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Impact of Reversible Visual Impairment on Cognition and Blood-Brain Barrier Integrity

A dissertation submitted to the University of Dublin for the
degree of Doctor of Philosophy (PhD)

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2026

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Angela Garcia-Gallardo

December 2025

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*Le dedico esta tesis a mi familia,
a quienes están conmigo y a quienes,
aunque ya no están,
siguen siendo parte de mí.*

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SUMMARY

Keywords: cataract surgery; visual impairment; cognition; blood–brain barrier; DCE-MRI; brain ageing; proteomics; sensory deprivation; ageing brain; neurovascular integrity.

Background

Age-related visual impairment represents one of the most common and potentially modifiable sensory risk factors for cognitive decline and dementia. Cataract, the leading cause of reversible blindness worldwide, causes gradual sensory deprivation that increases perceptual effort and cognitive load. The removal of cataract offers a unique clinical model in which sensory input is restored without direct central nervous system intervention, allowing investigation of the downstream effects of visual restoration on cognition, brain physiology, and systemic biomarkers. This doctoral study aimed to examine the impact of cataract-related visual impairment and its surgical correction on cognitive trajectories, blood–brain barrier (BBB) integrity, structural and brain-ageing indices, and plasma proteomic profiles in older adults.

Aims

The study was designed to: 1) characterise baseline relationships between visual function, cognition, and BBB permeability; 2) evaluate longitudinal change in cognitive performance, BBB indices, structural MRI, and proteomic markers following cataract surgery; and 3) explore whether post-operative improvement in sensory input is associated with attenuation of age-related BBB or brain-ageing trajectories.

Methods

Twenty adults scheduled for bilateral cataract surgery were prospectively enrolled; eighteen completed the full longitudinal protocol. Participants underwent assessments at baseline (T0), three months (T1), and nine months (T2) post-surgery. Best-corrected visual acuity (VA) was recorded binocularly using logMAR; cataract type and severity were graded using the Lens Opacities Classification System II. Cognitive evaluation used the Repeatable Battery for the Assessment of Neuropsychological Status-Update (RBANS-U), with percentile-ranked Total and Index scores; affective state was measured with the Beck Depression Inventory-II (BDI-II) and the State–Trait Anxiety Inventory (STAI-Y1/Y2). MRI protocols included dynamic contrast-enhanced MRI (DCE-MRI) for BBB permeability (whole-brain Ktrans, percentage of suprathreshold voxels, and 95th percentile leakage slope), volumetric and cortical-thickness pipelines (volBrain), and brain-age modelling. Longitudinal analyses used linear mixed-effects models (REML) with Tukey-adjusted pairwise comparisons; non-parametric tests were applied when assumptions were violated. Proteomic profiling was performed on plasma samples at each timepoint using label-free liquid chromatography–mass spectrometry (LC–MS/MS) and analysed with limma (empirical Bayes moderation). Differentially expressed proteins (DEPs) were defined by false-discovery-rate (FDR) adjusted p-value < 0.05 and $|\log_2 \text{fold change}| \geq 0.3$. Correlation analyses explored inter-relations among visual, cognitive, BBB, and structural variables, with VA–BBB forest plots generated using Snellen quotient units to preserve intuitive directionality.

Results

Cataract surgery produced robust and sustained improvement in VA. Cognitive performance improved significantly across the cohort: RBANS-U Total percentile increased from median 24th at T0 to 39th at T1 and 46th at T2. All cognitive domains showed parallel upward trends, most prominently Immediate and Delayed Memory. Depressive and anxiety scores remained mild and stable, supporting that cognitive gains were not attributable to reduced test anxiety or mood change.

DCE-MRI revealed relative stability of threshold-based BBB indices but a statistically significant reduction in whole-brain K_{trans} from T0 to T2. Exploratory maps showed the largest non-significant decreases in temporal lobes, including hippocampal regions typically vulnerable to age-related leakage. Whole-brain, grey-matter, and white-matter volumes expressed as %TIV, and lobar cortical thickness, remained tightly clustered with no significant change across T0–T2. Brain-age analysis revealed a significant reduction in the rate of brain ageing over the same period, indicating deceleration relative to expected age-related trajectories. Correlation matrices confirmed coherent positive inter-relations among RBANS-U domains and weak negative trends between improved VA and BBB permeability, without reaching corrected significance.

Proteomic profiling quantified 664 proteins across paired longitudinal samples. Differential-abundance analysis identified several proteins meeting FDR and fold-change criteria—NPM2, SAE1, GALE, and CATD—implicated in cellular homeostasis, protein turnover, and metabolic regulation. No significant differences were observed in canonical circulating BBB markers such as GFAP or VEGF. The overall proteomic pattern suggested subtle systemic recalibration following visual restoration rather than inflammatory activation. No single protein change correlated strongly with imaging or cognitive measures, consistent with the exploratory nature of this component.

Conclusions

In older adults, correction of cataract-related visual impairment was followed by measurable cognitive improvement and a modest but statistically significant reduction in BBB permeability, accompanied by relative stability of brain structure and deceleration of the estimated brain-ageing rate. These findings collectively suggest that sensory restoration may mitigate some aspects of neurovascular and cognitive ageing, possibly by reducing sustained perceptual load and restoring efficient environmental engagement. Although causality cannot be inferred in a small observational cohort, the directionally consistent multimodal evidence, from cognition, DCE-MRI, brain-age modelling, and plasma proteomics, supports the hypothesis that sensory input integrity contributes to maintenance of brain health in ageing. Future studies with larger samples, control groups, and extended follow-up are warranted to confirm these findings and clarify the mechanistic links between sensory restoration, BBB function, and cognitive resilience.

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I confirm that this work was undertaken during my period of registration for the degree and forms part of the research submitted for examination.

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ABBREVIATIONS

Aβ – Amyloid Beta (B-Amyloid)	GBD – Global Burden of Disease
Aβ_{1-40/42} – Amyloid Beta Peptide 1–40/42	GDPR – General Data Protection Regulation
AAO – American Academy of Ophthalmology	GFAP – Glial Fibrillary Acidic Protein
ABC – ATP-binding cassette	GLUT-1 – Glucose Transporter 1
ACE-R – Addenbrooke’s Cognitive Examination–Revised	HD – Huntington’s Disease
AD – Alzheimer Disease	HSE – Health Service Executive
AMD – Age-Related Macular Degeneration	ICAM-1 – Intercellular Adhesion Molecule-1
AJs – Adherens Junctions	IgG – Immunoglobulin G
ALS – Amyotrophic Lateral Sclerosis	IL-1/β/6/33 – Interleukin-1/ β /6/33
ApoE – Apolipoprotein E	IM – Immediate Memory (RBANS-U Index)
ApoE2/3/4 – Apolipoprotein E E2/3/4 Isoform	IOL – Intraocular Lens
APOE – Apolipoprotein E (Gene)	IOP – Intraocular Pressure
APP – Amyloid Precursor Protein	JAM-A/B/C – Junctional Adhesion Molecule-A/B/C
AQP4 – Aquaporin-4	Ktrans – Volume Transfer Constant (DCE-MRI permeability parameter)
BBB – Blood–Brain Barrier	LC-MS/MS – Liquid Chromatography–Tandem Mass Spectrometry
BDI-II – Beck Depression Inventory–II	LBD – Lewy Body Disease
BM – Basement Membrane	LDL – Low-Density Lipoprotein (Cholesterol)
BMECs – Brain Microvascular Endothelial Cells	LMIC – Low- And Middle-Income Countries
CAMI – Centre for Advanced Medical Imaging	LOCS – Lens Opacities Classification System
CAMs – Cell Adhesion Molecules	logMAR – Logarithm Of The Minimum Angle Of Resolution
CDC – Centres for Disease Control and Prevention	LRP1 – Low-Density Lipoprotein Receptor-Related Protein 1
CI – Confidence Interval	MCI – Mild Cognitive Impairment
CKD – Chronic kidney disease	MMSE – Mini-Mental State Examination
CME – Cystoid Macular Oedema	MNI – Montreal Neurological Institute
CNS – Central Nervous System	MoCA – Montreal Cognitive Assessment
COPD – Chronic Obstructive Pulmonary Disease	MMP (-2/9) – Matrix Metalloproteinase (-2/9)
CPAP – Continuous Positive Airway Pressure	MRI – Magnetic Resonance Imaging
CSF – Cerebrospinal Fluid	MS – Multiple Sclerosis; also Mass Spectrometry (Depending On Context)
CSVD – Cerebral Small-Vessel Disease	MSVI – Moderate To Severe Vision Impairment
Da – Dalton	Nd:YAG – neodymium-doped yttrium aluminium garnet
DCE-MRI – Dynamic Contrast-Enhanced Magnetic Resonance Imaging	NIA-AA – National Institute on Aging and Alzheimer’s Association
DEP(s) – Differentially Expressed Protein(S)	NMDA – N-methyl-D-aspartate (receptor)
DIA – Data-Independent Acquisition	NVC – Neurovascular Coupling
DIA-NN – Data-Independent Acquisition By Neural Networks	NVU – Neurovascular Unit
DM – Delayed Memory (RBANS-U index)	OCT – Optical Coherence Tomography
DMN – Default Mode Network	OCT-A – Optical Coherence Tomography Angiography
DRS – Dementia Rating Scale	OSA – Obstructive Sleep Apnoea
DSM – Diagnostic and Statistical Manual of Mental Disorders	P-gp – P-Glycoprotein
EC – Endothelial Cell	PD – Parkinson’s Disease
ECM – Extracellular Matrix	PECAM-1 – Platelet Endothelial Cell Adhesion Molecule-1
FDR – False Discovery Rate	PET – Positron Emission Tomography
FLAIR – Fluid-Attenuated Inversion Recovery	PIL – Participant Information Leaflet
FLACS – Femtosecond Laser–Assisted Cataract Surgery	PSC – Posterior Subcapsular (cataract)
FPN – Frontoparietal Control Network	PV – Progressive Vision
FTD – Frontotemporal Lobar Degeneration	
G*Power – Power Analysis Software	
GABA – Gamma-Aminobutyric Acid	
GAT – Goldmann Applanation Tonometry	

RAGE – Receptor for Advanced Glycation End Products

RBANS-U – Repeatable Battery for the Assessment of Neuropsychological Status–Update

REML – Restricted Maximum Likelihood

ROI – Region Of Interest

SAGE – Study on Global AGEing and Adult Health

SCD – Subjective Cognitive Decline

SD – Standard Deviation

SE – Status Epilepticus

SEM – Standard Error of The Mean

sICAM-1 – Soluble Intercellular Adhesion Molecule-1

SICS – Small-Incision Cataract Surgery

SJH – St James’s Hospital

sPDGFR β – Soluble Platelet-Derived Growth Factor Receptor Beta

STAI-Y – State–Trait Anxiety Inventory (Form Y)

STAI-Y1 – State–Trait Anxiety Inventory, Form Y, State subscale

STAI-Y2 – State–Trait Anxiety Inventory, Form Y, Trait subscale

sVCAM-1 – Soluble Vascular Cell Adhesion Molecule-1

SWATH – Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH–DIA mode)

Tau – Microtubule-Associated Protein Tau

T0 – Baseline timepoint before cataract surgery (study timepoint)

T1 – Follow-up timepoint approximately 3 months after surgery (study timepoint)

T2 – Follow-up timepoint approximately 9 months after surgery (study timepoint)

TBI – Traumatic Brain Injury

TCD – Trinity College Dublin

TDP-43 – TAR DNA-binding protein 43

TE – Time To Echo

TEER – Transendothelial Electrical Resistance

TEM – Transendothelial Migration

THT – Therapeutic Hypothermia Therapy

TIMPs – Tissue Inhibitors Of Metalloproteinases

TIV – Total Intracranial Volume

TJ – Tight Junction

TLE – Temporal Lobe Epilepsy

TNF- α – Tumour Necrosis Factor-Alpha

TR – Repetition Time

UHPLC – Ultra-High-Performance Liquid Chromatography

UV-B – ultraviolet-B

VA – Visual Acuity

V/C – Visuospatial/Constructional (RBANS-U index)

VE-cadherin – Vascular Endothelial Cadherin

VEGF – Vascular Endothelial Growth Factor

VFA – Variable Flip Angle

Vol2Brain – Volbrain Structural Pipeline

VolBrain – Online automated brain MRI volumetry and brain-age estimation platform

CHAPTER 1: INTRODUCTION

PART 1: UNDERSTANDING THE BLOOD–BRAIN BARRIER: FROM PHYSIOLOGY TO PATHOLOGY

1.1.1 Introduction

The brain is a complex and sophisticated organ with dynamic functions that make it the most metabolically demanding organ in the body (Schaeffer and Iadecola, 2021). Despite representing only 2% of the total body weight, the human brain consumes approximately 20% of the total oxygen and glucose available in the body (Dion-Albert et al., 2023; Kisler et al., 2017).

Given its metabolic needs, the brain possesses more than 600 km of vessels that provide nutrients, remove waste and toxins, and maintain the homeostasis of the nervous tissue, among other functions (Dion-Albert et al., 2023; Schlageter et al., 1999). Such vessels are distributed in a manner that ensures a correlation of almost one capillary per neuron (Schlageter et al., 1999), allowing a direct response to the changing blood supply needs of the neurons. This phenomenon is known as neurovascular coupling (Alahmari, 2021).

These brain vessels possess unique properties that distinguish them from the rest of the circulatory system and isolate the neural tissue, creating a multilayered barrier capable of strict control over influx and efflux, known as the blood–brain barrier (BBB) (Obermeier et al., 2013).

1.1.2 The Discovery

The existence of the BBB was first documented by Humphrey Ridley in the book *Anatomy of the Brain*, published in 1695, after observing that cerebral vessels were less permeable to beeswax and mercury compared to other bodily tissues (Ridley, 1695). Almost two centuries later, in 1885, Paul Ehrlich and his student, Edwin Goldman, revived this concept through their experimental findings. Ehrlich described that “after intravenous dye injection, the brain and spinal cord remained white as snow while peripheral organs were readily stained” (Ehrlich, 1885), demonstrating the ability of the CNS to exclude certain molecules.

In 1900, Max Lewandowsky confirmed these findings, concluding that “brain capillaries must prevent certain molecules from entering the brain,” a concept that he called *Bluthirnschranke*, the German word for blood–brain barrier (Lewandowsky, 1900). Finally, in 1967, with the invention of electron microscopy, Thomas S. Reese and Morris J. Karnovsky first described a morphological correlation to these barrier properties, identifying the presence of tight junctions connecting the overlapping endothelial cells (Reese and Karnovsky, 1967). From then on, BBB knowledge has continued to grow, fed by an endless number of completed and ongoing studies.

1.1.3 The Blood–Brain Barrier

The blood–brain barrier (BBB), present in most vertebrates and not exclusive to humans (Sweeney et al., 2018), is a dynamic, tightly controlled, and highly selective microvascular system with unique properties restricted to the CNS circulation (Obermeier et al., 2013). It consists of cellular and non-cellular vascular and neural components that support the brain microvascular endothelial cells (ECs) in their role of creating

a structural, transport, and metabolic barrier that limits the entry of foreign and unwanted molecules and cells, safeguards nutrient distribution, and controls the neuronal extracellular environment (Alahmari, 2021; Obermeier et al., 2013), maintaining a balanced microenvironment that protects the neurons (Zhao et al., 2022).

Acting as the primary gatekeepers, ECs interact dynamically with pericytes, astrocytes, basement membranes, microglial cells, and neurons. This collective interaction forms the neurovascular unit (NVU) and constitutes the building block of the BBB (Zhao et al., 2022).

1.1.3.1 BBB Function

Over the past century, as research in the field has expanded, the understanding of the BBB has evolved from a “brick wall” concept to an active, robust, regulatory, and dynamic multicomponent unit that adapts to meet CNS and systemic demands under physiological and pathological conditions (Lampron et al., 2013). BBB modulation is influenced by the crosstalk between NVU components, vascular and immune elements, as well as neurons. Recent studies using single-cell sequencing have revealed vascular changes occurring in response to neuronal activity (Hrvatin et al., 2018; Nishijima et al., 2010).

The BBB maintains CNS homeostasis by regulating the influx and efflux of ions, oxygen, and nutrients. It tightly controls molecular and cellular migration, preventing non-meaningful crosstalk (Abbott et al., 2006; Bernacki et al., 2008), and restricting the entry of blood cells, plasma components, toxins, and pathogens that could damage the brain environment (Harati et al., 2012; Zhao et al., 2015). Additionally, the BBB regulates the bidirectional water flow between the bloodstream and the extracellular space, ensuring proper volume balance (Rasmussen et al., 2022).

It has been proposed that there may be heterogeneity in the BBB across different functional regions of the brain, such as the cortex, hippocampus, white matter, and basal ganglia. This differentiation could contribute to the specialised functions of each region, though there is currently insufficient data to fully support this hypothesis (Profaci et al., 2020).

1.1.3.2 Neurovascular Unit Components

1.1.3.2.1 Endothelial Cells (ECs)

In the CNS, ECs create a continuous lining of highly specialised barrier cells, where the smallest capillary circumference is formed by a single EC (Profaci et al., 2020). Brain ECs are simple squamous epithelial cells characterised by a unique phenotype that makes them different from their peers in the rest of the circulatory system (Aird, 2007). They are non-fenestrated, rich in mitochondria, with luminal/abluminal polarisation, abundant junctional adhesion molecules (JAMs), and tight junctions (TJs) with high electrical resistance that regulate the paracellular movement of ions, macromolecules, and polar solutes. These cells also have specific transporters and receptors controlling transcellular molecule flow and exhibit a notably low level of transcytotic vesicle activity (Michalicova et al., 2020).

The luminal aspect of the ECs is covered by the glycocalyx, a dense coat composed of glycoproteins and glycolipids that restricts the interaction of ECs with large molecules (Pillinger and Kam, 2017), but that can

be permeated by smaller solutes like fluorescein (376 daltons, Da) and Alexa Fluor (643 Da) (Kutuzov et al., 2018). As expected, glycocalyx degradation has a negative impact on BBB integrity, being associated with more severe BBB leakage (DellaValle et al., 2019; Zhu et al., 2018). On the contralateral aspect of the ECs lies the basal lamina, or basement membrane (BM), a matrix of laminins, fibronectin, collagens, tenascin, and proteoglycans encasing the ECs and pericytes that acts as a selective binding interface for molecules and cell migration (Del Zoppo et al., 2006).

CNS EC TJs also exhibit features distinct from those in peripheral vessels. They are more abundant and 50–100 times closer together, resulting in extremely high transendothelial electrical resistance (TEER) (Nagasawa et al., 2006).

Despite being considered the core element of the BBB, the unique characteristics of ECs are the result of regulation carried out by the intricate molecular crosstalk network between the other cells in the NVU (Alvarez et al., 2013), required for BBB formation during embryonic development and maintenance of its integrity in adulthood (Keaney and Campbell, 2015).

1.1.3.2.2 Pericytes

Pericytes are multifunctional perivascular cells that wrap their finger-like projections around ECs at the capillary level (Dore-Duffy, 2008). As they play a significant role in the stability and integrity of the NVU, and therefore the BBB, the pericyte concentration and coverage over the endothelial walls of the CNS are much higher than in the peripheral circulation (Winkler et al., 2011). The pericyte-to-EC ratio has been shown to correlate directly with cerebral blood flow and barrier integrity (Delaney and Campbell, 2017).

Within the NVU, pericytes are located between the ECs and the BM, surrounded by astrocytic end-feet, microglia, and neurons. Pericytes place themselves at EC junctions and face the luminal aspect of astrocytic end-feet (Bertrand et al., 2019). This positioning allows them to perform their fundamental role in BBB development, growth, stability, and maintenance (Daneman and Prat, 2015) as they participate in the regulation of angiogenesis, “barriergenesis”, vascular remodelling, and vascular tone (Sweeney et al., 2016; Winkler et al., 2011).

Pericytes control the formation of TJs and transendothelial vesicle traffic (Dyrna et al., 2013), guide astrocytic foot processes to vessel walls and mediate the polarisation of astrocytic end-feet (Delaney and Campbell, 2017). Pericytes are key modulators of extracellular matrix protein secretion and levels, have a high phagocytic activity for clearing harmful foreign molecules (Sweeney et al., 2016), and actively suppress genes increasing vascular permeability or infiltration of blood-derived leukocytes (Daneman and Prat, 2015; Dyrna et al., 2013).

With ageing, there is pericyte loss and a subsequent reduction of TJ proteins (Dyrna et al., 2013). This leads to increased vascular permeability due to paracellular leakage that might contribute to the accumulation of toxic molecules in the vascular walls and CNS extracellular space, interfering with microvascular perfusion and resulting in neurodegeneration (Bell et al., 2010; Winkler et al., 2011).

1.1.3.2.3 Astrocytes

Astrocytes, a star-shaped subtype of glial cells, are the most abundant cell type in the vertebrate CNS (Vasile et al., 2017). Their strategic position between neurons and the other cells in the NVU allows them to protect neuronal microenvironment homeostasis (Dyrna et al., 2013). Astrocytes interact with ECs through polarised end-feet projections that collectively ensheath the abluminal aspect of the cerebral microvessels (Alvarez et al., 2011a). This interaction is fundamental to providing endothelial signalling to the capillary segment, modulating blood flow, and regulating local immune responses through the downregulation of pro-inflammatory molecules in ECs (Dyrna et al., 2013).

Astrocytes are crucial elements within the NVU, both for preserving BBB integrity and ensuring neuronal well-being. They release molecules involved in enhancing and maintaining the tightness and optimal function of the barrier (Michinaga and Koyama, 2019), as well as in repairing and reducing BBB dysfunction after a brain injury (Zlokovic, 2008). Along with ECs, they synchronise metabolite levels with cerebral blood flow and regulate brain water content through aquaporin-4 (AQP4), the dominant perivascular water channel located in astrocytic end-feet (Alvarez et al., 2011a; Zlokovic, 2008).

In their neuroprotective role, astrocytes guide the migration of developing neurons (Alahmari, 2021), promote synapse formation (Vasile et al., 2017), control neurotransmitter concentrations and their metabolism, regulate ion concentrations, and maintain the balance of the neural microenvironment (Delaney and Campbell, 2017). Despite not conducting electrical signals like neurons, astrocytes enfold synapses, modulate synaptic transmission, and ensure the continued function of neurons (Vasile et al., 2017).

1.1.3.2.4 Basement Membrane (BM)

The BBB basement membrane is a dense, sheet-like extracellular matrix (ECM) composed of proteins secreted by ECs, pericytes, and astrocytes (Xu et al., 2018). It is constructed from multiple types of highly cross-linked glycoproteins, such as laminins 4 and 5, collagen IV, fibronectin, nidogen, perlecan, and proteoglycans, among other molecules. The BM consists of two layers: an inner vascular layer produced by ECs and pericytes, and an outer glial one, synthesised by astrocytes. These layers are fused at the capillary level but separate on larger vessels (Sorokin, 2010).

The BM plays a critical role in BBB permeability, not only by serving as a physical barrier and scaffold (Xu et al., 2018) but also due to its dynamic properties. It displays selective permeability to solutes and cells, modulates cell signalling and communication among NVU components (Yao et al., 2014), helps to regulate pericyte differentiation, guides the migration and proliferation of cells involved in barrier repair, participates in neuroimmune surveillance (Engelhardt and Ransohoff, 2012), and contributes to leukocyte recruitment during neuroinflammation (Sixt et al., 2001).

The BM's metabolism and degradation are mediated by matrix metalloproteinases (MMPs), which are fundamental enzymes for BBB integrity. In several neurological conditions, MMP induction has been

shown to directly compromise the BBB, increasing permeability and facilitating leukocyte infiltration (Rempe et al., 2016).

Over time, the elasticity and thickness of the BM change, along with its susceptibility to degradation, thereby increasing the BBB's vulnerability during ageing and especially in response to CNS damage (Delaney and Campbell, 2017).

1.1.3.2.5 Microglia

Microglia are another type of glial cell distributed throughout the CNS, where they play a vital role in the innate immune response (Al-Onaizi et al., 2020). Derived from yolk sac haematopoietic progenitor cells, they migrate to the CNS during foetal development and mature there (Ginhoux and Prinz, 2015). Microglia have a highly ramified appearance and constitute approximately 5–20% of all the glial cell population (Ginhoux et al., 2013).

Located in proximity to ECs, microglia conduct immune surveillance and act as the CNS's main line of defence after crossing the BBB, phagocytosing harmful particles (Streit, 2005). As part of the NVU, they also contribute to the resealing of the BBB after a vascular injury (Fernández-López et al., 2014) by stimulating TJ expression, and they assist in the repair of injured brain tissue (Michell-Robinson et al., 2015). Activated microglia can exist in two states: those activated through the M1 pathway release pro-inflammatory cytokines, whereas those activated through the M2 pathway are involved in tissue repair and activation of neurotrophic pathways (Guo et al., 2022).

Emerging research on BBB breakdown and neuroinflammation in CNS pathologies, such as multiple sclerosis (MS) and Alzheimer disease (AD), suggests that microglial activation may be both a cause and a consequence of BBB dysfunction (Guo et al., 2022).

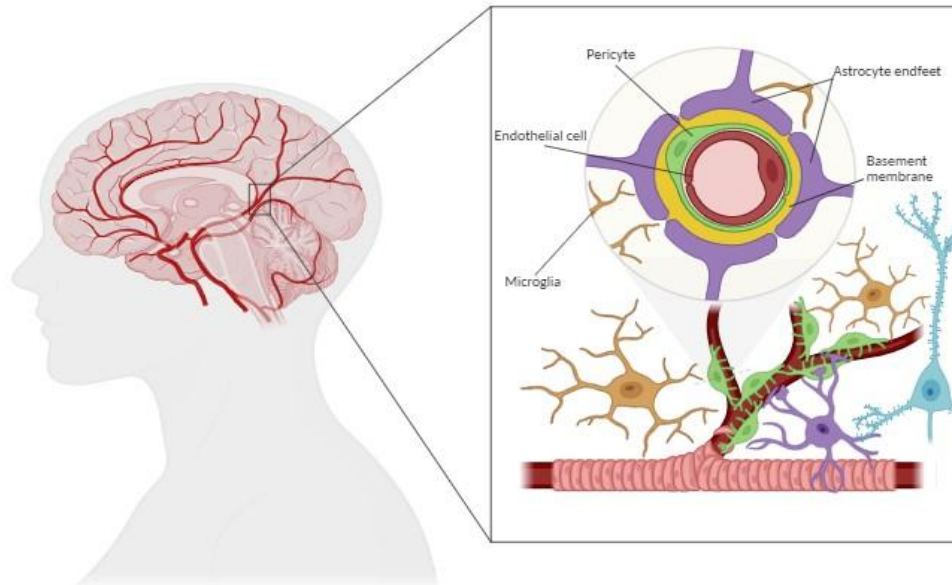


Figure 1.1. Components of the Neurovascular Unit.

1.1.3.3 BBB Main Molecules

CNS endothelial cells (ECs) feature highly specialised paracellular and transcellular transporters, along with enzymes that degrade unwanted substances in the blood that establish contact with the BBB.

The functional integrity of the paracellular gap is controlled by multiprotein complexes located between neighbouring ECs and is responsible for interconnecting them structurally and functionally. Disruption of EC junctional proteins can compromise BBB integrity, allowing harmful substances to enter the brain and potentially causing neuroinflammation or toxicity (Alvarez et al., 2013; Yasuda and Mochizuki, 2010).

1.1.3.3.1 Tight Junctions (TJs)

TJ protein complexes are constituted by an ensemble of multiple families of endothelial-specific transmembrane proteins connected to the EC cytoskeleton through anchoring cytoplasmic molecules. These TJs are essential for CNS homeostasis, as their interactions modulate the paracellular movement of molecules and ions, support the maintenance of EC polarity (Burek et al., 2019), increase the transendothelial electrical resistance (TEER) (Liebner et al., 2018), and participate in the cell signalling process within NVU components (Haseloff et al., 2015). TJ-associated proteins can alter BBB permeability under both normal and pathological conditions.

1.1.3.3.1.1 Claudins: The claudin family is composed of 24 transmembrane proteins with a molecular weight of 20–27 kDa (Hawkins and Davis, 2005). Depending on the specific requirements of the tissue, cells expressing claudins often display multiple isoforms of these proteins, enabling the formation of TJs of varying sizes (Dyrna et al., 2013). In the CNS, claudins are considered the keystone of TJs and the primary factor determining the paracellular tightness between ECs (Yang et al., 2020).

The claudins expressed in CNS ECs are predominantly claudin-5 and claudin-12 (Cardoso et al., 2010). Although claudin-1 and claudin-3 have been shown to have an important role in sealing the BBB (Brunner et al., 2020), claudin-5 is by far the most abundant and dominant TJ component in the CNS, expressed at least 500-fold more than other family members (Nitta et al., 2003). Its paracellular sealing function is the result of its association with other proteins on the adjacent EC surface, creating a cell-to-cell adhesion mechanism that reduces the intercellular distance to almost zero, thereby helping to maintain the integrity of the vessel structure (Greene et al., 2019).

Claudin-5 is necessary for the embryonic development of TJs and the maintenance of BBB integrity throughout life. A congenital claudin-5 deficit causes early postnatal brain swelling and death (Engelhardt and Liebner, 2014). Downregulation or altered expression of claudin-5 after BBB formation is associated with increased BBB permeability, infiltration of immune cells into the CNS, and neuroinflammation (Greene et al., 2019).

1.1.3.3.1.2 Occludin: This 65 kDa transmembrane protein was the first TJ protein discovered (Furuse et al., 1993). It is extensively expressed in CNS endothelium (Hirase et al., 1997) and, together with claudins and zonula occludens-1 (ZO-1), is a staple component of the TJ. Occludin participates in the modulation of EC paracellular permeability (Sakakibara et al., 1997), not only through its junctional activity (Huber et al., 2001) but also by regulating the formation, maintenance, and functioning of other TJs (Yang et al., 2020). Additionally, occludin contributes to the regulation of EC differentiation and signal transduction (Erickson et al., 2007). Unlike claudin-5, congenital occludin deficiency is compatible with life (Delaney and Campbell, 2017).

1.1.3.3.1.3 Junctional Adhesion Molecules (JAM-A, JAM-B, JAM-C): Junctional Adhesion Molecules (JAMs) are transmembrane proteins, members of the immunoglobulin superfamily with a molecular weight of about 40 kDa. JAM-A is localised at endothelial and epithelial cell gaps (Martín-Padura et al., 1998), whereas JAM-B and JAM-C are exclusively present in ECs (Aurrand-Lions et al., 2001). As part of the TJs, JAMs participate in cell-to-cell adhesion, as well as in the development and preservation of other components of TJs (Pimentel et al., 2020).

JAM proteins are also implicated in establishing the apical–basal EC axis and play a prime role in the regulation of immune cell traffic through the BBB by modulating leukocyte–EC interaction, leukocyte adhesion, and migration (Johnson-Léger and Imhof, 2003).

1.1.3.3.1.4 Zonula Occludens: The Zonula Occludens (ZO) family members are membrane-associated intracytoplasmic proteins with a molecular mass ranging from 130 to 220 kDa (Stevenson et al., 1986). These molecules function as scaffolding elements, providing the structural foundation for the formation of functional, stable, and lasting TJs. ZO proteins link and bind to various TJ components, anchoring them to the EC actin cytoskeleton (Fanning et al., 1998). ZO proteins also play important roles in cellular signal regulation, such as cell proliferation and motility (Gumbiner et al., 1991).

There are three types of ZO proteins: ZO-1, ZO-2, and ZO-3. ZO-1 was the first described cytoplasmic component identified as an anchor for TJs. It interacts with claudin-5, occludin, and the JAM family (Shin and Margolis, 2006). ZO-1 has also been found to associate with other junctional proteins, such as those in adherens junctions and gap junctions (Itoh et al., 1993). The dissociation of ZO-1 from its junctional proteins directly correlates with increased BBB permeability (Fischer et al., 2002). Recent studies suggest that in the absence of ZO-1, ZO-2 can compensate, forming morphologically normal TJs (Umeda et al., 2004).

1.1.3.3.2 Adherens Junctions (AJs)

AJs are protein complexes located on the abluminal side of ECs, below TJs. Structurally similar to TJs, AJs consist of transmembrane proteins, called cadherins, and intracytoplasmic catenins (Guo et al., 2007).

Cadherins are responsible for cell-to-cell and cell-to-matrix adhesion. In the CNS endothelium, vascular endothelial (VE)-cadherin is the predominant AJ protein (Stamatovic et al., 2016). Catenins, including p120, β -catenin, and plakoglobin, act as scaffolding proteins, linking the transmembrane portion of the AJ complex to ZO-1 and the actin cytoskeleton (Stamatovic et al., 2016). In addition to their structural role, catenins also participate in regulating cellular signalling processes (Guo et al., 2007). The crosstalk between components of AJs and TJs is essential for establishing and regulating the paracellular barrier (Keaney and Campbell, 2015).

