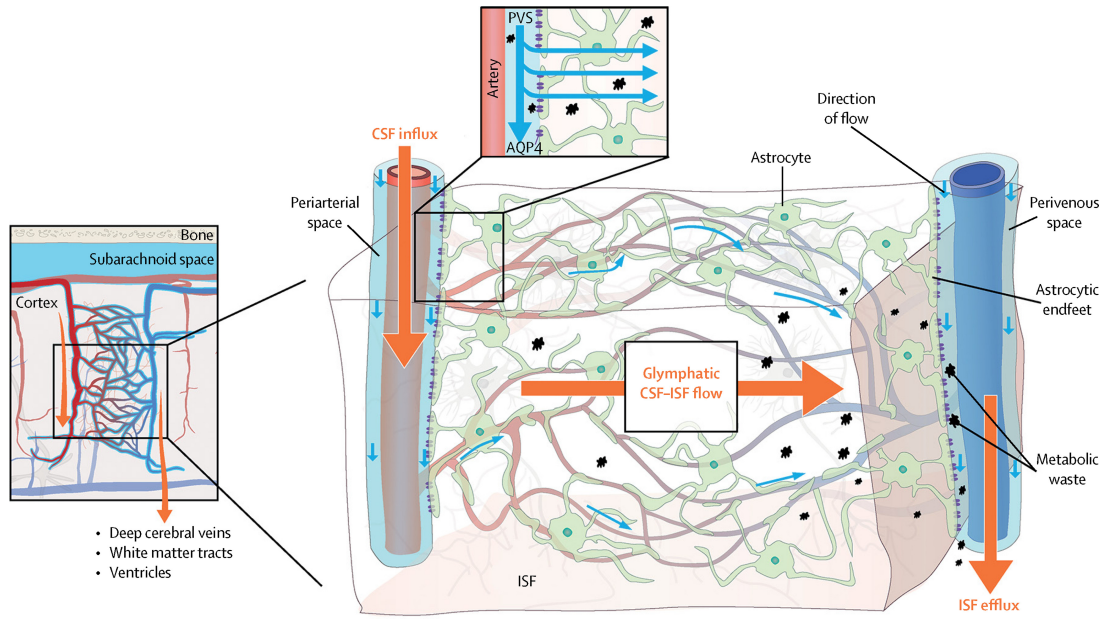


Figure 2.3: Schematic illustration of the glymphatic system and the “common space” hypothesis. CSF flows from the subarachnoid space into periarterial spaces, exchanges with ISF through astrocytic AQP4 channels, and subsequently drains along perivenous pathways, contributing to metabolic waste clearance from the brain parenchyma [41].

Ageing has been associated with a decline in glymphatic function, resulting from mechanisms such as loss of AQP4 polarisation, reduced CSF production, decreased arterial pulsatility, and reactive gliosis [35]. In AD, this impairment is exacerbated, leading to insufficient clearance of metabolic waste such as amyloid- β . The accumulation of these proteins within perivascular pathways further disrupts vascular function and glymphatic flow, creating a self-reinforcing cycle of impaired clearance and protein aggregation.

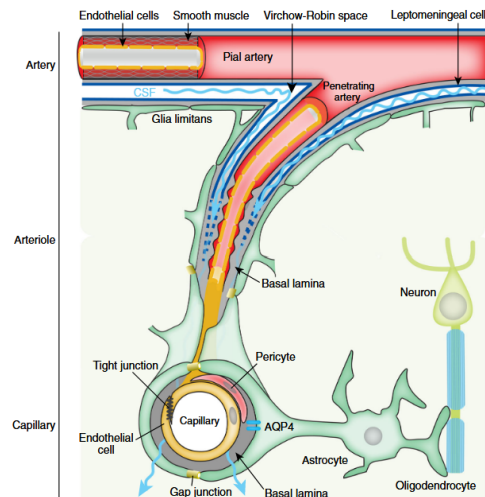


Figure 2.4: Neurovascular unit illustrating the bidirectional communication between the microvasculature and neurons, with astrocytes acting as intermediaries. Astrocytic vascular endfeet expressing AQP4 surround the entire vasculature and form the boundary of the perivascular space [35].

One major structural consequence of this dysfunction is the enlargement of perivascular

spaces (ePVS). Reduced CSF flow leads to fluid stagnation and dilation of these spaces, which become visible on MRI. In parallel, impaired interactions between vascular pulsatility, astrocytic regulation, and interstitial flow lead to reduced solute transport and further fluid accumulation [42]. These processes contribute to the preferential accumulation of larger molecules such as amyloid- β around cerebral vessels, as illustrated in Figure 2.5. Although ePVS enlargement is more frequent in AD, it is not disease-specific and may also be observed in other neurodegenerative conditions [35].

In parallel, the CP also contributes to glymphatic and immune dysfunction in AD. Structural and functional alterations of the CP (including chronic inflammation and impaired immune regulation) reduce the clearance of CSF proteins and promote neuroinflammation. These changes are associated with CP enlargement, likely reflecting tissue remodelling and altered CSF dynamics, and have been linked to cognitive decline [43, 44, 45].

These changes are also associated with alterations in brain fluid homeostasis, including increased extracellular free water, reflecting impaired drainage and disrupted glymphatic circulation [5, 4]. Although the GS remains incompletely understood, MRI provides a non-invasive tool to investigate these processes in vivo.

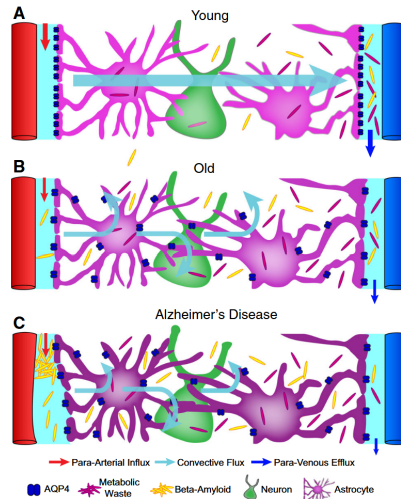


Figure 2.5: Schematic representation of glymphatic dysfunction across normal ageing and Alzheimer's disease, illustrating the progressive accumulation of solutes such as amyloid- β around cerebral vessels as perivascular clearance becomes impaired [35].

Chapter 3

Neuroimaging of the Glymphatic System: State of the Art

Until the early 2000s, AD could only be definitively diagnosed post-mortem through histopathological examination of brain tissue. Advances in research have since enabled the development of in vivo biomarkers, including positron emission tomography (PET), MRI, and more recently, blood-based markers, allowing investigation of disease-related alterations [26]. In the context of glymphatic dysfunction, MRI enables non-invasive microstructural investigation and indirect assessment of CSF dynamics through multiple complementary biomarkers [31].

This chapter reviews the four MRI-based biomarkers investigated in the present work to study glymphatic dysfunction in an AD cohort: CP volume, ePVS burden, the diffusion tensor image analysis along the perivascular space (DTI-ALPS) index, and NODDI metrics. For each biomarker, the main methodological approaches and image-processing strategies previously reported in the literature are presented and discussed.

3.1 Choroid Plexus Volume

As discussed in Section 2.2 of Chapter 2, CP enlargement has been proposed as a potential marker of glymphatic dysfunction and altered CSF regulation. Investigating this relationship in AD therefore requires reliable CP segmentation.

The CP can be visualised on structural MRI, particularly on T1-weighted and 3D FLAIR sequences, where it appears hyperintense relative to CSF due to its vascular composition [46, 47]. However, accurate volumetric quantification remains challenging because of its small size, irregular morphology, proximity to the ventricular wall, and limited contrast with surrounding tissues [46, 44]. Although manual delineation is considered the reference standard, it is time-consuming and difficult to apply to large datasets.

Atlas-based tools such as FreeSurfer [48] are frequently used for automated anatomical segmentation, but their performance for CP delineation remains limited. Incomplete coverage and partial misclassification of ventricular structures often reduce agreement with manual annotations, particularly in pathological brains. This is illustrated in Figure 3.1b [46, 47].

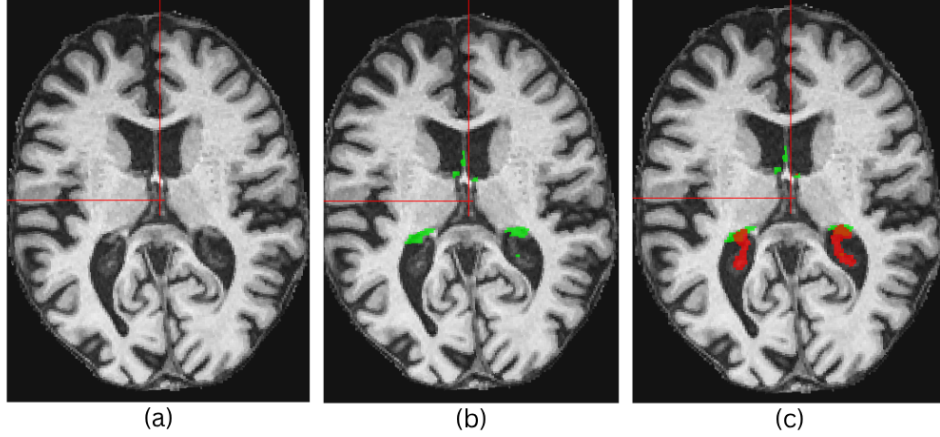


Figure 3.1: Visualisation of the choroid plexus on structural MRI. (a) Native T1-weighted image showing CP signal within the lateral ventricles. (b) Approximate CP localisation obtained from FreeSurfer `aseg` segmentation. (c) Refined CP segmentation obtained using the two-step U-Net framework proposed by Yazdan-Panah et al. [46].

To improve segmentation accuracy, intensity-based approaches using Bayesian Gaussian Mixture Model (GMM) have been proposed. Tadayon et al. introduced a two-stage GMM framework in which voxel intensity distributions within the ventricular region are iteratively modelled to distinguish CSF from CP tissue [47]. A first two-component Bayesian GMM identifies high-intensity candidate voxels within a ventricular mask, followed by spatial smoothing. A second three-component GMM is then applied, and the highest-intensity cluster is retained as the final CP segmentation. Compared with FreeSurfer, this approach showed improved agreement with manual delineations, although performance remained reduced in diseased subjects due to increased anatomical variability and lower tissue contrast [47]. Zhou et al. later introduced a region-informed one-GMM specifically designed for Alzheimer’s disease cohorts [44]. By combining intensity information with anatomical priors derived from FreeSurfer segmentations, this method improves robustness in subjects with ventricular enlargement and achieves stronger agreement with manual annotations while maintaining low computational complexity.

Deep learning approaches, including 3D U-Net architectures and multilayer perceptrons, have also been explored for CP segmentation [46]. Yazdan-Panah et al. proposed a two-step 3D U-Net framework operating directly on T1-weighted MRI [46]. A first network identifies candidate CP regions from a low-resolution whole-brain image, while a second network refines segmentation on high-resolution local patches. Final predictions are merged using Hann windowing to reconstruct the full CP mask, illustrated in Figure 3.1c as a comparison with FreeSurfer. This hierarchical strategy requires only minimal preprocessing and avoids registration to standard space or prior ventricular segmentation, improving robustness to anatomical variability while maintaining good agreement with manual annotations [46].

3.2 Enlarged Perivascular Spaces

As discussed in Section 2.2 of Chapter 2, enlargement of PVS has been proposed as a potential marker of glymphatic dysfunction. Reliable identification of ePVS is therefore essential to investigate their relationship with Alzheimer’s disease and altered brain fluid dynamics.

PVS become visible on MRI only when enlarged [42]. Because they contain cerebrospinal fluid, ePVS appear hyperintense on T2-weighted images, hypointense on T1-weighted imaging, and suppressed on FLAIR sequences. They are therefore observed as small (< 3 mm diameter) linear or punctate structures that follow the trajectory of penetrating vessels. Figure 3.2 illustrates this orientation dependence, showing the same ePVS appearing punctate in the axial plane and tubular in the sagittal plane. ePVS are most commonly evaluated in the centrum semiovale (CSO) and basal ganglia (BG), two regions associated with distinct pathological mechanisms [49]. Their identification remains challenging due to their small size relative to clinical voxel resolution, limited contrast with surrounding white matter (WM), and variable morphology influenced by partial-volume effects. In addition, ePVS may resemble other MRI-visible abnormalities, such as WM hyperintensities or lacunar infarcts, further complicating accurate differentiation [50].

Historically, ePVS burden has been evaluated using visual rating scales based on the number of visible structures within predefined anatomical regions. A commonly used scoring system assigns grades from 0 to 4, corresponding to the number of visible ePVS: 0 for no visible spaces, 1 for 1–10 ePVS, 2 for 11–20, 3 for 21–40, and 4 for more than 40 visible spaces [49]. Although simple and clinically interpretable, these ratings remain semi-quantitative, observer-dependent, and limited to selected slices rather than whole-brain assessment. Another important limitation is that these scales only account for the number of visible ePVS and not their volume. Consequently, two subjects may receive the same visual score despite having substantially different total ePVS volumes, potentially reflecting different degrees of underlying pathology.

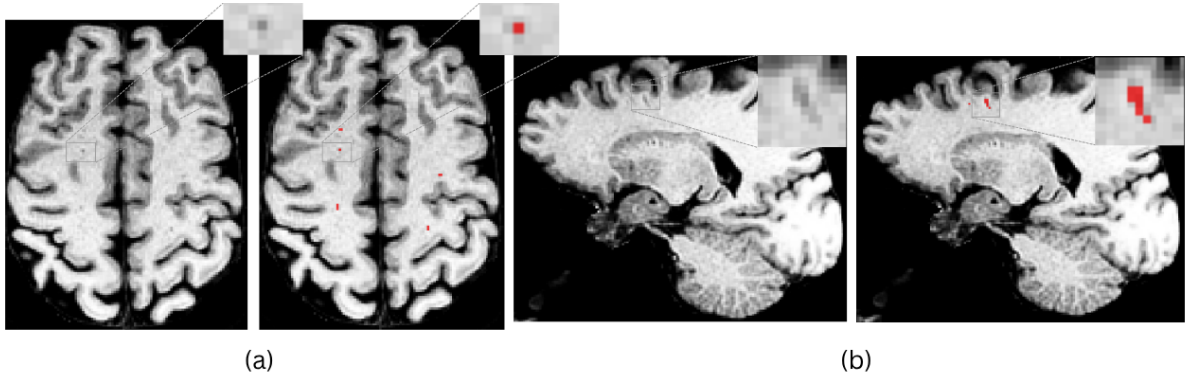


Figure 3.2: Illustration of ePVS masks in CSO region on combined T1-weighted and FLAIR image. (a) In the axial view, a representative ePVS appears as a small rounded structure. (b) In the sagittal view the same structure is visible as a tubular trajectory following the course of the vessel. Multiple additional ePVS are also visible throughout the surrounding WM.

To overcome these limitations, automated and semi-automated segmentation methods have been developed. Early approaches relied on object-based analyses. Descombes et al. modelled ePVS as tubular structures using a probabilistic object-based framework integrating intensity, shape, and spatial organisation [51]. Later, Schwartz et al. proposed the MAPS-T1 framework, which detects ePVS in WM using local intensity homogeneity on T1-weighted MRI combined with FLAIR-based exclusion of WM hyperintensities [50].

A widely adopted family of methods relies on vessel enhancement filtering. Ballerini et al. introduced a Frangi-based vesselness framework that enhances tubular structures on T2-weighted MRI prior to thresholding and morphological refinement [52]. This geometry-driven approach demonstrated good reproducibility and scalability for large neuroimaging cohorts, although performance may remain sensitive to image quality and false positives

from elongated lesions or vascular structures.

More recently, machine learning and deep learning approaches have enabled voxel-wise whole-brain quantification by learning spatial and intensity patterns directly from MRI data. Zhang et al. combined vascular filtering outputs with a structured random forest classifier to improve distinction between ePVS and background tissue [53], while Lian et al. proposed a multi-scale convolutional neural network (CNN) that iteratively refined segmentation using contextual information from previous predictions [54]. Compared with conventional filtering techniques, deep learning methods generally improve reproducibility and sensitivity, but remain dependent on annotated datasets.

3.3 DTI-ALPS index

PVS fluid dynamics can be indirectly investigated using diffusion MRI (dMRI) [42]. Because PVS are elongated fluid-filled structures surrounding cerebral vessels, water diffusion is expected to be preferentially oriented along their longitudinal axis. This anisotropic organisation provides the basis for DTI-ALPS, introduced by Taoka et al. [39].

The DTI-ALPS method exploits the orthogonal arrangement between medullary vessels and surrounding WM fibres near the lateral ventricles. In this region, PVS predominantly follow the right-left (x -axis) direction, whereas projection fibres are mainly oriented along the superior-inferior (z -axis) direction and association fibres along the anterior-posterior (y -axis). This configuration enables estimation of diffusivity along PVS from diffusion components measured perpendicular to the dominant fiber orientations, as illustrated in Figure 3.3. In the original implementation, spherical regions of interest (ROIs) were manually placed on colour-coded FA maps within projection and association fiber regions adjacent to medullary veins [39].

The ALPS index is defined as:

$$\text{ALPS-index} = \frac{\text{mean}(D_x^{\text{proj}}, D_x^{\text{assoc}})}{\text{mean}(D_y^{\text{proj}}, D_z^{\text{assoc}})}, \quad (3.1)$$

where D_x^{proj} and D_x^{assoc} correspond to diffusivities along the presumed PVS direction within projection and association fiber regions, while D_y^{proj} and D_z^{assoc} represent diffusivities perpendicular to the PVS. Higher ALPS values are interpreted as reflecting greater diffusivity along PVS, whereas lower values may indicate reduced fluid mobility within these pathways.

Reduced ALPS values have been reported in several neurodegenerative disorders, including AD and MCI, and have been associated with poorer cognitive performance and structural alterations such as hippocampal atrophy [39, 55, 56]. Based on its anatomical rationale, ALPS has been proposed as an indirect marker of glymphatic transport [39, 55].

Despite increasing use, several methodological limitations influence ALPS reproducibility. A first limitation concerns image orientation. Because diffusivities are measured along fixed image axes, head positioning relative to the scanner may alter ALPS values. Tatekawa et al. demonstrated that head orientation affects diffusion measurements and proposed tensor reorientation through registration to standard space to improve reproducibility [57]. Their implementation retained manually positioned spherical ROIs located in projection and association fiber regions near the lateral ventricles.

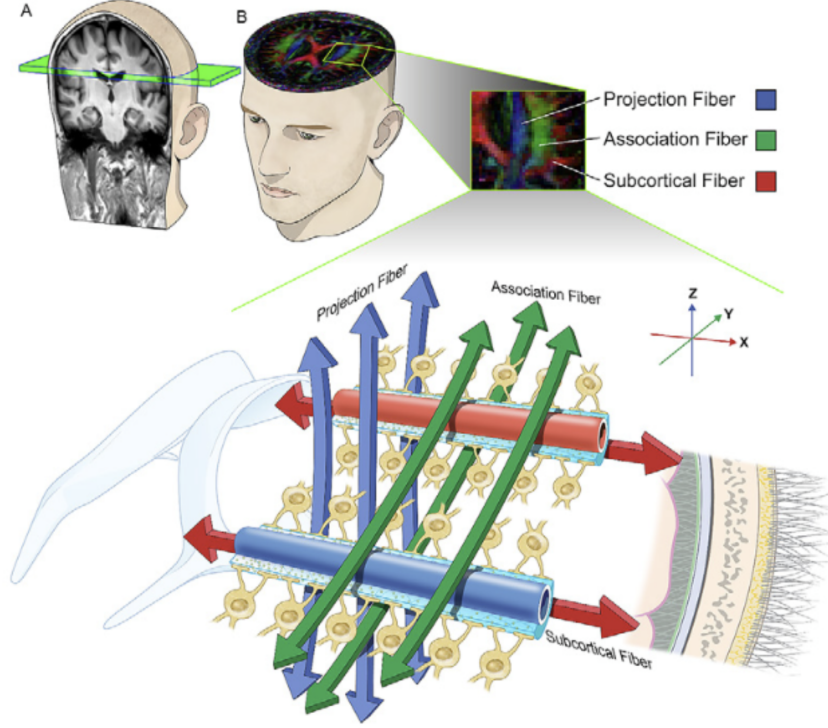


Figure 3.3: Schematic representation of the spatial relationship between PVS and surrounding WM fiber orientations. Diffusion along the longitudinal axis of PVS can be distinguished from diffusion components associated with projection, association, and subcortical fibres. Adapted from [58].

ROI definition represents another major source of variability. Ulloa et al. showed that ROI size, shape, and orientation substantially influence ALPS measurements [59]. Squared ROIs produced more consistent results, likely due to improved alignment with the voxel grid and reduced partial-volume effects. Larger ROIs improved stability through spatial averaging, whereas elongated or asymmetric ROIs increased variability by including neighbouring tissue compartments.

To reduce operator dependence, Li et al. proposed an automated ALPS framework [56]. In this approach, diffusion data are aligned to a standard reference space named Montreal Neurological Institute (MNI)¹ using FA registration, while anatomical priors are projected back into subject space to preserve native diffusion information [60]. Spherical ROIs are initialised at predefined MNI coordinates near the lateral ventricles: $(\pm 37, -12, 24)$ and $(\pm 26, -12, 24)$. These ROIs are then constrained using projection fiber masks from the corticofugal corona radiata and association fiber masks from the superior longitudinal fasciculus derived from the Johns Hopkins University (JHU)-ICBM white matter atlas² [60]. This automated strategy demonstrated good agreement with manual placement while improving reproducibility and scalability.

More fundamentally, ALPS remains an indirect biomarker of glymphatic activity. Because it reflects diffusion properties rather than direct fluid flow, measurements may also be influenced by WM microstructure, extracellular organisation, oedema, and tissue degeneration [56]. In addition, acquisition parameters, preprocessing choices, and scanner-

¹The MNI space is a standardized anatomical reference space derived within the International Consortium for Brain Mapping (ICBM) project from averaged MRI scans of healthy subjects [60].

²The JHU-ICBM white matter atlas is a probabilistic dMRI atlas of major white matter tracts derived from healthy subjects and registered to the standard MNI anatomical space [60].

specific factors contribute to variability across studies [57].

3.4 Advanced dMRI-derived metrics

Additionally, DWI, described in detail in Section 1.3, provides indirect markers of brain microstructure and water diffusion properties. Different diffusion models capture complementary aspects of tissue organisation and extracellular fluid composition, and several research groups have investigated how these markers vary in neurodegenerative disorders such as AD.

Conventional DTI metrics are susceptible to partial-volume effects, particularly in regions containing extracellular fluid or CSF contamination. To address this limitation, Pasternak et al. introduced free-water imaging (FWI) , a post-processing framework based on a bi-compartment model that separates tissue diffusion from isotropic extracellular water [61]. FWI estimates the extracellular free-water fraction, reflecting interstitial fluid content while improving sensitivity to extracellular fluid changes.

Bergamino et al. compared standard and FW-corrected diffusion metrics in AD and MCI. They reported increased FW in several WM regions, including the fornix, hippocampal pathways, cingulum, corpus callosum, thalamic radiations, and periventricular tissue [62, 63]. According to the Bergamino and Pasternak research groups, these increases may reflect extracellular fluid accumulation associated with neuroinflammation, oedema, tissue degeneration, or impaired clearance mechanisms [62, 61]. Elevated FW has also been associated with cognitive decline, particularly in limbic and periventricular WM pathways [62]. Nevertheless, these interpretations remain indirect, as the FW model captures extracellular diffusion changes that may arise from multiple biological processes rather than a single pathological mechanism.

More advanced diffusion models, such as NODDI, were specifically developed to characterise neurite architecture and therefore provide a more tissue-specific description of microstructure [22]. According to the framework introduced by Zhang et al., NODDI-derived metrics provide compartment-specific characterisation of neurite organisation beyond conventional diffusion tensor measures. Reduced NDI and ODI have been reported in neurodegenerative diseases and are interpreted as reflecting axonal loss, dendritic degeneration, and reduced tissue complexity. These alterations have been particularly observed in the fornix, cingulum, periventricular WM, temporo-limbic pathways, and fronto-parietal tracts, where they have been associated with cognitive impairment [64].

Because the GS contributes to fluid exchange between CSF and ISF, altered glymphatic transport may influence extracellular water content [39, 55]. However, the relationship between FW measurements and glymphatic function remains indirect and has not been formally established. FW should therefore be interpreted as a non-specific marker of extracellular fluid alterations.

Chapter 4

Materials & Methods

4.1 Data Acquisition

4.1.1 MRI Acquisition

MRI data were acquired at Saint-Luc University Clinics using a 3T SIGNA™ Premier MRI scanner (GE Healthcare, Chicago, IL, USA). The dataset included T_1 -weighted, T_2 -weighted, FLAIR, and diffusion-weighted MRI acquisitions obtained using different clinical protocols.

The distribution of the main acquisition protocols is shown in Figure 4.1, and the corresponding acquisition parameters are summarised in Table 4.1.

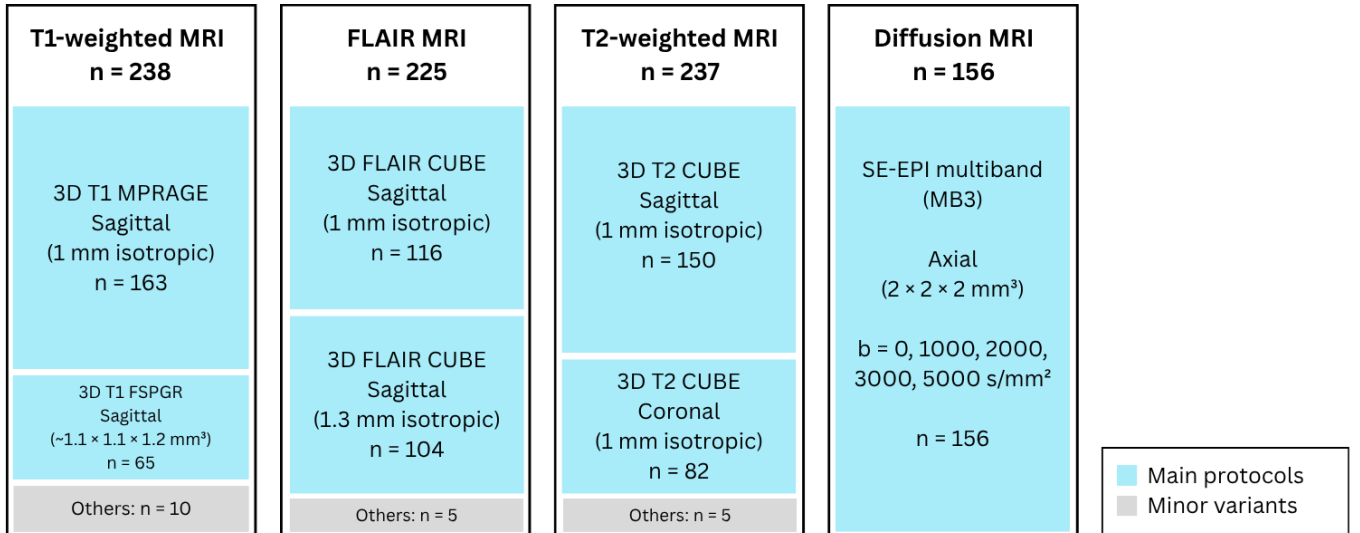


Figure 4.1: Distribution of the MRI acquisition protocols across the $n = 240$ available MRI scans. For each modality, the number of acquisitions is given per sequence type and orientation, with the main protocols shown in blue and minor variants in grey.

T_1 -weighted imaging

T_1 -weighted images were mainly acquired using either a 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence or a 3D fast spoiled gradient echo (FSPGR) sequence.

All T_1 -weighted acquisitions were obtained in sagittal orientation and provided whole-brain coverage with approximately millimetric spatial resolution.

FLAIR imaging

FLAIR images were acquired using 3D sagittal T_2 -FLAIR CUBE sequences with isotropic spatial resolution of either 1 or 1.3 mm. Five subjects were scanned using alternative protocols, including minor variants or thicker-slice acquisitions.

T_2 -weighted imaging

T_2 -weighted images were primarily acquired using 3D fast spin-echo CUBE sequences with isotropic spatial resolution. Most acquisitions ($n=150$) were obtained in sagittal orientation, while a substantial subset ($n=82$) was acquired in coronal orientation. Because these images were isotropic, differences in acquisition orientation did not affect downstream analyses, as volumes could be reformatted in any plane.

Diffusion-weighted imaging

Diffusion MRI data were acquired using a multi-shell spin-echo echo-planar imaging sequence with multiband acceleration. The protocol included multiple b-values ($b = 0, 1000, 2000, 3000$, and 5000 s/mm²), with a total of 167 volumes including non-diffusion-weighted and diffusion-weighted images. Gradient information was provided in standard `.bval` and `.bvec` files. During preprocessing, diffusion data were resampled to 2 mm isotropic resolution.

Table 4.1: Main MRI acquisition parameters used in the cohort. TR: repetition time; TE: echo time; TI: inversion time; α : flip angle; MB: multiband; iso.: isotropic.

Modality	Sequence	Orient.	Resolution	TR [ms]	TE [ms]	TI [ms]	α [°]
T_1	3D MPRAGE	Sag.	1 mm iso.	2186	2.94	900	8
T_1	3D FSPGR	Sag.	1.1×1.1×1.2	7.17	2.90	400	11
T_2	3D CUBE	Sag.	1 mm iso.	2500–3200	90–94	–	90
T_2	3D CUBE	Cor.	1 mm iso.	2500–3200	85–90	–	90
FLAIR	3D T_2 -FLAIR CUBE	Sag.	1–1.3 mm iso.	5000	143	1559	90
DWI	SE-EPI MB	Axial	2×2×2	4837	78.6	–	–

Data organisation followed a structure closely inspired by the Brain Imaging Data Structure (BIDS) [65] standard to facilitate reproducibility and automated processing pipelines. The dataset was managed using the BIDS Managing and Analysis Tool (BMAT) software framework [66], which provides tools for standardised organisation and processing of neuroimaging data.

4.1.2 Cognitive Assessment

Cognitive performance was assessed using a standardised neuropsychological battery administered at Saint-Luc University Clinics, typically within six months following MRI acquisition.

Global cognitive function was assessed using the Mini-Mental State Examination (MMSE; maximum score: 30), a widely used cognitive screening tool providing an overall measure of cognitive impairment across domains including orientation, memory, language, attention,

and visuoconstructive functioning. Although widely used for cognitive screening, the MMSE does not provide a detailed assessment of specific cognitive domains [67].

Neuropsychological assessment further evaluated four cognitive domains: memory, executive functions, language, and visuospatial abilities. Composite cognitive scores were computed by averaging standardised Z-scores obtained from several neuropsychological measures within each domain [68]. Z-scores were calculated relative to an independent cohort of cognitively stable amyloid-negative individuals from the UCL-2010-412 study.

- **Memory composite score**

Episodic verbal memory was assessed using the French version of the Free and Cued Selective Reminding Test (FCSRT) [69]. This test evaluates memory encoding, storage, and retrieval processes and is particularly sensitive to hippocampal dysfunction associated with Alzheimer’s disease. The memory composite score was computed from immediate recall, total recall with cueing, and delayed recall measures.

- **Executive composite score**

Executive functions involve adaptation to novel situations, cognitive flexibility, task switching, inhibition, planning, and organisation. These abilities were assessed using the Trail Making Test (TMT) parts A and B [70] and Luria’s Graphic Sequences. In the TMT, participants connect numbered circles sequentially (part A) and alternate between numbers and letters (part B). Completion time and errors were used as measures of processing speed and executive functioning.

- **Language composite score**

Language abilities were evaluated using semantic verbal fluency, phonemic verbal fluency, and oral naming tasks [71]. In semantic fluency tasks, participants were asked to generate as many words as possible belonging to a given category (e.g., animals) within a fixed time interval. In phonemic fluency tasks, participants generated words beginning with a specific letter. Oral naming abilities were assessed using line drawings of common objects that participants had to identify verbally. These tasks evaluate lexical access, semantic memory, verbal production, and language organisation. Impairment is commonly observed in AD.

- **Visuospatial composite score**

Visuospatial abilities were assessed using the Clock Drawing Test [72] and constructional praxis tasks from the CERAD battery [71]. Participants were asked to spontaneously draw a clock with correctly positioned numbers and hands, and to reproduce geometric figures. These tests evaluate visuoconstructive abilities, spatial organisation, planning, and praxis.

Based on these domain-specific scores, participants were initially categorised in three groups, as CN when no cognitive domains were impaired, as MCI when at least one domain was impaired, or as dementia when cognitive impairment was associated with significant functional decline. This initial classification relied on cognitive performance measures only and did not account for AD biomarkers. In addition to the domain-specific composite scores, a **global cognitive composite score** was computed by combining the standardised scores from the memory, executive, language, and visuospatial domains, providing an overall measure of cognitive performance.

Diagnostic categories were refined by combining cognitive assessment with amyloid status, determined by lumbar puncture or amyloid PET (^{18}F flutemetamol or ^{11}C PiB) on the

Centiloid scale [73]. Participants were classified as $A\beta$ positive for a Centiloid value above 25 or CSF $A\beta_{42}$ below 437 pg/mL [74]. Crossing cognitive stage (CN, MCI, dementia) with amyloid status defines six groups, separating the AD continuum from non-AD profiles (Table 4.2).

Table 4.2: Combined cognitive and amyloid-status classification used in the study.

Group	Cognitive status	Amyloid status	Interpretation
$A\beta$ – CN	Cognitively normal	Amyloid-negative	Normal aging
$A\beta$ + CN	Cognitively normal	Amyloid-positive	Preclinical Alzheimer’s disease
$A\beta$ – MCI	Mild cognitive impairment	Amyloid-negative	Non-AD MCI
$A\beta$ + MCI	Mild cognitive impairment	Amyloid-positive	Prodromal Alzheimer’s disease
$A\beta$ – DEM	Dementia	Amyloid-negative	Non-AD dementia
$A\beta$ + DEM	Dementia	Amyloid-positive	Alzheimer’s disease dementia

4.1.3 Population Description

All demographic, clinical, cognitive, and MRI information was centralised in a spreadsheet database, including subject identifiers, assessment and acquisition dates, CSF biomarkers, cognitive composite scores, and clinical diagnoses. This facilitated the matching between MRI and cognitive data. Among the available data, 117 participant visits had both MRI scans and all the clinical information required for the present analyses and were therefore included in the main cohort. Some participants had more than one MRI acquisition, but the follow-up data were too irregular to perform longitudinal analyses. Therefore, the analyses were conducted at the acquisition level, with each visit considered separately. These acquisitions were classified into three diagnostic groups according to the procedure described in Section 4.1.2, resulting in 26 CN, 64 MCI, and 27 dementia cases. Amyloid status was available for 115 of the 117 acquisitions, yielding 74 amyloid-positive and 41 amyloid-negative cases, while two acquisitions had unknown amyloid status (Figure 4.2).

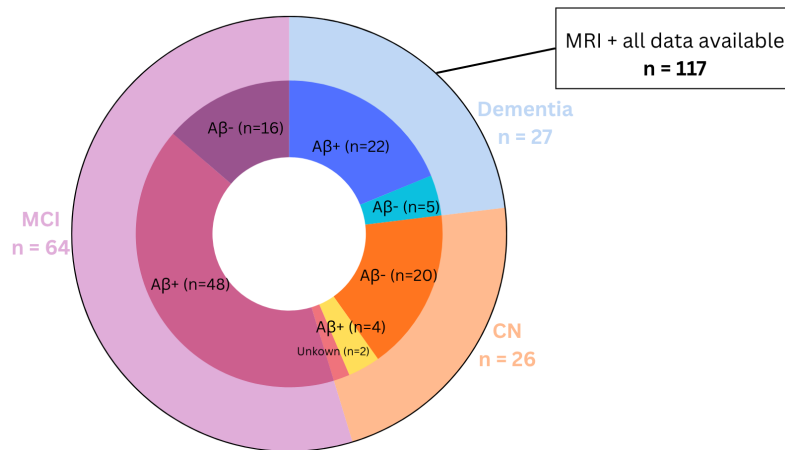


Figure 4.2: Composition of the study cohort. The outer black ring indicates the total number of subjects retained for the main analyses with both MRI scans and corresponding data available. The outer ring shows the distribution across the three diagnostic groups: (CN, MCI, dementia), and the inner ring further divides each group by amyloid status ($A\beta$ +, $A\beta$ –, or unknown). Values are expressed as numbers of scans available.

Demographic and cognitive characteristics of the final cohort are summarised in Table 4.3.

Potential demographic imbalances were assessed to identify differences in age, sex distribution, and education level that could influence subsequent analyses and to determine which covariates should be included for statistical adjustment. Two types of group comparisons were performed. First, demographic differences between diagnostic groups (CN, MCI, and dementia) were assessed. Sex distribution was compared using a Chi-square test, while age and years of education were compared using Analysis of Covariance (ANCOVA) models. Second, demographic differences between males and females were also examined. Differences in age and years of education between sexes were assessed using ANCOVA models adjusted for diagnostic group. The results of these analyses are presented in Chapter 5.

Table 4.3: Demographic, amyloid status, and cognitive characteristics of the study cohort. Values are reported as mean $\pm$ standard deviation unless otherwise specified.

	CN	MCI	Dementia
N	26	64	27
Amyloid+ / Amyloid- / Unknown	4 / 20 / 2	48 / 16 / 0	22 / 5 / 0
Age (years)	71.12 $\pm$ 8.16	76.39 $\pm$ 9.13	73.85 $\pm$ 7.99
Female / Male	14 / 12	33 / 31	14 / 13
Education (years)	16.83 $\pm$ 3.67	15.61 $\pm$ 3.70	13.48 $\pm$ 3.83
MMSE	29.28 $\pm$ 0.74	26.40 $\pm$ 1.75	19.52 $\pm$ 2.86
Global cognition	0.10 $\pm$ 0.43	-1.27 $\pm$ 0.86	-3.19 $\pm$ 1.46

4.2 Data Preprocessing

4.2.1 Diffusion MRI Preprocessing

Diffusion MRI data are affected by both general MRI-related artefacts and diffusion-specific distortions. Common MRI artefacts include thermal noise, subject motion, susceptibility-induced geometric distortions, and Gibbs ringing artefacts, while diffusion MRI is additionally sensitive to eddy-current distortions arising from strong diffusion gradients, diffusion-gradient-induced motion, and geometric distortions related to echo-planar imaging (EPI).

To correct for these artefacts, preprocessing was performed using the ElikoPy pipeline [75, 76], which integrates standard correction procedures into a unified workflow. As illustrated in Figure 4.3, the pipeline begins with skull stripping followed by Marchenko–Pastur Principal Component Analysis (MP-PCA) denoising to reduce thermal noise. When required, Gibbs ringing artefacts were corrected to reduce oscillations near sharp tissue boundaries. Susceptibility-induced distortions are estimated using Synb0-DISCO, which synthesizes an undistorted b_0 image from the T_1 -weighted image when reverse phase-encoded acquisitions are unavailable. This synthetic image is subsequently used within a Topup framework to estimate the off-resonance field.

Eddy-current distortions and subject motion are corrected using the FMRIB Software Library (FSL)’s `eddy` tool (CUDA/OpenMP implementation) [77], which additionally accounts for slice-to-volume and volume-to-volume motion. Finally, bias field correction is applied to compensate for intensity inhomogeneities, resulting in fully preprocessed diffusion data.

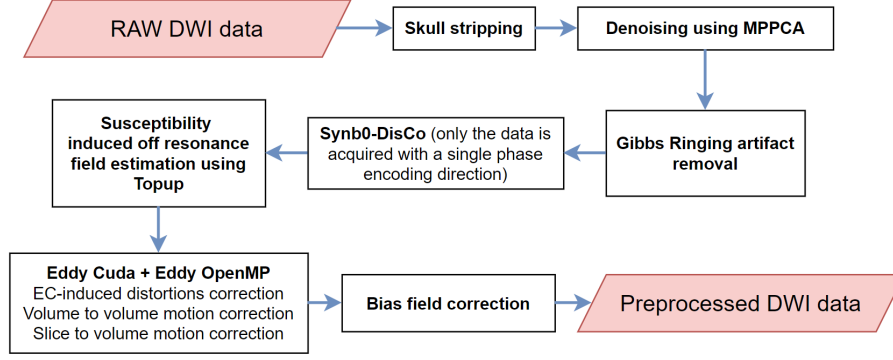


Figure 4.3: Overview of the diffusion MRI preprocessing pipeline implemented in ElikoPy [78].

4.2.2 Structural MRI Preprocessing

Structural T_1 -weighted images were processed using FreeSurfer (version 7.4.1) [48] through the automated `recon-all` pipeline, which performs skull stripping, tissue segmentation, cortical surface reconstruction, and anatomical labelling. Two principal outputs were used in this study: the `aseg`¹ segmentation for subcortical and ventricular structures, and the `aparc`² parcellation for cortical regions [79, 80].

For subjects with multiple time points, the longitudinal FreeSurfer pipeline was additionally applied. A subject-specific template was first created using `recon-all -base`, after which each session was reprocessed with `recon-all -long`. This helped make anatomical volumes more consistent across visits, especially intracranial volume. Although intracranial volume is expected to remain stable within the same adult subject, small differences can arise between MRI sessions due to image quality, head positioning, and segmentation variability.

4.2.3 Image Registration

Image and atlas registration were performed to align the different MRI modalities and standard-space atlases with each subject’s images. This allowed information from structural, FLAIR, and diffusion MRI to be combined in the following analyses, and allowed the required segmentations and regions of interest to be obtained in each patient’s own image space.

All registrations were performed using a custom pipeline based on the `regis.core` module from the `pilab-regis` package, which provides simplified access to registration algorithms implemented in DIPY (Diffusion Imaging in Python) [81].

Different transformations were applied depending on the anatomical relationship between modalities:

- The `aseg` and `aparc` segmentations were registered to the FLAIR images using a rigid transformation, as both images came from the same subject and session. This

¹`aseg` refers to the *automated anatomical segmentation* produced by FreeSurfer, which labels subcortical grey matter, ventricular structures, white matter, and other major anatomical regions from structural MRI data [79].

²`aparc` refers to the *automated cortical parcellation* produced by FreeSurfer, which assigns cortical surface regions to anatomical labels based on gyral and sulcal features [80].

corrected for differences in head position while preserving the anatomical labels.

- The `aseg` and `aparc` segmentations were mapped to the diffusion space, defined by the FA images, using a diffeomorphic transformation. Diffusion MRI is more prone to geometric distortions related to echo-planar imaging, so a non-linear transformation was required to achieve accurate alignment with the structural images.
- The JHU-ICBM white matter labels were registered into each subject’s native diffusion space. To this end, the MNI FA template (HCP1065)³ was first registered to the subject’s FA image using a diffeomorphic transformation, and the resulting deformation was then applied to the JHU label atlas. The FA template was used to drive the intensity-based registration, whereas the label atlas was only transported by the estimated transformation, since label maps cannot be registered directly.

Figure 4.4 illustrates an example of registration for one subject, showing the native FLAIR image, the original FreeSurfer segmentation, and the propagated `aseg` labels after alignment to FLAIR space.

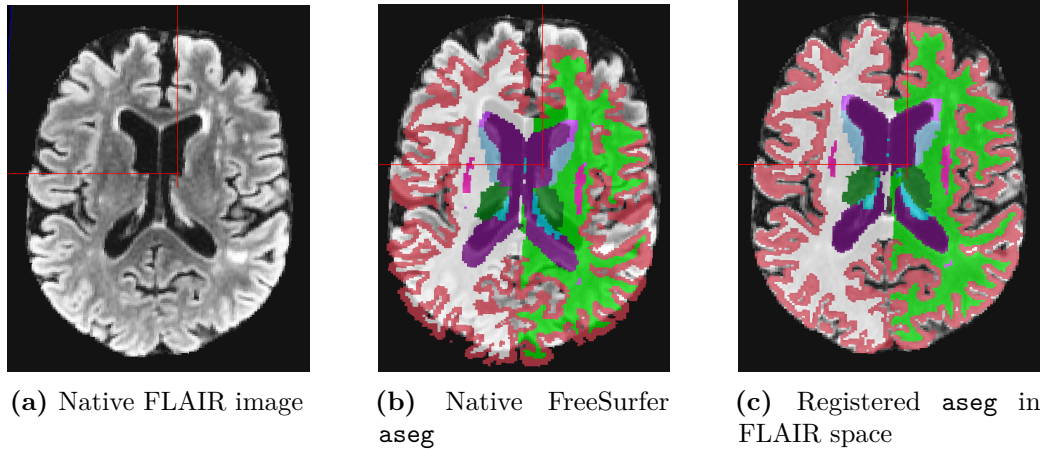


Figure 4.4: Example of image registration. From left to right: native FLAIR image, original FreeSurfer `aseg`, and propagated `aseg` labels after registration into FLAIR space.

Registration Quality Control

To ensure reliable alignment between the `aseg` segmentation and the FLAIR image, an automated quality control (QC) step was performed. This QC checked whether CSF-like low-intensity voxels around the ventricles matched the registered ventricular labels. Cases with an excessive overlap between CSF-like voxels and WM labels were flagged as potential misregistrations.

Using this approach, 10 subjects were flagged as potential failures. All flagged cases were subsequently reviewed through visual inspection. Most cases showed only minor or localized misalignment, with no impact on subsequent analyses. However, two subjects presented clear registration failures, as illustrated in Figures 4.5a and 4.5b. One case was likely related to severe ventricular enlargement, which led to poor alignment of the `aseg` segmentation, while the other showed noticeable misregistration artefacts. Overall, these

³The HCP1065 template is an FA template distributed with FSL and derived from diffusion MRI data of 1065 subjects from the Human Connectome Project (HCP) [82].

results indicate that the registration procedure was generally robust, with only these two cases requiring exclusion from subsequent analyses.