1.1.3.3.3 Transport Pathways and Molecules

Due to its high selectivity, only a limited number of molecules can freely cross the BBB. Features such as a strong affinity for hydrogen bond formation, flexible rotatable bonds, and an extensive polar surface area generally prevent particles from entering the CNS (Bodor and Buchwald, 2002). Passive diffusion is restricted to water, gases, particles with fewer than 10 hydrogen bonds (Patching, 2017), and lipophilic solutes smaller than 180 Da (Liu et al., 2004).

Hydrophilic nutrients and metabolites with structures that do not permeate the BBB require transcellular transport systems. Essential molecules, like glucose, fatty acids, and amino acids, move across the BBB through carrier-mediated transport mechanisms (Miller, 2015). CNS endothelium is densely populated with transporters. By relying on active transport, the BBB maintains strict regulation over molecular traffic. Transporters can be divided into two distinct classes: solute carriers and efflux transporters (Bouldin and Krigman, 1975; Ghersi-Egea et al., 2015).

1.1.3.3.3.1 Paracellular Transport: This involves the movement of small, soluble, extracellular molecules through the gaps between ECs, driven by a concentration gradient from the bloodstream to the brain tissue. TJs, acting as gatekeepers of the intercellular space, limit the passage of hydrophilic molecules larger than 2–4 Å (Chen and Liu, 2012).

1.1.3.3.3.2 Transcellular Transport: This process refers to the transit of solutes across ECs. Gaseous molecules such as oxygen, carbon dioxide, nitrogen, and alcohol traverse the BBB through this mechanism

(Verscheijden et al., 2019). Although small lipophilic particles, such as steroids, can move freely through ECs, their traffic is regulated by efflux pumps, the ATP-binding cassette (ABC) family of transporters (Hindle et al., 2017). These pumps attach to and remove excessive, unwanted, or potentially harmful liposoluble substrates, both xenobiotic and endogenous, back into the bloodstream (Campos-Bedolla et al., 2014).

1.1.3.3.3.3 Transporter Protein Pathway (Carriers): This active transport mechanism involves both solute carriers and ABC transporters. Solute carriers, expressed in EC membranes, facilitate bidirectional movement of metabolites, such as glucose, amino acids, ions, and fatty acids, across ECs and between the bloodstream and the CNS (Shimizu et al., 2008).

ABC transporters, primarily located on the luminal aspect of ECs and also present on pericytes and astrocytic end-feet, actively move a broad range of substrates against their concentration gradients, returning them to the systemic circulation (Patel and Patel, 2017).

1.1.3.3.3.4 Transcytosis: ECs employ multiple specific and unspecific transcytotic mechanisms to facilitate the transport of other hydrophilic macromolecules across the BBB and into the CNS (Zhang et al., 2020). The transcytosis pathway comprises three key steps: endocytosis, intracellular vesicular trafficking (during which lysosomal degradation is bypassed, a unique feature of BBB endothelium), and exocytosis. This vesicle-mediated process includes receptor-mediated and adsorptive-mediated transcytosis (Miller, 2015).

- *Receptor-mediated transcytosis:* In this process, specific receptors on the luminal surface of ECs bind to ligands, initiating internalisation and subsequent transport across the cell to the abluminal side (Pardridge, 2005). This mechanism is responsible for the entry of peptides and large molecules, like insulin, transferrin, and low-density lipoproteins, into the brain (Ding et al., 2020).
- *Adsorptive-mediated transcytosis:* This pathway facilitates the movement of macromolecules and charged nanoparticles across the BBB via electrostatic interactions between the ligands and the EC glycocalyx. This method supports the binding and uptake of cationic molecules, lipids, albumin, and nanoparticles on the luminal surface of ECs (Ding et al., 2020; Pardridge, 2005).

1.1.3.3.3.5 Cell-mediated Transport: In this pathway, molecules such as drugs cross the BBB encapsulated in liposomes within peripheral blood leukocytes as they migrate into the CNS, both under physiological and pathological conditions (Ransohoff and Engelhardt, 2012).

1.1.3.3.3.6 Transendothelial Leukocyte Migration: The CNS is considered an immune-privileged organ due to minimal leukocyte infiltration under healthy conditions, as a result of the modulatory role played by the BBB. Immune surveillance occurs continuously at the blood–cerebrospinal fluid (CSF) interfaces, including the meninges and choroid plexus (Greenwood et al., 1995).

During immunopathological conditions, regional leukocyte infiltration into the CNS occurs in response to danger signals expressed by NVU components. The transendothelial migration (TEM) of immune cells

across the BBB is a dynamic process initiated by activation of ECs, leading to the release of pro-inflammatory molecules (Carman and Martinelli, 2015) and upregulation of cell adhesion molecules on their luminal surfaces. This process is followed by leukocyte tethering, rolling, crawling, arrest, and diapedesis across ECs (Winger et al., 2014). Although its precise mechanism remains unclear, it is known to involve TJ remodelling and reorganisation of the endothelial cytoskeleton (Carman and Martinelli, 2015).

1.1.4 BBB Dysfunction

As a dynamic system composed of multiple interacting components, even a minor change in one of the BBB elements can lead to altered function and loss of neural homeostasis (Profaci et al., 2020). BBB dysfunction may result from structural damage, such as direct destruction of ECs, or from pathological processes including inflammation, hypoxia, excitotoxicity, lipid peroxidation, and calcium-mediated injury (Sun et al., 2019). Compromise of BBB integrity triggers ion dysregulation, oedema, neuroinflammation, and neurotoxicity, which elevate intracranial pressure and cause cellular dysfunction and degeneration, particularly in neurons (Shen et al., 2019).

BBB disruption is associated with CNS diseases such as AD, MS, stroke, epilepsy, encephalitis, traumatic brain injury (TBI), and amyotrophic lateral sclerosis (ALS) (Profaci et al., 2020). In some of these conditions, BBB impairment has been identified as an early and significant factor in disease pathogenesis, while in others its role remains controversial and subject to ongoing investigation.

Additionally, BBB compromise can result from physiological processes like ageing and menopause, making the CNS more vulnerable to external insults (Delaney and Campbell, 2017).

Factors like age, sex, temperature, and stress can promote BBB disruption. Initial damage is often mediated by cytokines and inflammatory molecules, including tumour necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin-1 (IL-1), interleukin-6 (IL-6), reactive oxygen and nitrogen species (ROS and RNS), MMPs, bradykinin, substance P, thrombin, and endothelin-1 (Almutairi et al., 2016). These agents target different cellular and molecular components of the BBB, leading to structural and functional compromise (Zhao et al., 2022).

Following BBB disruption, paracellular and transcellular permeability increase, driven by dysregulation of TJs, the EC cytoskeleton, transporters, transcytosis mechanisms, and gene expression. Oxidative damage occurs to proteins, lipids, and DNA (Pun et al., 2009). At the same time, immune cells infiltrate the CNS due to the upregulation of adhesion molecules and pro-inflammatory mediators, after a cascade of aberrant cell signalling (Kvichansky et al., 2019). All this causes fluid extravasation, neuroinflammation, and neuronal dysfunction, further exacerbating the damage caused by the initial insult (Takeshita and Ransohoff, 2012).

The mechanisms behind BBB failure and its involvement in the onset and progression of diseases remain poorly understood but are under investigation.

1.1.4.1 BBB Biomarkers

Quantification and analysis of BBB-associated molecules, along with advanced imaging techniques, are nowadays used as biomarkers to assess BBB integrity and functioning (Hussain et al., 2021). The discovery and understanding of these biomarkers have progressed with advances in molecular biology, immunoassays, and neuroimaging, establishing them as reliable indicators of BBB status (Hussain et al., 2021). These biomarkers hold potential for understanding, diagnosing, and monitoring CNS diseases where BBB disruption is a hallmark, such as AD, MS, and epilepsy. Outlined below are some of the main serum biomarkers of BBB dysfunction:

1.1.4.1.1 Brain Microvascular Endothelial Cells (BMECs): Any injury to the BBB can cause ECs to exfoliate and circulate in the bloodstream. As these cells are key components of the BBB, the quantification of circulating BMECs in the blood may serve as an indicator of the extent of BBB degradation (Huang et al., 2013).

1.1.4.1.2 Cellular Fibronectin (c-Fn): ECs produce and release c-Fn, which is essential for maintaining BM stability. When there is a BM rupture, c-Fn is released into the bloodstream, promoting leukocyte migration to the damaged area (Miao and Liao, 2014). Since c-Fn is primarily located within ECs and along the luminal side of the BM, elevated plasma c-Fn levels serve as a marker of endothelial damage at the BBB (Li et al., 2018).

1.1.4.1.3 Soluble Cell Adhesion Molecules (CAMs): Soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), endothelial-leukocyte adhesion molecule-1 (E-selectin), and P-selectin are involved in leukocyte adhesion and migration through the BBB. These molecules are upregulated in the event of BBB compromise, indicating enhanced immune cell trafficking across the barrier and neuroinflammation (D'Ambrosio et al., 2015).

1.1.4.1.4 S100B: This calcium-binding protein participates in cell differentiation, migration, and cognitive processes such as learning and memory (Delaney and Campbell, 2017). It is primarily produced by astrocytic end-feet, and its levels rise significantly following a BBB injury, as it is released into the bloodstream and CSF (Kadry et al., 2020).

1.1.4.1.5 Glial Fibrillary Acidic Protein (GFAP): GFAP is a cytoskeletal structural component, primarily expressed by astrocytes. It serves as a marker of astrocytic activation or injury. Elevated levels of GFAP in serum or CSF indicate astrocyte damage and are associated with BBB disruption. GFAP quantification is particularly useful for assessing the severity of BBB compromise and the extent of glial response (Kadry et al., 2020).

1.1.4.1.6 Vascular Endothelial Growth Factor (VEGF): This is a key signalling protein involved in angiogenesis promotion, vascular permeability, and barrier integrity. When produced by astrocytes, VEGF supports BBB maintenance and angiogenesis, while when secreted by ECs, it contributes to vascular permeability and repair (Profaci et al., 2020). VEGF levels increase in response to hypoxic, inflammatory,

or traumatic stimuli, and its presence can disrupt BBB integrity. Since its levels change rapidly in response to injury or disease progression, it provides a real-time reflection of BBB status (Zhao et al., 2022).

1.1.4.1.7 Albumin Quotient (CSF/Serum Albumin Ratio): Human serum albumin accounts for approximately 50% of all blood plasma proteins. While its primary role is to regulate blood oncotic pressure, it also binds to and transports various ligands. Under normal physiological conditions, albumin levels in the CSF are low. However, in cases of BBB dysfunction, increased permeability allows albumin to pass into the CNS. The CSF-to-serum albumin ratio is a well-established biomarker in clinical and research settings to assess BBB integrity, with elevated levels indicating increased BBB permeability (Wallin et al., 2018).

1.1.4.1.8 Matrix Metalloproteinases (MMPs): These endopeptidases play an important role in the physiological processes of tissue development and regeneration, including cell migration, angiogenesis, tissue formation, neuronal network formation, and BBB remodelling. They are also implicated in pathophysiological processes, like healing and inflammation. MMPs facilitate the degradation of TJ-related molecules and BM substrates. Under normal physiological conditions, MMP activity is tightly regulated; however, in pathological states, the overexpression of MMPs, specifically MMP-2 and MMP-9, can lead to barrier disruption (Kumar and Sandhir, 2022). As a result, there is increased permeability that allows harmful molecules and immune cells to infiltrate the CNS, contributing to neuroinflammation and further tissue damage. MMPs have been recognised as both biomarkers and therapeutic targets in conditions where BBB dysfunction is a central feature (Hussain et al., 2021).

1.1.4.1.9 Dynamic Contrast-Enhanced Magnetic Resonance Imaging (DCE-MRI): While serum biomarkers can reliably indicate and quantify BBB leakage, they do not provide information on the specific location of the disruption, or whether the permeability is regional or global. Recently developed DCE-MRI, an MRI technique employing gadolinium-based contrast agents, allows for the detection, localisation, and quantification of BBB leakage (Raja et al., 2018).

Gadolinium-based contrast agents enhance the visibility of blood vessels and permeable tissues during imaging. Under normal conditions, these agents do not cross an intact BBB due to their hydrophilic and polar properties, as well as their reliance on albumin transport. However, they may extravasate from the bloodstream into brain tissue when the BBB is compromised, even partially (Löscher and Friedman, 2020). DCE-MRI evaluates BBB permeability by measuring the rate and extent of contrast agent penetration into brain tissues, providing valuable information about its distribution and concentration. This non-invasive imaging technique, increasingly utilised in research and clinical settings, offers real-time data on vascular dynamics and BBB changes over time, enabling the monitoring of disease progression and therapeutic response (Raja et al., 2018).

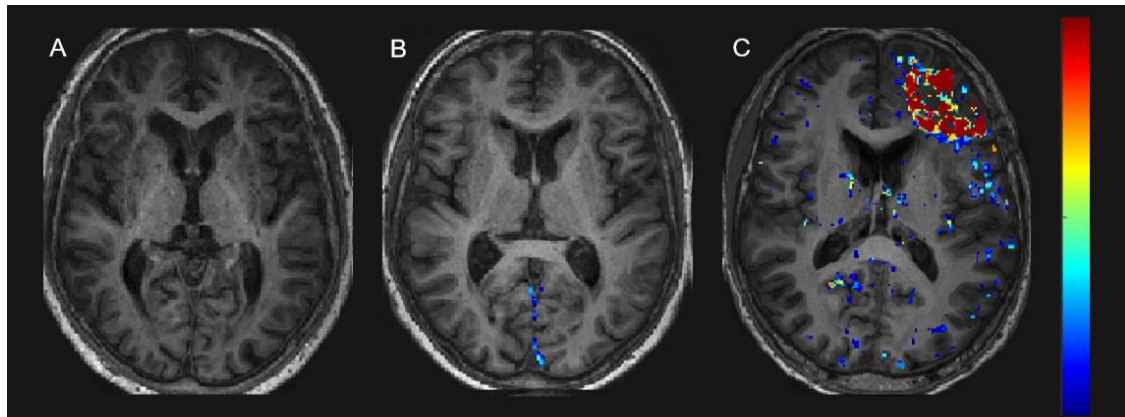


Figure 1.2. DCE-MRI images illustrating different patterns of BBB leakage. (A) MRI of a young individual with no evidence of BBB dysfunction. (B) Mild leakage was observed in the medial-posterior cortex of a healthy ageing individual. (C) Significant disruption of the BBB in the left frontal region, seen in a patient with a high-grade glioma. The colour scale indicates the magnitude of BBB permeability, with cooler colours (blue/purple) representing lower permeability values and warmer colours (green/yellow/red) representing higher permeability values.

1.1.5 BBB Under Physiological Conditions

1.1.5.1 BBB and Sex

Biological sex strongly influences the permeability, integrity, and response of the BBB to stress and injury. Sex hormones, particularly oestrogen and testosterone, are key regulators of BBB structure and function. Oestrogen enhances BBB integrity, while testosterone is necessary for its normal functioning. These hormones modulate the activity of TJ and BBB transport proteins, and influence the inflammatory response profile (Erickson et al., 2018), as well as facilitating cell communication. Such differences might contribute to potential sex-specific susceptibilities to various neurological and psychiatric conditions, including the development and progression of neurodegenerative diseases (Moon et al., 2021).

In general, most research has shown that females tend to exhibit better BBB integrity compared to males. Oestrogen exerts a protective role in maintaining CNS vasculature and BBB integrity. Its effects are mediated through membrane-bound oestrogen receptors α and β (ER α and ER β), expressed in brain endothelium. Oestrogen enhances nitric oxide (NO) production, which dilates cerebral vasculature, improving blood flow in response to metabolic demands (Weber and Clyne, 2021). At the BBB level, oestrogen promotes EC preservation, upregulates TJ protein expression (Maggioli et al., 2016; Pellegrini et al., 2023), and reduces TJ disruption (Shin et al., 2016). An animal study found that female mice have higher mRNA expression of claudin-1, claudin-5, claudin-12, occludin, JAM-A, and ZO-1 compared to males (Kamm, 2019; Weber and Clyne, 2021). Oestrogen is also a potent astrocyte regulator. At baseline, it affects the growth of astrocyte processes (Garcia-Segura et al., 1989) and modulates intracellular calcium levels (Chaban et al., 2004), influencing cell-to-cell communication within the BBB. Oestrogen's action on astrocytes is linked to several neuroprotective pathways, including reduced neuroinflammation and maintenance of neuronal homeostasis (Moon et al., 2021).

Oestrogen mitigates BBB inflammation by upregulating annexin A1 (ANXA1), an anti-inflammatory mediator, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). This promotes TJ protein expression and reduces lymphocyte migration across the barrier (Maggioli et al., 2016). Furthermore, oestrogen attenuates the expression of MMPs, helping to preserve TJ and BM integrity and maintaining BBB stability (Na et al., 2015).

During menopause or in conditions like ovariectomy, oestrogen-dependent NO production declines, potentially contributing to the deterioration of female cerebrovascular health and a marked increase in BBB permeability (Wilson et al., 2008). In animal models, ovariectomised female mice display drastically higher levels of brain pro-inflammatory cytokines, suggesting that low oestrogen levels may drive BBB breakdown and neuroinflammation (Dion-Albert et al., 2022). Although associated with increased cardiovascular morbidity and risk for stroke in some studies (Wilson et al., 1985), oestrogen replacement therapy in postmenopausal females has been shown to restore BBB integrity (Dion-Albert et al., 2022).

Oestrogen levels fluctuate significantly with age and reproductive cycles, adding complexity to research into its role in BBB regulation. The mechanisms by which fluctuations in oestrogen levels affect BBB permeability over time, age, and dosage remain to be fully elucidated (Dion-Albert et al., 2022).

In contrast to oestrogen, fewer studies have explored the impact of androgens on neurovascular function. Research on testosterone's role in vascular health has shown that it promotes endothelial and smooth muscle cell proliferation and migration (Wilson et al., 1985). Growing evidence suggests that lower testosterone levels in men exacerbate cardiovascular disease and increase stroke risk (Campelo et al., 2012).

Under physiological conditions, testosterone plays a protective role in BBB integrity. In animal models, chronic testosterone depletion leads to increased BBB permeability, reduced expression of claudin-5 and ZO-1, and TJ disorganisation. This permeability is associated with neuroinflammation, evidenced by the upregulation of inflammatory markers (Muller et al., 2004). Under pathological conditions, such as stroke, testosterone has been shown to impair BBB integrity by downregulating claudin-5 and upregulating pro-inflammatory cytokines (Atallah et al., 2017). However, testosterone supplementation in castrated male animals restored BBB integrity and reduced inflammation, further highlighting its protective role (Abi-Ghanem et al., 2020).

Excessive testosterone levels may negatively affect BBB integrity. For example, a study examining the effects of endurance training combined with testosterone supplementation in rats revealed that testosterone treatment reduced the expression of claudin-5, occludin, and VE-cadherin compared to non-supplemented animals, suggesting potential adverse impacts on barrier function (Nierwińska et al., 2019).

1.1.5.2 BBB and Temperature

In humans, normal body temperature oscillates between 36 and 37.5 °C (Rzechorzek et al., 2022), while brain temperature tends to be slightly higher, around 38.5 °C, due to the brain's high metabolic rate (Wang

et al., 2016). Recent studies suggest that brain temperature varies by region and age, following a circadian rhythm that reaches its lowest point at night (Rzechorzek et al., 2022).

Hypothermia is defined as a core body temperature below 35 °C (Cheshire Jr., 2016), and is commonly caused by exposure to cold weather, water immersion, drug-induced disruptions in thermoregulation, metabolic and endocrine disorders like hypothyroidism and adrenal insufficiency, and trauma, due to blood loss and shock (McCullough and Arora, 2004). Hyperthermia, on the other hand, occurs when body temperature exceeds 40.5 °C. It is often triggered by infections, environmental heat exposure, physical activity, adverse drug reactions to sympathomimetics, anticholinergics, and serotonergic agents, and medical conditions such as malignant hyperthermia and heat stroke (Cheshire Jr., 2016).

Temperature plays a critical role in regulating BBB permeability, altering water homeostasis and the structure and viability of CNS cells, which are sensitive to thermal damage. These effects are highly dependent on the duration of temperature exposure (Laptook, 2014).

The impact of hypothermia on neuronal tissue and the BBB has been predominantly studied in the setting of brain injuries like TBI, ischaemic encephalopathy, and stroke (Yenari and Hemmen, 2010). Therapeutic hypothermia therapy (THT), achieved through systemic cooling or selective head cooling, has been shown to protect BBB integrity. Studies suggest that reducing brain temperature to between 32 and 34 °C for 12–24 h can provide neuroprotection by enhancing BBB function, lowering cerebral metabolic rates, and reducing inflammation and apoptosis (Bhatt et al., 2020; Kuczynski et al., 2020).

THT started within one hour of a hypoxic–ischaemic event and concomitant with reperfusion treatment has been shown to reduce the expression of MMP-2, MMP-9, and VEGF, while promoting the activity of tissue inhibitors of metalloproteinases (TIMPs). This supports the preservation of TJs, AJs, cell–BM integrity, and overall BBB structure (Laptook, 2014; Li et al., 2017). Additionally, pericyte morphology and their umbrella-like coverage over EC gaps are maintained, which promotes the maintenance of vascular perfusion to ischaemic tissue (Laptook, 2014).

Extreme hypothermia can cause BBB disruption and selective glial activation due to brain dehydration (Kiyatkin and Sharma, 2009).

Hyperthermia generally has detrimental effects on the CNS, increasing cerebral metabolic rate, oxidative stress, and inflammation (Shepley et al., 1985). Studies in animals, where subjects are exposed to prolonged whole-body hyperthermia starting at 38 °C, showed BBB disruption, with increased paracellular transport in multiple brain regions, as well as microhaemorrhages in the youngest subjects, but not in adult ones (Zhao et al., 2023). Temperatures above 38.5 °C result in increased albumin and GFAP immunoreactivity, indicating astrocytic activation and BBB breakdown. Peak damage occurs at temperatures of 41–42 °C, marked by a substantial increase in brain water and electrolytes, as well as cellular abnormalities indicative of acute oedema and irreversible damage (Kiyatkin and Sharma, 2009). In vitro studies have revealed that cell death occurs at temperatures above 42 °C, though viability varies depending on cell type and duration of heat exposure (Zhao et al., 2023).

Therapeutic hyperthermia involves controlled increases in temperature and is utilised for the treatment of various diseases. Systemic therapy typically raises body temperature by 1–1.5 °C, which increases blood flow and might benefit prevention and treatment of cardiovascular diseases, particularly heart failure (Ye et al., 2020). In neuro-oncology, local hyperthermia therapy, including hyperthermic chemotherapy and radiofrequency ablation, is employed to treat brain tumours by inducing cell death and increasing BBB permeability in the targeted regions (Skandalakis et al., 2020).

1.1.5.3 BBB and Stress

Stress is characterised by the disruption of homeodynamic balance in response to physical, psychological, intrinsic, or extrinsic stimuli perceived as challenging. The stress response system helps to restore balance by adjusting energy distribution to meet the organism's needs. While short-term stress promotes adaptive responses, prolonged or excessive stress can overwhelm the system's ability to regulate itself, leading to maladaptive effects and a persistently imbalanced state (Agorastos and Chrousos, 2022).

Psychosocial stress affects all bodily systems, and the BBB is not immune to its effects. The hypothalamic–pituitary–adrenal axis and inflammatory signalling pathways mediate stress-induced BBB disruption. Elevated cortisol levels and pro-inflammatory cytokines prompt a series of NVU alterations that affect BBB dynamics, increasing its permeability (Welcome and Mastorakis, 2020). Notably, stress downregulates claudin-5 expression in ECs, directly compromising TJ integrity. This effect shows a sex-dependent topographic pattern in animal models and post-mortem studies (Dion-Albert et al., 2022). In females, stress-induced loss of claudin-5 occurs predominantly in the prefrontal cortex, responsible for executive function and decision-making, whereas in males it primarily affects the nucleus accumbens, fundamental in modulating stress responses and mood disorders (Dion-Albert et al., 2023).

Stress also induces pericyte proliferation, which exhibits a dysfunctional reduction in the expression of key markers, like α 1-integrin, essential for maintaining BBB stability. This leads to hindered pericyte–endothelium interaction, further perpetuating neurovascular dysfunction (Bhowmick et al., 2019). Astrocytic regulation of neuronal excitability and synaptic transmission is also affected by stress, which induces a reduction of glutamate uptake, leading to excitotoxicity and neuronal damage (David et al., 2009). In addition, downregulation of potassium (K^+) channel Kir4.1 increases extracellular K^+ , promoting neuronal depolarisation and hyperexcitability (Nwaobi et al., 2016). Stress also induces astrocytic overexpression of VEGF and cytokines, exacerbating barrier disruption and inflammatory signalling (Dion-Albert et al., 2023). Microglia activate and adopt a pro-inflammatory phenotype when facing stress, increasing the expression of inflammatory cytokines and oxidative stress markers. Additionally, microglia can phagocytose astrocytic end-feet, further compromising BBB function (Ronaldson and Davis, 2020).

Chronic social stress and sub-chronic variable stress induce sex-specific changes in the BBB, with females showcasing more pronounced BBB leakiness and associated depressive behaviours (Dion-Albert et al., 2022). Chronic stress and sustained BBB disruption can lead to cortical atrophy, particularly in the limbic system (Sathyanesan et al., 2017), impaired neurogenesis, and cognitive deficits (Lee et al., 2018).

However, studies in animal models have shown that treatment with the antidepressant imipramine for 35 days restored BBB integrity despite chronic stress exposure (Dion-Albert et al., 2022).

1.1.5.4 BBB and Ageing

Ageing is a time-related physiological process, consisting of the deterioration of cellular functions necessary for survival and reproduction, ultimately resulting in organ failure and death. A poorly preserved cellular identity, the accumulation of errors in DNA replication and repair, and reduced metabolic clearance create a stressed and vulnerable cellular environment (Vijg, 2000). In the CNS, ageing causes significant changes in the vasculature. Capillary density decreases (Reeson et al., 2018), along with neovascularisation capacity (Rivard et al., 2000) and overall cerebral blood flow (Tarumi and Zhang, 2018). In addition to the direct effects of ageing on NVU cellular components, the regulatory function of the BBB declines, increasing its permeability and disrupting CNS homeostasis (Hyman et al., 2012).

Even in healthy ageing, the BBB can exhibit degenerative features. CNS endothelium becomes progressively dysfunctional, losing its secretory capacity and ability to supply growth and nutritional factors to the brain (Marques et al., 2013). There is a decline in the expression of occludin, claudin-5, and ZO-1, leading to TJ breakdown (Elahy et al., 2015). Simultaneously, TJ- and basal lamina-degrading enzymes become overactive (Costea et al., 2019). Impaired paracellular and transcellular transport regulation allows the entrance of molecules such as albumin, fibrinogen, IgG, and autoantibodies into the brain (Montagne et al., 2017). Additionally, defective efflux transporters fail to clear these substances, leading to the build-up of toxic molecules (Xu et al., 2022). The BM thickens, and the expression of its components changes, with increased production of collagen and MMP-9 (Candiello et al., 2010). The pericyte population is reduced as a consequence of suppression of cell viability and upregulation of apoptosis (Schultz et al., 2018). Pericytes also undergo structural changes, including enlarged mitochondria, vesicular inclusions, and foamy transformations (Hussain et al., 2021). These senile pericytes generate ROS and RNS, exacerbating oxidative stress and amplifying BBB dysregulation (Sweeney et al., 2016). Astrocytic end-feet also display age-related changes. While their size increases, their vascular coverage diminishes, alongside reduced expression of AQP4. This is accompanied by elevated GFAP expression, indicating increased reactive astrogliosis (Middeldorp and Hol, 2011).

Ageing is further marked by a chronic, low-grade inflammatory state, which contributes to BBB dysfunction. Elevated levels of pro-inflammatory cytokines such as IFN- γ , IL-1 β , IL-6, and TNF- α , along with activated microglial and peripheral immune cells, have been identified in uninjured aged brains, resembling the inflammatory state after a TBI (Kumagai et al., 2007). In this aged CNS, the response to an insult is more severe, due to amplification of a pro-inflammatory response and attenuation of its anti-inflammatory counterpart (Delaney and Campbell, 2017).

The hippocampus has been identified as one of the first brain regions to experience BBB breakdown in ageing and cognitively normal populations, particularly in the CA1 and dentate gyrus areas (Montagne et al., 2015). This is thought to be related to baseline hippocampal BBB vulnerability due to a dual blood supply from anterior and posterior cerebral circulation and greater neuronal sensitivity to ageing and damage (Ivanidze et al., 2019). However, the extent of BBB leakage is small and variable among individuals

(Knopp et al., 2023). Further studies have reported variable BBB leakage, with some findings showing increased permeability in the orbitofrontal and cingulate cortex (Verheggen et al., 2020), and occipital white matter and thalamus (Ha et al., 2021). It has also been suggested that BBB disruption increases during normal ageing secondarily to white matter integrity loss and a decrease in cortical thickness (Wang et al., 2003).

In healthy individuals, these age-related BBB changes are generally mild and occur as part of a physiological process. While they do not cause pathology on their own, they may predispose the CNS to chronic inflammation and long-term damage, increasing the risk of developing age-associated neuropsychiatric and degenerative disorders (Delaney and Campbell, 2017).

1.1.6 BBB Under Pathological Conditions

AD, MS, and epilepsy are among the neurological conditions with the highest incidence and prevalence worldwide. While the pathophysiology of these disorders continues to be elucidated, it is clear that BBB dysfunction plays a significant role, either by contributing to disease onset or as a consequence of its development.

1.1.6.1 BBB and Alzheimer Disease

Alzheimer disease (AD) is an irreversible neurodegenerative condition and the leading cause of dementia worldwide, accounting for approximately 60–70% of all cases. AD commonly manifests after age 60 and progresses over 8–12 years. Clinically, it is characterised by amnesic cognitive decline and progressively impaired daily functioning. The disease is defined by the accumulation of β -amyloid ($A\beta$) plaques and neurofibrillary tangles in the brain, which result in neuronal death, synaptic loss, and brain atrophy, corresponding to its clinical manifestations (Kamatham et al., 2024). AD development involves a complex interaction between genetic and environmental factors (Bagheri et al., 2024).

At the molecular level, two key pathological features of AD have been identified: the accumulation of β -amyloid plaques and the formation of tau neurofibrillary tangles. $A\beta$ plaques are extracellular deposits primarily composed of $A\beta$, a peptide derived from the amyloid precursor protein (APP). Recent studies suggest that, in low concentrations, $A\beta$ modulates synaptic plasticity and memory (Puzzo and Arancio, 2013). Among $A\beta_{1-40}$ and $A\beta_{1-42}$, the most common peptide forms (Sehar et al., 2022), $A\beta_{1-42}$ is especially important in AD pathogenesis, as it is the species that aggregates extracellularly, triggering a cascade of toxic events leading to synaptic dysfunction, neuronal death, and neurodegeneration (Sehar et al., 2022). The precise mechanisms leading to pathological $A\beta$ accumulation and AD pathology remain an area of active investigation.

While $A\beta$ drives extracellular pathology, intracellular changes are dominated by tubulin-associated unit (tau) protein dysfunction. Tau is a cytoskeletal protein with a primary role in maintaining the stability of microtubules in neuronal axons. Tau is abundant in the CNS, predominantly in the cerebral cortex (Shin et al., 1991). In AD, tau becomes hyperphosphorylated, losing its stabilising ability and aggregating in intracellular neurofibrillary tangles, impairing synaptic function and promoting neuronal death (Alonso and Cohen, 2018).

Beyond these protein abnormalities, AD is also characterised by early disruptions in the BBB, which often precede clinical symptoms and neurodegeneration (Nation et al., 2019). Several mechanisms contribute to BBB breakdown in AD, including oxidative stress, inflammation, and alterations in key transporters and receptors, all of which favour the accumulation of A β and tau proteins. BBB dysfunction not only amplifies but also perpetuates this pathological cycle (Bell et al., 2012). One of the most significant genetic risk factors for both BBB breakdown and A β accumulation is the presence of the apolipoprotein E- ϵ 4 (ApoE4) allele (Perry et al., 2002).

The role of ApoE4 is closely tied to its interactions with other proteins, such as the low-density lipoprotein receptor-related protein-1 (LRP-1). ApoE is, physiologically, a lipid transport protein. The ApoE gene consists of three alleles, ϵ 2, ϵ 3, and ϵ 4, translated into respective isoforms. ApoE3 is the most abundant isoform in humans (77.9%), while ApoE4 and ApoE2 distributions are 13.7 and 8.4%, respectively (Farrer et al., 1997). Astrocytes produce ApoE in the CNS, while the liver does so for the rest of the body. ApoE plays an essential role in maintaining BBB integrity by interacting with LRP-1 on pericytes to modulate MMP activity (Bell et al., 2012; Deane et al., 2004). In individuals carrying the ApoE4 allele, accelerated degeneration of pericytes, along with overexpression of cyclophilin A and MMP-9, leads to BBB disruption. This has been demonstrated in animal models expressing human APOE4, where cerebral vasculature shows a thinner BM, reduced TJ expression, and a compromised BBB (Bell et al., 2012). Additionally, ApoE4 binds to A β , further hindering its clearance, and, together with LRP-1 downregulation, promotes A β accumulation (Deane et al., 2008).