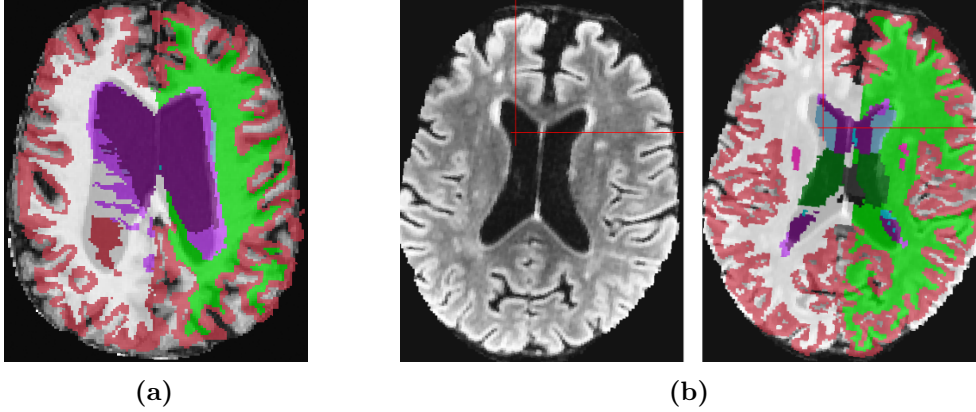


Figure 4.5: Registration failures detected during visual quality control. (a) The `aseg` segmentation was not correctly generated, likely due to severe ventricular enlargement. (b) The ventricular label shown in purple does not fully cover the CSF-like ventricular region, with part of this area incorrectly assigned to the caudate label shown in blue-grey.

4.3 Data Processing

4.3.1 Diffusion Model Reconstruction

Following preprocessing, several diffusion models were fitted to the data. First, the DTI model was estimated to derive the classical diffusion metrics described in Chapter 1, Section 1.3.1. In addition, the NODDI model was computed to obtain complementary microstructural measures. Among the NODDI-derived metrics, two volume fractions were retained for the subsequent analyses. The isotropic (free-water) fraction is denoted FISO ($\equiv f_{fw}$). The intra-neurite fraction, denoted FINTRA, was recovered as the raw intra-neurite signal fraction f_{intra} of the three-compartment model ($f_{intra} + f_{extra} + f_{fw} = 1$) introduced in Section 1.3.2.

4.3.2 Pipelines for Biomarker Assessment

Building upon the state-of-the-art review presented in Chapter 3, dedicated processing pipelines were implemented to quantify each biomarker in the present dataset. The related codes can be found on the project GitHub repository.⁴ Rather than reproducing a single published workflow, the pipelines were designed by adapting methodological components reported in the literature, with emphasis on approaches considered robust, reproducible, and compatible with the available imaging data. All pipeline parameters were empirically selected and kept constant across subjects.

⁴https://github.com/kayserl/Glymphatic_AD_tool

CP Segmentation

CP segmentation was performed using a custom Python implementation inspired by the One-GMM framework proposed by Zhou et al. [44] and described in Section 3.1. The pipeline was reimplemented in Python to integrate within the present workflow. The procedure followed three stages: (i) definition and refinement of an anatomical candidate region, (ii) intensity-based classification of CP tissue using a GMM, and (iii) final refinement and volume normalisation.

Candidate region. Anatomical priors derived from FreeSurfer `aseg` segmentations were used to define a candidate region combining the lateral ventricle (LV) and CP labels, processed independently for the left and right hemispheres to account for anatomical variability. The initial LV-CP mask was refined using a single binary erosion with a spherical structuring element (one iteration, radius of one voxel), followed by extraction of the largest connected component. This step removed boundary voxels and reduced partial-volume effects while preserving the core ventricular region containing CP tissue. This was particularly important because the `aseg` segmentation may misclassify voxels at the LV borders (Figure 4.6a-c). These erosion parameters were kept conservative to avoid removing CP voxels located along the ventricular borders.

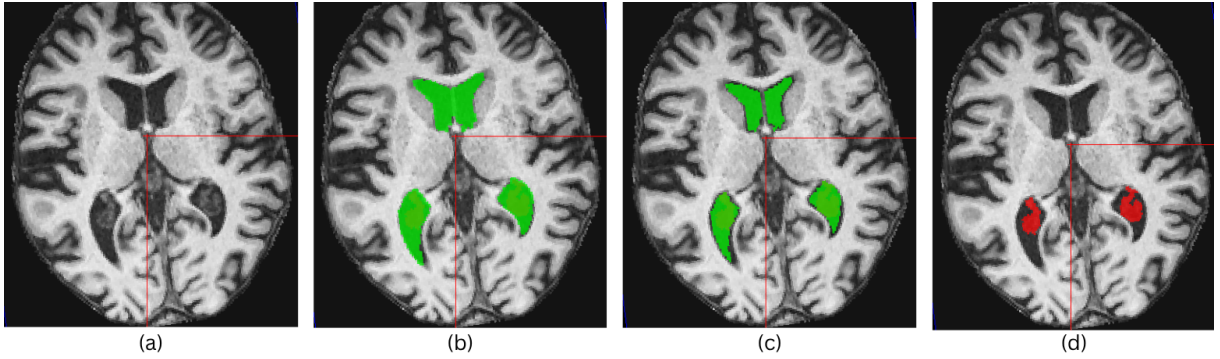


Figure 4.6: Refinement of the LV-CP candidate mask. (a) Raw T_1 -weighted image. (b) Initial LV-CP mask including boundary voxels and WM contamination. (c) Refined mask after erosion and extraction of the largest connected component, resulting in a cleaner candidate region that better isolates the ventricular space. (d) Final CP segmentation obtained using the GMM within the refined region, followed by expansion and morphological closing to improve spatial continuity.

GMM classification. A GMM was then fitted to the T_1 -weighted intensities within the refined mask. Four components were used instead of the three of the original method, which were found insufficient to separate CSF, partial-volume voxels, and CP tissue. Initial Gaussian means were set from the 10th, 40th, 70th, and 90th intensity percentiles, and the model parameters were optimised using the Expectation-Maximization algorithm until convergence or until the maximum number of iterations was reached. The component with the highest mean intensity was retained as the CP class.

Refinement and normalisation. Because the selection of a single class was not sufficient to capture partial-volume effects, whereas the inclusion of several classes led to over-segmentation, an expansion step incorporated neighbouring voxels of the candidate region whose intensity exceeded the 15th percentile of the selected class. Morphological closing with a spherical kernel (radius of one voxel) was then applied to improve spatial continuity, and only the largest connected component was retained. Left and right masks were finally combined into the bilateral CP segmentation (Figure 4.6d).

Finally, CP volume was computed from the final binary mask and normalised by the intracranial volume (ICV), extracted from the FreeSurfer `aseg.stats` file using the `EstimatedTotalIntraCranialVol` measure, and expressed both as a fraction of ICV and as volume per 1000 units of ICV.

DTI-ALPS Index Computation

The DTI-ALPS index was computed from the DTI maps using automated ROI placement in native FA space. The physiological interpretation and anatomical principles underlying the method are presented in Section 3.3. The pipeline comprised three steps: (i) automated definition of the projection and association ROIs, (ii) orientation correction of the diffusion tensors, and (iii) computation of the directional diffusivities and of the ALPS index.

ROI definition. Subject-specific ROIs were generated from predefined MNI coordinates proposed by Li et al. [56]: $(37, -12, 24)$, $(26, -12, 24)$, $(-26, -12, 24)$, and $(-37, -12, 24)$, corresponding to bilateral association and projection fibre regions. These coordinates were first transformed from MNI space to the subject’s FA space using the registration between the MNI FA template and the subject FA image, as described in Section 4.2.3. The transformed coordinates were then converted into voxel indices using the inverse affine matrix of the subject FA image. A $3 \times 3 \times 3$ voxel cubic ROI was generated around each location. This ROI size and shape were selected following the recommendations of Ulloa et al. [59] to standardise measurements across subjects while having enough information for the mean. To improve anatomical specificity, each cubic ROI was intersected with the corresponding JHU-derived fibre mask previously registered to the subject FA image. These masks corresponded to the superior longitudinal fasciculus for association fibres and the corona radiata for projection fibres, following Li et al. [56]. This step retained only voxels located within the expected fibre regions and reduced contamination from neighbouring tissues (Figure 4.7a).

Orientation correction. After ROI placement, directional diffusivities were extracted from the diffusion tensors in the subject’s native diffusion space. However, the voxel axes of each image do not necessarily correspond to the anatomical right-left, anterior-posterior, and superior-inferior directions, because head position and acquisition orientation can vary between subjects [57]. Therefore, before extracting directional diffusivities, the anatomical axes defined in MNI space were converted into each subject’s voxel coordinate system, using a change-of-basis approach (Figure 4.7b). The rotational component of the registration between the subject FA image and the MNI FA template was extracted using singular value decomposition, in order to remove residual scaling or shear. This rotation was then combined with the voxel-to-world orientation encoded in the diffusion-tensor affine matrix to express the MNI anatomical axes in the subject’s voxel space. This correction allowed diffusivities to be measured along consistent anatomical directions across subjects without resampling the diffusion tensors.

Diffusivity and index computation. Once the anatomical directions had been expressed in the subject’s voxel space, the diffusion tensor in each voxel was evaluated along these three directions. For a given unit vector $\mathbf{u}$, the corresponding directional diffusivity was computed as

$$D_{\mathbf{u}} = \mathbf{u}^T \mathbf{D} \mathbf{u}, \quad (4.1)$$

where $\mathbf{D}$ denotes the voxelwise diffusion tensor. This yielded three diffusivity maps per subject, corresponding to the right-left, anterior-posterior, and superior-inferior anatomical directions. Mean diffusivities were then extracted from the projection and

association ROIs in each hemisphere. The right and left ALPS indices were computed separately using the conventional DTI-ALPS formula introduced by Taoka et al. [39] and described in Section 3.3 (Equation 3.1). A global ALPS value was finally obtained by averaging the right and left indices.

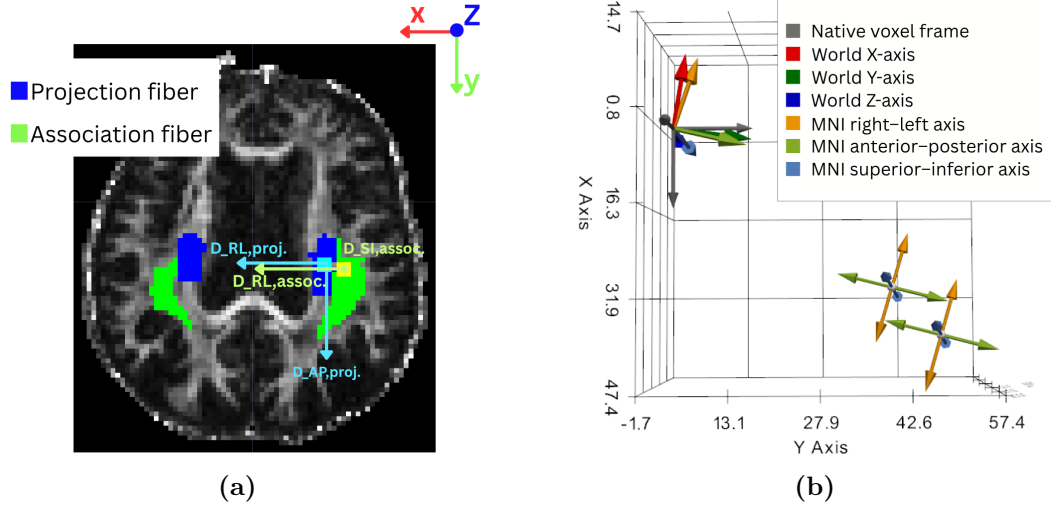


Figure 4.7: Automated ROI definition and orientation correction for DTI-ALPS computation. (a) Directional diffusivities used in the ALPS formula, overlaid on a subject FA map. The right-left diffusivities measured in the projection and association ROIs form the numerator of the index, while D_{AP} in the projection ROI and D_{SI} in the association ROI form the denominator. The coordinate axes indicate the anatomical right-left (x), anterior-posterior (y), and superior-inferior (z) directions, with the superior-inferior direction oriented through the axial slice. (b) Illustration of the change-of-basis used to express the anatomical directions in the subject's native voxel space before extracting directional diffusivities.

EPVS Segmentation

ePVS were segmented using a custom Python pipeline combining anatomical priors derived from FreeSurfer parcellation with intensity-based enhancement of tubular structures on structural MRI, following several strategies reported in the literature (Section 3.2). The aim was to detect ePVS within anatomically plausible regions (CSO and BG) while limiting common sources of false positives such as white matter hyperintensities (WMH), ventricular borders, and partial-volume effects.

Image preparation. The pipeline used both T_1 -weighted and FLAIR acquisitions, as ePVS appear hypointense on T_1 -weighted images and can be distinguished from WMH on FLAIR. After registration and intensity normalisation, the T_1 -weighted and FLAIR images were summed voxel-wise to create a single multimodal contrast image, following the approaches proposed by Schwartz and Rashid [50, 83]. This combined image was used to improve ePVS visibility, reduce inter-subject intensity variability, and limit confusion with WMH.

Anatomical masks. WM, BG, and CSO masks were derived from the FreeSurfer `aseg` segmentation previously registered to T_1 space (Section 4.2.3). The CSO region was defined as the superior portion of WM above the corpus callosum, facilitating reproducibility, with erosion applied to reduce border-related partial-volume effects that may mimic linear structures (blue mask in Figures 4.8 and 4.9a). The BG mask (red in Figures 4.8 and 4.10a) was generated from the caudate, putamen, pallidum, thalamus, and nucleus accumbens

labels.



Figure 4.8: Anatomical masks used for ePVS segmentation. CSO region is shown in blue, while BG region is shown in red on a sagittal T_1 -weighted MRI slice.

Tubular enhancement and detection. A multi-scale Frangi filter was applied to the combined T_1 +FLAIR image to enhance tubular structures compatible with ePVS. This filter uses the local curvature of the image, derived from the Hessian matrix, to identify elongated intensity patterns. Several Gaussian scales ($\text{sigmas} = [0.5, 0.7, 1.0]$) were used to detect structures of different diameters. The resulting vesselness map was restricted to the BG and CSO masks, and then thresholded to obtain a binary candidate mask. A threshold of 0.22 was used, based on values from the literature and refined by visual quality control. Lower thresholds produced more false positives, whereas higher thresholds missed smaller ePVS. Finally, connected-component filtering was applied to remove unlikely detections. Only components extending over at least 4 voxels along one image axis were retained. This preserved elongated structures while removing small clusters caused by noise or isolated Frangi responses (Figures 4.9 and 4.10).

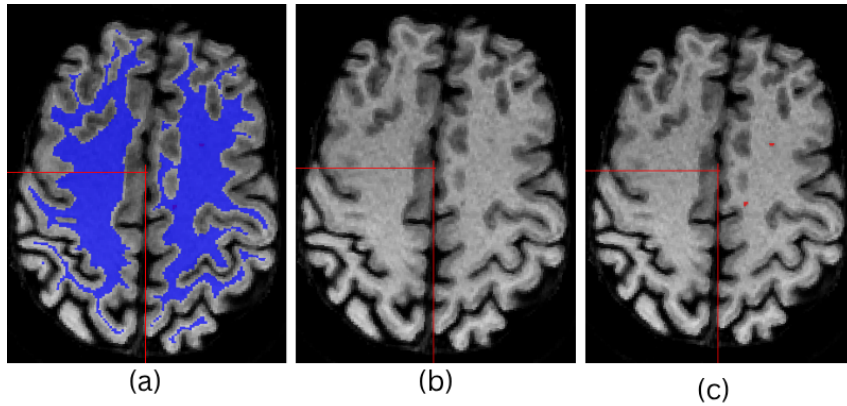


Figure 4.9: Example of the proposed ePVS segmentation pipeline in the CSO region. (a) Illustration of the anatomical CSO mask. (b) The corresponding T_1 +FLAIR image. (c) The final ePVS segmentation.

Quantification. The final binary ePVS masks were quantified by counting connected voxel clusters separately in the left and right hemispheres for both the BG and CSO regions. For each region, the highest hemispheric count was retained. For validation against expert visual assessments, these counts were converted into visual rating scores using the scale described in Section 3.2. The main analyses, however, used the continuous automated counts rather than the derived visual scores.

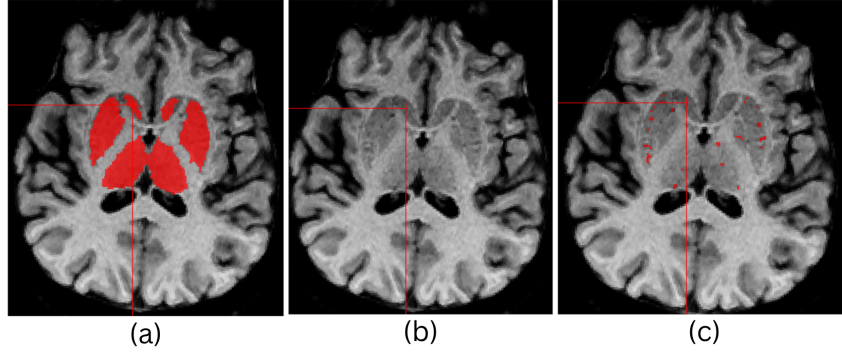


Figure 4.10: Example of the proposed ePVS segmentation pipeline in the BG region. (a) Illustration of the anatomical BG mask. (b) corresponding T_1 +FLAIR image. (c) Final ePVS segmentation. (d) Sagittal view including CSO and BG masks.

NODDI-derived Metrics

The present pipeline focused on the raw NODDI signal fractions FISO (f_{iso}) and FINTRA (f_{intra}), rather than on NDI and ODI described in Section 1.3.2. FISO was retained as a marker of free-water diffusion, which is directly relevant to fluid-related alterations. FINTRA was preferred over NDI because it represents the intra-neurite fraction of the whole voxel, whereas NDI is normalised to the tissue compartment only and is therefore less directly comparable when free-water content varies.

NODDI metrics were extracted from the maps computed during diffusion preprocessing (Section 4.3.1) in subject-specific diffusion space. Regional extraction was based on FreeSurfer segmentations and JHU white matter atlas labels registered to each subject’s FA space (Section 4.2.3). The selected regions were chosen among those consistently reported in the literature (Section 3.4) in relation to Alzheimer’s disease and cognitive decline. They included grey matter ROIs, namely the hippocampus, amygdala, parahippocampal cortex, entorhinal cortex, precuneus, and cingulate subdivisions [64], as well as WM tracts from the JHU atlas, including the cingulum, uncinate fasciculus, superior longitudinal fasciculus, and fornix. For each ROI, left and right hemisphere labels were merged into bilateral masks to improve measurement robustness and statistical power [84, 85]. Finally, mean and standard deviation values of FISO and FINTRA were extracted within each bilateral mask.

4.4 Experiments

The following statistical analyses were performed in Python (version 3.11.13) using the `statsmodels` library for OLS regression and ANCOVA, `pingouin` for partial correlations, and `scipy` for the Shapiro–Wilk and Levene tests.

4.4.1 Biomarker Validation Strategy

Two strategies were used to assess the reliability of the proposed imaging biomarkers. Validation was performed only for CP volume and ePVS burden, as these were the only biomarkers for which reference data were available in this cohort. For the remaining

biomarkers, namely ALPS and NODDI-derived metrics, there was no direct ground truth. Their interpretation relied on comparison with previous findings from the literature.

Validation of Choroid Plexus Segmentation

CP segmentation validation was performed by comparing the proposed GMM-based method with the U-Net framework developed by Yazdan-Panah et al. [46] (Section 3.1). This U-Net method was applied to the present cohort and was used as the reference segmentation for validation. Only subjects available in both segmentation outputs were included in the analysis. CP volumes were normalised by ICV and expressed as CP volume per 1000 mm³ of ICV.

Volumetric agreement between both methods was assessed using Pearson correlation analysis. A linear regression model was additionally fitted to evaluate the global relationship between the two segmentation approaches.

Spatial overlap between CP masks was evaluated using the Jaccard similarity coefficient. Subjects with a Jaccard index below 0.3 were flagged for visual inspection. This threshold was chosen as criterion, because cases around this value showed variable segmentation quality, with some masks still being acceptable and others corresponding to clear segmentation errors. The threshold was therefore not used as an automatic exclusion rule, but rather to identify cases requiring manual review. Visual inspection was then performed to determine whether the disagreement originated from the proposed segmentation pipeline or from the reference U-Net framework, and to decide whether the subject could be reliably retained for subsequent analyses.

Validation of Automated ePVS Quantification

For ePVS, automated counts were compared with manual visual ratings performed by an experienced rater from Saint-Luc University Clinics. Because visual ratings are discrete and slice-based, whereas the automated pipeline provides continuous whole-volume counts, this comparison was used to assess overall consistency rather than absolute equivalence. Subsequent analyses were performed using the continuous automated ePVS counts.

Manual assessment was performed in the BG and CSO regions separately using the conventional visual rating procedure described in Section 3.2. For each participant, the slices exhibiting the highest visible ePVS burden were identified and visually rated within each anatomical region. In parallel, automated quantification was obtained using the proposed 3D segmentation pipeline (Section 4.3.2).

Agreement between manual and automated measurements was evaluated using Spearman correlation analyses, as the visual ratings were ordinal scores. Confusion matrices were also used to examine agreements and disagreements between the manual and automated rating scores.

4.4.2 Biomarker Analysis Strategy

The following strategy was applied consistently across all imaging biomarkers investigated in the present study.

Inclusion Criteria and Outliers Removal

Only participants with available biomarker values, cognitive scores, age, sex, and years of education were included in the analyses. For the CP biomarker, excluded subjects corresponded to visually identified segmentation failures during the validation procedure. For the other biomarkers, outlier detection was systematically applied using the

interquartile range (IQR) method in order to identify extreme values potentially related to methodological limitations. This procedure was applied both to imaging biomarkers and cognitive scores. Indeed, unusually low cognitive performances may occasionally reflect non-biological factors such as fatigue during assessment or misunderstanding of instructions, potentially leading to scores not fully representative of the participant's actual cognitive status.

Subjects with values outside the interval

$$[Q_1 - 1.5 \times IQR, Q_3 + 1.5 \times IQR]$$

where Q_1 and Q_3 are the first and third quartiles and $IQR = Q_3 - Q_1$, were considered outliers according to the conventional Tukey criterion. Analyses were repeated with and without outlier exclusion to evaluate the influence of extreme values on the observed associations.

Associations Between Imaging Biomarkers and Age

Because age is a major factor in Alzheimer's disease and may also influence glymphatic-related imaging biomarkers, associations between imaging biomarkers and age were investigated using correlation and regression models.

Ordinary least squares (OLS) regression was used to investigate associations between age and each imaging biomarker. All models included education level and sex as covariates, except where one of these variables defined the stratification. Three complementary analyses were performed.

First, a main-effect model estimated the overall association between age and the biomarker while adjusting for sex and education:

$$\text{Biomarker} \sim \text{Age} + \text{Sex} + \text{Education}.$$

Second, interaction models were used to test whether the association with age differed according to sex or amyloid status:

$$\text{Biomarker} \sim \text{Age} * \text{Sex} + \text{Education},$$

$$\text{Biomarker} \sim \text{Age} * \text{Amyloid status} + \text{Sex} + \text{Education}.$$

For each term, the regression coefficient (β) and its associated p -value were extracted.

Third, subgroup-specific age slopes were estimated for visualisation and interpretation. Biomarker values were first adjusted for the relevant covariates using a residualisation approach. In practice, a regression model was used to estimate the part of each biomarker explained by the covariates. This covariate-related component was then removed from the original biomarker values, and the group mean was added back to keep the adjusted values on a comparable scale. A separate linear regression was then fitted within each subgroup.

For multi-region biomarkers, such as NODDI-derived metrics, analyses were performed separately within each anatomical region. Partial Pearson correlations with age were computed while controlling for sex and years of education, and results were summarised as heatmaps. Regions showing the strongest associations with age were then selected for further visualisation and investigation.

Associations Between Imaging Biomarkers and Cognitive Performance

Associations between biomarkers and cognitive performance were investigated using correlation and regression approaches.

First, partial Pearson correlations were computed between each biomarker and cognitive score while controlling for age, sex, and years of education. Both global and domain-specific cognitive associations were examined. Particular attention was given to the *global cognitive composite* score, as GS biomarkers represent indirect and non AD-specific imaging measures. MMSE associations were also explored, but were considered less sensitive to early cognitive impairment, as discussed in Section 4.1.2.

Second, group differences were assessed using ANCOVA, with diagnostic group (CN, MCI, dementia; defined in Section 4.1.3) as the main factor and age, sex, and education as covariates:

$$\text{Biomarker} \sim \text{Diagnostic group} + \text{Age} + \text{Sex} + \text{Education}.$$

Adjusted pairwise comparisons between groups were obtained by changing the reference category in the model, allowing comparisons of MCI versus CN, dementia versus CN, and dementia versus MCI. The analysis was then repeated within the amyloid-positive subgroup to focus on subjects with evidence of cerebral amyloid deposition. Adjusted group distributions were displayed using boxplots.

For NODDI-derived metrics, which were measured in many anatomical regions, an additional region-selection step was applied before group analyses. Partial correlations between each metric and cognitive scores were first summarised as heatmaps. For each metric, the three regions showing the strongest average cognitive associations were then selected for the subsequent ANCOVA analyses.

The Shapiro-Wilk and Levene tests were used to check model assumptions. The Shapiro-Wilk test assessed the normality of model residuals, while Levene’s test assessed equality of variances across diagnostic groups.

Associations Between CP Volume and DTI-ALPS

As a first attempt to explore the complementarity between biomarkers, CP volume and DTI-ALPS were analysed jointly. These two biomarkers were selected because they provide one quantitative value per subject, which makes direct comparison and joint modelling easier. Analyses were restricted to subjects with both CP and ALPS measurements available, using the same inclusion and outlier-removal procedures described above. Since the objective was to provide a first view of their potential combined relevance, the analysis was restricted to cognitive outcomes. Partial Pearson correlations between CP volume and ALPS were first computed while controlling for age, sex, and education. Regression models were then used to evaluate whether one biomarker added information beyond the other for explaining cognitive performance.

4.5 Technical Resources

Several technical resources and software tools were used throughout this work:

- Scientific articles were mainly accessed through UCLouvain web library and Zotero was used to organise bibliographic references.
- MRI preprocessing and biomarker extraction pipelines were run on the *Consortium des Équipements de Calcul Intensif (CÉCI)*⁵ infrastructure, which provides shared

⁵The CÉCI is funded by the Fonds de la Recherche Scientifique de Belgique (F.R.S.-FNRS) under Grant No. 2.5020.11 and by the Walloon Region.

Linux-based computing clusters managed with Slurm. These resources were used to process the large MRI datasets and intermediate files.

- ChatGPT (OpenAI) was used as a tool to support code readability, debugging, and improvement of English scientific writing.

Chapter 5

Results

5.1 Demographics

Before presenting the biomarker analyses, descriptive statistics were computed for the study sample using the available demographic variables (age, sex, and education), as detailed in Chapter 4, Section 4.1.3.

Sex distribution did not differ significantly across the three diagnostic groups ($\chi^2 = 0.04$, $p = 0.980$), whereas amyloid status differed markedly between groups ($\chi^2 = 36.19$, $p < 0.001$) (Figures B.1a and B.1b).

Age did not differ significantly between males and females ($p = 0.274$). CN participants were significantly younger than MCI participants ($p = 0.030$), whereas neither the CN-dementia nor the MCI-dementia comparison reached significance ($p = 0.435$ and $p = 0.195$, respectively; Figure B.2).

Education level differed along both dimensions. Male participants reported significantly more years of education than female participants ($p = 0.045$; Figure 5.1a). Dementia participants had significantly fewer years of education than both CN ($p = 0.007$) and MCI ($p = 0.039$) participants, whereas CN and MCI did not differ significantly (Figure 5.1b). Because age, sex, and education may influence both cognitive performance and biomarker measures, their effects were controlled for in all subsequent analyses.

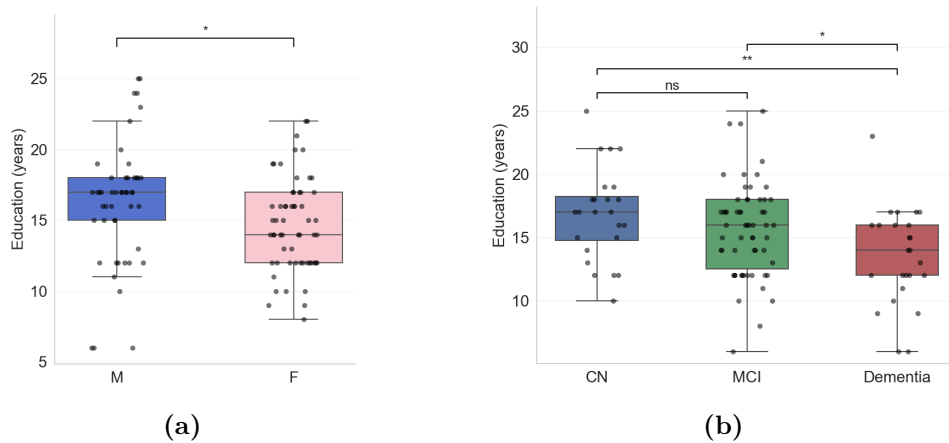


Figure 5.1: Education level distribution according to sex (a) and across diagnostic groups (b). Significance levels: (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = non-significant).

5.2 Biomarker Validation

5.2.1 Validation of CP segmentation

Following the validation procedure described in Section 4.4.1, a total of 81 subjects were available for comparison between the proposed GMM-based segmentation and the reference U-Net framework. Volumetric comparison including all subjects showed a weak but significant correlation between both methods (Pearson’s $r = 0.26$, $p = 0.021$). Visual inspection of the correlation plot revealed several extreme outliers (Figure 5.2a). After exclusion of subjects presenting normalised CP volumes above 3 mm^3 per 1000 mm^3 of ICV ($n = 7$), the relationship between both methods became clearer (Figure 5.2b) (Pearson’s $r = 0.84$, $p < 0.001$).

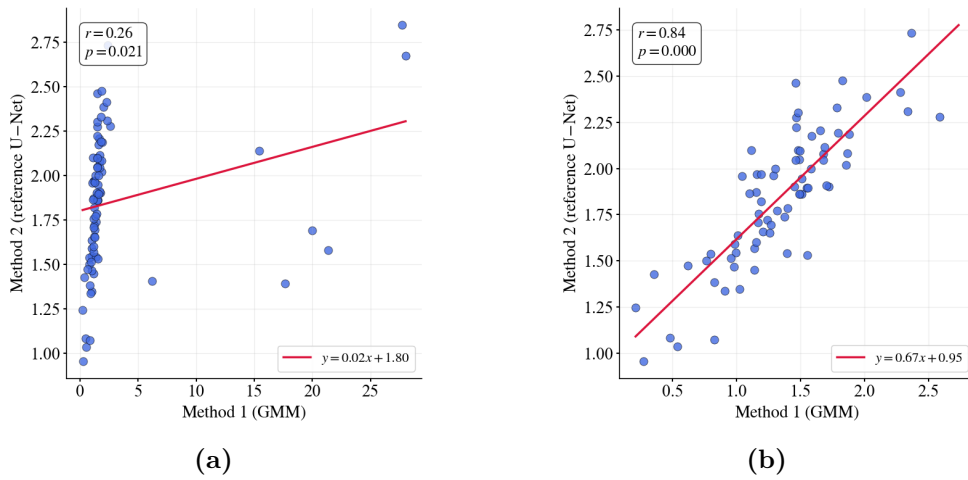


Figure 5.2: Validation of the proposed CP segmentation method with the reference U-Net framework. (a) Correlation before exclusion of outliers. (b) Correlation after exclusion of outliers.

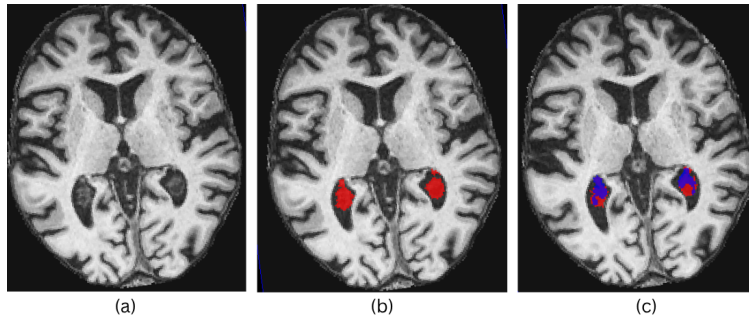


Figure 5.3: Representative example of CP segmentation comparison (Jaccard score = 0.55). (a) Native T1-weighted MRI used for segmentation, slice selected for visualisation of the CP in centre of the LV. (b) CP mask obtained with the proposed GMM-based segmentation method (red). (c) Superposition of the proposed GMM-based segmentation (red) and the reference U-Net segmentation (blue). In this example, the proposed method captures a larger extent of anatomically plausible CP tissue compared with the reference segmentation.

In addition to volumetric agreement, Jaccard similarity coefficients were analysed. Overall, good concordance was observed between both methods, with a mean Jaccard coefficient of 0.46 ± 0.14 . Figure 5.3 illustrates a representative comparison between the two segmentation approaches.

Subjects presenting a Jaccard index below 0.3 ($n = 18$) were further visually inspected to identify the origin of disagreement between methods. Based on this visual analysis comparing subjects with small Jaccard scores, 11 subjects were excluded from subsequent analyses due to segmentation failures.

5.2.2 Validation of Automated ePVS Quantification

A total of 43 subjects were available for comparison between automated and manual ePVS quantification methods. Agreement analyses were performed separately for the BG and CSO regions following the strategy described in Section 4.4.1.

In the BG, automated and manual ePVS scores showed no significant positive correlation (Spearman's $\rho = 0.215$, $p = 0.166$). In contrast, stronger agreement was observed in the CSO region, with a strong positive correlation (Spearman's $\rho = 0.767$, $p < 0.001$). Visual comparison of automated and manual scores is presented in Figure 5.4. In the CSO region, most subjects were distributed close to the identity line. Confusion matrices illustrating score distributions for both anatomical regions are shown in Figures 5.5a and 5.5b .

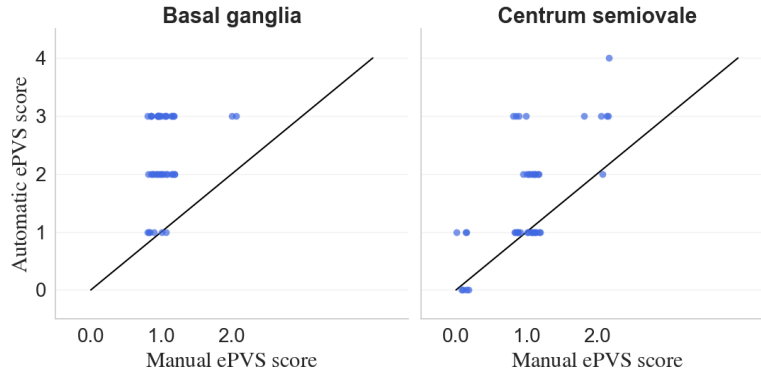


Figure 5.4: Comparison between automated and manual ePVS scores in BG and CSO regions. Each point represents one subject and the black diagonal line corresponds to the identity line.

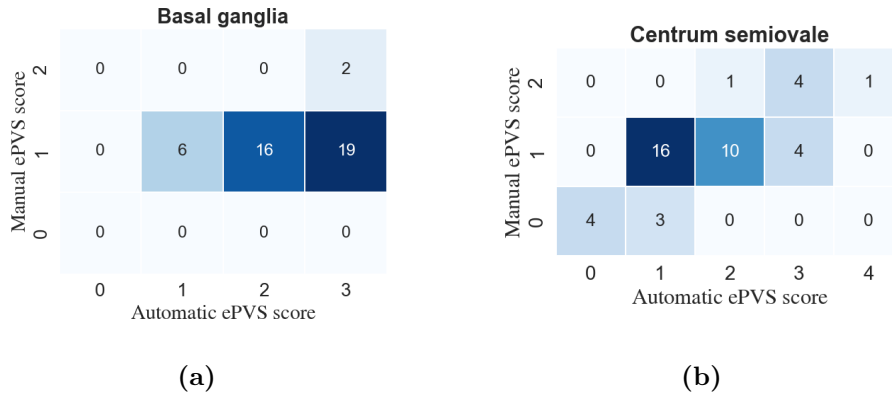


Figure 5.5: Confusion matrices comparing ePVS visual-like scores in the BG (a) and CSO (b) regions. Values represent the number of subjects for each score combination.

5.3 Biomarker Analysis

5.3.1 CP Volume

Based on the visual validation procedure described previously (see Section 5.2.1), 11 subjects were identified as having incorrect CP segmentations and excluded from the study. In addition, 3 participants identified as outliers for the global cognitive composite score were removed, resulting in a final sample of 103 subjects for all subsequent analyses.

Associations with Age

In a linear model adjusted for sex and education, CP volume was significantly and positively associated with age ($\beta = 0.0110$, $p = 0.034$), indicating that CP volume tends to increase across the lifespan. The age $\times$ sex interaction term was not significant ($\beta = -0.0003$, $p = 0.969$), and stratified analyses revealed comparable positive slopes in men ($\beta = 0.0107$, $p = 0.067$) and women ($\beta = 0.0110$, $p = 0.056$). These results suggest similar age-related trajectories of CP volume in both sexes (Figure 5.6a).

In contrast, amyloid status significantly modulated the age-related trajectory of CP volume. The age $\times$ amyloid interaction term was significant ($\beta = -0.0287$, $p = 0.013$), indicating that the age-related slope differed between amyloid-negative and amyloid-positive subjects. As illustrated in Figure 5.6b, stratified analyses showed a pronounced increase in CP volume with age in amyloid-negative subjects ($\beta = 0.0318$, $p < 0.001$, $R^2 = 0.324$), whereas no significant age-related change was observed in amyloid-positive participants ($\beta = 0.0050$, $p = 0.495$, $R^2 = 0.008$). Amyloid-positive subjects also tended to present larger CP volumes at younger ages.

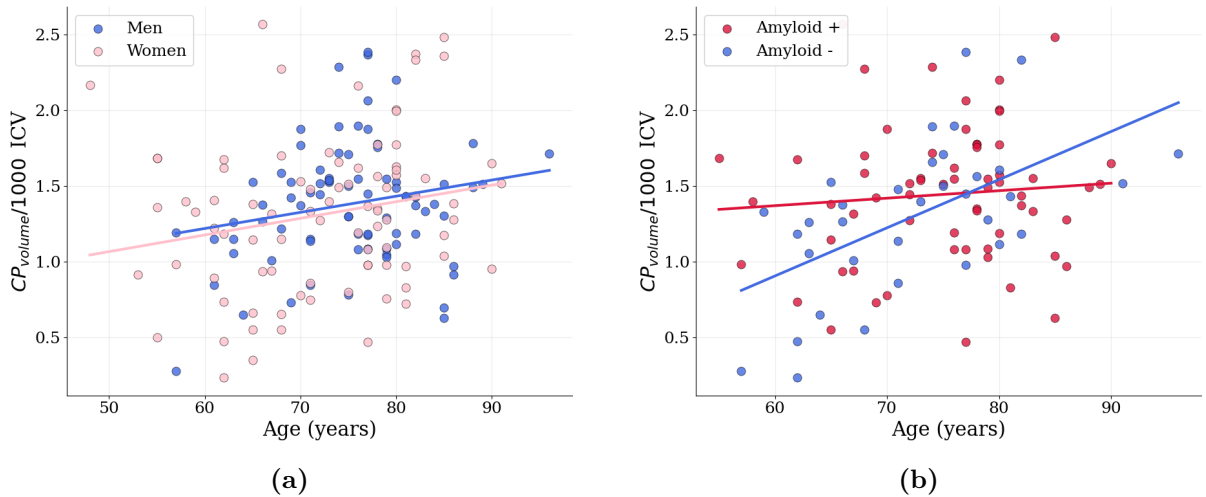


Figure 5.6: Age-related trajectories of normalised CP volume according to sex (a) and amyloid status (b) without outliers. Regression lines correspond to OLS linear regressions fitted separately within each subgroup.

Associations with Cognition

CP volume showed a significant negative association with the *global cognitive composite* score ($r = -0.282$, $p < 0.01$) (Figure 5.7a). However, the relatively scattered distribution of data points reflects inter-subject variability.

Group-level comparisons further supported these findings. Adjusted CP volume differed significantly across diagnostic groups (ANCOVA: $p = 0.010$), with dementia patients

exhibiting significantly larger CP volumes than CN participants ($\beta = 0.401$, $p = 0.008$) (Figure 5.7b). Although dementia patients also tended to show larger CP volumes than MCI participants, this difference did not reach statistical significance ($\beta = 0.239$, $p = 0.055$). A similar pattern was observed when analyses were restricted to the amyloid-positive subgroup, where dementia patients showed significantly larger CP volumes than CN participants ($\beta = 0.613$, $p = 0.037$). However, these subgroup results should be interpreted with caution given the very limited number of amyloid-positive CN participants ($n = 3$) (Supplementary Figure B.3).

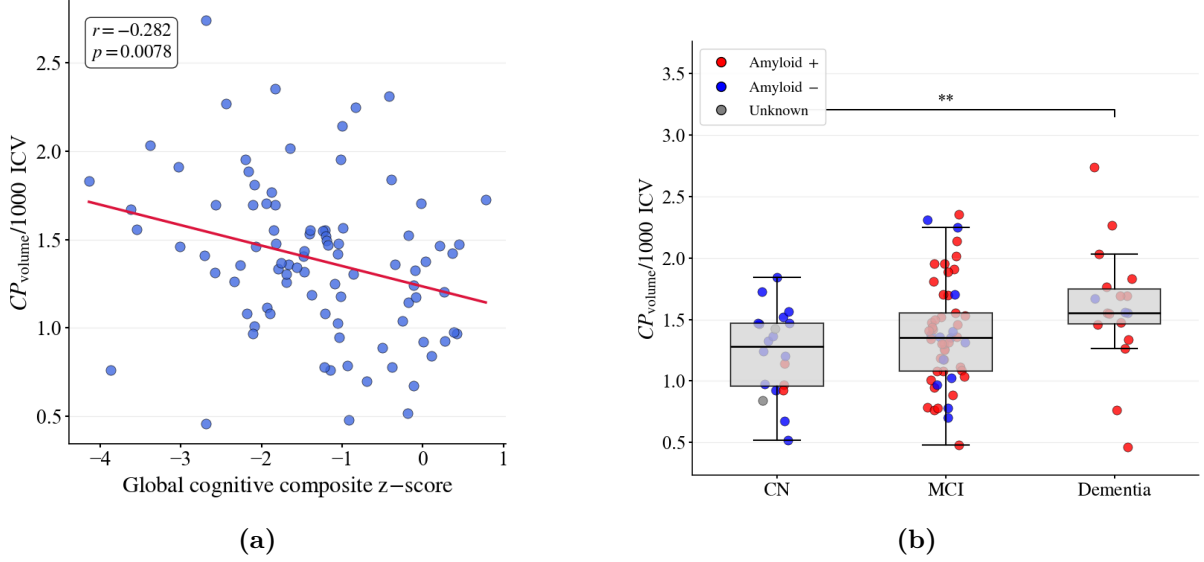


Figure 5.7: (a) Partial correlation between adjusted CP volume and the global cognitive composite score. (b) Adjusted CP volume distributions across diagnostic groups in the full cohort. Significant pairwise differences are indicated by star annotations (*: $p < 0.05$, **: $p < 0.01$).

5.3.2 DTI-ALPS Index

After outlier exclusion, the 2 subjects were removed for extreme cognition values and 1 additional participant was excluded due to an inconsistent DTI-ALPS value, resulting in 114 subjects for the analysis.

Associations with Age

The DTI-ALPS index showed a significant negative association with age ($\beta = -0.0043$, $p = 0.019$), indicating lower DTI-ALPS values with increasing age. However, the effect size remained modest ($R^2 = 0.037$). Interaction analyses did not reveal significant age-by-sex or age-by-amyloid interactions, indicating that the rate of age-related DTI-ALPS decline did not differ significantly between men and women, or between amyloid-positive and amyloid-negative participants (Table 5.1).

After IQR outlier removal, the age-related decline in DTI-ALPS was significant in men ($\beta = -0.0075$, $p = 0.014$), but not in women (Figure 5.8a). When stratified by amyloid status, both groups showed non-significant negative slopes. Nevertheless, amyloid-positive participants tended to show lower DTI-ALPS values across the age range than amyloid-negative participants (Figure 5.8b).

Table 5.1: Interaction effects between age and sex or amyloid status on DTI-ALPS index before and after IQR-based outlier exclusion.

Analysis	Before IQR removal		After IQR removal	
	β	p-value	β	p-value
Age $\times$ Sex	-0.0037	0.355	-0.0051	0.181
Age $\times$ Amyloid	0.0023	0.676	-0.0005	0.923

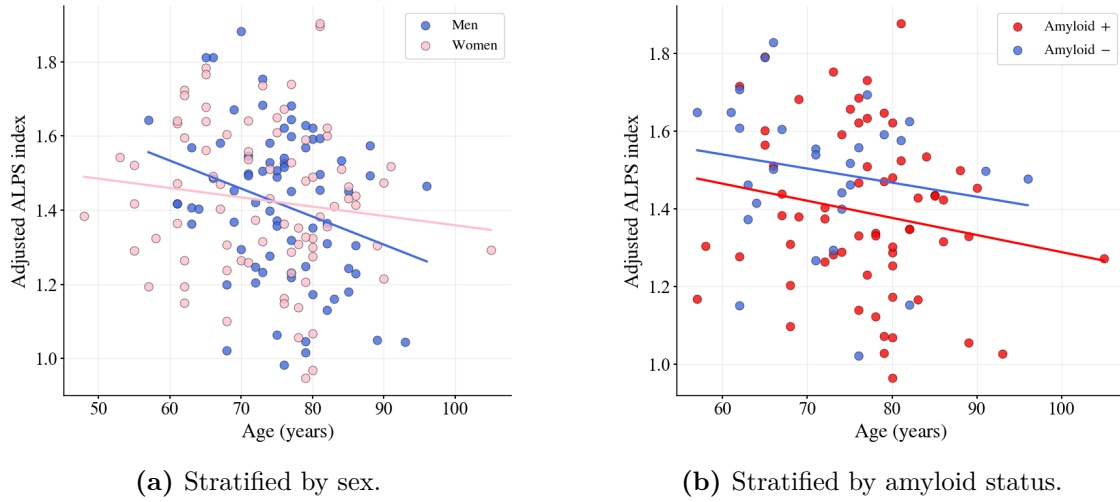


Figure 5.8: Age-related trajectories of the DTI-ALPS index according to sex (a) and amyloid status (b), after IQR-based outlier exclusion. Regression lines correspond to ordinary least squares linear regressions fitted separately within each subgroup.