LRP-1 is a primary receptor for A β clearance. It mediates uptake and internalisation of A β for degradation within neurons, astrocytes, microglia, and vascular smooth muscle cells, and also acts as an A β efflux transporter on the EC surface. Reduced LRP-1 levels have been observed in aged brains in humans and animal models. This downregulation is thought to be driven by oxidative stress and inflammation, contributing to A β retention in the brain (Deane et al., 2004; Storck et al., 2016).

In addition to LRP-1, other transport systems such as P-glycoprotein (P-gp) play a significant role in regulating A β levels at the BBB. Highly expressed on the luminal aspect of brain ECs, P-gp, also known as ABCB1, is an efflux pump with a multitude of substrates, including A β . Its role extends beyond the BBB to neurons, where it mediates the export of A β peptides (van Assema et al., 2012). P-gp deficiency in mouse models reduces A β clearance by half. Conversely, A β ₁₋₄₀ triggers P-gp internalisation, establishing a detrimental feedback loop that exacerbates pathology (Hartz et al., 2016). Studies have found that P-gp expression and activity are inversely correlated with ageing, A β deposition, and AD progression (van Assema et al., 2012).

The receptor for advanced glycation end products (RAGE), another key player in A β transport, is a transmembrane protein present in ECs, among other tissues like microglia and neurons, mediating the influx of A β from the bloodstream into the brain, working in opposition to LRP-1. RAGE expression is upregulated at the BBB of patients with AD as a consequence of its interaction with advanced glycation end products (AGEs) and A β . This leads to further A β accumulation, tau hyperphosphorylation,

neurofibrillary tangle formation, increased oxidative stress, and inflammation in ECs, exacerbating BBB breakdown and perpetuating neuronal degeneration (Deane et al., 2003).

As A β aggregates and plaque formation progresses, reactive inflammation follows, inducing perivascular aggregation of proteins, including fibrinogen, albumin, thrombin, and immunoglobulin G (IgG), while compromising tight junctions and degenerating ECs and pericytes (Kinnecom et al., 2007; Nelson et al., 2016). Glucose transporter protein (GLUT-1), enriched in CNS endothelium, is known to be repressed in brain microvessels of patients with AD, reducing glucose availability in the brain. Whether this reduction represents lower energy demands or primary BBB dysfunction remains unclear. Astrocytes in the AD brain also undergo morphological changes, with end-feet depolarisation, reduced expression, and altered distribution of AQP4, which is also responsible for facilitating A β clearance, further contributing to A β build-up and neuroinflammation (Hoshi et al., 2012; Rasmussen et al., 2018).

Microglia play a complex role in AD, as they are implicated in both the progression and potential mitigation of the disease. They activate in response to A β plaques and neurofibrillary tangles, leading to the release of pro-inflammatory cytokines, leukocyte migration across the BBB, and further neuroinflammation. This chronic inflammation exacerbates neuronal damage and contributes to disease progression. On the other hand, microglia phagocytose A β and tau, facilitating the clearance of these pathological molecules; however, in the later stages of AD, this mechanism fails (Merighi et al., 2022).

Emerging evidence suggests that BBB dysfunction in AD may begin in the hippocampus, further implicating this region in the early stages of the disease (Miners et al., 2019; Montagne et al., 2015). Some studies have proposed BBB dysfunction as an early biomarker of AD, given its presence before the onset of clinical symptoms and neurodegeneration (Montagne et al., 2015; Nation et al., 2019; van de Haar et al., 2016).

1.1.6.2 BBB and Multiple Sclerosis

Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory, and neurodegenerative disease of the CNS, characterised by demyelination and axonal damage. It affects the brain, spinal cord, and optic nerves while sparing structures of the peripheral nervous system. MS primarily affects young adults, with a mean age of onset between 20 and 30 years, and it is more prevalent in women. This disease is driven by both inflammatory and neurodegenerative processes, resulting in intermittent neurological symptoms, followed by the gradual accumulation of disability. Common clinical features of MS include motor and sensory deficits, facial paralysis, nystagmus, speech and vision impairment, ataxia, tremor, fatigue, and cognitive decline (McGinley et al., 2021).

MS is clinically classified into three main forms: relapsing–remitting (RR), primary progressive (PP), and secondary progressive (SP). RR-MS, the most common form, is characterised by “attacks” of new or worsening neurologic symptoms, known as relapses, followed by periods of partial or complete recovery, termed remissions. During remissions, symptoms might either resolve or become permanent. Over time, RR-MS often transitions into the SP stage, where functional decline becomes progressive (McGinley et al., 2021).

Despite extensive research, the exact cause of MS remains unclear; however, advances in drug therapies have highlighted the critical role of immune cell migration across the BBB in the pathophysiology of the disease. As BBB dysfunction is central to both the onset and progression of MS, it has been a focus of many studies. The NVU plays a key role in regulating immune cell infiltration into the brain, promoting the development of a CNS-specific immune response (Gaitán et al., 2011; Guttman et al., 2016).

During an MS attack, activated T cells, B cells, leukocytes, and peripheral macrophages infiltrate the brain through a disrupted BBB, targeting the myelin sheath surrounding axons (Sweeney et al., 2019). Cytotoxic factors such as pro-inflammatory cytokines, proteases, ROS, and RNS accumulate and contribute to myelin destruction. This breakdown of the BBB, while transient, often recurs in different locations over time, leading to irregular lesion development involving further BBB leakage, immunologically mediated demyelination, and axonal damage (Ortiz et al., 2014).

Activated immune cells enter the CNS perivascular space through postcapillary venules (Greter et al., 2005), where they express cell adhesion molecules (Carrithers et al., 2000) that activate the endothelium. Leukocytes then travel across endothelial surfaces in search of intercellular gaps, where they alter the expression and organisation of TJs, allowing firm adhesion and transendothelial migration (Hernández-Pedro et al., 2013), and later access the parenchyma by breaking down the BM through MMP action (Wu and Tsirka, 2009). This process activates astrocytes and damages both cellular and non-cellular components of the BBB (Schreiner et al., 2022).

Once inside the CNS, T cells recognising CNS-specific autoantigens are reactivated, leading to their clonal expansion and amplification of the immune response (Fletcher et al., 2010). These T cells, in collaboration with antigen-presenting cells (mainly perivascular macrophages), release cytokines, chemokines, and other inflammatory mediators that cause structural changes in the BBB. This promotes the secondary influx of immune cells, activates resident microglia, and perpetuates a cascade of demyelination and axonal damage (Ricci et al., 2009).

Although the exact triggers of inflammation in MS are not fully understood, it is widely accepted that environmental factors play a significant role in genetically predisposed individuals, activating a T-cell-mediated immune response against the CNS (Ricci et al., 2009).

Neuroimaging studies using contrast enhancement have demonstrated that BBB disruption is an early feature of MS (Alvarez et al., 2011b). While gadolinium enhancement is typically associated with active MS lesions, MRI scans have revealed BBB disruption in seemingly normal white matter even before lesion enhancement and in non-enhancing areas (Vos et al., 2005). These observations suggest that BBB dysfunction may precede visible lesion formation and could play a critical role in the early stages of disease development (Sweeney et al., 2019).

Increasing interest in the BBB as a therapeutic target for MS has led to the development of treatments aimed at either restoring BBB integrity or reducing local inflammatory responses and limiting CNS-specific immune cell infiltration (Ortiz et al., 2014).

1.1.6.3 BBB and Epilepsy

Epilepsy is a chronic neurological disease characterised by a predisposition to recurrent seizures (Fisher et al., 2014). Its aetiology is diverse and includes genetic, structural, metabolic, immune, infectious, and idiopathic causes (Thijs et al., 2019). Epilepsy can be focal, where seizures arise from networks limited to a specific cortical region, or generalised, involving both hemispheres from onset (Fisher et al., 2014).

Epilepsy affects individuals of all ages, sexes, and ethnicities, with a bimodal incidence peak in infancy and older age groups. Its impact on quality of life is substantial, stemming not only from the seizures but also from associated comorbidities, including cognitive deficits, mood disorders, and the side effects of treatment (Devinsky et al., 2018).

The relationship between epilepsy and the BBB is multifaceted. The concept of BBB dysfunction as a hallmark of a seizing brain is the product of extensive research on the correlation between epilepsy and BBB disruption (Löscher and Friedman, 2020). Whether BBB impairment is a cause, or a consequence of epileptic seizures remains to be completely elucidated but is believed to be a bidirectional relationship (Sweeney et al., 2019). Seizures can impair BBB integrity, while BBB dysfunction can facilitate epileptogenesis and exacerbate seizure activity. This is evident in conditions like neuroinfections, inflammation, stroke, and TBI, which compromise BBB integrity and can trigger seizures and lead to epilepsy (Oby and Janigro, 2006; van Vliet et al., 2007).

Acute epileptogenic insults, such as traumatic injury or hypoxic–ischaemic lesions, lead to the release of glutamate, pro-inflammatory mediators, and free radicals, which in turn induce endothelial activation, TJ disruption, increased MMP activity, and pericyte dysfunction, ultimately causing BBB breakdown. This activates resident glial cells and attracts leukocyte migration into the brain parenchyma, thereby expanding cerebrovascular inflammation and disturbing local homeostasis. Consequently, the spreading depolarisation threshold decreases, neurons become hyperexcitable, and seizures occur. Impaired glial function and neuronal excitability are further linked to synaptogenesis and neuroplasticity, processes that may contribute to epileptogenesis and cognitive deterioration (Löscher and Friedman, 2020).

Prolonged seizure activity, as in the case of status epilepticus (SE) in its most extreme expression, is followed by spreading cortical depression (van Vliet et al., 2015), to which brain vasculature responds with altered vascular tone. This causes either transient hyperperfusion in otherwise healthy tissue or severe hypoperfusion in areas at risk of progressive damage (Winkler et al., 2012), leading to cellular injury and BBB disruption.

While the exact mechanisms of epileptogenesis remain incompletely understood, it is well established that BBB dysfunction and brain inflammation lower the seizure threshold, creating a self-perpetuating cycle that drives epilepsy progression (van Vliet et al., 2015). However, BBB disruption alone is insufficient to induce seizure activity or cause epilepsy (van Vliet et al., 2015), as seen in other conditions like AD, MS, or stroke, where seizures can occur but are not a consistent feature. Epileptogenesis is believed to depend

not only on the nature and extent of BBB permeability increase but also on the affected brain networks, among other alterations (Löscher and Friedman, 2020).

In established epilepsy, several studies have documented peri-ictal and interictal BBB dysfunction, often correlating temporally and anatomically with the region of seizure onset (Rüber et al., 2018; Reiter et al., 2024). In temporal lobe epilepsy (TLE), the most common focal epileptic disorder in adults (Tatum, 2012), resected hippocampus reveals aberrant angiogenesis, increased microvascular density, loss of TJs, and increased permeability to IgG (Sweeney et al., 2019). In animal models of TLE, BBB leakage is observed not only in the hippocampus but also in the entorhinal and piriform cortices, thalamus, amygdala, septum, and substantia nigra, with consistent metabolic activation during seizures (van Vliet et al., 2015).

Other studies on epilepsy have shown extravasation of serum proteins like albumin, increased pinocytosis, TJ abnormalities, BM thickening, pericyte–microglia clustering, astrocytic activation accompanied by altered expression of K^+ channels, and AQP4 in epileptic brains (Löscher and Friedman, 2020), as well as efflux transporter overexpression, which may limit the penetration of antiepileptic drugs, contributing to drug resistance (Hartz et al., 2019).

A deeper understanding of these processes holds the potential to drive the development of novel therapeutic strategies that are disease-modifying rather than merely seizure-suppressive.

1.1.7 Conclusion

The BBB is a highly specialised and dynamic system, fundamental to CNS homeostasis. Its structural and functional complexity, resulting from the interplay of cellular and molecular components within the NVU, ensures precise regulation of nutrient transport, waste clearance, and neuronal protection. ECs, pericytes, astrocytes, and BMs form interconnected networks supported by TJs, transport systems, and signalling molecules. Together, these elements maintain the barrier's selective permeability, protecting the brain from external insults while preserving a stable microenvironment essential for neuronal function. This sophisticated orchestration highlights the BBB's evolutionary refinement to meet the unique metabolic demands of the brain, and its critical role as a gatekeeper for the CNS.

While this complexity enables the BBB to adapt to most physiological challenges, it is not immune to deterioration. Factors like ageing, hormonal imbalances, stress, and temperature extremes can weaken its integrity, increasing permeability and making the CNS more susceptible to damage. In ageing, for instance, a decline in the expression of TJ proteins, BM alterations, and chronic inflammation contributes to increased BBB permeability, making the CNS vulnerable to injury and disease.

BBB dysfunction is increasingly recognised as a key factor in the pathogenesis of numerous neurological disorders, emphasising its critical role in neural health. In AD, breakdown of the BBB contributes to the accumulation of β -amyloid plaques and neurofibrillary tangles, exacerbating neuronal degeneration. In MS, immune cell infiltration through a compromised BBB initiates demyelination and axonal damage, driving disease progression. Similarly, in epilepsy, BBB disruption promotes neuroinflammation and

excitotoxicity, lowering the seizure threshold and perpetuating epileptogenesis. These examples illustrate both the pathological consequences of barrier failure and its potential as a therapeutic target in some neurological disorders.

As our understanding of the BBB continues to grow, it remains a keystone of neuroscience research, representing both a challenge and an opportunity in the pursuit of strategies to protect and repair the CNS.

CHAPTER 1, PART 2: THE SPECTRUM OF COGNITIVE AGEING: FROM NORMAL BRAIN AGEING TO MILD COGNITIVE IMPAIRMENT

1.2.1 Ageing

Ageing is a biological process characterised by a progressive decline in several physiological functions resulting from the accumulation of molecular and cellular damage over time. This leads to a gradual decrease in physical and mental capacity, a growing risk of disease, and ultimately death. The changes that occur during the ageing process are not linear and loosely associated with a person's age in years. The diversity observed in older age is not random, but rather the result of the sum of multiple factors that impact the rate and magnitude of this process, including diet, lifestyle, genetics, inflammation, and co-existing illnesses, leading to such variability between individuals (Deary et al., 2022; World Health Organization (WHO), 2022). While some aspects of ageing are inevitable, a healthy lifestyle can promote well-being throughout life.

The World Health Organization (WHO) does not specify a single chronological threshold for the onset of ageing. However, many initiatives, including the Study on Global AGEing and Adult Health (SAGE), operationally define older adults as those aged 60 years and over, for global health policy and research purposes (Kowal et al., 2012).

According to the WHO, the global population is experiencing a significant demographic shift in age distribution with a tendency towards ageing. While in 2019 there were approximately 1 billion people aged 60 years and older, this number is projected to increase to 1.4 billion by 2030 and to 2.1 billion by 2050. Globally, life expectancy at birth reached 73.3 years in 2024, an increase of 8.4 years since 1995. Further reductions in mortality are projected to result in an average global longevity of 77.4 years by 2054 (United Nations, 2024).

Ageing is the greatest risk factor for developing neurodegenerative conditions, such as Alzheimer disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Although these conditions precipitate degenerative changes in the brain, their progression may be influenced by normal age-related changes (Juan et al., 2019).

1.2.1.1 The Ageing Brain

Multiple brain regions undergo structural and functional changes during the ageing process; however, these changes are not homogeneous among such areas (Juan et al., 2019). To date, promising developments in neuroscience have enabled the identification and understanding of such changes, although the studies available vary significantly in design, study population, and variables analysed. Changes identified so far include grey matter, white matter, and vascular tissue.

Grey matter volume loss found in older adults, which progresses after age 20, is not only a consequence of neuronal death but also of structural changes suffered by neurons. With ageing, neurons' morphology changes significantly: the length of dendritic spines decreases, along with the complexity of dendrite

arborisation and of synaptic density, which translates into decreased neuronal connections (Harada et al., 2013; Morrison and Baxter, 2012). Large volumetric changes have been documented in the putamen, nucleus accumbens, thalamus, corpus callosum, and frontal and temporal cortices (Fjell and Walhovd, 2010). These changes correlate with a reduction in processing speed, memory retrieval, and executive functions overall performance (Madden et al., 2009; O'Sullivan et al., 2001; Rogalski et al., 2012).

A great proportion of the grey matter atrophy occurs in the prefrontal cortex and is caused by the loss of dendritic thin spines, whereas the changes in the temporal lobes are moderate and involve mainly the hippocampus, which loses the large, perforated mushroom synapses that modulate learning and memory. The entorhinal cortex, on the other hand, while atrophic in AD, does not suffer major changes during normal ageing (Harada et al., 2013; Morrison and Baxter, 2012). It has also been proposed that the functionality of posterior cortical regions declines with age, particularly the ventral visual cortex, exhibiting less neural selectivity to inputs from the visual system (Park et al., 2004).

As one of the principal brain structures under study in this field, some suggest the hippocampus as the marker to differentiate “healthy” from “pathological” ageing, where the reduction in synaptic density and neuronal size in the dentate gyrus and subiculum is related to normal ageing, whereas degeneration of the hippocampus proper (Cornu Ammonis 1–4 (CA1–4)) and the entorhinal cortex are implicated in neurodegenerative diseases (Jagust, 2013).

Normal-age-related white matter changes are much more pronounced than grey matter changes. These changes are the result of a combination of structural, cellular, and molecular mechanisms, the most prominent contributors being progressive myelin loss, reduced capacity for myelin repair, axonal degeneration, and increased vulnerability to oxidative stress and mitochondrial dysfunction (Groh and Simons, 2025).

Changes in excitatory and inhibitory neurotransmitter activity participate in the destabilisation of synapses and, therefore, hinder the baseline cognitive performance. Several studies have reported a reduced activity of excitatory glutamatergic synapses in the hippocampus, caused by decreased expression of AMPA and NMDA receptors, presynaptic protein SNAP25, and postsynaptic protein PSD95, heavily involved in the learning and memory process (Adlard et al., 2010; Canas et al., 2009). The ageing hippocampus also exhibits a decreased inhibitory postsynaptic potential, mediated by GABA, which, by controlling the overall excitability of neural circuits, maintains the homeostatic regional balance (Rozycka and Liguz-Leczna, 2017).

Neuroplasticity in the ageing brain has the role of overcoming such alterations to promote homeostatic balance of excitatory and inhibitory activity within the synapse (Rozycka and Liguz-Leczna, 2017). However, several markers of synaptic plasticity, like calmodulin-dependent kinase II (CaMKII) and synaptophysin, show reduced levels in some animal studies (Adlard et al., 2014).

Ageing-related changes in the DNA structure and gene expression are the modulators of neuronal and neurovascular cells' morphological and functional changes. Ageing individuals undergo changes in histone

methylation, acetylation, and DNA demethylation, responsible for structural neuronal changes and synapse loss (Azpurua and Eaton, 2015). They also experience upregulation of genes involved in the stress response, inflammatory response, and metal ion homeostasis and downregulation of genes involved in neural plasticity, synaptic function, and inhibitory interneuron function (Bishop et al., 2010).

1.2.1.2 Cognitive Changes in Normal Ageing

Along with the structural brain alterations, ageing induces cognitive changes that reflect a gradual modification in neurobiological processes rather than frank pathology. These changes vary across individuals and domains affected, as they are the result of several factors, including synaptic integrity, neural network reorganisation, neuromodulatory tone, and compensatory mechanisms, all of which exhibit significant interindividual variability (McDonough, 2022).

Processing speed decline is considered to be universal to ageing, commencing in midlife and progressing linearly across the lifespan (Hedden and Gabrieli, 2004). This is closely related to alterations in white matter integrity, specifically in long-range association tracts, such as the superior longitudinal fasciculus and corpus callosum, and is the result of cumulative microvascular insults, gliosis, and myelin breakdown (Kennedy et al., 2009). Such delay in processing speed affects multiple other domains, including memory encoding, attentional control, and complex decision-making (Salthouse, 2011).

Executive functions, particularly cognitive flexibility, working memory, and inhibitory control, are among the earliest and most consistently impacted domains. These functions rely on prefrontal circuits, especially the dorsolateral prefrontal cortex, which undergoes structural atrophy and synaptic remodelling with age (Buckner, 2004). Functional imaging studies have shown reduced dorsolateral prefrontal cortex activation during executive tasks in older adults, often accompanied by compensatory recruitment of contralateral or posterior regions (Cabeza, 2002). Not all executive functions have a tendency towards impairment or decay at the same rate. Semantic fluency, abstraction, reasoning with familiar material, and concept formation remain stable and can even improve across the lifespan (Ballesteros et al., 2009; Reuter-Lorenz, 2002). In contrast, working memory and reasoning with novel information decline steadily across decades.

Episodic memory decline becomes more marked around the age of 70 (Celsis, 2000), with retrieval processes more vulnerable than encoding or retention (Hedden and Gabrieli, 2004). Recognition memory and semantic associations, on the other hand, are often preserved or decline only in late life (Hedden and Gabrieli, 2004). Functional MRI studies have revealed age-related reductions in hippocampal activation during encoding, along with increased reliance on prefrontal resources, considered to be compensatory redistribution (McDonough, 2022). Semantic memory, vocabulary, and general knowledge are often maintained or even enhanced, reflecting cumulative learning and potential compensatory reorganisation in cortical language regions (Meunier et al., 2009). Non-declarative memory, such as procedural learning and habit memory, remains relatively stable across the lifespan (Hedden and Gabrieli, 2004).

Visuospatial and constructional abilities decline over time, especially after age 70. Attention changes show domain-specific patterns. Simple auditory span is relatively preserved, whereas divided and selective

attention decline, especially when task demands are high or involve interference control (Harada et al., 2013).

Individual variability in the trajectory of cognitive ageing is substantial. Lifestyle factors such as physical activity, sleep quality, and cognitive engagement, as well as biological variables like cardiovascular health, systemic inflammation, and hormonal changes, among others, modulate susceptibility to age-related cognitive decline (Fard, 2019; Jin et al., 2022; Li, 2023; Zimmerman, 2021).

1.2.2 Mild Cognitive Impairment

Mild cognitive impairment (MCI) is considered to be the distinctive clinical syndrome that bridges the gap between normal ageing and dementia. It occupies a critical position along the cognitive continuum, which spans from age-consistent cognitive changes, through subjective cognitive decline (SCD), to MCI and eventually to dementia (Petersen et al., 2014). Beyond the expected (and mentioned) changes that occur during normal ageing, MCI refers to impairment that exceeds such changes but does not yet meet the functional criteria for dementia.

By definition, individuals with MCI experience a measurable decline in one or more cognitive domains, the most frequent one being memory, but maintain relative independence in daily functioning (Albert et al., 2011). Such a decline may be self-reported, reported by an informant, usually a close relative or friend, or observed objectively by a healthcare professional (Petersen et al., 1999). This stage is deemed as a potential therapeutic window, a point at which interventions may be implemented to delay or prevent progression to irreversible, neurodegenerative diseases (Jack et al., 2018).

MCI is a heterogeneous condition. Although it is considered a prodromal phase for dementia in many individuals, especially AD, it does not invariably progress. Some individuals with MCI remain stable over time, and a smaller proportion may even return to normal cognition (Petersen et al., 2004).

The term "mild cognitive impairment" was first introduced by Reisberg and colleagues in 1988, but it was Petersen and colleagues at the Mayo Clinic who formalised its clinical use in 1999. Their initial criteria focused on amnesic MCI, defined as a subjective memory complaint corroborated by objective impairment in memory performance, intact general cognition, preserved activities of daily living, and absence of dementia (Petersen et al., 1999).

In 2003, the Key Symposium in Stockholm led to the development of a more refined and inclusive typology of MCI, later published in 2004. This framework identified four subtypes:

1. Amnesic single-domain MCI, involving isolated memory impairment.
2. Non-amnesic single-domain MCI, affecting non-memory domains such as language, attention, executive functions, or visuospatial abilities.

3. Amnesic multiple-domain MCI, where memory and at least one other cognitive domain are impaired.
4. Non-amnesic multiple-domain MCI, involving deficits in multiple domains excluding memory (Petersen, 2004).

This classification acknowledged MCI's multiple aetiologies, and that non-amnesic variants, although less frequent, may precede non-Alzheimer dementias, such as frontotemporal or vascular dementia.

In 2011, the National Institute on Ageing and the Alzheimer's Association (NIA-AA) issued updated diagnostic criteria for "MCI due to AD." These criteria included:

1. Concern regarding a change in cognition, reported by the individual, informant, or clinician.
2. Impairment in one or more cognitive domains relative to age and education.
3. Preserved functional independence; and
4. No evidence of dementia (Albert et al., 2011).

Subsequently, the 2018 NIA-AA research framework proposed a syndrome staging model, placing MCI between the cognitively unimpaired and dementia stages. Since this framework distinguishes between clinical syndrome and underlying biology, it allows clinicians and researchers to describe patients as having MCI with or without Alzheimer pathophysiology, depending on biomarker status (Jack et al., 2018), and opens the way for its universal usage.

In parallel, the American Psychiatric Association, in the DSM-5, introduced the term mild neurocognitive disorder (mild NCD) to describe a syndrome functionally equivalent to MCI, but broader in scope. The DSM-5 criteria require:

1. Evidence of modest cognitive decline in one or more domains.
2. Maintenance of independence in daily activities.
3. No delirium.
4. Absence of a better explanatory condition, and
5. No dementia diagnosis (American Psychiatric Association, 2013).

Unlike the NIA-AA criteria, the DSM-5 definition was from the beginning aetiology-agnostic and did not presuppose AD as the underlying cause.

Although MCI is typically studied in older adults, advanced age is not a diagnostic requirement. However, its prevalence increases significantly after age 65. Among those with amnesic MCI, approximately 10% to 15% convert to dementia annually, although this progression is not universal (Petersen et al., 2014).

1.2.2.1 Epidemiology

MCI's prevalence varies considerably across studies, mainly due to differences in diagnostic criteria, methodology, and sample characteristics. The Alzheimer's Association Special Report estimates that between 12% and 18% of individuals aged 60 years or older meet criteria for MCI (Alzheimer's Association, 2025). Similar trends are observed in a population-based study from the United States, where approximately 22% of adults over 65 live with MCI (Loewenstein et al., 2022). As expected, this prevalence increases with advancing age. In a national study from China, rates rose from 19.4% in the 60–64 age group to 40% among those over 80 (Jia et al., 2022).

Despite such robust estimates, the actual prevalence of MCI is difficult to establish accurately. A systematic review encompassing international data reported prevalence figures ranging from 1.2% to 87%, underscoring how differences in study design, cultural context, and diagnostic thresholds shape reported rates (Sachdev et al., 2015). Even within more tightly defined cohorts, variability remains; prevalence in adults over 60 years of age is generally estimated at 6.7% to 25.2%, with higher rates in men and in those with lower educational attainment (Petersen et al., 2014).

Subtyping of MCI further refines these estimates. Amnesic single-domain MCI remains the most common phenotype, particularly in Western cohorts. Multi-domain amnesic MCI is less prevalent but carries the highest risk of progression to Alzheimer dementia (Petersen, 2004). In contrast, non-amnesic forms, both single and multi-domain, are less frequent but often precede non-Alzheimer dementias, including vascular and frontotemporal subtypes (Besson et al., 2015).

The MCI trajectory is equally heterogeneous among individuals. Longitudinal studies indicate that between 30% and 50% of individuals revert to normal cognition at follow-up, especially those with single-domain impairment, higher baseline cognitive performance, or absence of Alzheimer risk markers such as APOE ϵ 4 (Petersen et al., 2014). However, reversion does not guarantee long-term stability, as many individuals later redevelop impairment.

On the other hand, MCI is associated with an annual conversion to dementia risk of 5% to 10%, which is substantially higher than the 1% to 2% incidence observed in cognitively healthy older adults (Mitchell and Shiri-Feshki, 2009). Clinical settings often report even higher figures, reflecting referral bias. For example, analysis of the French National Alzheimer Database showed an annual progression rate of 13.7%, with amnesic MCI converting at nearly twice the rate (18.2%) of non-amnesic MCI (9.5%) (Besson et al., 2015). Neuropsychiatric symptoms also influence risk; individuals meeting criteria for mild behavioural impairment, defined as later-life onset of persistent (≥ 6 months), impactful new neuropsychiatric symptoms reflecting a change from longstanding personality/behaviour, not explained by a primary psychiatric disorder, show an annual conversion rate of 14.7%, compared to 8.3% among those without such symptoms (Ruthirakuhan et al., 2022).

Given the projected rise in global dementia prevalence to 153 million cases by 2050 (GBD 2019 Dementia Forecasting Collaborators, 2022), identifying and monitoring MCI represents a critical step in preventive neurology and translational research. We will discuss this later on.

1.2.2.2 Risk Factors for MCI Development

As previously established, MCI is not an aetiologic diagnosis but a syndrome. Although a significant proportion of MCI reflects a prodromal neurodegenerative cause, such as AD, non-degenerative and mixed-pathology mechanisms frequently trigger or modulate its presentation: vascular injury, metabolic dysfunction, sensory deficits, psychiatric comorbidity, and immune/inflammatory insults (Biessels and Despa, 2018; Debette and Markus, 2010; Livingston et al., 2024; Wardlaw et al., 2019). All these factors activate different pathways that conclude in shared mechanisms of neurovascular unit (NVU) disruption, blood-brain barrier (BBB) breakdown, neuroinflammation, oxidative stress, synaptic dysconnectivity, and network inefficiency, leading to cognitive phenotypes, whose trajectories may remain unchanged, decline, or recover, depending on both the underlying substrate and risk factors (Biessels and Despa, 2018; Livingston et al., 2024; Wardlaw et al., 2019).

Unlike non-modifiable determinants such as age, sex, or genetic predisposition (including APOE genotype, familial mutations, and sporadic mutations in genes like MAPT, GRN, and C9orf72), many aetiological contributors are modifiable (Livingston et al., 2024). Evidence from longitudinal cohorts and multidomain trials shows that addressing conditions such as hypertension, diabetes, sleep disorders, depression, sensory loss, physical inactivity, and social isolation, at different stages of life, not only reduces the incidence of cognitive decline but can also stabilise or even reverse MCI in some individuals (Livingston et al., 2024; Sanford, 2017). Understanding how these modifiable risk factors impact early neuropathology, network function, and cognitive trajectories is crucial, as it can lead to the identification of early biomarkers, facilitate risk stratification, and shape interventions with the potential to prevent and reverse decline.

Along with the growing number of studies describing potential modifiable factors that influence the onset and trajectory of MCI, the 2024 Lancet Commission on Dementia Prevention, Intervention, and Care has delineated 14 risk factors with the most consistent, statistically robust, and mechanistic evidence to date. These factors represent the strongest candidates for targeted intervention, offering a foundation for integrating their modification into the strategic approach for MCI prevention and management (Livingston et al., 2024).

Risk Factor	Life-Course Stage(s) Important	Epidemiological Strength*	Mechanistic Pathways Relevant to MCI
Low educational attainment / less education	Early life (<18 years) through midlife	Strong	Reduces cognitive reserve and increases the risk of dementia due to a reduced ability to compensate for neuropathology or vascular injury.
Hearing loss	Midlife through late life	Strong, with increasing support from recent studies	Increases cognitive effort, social isolation, and reduced sensory input, leading to downstream atrophy or network inefficiency. Possibly increases the risk of neuroinflammation and vascular compromise.
High low-density lipoprotein (LDL) cholesterol	Midlife	New evidence	Atherogenesis, increased risk of cerebrovascular disease, possible exacerbation of BBB permeability, and oxidative stress. Elevated central cholesterol homeostasis may also influence amyloid and tau metabolism.
Depression	Late life and possibly midlife	Recognized and increasing strength with recent studies	Increases hormones (glucocorticoids), and induces hypothalamic-pituitary-adrenal axis dysregulation, hippocampal atrophy, sleep disruption, and neuroinflammation. It can also reduce engagement in protective lifestyle behaviours.
Traumatic Brain Injury (TBI)	Any age, but midlife is especially important	Recognized and increasing strength with recent studies	Leads to acute injury (axonal shearing, BBB disruption), chronic neuroinflammation, possible accumulation of tau, microgliosis, and secondary degeneration, possibly affecting reserve. Recurrent or severe TBI may lead to persistent deficits.
Physical inactivity	Midlife and late life	Strong	Sedentary behaviour increases the risk of obesity, insulin resistance, and vascular stiffness. Exercise benefits cerebral blood flow, neurogenesis, and synaptic plasticity, and reduces vascular risk, improves metabolic regulation, and reduces inflammation. In MCI, maintaining physical activity can help delay progression.
Diabetes mellitus (type 2 diabetes)	Midlife	Strong	Chronic hyperglycaemia, glycaemic variability, advanced glycation end products, insulin resistance, microvascular damage, inflammation, oxidative stress. Associated with loss of hippocampal volume and white matter integrity. Executive and memory domains are often affected due to metabolic insult.
Smoking	Midlife and continuing into late life	Strong	Causes oxidative and metabolic stress, endothelial dysfunction, accelerates atherosclerosis, and increases the risk of microvascular damage, inflammation, and amyloid deposition.
Hypertension	Midlife (primarily) and continuing into late life	Strong and well-established	Drives large and small vessel disease, white matter hyperintensities, microinfarcts, reduced cerebral perfusion, and vascular remodelling, leading to fronto-subcortical network disruptions.
Obesity	Mainly midlife	Strong. The effect is partially mediated through metabolic syndrome	Influences insulin resistance, systemic inflammation, vascular risk, and dyslipidaemia. Adipose tissue secretes pro-inflammatory cytokines. It may also physically limit mobility and physical activity, worsening cognitive outcomes.

Excessive alcohol consumption	Midlife into late life	Strong	It can lead to thiamine deficiency, liver disease, oxidative stress, neurotoxic effects, increased risk of vascular damage, and hippocampal vulnerability. Even moderate but chronic drinking increases risk.
Social isolation	Across the life course, especially late life	Strong	Can increase stress, reduce cognitive stimulation, affect sleep and mood, all contributing to worse trajectories. Social engagement supports cognitive reserve, stimulates neural networks, and reduces the risk of depression and negative affect.
Air pollution	Across life, especially with mid to late life exposure	Recognized and increasing strength with recent studies	Fine particulate matter (NO ₂ , ozone, and other pollutants) can induce systemic inflammation, oxidative stress, and endothelial dysfunction; it may cross the BBB or cause vascular injury and worsen cerebrovascular health. Chronic exposure contributes to small vessel disease.
Vision loss (untreated)	Particularly late life	New evidence	Sensory deprivation, reduced engagement in cognitive and social activities, possibly altered circadian rhythm, and increased risk of depression/social isolation.

Table 1.1. Modifiable risk factors for MCI and Dementia prevention and stabilization from: 2024 Lancet Commission on Dementia Prevention, Intervention, and Care (Livingston et al., 2024)

1.2.2.3 Pathophysiological Correlations: How Modifiable Risk Factors Drive MCI

1.2.2.3.1 Neurovascular Unit Dysfunction and Cerebrovascular Disease: *Hypertension, high LDL cholesterol, diabetes, and smoking* promote small-vessel disease, cerebrovascular lesions, and white matter damage that disrupt, among other structures, fronto-subcortical circuits, especially those supporting executive function, attention, and processing speed. BBB breakdown allows blood-borne factors to produce neuroinflammation and synaptic damage (Biessels and Despa, 2018; Debette and Markus, 2010; Livingston et al., 2024; Wardlaw et al., 2019).

1.2.2.3.2 Metabolic Stress, Insulin Resistance, and Lipid Dysregulation: *Elevated LDL cholesterol, diabetes, and obesity* interfere with systemic metabolism; insulin resistance impairs glucose utilisation in the brain, reduces neurotrophic signalling, and increases oxidative stress. Dyslipidaemia hinders the amyloid generation/clearance dynamic but also causes vascular injury. These stresses diminish synaptic integrity and network connectivity and are associated with hippocampal and frontal atrophy (Biessels and Despa, 2018; Livingston et al., 2024; Wardlaw et al., 2019).

1.2.2.3.3 Sensory Deprivation and Reduced Cognitive/Social Input: *Untreated hearing and vision loss* reduces sensory input, induces overuse of cognitive resources, promotes social isolation, and increases the risks of depression. The loss of sensory input can alter neuroplasticity, degrade networks involved in visuospatial and attentional circuits, and potentially disrupt input to higher-order regions. Sensory deficits also interfere with compensatory cognitive strategies, worsening the severity of cognitive impairment disproportionately compared to the neurodegenerative pathology status, therefore making it more symptomatic (Diniz et al., 2013; Lin et al., 2023; Livingston et al., 2024).

1.2.2.3.4 Inflammation, Oxidative Stress, and Immune Dysregulation: *Obesity, diabetes, air pollution, smoking, and depression* provoke systemic inflammation, oxidative damage, and endothelial dysfunction. Chronic inflammation may prime microglial activation, which can exacerbate synaptic pruning or neurodegeneration. BBB disruption allows infiltration of blood components with immunogenic-toxic potential (Diniz et al., 2013; Livingston et al., 2024; Peters et al., 2019; Ruthirakuhan et al., 2022; Xie et al., 2024).

1.2.2.3.5 Lifestyle, Cognitive Reserve, and Behavioural Modulation: *Physical activity, cognitive engagement, social interaction, and education*, especially in early life, build cognitive reserve. Such reserve can buffer the functional impact of neurodegenerative conditions. Behavioural risk factors such as *alcohol misuse, smoking, traumatic brain injury (TBI), and physical inactivity* modulate multiple downstream biological pathways, contributing to oxidative stress, systemic inflammation, and metabolic dysregulation, while also impairing cerebrovascular health (Diniz et al., 2013; Livingston et al., 2024; Montagne et al., 2015; Ruthirakuhan et al., 2022).

While these contributors are universally accepted and acknowledged, recent studies suggest additional modifiable risk factors with promising data that could widen strategies for MCI prevention, stabilisation, and management. These factors are supported by an expanding evidence base, although the data remain less consistent and more heterogeneous than for those prioritised in the Lancet framework.

1.2.2.3.6 Sleep Disorders and Sleep Quality: Several recent studies report that sleep disorders, particularly sleep disordered breathing, such as obstructive sleep apnoea (OSA), are significantly associated with a higher prevalence of MCI. For example, in a study conducted on 387 patients with sleep apnoea, severe OSA, poor night-time sleep quality, and daytime sleepiness were correlated with elevated odds of having MCI (Lal et al., 2020). A broader population-based study showed that sleep disorders (including insufficient or excessive sleep duration, insomnia, and fragmented sleep) are risk factors for all-cause MCI/dementia. The suggested mechanisms include impaired clearance of amyloid β and tau, increased vascular risk, inflammation, endothelial dysfunction, oxidative stress, and sleep fragmentation, impairing network integrity (Bubu et al., 2017).

Evidence from observational studies and small pilot trials also suggests that treatment of OSA with continuous positive airway pressure (CPAP) may mitigate cognitive decline or delay the onset of dementia, though results remain mixed and larger trials are needed (Balk et al., 2024; Gosselin et al., 2019; Wang et al., 2020).

1.2.2.3.7 Extreme Sleep Duration: Both short and long sleep duration (outside of the normative 7 to 9 hours) have been associated with elevated risk of cognitive decline and incident MCI, even after adjusting for vascular and metabolic risks (Ma et al., 2020). Possible mechanisms include disturbed sleep architecture impairing memory consolidation, increased amyloid- β /tau deposition due to inefficient protein clearance, metabolic dysregulation, and chronic inflammation (Spira et al., 2014).

1.2.2.3.8 Socio-economic Status and Income Inequality: Growing evidence suggests that socio-economic factors, such as income, neighbourhood deprivation, and economic instability, are associated with risk of cognitive decline and MCI, independent of educational attainment (Liu et al., 2020; Rosická et al., 2024). Mechanisms include reduced access to healthcare and nutrition, unsafe environments, exposure to stress and pollutants, and higher allostatic load.

1.2.2.3.9 Dietary Pattern and Nutrition Beyond Obesity: While obesity is an established risk factor, specific dietary patterns, such as adherence to a Mediterranean diet, high consumption of fruits, vegetables, and fish, and low intake of processed foods, are associated with slower decline and lower MCI incidence (Martínez-Lapiscina et al., 2013; Singh et al., 2014). Micronutrient deficiencies are also implicated: vitamin B12 and vitamin D deficiency, low omega-3 fatty acids, and insufficient antioxidant intake have been correlated with poorer cognition, though evidence is still mixed (Etgen et al., 2012; Loeff and Walach, 2012; Lauer et al., 2022).

1.2.2.3.10 Toxic Exposures: Exposure to environmental toxins, including heavy metals (lead, mercury), pesticides, and persistent organic pollutants, has been associated with poorer executive and memory performance in epidemiological studies (Power et al., 2011). Elevated blood lead levels correlate with slower processing speed and greater cognitive impairment, even at low exposures. Indoor air pollution from biomass fuels and smoke also emerges as a contributor to cognitive decline (Huszár et al., 2024; Kulkarni and Grigg, 2021).

1.2.2.3.11 Chronic Systemic Diseases and Inflammation: Several chronic inflammatory and systemic diseases are increasingly implicated in MCI. Chronic obstructive pulmonary disease (COPD), heart failure, and autoimmune disorders contribute to cognitive decline through systemic hypoxaemia, vascular dysfunction, and inflammation (Ye et al., 2022; Cleutjens et al., 2014). Chronic kidney disease (CKD), particularly moderate-to-severe stages, is also associated with increased MCI risk via uraemic toxins, vascular injury, and metabolic disruption (Bobot et al., 2024; Bugnicourt et al., 2013).

1.2.2.4 The Pathophysiology of MCI

Since MCI represents a transitional state between normal ageing and dementia (of any aetiology), it is underpinned by diverse biological alterations that extend beyond the aforementioned ageing process and “classical” neurodegeneration features. While MCI is often a precursor of conditions like AD, it also arises from vascular, metabolic, inflammatory, and systemic mechanisms. These processes converge on a limited set of biological pathways: synaptic dysfunction, impaired neurovascular regulation, network disconnection, and maladaptive neuroinflammatory responses.

1.2.2.4.1 Synaptic and Neuronal Dysfunction

Structural changes underlying MCI are characterised not only by cortical atrophy or macroscopic vascular injury, but more profoundly by synaptic and neuronal alterations that compromise the efficiency of neural

circuits. These microscopic changes constitute the earliest substrate of cognitive decline, occurring well before widespread cell loss or cortical thinning (Fang et al., 2024; Jackson et al., 2019).

Across aetiologies, MCI is consistently associated with reduced synaptic density, impaired neurotransmission, and alterations in neuronal plasticity. Post-mortem analyses and in vivo imaging studies have demonstrated that synaptic loss, rather than neuronal death, is the strongest correlate of early cognitive decline (Fang et al., 2024; Jackson et al., 2019). Dendritic spine retraction, decreased ability to strengthen synaptic connections between neurons, known as long-term potentiation (LTP), and disruption of excitatory–inhibitory balance limit the efficacy of processes like encoding and retrieving information competently (Scheff et al., 2006; Selkoe, 2002).

Mitochondrial dysfunction and oxidative stress further weaken neuronal resilience in MCI. Reduced ATP production, increased reactive oxygen species, and dysregulated calcium handling interfere with synaptic signalling and contribute to dendritic degeneration (DuBoff et al., 2013). Collectively, these findings establish MCI as a biological state of neuronal vulnerability, where circuit efficiency is compromised despite relative preservation of gross anatomy.

Although synaptic compromise is a unifying hallmark of MCI, it is the product of different processes, depending on its aetiology, that converge on a final common pathway of synaptic inefficiency and neuronal stress.

Vascular MCI is the product of chronic hypoperfusion and microinfarcts that deprive neurons of oxygen and glucose, weakening synaptic signalling and leading to disconnection of white matter tracts critical for fronto-subcortical communication (Beishon et al., 2021; Wardlaw et al., 2019).

In metabolic-related MCI, such as type 2 diabetes and metabolic syndrome, insulin resistance reduces neuronal glucose uptake and LTP, while advanced glycation end-products disrupt dendritic integrity (Biessels and Despa, 2018). Systemic inflammation in chronic disease states, such as vascular disease, obesity, chronic infections, autoimmune-inflammatory diseases, CKD, or COPD primes microglia, making them overreactive to subsequent insults. In this sensitised state, microglia excessively prune synapses, disrupting neuroplasticity and LTP (Walker et al., 2019).

In psychiatric and stress-related MCI, chronic hypercortisolaemia, such as the one documented in late-life depression, induces dendritic spine loss and suppresses neurogenesis in hippocampal and prefrontal regions, contributing to progressive cognitive impairment (Duman et al., 2016).

In AD-related MCI, soluble amyloid- β oligomers disrupt NMDA receptor signalling and impair synaptic plasticity, while hyperphosphorylated tau destabilises microtubules and axonal transport (Merino-Serrais et al., 2013; Walsh and Selkoe, 2007). Early cholinergic deficits in basal forebrain projections further weaken attentional networks (Ballinger et al., 2016). In Lewy body disease (LBD), α -synuclein interferes with presynaptic vesicle cycling, impairing dopaminergic and cholinergic transmission (Kramer and Schulz-Schaeffer, 2007). In frontotemporal lobar degeneration (FTD), TDP-43 or tau pathology affects glutamatergic synapses (Liscic et al., 2008).

1.2.2.4.2 Neurovascular and Blood–Brain Barrier Dysfunction

MCI is recognized as a disorder of the neurovascular unit (NVU) and blood–brain barrier (BBB) as much as of neurons and synapses. Across aetiologies, failure of vascular-neuronal coupling and barrier integrity emerge early and measurably, preceding major structural changes like atrophy. These neurovascular changes degrade circuit energetics, amplify neuroinflammation, hinder waste clearance, and consequently interfere with the efficacy of cognitive networks (Denkinger et al., 2025; Nation et al., 2019; Tian et al., 2024).

A growing number of human-based research studies have shown that BBB dysfunction is already present at the time of MCI clinical manifestations, being more associated with some specific cognitive deficits, such as episodic memory and processing speed, and in many individuals, it even appears before traditional AD biomarkers or signs of neurodegeneration are detectable (Denkinger et al., 2025; Hergert et al., 2024; Montagne et al., 2015; Montagne et al., 2020; Moon et al., 2021; Nation et al., 2019; Tian et al., 2024).

As discussed in chapter one, the NVU integrates endothelial cells, pericytes, astrocyte end-feet, neurons, and extracellular matrix to enforce BBB selectivity, synchronise neurovascular coupling (NVC), so local blood flow matches the neural demand, and support transport and clearance of metabolites, such as amyloid β (A β) efflux via LRP1/P-glycoprotein and influx via RAGE (Iadecola, 2017; Sweeney, Ayyadurai, and Zlokovic, 2016; Zlokovic, 2011).

Healthy ageing brings subtle NVU-BBB alterations, already described, that remain clinically unremarkable. However, these age-amplified pathways appear to tip the NVU from resilience to failure. In the BBB of individuals with MCI, tight junctions are loosened, pericytes degenerate, astrocytic AQP4 polarity is lost, endothelial transporter expression is reduced, matrix metalloproteinase (MMP-9) is activated, and NVC regulation is weakened, setting the stage for cognitive impairment (Benveniste, Nedergaard, and Witte, 2019; Sweeney, Sagare, and Zlokovic, 2018). The NVU integrity and function are then compromised, transforming a selective, tightly regulated barrier into a semi-permeable one. As a result, plasma proteins such as fibrinogen, thrombin, and albumin penetrate the perineuronal space, triggering astrocytic and microglial activation, and sustaining chronic neuroinflammation (Bowman et al., 2018; Senatorov et al., 2019). The decline in pericyte support and the loss of astrocytic polarity reduce the metabolic and ionic homeostasis (Montagne et al., 2020), while impaired expression of efflux transporters such as LRP1 and P-glycoprotein diminishes clearance of toxic metabolites (Andjelkovic et al., 2023; Nation et al., 2019). At the same time, endothelial dysfunction weakens NVC synchrony, lowering the brain's capacity to match regional perfusion to neuronal activity (Liu et al., 2025; Rajeev et al., 2022). Together, these processes further weaken synaptic plasticity, impair myelin maintenance, and erode the connectivity of memory and executive networks (Freeze et al., 2020; Li et al., 2021).

This ageing brain to MCI progression is triggered by several factors, such as chronic microvascular endothelial dysfunction, pericyte degeneration, entry of neurotoxic substances through the barrier, and neuroinflammation, and exacerbated by factors such as hypertension, diabetes, chronic cerebral hypoperfusion, APOE4 genotype, and systemic inflammation. These exacerbate endothelial injury,

promote oxidative stress, and increase the activity of matrix metalloproteinases, further compromising BBB integrity and creating a vicious cycle of BBB dysfunction (Chen et al., 2024; Grammas, 2011; Huang et al., 2025; Nation et al., 2019; Preis et al., 2024; Raulin et al., 2022; Santisteban et al., 2023; Tarawneh et al., 2023).

This transition to MCI, marked by quantitatively greater, more extensive, and regionally selective increase in BBB permeability, has been broadly described on dynamic contrast-enhanced MRI (DCE-MRI) and fluid biomarkers-based studies (Denkinger et al., 2025; Montagne et al., 2015; Nation et al., 2019). One of the most consistent findings was medial temporal leakage, specifically in hippocampal and parahippocampal regions, which was in keeping with increased levels of cerebrospinal fluid (CSF) soluble platelet-derived growth factor receptor beta (SPDGFR β), a marker of pericyte injury-degeneration, and correlated clinically with impaired episodic memory performance but independent of A β /tau status (Montagne et al., 2015; Nation et al., 2019; van de Haar et al., 2016).

Vascular risk factors, including hypertension, diabetes, and dyslipidaemia, variably present in the current ageing population, instigate endothelial dysfunction, pericyte dropout, and oxidative/inflammatory stress, inducing BBB leakage that, at its highest dysfunction, parallels white matter damage burden, and correlates with slower processing speed and executive dysfunction resulting from fronto-subcortical networks disconnection (Biessels and Despa, 2018; Freeze et al., 2020; Hergert et al., 2024; Li et al., 2021; Rajeev et al., 2022).

Cerebral small-vessel disease (CSVD), including white matter hyperintensities, lacunes, and microbleeds, induces microvascular leakage of fibrinogen that activates microglia and causes oligodendrocyte injury, promoting demyelination and axonal loss. Consequently, the capillary flow becomes heterogeneous, and the perfusion of active microcircuits inefficient despite normal large-artery flow (Beishon et al., 2021). As CSVD burden worsens, damage becomes extensive and diffuse, but frontal white matter and basal ganglia remain as the principal areas affected in vascular MCI (Wardlaw et al., 2019).

In APOE4 carriers, even when still cognitively normal, pericyte-centric cascades (such as cyclophilin A–MMP-9) destabilise tight junctions and basement membrane, producing hippocampal BBB leakage, also independent of A β and tau burden, and predicting cognitive decline (Montagne et al., 2020; Moon et al., 2021).

In AD-spectrum MCI, BBB failure meets with proteinopathy, as described in Chapter One. Reduced LRP1/P-gp mediated A β efflux and pericyte-driven basement membrane degradation increase vascular and parenchymal A β deposit, while local inflammation amplifies tau-related network failure (Denkinger et al., 2025; Montagne et al., 2020; Nation et al., 2019; Preis et al., 2024; Wu et al., 2024). Besides the aforementioned medial temporal lobe, consistently the earliest and most affected region across aetiologies, the posterior cingulate and precuneus regions are disrupted in AD-MCI, in keeping with early default mode network (DMN) disconnection (Bai et al., 2009).

In non-AD neurodegenerative MCI, several types of misfolded proteins can injure the microvasculature and impair endothelial performance, producing selective regional perfusion and BBB permeability changes that correlate with non-amnestic phenotypes (Andjelkovic et al., 2023; Wu et al., 2024). In Lewy body and Parkinsonian MCI, α -synuclein accumulates at endothelial/perivascular sites, interfering with the transporters' performance and promoting local inflammation. Midbrain and brainstem nuclei exhibit BBB dysfunction features in patients with Parkinsonian MCI, linking vascular compromise to dopaminergic and cholinergic degeneration (Kortekaas et al., 2005). In FTD, TDP-43 and tau variants associate with microvascular degeneration and hypoperfusion, especially in frontal networks (Kawakami et al., 2019). These patterns yield non-amnestic MCI phenotypes where executive, language, and visuospatial deficits are predominant.

Systemic and CNS inflammatory mediators further increase BBB permeability and microglial reactivity, linking peripheral health to central barrier integrity (Andjelkovic et al., 2023; Bowman et al., 2018). Across aetiologies, the shared final pathway becomes: NVU failure $\rightarrow$ barrier permeability $\rightarrow$ inflammatory–metabolic stress $\rightarrow$ synaptic/network inefficiency.

BBB dysfunction is not only a causal contributor but also a marker of brain vulnerability, such as in the MCI case. As a biomarker, it is defined by measurable alterations in barrier integrity that occur before and during the symptomatic stage of cognitive decline. What makes BBB dysfunction particularly valuable in the MCI context is that it captures a pathological process that is both early and independent of classical Alzheimer markers, increasing the possibility of early detection, beyond A β and tau. As mentioned in the previous chapter, advanced imaging modalities such as DCE-MRI allow quantification of regional BBB permeability (Montagne et al., 2015; Montagne et al., 2020; Nation et al., 2019), and parallel fluid biomarkers, including CSF sPDGFR β , correlate with permeability changes, providing a complementary window into microvascular pathology (French et al., 2025; Nation et al., 2019).

As a biomarker, BBB also holds prognostic value. Longitudinal studies have demonstrated that individuals with increased BBB permeability are more likely to progress from MCI to dementia, even when A β and tau levels are not yet abnormal (Denkinger et al., 2025; Tian et al., 2024). As described earlier, in APOE4 carriers, who represent an AD high-risk population, hippocampal leakage is detectable years before clinical conversion, making BBB disruption a sensitive risk stratifier (Montagne et al., 2020; Moon et al., 2021).

1.2.2.4.3 Network Disconnection

Synaptic-neuronal and NVU-BBB dysfunctions produce alterations in connectivity, synchrony, and communication efficiency across brain regions and distributed systems, which translates into network-level disruptions. These network-based changes explain the heterogeneity of MCIs, with specific patterns and prognosis depending on the underlying biology.

The default mode network (DMN), anchored in the posterior cingulate cortex, precuneus, and medial temporal lobe, is particularly vulnerable in amnestic MCI. Studies using functional MRI have consistently demonstrated reduced connectivity between hippocampus and posterior cingulate cortex, correlating

with episodic memory, self-referential processing, and social cognition deficits (Bai et al., 2009), which reflect synaptic dysfunction in the hippocampal–cortical circuits, driven by amyloid- β and tau pathology in AD-related MCI, but also seen in vascular and metabolic forms where perfusion deficits and insulin resistance impair medial temporal lobe activity (Beishon et al., 2021; Biessels and Despa, 2018).

Non-amnestic forms of MCI frequently involve changes in the frontoparietal control network (FPN) and subcortical executive network. Vascular MCI, in particular, is associated with reduced connectivity between the prefrontal cortex, basal ganglia, and thalamus due to white matter small vessel disease (Wardlaw et al., 2019). These disruptions translate into slowed processing speed, attentional deficits, and impaired task switching, and correlate with the extent of vascular burden (Beishon et al., 2021).

In some MCI subtypes, especially those associated with psychiatric or behavioural symptoms, dysfunction extends to the salience network and limbic networks. These systems integrate emotional and cognitive processing; their disruption contributes to attentional instability, apathy, and affective dysregulation (Ruthirakuhan et al., 2022).

Beyond specific network deficiencies, large-scale connectivity studies emphasise the role of hub regions, such as the posterior cingulate, precuneus, and medial prefrontal cortex. These hubs integrate information across networks and depend heavily on synaptic efficiency. In MCI, hub failure results in a transition from an optimised architecture toward less efficient configurations (Stam et al., 2007; Zhao et al., 2012). This hub vulnerability explains why MCI patients show widespread but subtle deficits across multiple domains, even when structural atrophy or focal lesions are modest.

1.2.2.4.4 Neuroinflammation and Glial Responses

Neuroinflammation and glial activation are common denominators across all MCI aetiologies. Once BBB dysfunction or neuronal stress occurs, glial cells, primarily microglia and astrocytes, respond, initially in adaptive ways, but over time, shifting into maladaptive phenotypes that amplify damage, paving the way for cognitive decline (Chen et al., 2025).

As described in Chapter One, microglia respond to insults like misfolded proteins, hypoxia, vascular injury, metabolic dysregulation, or systemic inflammatory signals by transitioning from surveillance and homeostatic states to primed, activated states. When those states are sustained over time, microglia increase their secretion of pro-inflammatory cytokines, reactive oxygen and nitrogen species, as well as complement proteins, all of which exacerbate synaptic pruning, impair neuronal mitochondrial function, facilitate tau hyperphosphorylation (where tauopathy is present), and further destabilise network connectivity (Gao et al., 2023; Ghimire et al., 2024). Simultaneously, astrocytes undergo reactive changes: increasing GFAP and other markers expression, changing morphology, reducing expression of glutamate transporters, and diminishing their capacity to regulate ions, clear excitatory neurotransmitters, and deliver metabolic substrates. The loss of astrocytic trophic and homeostatic functions increases neuronal vulnerability (Chen et al., 2025; Garland et al., 2022).

The interaction between microglia and astrocytes is fundamental; not only do they respond to common upstream insults, but they modulate one another. For example, activated microglia may release factors such as IL-33, which influence astrocyte phenotypes; astrocyte-derived molecules, like S100B or cytokines, can in turn exacerbate or modulate microglial activation (Garland et al., 2022). Recent studies have suggested that this bidirectional regulation becomes dysregulated in MCI as a consequence of a homeostatic crosstalk deterioration, causing astrocytes to increasingly promote pro-inflammatory signalling, which pushes microglia into more deleterious states (Chen et al., 2025). This contributes to further synaptic injury, network disconnection, and ultimately, measurable cognitive decline.

Although common to all forms of MCI, different aetiologies show distinct profiles in glial and inflammatory responses. In MCI due to AD, early amyloid accumulation promotes microglial activation, leading to the emergence of disease-associated microglial states, which are initially protective but later become neurotoxic. Astrocytes surrounding amyloid plaques exhibit impaired glutamate clearance and reduced metabolic support, increasing excitotoxic stress (Chen et al., 2025; Gao et al., 2023). In non-AD neurodegenerative forms of MCI, such as those linked to LBD, PD, or FTD, microglia and astrocytes also shift from supportive to reactive states. Microglia respond to abnormal protein aggregates like α -synuclein or TDP-43 with pro-inflammatory activation, releasing cytokines and reactive species that aggravate synaptic stress and neuronal vulnerability. Astrocytes, in turn, exhibit reactive phenotypes marked by altered glutamate clearance, impaired metabolic support, and release of inflammatory mediators (Garcia-Revilla et al., 2022; Huang et al., 2025).

In vascular or mixed aetiologies, hypoperfusion, CSVD, and microinfarcts lead to BBB compromise and pericyte loss. This allows leakage of plasma proteins such as fibrinogen into the perineuronal space, which act as potent glial activators. Microglia become “primed” and respond excessively to secondary insults. Astrocytic end-feet surrounding cerebral vessels are also disrupted, impairing the NVC and molecular clearance (French et al., 2025; Garland et al., 2022).

In metabolic and systemic inflammatory conditions, previously listed, chronic peripheral inflammation promotes central glial activation. Elevated circulating cytokines correlate with increased brain glial reactivity and are associated with accelerated cognitive decline (Bieger et al., 2023; Lista et al., 2024). Moreover, insulin resistance and impaired glucose metabolism reduce the energetic capacity of both neurons and glia, weakening their resilience to injury. Astrocytic support is diminished, while pro-inflammatory phenotypes predominate in microglia, particularly in vulnerable regions such as the hippocampus and medial temporal lobe.

In sum, glial dysregulation acts as both a downstream response to neuronal injury and an upstream driver of continued pathology in MCI.

1.2.2.5 Diagnostic Process and Assessment Tools for MCI

The diagnosis of MCI is fundamentally clinical and requires a structured, stepwise approach that integrates history taking, collateral information, medical evaluation, and cognitive testing (Sanford, 2017). As the initial concern often arises in primary care or community settings, screening instruments are employed to detect possible impairment. Then, patients with suspicious findings are typically referred to specialised memory clinics or neurology services, where more comprehensive evaluations are conducted (Zhuang, Yang, and Gao, 2021).

The following are some of the most frequently used screening tests in everyday practice:

- **Mini-Mental State Examination (MMSE)** (Folstein et al., 1975): The MMSE is one of the most extensively used instruments worldwide. It evaluates orientation, registration, attention, immediate recall, language, and visuospatial construction in 5 to 10 minutes. While its brevity and extensive familiarity are advantages, its sensitivity to early impairment is limited, particularly in highly educated individuals, showcasing variable accuracy in detecting MCI. Sensitivity ranges between 13% and 97%, and specificity between 60% and 100%, depending on cut-offs (Zhuang, Yang, and Gao, 2021). Therefore, the MMSE is better suited for detecting more advanced impairment than for identifying subtle cognitive changes.
- **Montreal Cognitive Assessment (MoCA)** (Nasreddine et al., 2005): Developed to address the limitations of the MMSE, the MoCA evaluates a broader set of domains, including executive functions, visuospatial ability, memory, language, attention, and orientation. It has shown higher sensitivity to MCI, often ranging from 67% to 100%, though specificity varies between 50% and 95%, depending on the population and cut-offs (Nasreddine et al., 2005; Zhuang et al., 2021). While overall more comprehensive, MoCA performance is influenced by educational level, with those below 12 years of schooling often scoring lower, independent of pathology.
- **Addenbrooke's Cognitive Examination-Revised (ACE-R)** (Mioshi et al., 2006): A longer screening tool (15 to 20 minutes), ACE-R expands upon MMSE by including verbal fluency and memory subtests, offering greater sensitivity to MCI and early dementia. Its longer duration makes it less used in primary care.
- **Dementia Rating Scale (DRS)** (Matis, 1976): The DRS was originally designed for moderate dementia and includes attention, initiation, construction, conceptualisation, and memory. While robust in more advanced disease, it lacks the sensitivity needed for subtle deficits in MCI (Mossbarger, Whitney, Herman, and Mariner, 2012).

Despite contributing valuable information, these screening tools suffer from ceiling effects, variability in sensitivity and specificity, and educational or cultural bias. For this reason, abnormal or borderline screening results are best followed by comprehensive neuropsychological testing.

Formal neuropsychological evaluation provides a more detailed profile of cognitive function. It assesses memory, attention, executive functioning, visuospatial skills, and language through multiple tests, reducing the risk of mislabelling. This is particularly important, since even healthy older adults may score below the cut-off on a single test, raising the risk of a false-positive diagnosis if impairment is not demonstrated across multiple tests (Anderson, 2019). Neuropsychological testing also accounts for factors such as mood, medical history, sensory limitations, and educational background that may influence performance (Sanford, 2017).

1.2.2.5.1 RBANS: a Bridge Between Screening Tests and Full Batteries

Among a myriad of available assessment tools, the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) has proven especially valuable in the context of MCI. Developed in the late 1990s, RBANS was designed to detect and characterise abnormal cognitive decline with efficiency and precision (Randolph, Tierney, Mohr, and Chase, 1998). Administration requires between 20 and 30 minutes, which is significantly shorter than full batteries. It yields scaled scores across five domains: immediate memory, visuospatial/constructional skills, language, attention, and delayed memory.

RBANS has shown strong validity, correlating well with comprehensive instruments such as the Wechsler Adult Intelligence Scale-III (WAIS-III) and Wechsler Memory Scale (WMS), while maintaining sensitivity to subtle deficits often missed by MMSE or DRS (Gold, Queern, Iannone, and Buchanan, 1999). Patients with early impairment who perform within normal limits on brief assessments often demonstrate domain-specific weaknesses on RBANS (Randolph et al., 1998). Its design includes several alternate forms, allowing reliable serial assessments without significant practice effects, a critical advantage in both clinical follow-up and research trials (Shura et al., 2018).

Beyond dementia, RBANS has been validated across diverse clinical populations, including patients with schizophrenia, epilepsy, Parkinson's disease, and traumatic brain injury, reinforcing its adaptability (Shura et al., 2018). Embedded validity indices further support its clinical utility by providing in-test measures of response reliability.

In practice, RBANS occupies a niche between brief screeners and comprehensive neuropsychological batteries: it is sensitive, repeatable, portable, and efficient, making it a sensible option for diagnosis and longitudinal monitoring in MCI.

In general, cognitive evaluation tools are not only useful for diagnosis; they also provide standardised metrics for longitudinal follow-up, allowing clinicians and researchers to track stability, progression, or even return to normal cognition.

1.2.2.5.2 Broader Diagnostic Workup

Since cognitive testing only defines the presence of MCI, clinicians often perform additional assessments to identify the underlying aetiology and rule out reversible secondary causes. Neurological examination evaluates reflexes, eye movements, gait, and coordination to exclude structural or movement disorders.

Laboratory tests may reveal reversible causes, such as vitamin B12 deficiency or thyroid dysfunction. Structural imaging (MRI or CT) can identify strokes or tumours, while advanced modalities such as amyloid PET or CSF biomarkers can support a diagnosis of prodromal AD (Sanford, 2017). These tests clarify the cause but do not replace the clinical syndrome of MCI.