Associations with Cognition

A weak non-significant positive association was observed between the DTI-ALPS index and the *global cognitive composite* score, ($r = 0.112$, $p = 0.327$) (Figure B.4). The distribution of the points remained relatively scattered, suggesting inter-subject variability.

Group comparisons showed a trend toward lower DTI-ALPS values across diagnostic groups (ANCOVA: $p = 0.053$). Compared with CN participants, both dementia participants ($\beta = -0.162$, $p = 0.018$) and MCI participants ($\beta = -0.117$, $p = 0.042$) showed significantly lower DTI-ALPS values. When the analysis was restricted to the amyloid-positive subgroup, a similar pattern was observed, although it no longer reached statistical significance (Figures 5.9a and 5.9b).

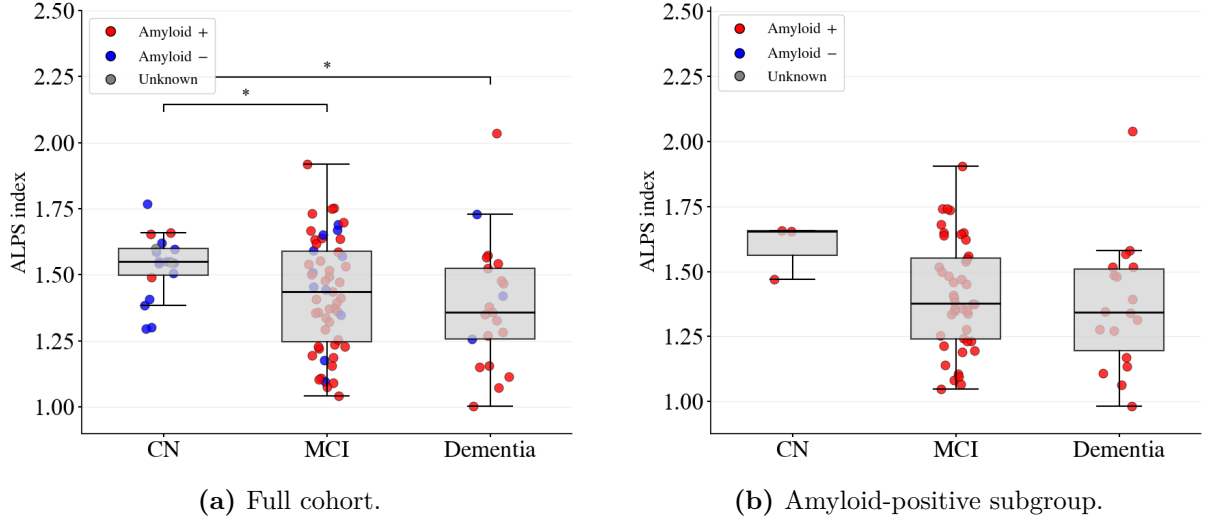


Figure 5.9: Distribution of DTI-ALPS index values across CN, MCI, and dementia groups in the full cohort (a) and restricted to the amyloid-positive subgroup (b). Significant pairwise differences are indicated by star annotations (*: $p < 0.05$, **: $p < 0.01$).

5.3.3 EPVS Burden

After outlier exclusion, 1 subject was identified as an outlier for BG ePVS counts and 2 additional subjects were identified as outliers for the *global cognitive composite* score using the IQR criterion, resulting in a final sample of 114 subjects. 5 subjects were identified as outliers for CSO ePVS counts and 2 additional subjects were excluded based on the *global cognitive composite* score, resulting in a final sample of 110 subjects.

Associations with Age

No significant interaction effects were observed between age and sex or age and amyloid status in either the BG or CSO, both before and after IQR-based outlier exclusion (Table 5.2). Likewise, sex-stratified and amyloid-stratified regressions did not reveal significant differences in age-related ePVS trajectories between subgroups. See Figures B.5 and B.6.

Table 5.2: Interaction effects between age and sex or amyloid status on continuous ePVS counts before and after IQR-based outlier exclusion.

Region	Analysis	Before IQR removal		After IQR removal	
		β	p-value	β	p-value
Basal ganglia	Age $\times$ Sex	0.3515	0.038	0.2281	0.161
	Age $\times$ Amyloid	-0.0345	0.861	-0.0487	0.805
Centrum semiovale	Age $\times$ Sex	-0.2009	0.345	-0.1237	0.499
	Age $\times$ Amyloid	0.0716	0.773	0.1515	0.523

Associations with Cognition

Partial correlation analyses revealed no significant associations between ePVS burden and *global cognitive composite* score. The correlations were positive but remained non-significant after IQR outlier removal in both regions (BG: $r = 0.037$, $p = 0.726$; CSO: $r = 0.140$, $p = 0.182$) (Figures 5.10a and 5.10b).

Group comparisons also showed no significant differences in ePVS count across CN, MCI, and dementia groups (Figure B.7). Although these trends did not reach statistical significance, both BG and CSO regions showed a tendency toward higher ePVS counts in dementia participants within the amyloid-positive subgroup (Figures 5.11a and 5.11b).

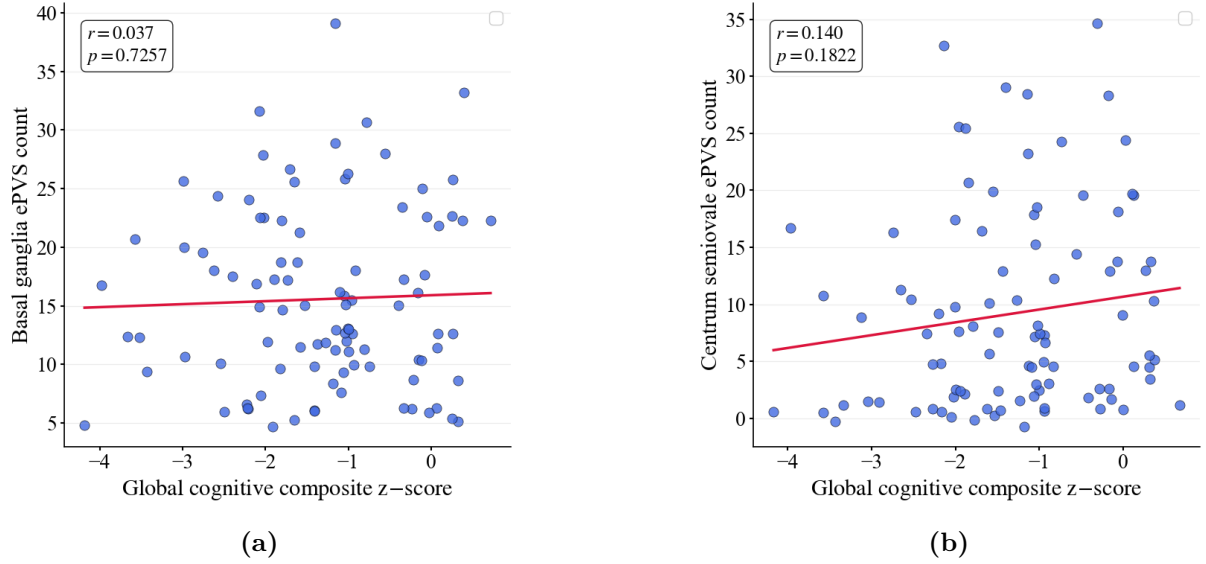


Figure 5.10: Partial correlation between BG (a) and CSO (b) ePVS count and the global cognitive composite score after adjustment for age, sex, and years of education.

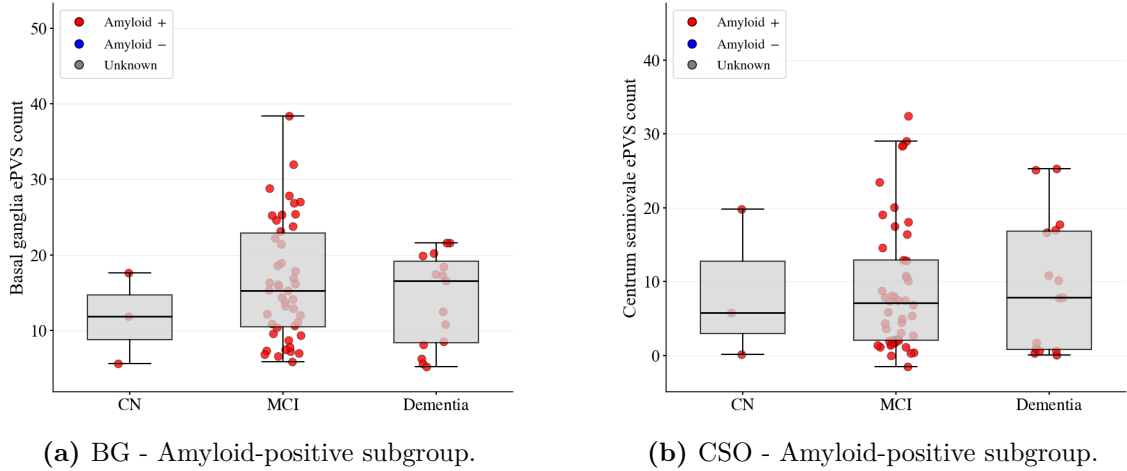


Figure 5.11: Adjusted ePVS count distributions across CN, MCI, and dementia groups restricted to the amyloid-positive subgroup, in the BG (a) and CSO (b). No significant group differences were observed.

5.3.4 NODDI Metrics

Associations with Age

Partial Pearson correlation analyses across all anatomical regions identified the uncinate fasciculus as the region showing the strongest age-related associations for both FISO and FINTRA (Figure B.8). As summarised in Table 5.3, these findings suggest that the observed age-related associations were robust to outlier removal and became slightly stronger after exclusion of extreme values.

Table 5.3: Effect of IQR-based outlier removal on age-related associations in the uncinate fasciculus.

Metric	Before IQR removal	After IQR removal
FISO	$r = 0.356, p < 0.001$	$r = 0.383, p < 0.001$
FINTRA	$r = -0.271, p < 0.001$	$r = -0.387, p < 0.001$

Given the robustness of these findings, subsequent analyses focused on the uncinate fasciculus after outlier removal. Detailed stratified analyses are summarised in Table 5.4.

Table 5.4: Stratified age-related associations for FISO and FINTRA in the uncinate fasciculus after IQR-based outlier removal.

Metric	Subgroup	β	p	R^2
FISO	Men	0.0066	< 0.001	0.309
FISO	Women	0.0044	0.004	0.151
FISO	Amyloid-positive	0.0049	< 0.001	0.182
FISO	Amyloid-negative	0.0044	0.013	0.177
FINTRA	Men	-0.0039	0.001	0.206
FINTRA	Women	-0.0025	0.028	0.091
FINTRA	Amyloid-positive	-0.0022	0.042	0.062
FINTRA	Amyloid-negative	-0.0038	0.003	0.257

As shown in Table 5.4, FISO increased significantly with age across all examined subgroups, whereas FINTRA decreased significantly with age. To test whether these age-related trajectories differed between subgroups, interaction models including Age $\times$ Sex and Age $\times$ Amyloid terms were fitted. None of these interaction terms reached significance. For FISO, the age slope did not differ significantly according to sex ($\beta = 0.0022, p = 0.302$) nor amyloid status ($\beta = 0.0004, p = 0.856$). The same pattern was observed for FINTRA (Age $\times$ Sex: $\beta = -0.0014, p = 0.384$; Age $\times$ Amyloid: $\beta = 0.0016, p = 0.331$).

Despite the absence of significant interactions, visual inspection of the stratified trajectories suggested subtle subgroup differences. Amyloid-positive participants tended to show globally higher FISO values across the entire age range (Figure 5.12a), and a less pronounced age-related decline in FINTRA compared with amyloid-negative participants (Figure 5.12b). These differences appeared to reflect an overall vertical shift between groups rather than a divergence in slope, which is consistent with the non-significant Age $\times$ Amyloid interaction term. In contrast, no comparable visual differences emerged between sex subgroups (Figures 5.12c and 5.12d).

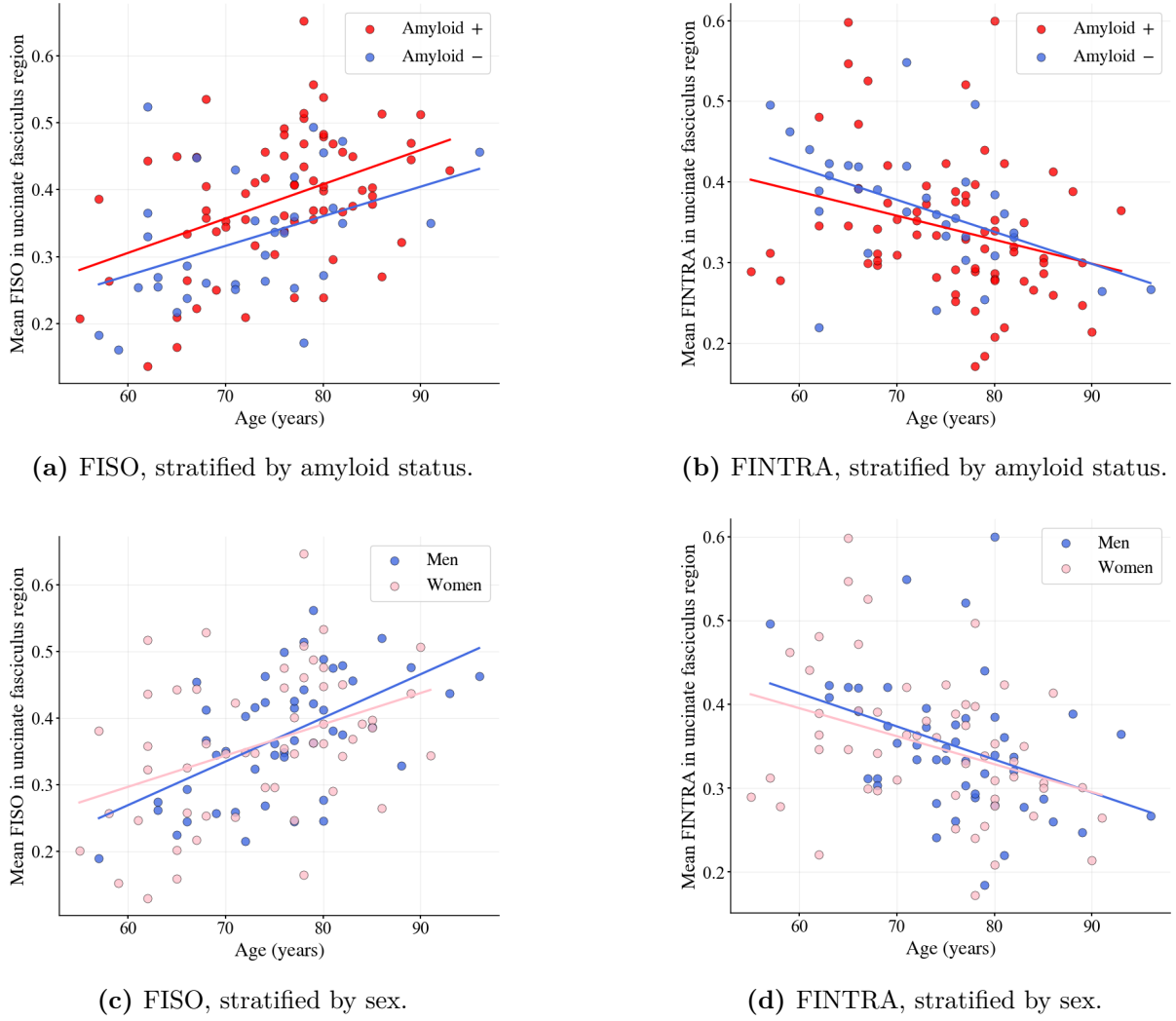


Figure 5.12: Age-related trajectories of FISO (a,c) and FINTRA (b,d) in the uncinate fasciculus, stratified by amyloid status (a,b) and sex (c,d) after covariate adjustment.

Associations with Cognition

Regions selected for detailed cognitive analyses were chosen based on partial Pearson correlations displayed in heatmaps across all anatomical regions and cognitive domains (Figure B.9).

For FISO, the selected regions were the hippocampus and cingulate isthmus, which showed the strongest and most consistent negative associations with cognition across all domains. For FINTRA, the precuneus and the fornix showed the strongest positive associations across cognitive domains. The cingulate isthmus was additionally investigated for FINTRA given its relevance to AD-related pathways, and the opportunity it provides for a direct comparison with the corresponding FISO findings within the same anatomical structure.

Hippocampal FISO

Within the hippocampus, FISO remained significantly negatively associated with *global cognitive composite* score after IQR-based outlier removal ($r = -0.338$, $p < 0.001$), consistent with the partial correlation shown in Supplementary Figure B.10a. Group comparisons revealed significantly higher hippocampal FISO values in MCI compared with CN participants ($\beta = 0.0306$, $p = 0.011$), and in dementia compared with both CN ($\beta = 0.0573$, $p < 0.001$) and MCI participants ($\beta = 0.0267$, $p = 0.039$) (Figure 5.13a). Within the amyloid-positive subgroup (Figure 5.13b), the overall gradient was attenuated,

with no pairwise comparison remaining significant after outlier removal, although the dementia versus MCI contrast approached significance ($p = 0.064$). Comparisons involving CN participants were limited by the small number of amyloid-positive CN subjects.

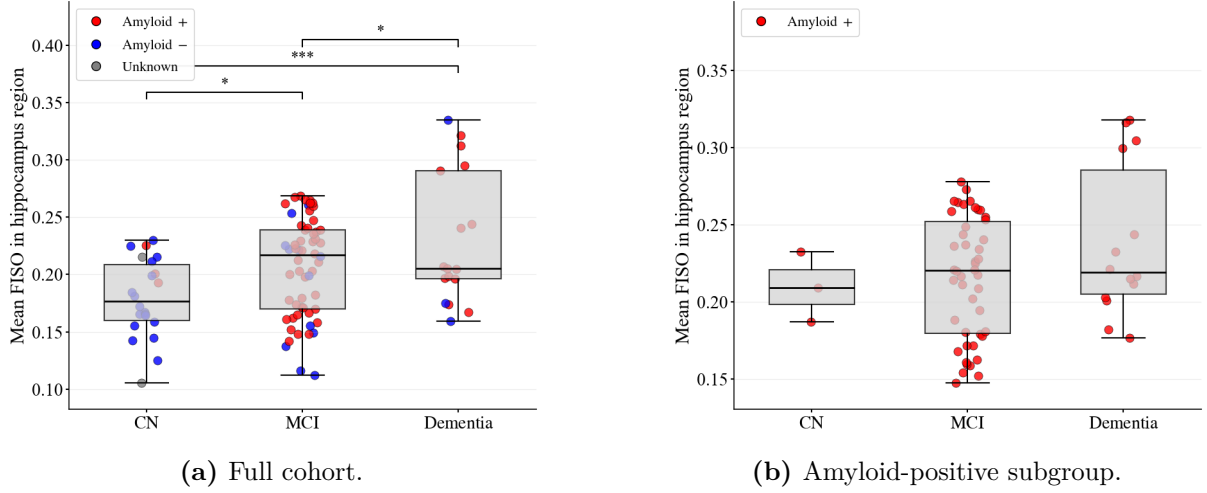


Figure 5.13: Hippocampal FISO distributions across CN, MCI, and dementia groups in the full cohort (a) and within the amyloid-positive subgroup (b), after IQR-based outlier removal. Significant pairwise differences are indicated by star annotations (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Fornix FINTRA

A total of 10 outliers were removed for the analysis, including eight metric-related outliers and two cognition-related outliers. After IQR-based outlier removal, the association with *global cognitive composite* did not reach significance ($r = 0.135$, $p = 0.119$) (Figure B.10b). Similarly, ANCOVA revealed no significant group differences (Figure 5.14a) either in the full cohort or within the amyloid-positive subgroup (Figure 5.14b), suggesting that the initial fornix FINTRA findings were substantially influenced by extreme observations before outlier removal.

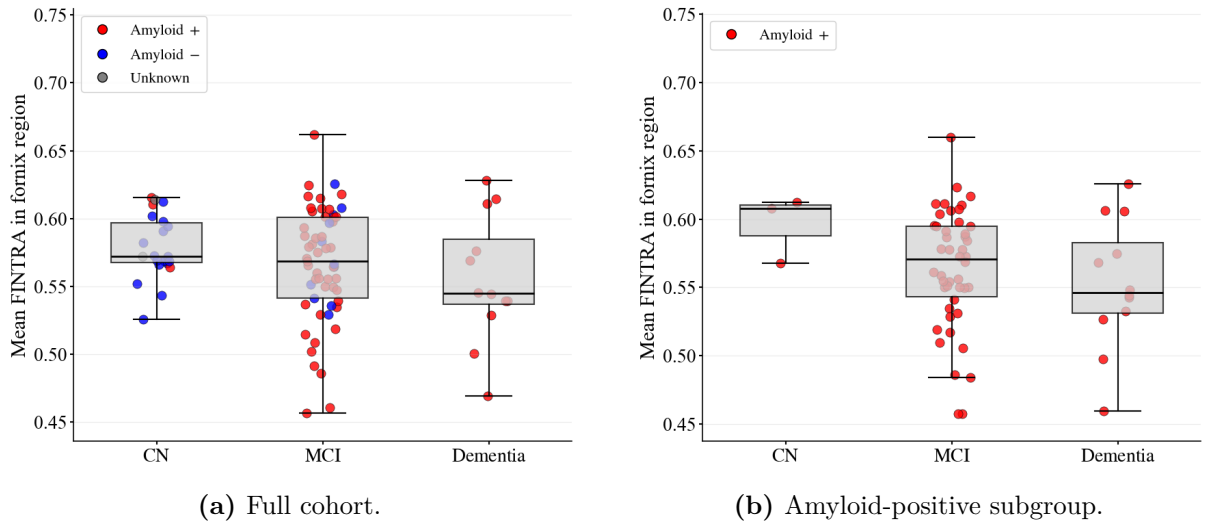


Figure 5.14: Fornix FINTRA distributions across CN, MCI, and dementia groups in the full cohort (a) and within the amyloid-positive subgroup (b), after IQR-based outlier removal. Significant pairwise differences are indicated by star annotations (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Cingulate isthmus FISO and FINTRA

Within the cingulate isthmus, both metrics showed associations with global cognition after outlier removal (Figure 5.15). FISO remained significantly negatively associated with *global cognitive composite* score ($r = -0.318$, $p < 0.001$), whereas the FINTRA association became weaker and no longer reached significance ($r = 0.110$, $p = 0.200$). Five outliers were removed for the FISO analysis (two metric-related and three cognition-related outliers), while four outliers were removed for FINTRA (one metric-related and three cognition-related outliers). Group comparisons revealed significantly increased cingulate FISO values in dementia participants compared with both CN ($\beta = 0.0583$, $p < 0.001$) and MCI participants ($\beta = 0.0360$, $p = 0.004$) (Figure 5.16a). In contrast, although FINTRA no longer showed a robust association with cognition, dementia participants still exhibited significantly lower values compared with MCI participants ($\beta = -0.0176$, $p = 0.039$) (Figure 5.16b).