1.2.2.6 MCI Prevention and Interventions

1.2.2.6.1 Early Diagnosis and Risk Factors Identification

Despite its prevalence and clinical significance, MCI remains globally underdiagnosed and undertreated in primary care and community settings (Sanford, 2017). This gap not only delays intervention but also closes off a critical opportunity to alter the disease trajectory. Since no curative treatments currently exist for most dementias, prevention, early diagnosis, and timely interventions represent the most practical strategies to combat cognitive decline.

Since the clinical course of MCI is highly heterogeneous, some individuals remain stable for years, some progress to dementia, and a meaningful subset may potentially revert to normal cognition (Roberts et al., 2014). Early identification of cognitive decline allows clinicians to detect modifiable factors that are known to influence prognosis (Petersen et al., 2018). This creates opportunities for structured cognitive interventions, cardiovascular risk management, and the promotion of neuroprotective behaviours such as physical activity, dietary improvement, and cognitive training, all of which have shown promise in slowing progression (Ngandu et al., 2015).

The current diagnostic practices for MCI are predominantly reactive and rely on patient or caregiver concern to prompt an evaluation. Unfortunately, this case-finding approach misses mildly affected individuals who could also benefit from early intervention. Since universal cognitive screening in older adults is controversial due to insufficient consensus on test specificity, risk of overdiagnosis, and resource allocation (Sanford, 2017), several experts advocate for at least routine cognitive surveillance in individuals with known risk factors, such as diabetes, hypertension, apolipoprotein E ϵ 4 (APOE4) genotype, history of stroke or TBI, and sensory deprivation, given their higher pre-test probability of impairment (Livingston et al., 2020).

Instead of a “screen all” versus “screen none” model, a stratified approach based on individualised risk profiles may offer a more practical solution. Risk-based screening models, integrating age, genetic predisposition, and modifiable exposures, could enable scalable early detection strategies with both clinical and economic efficiency.

1.2.2.6.2 Prevention and Stabilisation

Tackling modifiable risk factors represents one of the most practical and effective strategies for preventing and controlling MCI, as it targets mechanisms that are both common and amenable to change. Importantly, these interventions often have additional health benefits, such as improving cardiovascular

and metabolic outcomes, reducing frailty, and enhancing quality of life, which increases their viability and cost-effectiveness at the population level. By prioritising early identification and sustained management of modifiable risks, clinicians and policymakers can lessen the period of cognitive disability, reduce conversion to dementia, and support healthier and more active ageing trajectories.

1.2.2.6.2.1 Early Intervention Across the Life Course: Many risk exposures begin in early or midlife, including low educational attainment, midlife hypercholesterolaemia, hearing loss, and hypertension. Because the biological impact of these risks is cumulative and neuronal resilience declines with age, intervening early is critical.

In early life, equitable access to quality education and cognitive enrichment programmes builds cognitive reserve, which protects against later decline. In midlife, aggressive management of vascular and metabolic health is essential: regular screening for blood pressure, lipids, and glucose, followed by pharmacological or lifestyle interventions, can prevent the accumulation of CSVD and metabolic injury that later would manifest as MCI. Early detection and treatment of sensory impairments, through routine audiometry and vision checks, are practical and cost-effective strategies that reduce cognitive load and enhance reserve.

These measures are not only preventive but may stabilise trajectories in those already diagnosed with MCI, supporting the principle that risk modification at any stage of life provides tangible benefits, with the greatest gains when initiated earlier (Debette and Markus, 2010; Lin et al., 2023; Livingston et al., 2024).

1.2.2.6.2.2 Multicomponent Interventions: Interventions targeting several risk factors simultaneously, such as diet, physical activity, vascular and metabolic health, cognitive training, and social engagement, have shown the most promising slowing of cognitive decline in individuals with MCI. The FINGER trial (Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability) demonstrated that a two-year multidomain lifestyle programme significantly improved or stabilised cognitive function in older adults at risk for dementia, outperforming standard health advice (Ngandu et al., 2015). Participants receiving the intervention had better outcomes in global cognition, executive function, and processing speed, particularly when tailored to individual risk profiles and sustained over time.

The strength of this strategy lies not only in its biological efficacy but also in its potential for long-term adherence and personalisation. Interventions that combine physical, cognitive, and social components simultaneously address multiple brain health mechanisms, enhancing neuroplasticity, reducing vascular risk, mitigating depressive symptoms, and reinforcing healthy behaviours.

1.2.2.6.2.3 Sensory Correction: Routine screening for hearing and vision, particularly in older adults or individuals presenting with cognitive complaints, is essential. Interventions such as prescription glasses, cataract surgery, and hearing aids not only improve communication and functional independence but also preserve cognitive function in individuals at risk. Hearing-aid use, one of the most studied strategies, has been associated with slower rates of cognitive decline and reduced incidence of dementia, particularly when implemented early (Lin et al., 2023).

These interventions are deemed as highly cost-effective, especially when weighed against the long-term societal and healthcare costs of dementia-related disability (Lin et al., 2023; Livingston et al., 2024). Given that sensory deficits are highly prevalent, easily detectable, and largely reversible, systematic correction of hearing and vision should be considered a core component of MCI management and cognitive decline prevention strategies. This will be discussed extensively in the next chapter.

1.2.2.6.2.4 Cardiovascular and Metabolic Health Optimisation: Blood pressure control, LDL cholesterol lowering, glucose control in diabetics, and weight management are key targets. Beyond individual risk factor control, the cumulative burden of metabolic syndrome is strongly associated with impaired cognitive performance, so aggressive interventions aiming for cardiovascular optimisation not only reduce stroke and myocardial infarction risk but also promote better cerebral perfusion, reduced neuroinflammation, and preservation of white matter integrity, all of which are crucial in the MCI population (Debette and Markus, 2010; Livingston et al., 2024; Wardlaw et al., 2019).

1.2.2.6.2.5 Behavioural and Psychosocial Domains: Lifestyle behaviours and psychosocial health are pivotal, modifiable components of cognitive risk that exert their influence through multiple biological and environmental pathways. Smoking cessation, limiting harmful alcohol use, and promoting regular physical activity have consistently been associated with a lower incidence of cognitive decline and dementia. These behaviours attenuate vascular and metabolic risk factors, while also supporting structural brain health, including preserved hippocampal volume and white matter integrity. Physical activity, in particular, enhances cerebral blood flow and improves sleep quality and mood, all of which contribute to better cognitive resilience (Livingston et al., 2024).

Beyond somatic health, psychosocial factors such as untreated depression, loneliness, and lack of social engagement also play critical roles in cognitive decline. Interventions that effectively treat mood disorders can mitigate these effects and slow cognitive deterioration. Likewise, maintaining strong social networks and regular interpersonal engagement has been shown to enhance cognitive reserve and delay symptom onset in individuals with MCI (Diniz et al., 2013; Ruthirakuhan et al., 2022).

1.2.2.6.2.6 Environmental and Policy-Level Interventions: Addressing modifiable risk factors for MCI requires moving beyond individual behaviour change to tackle the broader environmental and structural determinants of brain health. Public health policies that lower air pollution have the potential to reduce oxidative stress, vascular injury, and inflammation. Urban planning measures such as expanding green spaces, regulating emissions, and improving ventilation in residential and occupational environments are emerging as scalable and cost-effective interventions (Kulkarni and Grigg, 2021; Livingston et al., 2024). These policies confer additional benefits to cardiovascular and respiratory health, making them even more impactful on ageing populations at risk of MCI.

Policy-level interventions are also critical for ensuring equitable access to protective resources that shape cognitive trajectories across the life course. Universal access to quality early-life education builds cognitive reserve, while lifelong learning programmes and adult literacy initiatives may help sustain it in mid- and late life. Publicly funded sensory health programmes that cover hearing aids, eyeglasses, and cataract

surgeries are cost-effective ways to reduce cognitive burden, especially in older adults (Lin et al., 2023; Livingston et al., 2024). Additionally, social policies that address income inequality, food insecurity, and access to healthcare can indirectly reduce many MCI risk factors, such as obesity, depression, and poor diabetes control. Mass awareness campaigns, centred on brain health promotion, healthy ageing, and the reversibility of some cognitive impairments, can shift the public perception and increase early intervention uptake.

These interventions highlight that MCI is not a static state, and its trajectory can often be influenced by timely action.

Return from MCI to normal cognition occurs in approximately 10 to 30% of cases, and most frequently when in the setting of reversible causes, such as medication side effects, sleep disorders, or psychiatric conditions (Roberts et al., 2014; Sachdev et al., 2014). Even in cases where full reversion is not possible, achieving clinical stability, understood as the absence of further decline over time, represents a significant therapeutic success. This stabilisation helps to preserve autonomy, limits caregiver burnout, and maintains quality of life.

As no definitive cure for MCI currently exists, management should focus on achievable goals: stopping progression, reducing modifiable risks, and maximising daily function. These strategies not only align with current evidence but also with what matters most to individuals living with MCI: maintaining independence, dignity, and well-being.

1.2.3 Conclusion

The progression from normal cognitive ageing to MCI reflects the interaction of age-related brain progressive structural, neurochemical, and functional changes with vascular, metabolic, inflammatory, and neurodegenerative processes. These factors converge on common mechanisms, including NVU and BBB dysfunction, synaptic compromise, network disconnection, and glial dysregulation, that gradually wear down cognitive performance.

MCI is neither inevitable nor uniformly progressive. Its trajectory is highly heterogeneous, ranging from stability to decline or, in some cases, reversal to normal cognition; this is particularly the case when reversible contributors are identified and addressed. Such variability reinforces the importance of surveillance and early diagnosis, especially in populations with increased predisposition and established risk factors.

Brain ageing is not a passive process but one shaped by cumulative exposures across the life course. By integrating clinical vigilance, individualised risk profiling, and multidomain interventions, it is possible to extend the period of cognitive health, delay further deterioration, and preserve independence and good quality of life. In the absence of curative therapies, this proactive and holistic approach represents the most effective path to mitigating the burden of cognitive decline.

CHAPTER 1, PART 3: SENSORY IMPAIRMENT IN AGEING: NEUROCOGNITIVE AND FUNCTIONAL IMPLICATIONS

The primary senses create the interface through which individuals perceive, interpret, and respond to the world. From early development through older adulthood, vision, hearing, touch, taste, and smell support language acquisition, spatial navigation, emotional regulation, social communication, and learning. Each sensory modality contributes uniquely to perceptual awareness and cognitive integration, with multisensory processing enhancing accuracy and responsiveness in daily life.

Among the senses, vision and hearing are especially vital. They are pivotal in how humans navigate complex social and physical environments: recognising faces, interpreting speech, reading, orienting in space, or detecting danger. Unlike the more subtle impact of olfactory, gustatory, or touch, less well-defined and studied deficits, impairments in hearing or vision often result in immediate and measurable consequences for communication, mobility, autonomy, and emotional well-being. Their importance becomes even more pronounced with age.

Ageing is frequently accompanied by a growing incidence of partial or complete, mono- or multimodal sensory loss. Since vision and hearing are fundamental for environmental interaction throughout life, their age-related deterioration and loss are not simply benign markers of ageing but predictors of cognitive impairment, mental health deterioration, and functional decline. Over 25% of adults over 70 experience hearing loss, and nearly one in six has visual impairment (Liu et al., 2022). These numbers increase dramatically in the eighth and ninth decades of life. Notably, 11% of adults over 80 have dual sensory loss, a condition consistently associated with even more severe cognitive and functional deterioration (Swenor et al., 2013).

1.3.1 Sensory Impairment and Cognitive Decline

Over the past two decades, hearing and visual sensory impairment (and deprivation) have gained recognition as potential modifiable risk factors for cognitive decline in the ageing population, leading to a growing body of research dedicated to understanding the relationship. Much of the current literature available has prioritised establishing/confirming, and quantifying such correlations, and only a limited number of studies have worked on finding the biological mechanisms for it. So far, although no singular causal mechanism has been definitively confirmed, several hypotheses have emerged:

- **Reduced neural stimulation:** When auditory or visual input is diminished, the corresponding primary cortices receive less external stimulation. Over time, this chronic underuse results in decreased synaptic activity and functional disconnection from downstream regions involved in higher-order cognitive tasks, such as the prefrontal cortex and hippocampus. Animal models and human neuroimaging studies have shown that sensory deprivation can trigger maladaptive neuroplasticity, leading to synaptic pruning, cortical thinning, and regional atrophy. In older adults, these changes add to the coexisting age-related degeneration, reducing the capacity for compensatory reorganisation (Völter et al., 2021).

- **Information degradation hypothesis:** As sensory signals become less clear, the brain must make a greater effort to decode them. Such a process consumes cognitive resources that would otherwise be directed toward memory formation, attention regulation, or problem-solving. For example, when an older adult struggles to understand muffled speech, their ability to remember what was said diminishes significantly. The cognitive system, already constrained by age-related decline in working memory and processing speed, becomes overloaded with perceptual decoding. This persistent reallocation of resources weakens performance across multiple domains and may accelerate cognitive decline (Pichora-Fuller et al., 2016).

- **Social isolation and depression:** Sensory impairment frequently interferes with social interaction. Hearing loss makes conversations exhausting or embarrassing, while vision loss can limit mobility and recognition of social cues. These barriers contribute to reduced participation in social networks, which in turn leads to isolation and loneliness. Both social isolation and depression have independently been associated with increased risk of cognitive impairment and dementia (Livingston et al., 2020), and sensory impairment may open the door to those stressors, commencing a cascade of reduced cognitive stimulation, emotional dysregulation, and eventually, structural brain changes.

- **Accelerated biological ageing:** Emerging research suggests that sensory loss may not only reflect ageing, but it can also exacerbate it at a cellular level. Studies have linked sensory impairment with biomarkers of accelerated biological ageing, such as chronic systemic inflammation, oxidative stress, and telomere shortening (Loughrey et al., 2018). These processes interfere with neurogenesis and contribute to vascular alterations that impair cerebral blood flow.

- **Common cause hypothesis:** Instead of viewing sensory impairment as a causal factor, this hypothesis suggests that both sensory and cognitive decline are parallel consequences of shared neurodegenerative processes. For instance, microvascular disease, β -amyloid accumulation, or general neuronal atrophy may simultaneously affect auditory/visual systems and cognitive circuits. In this model, the observed association between sensory loss and dementia reflects a broader age-related vulnerability to neurobiological deterioration. While this does not exclude the contributory role of sensory deprivation, it considers it as an early signal of central nervous system (CNS) ageing rather than a downstream effect alone (Lindenberger and Baltes, 1994).

These five mechanisms can be considered aetiological separately, but are most likely dynamically interconnected, operating in tandem rather than in isolation to shape cognitive trajectories in later life. Older adults with vision or hearing impairment often show accelerated decline in certain domains, such as episodic memory and executive function, and these effects extend beyond mere association: neuroimaging studies have shown that sensory loss, even in a monomodal fashion, is linked to cortical atrophy, specifically in prefrontal, temporal and hippocampal regions, areas involved in perception processing and cognition (Lin et al., 2014; Völter et al., 2021). Sensory deprivation can therefore induce structural and functional brain changes.

Cognitive impairment can also exacerbate the effects of sensory loss, as individuals with early cognitive decline may be less aware of, or less able to adapt to, sensory deficits. This bidirectional relationship generates a self-perpetuating cycle: sensory loss increases the cognitive load and reduces stimulation, while cognitive decline weakens the compensatory capacity, accelerating overall decline (Deal et al., 2017; Maharani et al., 2018).

Since the scope of this study is limited to a single sensory modality, I will further elaborate on this particular one.

1.3.2 Visual Impairment

Visual (or vision) impairment is defined by the Centers for Disease Control and Prevention (CDC) as a functional limitation of the eye or vision system. It comprehends a wide spectrum of visual deficits that range from mild loss of visual clarity to complete blindness, involving alterations in one or more of the different aspects of visual function: visual acuity, visual fields, contrast sensitivity, central visual processing, etc. In the absence of treatment, such as corrective eyewear, medication, or surgical interventions, it may interfere with the individual's mobility, spatial awareness, independence in performing everyday activities, and quality of life (CDC, 2021).

The World Health Organization (WHO) classifies visual impairment based on presenting visual acuity in the better-seeing eye using Snellen fractions. In this fraction, the top number is the distance in metres at which the test is conducted, and the bottom one is the distance at which a person with normal vision can read the same line:

- Mild visual impairment: Worse than 6/12 to 6/18
- Moderate visual impairment: Worse than 6/18 to 6/60
- Severe visual impairment: Worse than 6/60 to 3/60
- Blindness: Worse than 3/60, or corresponding visual field narrowing to less than 10 degrees (WHO, 2019).

This classification system enables consistent clinical evaluation and facilitates standardised global monitoring of vision loss.

1.3.2.1 The Global Burden of Vision Impairment

Visual impairment represents one of the most pressing and, perhaps, under-addressed public health challenges. Recent global estimations indicate that approximately 43.3 million people are blind, and an additional 295 million live with moderate to severe vision impairment (MSVI). When mild visual loss is included, the total number of visually impaired individuals worldwide exceeds 1.35 billion, a number that has doubled over the past three decades (Que et al., 2025).

Such a rise is not the product of increased incidence of eye diseases but rather of shifts in demographic structures. Between 1990 and 2021, global population growth and increased life expectancy have

considerably expanded the number of older adults exposed to progressive ocular conditions. Projections for the next quarter century suggest a continued rise, with blindness expected to affect 61 million people by 2050, and MSVI projected to exceed 834 million (Que et al., 2025). This trajectory reflects the cumulative burden of untreated or inadequately treated eye conditions over time.

Older adults bear a disproportionate share of the global burden of vision loss. While the estimated 1.35 billion visually impaired individuals include all age groups, prevalence increases sharply with advancing age. Vision impairment becomes markedly more common among individuals aged 60 and older as a result of the cumulative effects of chronic and progressive eye conditions such as cataracts, age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy (WHO, 2019).

Global data from the Vision Loss Expert Group (2021) indicate that in adults aged 65 and above, the age-standardised prevalence of visual impairment has remained relatively stable over the past three decades, but the absolute number of affected individuals has grown significantly due to global population ageing (Global Burden of Disease (GBD) 2021; Han et al., 2023). This demographic shift highlights the need to address age-related vision loss not only as a clinical challenge but also as a public health priority with far-reaching consequences for autonomy, mental health, and cognitive ageing.

1.3.2.2 Leading Causes of Vision Impairment in Older Adults

Vision impairment in ageing populations arises from a distinct set of pathologies, most of which are either preventable, correctable, or modifiable in their progression. The five most common causes include:

- **Uncorrected refractive errors.** As the leading cause of visual impairment globally, representing 43% of all vision impairment cases (WHO, 2019), conditions such as myopia, hyperopia, and astigmatism can usually be corrected with spectacles, contact lenses, or refractive surgery. Unfortunately, millions lack access to basic vision screening and corrective lenses, especially in rural and low-resource settings.
- **Cataracts.** Accounting for 39.6% of global blindness and 28.3% of MSVI (GBD, 2021), cataracts remain the leading cause of blindness worldwide. Their prevalence rises sharply with age and disproportionately affects women, rural residents, and those with limited access to surgical care. Although highly treatable with surgery, cataracts continue to account for a significant share of global visual disability due to health system limitations and late-stage presentation (WHO, 2019).
- **Glaucoma.** This is the second leading cause of irreversible blindness globally. It affects an estimated 76 million people worldwide, a number projected to rise to 111.8 million by 2040. The condition often progresses silently until significant vision loss has occurred, making early detection and long-term monitoring essential. Older age, African or Asian descent, and a family history of glaucoma are key risk factors (Tham et al., 2014).
- **Age-related macular degeneration (AMD).** AMD is the leading cause of irreversible central vision loss in high-income countries, affecting over 196 million people globally (Wong et al., 2014). Its prevalence increases exponentially with age, particularly after age 75. While dry AMD progresses slowly and may be

managed with lifestyle modifications, wet AMD can cause rapid vision loss and requires timely intravitreal therapy (GBD, 2021). The increasing ageing population in Europe, North America, and East Asia makes AMD a growing public health concern.

– **Diabetic retinopathy.** Diabetic retinopathy affects approximately one in three people with diabetes and is responsible for 4.8% of global blindness (Yau et al., 2012). As diabetes prevalence increases, especially in middle-income countries undergoing nutritional and demographic transitions, diabetic eye disease is emerging as a key driver of preventable vision loss. Poor glycaemic control, duration of diabetes, and lack of retinal screening are critical modifiable risk factors (Livingston et al., 2024).

These conditions, though diverse in aetiology and progression, share a critical feature: most are preventable, treatable, or manageable with early detection and access to appropriate care. As global ageing accelerates, failing to address these vision-related diseases not only increases disability but also compounds broader age-related vulnerabilities, among which lies the susceptibility to cognitive decline.

1.3.3 Cataracts in Ageing: Biology, Burden, And Systemic Consequences

1.3.3.1 What is a Cataract?

The crystalline lens is a transparent, avascular, biconvex tissue, suspended posteriorly to the iris by zonular fibres and enveloped by an elastic basement membrane known as the lens capsule. It contributes to approximately one-third of the eye's refractive power and can alter its curvature to provide accommodation, sharpening retinal focus across distances (Chen et al., 2025). Its optical clarity depends on the extreme order of long-lived structural proteins, called α -, β -, and γ -crystallins, the lack of organelles in mature lens fibres, tightly regulated water content, and robust antioxidant/ion-transport systems sustained by the aqueous humour (Liu et al., 2017). With ageing, accommodative amplitude declines (presbyopia), and the molecular systems that maintain transparency become increasingly vulnerable to oxidative and metabolic stressors (Liu et al., 2017).

A cataract is an opacification of the crystalline lens that increases intraocular light scatter and reduces retinal image quality, causing progressive blur, glare, and contrast loss. At a molecular level, cumulative oxidative stress, post-translational protein modification, and protein homeostasis failure drive crystallins unfolding/aggregation, phase separation, and crosslinking. Reduced glutathione cycling and mitochondrial dysfunction in lens epithelium further accelerate these changes (Liu et al., 2017; WHO, 2019). Although age is the dominant driver, cataractogenesis is multifactorial: diabetes (glycation/oxidative injury), smoking (oxidants), ultraviolet-B (UV-B) exposure (photochemical damage), corticosteroid therapy (posterior subcapsular changes), ionising radiation, ocular inflammation or trauma, and genetic and metabolic disorders all contribute (Chen et al., 2025; WHO, 2019).

1.3.3.2 Cataracts as an Epidemiological Problem

Cataracts represent the single most frequent cause of age-related blindness globally. Despite being highly treatable through surgery, they continue to impose a massive burden. Globally, 17 million blind individuals and 83.5 million with MSVI have vision loss directly attributable to cataracts (GBD, 2021).

Between 1990 and 2019, the crude prevalence of cataract-related impairment increased from approximately 791 to around 1,254 per 100,000 individuals, primarily due to population growth and demographic ageing (Han et al., 2023). The age-standardised prevalence, however, has remained stable or declined slightly in some regions, indicating that surgical interventions are working but not keeping up with the demand.

Cataract burden is unequally distributed across regions and populations. In China, prevalence reaches 73% among those aged 85 to 89, compared to just 7% in individuals aged 45 to 49. In Nigeria, cataract surgical coverage is under 40%, with even lower rates in pastoral communities. Countries like Brazil and Mexico have made significant strides through public health initiatives and large-scale cataract campaigns, yet rural populations continue to face logistical and financial barriers. Rural areas worldwide endure extreme challenges, including fewer surgical centres, fewer trained eye care professionals, and socioeconomic barriers to care. Gender disparities are also pronounced, with women significantly less likely to access surgery due to social, financial, and cultural barriers. In Pakistan, female surgical coverage is 75% versus 94% in men, and unoperated cataract blindness is three times higher among women over age 50 (WHO, 2019).

High-income countries, such as the United States and Canada, offer high surgical coverage, but some barriers are prevalent. In the United States, access is often tied to insurance status, leaving uninsured or underinsured individuals, particularly in rural or low-income communities, with limited options and displaying tendencies similar to those in low- and middle-income countries (LMIC). In Canada, universal healthcare ensures coverage, but patients may face long wait times due to resource constraints (WHO, 2019).

Europe has achieved relatively high surgical coverage, although some gaps remain. Ireland presents a particularly instructive case. Despite the public funding of cataract surgery through the Health Service Executive (HSE), waiting lists are among the longest in Western Europe, with patients facing delays of up to two years for surgery. Access to care varies widely across regions, with rural and less densely populated areas often having fewer surgical centres and ophthalmologists and forcing patients to travel long distances or remain untreated. Adding to the challenge is the limited availability of comprehensive and timely national data on cataract prevalence and surgical outcomes, making it difficult to fully understand the scale of unmet need and to allocate resources effectively (HSE, 2025; Oireachtas, 2024).

1.3.3.3 Risk Factors for Cataract Formation

Ageing remains the strongest and main non-modifiable risk factor for cataract development. Cataract prevalence and severity increase sharply with each decade of life after age 60, as a result of the weakening

of oxidative and metabolic defences. In many populations, over 70% of individuals aged 85 and older are affected by cataracts (WHO, 2019). Biological sex is also an important factor, as women are more likely to develop cataracts and are less likely to receive surgical treatment. Biological factors, such as hormonal influences and longevity, partly explain the increased prevalence. However, social determinants, including reduced healthcare access, lower income, and caregiving responsibilities, contribute significantly to under-treatment. This is especially evident in LMICs, where women face higher rates of unoperated cataracts (WHO, 2019).

Several modifiable and associated risk factors have been identified, including UV-B exposure, cigarette smoking, diabetes mellitus, and corticosteroid use (both topical and systemic), which have the strongest epidemiological support (Chen et al., 2025; WHO, 2019). Other correlations described associate cataracts with the presence of hypertension, obesity, and metabolic syndrome, heavy alcohol intake, and some medication use, including phenothiazine antipsychotics, miotics, amiodarone, psoralen, tamoxifen, and statins, with different levels of evidence and, in some instances, mixed/contradictory findings.

From an ophthalmological point of view, high myopia, prior vitrectomy, uveitis, pseudoexfoliation, and ocular irradiation are also associated with cataract development. Congenital cataracts reflect genetic and metabolic causes, as well as intrauterine infections such as rubella (Chen et al., 2025; Liu et al., 2017).

Despite extensive study of nutritional and antioxidant preventative strategies, no pharmacological approach has been convincing so far, and practical measures remain UV protection, smoking cessation, and cardiometabolic risk control (Chen et al., 2025; WHO, 2019).

1.3.3.4 Types of Cataracts

Age-related cataracts are classified based on the anatomic location of opacification of the lens on the slit-lamp exam:

- **Nuclear sclerotic.** It forms in the nucleus of the lens, centrally, causing its opacification and sclerosis. It typically triggers myopic shift with progressive reduction in distance visual acuity, as well as colour desaturation (Chen et al., 2025; Liu et al., 2017).
- **Cortical.** Appears as white, radial, spoke-like (or star-shaped) opacities in the outer edge, or cortex, of the lens. Associated glare and monocular diplopia are common, with astigmatic blur due to spokes appearing in the visual field, and impaired depth perception (Chen et al., 2025).
- **Posterior subcapsular (PSC).** This plaque-like opacity forms at the posterior aspect of the lens capsule. It produces disproportionate and potentially disabling glare, contrast loss, and near-vision impairment, despite relatively preserved Snellen acuity, and often advances rapidly. It is commonly associated with steroid use, diabetes, and radiation exposure (Chen et al., 2025; Liu et al., 2017).

Less common patterns include anterior subcapsular, anterior/posterior polar, and mixed morphologies (Chen et al., 2025).

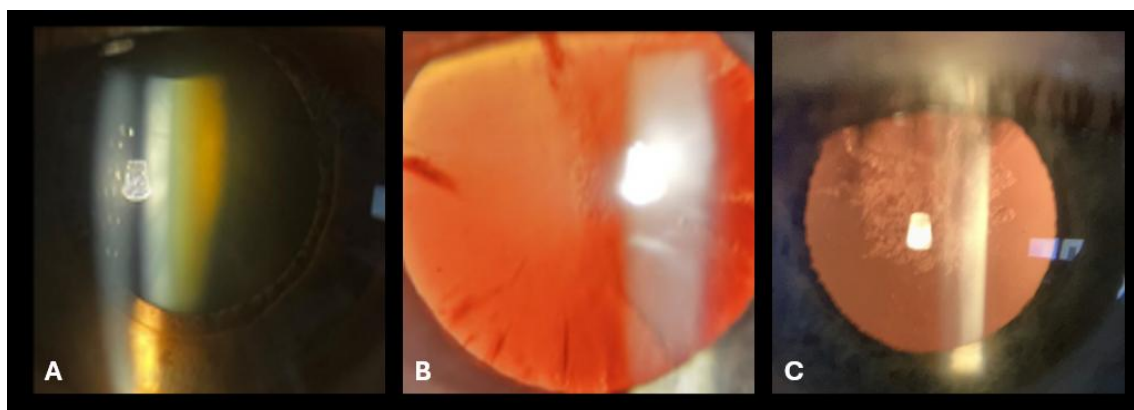


Figure 1.3. Types of age-related cataracts: (A) Nuclear sclerotic. (B) Cortical. (C) Posterior Subcapsular.

1.3.3.5 Cataract Grading System

Cataract grading systems ensure consistent documentation and allow for research comparability. The Lens Opacities Classification System (LOCS), currently in use, uses photographic standards to grade nuclear colour/opalescence, cortical, and PSC changes. While LOCS II (1989) provided reproducible photographic references and improved inter-observer agreement over earlier schemes (Chylack et al., 1989), LOCS III (1993) expanded the dynamic grading ranges and refined photographic standards, enhancing sensitivity and reproducibility. Currently, LOCS III is the most widely used classification system for epidemiology and clinical trials (Chylack et al., 1993; Liu et al., 2017).

1.3.3.6 Symptoms, Progression, and Systemic Implications

Cataracts are typically progressive and painless. By scattering and absorbing light before it reaches the retina, cataracts reduce the signal-to-noise ratio of visual input, eroding the quality of the perceived image. Early cataract manifestations include disabling glare (oncoming headlights, bright sunlight), reduced contrast sensitivity and colour desaturation, halos/starbursts, and monocular diplopia. As the lens opacification advances, visual acuity declines at near, distance, or both. Nuclear cataracts often cause myopic shift that degrades distance vision first, whereas PSC usually impairs near tasks and bright-light tolerance significantly. In advanced disease, dense opacification results in severe visual impairment and, if untreated, blindness (Chen et al., 2025; WHO, 2019).

In older adults, this visual deterioration raises cognitive effort for everyday perception, elevates postural and navigational demands, and amplifies risk of falls, fractures, depression, and loss of independence, all of which compound frailty and can accelerate institutionalisation (WHO, 2019).

1.3.3.7 Cataract Screening

Although there is no global structured screening programme for early cataract detection and management in place yet, some institutions have already established their recommendations. For example, for asymptomatic adults, the American Academy of Ophthalmology (AAO) recommends a baseline exam at

approximately age 40, and every 1–2 years after age 65, to detect not only cataracts but also other age-related conditions, such as glaucoma and AMD (AAO, 2022; Chen et al., 2025). At the health-system level, the WHO advocates for Integrated People-Centred Eye Care (IPEC) services to ensure universal access to diagnosis and management of eye conditions, including cataracts, as well as for tracking effective cataract surgical coverage as part of universal health coverage, aiming to measure not only who gets surgery, but also who attains good postoperative vision (WHO, 2019).

1.3.3.8 Diagnostic Process and Management

A comprehensive eye examination with slit-lamp biomicroscopy after dilation is the diagnostic standard for cataract detection. Visual function should also be assessed beyond high-contrast Snellen acuity, to include contrast sensitivity and glare testing, especially when symptoms outdo chart performance (Chen et al., 2025). If surgical management is planned, a preoperative macular optical coherence tomography (OCT) is valuable to uncover occult retinal disease (e.g. epiretinal membrane, macular oedema) that may limit visual prognosis (Liu et al., 2017).

Once diagnosed, and before surgery if it is not yet deemed necessary, management includes optimisation of corrective eyewear for refractive shifts, enhancing lighting/contrast, and considering low-vision devices to maintain independence (Chen et al., 2025; WHO, 2019). The indication for surgery is fundamentally functional, proceeding to intervene when cataract-related visual symptoms interfere with activities of daily living or safety (e.g. night driving, falls), and not driven by visual acuity alone. Since PSC can devastate glare tolerance and near performance with relatively preserved Snellen acuity, patients may undergo surgery even with “20/20” visual acuity if their functional disability is substantial (Chen et al., 2025). Additional indications include cataract-induced anisometropia, lens-induced glaucoma/inflammation, or when opacity obstructs the diagnosis and management of posterior segment diseases (Liu et al., 2017).

1.3.3.9 Cataract Surgery

Cataract extraction is typically performed as an outpatient procedure under topical or local anaesthesia.

– **Phacoemulsification (phaco).** The predominant cataract technique worldwide uses a microincision and continuous curvilinear capsulorhexis, followed by ultrasonic nuclear emulsification with aspiration, cortical clean-up, and implantation of a foldable intraocular lens (IOL) in the capsular bag; the self-sealing wound typically requires no sutures (Liu et al., 2017). It accounts for the majority of cataract operations, especially in high-income settings, with an estimated 17.7 million procedures reported in 2018 (Bali et al., 2022).

An uncomplicated phaco usually takes between 10 and 20 minutes per eye. Functional visual improvement is expected to occur within days, and refractive stabilisation takes approximately two weeks. Corrective eyewear, if needed, should be reviewed after this period (Liu et al., 2017). Activity restrictions are minimal after this small-incision surgery (Chen et al., 2025).

– **Small-incision cataract surgery (SICS).** A manual extracapsular technique uses a self-sealing scleral tunnel to retrieve the nucleus, followed by cortical aspiration and posterior-chamber IOL implantation. It tends to be favoured in LMICs and high-volume outreach because it is fast, low-cost, and equipment-light, with excellent visual outcomes when done by experienced hands (Liu et al., 2017).

– **Femtosecond laser–assisted cataract surgery (FLACS).** Uses a femtosecond laser to create clear-corneal incisions, a precise anterior capsulotomy, and nuclear pre-fragmentation, after which standard phacoemulsification and IOL implantation are completed. Its use is limited and is mostly employed in selected cases, such as weak zonules, a shallow anterior chamber, or for certain premium-IOL cases. Comparative studies have shown no consistent clinical advantage over conventional phaco for routine surgery and higher cost, so most systems reserve FLACS for refractive or premium indications rather than standard care (Liu et al., 2017).

1.3.3.10 Surgical Complications

Serious adverse events are uncommon with modern cataract surgery. Intraoperatively, posterior capsule rupture with vitreous loss occurs in approximately 1% of cases, increasing the risk of cystoid macular oedema (CME) and endophthalmitis, and potentially limiting IOL selection options. Early postoperative risks include infectious endophthalmitis in 0.09 to 0.17% of cases within 30 days, toxic anterior segment syndrome, a sterile inflammation responsive to steroids, in 0.22% of cases, and CME after uncomplicated phaco in 0.1 to 3.8% of patients (Liu et al., 2017). The most common late-onset complication is posterior capsular opacification, affecting between 7 and 23% of cases by 5 years, and typically managed with outpatient laser capsulotomy (neodymium-doped yttrium aluminium garnet (Nd:YAG)) (Liu et al., 2017).

Overall, large registries show that more than 90% of patients achieve 20/40 or better corrected distance vision postoperatively, with very low rates of vision-threatening events when current standards of care are met (Liu et al., 2017).

1.3.3.11 Quality of Life, Functional Recovery

Cataract surgery is among the most cost-effective medical procedures, delivering substantial benefits, such as increased productivity, reduced care dependency, and enhanced quality of life. It delivers substantial, patient-centred benefits, including sharper distance vision, better contrast sensitivity, enhanced colour perception and binocular function, and greater ease with night driving and reading. The procedure also reduces glare, lowers the risk of falls and depressive symptoms, and supports daily functioning, mobility, and social participation (WHO, 2019). Second-eye surgery adds further gains in visual function and daily functioning and remains cost-effective in health-economic analyses (Liu et al., 2017). Observational studies also suggest associations with lower rates of falls, fractures, and, in some populations, a reduced risk of developing dementia. These findings align with sensory–cognitive models, although causal associations remain to be fully proven (Liu et al., 2017; WHO, 2019).

1.3.4 Cataracts and Cognitive Impairment: Mechanisms, Brain Changes, and the Impact of Surgery

Cataracts exemplify a “low-signal visual state”, where retinal inputs arrive dimmer, noisier, and temporally distorted as the opacified lens scatters and absorbs light. In ageing adults, that degraded input does not exclusively affect the eye and its sensory capacity. Multiple research studies, including those detailed below, have shown that visual sensory deprivation, such as that from cataracts, reshapes everyday cognition by interfering with how efficiently attention is used, how much working memory is stressed by visually demanding tasks, and how executive function fatigue is accelerated from sustained compensation.

Below is a synthesis of current knowledge and remaining uncertainties about the cognitive impact of cataracts, including the magnitude of their effect, the neurobiological pathways linking lens opacity to brain changes, and the functional and structural outcomes of cataract surgery and vision correction.

1.3.4.1 Cognitive Impact of Cataracts

As outlined in the sensory impairment section, multiple observational cohorts and systematic reviews have consistently correlated late-life visual impairment with accelerated cognitive decline and increased dementia risk, and cataracts, as the leading cause of MSVI and blindness in this age group, contribute substantially to this association.

Accumulating evidence indicates that older adults with visual deterioration perform worse on global cognitive tests and experience faster decline in specific domains, like executive functions and episodic memory, compared to peers with intact vision. This effect remains significant after controlling for age, education, and comorbidities (Nagarajan et al., 2022). In the specific case of cataracts, meta-analytic data suggest a higher incidence of cognitive impairment in those with cataracts compared with those without them (Xiong et al., 2023). The absolute effect size varies by diagnostic instruments used and follow-up frequency and timing, but the overall consistent finding of a decrement in combined cognitive scores at baseline and a steeper decline over time remains (Nagarajan et al., 2022; Xiong et al., 2023).

Knowing the deleterious effects of cataracts, a central question to address is whether reversing sensory loss can alter such cognitive trajectories. The most compelling answers to date come from epidemiological studies: in a large, well-characterised North American cohort followed over three decades, cataract surgery was linked to a significantly lower risk of developing dementia compared to matched peers who did not undergo the procedure (adjusted hazard ratio ≈ 0.71 ; about a 29% reduction in risk) (Lee et al., 2022). Consistent with this, a systematic review and meta-analysis reports similarly protective associations for both all-cause dementia and Alzheimer disease (Zhang et al., 2023). While these findings are observational and cannot establish direct causality, the consistency of the identified association has attracted attention towards the underlying mechanisms of it.

In Anstey and colleagues’ study, immediate postoperative neuropsychological re-testing in otherwise healthy adults showed modest to no improvement, whereas several older trials reported little short-term changes on global assessments (Anstey et al., 2006). On the other hand, longer-term evaluations have shown different and more positive results, suggesting that the dementia risk accumulated over years may

be more responsive to restored sensory input and subsequent mobility, sleep, and social engagement improvements than what could be identified through brief postoperative changes on cognitive tests (Nagarajan et al., 2022). More recent studies have focused on identifying domain-specific benefits, such as improvements in processing speed and attention, and subgroup analysis gains, like in those starting with poorer cognition, rather than expecting large, dramatic gains across all tests within weeks (Nagarajan et al., 2022; Yeo et al., 2024).

1.3.4.2 Neurobiological Pathways Linking Lens Opacity to Brain Changes

Mechanistically, while no specific causal link has been proven, existing hypotheses offer a compelling multidimensional framework connecting cataracts to biological changes leading to cognitive decline. Besides the aforementioned theories of reduced neural stimulation and plasticity, information degradation and cognitive overload, social isolation and depression, accelerated biological ageing, and the common cause hypothesis, proposed for both hearing and vision loss, two specific hypotheses have been suggested, directly linking cataracts to cognitive impairment. These are still under scrutiny as evidence is still emerging.

- **Disruption of sleep and circadian physiology.** The ageing lenses, brunescent and opaque as cataracts progress, filter out short-wavelength (blue) light, which weakens the brain's ability to receive circadian signals. This reduces light-driven melatonin suppression, leading to impaired circadian alignment and poorer sleep quality. Since sleep plays a significant role in memory consolidation and brain waste clearance, this pathway impacts everyday performance and potentially accelerates degenerative changes due to the accumulation of neurotoxic molecules. After cataract surgery, sensitivity to light often improves, circadian rhythms stabilise, and sleep metrics tend to recover. Notably, intraocular lenses (IOLs) that transmit blue and UV light may yield better cognitive and sleep outcomes than blue-blocking designs, according to several small studies (Brøndsted et al., 2015; Chellappa et al., 2019; Wei et al., 2013).

- **Shared risks and vascular burden.** Both cataracts and cognitive decline share a range of common risk factors, specifically those associated with ageing and cardiometabolic health, including diabetes, hypertension, hyperlipidaemia, and smoking. These factors contribute not only to lens opacification but also to cerebrovascular compromise, making the vascular system a potential shared pathway linking ocular and cognitive ageing. Cataracts then may serve as a visible biomarker of cumulative vascular and oxidative stress that also affects the brain. Supporting this, genetic and epidemiological studies have reported associations between cataracts and smaller brain volumes, white matter changes, and higher cerebrovascular burden (Ferguson et al., 2024; Ma et al., 2023). These findings suggest that the relationship is not purely confounded by age but may reflect a partially causal link through shared vascular pathology.

1.3.4.3 Brain and Cognitive Changes in Cataract-Related Sensory Deprivation

Supporting the cataracts–cognitive decline relationship, neuroimaging studies have documented evidence that prolonged visual impairment may lead to cortical atrophy and functional reorganisation. Before

cataract surgery occurs, older adults with significant vision loss exhibit reduced local processing capacity and diminished neural engagement not only in primary visual cortices but also in higher-order cognitive regions, including the superior parietal lobule and precuneus, as shown in functional neuroimaging studies (Lin et al., 2018).

Grey matter volume reductions have also been observed across occipital, parietal, and temporal cortices. Large-scale population data associate cataracts and poorer visual acuity with lower total and grey matter brain volume, as well as increased white matter hyperintensities, suggesting widespread neuroanatomical vulnerability (Ferguson et al., 2024).

Certain regions appear particularly sensitive to chronic visual deprivation: thinning of the anterior calcarine cortex, for example, correlates with diminished visual acuity and contrast sensitivity, which in turn relate to slower processing speed and reduced visuospatial perception performance. Involvement of the insula, a hub for salience and attentional reallocation, further points to the disruption of associative networks beyond the striate cortex (Chamard et al., 2022; Griffis et al., 2016). These structural alterations align with frequently reported cognitive deficits in visually impaired older adults, including decline in processing speed, executive function, visuospatial attention, and working memory (Hong et al., 2024; Nagarajan et al., 2022). Notably, some of these cognitive changes, such as visuospatial attention deficits and executive dysfunction, have been directly linked to reduced grey matter volume and altered activity in the superior parietal lobule, precuneus, and other regions implicated in attention and control processes (Ferguson et al., 2024; Lin et al., 2018).

1.3.4.4 Functional and Structural Outcomes of Cataract Surgery and Vision Correction

Cataract surgery, while intended primarily as a sensory intervention, induces measurable structural and functional changes in the brain, most of which have been described within visual and attention-related networks. Some longitudinal neuroimaging studies have shown that visual restoration triggers cortical plasticity. Within approximately six weeks after unilateral extraction, grey matter thickening has been detected in the contralateral secondary visual cortex (V2), with the magnitude of change correlating with postoperative binocular acuity symmetry, possibly a proxy for restored binocular integration (Lou et al., 2013). Over the subsequent months, broader recovery has been observed, including increased spontaneous activity in regions such as primary visual cortex (V1), lingual gyrus, cuneus, precuneus, and superior parietal lobule, along with grey matter gains in the middle occipital gyrus, fusiform gyrus, posterior parietal cortex, and middle temporal gyrus (Lin et al., 2018). These areas support core visual functions, including object recognition, facial recognition, spatial mapping, and attentional control, and their recovery aligns closely with patients' subjective reports of improved contrast, reduced glare, faster visual search, and better reading speed after surgery.

The impact of surgery extends beyond the visual system. These neural shifts appear to reduce the perceptual and cognitive load associated with vision-dependent tasks, contributing to improved dual-task gait performance, increased mobility, and better sleep, factors that reinforce cognitive resilience in older adults (Maharani et al., 2018). While grey matter plasticity is well documented in the visual cortex,

evidence from sight-restoration studies in congenitally blind populations suggests that white matter integrity may also improve post-intervention (Pedersini et al., 2023). Although extrapolations to age-related cataract must be cautious, given the differing developmental contexts, evidence points toward the brain's capacity for structural recovery following restored input. However, other studies highlight that behavioural gains can precede detectable structural changes, particularly in association cortices, indicating region-specific timelines of neuroplasticity (Ashtari et al., 2023).

In terms of cognitive performance, the effects of cataract surgery are documented to be most evident in specific domains rather than global cognitive metrics. While changes on broad screening instruments such as the Mini-Mental State Examination (MMSE) tend to be small, often diluted by ceiling effects and practice bias (Anstey et al., 2006), more targeted testing reveals meaningful short-term improvements in processing speed, sustained attention, and episodic memory, particularly when assessments are vision-normalised and follow-up extends beyond the immediate postoperative period (Maharani et al., 2018; Yeo et al., 2024). These benefits may be greater in subgroups with poorer baseline vision, mild cognitive impairment, or advanced age. Meta-analytic estimates suggest an average cognitive test improvement of 4% post-surgery (Yeo et al., 2024), although larger individual gains likely occur. Notably, epidemiological data show that cataract surgery is associated with a significantly reduced risk of long-term cognitive decline, with studies reporting a 29% lower hazard of dementia compared to non-surgical peers (Lee et al., 2022).

Mechanistically, these cognitive gains may be mediated not only by direct improvements in visual input and visual cortex function but also through collateral pathways and functional gains. For example, enhanced sleep quality and improved gait safety, both improved after cataract surgery (Brøndsted et al., 2015; Chellappa et al., 2019), are tightly coupled to attentional stability and executive control in older adults. These interconnected outcomes may represent the functional bridge between restored sensory signal-to-noise ratio and broader neurocognitive protection. At a neural level, changes have been most consistently detected in early visual areas (V1, V2), visuospatial attention networks (e.g. superior parietal lobule, precuneus), and salience-related hubs like the insula, which may undergo adaptive reorganisation in response to sensory deprivation and recovery (Chamard et al., 2022).

At a microscale, direct in vivo data on the biochemical and neurophysiological dynamics of cataract-related plasticity remain limited. Some magnetic resonance (MR) spectroscopy and blood-oxygen-level-dependent (BOLD) variability studies imply that visual input restoration may influence cortical inhibitory tone and signal variability, parameters often associated with efficient information processing in ageing brains (Elshout et al., 2021). Though histological evidence specific to age-related cataract is currently lacking, emerging imaging data confirm that the adult brain retains functional and structural plasticity well into older age, particularly in response to restored sensory input.

1.3.4.5 Remaining Uncertainties

Despite the current interest in the vascular mechanisms of cognitive ageing, no human study has identified, to date, any cataract-related changes in the blood–brain barrier (BBB), neurovascular unit

(NVU), or neurovascular coupling (NVC). The NVU's role of matching neural activity to local blood flow and preserving BBB integrity is well established (Iadecola, 2017; Kaplan et al., 2020), but in cataract cohorts, the identified and reproducible findings remain macroscopic and functional: altered activation and grey-matter metrics that track degraded visual input, rather than microvascular injury. Any vascular or coupling effects, if present, are inferred to be indirect, more likely downstream of reduced activity or cardiometabolic comorbidity than a direct consequence. Population genetics and imaging research link vision loss to smaller brain volumes and higher vascular dementia risk, but these patterns point to shared vascular risks rather than cataract-specific BBB disruption (Ferguson et al., 2024; Ma et al., 2023). This highlights a critical research gap: defining whether subtle BBB/NVU/NVC changes accompany cognitive gains after cataract surgery, and, if so, where and how they operate; this question constitutes a central focus of the present study.

1.3.5 Conclusion

Across late life, failing senses reshape cognition, functionality, and mood. Vision and hearing dominate the landscape as they participate in communication, mobility, safety, and social participation. Evidence supports a multifactorial causation model where degraded sensory input increases perceptual effort and diverts resources from memory and executive control, reduced engagement fosters isolation and depression, and ageing biology limits compensation. Within this frame, cataract stands out for its prevalence, preventability, and restorability, making it a high-yield target for brain-health strategies.

Cataract remains the leading cause of blindness and MSVI worldwide in the ageing population. Clinically, cataract is more than a fall in high-contrast acuity: early glare and contrast deficits degrade the individual's real-world functioning and corrode performance in the daily activities that sustain cognitive health. Surgical correction through small-incision phacoemulsification (and SICS where appropriate) is brief, safe, and cost-effective.

Restoring visual signal-to-noise has benefits beyond vision and functionality. Surgery reduces compensatory cognitive load, improves mobility and sleep, and is associated with slower cognitive decline and lower incidence of dementia at a population scale. Postoperatively, visual and attention networks show functional reactivation and regionally specific structural plasticity, consistent with preserved adaptability in later life. Although short-term global test changes are modest, vision-normalised measures detect meaningful gains in processing speed, attention, and episodic memory.

To date, important uncertainties remain, as cataract-related changes in the BBB/NVU/NVC associated with structural adaptations have not been studied in humans yet. Current research data favour macrostructural cognitive, behavioural, and circadian mechanisms, with shared vascular risk as background context.

CHAPTER 2: THESIS HYPOTHESIS, AIMS & OBJECTIVES

2.1 Introduction

Sensory deprivation, even when partial and gradual, places an extra burden on an individual's cognition, as it increases perceptual effort and reduces the quality and reliability of the environmental input. In older adults, this occurs on a background of existing neural vulnerability, where compensatory mechanisms are already working extensively. Cataract-related visual impairment is one of the most common forms of sensory deprivation in later life and has been associated with reduced quality of life, loss of independence, and a higher risk of functional and cognitive decline. From a neurobiological perspective, prolonged visual degradation is considered to be more than a simple peripheral problem of the lens; it is a chronic alteration in the quality of sensory information reaching cortical networks that support attention, memory, language, and visuospatial processing.

In parallel, the integrity of the blood–brain barrier (BBB) and the cerebral microvasculature is increasingly recognised as central to brain ageing and cognitive status. Low-grade BBB leakage and subtle vascular dysregulation have been described in healthy older adults, in preclinical stages of several neurodegenerative conditions, and progressively more marked in individuals with established mild cognitive impairment (MCI). Dynamic contrast-enhanced MRI (DCE-MRI) now allows in vivo quantification of such leakage, providing a window on BBB behaviour that can be examined alongside detailed neuropsychological profiles. When combined with standardised cognitive assessment, these tools offer an opportunity to explore how sensory loss and subsequent sensory restoration through cataract surgery may relate not only to cognitive performance but also to BBB function and broader neurovascular health in the ageing brain.

In this project, we followed older adults scheduled for bilateral cataract surgery at three timepoints: before surgery (T0), approximately 3 months after surgery (T1), and approximately 9 months after surgery (T2). This work was designed to address three broad questions:

1. How cataract-related visual impairment relates to cognition and brain vascular physiology and macrostructure at baseline.
2. How cognition changes after restoration of best-corrected vision; and
3. How BBB permeability, brain structure, and proteomic markers behave in parallel.

The study did not require participants to have cognitive complaints or a diagnosed cognitive disorder, as the emphasis was on the cognitive changes that occurred over time rather than on pre-existing impairment. As a result, the sample was deliberately heterogeneous, with participants ranging from 60 to their early eighties and covering a wide range of baseline cognitive profiles.

It is important to note that, although prior literature has established that visual impairment is a modifiable risk factor for cognitive decline, the mechanisms underlying this association remain incompletely understood. The present study does not challenge the established epidemiological relationship; instead,

it seeks to clarify how sensory deprivation may influence cognitive systems and cerebrovascular integrity, and whether restoration of high-quality visual input reverses these effects.

2.2 Primary Hypothesis and Overall Aim

The main working hypothesis of this study is that partial sensory deprivation due to cataract-related visual impairment has a negative impact on the cognitive profile of older adults, even in the absence of manifest cognitive complaints, and that this is accompanied by changes in BBB permeability. We further hypothesised that restoration of best-corrected vision after bilateral cataract surgery would be associated with measurable improvement or stabilisation in cognition and BBB-related measures, rather than continued deterioration over time.

More specifically, we hypothesised that:

1. At baseline, worse visual acuity (VA) and greater cataract burden would be associated with a less favourable cognitive profile in this ageing cohort, independently of other major risk factors.
2. BBB permeability would show alterations potentially related to visual sensory deprivation, cognitive vulnerability, or a combination of both.
3. After surgery, and once best-corrected vision was restored, both cognitive performance and BBB metrics would show quantifiable improvement or stabilisation at T2.

The overall aim of the project was to characterise longitudinal changes in visual function, cognition, and brain physiology in older adults undergoing bilateral cataract surgery, and to explore possible links between sensory input, cognitive performance, and BBB integrity.

In order to test this multifaceted hypothesis, several aims for this project were established. These are defined below.

2.3 Aim 1: To Establish Baseline Cataract-Related Visual, Cognitive, and Brain Status

To determine whether cataract-related visual deprivation at baseline is already reflected in cognitive performance and brain status before any surgical intervention.

2.3.1 Specific Objectives

1. To characterise cataract severity by type (nuclear, cortical, posterior subcapsular (PSC)) and grade in each eye, and to measure best-corrected VA (logMAR) for each eye at T0 in adults aged 60 years and above.
2. To examine whether baseline visual impairment is associated with lower global cognition and specific Repeatable Battery for the Assessment of Neuropsychological Status-Update (RBANS-U) domain scores at T0.

3. To explore whether baseline visual parameters (VA and cataract grading) show any relationship with BBB permeability measures and structural brain indices, including total intracranial volume (TIV), regional volumes, cortical thickness, and brain-age estimates at T0.

2.3.2 Rationale and Approach

This aim focuses on the starting point of the cohort, before any change in visual input is achieved. It asks whether the degree of visual impairment from cataracts is already mirrored in cognitive performance and in macro- and microvascular brain status. Cataracts were graded per eye for nuclear, cortical, and PSC components, and VA was recorded in logMAR units under best-corrected conditions. Baseline cognition was assessed with the RBANS-U, and MRI was used to obtain DCE-derived BBB metrics (Ktrans, percentage of disrupted voxels, 95th percentile leakage) alongside volumetric, cortical-thickness, and brain-age measures. Cross-sectional associations between visual function, cognition, and brain indices at T0 were examined using correlation analyses and graphical tools (heatmaps and forest plots), in order to establish whether cataract-related sensory deprivation is already linked to cognitive and neurovascular vulnerability prior to surgery.

2.4 Aim 2: To Characterise Longitudinal Cognitive Trajectories Following Restoration of Best-Corrected Vision

To determine whether restoring best-corrected vision with bilateral cataract surgery is accompanied by measurable changes in cognitive performance over time, and whether these trajectories differ in individuals with MCI.

2.4.1 Specific Objectives

1. To track longitudinal changes in global cognitive performance using the RBANS-U Total Scale percentile scores across T0, T1, and T2.
2. To examine changes in individual RBANS-U domains (Immediate Memory, Visuospatial/Constructional, Language, Attention, and Delayed Memory) and their respective subtests over the same time period.
3. To characterise cognitive trajectories in the subgroup of participants who met criteria for MCI at baseline, and to compare their pattern of change with that of the full cohort.
4. To monitor mood and anxiety using the Beck Depression Inventory-II (BDI-II) and the State–Trait Anxiety Inventory (STAI-Y1 and STAI-Y2) across T0, T1, and T2, as potential modifiers of cognitive performance.

2.4.2 Rationale and Approach

This aim addresses the core clinical question of the thesis: whether cognitive performance changes after visual input is restored, and whether any such changes are evident beyond practice effects and affective

fluctuation. Participants completed RBANS-U at T0, T1, and T2. Group-level changes in global and domain scores were assessed using repeated-measures statistics with appropriate post hoc tests. The MCI subgroup was analysed separately to test whether individuals with more vulnerable baseline cognitive profiles exhibit different or amplified trajectories compared with the overall sample. BDI-II and STAI-Y1/STAI-Y2 scores were tracked across all timepoints, providing a context in which to interpret cognitive changes and to assess whether improvements (or declines) in cognition are more likely to reflect sensory and neurobiological factors than shifts in mood or anxiety.

2.5 Aim 3: To Examine Neurovascular, Structural Brain, Brain-Age, and Proteomic Changes Accompanying Visual Restoration

To investigate longitudinal changes in BBB permeability, structural brain measures, and circulating proteomic profiles, and to explore how these potential mechanisms relate to visual and cognitive trajectories following cataract surgery.

2.5.1 Specific Objectives

1. To quantify whole-brain and regional BBB permeability using DCE-MRI at T0, T1, and T2, including percentage of disrupted voxels, 95th percentile leakage, and Ktrans, and to characterise regional patterns across all brain lobes.
2. To assess longitudinal changes in brain structure, including TIV, grey and white matter volumes, regional TIV, and regional cortical thickness across lobes, between T0, T1, and T2.
3. To estimate brain-age at T0 and T2 using the VolBrain BrainAGE pipeline, by computing a brain-age/chronological-age ratio, and to evaluate whether this Rate of Brain Ageing changes over time at the group level.
4. To explore whether changes in BBB permeability, structural indices, and brain-age measures correlate with changes in cognition and VA.
5. To identify serum proteins that change in expression over time, and to consider how these might relate to BBB status, structural brain measures, and cognitive outcomes.

2.5.2 Rationale and Approach

This aim focuses on the biological substrates through which a peripheral intervention, cataract surgery, might influence the ageing brain. Changes in visual input could modulate neurovascular coupling, metabolic demand, and inflammatory tone, with downstream effects on BBB function and brain structures. DCE-MRI was used to quantify BBB leakage globally and by lobe, and regional values were also expressed relative to whole-brain disruption (region/whole-brain ratios) to account for global age-related permeability. Structural MRI provided volumetric and cortical thickness measures as a macrostructural backdrop against which more subtle vascular and biological changes could be interpreted. Brain-age was estimated at T0 and T2 to determine whether the brain appeared biologically older or younger than

expected for each participant's chronological age, and whether this ratio shifted following visual restoration. Proteomic analyses were used to screen for candidate circulating markers, particularly vascular, endothelial, and inflammatory proteins, that might mirror or mediate neurovascular changes. Statistical analyses included repeated-measures comparisons and correlation models, focusing on whether BBB, structural, brain-age, and proteomic changes aligned with visual and cognitive trajectories rather than with overt macroscopic atrophy alone.

CHAPTER 3: METHODOLOGY

3.1 Study Overview

This study was designed as a prospective, single-centre observational cohort study conducted within the Progressive Vision Research Program. All participants were recruited from Progressive Vision (PV), a private ophthalmology clinic collaborating with the Campbell Neurovascular Genetics Laboratory at Trinity College Dublin (TCD). Study visits took place at Progressive Vision, at St James's Hospital (SJH), and at the Centre for Advanced Medical Imaging (CAMI) located on the St James's Hospital campus in Dublin, Ireland. Protocol development began in July 2022, and participant follow-up concluded in July 2025.

Ethical and regulatory oversight was provided by the St James's Hospital–Tallaght University Hospital Joint Research Ethics Committee. Provisional approval was obtained in November 2022, with full approval attained in January 2023 (Project ID: 2211). The study was also registered with the St James's Hospital Research and Innovation Programme (Project ID: 7747). The legal basis for the study was informed consent, in line with the requirements of the General Data Protection Regulation (GDPR) (EU Regulation 2016/679). All participants received verbal and written study information and were given 48 to 72 hours to consider enrolment before signing written consent. All procedures were conducted in accordance with the Declaration of Helsinki. Transportation, food, and drinks were provided for every study visit.

Recruitment began on March 22, 2023, and closed on May 15, 2024. Data collection was completed and locked on July 17, 2025, following the final participant visit.

Participants fulfilling the inclusion criteria were enrolled immediately after a confirmed diagnosis of bilateral age-related cataracts, established through routine clinical practice at Progressive Vision. Cataracts were diagnosed using the Lens Opacities Classification System II (LOCS II) grading scale, in line with the clinic's standard protocol.

Longitudinal follow-up consisted of three planned timepoints: baseline (T0), 3 months (T1), and 9 months (T2) after second-eye surgery. Each participant acted as their own control. Visit windows were harmonised, and no participant completed a follow-up visit earlier than the required three-month and nine-month landmarks. A window of one to two months after each scheduled timepoint was permitted to accommodate participants' availability. All appointments were arranged flexibly to ensure full protocol completion while maintaining the temporal structure of the study. The three study visits included the following assessments:

- T0 (baseline) was completed at PV and included questionnaires covering baseline demographic and medical history, along with neurocognitive and affective testing using the Repeatable Battery for the Assessment of Neuropsychological Status–Update (RBANS-U) version A or B, the Beck Depression Inventory–II (BDI-II), and the State-Trait Anxiety Inventory, Form Y (STAI-Y). Participants underwent a

complete ophthalmic assessment, including LOCS II cataract grading and photography, followed by retinal imaging with Optical Coherence Tomography (OCT). Within seven days of the T0 visit, participants attended CAMI for Magnetic Resonance Imaging (MRI), including Dynamic Contrast-Enhanced MRI (DCE-MRI), and provided venous blood samples collected into two serum tubes.

– T1 occurred three months after completion of the second eye surgery. The assessment protocol was the same as baseline, except for the questionnaire, which focused on interval changes in demographics or health status, and the RBANS-U form, which was the alternative version to that used at T0. All imaging and ophthalmic assessments were unchanged.

– T2 occurred nine months after completion of the second eye surgery. Procedures again matched the prior timepoints. The same follow-up questionnaire used at T1 was administered, and the RBANS-U form was the same one used at T0.

The primary aim of the study was to determine whether restoration of best-corrected vision following bilateral cataract surgery was associated with longitudinal changes in global cognition, blood–brain barrier (BBB) permeability, and brain structure. Specific objectives were to track cognitive trajectories using the RBANS-U, to characterise alterations in BBB permeability using DCE-MRI and circulating biomarkers relevant to neurovascular function, and to describe accompanying changes in global and regional brain macrostructure and brain-age estimates.

Primary outcomes were prespecified as the change in RBANS-U Total Scale percentile scores across T0, T1, and T2, the change in BBB permeability metrics derived from DCE-MRI (percentage of suprathreshold voxels, 95th percentile leakage, and Ktrans), and the rate of brain ageing between T0 and T2. Secondary outcomes included changes in individual RBANS-U domain scores, lobar BBB permeability indices (frontal, temporal, parietal, occipital), extended structural MRI measures such as global and lobar grey and white matter volumes and regional cortical thickness, and exploratory longitudinal changes in circulating proteins with putative vascular, endothelial, or inflammatory roles.

3.2 Participants

3.2.1 Recruitment

Participants were recruited after receiving a diagnosis of bilateral age-related cataracts at Progressive Vision. Patients were referred to PV by their general practitioner because of visual complaints. As part of routine clinical practice at PV, all new patients were asked whether they would be open to hearing about research opportunities if eligible.

Patients were approached with the study offer immediately after cataract diagnosis and placement on the surgical waiting list. Cataract severity itself was not an inclusion or exclusion criterion, provided the cataracts were age-related and expected to benefit from surgery. As part of the surgical pathway,

individuals were scheduled for a series of preoperative assessments at PV. When a patient agreed to hear more about the study, the researcher organised an in-person discussion to coincide with one of these scheduled clinical appointments. During this meeting, the study purpose and procedures were explained, and questions were addressed. Patients received a Participant Information Leaflet (PIL) and were given 48 to 72 hours to consider participation, based on their preference. A follow-up call confirmed their decision. Those who agreed were booked for a visit, scheduled within four weeks of the information meeting and always at least two weeks before their first cataract surgery. Written informed consent was obtained before any study procedures were performed.

Recruitment was consecutive and included all eligible patients who consented to be contacted for research. Recruitment was paused only during holiday periods and the researcher's annual leave. Screening logs were maintained throughout the entire recruitment period.

3.2.2 Inclusion criteria

- Age older than 60 years.
- Bilateral age-related cataracts diagnosed at PV.
- Subjective visual impairment symptoms.
- Planned staged bilateral cataract extraction (usual interval three to six months between procedures).
- Sufficient English proficiency to complete all assessments.
- No requirement for cognitive symptoms. Eligibility was independent of baseline cognitive performance, as the study aimed to evaluate cognitive change rather than pre-existing status.

3.2.3 Exclusion criteria

- Neurologic or psychiatric conditions likely to interfere with study completion: dementia, schizophrenia, bipolar disorder, personality disorder, or drug or alcohol dependence.
- Ophthalmic conditions predicted to limit postoperative improvement or confound measurement: significant diabetic retinopathy requiring intervention during follow-up, active intraocular inflammation, vitreous haemorrhage, macula involving retinal pigment epithelial tears, subfoveal fibrosis or atrophy, submacular surgery, prior vitrectomy, history of rhegmatogenous retinal detachment or macular hole stage 3 or 4, large subretinal haemorrhage involving the fovea, aphakia or absent posterior capsule, uncontrolled glaucoma with pressures 30 mmHg or higher despite therapy, prior glaucoma filtering surgery, and prior corneal transplant. Recent photocoagulation within one month, subfoveal focal laser, prior intravitreal drug or device administration, or submacular procedures were also exclusionary.
- Imaging contraindications: inability to undergo MRI, contraindication to MRI contrast, or contraindication to pharmacological pupil dilation.
- Other exclusions: active systemic infection or inability to comply with assessments or follow-up.