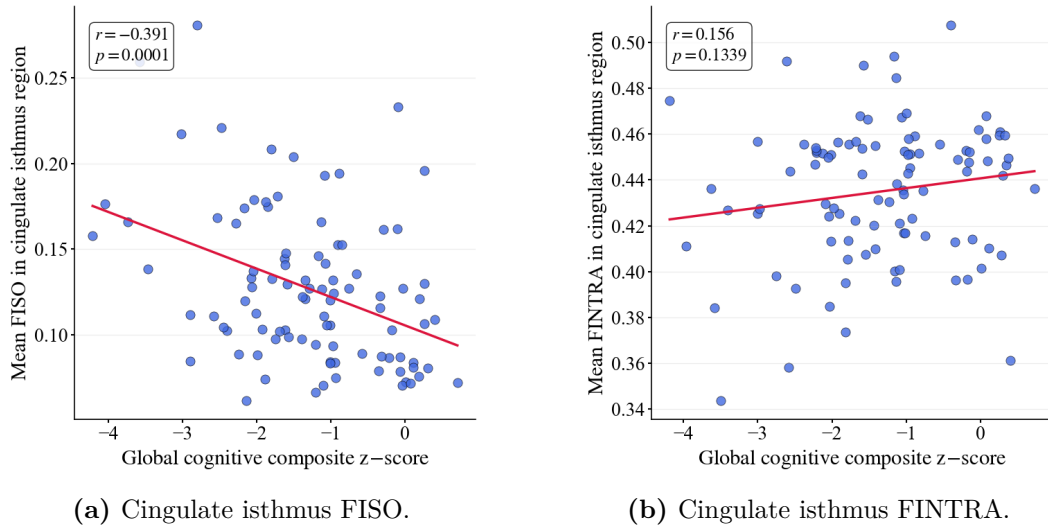


Figure 5.15: Partial correlations between the global cognitive composite score and cingulate isthmus FISO (a) and FINTRA (b), after adjustment for age, sex, years of education and after IQR-based outlier removal.

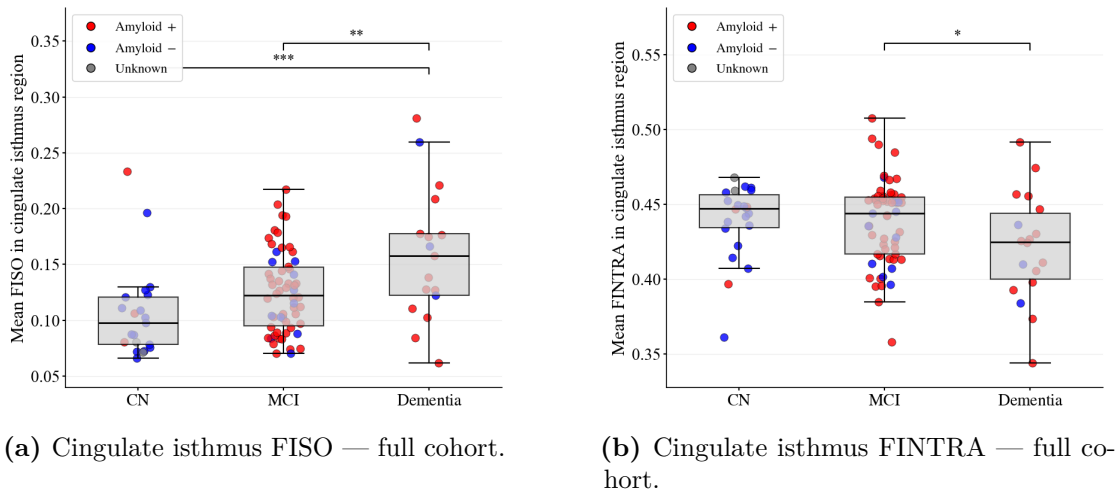


Figure 5.16: Cingulate isthmus FISO (a) and FINTRA (b) distributions across CN, MCI, and dementia groups in the full cohort after IQR-based outlier removal. Significant pairwise differences are indicated by star annotations (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Within the amyloid-positive subgroup, the dementia versus MCI contrast remained significant for cingulate FISO ($\beta = 0.0302$, $p = 0.042$), whereas the corresponding FINTRA comparison no longer reached statistical significance ($\beta = -0.0180$, $p = 0.081$). Comparisons involving CN participants did not reach significance in this subgroup, likely reflecting the reduced number of available amyloid-positive subjects (Supplementary Figures B.11a and B.11b).

5.3.5 Combined CP & DTI-ALPS Cognitive Models

CP volume and DTI-ALPS index showed only weak associations after adjustment for age, sex, and years of education, both in the full cohort ($r = -0.18$, $p = 0.12$) and in the amyloid-positive subgroup ($r = -0.26$, $p = 0.07$).

Adding CP volume to models already including DTI-ALPS significantly improved the association with *global cognitive composite* in both the whole cohort ($\Delta R^2 = 0.063$, $p = 0.022$) and amyloid-positive subjects ($\Delta R^2 = 0.088$, $p = 0.028$). In contrast, adding DTI-ALPS to models already including CP volume did not significantly improve the explained cognitive variance, either in the whole cohort ($\Delta R^2 = 0.023$, $p = 0.162$) or in the amyloid-positive subgroup ($\Delta R^2 = 0.018$, $p = 0.312$).

5.4 Biomarker Associations Across Cognitive Domains

Beyond the *global cognitive composite*, partial correlations were also examined across the individual cognitive domains in order to better describe how each biomarker relates to cognition. The corresponding coefficients, adjusted for age, sex, and years of education, are reported in Table 5.5. The FISO metrics in the hippocampus showed the most widespread associations, whereas ePVS counts were not significantly related to any cognitive measure.

Table 5.5: Partial correlation coefficients (r) between cognitive scores and imaging biomarkers. Partial correlations were adjusted for age, sex, and years of education. Black text indicates non-significant associations ($p \geq 0.05$); green text indicates $p < 0.05$; orange text indicates $p < 0.01$; red text indicates $p < 0.001$.

Biomarker	MMSE	Memory	Language	Executive	Visuospatial	Global
DTI-ALPS	0.213	0.222	0.235	0.075	0.192	0.213
CP volume	-0.319	-0.134	-0.242	-0.250	-0.216	-0.282
ePVS BG	0.060	-0.043	0.002	0.076	0.101	0.037
ePVS CSO	0.091	0.065	0.127	0.141	0.082	0.140
FISO – hippocampus	-0.329	-0.356	-0.286	-0.308	-0.243	-0.338
FISO – cingulate isthmus	-0.303	-0.213	-0.257	-0.198	-0.250	-0.318
FISO – parahippocampus	-0.250	-0.162	-0.169	-0.127	-0.302	-0.264
FINTRA – fornix	0.311	0.140	0.221	0.223	0.123	0.135
FINTRA – precuneus	0.276	0.063	0.235	0.032	0.104	0.173
FINTRA – cingulate isthmus	0.236	-0.005	0.108	0.048	0.195	0.110

Chapter 6

Discussion

6.1 Methods Validation

Before interpreting the biomarker findings, the present discussion will first address the validation of the CP segmentation and automated ePVS quantification methods presented in Section 5.2. This validation step is essential for assessing the confidence that can be placed in the subsequent analyses.

6.1.1 Choroid Plexus Segmentation

The proposed GMM-based segmentation showed limited agreement with the reference U-Net when all subjects were included, but this agreement markedly improved after outlier removal. This indicates that the global performance was strongly influenced by a small number of segmentation failures, which therefore required particular attention. The Jaccard similarity coefficients provided complementary spatial information: while they indicated an overall acceptable overlap between the two methods, low values (<0.3) helped identify problematic cases and guided the analysis of the underlying segmentation errors.

Visual inspection of these flagged cases revealed two distinct sources of failure. First, some disagreements originated from inaccurate U-Net predictions, where the predicted CP mask extended outside the anatomically expected CP region (Figure 6.1a). Second, failures also originated from the GMM decomposition step. In several subjects, the LV-CP mask became excessively refined after erosion, leaving insufficient intensity variability for reliable separation between CSF and CP components. In these cases, the GMM could not perform a meaningful decomposition and incorrectly classified the whole ventricular region as CP tissue (Figure 6.1b).

Compared with the reference U-Net approach, the proposed method combines anatomical priors with intensity-based refinement. This provides additional control over the segmentation process when CP intensity remains distinguishable from surrounding CSF. However, this strategy also means that the method depends strongly on the quality of the anatomical masks used at the beginning of the pipeline. Since the GMM decomposition relies on ventricular and CP masks derived from `aseg` and from the registration strategy, inaccuracies in these masks can directly affect the definition of the LV-CP candidate region and compromise the subsequent intensity-based separation. In practice, errors in the initial anatomical masks required additional erosion and refinement steps to reduce the

candidate region as described in Section 4.3.2. While this improved the segmentation in many cases, it also introduced a risk of excessive refinement, as described above. This limitation was particularly observed in subjects with severe ventricular enlargement and small CP structures. Although these subjects were excluded from the final analyses, it should be kept in mind that such extreme anatomical configurations may themselves carry important pathological information, as they could reflect specific disease stages.

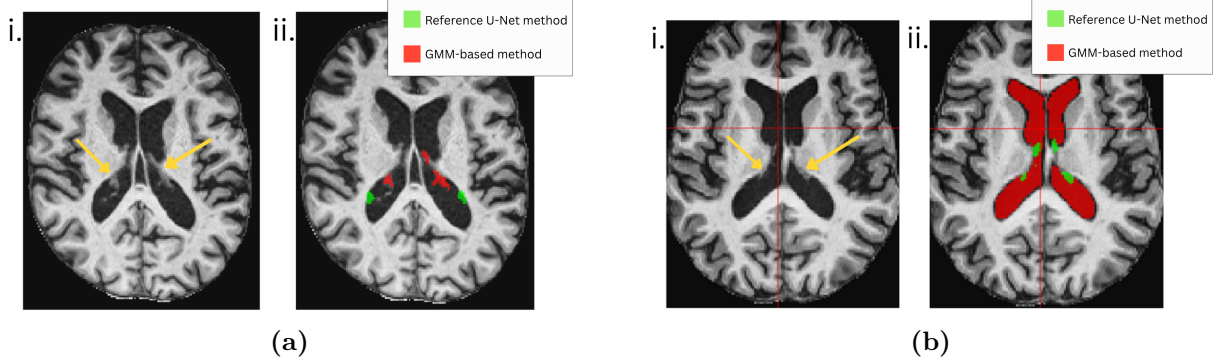


Figure 6.1: Examples of segmentation inconsistencies between the proposed GMM-based method (red) and the reference U-Net segmentation (green). For each example, the left panel (i) shows the native T_1 -weighted MRI slice without segmentation masks for better anatomical visualisation. The right panel (ii) shows the same slice with both segmentation masks overlaid. Arrows point to the CP. (a) The reference U-Net segmentation appears spatially misaligned, whereas the GMM-based segmentation remains anatomically consistent. (b) Refinement of the LV-CP candidate region was excessively restrictive, resulting in failure of the GMM segmentation.

6.1.2 Automated ePVS Quantification

The performance of the automated ePVS segmentation differed between the BG and the CSO. In the BG, the Spearman correlation with visual ratings was not statistically significant. However, the confusion matrix (Fig. 5.5a) showed that most disagreements remained close to the diagonal. This can be explained because the visual assessment was based on a limited number of slices, whereas the proposed method relied on volumetric whole-region quantification, which may provide a more sensitive estimation of ePVS burden.

In contrast, stronger correlations were observed in the CSO together with a clearer diagonal structure in the confusion matrix (Fig. 5.5b), indicating better agreement between automated and visual ratings. This improved performance may be explained by the higher number and greater spatial distribution of ePVS within the WM. In addition, the elongated tubular morphology of ePVS in this region may be more effectively captured by vessel-enhancement filters such as the Frangi filter used in the present pipeline.

Finally, the validation performed here relied on ratings from a single expert rater. Establishing inter-rater reliability would further strengthen the reference standard for evaluating automated methods. Nevertheless, although the agreement between manual and automated counts remained limited, the automated pipeline was still used for the subsequent analyses by relying on continuous count measurements rather than categorical scores ranging from 1 to 4. Using continuous measurements may increase sensitivity to subtle inter-subject variations in ePVS burden and could therefore provide more detailed information when investigating potential associations with cognition and disease progression.

6.2 Individual Biomarker Analysis

Having discussed the validation of the CP and ePVS methods, the discussion now turns to the interpretation of the individual biomarker findings. Detailed results for this section are presented in Section 5.3.

6.2.1 CP Volume

CP volume showed a significant positive association with age in the overall cohort. However, this association was no longer significant when analyses were restricted to amyloid-positive subjects, suggesting that CP enlargement in these subjects may not be primarily driven by normal aging processes. In addition, dementia subjects presented larger CP volumes compared with CN participants, including within the amyloid-positive subgroup. Moreover, CP volume was negatively associated with *global cognitive score*. Together, these observations suggest that CP enlargement may be associated with disease-related cognitive decline rather than age alone.

Several mechanisms may explain these observations. One possible explanation is neuroinflammation, as increasing evidence suggests that the CP actively participates in immune regulation and inflammatory signalling [86]. Reynolds et al. also suggested that neuroinflammatory and neurodegenerative alterations may not represent fully distinct processes, and may contribute together to disease progression. However, CP enlargement may also partly reflect secondary anatomical changes associated with brain atrophy and ventricular expansion during neurodegeneration, where enlarged ventricles could simply provide additional space for CP expansion. Distinguishing between inflammatory enlargement and passive structural expansion therefore remains challenging and requires further investigation. Future studies comparing Alzheimer’s disease with more inflammatory disorders such as multiple sclerosis could help clarify the biological significance of CP enlargement.

6.2.2 DTI-ALPS Index

The ALPS index showed a significant negative association with age, with amyloid-positive participants tending to show lower values than amyloid-negative participants, although this difference did not reach statistical significance. In the full cohort, significant differences were observed between cognitively normal subjects and more impaired groups, whereas these differences disappeared when analyses were restricted to amyloid-positive subjects only.

These findings may suggest that the ALPS index captures alterations related to the beginning of the disease continuum. However, although the ALPS index was developed to reflect glymphatic fluid flow, the lack of significant findings within the amyloid-positive subgroup suggests that it may also be influenced by broader brain changes rather than by amyloid-related glymphatic dysfunction alone. Studies have emphasised that ALPS should be interpreted cautiously as an indirect biomarker influenced by multiple factors beyond glymphatic function itself. White matter degeneration, fibre disorganisation, vascular alterations, extracellular free-water accumulation, and partial-volume effects may all contribute to ALPS variability independently of fluid clearance mechanisms [56, 87]. This

interpretation is further supported by large-cohort studies showing stronger associations between ALPS and vascular burden than with Alzheimer’s-specific pathology [88].

6.2.3 EPVS Burden

No significant association was observed between ePVS burden and age in either the BG or CSO. Similarly, no significant associations were found with *global cognitive composite* or along the AD continuum. Although statistical significance was not reached within the amyloid-positive subgroup, a progressive visual increase in ePVS burden across disease stages could still be observed.

The present results should be considered in the context of the heterogeneous literature on ePVS. Increased ePVS burden with aging and AD has often been reported, particularly in the CSO [89, 90], but this association is not consistently observed across studies, others have found only weak or absent associations [38]. In the present work, no clear increase in ePVS burden was observed and several factors may help explain this absence of association.

First, the present cohort exhibited a relatively limited visible ePVS burden overall, as reflected by the low range of visual ratings observed during validation (Section 6.1.2), with CSO visual scores ranging only from 0 to 2 despite the scale extending to 4. This may indicate that the cohort was not fully representative, potentially limiting the ability to detect the expected group differences. Second, MRI-visible ePVS represent only a static anatomical snapshot of a highly dynamic physiological system: glymphatic activity, and therefore PVS size, is thought to be strongly modulated by sleep, vascular pulsatility, and cerebrospinal fluid circulation [90]. Automated ePVS counts may therefore not reliably reflect the true efficiency of perivascular fluid transport. Third, the limited agreement between automated and manual counts reported in the validation section (Section 6.1.2) suggests that methodological factors may also have contributed to the associations observed here.

6.2.4 NODDI-derived Metrics

Because the GS is closely related to fluid exchange within the brain, NODDI was included as a diffusion MRI-based biomarker that may help capture microstructural alterations associated with aging and neurodegeneration. The present analyses focused on the regions showing the strongest and most consistent associations with age and cognition (see Heatmaps B.8 and B.9). These included the uncinate fasciculus for the age-related association, the hippocampus and cingulate isthmus for FISO, and the fornix and cingulate isthmus for FINTRA. These regions are strongly involved in memory and cognitive processing and are known to be particularly vulnerable in AD. The hippocampus plays a central role in memory formation and is one of the earliest regions affected by AD-related neurodegeneration. The fornix is the main WM tract connecting the hippocampus to other limbic structures, while the cingulate isthmus and the uncinate fasciculus are involved in large-scale memory and cognitive networks linking temporal and frontal brain regions. Their early involvement in AD therefore makes them particularly relevant for investigating microstructural alterations across the disease continuum.

Strong age-related associations were observed in the cingulate isthmus, with increasing FISO and decreasing FINTRA values. In addition, higher hippocampal FISO values were significantly associated with lower *global cognitive composite* score. Along the AD

continuum, FISO progressively increased in the hippocampus, while more pronounced alterations in both FISO and FINTRA were observed in the uncinate fasciculus, particularly between MCI and dementia stages. Interestingly, several associations became weaker when analyses were restricted to amyloid-positive subjects only.

The stronger and more consistent associations observed for FISO compared with FINTRA may suggest that FISO captures more global extracellular alterations occurring during neurodegeneration. Increased FISO likely reflects broader changes in extracellular water content, associated with tissue loss and expansion of the extracellular space. In contrast, FINTRA reflects hindered diffusion within the intracellular compartment, which makes its interpretation more complex. This metric may be influenced by several processes occurring simultaneously, including axonal degeneration, neuronal loss, inflammatory processes, and alterations in tissue organisation. These mechanisms may affect intracellular diffusion properties in different ways, leading to more heterogeneous FINTRA variations across regions and disease stages. These observations therefore suggest that NODDI-derived metrics may provide complementary information regarding tissue microstructure and fluid-related alterations occurring during neurodegeneration. However, their relationship with glymphatic dysfunction remains indirect and requires cautious interpretation.

6.3 Cross-Biomarker Analysis

After having discussed each biomarker individually, this section will now examine their relationships with one another. The analysis first considers whether CP volume and DTI-ALPS provide overlapping or distinct information when included in the same cognitive model. It then examines how the full set of biomarkers differs in its associations across the individual cognitive domains.

6.3.1 Relationships Between CP Volume & DTI-ALPS Index

CP volume and DTI-ALPS were selected because previous analyses suggested partially distinct association profiles: ALPS alterations appeared more evident in early group comparisons, whereas CP volume showed clearer differences across the clinical continuum.

After adjustment for covariates, CP volume and ALPS index showed only weak and non-significant correlations, both in the full cohort and in the amyloid-positive subgroup. This suggests limited redundancy between the two biomarkers and indicates that they may reflect different aspects of brain fluid regulation. In line with this interpretation, adding CP volume to models already including ALPS significantly improved the association with *global cognitive score* performance, whereas adding ALPS to models already including CP volume did not. This suggests that CP-related alterations may capture cognitive changes that are not fully reflected by ALPS measurements alone.

One possible explanation is that ALPS may be sensitive to broad diffusion changes that occur early in the disease process. However, as discussed in Section 6.2, ALPS may not reflect glymphatic function alone and may also be influenced by WM integrity, vascular alterations, and other global microstructural changes. CP enlargement, in contrast, may become more visible at later disease stages, when ventricular expansion and structural remodelling are more pronounced. In the present cohort, subjects with small CP volumes and large ventricles were often removed because these anatomical configurations frequently

led to segmentation failures, as discussed in Section 6.1. As a result, atypical early CP alterations may have been underrepresented, whereas larger CP enlargement remained easier to detect at later stages. However, these interpretations remain hypothetical and require further investigation.

6.3.2 Relationships with Different Cognitive Domains

While previous analyses focused on the *global cognitive composite*, examining biomarker associations across individual cognitive domains (Table 5.5) reveals different patterns that may help characterise the biological processes captured by each marker.

Among the NODDI-derived biomarkers, FISO showed the broadest cognitive associations, being significantly correlated with nearly all domains and most strongly with *global cognitive composite*. This widespread pattern may suggest that increased free-water diffusion reflects relatively diffuse microstructural or extracellular alterations affecting multiple cognitive functions simultaneously, suggesting that FISO is a more global marker of neurodegenerative tissue changes.

In contrast, FINTRA alterations appeared more regionally specific. Within the fornix, lower FINTRA was most strongly associated with MMSE and, to a lesser but still significant extent, with language and executive performance, whereas no significant association was observed with the *global cognitive composite*. A similar pattern was observed in the cingulate isthmus, where FINTRA was significantly associated with visuospatial performance despite the absence of an association with the *global cognitive composite*.

The cingulate isthmus, which is part of the cingulum bundle, connects posterior parietal regions to the hippocampus and is involved in visuospatial and memory-related processing [91]. The consistency between the affected region and the associated cognitive domain may suggest an early disruption of specific cognitive pathways before more generalised impairment becomes detectable. These domain-specific associations suggest that FINTRA may reflect localised microstructural or network changes. Such alterations may be related to specific cognitive functions without necessarily producing a measurable effect on the *global cognitive composite*, in contrast to FISO, which showed broader associations across cognitive domains.

The strong association observed with MMSE may further indicate that these microstructural alterations are already present when subtle global cognitive changes begin to emerge. This interpretation is consistent with previous studies showing that the cingulum is among the WM tracts most sensitive to early AD-related microstructural alterations [92, 91].

In parallel, CP volume showed a different pattern of cognitive associations. CP enlargement was significantly associated with executive, language, and visuospatial deficits, whereas memory was the only cognitive domain without a significant association. This may suggest that CP enlargement is not primarily related to the processes driving memory impairment in AD. Instead, it may reflect larger anatomical changes that become visible later in the disease course and may then be linked to disease progression, such as ventricular enlargement or neuroinflammatory remodelling.