3.3 Study Procedures and Timeline

3.3.1 Visit schedule

Each participant completed three study visits. These comprised a baseline pre-surgery visit (T0), conducted between three and one month before first eye surgery; a first follow-up (T1), scheduled for three months after completion of second eye surgery and occurring in most participants between six and nine months after T0, determined by the timing of their surgeries; and a final visit (T2), scheduled for nine months after second eye surgery, approximately six months after T1 and typically between 12 and 18 months after T0. All visits were arranged in accordance with participant availability.

All visits followed the same structure with minimal variations. The initial part of each visit took place in Clinical Room 4 at PV, with standardised lighting, and temperature adjusted to participant comfort. PV staff were aware of assessments in progress, and the room door remained closed throughout to avoid interruptions. All appointments were scheduled on Fridays between 10:00 and 14:00h. The full PV visit required approximately two hours. Participants were advised to bring their current glasses and to use sunglasses after pupil dilation. Driving was prohibited after dilation, and transportation was arranged when needed. At the start of each visit, the researcher ensured the participant had the opportunity to use the restroom and provided tea, coffee, or water according to preference.

The second portion of each visit was completed at SJH, where venous blood sampling and cannulation took place in a consultation room within the Memory Service. Participants were then escorted to CAMI for MRI, including DCE-MRI. This part of the visit typically lasted 60 to 90 minutes and did not require any specific restrictions.

3.3.1.1 T0 Procedures

The PV section of the T0 visit included final confirmation of informed consent and discussion of any remaining questions. Participants completed a structured demographic and full medical history questionnaire administered by the researcher, which required 15 to 20 minutes. Neurocognitive assessment was then performed using the RBANS-U (Form A or Form B), administered by the researcher, a neurologist trained in neurocognitive evaluation, following the standardised administration script and guidelines outlined in the RBANS manual and stimulus booklet (30 to 45 minutes). Participants then completed the BDI-II and STAI-Y independently in the clinic room. If visual or physical limitations prevented independent completion, the researcher read each question aloud and recorded responses. These forms required approximately 10 to 15 minutes. Once RBANS-U testing was completed, rest breaks were offered to minimise fatigue.

Following cognitive assessment, participants underwent a complete ophthalmic examination. A PV optometrist assessed monocular visual acuity (VA) under habitual correction, after which topical corneal anaesthesia and fluorescein staining were applied using fluorescein sodium 0.25% with lidocaine hydrochloride 4% eye drops (Minims Lidocaine & Fluorescein, Bausch & Lomb). If no contraindications

were present after slit lamp examination, intraocular pressure (IOP) was measured using Goldmann Applanation Tonometry (GAT). When IOP was below 20 mmHg in both eyes, pharmacological mydriasis was achieved using tropicamide 0.5% single-use drops (Minims Tropicamide 0.5 percent, Bausch & Lomb).

Once dilation was complete, cataract grading was performed by the PV lead senior optometrist or research ophthalmologist using the LOCS II scale for nuclear, cortical, and posterior subcapsular cataracts, followed by standardised photography of both eyes. Images were reviewed immediately to ensure adequate quality and then stored on the research computer. Participants were then moved to Test Room 2 for retinal imaging. Ultra-widefield retinal photographs were obtained using the Optomap retinal scanning system (Optos plc, Dunfermline, UK). OCT was then performed on both eyes.

At the SJH visit, carried out within seven days of the PV visit, a 20-gauge cannula was inserted following HSE guidance on venepuncture and peripheral IV cannulation (HSE ONMSD, 2017), and two p tubes of blood were collected. Samples were transported to and processed in the Campbell Neurovascular Genetics Laboratory at TCD. Participants were then escorted to CAMI for MRI and DCE-MRI acquisition. Imaging was performed on a Philips Achieva 3.0T TX scanner using identical sequence parameters across all visits. Following imaging, participants were discharged home.

3.3.1.2 T1 and T2 Procedures

At three months (T1) and nine months (T2) after completion of second-eye surgery visits, participants returned to PV and SJH for identical assessments to those performed at T0. Visit procedures were unchanged except for:

- Questionnaires: At T1 and T2, participants completed follow-up questionnaires documenting any changes in demographic, medical, or care status since the prior visit.
- RBANS-U Versioning: At T1, participants completed the alternate RBANS-U form to the one used at T0 (Form B if Form A was used at T0, and vice versa). At T2, participants completed the same RBANS-U form used at T0. This counterbalancing ensured adequate washout and minimized learning or exposure effects.

3.3.2 Cataract Surgery

All recruited participants underwent standard phacoemulsification with implantation of a monofocal intraocular lens (IOL). All procedures were performed by the same surgeon, Mr Mark Cahill, Consultant Ophthalmic Surgeon and Clinical Director of PV. IOL selection was based on biometry and the participant's visual needs. All IOLs were monofocal aspheric lenses from the ZEISS CT ASPHINA or CT LUCIA family (Carl Zeiss Meditec, Germany). The more severely affected eye was operated first in all cases.

3.3.3 Standardisation of Procedures

The order of investigations was predefined in the protocol to minimise fatigue and to prevent cognitive performance from being affected by prolonged testing. RBANS-U form alternation (A/B) was implemented to reduce learning effects, and a minimum six-month interval between study visits further reduced

exposure and practice effects on test performance. If a participant had been unable to complete a test (which did not occur), the visit would have been paused or rescheduled depending on the participant's condition.

All visits were conducted by the researcher. All cognitive and affective assessments, as well as blood sampling and cannula insertion, were performed by the same researcher, a neurologist with training in neurocognitive evaluation. The researcher was not blinded to previous assessments but followed a standardised administration protocol.

Visual assessment components were performed by optometrists or ophthalmologists, with the researcher present to ensure strict adherence to protocol and accurate data recording. All ophthalmic examinations were carried out by trained PV optometrists, and cataract grading was completed by either the PV lead optometrist or the research ophthalmologist.

All visits used the same rooms and equipment to maximise consistency across timepoints.

3.4 Assessments and Outcomes

3.4.1 Participants' Questionnaire: Demographic and Clinical Characterisation

Demographic and clinical data were captured using a standardised questionnaire administered as a structured interview at baseline (T0). The questionnaire collected information across three domains: demographic characteristics, medical background, and family history. Questionnaires were administered by the researcher using a standardised script to ensure consistency across visits. Responses were recorded on standardised case-report forms and subsequently entered into a secure study database. All questionnaires were completed in English; no interpreter support was required. No missing or unknown responses were recorded in this cohort.

Demographic variables recorded at T0 included age; biological sex (male/female); country of origin (Irish vs. non-Irish); handedness; highest level of education achieved (unable to read and write, primary education, secondary education, third-level education, postgraduate); occupational status (employed vs. retired, and if retired, duration of retirement); marital status (single, married, co-habiting, separated, divorced, widowed); living arrangement/cohabitation status (living alone, living with one person, or living with more than one person, and if so, number of cohabitants); and lifestyle/leisure indicators, including participation in leisure/social activities, hobbies, and sport/exercise (each recorded as yes/no and, if yes, frequency as an open number of times per week).

Medical background variables were self-reported by participants and corroborated where possible using referral information to PV and the medication list obtained at interview. Variables included hypertension; diabetes mellitus (type 1, type 2, or none); dyslipidaemia (hypercholesterolaemia, hypertriglyceridaemia, or none); history of high BMI/overweight; thyroid disease (hyperthyroidism, hypothyroidism, or none);

cardiovascular disease (myocardial infarction, angina, heart failure, arrhythmia, other specified cardiovascular condition, or none); cerebrovascular disease (brain infarct, brain haemorrhage, transient ischaemic attack, or none); epilepsy and/or antiseizure medication use; depression, anxiety, or other mental health conditions; hearing problems and hearing-aid use; insomnia or other sleep disorders; history of traumatic brain injury; smoking status (current smoker with quantity/former smoker with time since cessation/never smoker), and alcohol intake (frequency and estimated Irish units per week, or none).

Family history variables were self-reported and documented the presence of neurological disease in first, second, and third-degree relatives, including Alzheimer's disease, vascular dementia, Lewy body dementia, frontotemporal dementia, Parkinson's disease, motor neuron disease, prion disease, Huntington's disease, and epilepsy.

Collectively, these variables were obtained to provide a comprehensive demographic and clinical characterisation of the study cohort, capturing established and potential modifiable and non-modifiable risk factors for cognitive decline or dementia, and identifying potential confounders associated with reduced cognitive performance or longitudinal cognitive change. These variables were prespecified as candidate covariates in exploratory models of cognitive outcomes.

3.4.1.1 Follow-up Questionnaire (T1 and T2)

At T1 (3 months post-second eye surgery) and T2 (9 months post-second eye surgery), participants completed a brief follow-up questionnaire to capture interval changes since the preceding timepoint. Follow-up questionnaires were completed at each study timepoint prior to cognitive testing. Variables reassessed included occupational status, marital status, living arrangement/cohabitation status, and lifestyle/leisure indicators (leisure/social activities, hobbies, and sport/exercise; recorded as yes/no and, if yes, open-number frequency per week). Participants were additionally asked to report any new diagnoses or significant health changes since the last visit (yes/no, with details if yes), an updated medication list (names), and current smoking and alcohol use/status.

3.4.2 Neurocognitive and Affective Assessment

At each study timepoint (T0, T1, T2), participants completed a standardised neurocognitive and affective assessment protocol. The fixed administration order was questionnaires → RBANS-U → BDI-II → STAI-Y. All assessments were conducted by the researcher. Testing took place in the same clinic room and under equivalent conditions at all visits, as detailed in previous sections. Participants used their usual corrective glasses (if applicable) and hearing aids (if applicable) during testing; where residual hearing limitations were evident, instructions were repeated in a louder and clearer manner in a consistent, supportive way.

Blinding to timepoint and surgical status was not feasible. To minimise examiner and expectation effects, assessments were administered and scored by a single reviewer across all visits, following a standardised script, with strict alternation of RBANS-U parallel forms within participants. Before commencing the

protocol, participants were offered the opportunity to use restroom facilities and to have a drink of their choice; this was offered again after completion of the RBANS-U. No assessments were interrupted once initiated.

3.4.2.1 Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

Global cognitive performance was assessed using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), a brief, individually administered multi-domain cognitive instrument designed to detect and characterise cognitive performance decline efficiently (Randolph et al., 1998). RBANS has demonstrated validity across neurodegenerative, neurological, and psychiatric populations, including cohorts with mild cognitive impairment and Alzheimer's disease, where it captures the characteristic amnesic profile (Duff et al., 2010; Randolph et al., 1998; Schatz, 2018).

RBANS has been adopted internationally, with over 25 linguistically and culturally validated translations and normative datasets covering adult age ranges, education levels, and specific demographic groups (Pearson Clinical, 2015; Schatz, 2018). A revised updated version, RBANS-Update (RBANS-U), retains the original 12-subtest/5-Index structure while providing updated materials, expanded norms, and four parallel English forms (A–D) to support repeat testing in longitudinal designs (Pearson Clinical, 2015). Evidence from clinical validation studies indicates that RBANS demonstrates good test–retest reliability and internal consistency, with reported diagnostic performance in the approximate range of sensitivity 0.64, specificity 0.90, and positive/negative predictive values 0.87 and 0.72, respectively; these values vary according to the population studied and the cut-offs applied (Garrett et al., 2021).

In this study, the 2015 European RBANS-U adaptation (Pearson stimulus booklet, record forms, and manual norms) was used. RBANS-U administration required approximately 25–30 minutes and minimal materials (record form, pencil, and stimulus booklet) (Pearson Clinical, 2015; Randolph et al., 1998). Forms A and B were strictly alternated within participants (A→B→A or B→A→B) across T0–T2 to minimise learning effects. Raw scores were hand-scored and converted to Index and Total Scale standard scores, and subsequently to normative percentile ranks using the manual's tables, with all scoring performed by the researcher.

Domain structure and subtest specifications

RBANS/RBANS-U comprises 12 subtests generating a Total Scale score and five Index scores: Immediate Memory, Visuospatial/Constructional, Language, Attention, and Delayed Memory (Randolph et al., 1998; Schatz, 2018). The domains and their subtests are:

- Immediate Memory Index: assesses initial encoding and short-term learning of new verbal material.
 - *List Learning*: participants hear a 10-word list across repeated learning trials and recall as many items as possible after each trial. Captures verbal learning efficiency, immediate recall capacity, acquisition tendencies across trials, and intrusion/perseveration propensities.

- *Story Memory*: participants listen to a brief story and immediately reproduce it. Probes contextual verbal encoding, semantic organisation, and immediate narrative recall.
- Visuospatial/Constructional Index: evaluates visuo-perceptual processing and constructional praxis.
 - *Figure Copy*: copying of a complex geometric figure. Scores reflect visuo-constructional ability, spatial planning, and integration of visual parts into a coherent whole.
 - *Line Orientation*: matching the orientation of angled lines to a reference array. Measures basic visuospatial perception and orientation judgment with minimal motor/executive demand.
- Language Index: captures expressive language and lexical/semantic retrieval.
 - *Picture Naming*: confrontation naming of pictured objects, indexing word-finding, semantic retrieval, and expressive vocabulary.
 - *Semantic Fluency*: timed generation of elements of a specific category, assessing semantic network access, lexical productivity, and retrieval speed.
- Attention Index: measures auditory attention/working span and speeded visual processing.
 - *Digit Span*: repetition of progressively longer digit strings, assessing auditory attention, immediate working-memory span, and maintenance under increasing load.
 - *Coding*: a timed symbol-to-number transcription task, measuring processing speed, sustained attention, visual scanning, and visuomotor efficiency.
- Delayed Memory Index: assesses retention and retrieval of previously learned material after a delay across verbal and visual modalities.
 - *List Recall*: delayed free recall of the word list, examining uncued delayed verbal retrieval.
 - *List Recognition*: recognition of target words among distractors, assessing delayed verbal recognition and discrimination.
 - *Story Recall*: delayed reproduction of the story, measuring retention of contextual verbal information.
 - *Figure Recall*: delayed reproduction of the geometric figure from memory, assessing delayed visuospatial memory.

Together, the subtests provide a structured profile of global cognition and domain-specific functioning across memory, attention/processing speed, language, and visuospatial abilities (Randolph et al., 1998; Schatz, 2018).

Interpretation: RBANS-U Index scores are standardised to normative expectations (mean 100, standard deviation (SD) 15) with percentile ranks (Pearson Clinical, 2015; Randolph et al., 1998). In keeping with commonly used neuropsychological operationalisations of mild cognitive impairment/mild neurocognitive

disorder, performance ≤ 10 th percentile in one or more domains was considered consistent with MCI (Duff et al., 2010; Sachdev et al., 2014).

Percentile-based performance bands were categorised as: >75 th (high-average), 25th–75th (average), 10th–24th (low-average), and <10 th (impaired). Longitudinal analyses evaluated change across T0, T1, and T2 in RBANS normative percentile ranks for both the Total Scale score and each of the five Index scores.

In the statistical analyses, RBANS-U Total Scale percentile scores were treated as the primary cognitive outcome, with the five RBANS-U Index percentile scores (Immediate Memory, Visuospatial/Constructional, Language, Attention, Delayed Memory) analysed as prespecified secondary outcomes. Both standard scores and normative percentile ranks were summarised at each timepoint (T0, T1, T2), and longitudinal analyses focused on within-participant change across visits. RBANS-U measures were examined in relation to visual, MRI-based BBB, structural brain, and serum proteomic variables as key explanatory and associated measures.

3.4.2.2 Depression and Anxiety Profiling

Affective status was assessed at each visit to identify previously undiagnosed or insufficiently controlled mood and anxiety symptoms that might interfere with cognitive test performance, and to track potential changes across timepoints that could also be reflected in RBANS performance. Self-report inventories (BDI-II and STAI-Y) were selected to maximise participant comfort when disclosing affective symptoms. Participants were encouraged to answer the questionnaires independently; the researcher only assisted when a participant did not understand a specific item or question, or had visual, cognitive, or educational limitations that could compromise comprehension and accurate responding.

After the RBANS-U was administered, participants who were able to work independently were typically left alone in the assessment room for approximately 15–20 minutes to fill in the affective questionnaires. For those requiring assistance, the researcher went through the inventories with them after a brief pause following RBANS-U, reading items aloud and recording answers, while avoiding any additional questioning beyond the standard instructions. This approach was applied consistently across all timepoints (T0, T1, T2).

3.4.2.2.1 Beck Depression Inventory–II (BDI-II)

Depressive symptoms were assessed with the Beck Depression Inventory–II (BDI-II), a self-report instrument developed as a revision of the original Beck Depression Inventory to align with DSM-IV criteria for major depressive disorder (Beck et al., 1996; Wang & Gorenstein, 2013). Each item is rated on a 0–3 scale, producing a total score range of 0–63, with higher scores reflecting greater depressive symptom severity.

The 21 items included cover core cognitive, affective, and somatic features of depression, including sadness, pessimism, self-dislike, guilt, self-criticalness, suicidal thoughts, agitation, anhedonia, indecisiveness, fatigue, sleep disturbance, appetite change, and loss of interest in sex (Park et al., 2020; Wang & Gorenstein, 2013). The BDI-II is validated for adolescents and adults (≥ 13 years) and is widely used in clinical, research, and community settings to screen for depression, quantify symptom severity, and monitor treatment response (Arnau et al., 2001; Beck et al., 1996; Wang & Gorenstein, 2013).

Across diverse populations, including primary care, medical, community, and psychiatric samples, the BDI-II has demonstrated high internal consistency (Cronbach's α typically ≥ 0.85), good test-retest reliability, and robust convergent and criterion validity against diagnostic interviews and other depression scales (Arnau et al., 2001; Park et al., 2020; Richter et al., 1998; Saal et al., 2018; Wang & Gorenstein, 2013). A diagnostic meta-analysis of BDI-II cut-offs reported that a threshold around 14–15 has a sensitivity of 0.86 and specificity of 0.78 across heterogeneous settings, while emphasising that optimal cut points are population- and context-dependent (von Glischinski et al., 2019).

In this study, the BDI-II was administered at each timepoint (T0, T1, T2) following RBANS-U. Participants completed the questionnaire in paper-and-pencil format in English. When self-completed by the participant, the researcher visually inspected each BDI-II form to ensure that all items had been completed; forms were checked only for completeness, and scoring was performed later from the stored paper forms. As a result, there were no missing BDI-II data in this cohort. Although no participant in this study endorsed severe depressive symptoms or suicidality on the BDI-II, the protocol specified that any such responses would be discussed with the participant and, where appropriate, they would be advised to contact their general practitioner or be referred for further clinical evaluation.

Scoring and Interpretation: Each participant's responses were summed to derive a BDI-II Total score (0–63). For descriptive characterisation of depressive symptom burden in the cohort, BDI-II scores were grouped into severity bands as follows:

- 1–10: normal/minimal depressive symptoms
- 11–16: mild depression
- 17–20: borderline clinical depression
- 21–30: moderate depression
- 31–40: severe depression
- >40 : extreme depression

These ranges fall within the spectrum of BDI-II cut-offs and severity thresholds reported in the literature for clinical and general-population samples (Dolle et al., 2012; Lasa et al., 2000; von Glischinski et al., 2019; Wang & Gorenstein, 2013).

For statistical purposes, BDI-II Total scores were treated as a continuous measure of depressive symptom severity. These scores were summarised at each timepoint and included as prespecified covariates in exploratory models of cognitive outcomes, in order to adjust for the potential impact of depressive symptoms on trajectories of neurocognitive performance.

3.4.2.2 State–Trait Anxiety Inventory, Form Y (STAI-Y)

Anxiety symptoms were assessed using the State–Trait Anxiety Inventory, Form Y (STAI-Y), which comprises two parallel 20-item self-report scales: the State Anxiety scale (STAI-Y1), indexing transient, situational anxiety (“how you feel right now”), and the Trait Anxiety scale (STAI-Y2), assessing general anxiety tendency (“how you generally feel”) (Forrest et al., 2021).

Items cover feelings such as tension, apprehension, nervousness, worry, calmness, satisfaction, and self-confidence. Each item is rated on a 4-point Likert-type scale, producing separate total scores for state and trait anxiety from 20 to 80, with higher scores indicating greater anxiety.

STAI-Y is a revision of the original State–Trait Anxiety Inventory and has been extensively used to distinguish between temporary state anxiety and more enduring trait anxiety in adolescents and adults (Bieling et al., 1998; Elwood et al., 2012; Forrest et al., 2021). It is widely applied in clinical, community, and research settings to screen for anxiety, quantify symptom severity, and monitor change over time or in response to interventions (Elwood et al., 2012; Grös et al., 2007; Knowles & Olatunji, 2020). Psychometric studies consistently indicate excellent internal consistency (Cronbach’s α typically 0.90–0.94 for both state and trait scales) and good test–retest reliability for trait anxiety over intervals ranging from several months to multiple years (Guillén-Riquelme & Buela-Casal, 2011; Klingelhöfer-Jens et al., 2025).

Diagnostic accuracy studies using ROC curve analysis of the full 40-item STAI-Y in adult clinical samples have demonstrated acceptable screening performance: in a mixed anxiety-disorder versus healthy-control sample, Ercan et al. (2015) identified optimal cut-offs of ≥ 41 for state anxiety and ≥ 44 for trait anxiety, offering sensitivities of 78.3% and 93.5% and specificities of 71.2% and 57.4%, respectively; in a specialist epilepsy clinic, Wiglusz et al. (2019) reported that a STAI-T threshold of ≥ 52 provided sensitivity of 81.3% and specificity of 77.5% for DSM-IV anxiety disorders. Taken together, these findings indicate that, although optimal thresholds vary by population and context, the standard full-length STAI-Y offers reasonably good diagnostic accuracy for clinically significant anxiety.

In this study, both STAI-Y1 (state) and STAI-Y2 (trait) were administered at each timepoint (T0, T1, T2), following the fixed sequence RBANS-U $\rightarrow$ BDI-II $\rightarrow$ STAI-Y. Questionnaires were completed in paper-and-pencil format in English. Participants were asked to respond independently whenever possible, and were only assisted when unable to do so, following the same principles described for BDI-II.

As with BDI-II forms, the researcher visually checked each STAI-Y1 and STAI-Y2 form to confirm that all items had been answered; forms were checked only for completeness at the time of the visit, and scoring was carried out later from the stored paper records. As a result, there were no missing STAI-Y data in this cohort. Although no participant scored in an extreme anxiety range, the same safety principle applied as for the BDI-II: if markedly elevated anxiety scores suggestive of significant clinical concern had emerged, these would have been discussed with the participant and, where appropriate, they would have been advised to contact their general practitioner or referred for further clinical evaluation.

Scoring and Interpretation: For each participant and timepoint, separate STAI-Y1 (state) and STAI-Y2 (trait) Total scores (range 20–80) were calculated by summing item scores. For descriptive interpretation of state anxiety, the following a priori bands were used:

- 20–37: low/no anxiety
- 38–44: moderate anxiety
- 45–80: high anxiety

Trait anxiety scores (STAI-Y2) were interpreted analogously as indices of low, moderate, or high trait anxiety, acknowledging that STAI-Y2 reflects a broader negative affectivity dimension.

For analysis, STAI-Y1 and STAI-Y2 Total scores were treated as continuous measures of state and trait anxiety, respectively. They were summarised descriptively at each timepoint and prespecified as candidate covariates, analogous to BDI-II Total scores, in exploratory models of cognitive outcomes, to allow adjustment for the potential influence of both transient and enduring anxiety on neurocognitive performance trajectories.

3.4.3 Ophthalmic Assessment

3.4.3.1 Visual Acuity

Visual acuity (VA) was assessed at each visit in Clinical Room 4 at PV under standardised conditions. Testing was performed at 6 m using a distance chart set out in logMAR format, with equivalent Snellen values also displayed. The same chart, testing distance, and room were used for all visits. Room lights were dimmed, and the chart was uniformly illuminated.

VA was always measured after completion of the neurocognitive and affective assessments and before instillation of any ocular drops. At every visit, VA was assessed in both eyes monocularly and then binocularly. For each eye, unaided VA, pinhole VA, VA under habitual correction, and best corrected VA following refraction were obtained by the PV lead optometrist or the research ophthalmologist. Best corrected VA was derived using standard subjective refraction according to routine PV clinical practice. Participants were assessed wearing their habitual glasses, which were adjusted over time as part of routine care; therefore, VA at T1 and T2 corresponded to their then-current glasses, if any, rather than those used at T0.

For this study, the primary VA variable was best corrected VA, analysed at each eye level. Pre-surgery best corrected VA for each eye at T0 served as the baseline value, and best corrected VA at T2 for each eye was treated as the main post-surgical value for longitudinal comparison. Unaided and pinhole VA were retained for descriptive and safety purposes but were not used as primary analytic outcomes. All VA values used in the analyses were available in both logMAR units and Snellen fractions.

The logMAR (logarithm of the minimum angle of resolution) scale expresses VA as the base-10 logarithm of the minimum resolvable angle in minutes of arc. A minimum angle of resolution (MAR) of 1 minute corresponds to logMAR 0.0, whereas a MAR of 2 minutes corresponds to logMAR 0.3. Lower logMAR values indicate better acuity. LogMAR letter charts are designed with a geometric progression of optotype size, equal numbers of optotypes per line, and standardised spacing, and are scored letter by letter. These features provide improved precision, sensitivity to small changes, and test–retest repeatability compared with traditional Snellen charts, and produce an approximately linear scale that is well suited to parametric statistical analysis (Bailey and Lovie-Kitchin, 2013; Bourne et al., 2003; Hazel and Elliott, 2002; Rosser et al., 2001). For these reasons, logMAR has become a preferred format for reporting VA in clinical trials and epidemiological research, with Snellen equivalents often provided alongside to aid clinical interpretability (Hussain et al., 2006; Tsou and Bressler, 2017).

LogMAR	Snellen (m)	Snellen (ft)
−0.30	6/3	20/10
−0.20	6/3.8	20/12.5
−0.10	6/4.8–6/5	20/16
0.00	6/6	20/20
0.10	6/7.5	20/25
0.20	6/9–6/9.5	20/32
0.30	6/12	20/40
0.40	6/15	20/50
0.50	6/18–6/19	20/63
0.60	6/24	20/80
0.70	6/30	20/100
0.80	6/38	20/125
0.90	6/48	20/160
1.00	6/60	20/200

Table 3.1. LogMAR ↔ Snellen conversion chart

VA was expressed in logMAR units as the study standard. The exception was VA–BBB correlation forest plots (Chapter 8, Sections 8.8–8.9), for which VA was expressed as a Snellen fraction quotient (decimal acuity), where higher values (approaching 1.0) indicate better acuity and lower values (approaching 0) indicate worse acuity. This was done to preserve intuitive directionality when interpreting correlation coefficients.

3.4.3.2 Anterior Segment Examination, Intraocular Pressure, and Mydriasis

Following the VA assessment, participants underwent a standardised anterior segment examination. The PV lead optometrist or the research ophthalmologist performed slit-lamp biomicroscopy after topical corneal anaesthesia and diagnostic staining using combined Fluorescein Sodium 0.25% and Lidocaine Hydrochloride 4% eye drops (Minims Lidocaine & Fluorescein, Bausch & Lomb). If no contraindications were identified on slit-lamp examination, IOP was measured in both eyes using GAT. When IOP was below 20 mmHg in both eyes, and there were no contraindications to dilation, pharmacological mydriasis was induced with Tropicamide 0.5% single-use drops (Minims Tropicamide 0.5%, Bausch & Lomb). The order of testing was identical for all participants and all visits: VA, slit lamp and fluorescein examination, IOP measurement, and then mydriasis.

Anterior segment findings, IOP, and concurrent ocular pathology were documented for descriptive and safety purposes and were used to identify eyes with comorbid conditions that might confound visual outcomes or retinal imaging interpretation. These variables were not used as primary analytic outcomes.

3.4.3.3 Cataract Grading with LOCS II

After full mydriasis at T0, cataracts were graded in each eye using the Lens Opacities Classification System II (LOCS II). It is a semi-quantitative photographic grading system that uses standardised slit-lamp and retro illumination images to classify four principal lens features: nuclear colour and opalescence, cortical opacity, and posterior subcapsular opacity (Gali et al., 2019; Magno et al., 1993; Miglior et al., 1992). Each feature is graded by comparing the lens's clinical appearance at the slit lamp with reference photographs representing increasing severity, and assigning the score that best matches the standard. LOCS II was developed to support reproducible documentation of cataract severity and progression and has demonstrated good inter- and intra-observer reproducibility and sensitivity to change, particularly in early to moderate disease (Magno et al., 1993; Miglior et al., 1992).

In this study, nuclear colour and opalescence, cortical cataract, and posterior subcapsular cataract were graded separately for each eye. Grading was performed by the PV lead optometrist or the research ophthalmologist using a LOCS II visual aid at the slit lamp, with standardised photographic documentation of each lens. When uncertainty occurred, the optometrist and ophthalmologist reviewed the case together and agreed on a consensus grade to maximise internal consistency. LOCS II scores were recorded as separate component grades per eye rather than combined into a composite score.

LOCS II grading was performed only at T0, before cataract surgery, and was used as a baseline index of cataract severity. Grading was not repeated postoperatively because the native crystalline lens had been removed and replaced with a clear intraocular lens, and no residual cataract was expected after surgery. Although LOCS III is now the most widely used classification system in contemporary research (Chylack et al., 1993; Gali et al., 2019; Cicinelli et al., 2023), LOCS II remained the established system in PV practice. Using LOCS II in this project preserved continuity with routine clinical practice, facilitating existing staff training and familiarity, and avoiding potential misclassification associated with introducing a new and less familiar system.

LOCS II grades were used both descriptively and analytically. At the eye level, LOCS II scores were treated as continuous indices of cataract severity and also categorised by cataract type and severity band. For exploratory multivariable analyses, baseline cataract severity quantified by LOCS II was included as a covariate to adjust for potential confounding of visual and cognitive outcomes.

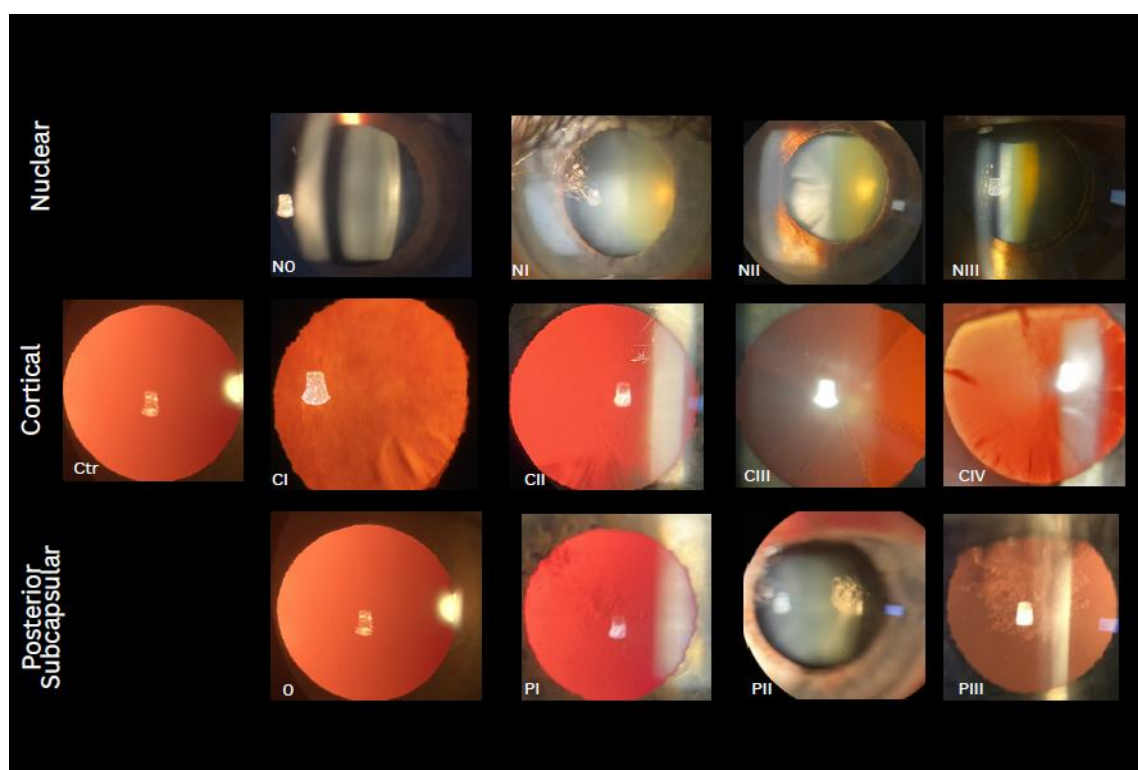


Figure 3.1. Lens Opacities Classification System II (LOCS II), used for cataract grading.