This interpretation is consistent with the findings discussed in Section 6.2. It is also supported by the group comparisons reported in Section 5.3.1, where CP volume differences were most apparent between CN and dementia participants, rather than between CN and MCI. This suggests that CP enlargement becomes more prominent only once the disease

has progressed beyond its earliest stages.

The ALPS index showed another pattern of associations. It was weakly but significantly related to memory, language, MMSE, and the *global cognitive composite*, whereas no significant association was observed with executive or visuospatial functions. This contrasted with the pattern observed for CP volume. The modest effect sizes across domains may reflect the fact that ALPS is not specific to a single biological process, as discussed previously in Section 6.2. Instead, it may be influenced by several diffuse brain changes that collectively affect cognitive performance without being strongly tied to one specific cognitive domain.

Interestingly, unlike CP volume, ALPS remained associated with the memory composite. This supports the interpretation proposed previously in Section 6.3.1, that CP volume and ALPS may reflect different stages or altered brain fluid regulation during disease progression.

Finally, ePVS burden showed no significant association with any cognitive domain, which may be consistent with the interpretation proposed in Section 6.2.

Overall, these findings suggest that the investigated biomarkers are not redundant in their associations with cognition. Rather, they may capture complementary aspects of brain fluid regulation and tissue alteration, which could be interpreted within the broader framework of the glymphatic system. However, these interpretations remain speculative and should be considered with caution, particularly given the indirect nature of the biomarkers and the methodological limitations.

6.4 Limitations

To complete the discussion, several limitations of the present work should be acknowledged.

The GS remains a relatively recent and still evolving field of research. As a result, many of the currently proposed biomarkers and even some interpretations found in the literature should be considered with caution. This is well illustrated by the DTI-ALPS method, whose interpretation and physiological meaning are still being actively discussed and refined in the literature itself.

Beyond this conceptual limitation, several methodological factors may have affected the results. All pipelines required image registration and despite quality control, small local inaccuracies may have persisted and propagated downstream, affecting the final biomarker estimates. Beyond this shared limitation, each pipeline presented specific weaknesses:

- **CP segmentation.** The proposed CP segmentation pipeline was strongly dependent on the quality of the initial ventricular segmentation and on the contrast of the T1-weighted images. The GMM refinement step was particularly sensitive to the definition of the ventricular search region as highlighted in Section 6.1.1. The reliability of this method therefore depends not only on the refinement step itself, but also on the quality of the upstream preprocessing, segmentation, and registration steps.
- **DTI-ALPS.** The ALPS index relies on the assumption that projection fibres, association fibres, and perivascular spaces remain approximately orthogonal within the selected ROIs. However, this anatomical configuration may be altered by local

variability, WM lesions, ventricular enlargement, atrophy, or crossing fibres, which could bias diffusivity estimates. Even after correcting for scanner orientation, this assumption may not hold equally across all subjects, since local fibre organisation varies independently of head position. In addition, the metric remains sensitive to acquisition quality, tensor estimation, and ROI placement. Since the ALPS value is computed from a small region, it may also not fully represent the surrounding tissue or the whole-brain glymphatic status. Finally, no direct validation method is currently available to confirm that ALPS specifically reflects glymphatic activity in vivo.

- **ePVS detection.** As discussed in Section 6.2.3, the present cohort showed a low overall ePVS burden, which may have limited the variability available to detect associations with cognition or amyloid status. Beyond this cohort-related limitation, the automated detection pipeline may also have been affected by the contrast-enhancement strategy combining T1 and FLAIR images. Although this approach was intended to improve ePVS visibility, it may have reduced the anatomical specificity of the detected structures by enhancing vessel-like artifacts, potentially contributing to false-positive detections.
- **NODDI metrics.** NODDI relies on a simplified biophysical model with fixed compartment assumptions that may not fully capture pathological tissue complexity. FINTRA reflects hindered diffusion rather than direct neurite loss. Similarly, the FISO metric, used here as a proxy for extracellular free-water accumulation, lacks biological specificity. It remains difficult to determine precisely what these metrics reflect biologically, as the observed diffusion changes could result from a mixture of different processes.

None of the investigated biomarkers directly measures glymphatic flow or CSF clearance. They should therefore be interpreted as indirect surrogate markers rather than direct indicators of glymphatic dysfunction. Moreover, they are not specific to AD, since similar alterations may occur in the context of normal aging, cerebrovascular disease, neuroinflammation, or other neurodegenerative conditions.

In addition, the study did not include direct comparisons with established markers of neurodegeneration relevant to AD, such as hippocampal volume or ventricular enlargement. Such comparisons would have helped to establish more specific links with the disease. Similarly, more advanced statistical analyses exploring interactions or combined effects between biomarkers could have clarified whether these markers reflect complementary or overlapping pathological processes.

Although some subjects had longitudinal MRI follow-up with multiple imaging sessions available, the number of repeated acquisitions and the duration of follow-up were not sufficient to perform robust longitudinal analyses. Thus, the cross-sectional design of the study also limits the interpretation of the observed associations.

Last but not least, patient classification into clinical subgroups also introduced variability. Different criteria for defining amyloid positivity or cognitive impairment, including which clinical scores or thresholds were applied, and which rater reviewed the classification can yield different groupings and influence the results. In addition, the clinical database was manually compiled into an Excel datasheet and reviewed to ensure consistency across subjects. Although this step enabled the data to be gathered, it also led to the exclusion of several participants because of missing, incomplete, or inconsistent information. Finally,

the overall sample size remained moderate ($N = 117$ with available amyloid status), and some subgroups were particularly small. The amyloid-positive CN subgroup included only four subjects, substantially limiting statistical power and the robustness of subgroup comparisons.

6.5 Future Perspectives

The limitations described in Section 6.4 highlight the importance of extending this work further, especially given that the GS remains a relatively recent and still evolving field of research. Some possible future perspectives are discussed below.

First, a natural next step would be to further investigate the relationships between the studied biomarkers and other well-established markers of neurodegeneration in AD, such as hippocampal atrophy or ventricular enlargement. Additional statistical analyses exploring interactions or relationships between biomarkers could also help clarify the observed associations and potentially provide further insight into the underlying mechanisms.

Second, many of the physiological and pathological mechanisms underlying glymphatic dysfunction remain poorly understood. Additional biological and experimental studies will therefore be necessary to better understand the role of brain fluid regulation in AD. More direct validation approaches, such as tracer-based or fluorescent marker studies, could also help better confirm whether the investigated MRI biomarkers truly reflect glymphatic-related processes in humans.

Third, several methodological improvements remain possible. For example, alternative ePVS segmentation approaches using different vessel-enhancement filters, such as Meijering filters or more advanced shape-based detection strategies, could potentially improve detection robustness and sensitivity to cognitive alterations. Future MRI developments may also provide more specific tools to investigate these mechanisms. More specialised acquisition sequences designed to study cerebrospinal fluid dynamics, perivascular flow, or brain fluid exchange could provide more direct measurements of glymphatic-related activity. In addition, because perivascular spaces and fluid circulation may vary dynamically over time, functional MRI approaches could help investigate how these mechanisms evolve under different physiological conditions.

Fourth, longitudinal studies following subjects over time would be important to better understand how these biomarkers evolve during disease progression and whether some alterations may appear earlier than others.

Finally, future work on the GS will likely require strong multidisciplinary collaborations between clinicians, neuroscientists, physicists, and imaging researchers. Such collaborations will be essential to better interpret these biomarkers and further improve our understanding of GS dysfunction in Alzheimer's disease.

Conclusion

Alzheimer’s Disease (AD) is a complex neurodegenerative disorder whose mechanisms remain only partially understood. In recent years, increasing attention has been directed toward the potential role of glymphatic dysfunction and impaired brain waste clearance in the progression of neurodegeneration. Although the glymphatic system remains difficult to investigate directly in humans and its exact mechanisms are still debated, MRI provides a valuable non-invasive tool to explore structural and microstructural alterations potentially related to these processes.

In this context, the present work investigated four MRI biomarkers potentially associated with glymphatic dysfunction in a cohort spanning the AD continuum and stratified according to amyloid status. The study focused on choroid plexus (CP) volume, enlarged perivascular spaces (ePVS), the DTI-ALPS index, and NODDI-derived metrics.

From a methodological perspective, this work developed and validated dedicated processing pipelines for the investigated biomarkers, including a hybrid GMM-based CP segmentation method, a complete DTI-ALPS implementation designed to address some of the method’s main limitations, and an automated ePVS quantification pipeline. NODDI metrics were extracted using the Elikopy workflow. These pipelines allowed the biomarkers to be analysed individually and jointly, in order to assess whether they provide complementary information regarding cognitive decline.

Overall, the results suggest that some biomarkers were more strongly associated with global cognitive decline, whereas others showed more specific relationships with particular cognitive domains or disease stages. The investigated biomarkers therefore appear to provide complementary but distinct information regarding brain alterations associated with aging, amyloid pathology, and cognitive decline. Increased choroid plexus volume was consistently associated with reduced cognitive performance, supporting previous observations linking CP alterations to neurodegeneration. However, the physiological and pathological mechanisms underlying these associations could not be directly assessed within the scope of this work and remain incompletely understood.

Although the DTI-ALPS index showed several trends associated with cognition and disease progression, the study also highlighted important methodological limitations of the approach. Because the method relies on small ROIs placed at fixed anatomical locations, the measurements remain sensitive to local anatomical variability, white matter alterations, and ROI positioning, which limits the robustness and interpretation of the results.

The automated ePVS quantification pipeline also presented important limitations. The obtained results showed limited robustness and consistency, suggesting that the current implementation remains highly sensitive to preprocessing choices, anatomical variability, and imaging quality. In addition, the cohort may not have contained enough ePVS

burden to properly evaluate the performance of the pipeline. These limitations make the interpretation of the ePVS analyses more difficult and indicate that further methodological improvements and validation would be necessary before drawing strong conclusions from these measurements.

Among the NODDI-derived biomarkers, FISO appeared as a relatively global marker associated with widespread tissue alterations and cognitive decline. Nevertheless, the analyses still highlighted regions known to be strongly involved in AD, such as hippocampal and limbic structures, which consistently appeared among the most affected regions. In contrast, FINTRA showed more specific and localized associations with particular cognitive domains in anatomically relevant regions. Although this may appear less intuitive at first glance, these findings suggest that FINTRA could potentially provide important information regarding microstructural degeneration within critical white matter pathways involved in cognitive impairment.

More broadly, this work contributes to the growing effort to better understand the relationship between brain fluid regulation, glymphatic dysfunction, and neurodegeneration in AD. Although these biomarkers do not directly measure glymphatic flow, they may still provide indirect information about impaired clearance mechanisms and altered brain homeostasis. Future studies using longitudinal analyses, larger cohorts, comparisons with established AD biomarkers, and experimental confirmation through more direct approaches such as fluorescent tracer studies will however remain necessary to better clarify the specificity and clinical relevance of these biomarkers.

Finally, this work fits into a broader effort to develop non-invasive imaging tools capable of detecting glymphatic-related alterations early in the course of AD. If impaired brain waste clearance contributes to disease progression, these biomarkers could eventually, one day, support earlier diagnosis and open new avenues for therapeutic intervention.

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Appendix A

Signal detection and image acquisition

The transverse magnetization is detected using a receiver coil (antenna). As the transverse magnetization precesses around the static magnetic field B_0 , it induces an oscillating voltage in the receiving coil.

The detected signal corresponds to a sinusoidal oscillation whose amplitude decays exponentially with time due to transverse relaxation (T_2^*). This signal is known as the *Free Induction Decay* (FID) Figure 1.4b and represents the fundamental signal measured in MRI.

The temporal evolution of the magnetization vector in the rotating reference frame is described by the Bloch equations. In their component form, they can be written as:

$$\begin{cases} \frac{dM_x}{dt} = -\frac{M_x}{T_2} + \Delta\omega M_y - \gamma B_{1y} M_z \\ \frac{dM_y}{dt} = -\Delta\omega M_x - \frac{M_y}{T_2} + \gamma B_{1x} M_z \\ \frac{dM_z}{dt} = \gamma B_{1y} M_x - \gamma B_{1x} M_y + \frac{M_0 - M_z}{T_1} \end{cases} \quad (\text{A.1})$$

where M_x , M_y , and M_z represent the components of the magnetization vector, T_1 and T_2 are the longitudinal and transverse relaxation times, γ is the gyromagnetic ratio, and B_1 represents the applied radiofrequency field.

These equations describe the combined effects of precession and relaxation of the magnetization vector.

Depending on the experimental conditions:

- $\Delta\omega \neq 0$: precession occurs around the effective magnetic field B_{eff}
- $\Delta\omega = 0$: precession occurs around the RF field B_1
- $B_1 = 0$: precession occurs around the static magnetic field B_0 (laboratory frame)

Spatial encoding using magnetic field gradients

After RF excitation, the MRI signal must be spatially encoded in order to determine where it originates within the body. This spatial localization is achieved using magnetic field gradients.

Three types of gradients are used during MRI acquisition.

Slice selection gradient

During RF excitation, a slice-selection gradient G_{ss} is applied along the cranio-caudal direction (typically the z -axis). Because the Larmor frequency varies with position, only spins whose resonance frequency matches the RF pulse are excited. This allows selection of a specific slice within the body as in Figure A.1.

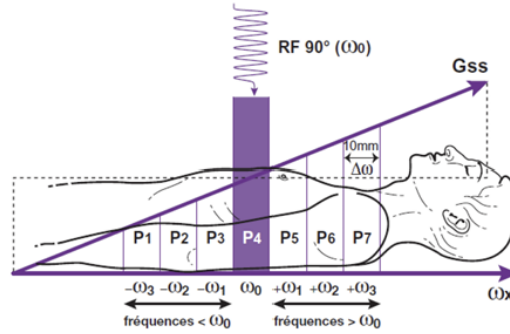


Figure A.1: Principle of slice selection in MRI. A frequency-selective RF pulse (90°, centred on ω_0) is applied together with a slice-selection gradient G_{ss} along the head-foot axis. This gradient makes the Larmor frequency vary linearly with position, so that spins located at different positions precess at different frequencies. Only the spins whose resonance frequency lies within the RF bandwidth are excited. In this example, the selected slice is centred on P4, where the resonance frequency matches ω_0 .

Frequency encoding gradient

During signal readout, a gradient is applied along one spatial direction (e.g. x). Spins located at different positions experience slightly different magnetic fields and therefore precess at different frequencies. The received signal contains multiple frequencies that correspond to different spatial locations. Once the gradient is turned off, all protons return to their original frequency (ω_0), so the frequency differences must be measured while the gradient is active.

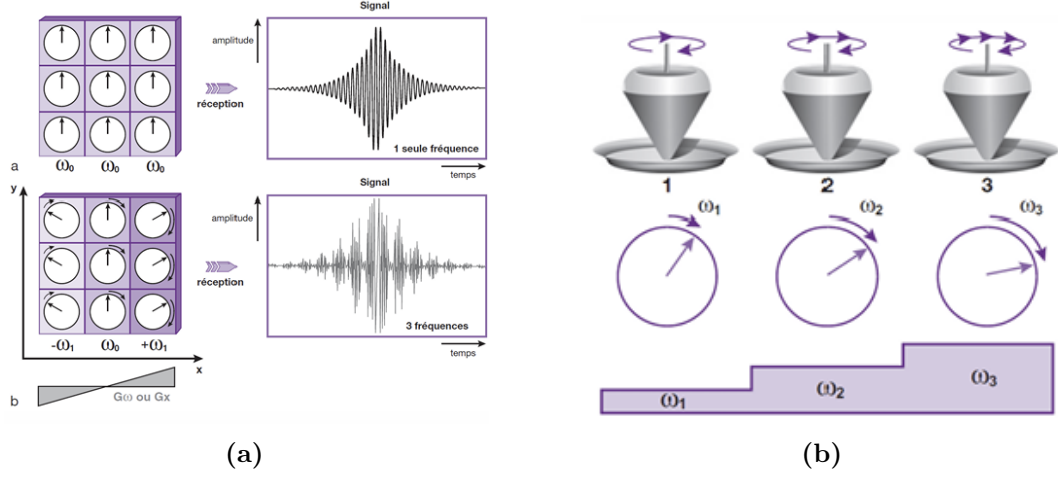


Figure A.2: Frequency encoding in MRI. (a) Effect of a frequency-encoding gradient on the received signal. Without a gradient (top), all spins precess at the same frequency ω_0 (clocks in phase), and the received signal contains a single frequency. When a gradient G_x is applied along x (bottom), spins precess at position-dependent frequencies ($-\omega_1$, ω_0 , $+\omega_1$), so the received signal becomes a superposition of several frequencies, each encoding a spatial position. (b) Underlying mechanism: the gradient makes the local Larmor frequency increase stepwise with position ($\omega_1 < \omega_2 < \omega_3$), so that spins at different locations precess at different rates, translating spatial position into precession frequency.

Phase encoding gradient

A second gradient is briefly applied along another direction (e.g. y). This gradient causes spins at different positions to accumulate different phases. After the gradient is turned off, spins return to the same frequency but retain the phase differences created during the gradient. These phase shifts encode spatial information along this direction.

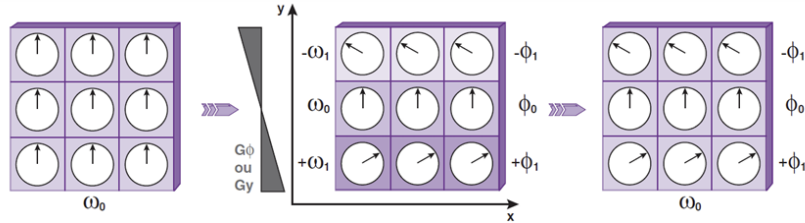


Figure A.3: Phase encoding in MRI. Initially (left), all spins precess in phase at the same frequency ω_0 . A gradient G_y is then briefly applied along y (middle): during this interval, spins precess at position-dependent frequencies ($-\omega_1$, ω_0 , $+\omega_1$) and therefore accumulate position-dependent phase shifts ($-\phi_1$, ϕ_0 , $+\phi_1$), shown by the differing clock orientations. Once the gradient is switched off (right), all spins precess again at the same frequency ω_0 , but retain the phase differences acquired during the gradient, so that position along y is now encoded in the phase.

Because the same frequencies are measured during each signal acquisition, the amplitude of the phase encoding gradient must be modified for each repetition of the sequence. This allows different spatial information to be collected during each acquisition.

This process is referred to as *incrementing the phase-encoding gradient*.

K-space representation

The MRI signal is acquired in the spatial frequency domain called *k-space*. The k-space coordinates are determined by the application of the different encoding gradients. During acquisition, each repetition fills one line of k-space using the frequency encoding gradient while the phase encoding gradient is incremented between repetitions. Once the entire k-space matrix is filled, the final image is obtained by applying an inverse Fourier transform.

Appendix B

Supplementary Figures

B.1 Demographics

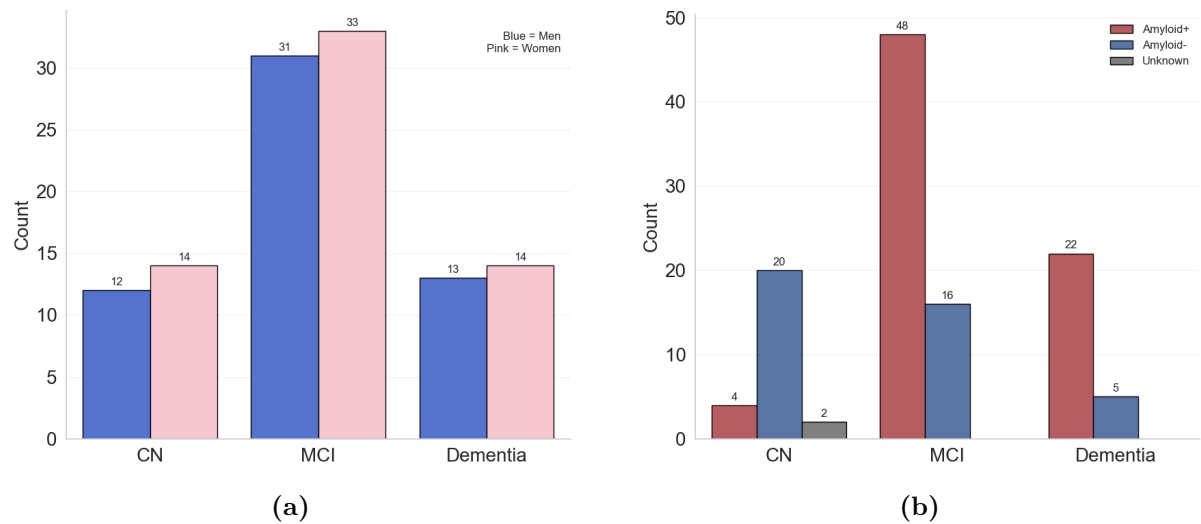


Figure B.1: Distribution across the three diagnostic groups (CN, MCI, dementia) in the main cohort ($n = 117$). (a) Sex distribution (men in blue, women in pink). (b) Amyloid status distribution (Amyloid+, Amyloid-, or unknown). The number of participants is indicated above each bar.

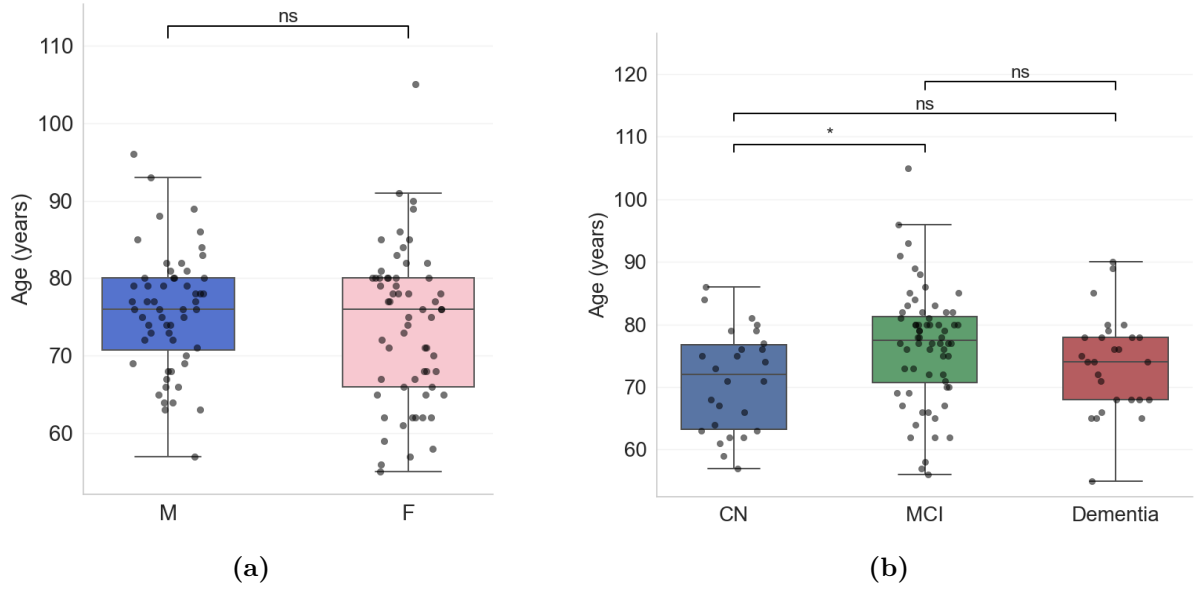


Figure B.2: Age distribution in the main cohort ($n = 117$). (a) Age distribution by sex (M, F). (b) Age distribution across the three diagnostic groups (CN, MCI, dementia); significance of pairwise comparisons: (* $p < 0.05$, ns = not significant). In each boxplot, the central line is the median, the box the interquartile range, and individual points the subjects.

B.2 CP Analyses

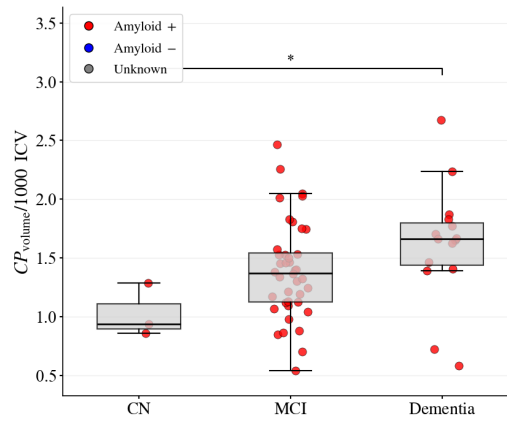


Figure B.3: Adjusted CP volume distributions across diagnostic groups, restricted to the amyloid-positive subgroup. Significant pairwise differences are indicated by star annotations (*: $p < 0.05$, **: $p < 0.01$).

B.3 DTI-ALPS Analyses

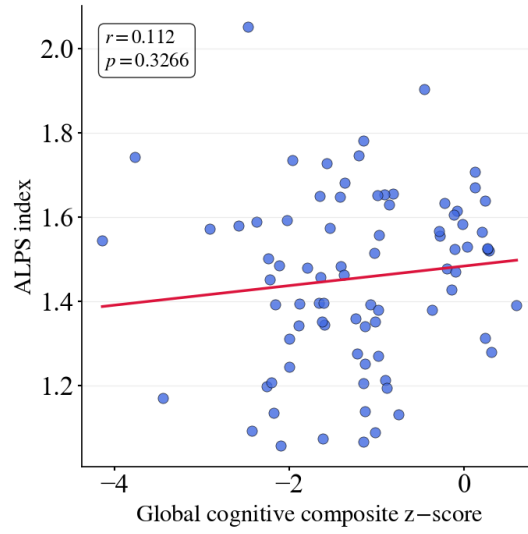


Figure B.4: Partial correlation between the DTI-ALPS index and the global cognitive composite score after adjustment for age, sex, and years of education ($r = 0.112$, $p = 0.326$).

B.4 EPVS Analyses

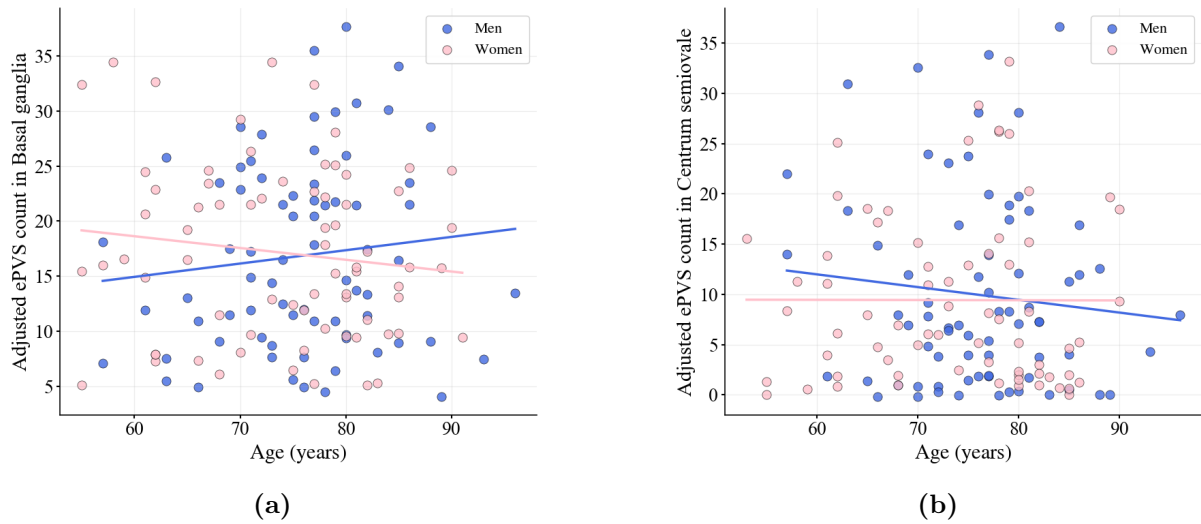


Figure B.5: Age-related ePVS trajectories stratified by sex in BG (a) and CSO (b) regions.

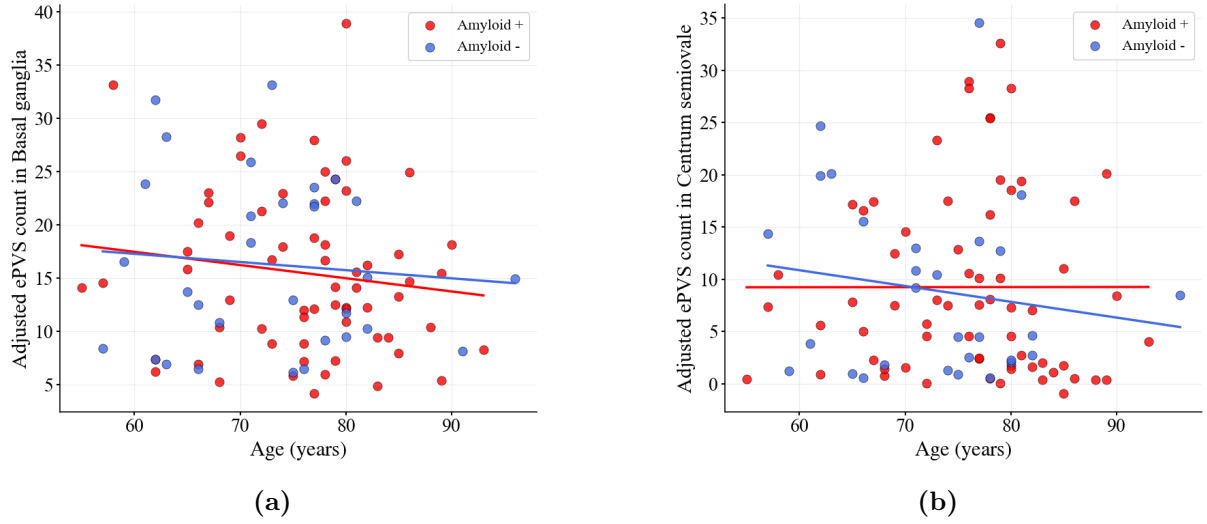


Figure B.6: Age-related ePVS trajectories within amyloid subgroups in BG (a) and CSO (b) regions.

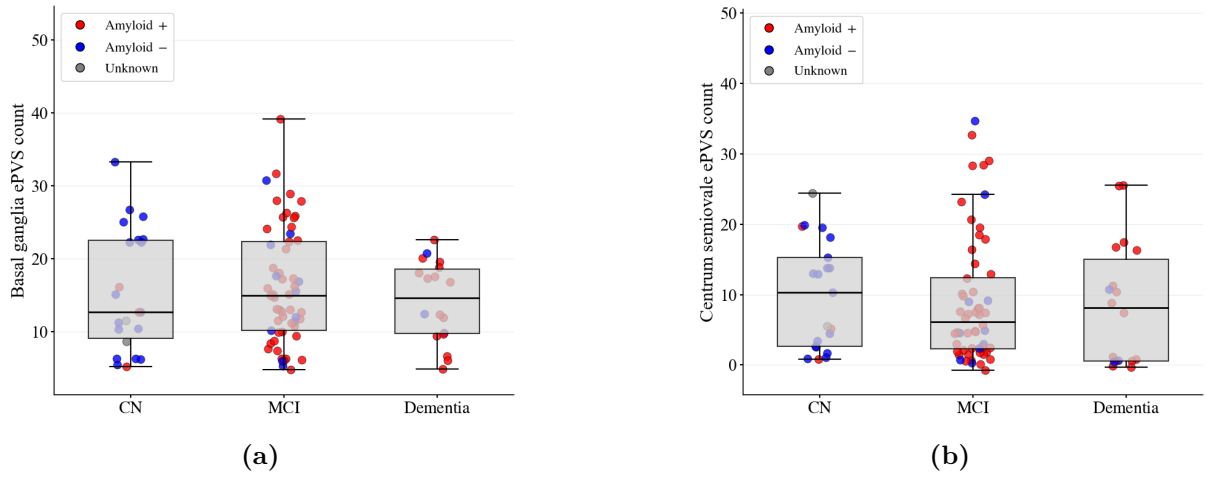
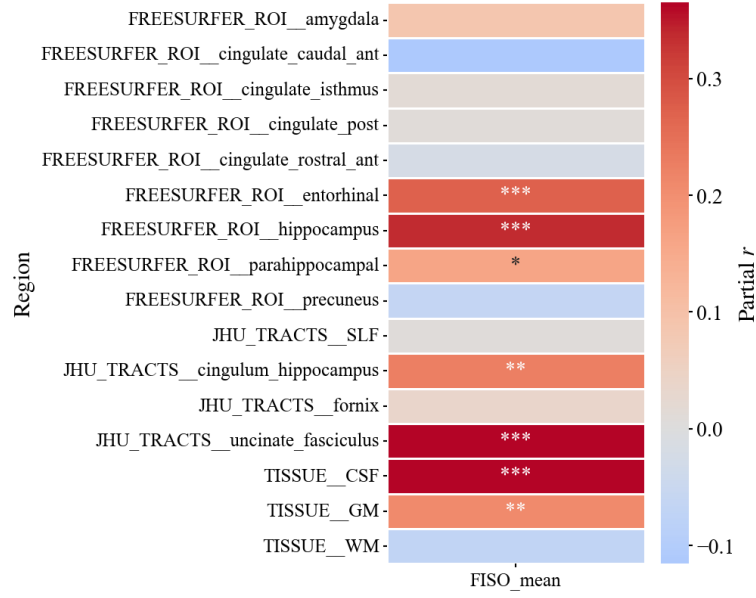
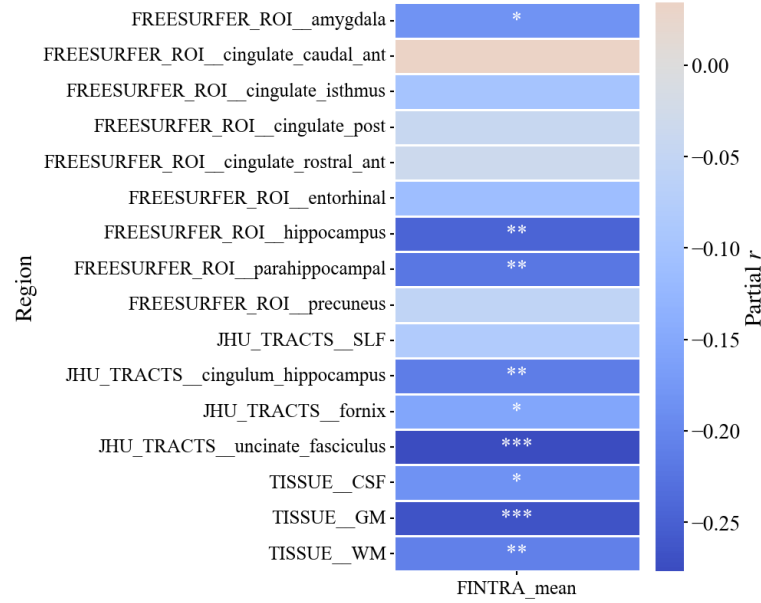


Figure B.7: (a) Adjusted BG and (b) CSO ePVS count distributions across CN, MCI, and dementia groups.

B.5 Noddi Analyses

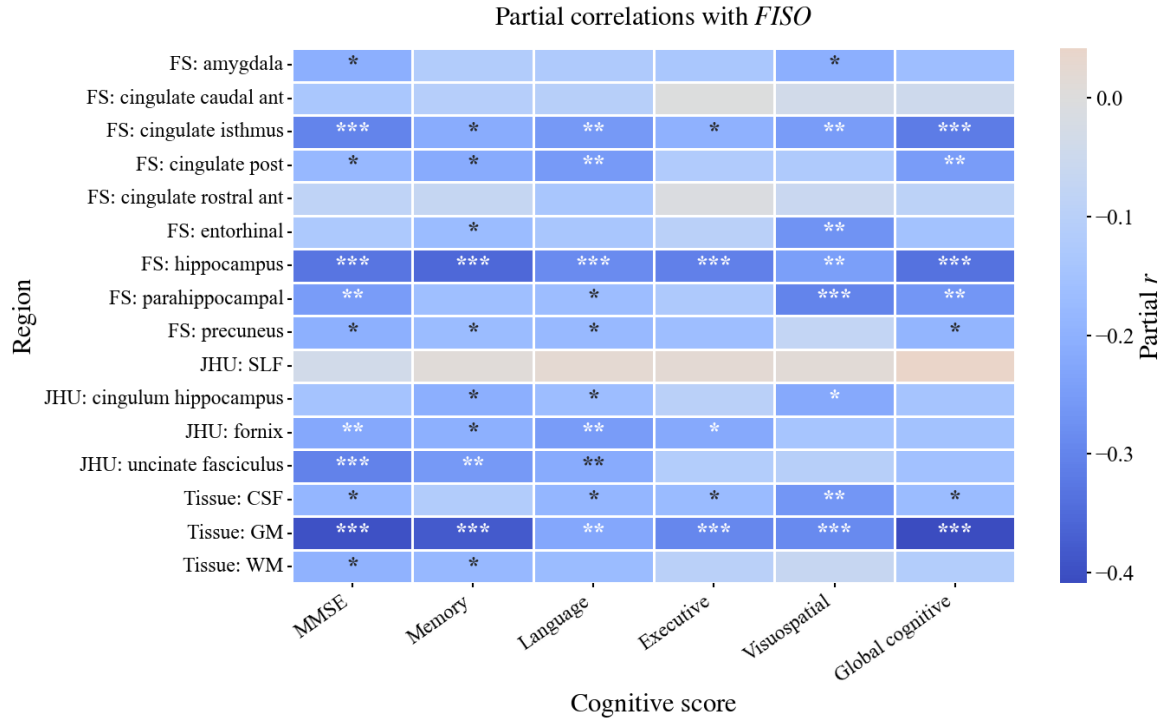


(a) FISO associations with age.

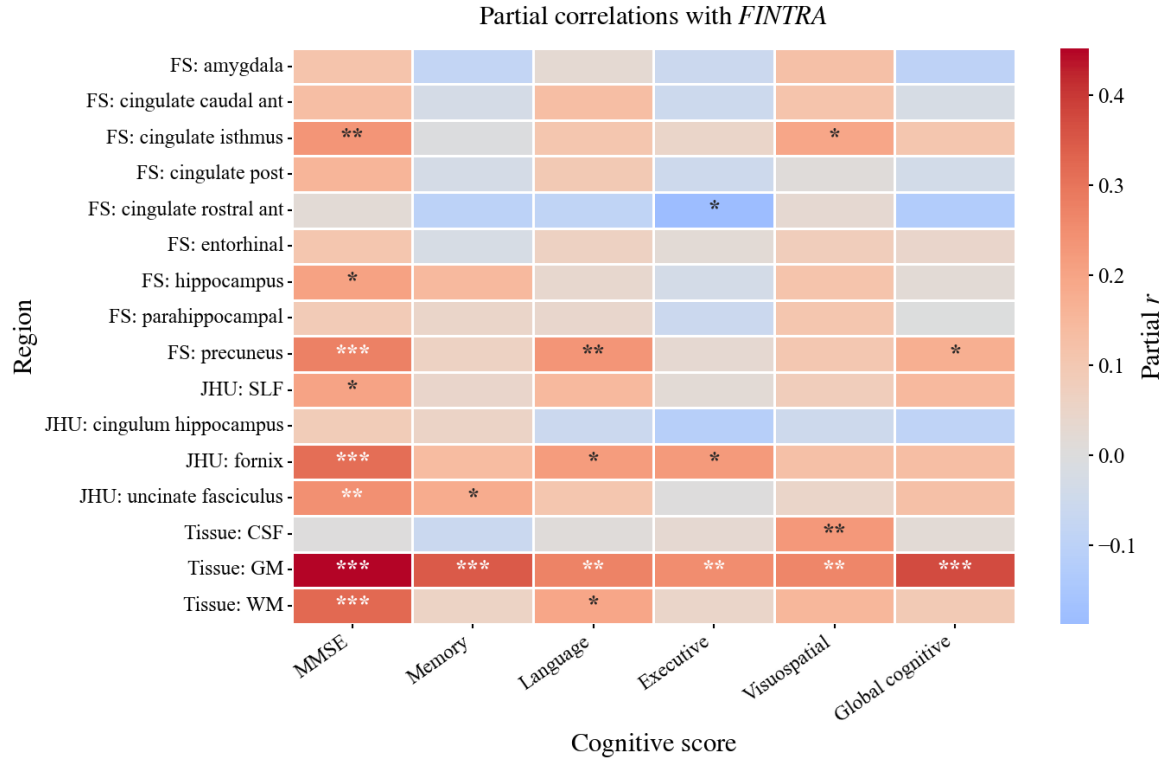


(b) FINTRA associations with age.

Figure B.8: Heatmaps of partial Pearson correlation coefficients between age and NODDI-derived metrics across anatomical regions, controlling for sex and years of education. Significant associations are indicated by star annotations (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).



(a) FISO correlations with cognitive scores.



(b) FINTRA correlations with cognitive scores.

Figure B.9: Partial correlations between cognitive composite scores and regional NODDI metrics after adjustment for age, sex, and years of education. Asterisks indicate significance levels (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

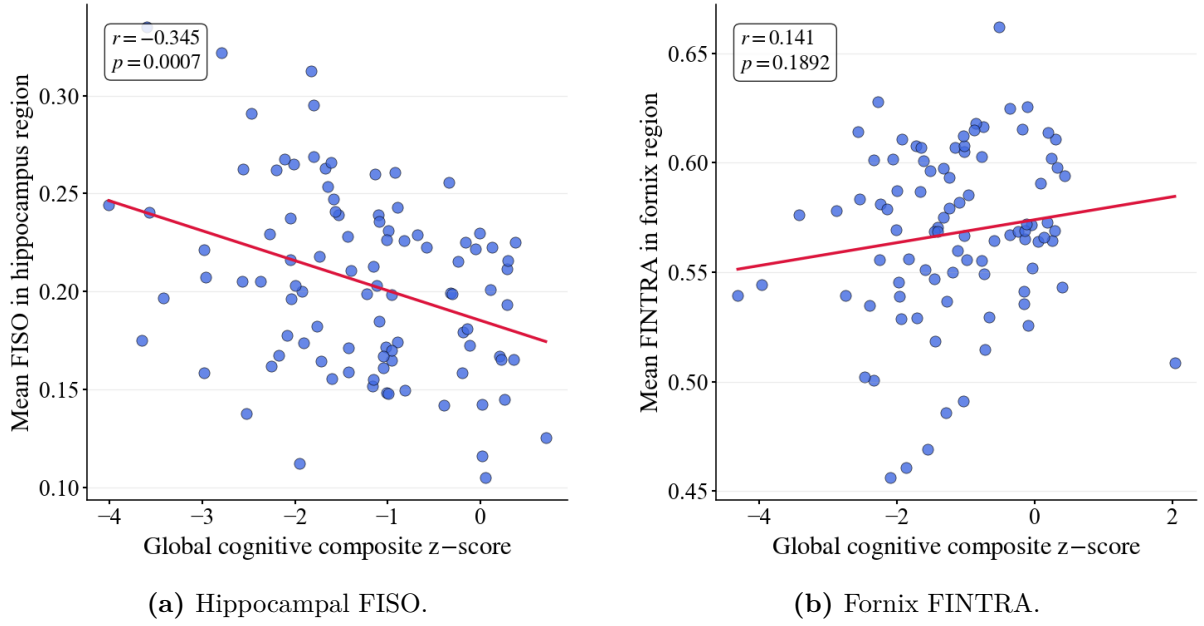


Figure B.10: Partial correlations between the global cognitive composite score and hippocampal FISO (a) and fornix FINTRA (b), after adjustment for age, sex, and years of education.

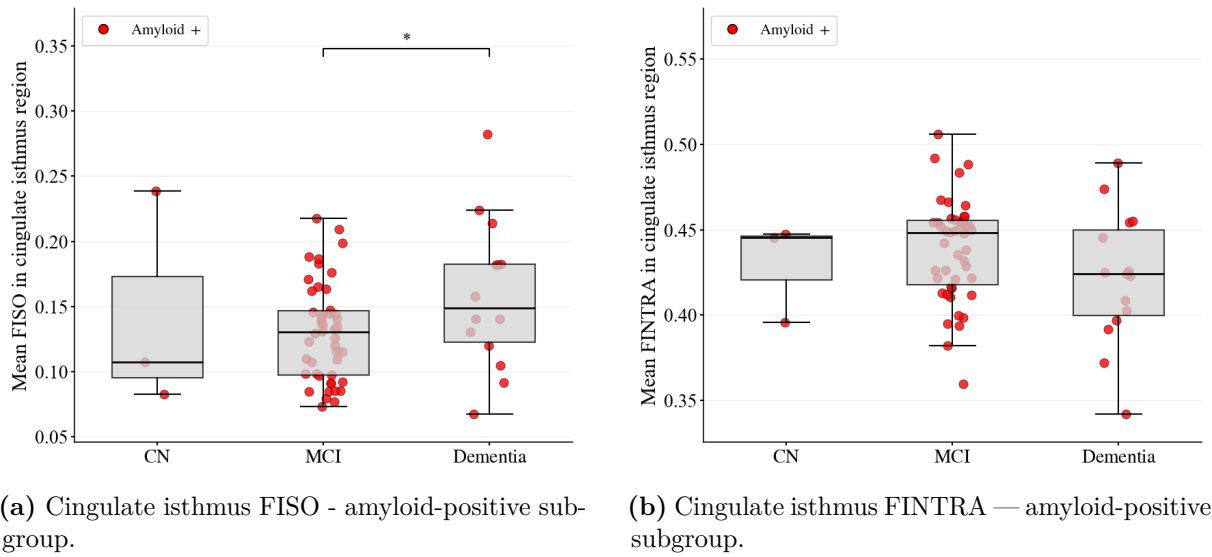


Figure B.11: Cingulate isthmus FISO (a) and FINTRA (b) distributions across CN, MCI, and dementia groups, restricted to the amyloid-positive subgroup.

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