3.4.3.4 Retinal Imaging and Optical Coherence Tomography

Retinal imaging and structural optical coherence tomography (OCT) were acquired at each visit using an Optos Silverstone ultra-widefield scanning laser ophthalmoscope with integrated swept-source OCT (SS-OCT) (Optos plc, Queensferry House, Carnegie Campus, Dunfermline, UK). This system provides ultra-widefield colour retinal images of up to 200° in a single capture (Optomap) and 1050 nm SS-OCT imaging

with axial resolution < 7 µm, transverse resolution < 20 µm in tissue, and an A-scan rate up to 100 kHz, permitting targeted OCT acquisition at any location within the ultra-widefield image.

After dilation and cataract grading at T0, and after dilation at T1 and T2, ultra-widefield colour and autofluorescence images of each eye were obtained. A 6 × 6 mm macular cube SS-OCT scan was then acquired for each eye, supplemented by a higher-resolution raster line scan across the fovea. OCT images were reviewed qualitatively by the PV lead optometrist to confirm retinal and optic nerve structural integrity and to exclude macular or optic nerve pathology that might influence visual function or confound interpretation of retinal vascular findings (such as advanced age-related macular degeneration, macular oedema, epiretinal membrane, or advanced glaucomatous damage).

All OCT images were checked immediately for artefact, segmentation failure, or motion degradation, and were repeated within the same visit if image quality was inadequate. Quantitative OCT metrics (such as central macular thickness or peripapillary retinal nerve fibre layer thickness) were not extracted for this study and were not included as analytic variables. OCT, therefore, served a descriptive and safety role, ensuring structural suitability of the eyes for inclusion, rather than providing primary outcome data. No OCT-angiography (OCT-A) was performed as part of this protocol.

During follow-up, one participant developed cystoid macular oedema following cataract surgery. Their follow-up assessments were postponed until resolution, after which they completed the protocol at T2 and were included in the final analyses. One participant had pre-existing dry age-related macular degeneration, which was monitored and did not show clinically significant progression during the study; their data were also retained in the analyses. No additional sight-threatening macular or optic nerve complications occurred during follow-up that required exclusion of eyes from analysis.

3.4.3.5 Consistency Across Timepoints and Analytic Role of Ophthalmic Variables

All ophthalmic assessments: VA, slit-lamp and anterior segment examination, IOP measurement, LOCS II grading, ultra-widefield imaging, and OCT, were performed at T0, T1, and T2 using the same equipment, the same rooms within PV, and the same imaging platform. The order of testing was identical for all participants and at all visits: VA; slit-lamp and fluorescein examination; IOP measurement; pharmacological dilation; LOCS II grading and lens photography (T0 only); ultra-widefield imaging; and finally, OCT.

VA, slit-lamp examination, and IOP measurement were performed by experienced PV optometrists or by the research ophthalmologist. LOCS II grading and review of retinal images were undertaken by the PV lead optometrist or the research ophthalmologist. In most cases, ultra-widefield imaging and OCT acquisitions were performed by the research ophthalmologist; when this was not possible, a PV technician performed imaging under direct supervision of the researcher following a written protocol, with immediate image-quality review and re-acquisition if necessary. The image reader was not blinded to

timepoint or surgical status, so some expectation bias was possible; however, predefined grading criteria and the use of the same experienced graders at all timepoints were intended to reduce this risk.

In the statistical analyses, the best corrected monocular logMAR VA at the eye level was treated as the visual outcome. Baseline cataract severity graded by LOCS II and best corrected VA were included as covariates in the exploratory multivariable models. Slit-lamp findings, IOP, and OCT structural findings were used for descriptive characterisation and safety monitoring and were not included as primary outcome variables.

3.4.4 MRI Acquisition and Analysis

All brain MRIs were performed at CAMI, St James's Hospital, Dublin, using a 3.0 Tesla Philips Achieva TX system (Philips Healthcare, Best, The Netherlands) equipped with a 32-channel SENSE head coil. The same scanner, software release (5.7 rev. 01), and protocol were used for all participants and at all three timepoints. MRI visits were scheduled between 10:00 and 15:00h and, as described earlier, occurred within seven days of the corresponding PV visit wherever possible. Participants were positioned supine with foam padding to minimise head motion and were monitored throughout via in-scanner audio-visual communication.

The imaging session followed a fixed sequence order: localiser images, structural sequences (T1-weighted, T2-weighted, and fluid-attenuated inversion recovery (FLAIR)), pre-contrast T1 mapping using a variable flip angle (VFA) method, and finally dynamic contrast-enhanced MRI (DCE-MRI). All sequences were acquired at T0, T1, and T2, provided no new contraindications had arisen.

3.4.4.1 Structural MRI: Hardware and Core Sequences

The structural protocol consisted of:

- **T1-Weighted Anatomical Imaging:** Three-dimensional (3D) gradient-echo acquisition (time to echo (TE) / repetition time (TR) = 3 / 6.7 ms; acquisition matrix 268 × 266; voxel size 0.83 × 0.83 × 0.9 mm). These images provided high-resolution whole-brain anatomy for visual assessment, volumetric analysis, and registration.
- **T2-Weighted Imaging:** Fast spin-echo sequence (TE/TR = 80 / 3000 ms; in-plane resolution 0.45 × 0.45 mm; slice thickness 4.0 mm). T2-weighted images were used to detect tissue abnormalities such as lacunar infarcts, prior haemorrhage, or other focal lesions.
- **FLAIR:** TE/TR = 125 / 11 000 ms; voxel size 0.45 × 0.45 × 4.0 mm. FLAIR images were acquired to evaluate white matter hyperintensities and other T2-hyperintense lesions.

White matter hyperintensity burden was reviewed qualitatively as part of the neuroradiological safety assessment but was not included as a formal imaging outcome variable in the present analyses.

3.4.4.2 Pre-Contrast T1 (Sequence) Mapping

Pre-contrast longitudinal relaxation time (T10) was estimated using a VFA 3D T1-weighted fast field echo (T1w-FFE) sequence. VFA is a T1-mapping method that acquires a series of T1-weighted images with identical TE and TR but different flip angles; because the steady-state T1-weighted signal depends systematically on flip angle, the set of signals can be used to solve for the underlying T1 in each voxel. In this study, the VFA sequence had TE/TR = 2.78 / 5.67 ms, acquisition matrix 240 × 184, voxel size 0.68 × 0.68 × 5 mm, and was acquired at five flip angles (10°, 15°, 20°, 25°, 30°).

For each voxel, signal intensities from the five flip-angle images were fitted to the spoiled gradient-echo signal equation to obtain a pre-contrast T10 value. These T10 maps were then used to convert DCE-MRI signal changes into gadolinium concentration–time curves on a voxelwise basis, following standard dynamic contrast-enhanced MRI principles (Larsson et al., 2009).

3.4.4.3 Dynamic Contrast-Enhanced MRI (DCE-MRI) Acquisition

DCE-MRI was used to quantify subtle BBB leakage. DCE-MRI is a T1-weighted technique in which repeated images are acquired before and after intravenous administration of a gadolinium-based contrast agent, allowing the time-course of contrast passage through the vasculature and into brain tissue to be measured. In regions with intact BBB, signal enhancement is largely intravascular and decays quickly; in regions with BBB disruption, contrast slowly accumulates in the extravascular–extracellular space, producing a gradual, late-phase increase in T1-weighted signal (Montagne et al., 2015; Nation et al., 2019; van de Haar et al., 2016; Wardlaw et al., 2017; Zhang et al., 2017).

DCE-MRI was acquired using an axial 3D T1w-FFE sequence (TE/TR = 2.78 / 5.6 ms; acquisition matrix 240 × 184; voxel size 0.68 × 0.68 × 5 mm; flip angle 20°). A total of 61 temporal repetitions were obtained with a temporal resolution (Δt) of approximately 22.2 seconds, yielding a dynamic acquisition time of around 22.6 minutes, in addition to brief magnet ramp-up and ramp-down periods.

After acquisition of three pre-contrast DCE volumes, an intravenous bolus of gadoteric acid (Dotarem, 279.32 mg/mL = 0.5 mmol/mL) was administered via an automatic injector at 1 mL/kg (maximum 10 mL for participants ≥ 100 kg), followed by a 10 mL saline flush. Injection and flush were delivered at a constant rate of 4 mL/s through a peripheral cannula placed in the arm. Participants remained in the scanner throughout the DCE-MRI sequence.

Each of the 61 DCE timeframes was subjected to automated rigid realignment to a reference volume and to visual inspection by the research imaging team. Frames with clear motion artefacts were excluded from subsequent analysis. A predefined quality-control rule stated that if more than 10% of frames in a given DCE series were corrupted by motion, the entire scan would be excluded. No scan met this exclusion threshold. One participant did not undergo DCE-MRI due to a new medical contraindication to prolonged MRI; their structural MRI data were still acquired and included. No participant experienced claustrophobia

or was unable to complete the structural MRI sequences. In line with CAMI protocol, routine renal function testing prior to gadolinium administration was not required, and no contrast-related adverse events were observed.

All clinical scans were initially reviewed by a radiology specialist at CAMI for safety and incidental findings. Research-level DCE and structural processing were performed by a neurologist and a neuroscientist working together. The research analysts were not blinded to timepoint or surgical status.

3.4.4.4 DCE-MRI Modelling and BBB Leakage Metrics

Classical tracer-kinetic analysis of DCE-MRI fits tissue concentration–time curves to compartmental models such as the Tofts or Extended Tofts model, which require an arterial (or vascular) input function and yield parameters including the transfer constant K_{trans} (plasma–tissue transfer), v_e (extravascular–extracellular volume fraction), and v_p (plasma volume fraction) (Larsson et al., 2009). These model-based approaches are particularly sensitive to the early upslope of the curve and are influenced by both permeability and blood flow. In parallel, late-phase slope methods focus on the near-linear segment of the post-contrast curve and capture slow, ongoing accumulation of contrast that reflects low-grade BBB leakage (O’Keeffe et al., 2020; Veksler et al., 2020; Weissberg et al., 2014).

In the present study, we derived two complementary BBB permeability metrics:

1. **Model-based K_{trans} .** For each voxel, the full gadolinium concentration-time curve from the entire DCE acquisition was fitted with a standard tracer-kinetic model of the Tofts type (Larsson et al., 2009). In this framework, the observed tissue concentration is modelled as the result of contrast moving from the vascular space into, and sometimes back from, the extravascular–extracellular space, using a vascular input function to describe the contrast concentration in blood. Fitting this model produces the transfer constant K_{trans} , which quantifies the rate at which contrast moves from plasma into the extravascular–extracellular space (min^{-1}). Higher K_{trans} values indicate more rapid transfer and are interpreted as reflecting increased BBB permeability and/or increased surface area for exchange, whereas values close to zero are consistent with an intact BBB (Larsson et al., 2009). Voxelwise K_{trans} maps were generated across the whole brain and used to derive global and regional (lobar) summary measures.
2. **Late-phase normalised leakage slope.** In parallel, a late-phase leakage index was derived using the method described by Weissberg, O’Keeffe, and Veksler (O’Keeffe et al., 2020; Veksler et al., 2020; Weissberg et al., 2014). After conversion of signal to concentration-time curves, the slow, approximately linear segment of each voxel’s curve between minutes 6 and 22 after contrast arrival was identified. A simple linear regression line was then fitted to this time window, and the slope of this line was taken as the voxel’s late-phase accumulation rate, with slopes near zero indicating minimal ongoing accumulation and positive slopes indicating slow, continuous leakage. To reduce interindividual variability in cardiac output and bolus delivery, each voxel’s slope was normalised to

the slope measured in a venous reference region within the superior sagittal sinus, generating a dimensionless normalised leakage index. This approach complements K_{trans} by emphasising persistent, low-grade accumulation in the late post-contrast phase, which is particularly relevant for detecting subtle BBB dysfunction in non-lesional tissue (O’Keeffe et al., 2020; Veksler et al., 2020; Weissberg et al., 2014).

All image pre-processing and BBB quantification were implemented in MATLAB using SPM12 and custom scripts based on the pipeline described by Weissberg, O’Keeffe, and Veksler (O’Keeffe et al., 2020; Veksler et al., 2020; Weissberg et al., 2014). In brief, each participant’s T1-weighted anatomical image was segmented into grey matter, white matter, and cerebrospinal fluid and normalised to Montreal Neurological Institute (MNI) space using SPM12’s unified segmentation–normalisation framework. The full DCE series was rigidly realigned to correct for intra-series motion and co-registered to the native-space T1 image; the resulting transformation and the T1-to-MNI transformation were then applied to the DCE data so that all subsequent analyses were performed in MNI space.

Using the VFA-derived T10 map and the known sequence parameters, T1-weighted signal intensities from the DCE series were converted voxelwise into gadolinium concentration–time curves using the standard spoiled gradient-echo signal equation (Larsson et al., 2009). For each voxel, this yielded a continuous concentration-time profile across the full 22.6-minute acquisition.

To derive the late-phase leakage metric operationally, a simple linear regression model was fitted to the approximately linear portion of each voxel’s concentration–time curve between minutes 6 and 22 after contrast arrival, and the slope of this fit was taken as the voxel’s late-phase leakage rate (O’Keeffe et al., 2020; Veksler et al., 2020; Weissberg et al., 2014). Each voxel’s slope was then normalised to the slope measured in a venous reference region within the superior sagittal sinus, producing the dimensionless, normalised leakage index described above and reducing variability arising from interindividual differences in cardiac output and bolus delivery.

In parallel, voxelwise concentration-time curves were fitted with a standard tracer-kinetic model to estimate K_{trans} , providing the complementary permeability parameter that captured the rate of contrast transfer from plasma to the extravascular–extracellular space over the full DCE acquisition (Larsson et al., 2009). The resulting normalised slope maps and K_{trans} maps were visually inspected for artefacts and misregistration, and masks were applied to exclude non-brain voxels and large vessels before calculation of global and regional BBB metrics.

Thresholds for “high” permeability were defined using a control-derived normative distribution. Specifically, the 95th percentile of normalised voxelwise slopes in a previously characterised healthy control cohort scanned with the same protocol in the Friedman laboratory was used as the suprathreshold cut-off (Weissberg et al., 2014). For each participant and timepoint in the present study, voxels with

normalised leakage indices exceeding this threshold were designated suprathreshold and interpreted as exhibiting abnormally increased BBB permeability.

A global BBB leakage index was defined as the percentage of suprathreshold voxels across the whole brain. In parallel, global and regional mean Ktrans values were derived from the Ktrans maps. These model-based and slope-based indices were treated as complementary BBB measures; both entered the statistical analysis plan as prespecified BBB imaging outcomes.

3.4.4.5 Regionalisation of BBB Leakage

To characterise the anatomical distribution of BBB leakage, normalised leakage maps and Ktrans maps were further summarised by lobe and hemisphere. For each participant and timepoint, frontal, temporal, parietal, and occipital lobes were delineated separately in the left and right hemispheres. Lobar regions of interest (ROIs) were defined manually in MNI space by the research team using standard neuroanatomical boundaries and the T1-weighted images as reference. ROIs were drawn individually for each lobe, hemisphere, participant, and timepoint. All lobar ROIs were drawn by the same analyst to maximise internal consistency.

Within each lobar ROI, the following regional BBB measures were calculated:

- Mean normalised leakage slope, obtained as the average voxelwise normalised slope within that lobe.
- Percentage of suprathreshold voxels within that lobe, representing the regional burden of abnormal BBB leakage.
- Mean lobar Ktrans, calculated as the average voxelwise Ktrans within that lobe.

Prespecified BBB imaging outcomes were the global percentage of suprathreshold voxels and the 95th percentile of the slope-derived leakage distribution, derived from the DCE-MRI maps. Global mean Ktrans was also quantified as a secondary BBB imaging outcome. Lobar slope-based measures were examined as regional extensions of the primary indices of BBB dysfunction, and occipital Ktrans estimates were analysed as an exploratory region of interest.

3.4.4.6 Structural Volumetry, Cortical Thickness, and Biological Brain Age

T1-weighted anatomical images were also used to obtain quantitative measures of brain structure. All T1-weighted scans were anonymised and uploaded to the VolBrain online brain volumetry platform (<https://www.volbrain.net/>) and processed with the Vol2Brain v1.0 pipeline (Manjón & Coupé, 2016). Vol2Brain performs automated whole-brain parcellation and cortical surface reconstruction in MNI space and returns expert-labelled regional volumes and cortical thickness measures.

Total intracranial volume (TIV) was defined as the sum of grey matter, white matter, and cerebrospinal fluid volumes. All regional volumes were normalised to TIV and expressed as a percentage of TIV (%TIV)

to adjust for interindividual differences in head size (Manjón & Coupé, 2016). For each participant and timepoint, the following volumetric measures were extracted:

- Total brain volume (%TIV).
- Right and left cerebral grey matter and cerebral white matter volumes (%TIV).
- Lobar volumes (%TIV) for frontal, temporal, parietal, and occipital lobes.

Cortical thickness was obtained for the same lobar territories (frontal, temporal, parietal, occipital) in each hemisphere using VolBrain's cortical thickness pipeline. All VolBrain outputs (native T1 sequence, segmentations, and parcellation overlays) were visually inspected for segmentation and labelling accuracy; no scans required exclusion due to segmentation failure.

To estimate biological ageing at the regional level, Vol2Brain outputs were further processed using BrainStructureAges, which returns a predicted "brain age" for each labelled structure, along with its corresponding volume. For each participant, regional brain age estimates were derived for the parcellated cortical and subcortical regions, and deviations between predicted brain age and chronological age were calculated. Brain age indices were treated as ancillary, hypothesis-generating measures rather than primary outcomes.

Global structural MRI outcomes were prespecified as total brain volume, total grey matter volume, and total white matter volume, each normalised to TIV. These were treated as co-primary structural MRI endpoints. Secondary structural outcomes included lobar volumes and cortical thickness metrics. Brain-age indices derived from the volBrain BrainAGE pipeline, particularly the brain-age/chronological-age ratio, were analysed as secondary, complementary measures of structural brain ageing.

3.4.5 Serum Proteomics

3.4.5.1 Blood Sampling and Serum Processing

At each study timepoint (T0, T1, T2), venous blood was drawn at the CAMI/SJH visit. For each participant, 10 mL of blood was collected into serum separator tubes (two "gold-cap" tubes). Samples were transported on the same day to the Campbell Neurovascular Genetics Laboratory (TCD) and processed following standard laboratory procedures. Serum was separated by centrifugation within three hours of collection (in most cases, within two hours), aliquoted, and frozen. No sample showed visible haemolysis, and therefore no aliquots were excluded on pre-analytical grounds.

For proteomic analysis, serum samples from all participants who completed longitudinal follow-up ($n = 18$) were included at all three timepoints. Aliquots destined for proteomics were batched and shipped on dry ice to the collaborating laboratory at Hiroshima University, Japan, where all mass spectrometry (MS) work was performed. Each sample was thawed once immediately prior to digestion.

3.4.5.2 Protein Digestion and Peptide Preparation

Serum digests were prepared using a standardised in-solution digestion protocol supplied by the collaborating proteomics laboratory. For each sample, 5 μL of serum (corresponding to approximately 50 μg total protein) was diluted four-fold with 0.1 M Tris-HCl (pH 8.5). A 5 μL aliquot of this diluted serum was then mixed with 10 μL of 8.0 M urea in 0.1 M Tris-HCl (pH 8.5) to denature proteins.

Proteins were reduced by adding 2.5 μL of 20 mg/mL dithiothreitol (DTT) in 0.1 M Tris-HCl (pH 8.5) and incubating for 1 hour at room temperature, followed by alkylation with 2.5 μL of 50 mg/mL iodoacetamide in 0.1 M Tris-HCl (pH 8.5) for 1 hour at room temperature in the dark.

Initial digestion was performed with 1.0 μL of lysyl endopeptidase (Lys-C; 0.5 $\mu\text{g}/\mu\text{L}$; FUJIFILM Wako Pure Chemical Corporation, #129-02541) for 3 hours at 30 °C. Urea concentration was then reduced by adding 78 μL of 0.1 M Tris-HCl (pH 8.5), after which an overnight trypsin digestion was carried out using 1.0 μL of sequencing-grade trypsin (0.5 $\mu\text{g}/\mu\text{L}$; Promega, #V5113) at 37 °C.

Following digestion, peptides were acidified by adding 6 μL of 10% trifluoroacetic acid. Samples were then desalted using Empore SDB-XD StageTips (3M, #5065-64045). Eluted peptides were dried by vacuum centrifugation and resuspended in 2% acetonitrile (ACN) with 0.1% formic acid prior to LC-MS/MS analysis.

3.4.5.3 Liquid Chromatography–Tandem Mass Spectrometry Acquisition

Peptide samples (0.25 $\mu\text{g}/\mu\text{L}$; 250 ng per injection) were analysed by nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS) using a ZenoTOF 7600 mass spectrometer (SCIEX) coupled to a Vanquish Neo UHPLC system (Thermo Fisher Scientific).

Peptides were first loaded onto a C18 trap column (PepMap Neo C18, 5 μm , 0.3 $\times$ 5 mm; Thermo Fisher Scientific, #174500) and then separated on a C18 analytical column (Double nanoViper PepMap Neo C18, 2 μm , 75 μm $\times$ 150 mm; Thermo Fisher Scientific, #DNV75150PN). Chromatographic separation was achieved with a linear gradient from 2% to 35% solvent B over 120 minutes at a flow rate of 300 nL/min (solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in ACN). Between samples, the column was washed with 90% solvent B for 10 minutes, followed by re-equilibration at 2% solvent B for 20 minutes to minimise carry-over.

Eluting peptides were ionised by electrospray and acquired in Zeno narrow SWATH–data-independent acquisition (DIA) mode. The DIA method comprised 155 variable-width isolation windows covering the 400–1000 m/z range, with an accumulation time of 0.025 seconds per window and a total cycle time of approximately 4.7 seconds. This data-independent acquisition strategy allowed consistent, high-coverage quantification of a wide range of serum proteins across all timepoints in all participants.

3.4.5.4 Protein Identification, Quantification, and Quality Control

Raw DIA data were processed using DIA-NN (Data-Independent Acquisition by Neural Networks; version 2.0.2) in library-free mode against a human UniProt FASTA database (one canonical protein sequence per gene), with default settings for narrow-window SWATH data (Demichev et al., 2020). DIA-NN uses neural network-based scoring and interference correction to improve peptide identification and quantification in complex DIA datasets, allowing sensitive and robust label-free protein quantification (Demichev et al., 2020).

For each sample, DIA-NN produced protein-level intensity estimates after retention-time alignment, precursor deconvolution, and interference correction. Only proteins consistently identified across the majority of samples were retained for downstream statistical analysis to reduce the influence of spurious, low-confidence detections. All samples from the three timepoints were run and processed in a single analytical batch to minimise batch effects.

3.4.5.5 Analytical Role of Serum Proteomics in the Study

In this project, serum proteomics was prespecified as a secondary, exploratory outcome rather than a primary endpoint. The intent was to characterise longitudinal changes in circulating proteins with putative vascular, endothelial, or inflammatory roles across T0, T1, and T2, and to explore how these changes related to MRI-derived measures of BBB integrity and cognitive performance.

Protein intensity estimates generated by DIA-NN were exported for downstream statistical analysis and integrated with clinical, cognitive, and imaging datasets. Detailed statistical procedures, including normalisation, handling of repeated measures, and the modelling of associations between proteomic, imaging, and cognitive outcomes, are described in the Statistical Analysis section of this chapter.

Classic BBB-related proteins, such as glial fibrillary acidic protein (GFAP), vascular endothelial growth factor (VEGF), and several other canonical injury markers, were not reliably detected above the noise floor in these serum datasets. This likely reflected their low circulating concentrations relative to the broader serum proteome in this older, non-acute cohort. This limitation was explicitly considered when interpreting the proteomic findings, which were used primarily to generate hypotheses for future targeted validation rather than to provide definitive biomarker conclusions.

3.5 Statistical Analysis

3.5.1 Data Management and Software

All datasets were initially collected and anonymised in Microsoft Excel (Microsoft Corporation) before export for analysis. Statistical processing and graphical visualisation were performed primarily in GraphPad Prism 10 (GraphPad Software, San Diego, CA; academic licence via the Campbell Neurovascular Unit Laboratory, Trinity College Dublin).

Prism was used for descriptive summaries, assumption checking, hypothesis testing, and generation of bar graphs with individual data points, line plots, scatter plots, paired “spaghetti” plots, supplementary Bland–Altman plots for selected measures, and correlation heatmaps. Forest plots summarising correlation coefficients (for example, between visual parameters, BBB metrics, and RBANS scores) were generated in R (R Foundation for Statistical Computing) using RStudio as the integrated development environment. Figures were formatted in monochrome and colourblind-safe palettes suitable for print and on-screen viewing. Where appropriate, graphs display individual participant values, group means, and standard deviation (SD) error bars. Exact p-values are reported in figure legends and/or in the corresponding Results text. Unless otherwise specified, all tests were two-tailed, and significance was set at $\alpha = 0.05$.

3.5.2 Sample Size and Power

A priori power calculations were conducted using G*Power (version 3.1; Heinrich-Heine-Universität Düsseldorf) for a within-subject (repeated-measures) design. With $\alpha = 0.05$ and power $(1-\beta) = 0.90$, and assuming a moderate within-subject effect size for change in the primary longitudinal outcomes (cognitive performance and MRI-based measures of BBB permeability), the required minimum sample size was 20 participants.

To meet this target, 20 individuals were initially enrolled. Two participants did not complete follow-up assessments (one due to death from an unrelated cause, and one due to mobility limitations following an unrelated spinal procedure), and their data were not used in the analysis. The final longitudinal cohort included 18 participants. This modest drop-out slightly reduced the theoretical power relative to the original calculation, particularly for detecting small effect sizes, and is acknowledged when interpreting marginal findings.

For the DCE-MRI component, an additional limitation occurred: one participant had a significant medical contraindication to prolonged exposure in the MRI scanner and did not undergo DCE acquisition. Analyses involving BBB permeability indices (Ktrans, percentage of suprathreshold voxels, 95th percentile of the leakage-slope distribution) were therefore conducted on $n = 17$ participants. All other non-DCE analyses (RBANS, structural brain volumes, regional cortical thickness, brain-age indices, visual parameters, and serum proteomics) were performed on the full $n = 18$ sample unless otherwise specified.

3.5.3 Data Preparation and Assumption Testing

Before performing inferential statistics, all continuous variables were examined for distributional properties and outliers. Variables included:

- Visual parameters (i.e., best corrected VA under LogMAR notation)
- RBANS Total Scale and Index percentile scores
- BDI-II and STAI-Y scores
- DCE-MRI BBB permeability indices (global and lobar Ktrans, global percentage of suprathreshold voxels, 95th percentile leakage slope)

- MRI-derived structural measures (whole-brain, grey matter, white matter volumes, lobar volumes, cortical thickness)
- Brain-age indices (i.e., brain-age/chronological-age ratio)
- Serum proteomic protein-intensity measures (for summary and exploratory analyses).

Normality was assessed using a combination of D’Agostino–Pearson omnibus test, Shapiro–Wilk test, Anderson–Darling test, Kolmogorov–Smirnov test, together with inspection of histograms and Q–Q plots where relevant, recognising the limited power of formal normality tests in small samples. Variables that satisfied normality criteria at $\alpha = 0.05$ were analysed using parametric methods; those that did not were analysed using non-parametric alternatives.

Data completeness was reviewed at each timepoint. For analyses requiring paired values at two timepoints (e.g., paired t-tests or Wilcoxon matched-pairs tests), participants were included only when they had valid data at both timepoints. For analyses spanning all three timepoints (T0, T1, T2), mixed-effects procedures were used where appropriate to accommodate occasional missing values without imputation. Correlation analyses used pairwise-available data; therefore, the effective sample size contributing to individual coefficients may vary.

3.5.4 Longitudinal Comparisons Across Timepoints

Longitudinal change in outcomes assessed at T0, T1, and T2 was evaluated primarily using mixed-effects models, fitted using restricted maximum likelihood (REML). In these models, timepoint (T0, T1, T2) was specified as a fixed effect and participant as a random effect (random intercept), accounting for within-subject correlation and allowing inclusion of participants with minor timepoint-specific missingness. Where an overall time effect was detected, Tukey-adjusted post hoc pairwise comparisons were performed (T0 vs T1, T0 vs T2, T1 vs T2). Statistical significance was set at $\alpha = 0.05$ unless otherwise.

3.5.4.1 Cognitive, Visual, and Affective Measures

RBANS-U Total Scale and domain percentile trajectories, and affective measures (BDI-II; STAI-Y1 and STAI-Y2), were analysed longitudinally using the mixed-effects framework described above, with multiplicity-adjusted post hoc contrasts where appropriate. Baseline cognitive domain profile at T0 (i.e., differences between RBANS-U domain percentile scores within participants) was assessed using one-way repeated-measures ANOVA with Tukey’s multiple comparisons test.

For the prespecified low-performing subgroup (defined as scoring at or below the 10th percentile in at least one RBANS-U domain at T0), RBANS-U Total and domain percentile scores were analysed across the three timepoints using one-way repeated-measures ANOVA with Tukey’s post hoc tests ($\alpha = 0.05$), consistent with the Results chapter reporting for this subgroup.

Best-corrected VA was analysed at the eye level using logMAR values, where higher logMAR values indicate worse vision. Given the small sample size and distributional considerations for VA change, planned within-

participant longitudinal comparisons were assessed using Wilcoxon matched-pairs signed-rank tests. Inter-eye concordance was assessed using Spearman correlation; where stated in the Results, one-tailed p-values were used for the directional hypothesis of positive concordance.

3.5.4.2 Structural MRI, Brain Age, and BBB Permeability

Structural MRI measures (including total brain/grey matter/white matter %TIV, lobar %TIV, and lobar cortical thickness) were evaluated longitudinally using mixed-effects models with timepoint as a fixed effect and participant as a random effect, followed by Tukey-adjusted pairwise comparisons.

Biological brain age and derived brain-age indices were examined primarily for change from T0 to T2. The Rate of Brain Ageing index was defined as the ratio of predicted brain age to chronological age for each participant. T0–T2 change in brain age metrics was assessed using paired-samples t-tests, where distributional checks supported approximate normality, with mean differences and 95% confidence intervals reported.

For DCE-MRI BBB indices, whole-brain % BBB disruption and whole-brain 95th percentile linear leakage (WB 95th Linear) were analysed across T0, T1, and T2 using mixed-effects models with Tukey-adjusted pairwise contrasts. Whole-brain Ktrans was presented across all three timepoints to characterise the perioperative trajectory; however, because of deviation from normality at T2, within-participant change from T0 to T2 was assessed using a two-tailed Wilcoxon matched-pairs signed-rank test. Where subgroup analyses were restricted to specific baseline leakage patterns (e.g., occipital-predominant voxelwise patterns) and non-normality persisted, paired non-parametric tests were used for the prespecified T0–T2 contrast.

3.5.5 Associations Between BBB Integrity, Structural Change, Vision, and Cognition

Associations among BBB integrity indices, structural MRI measures, visual function, and cognitive performance were explored at each timepoint and, where appropriate, for change scores (e.g., T0–T2). Correlations were examined between BBB indices (whole-brain % BBB disruption, whole-brain Ktrans, WB 95th Linear), structural MRI measures (global and lobar volumes, cortical thickness), visual measures (VA), RBANS-U Total and domain percentile scores, and affective measures (BDI-II, STAI-Y).

Correlation analyses used Pearson's correlation coefficient for approximately normally distributed continuous variables and Spearman's rank correlation coefficient for non-normal or ordinal variables. Correlation matrices were visualised as heatmaps in Prism, and selected sets of correlations were summarised in forest plots generated in R; effective sample sizes for individual coefficients were reported where relevant.

VA was reported descriptively in logMAR units as the study standard; however, for VA–BBB correlation forest plots (Chapter 8, Sections 8.8–8.9), VA was expressed as a Snellen fraction quotient (decimal acuity),

where higher values (approaching 1.0) indicate better acuity and lower values (approaching 0) indicate worse acuity, to preserve intuitive directionality when interpreting correlation coefficients.

For larger families of correlations, the Benjamini–Hochberg false discovery rate (FDR) procedure was applied in selected analyses as specified in the Results. These association analyses were prespecified as exploratory and were interpreted primarily in terms of effect size and directional consistency rather than as confirmatory evidence.

3.5.6 Proteomic Analyses

Serum proteomic data consisted of label-free intensity estimates for a large number of proteins quantified via nano–LC–MS/MS and processed using DIA-NN. Downstream statistical analyses focused on longitudinal differential abundance and on exploratory associations with imaging and cognitive measures.

3.5.6.1 Differential Protein Abundance

For each protein consistently detected across the majority of participants and timepoints, differential expression between timepoints was assessed using linear modelling with empirical Bayes moderation as implemented in the limma package in R. Pairwise contrasts included T1 vs T0 (early post-surgical change) and T2 vs T0 (later post-surgical change).

For each contrast, $\log_2$ fold change ($\log_2FC$) and associated p values were estimated. Given the modest cohort size and the exploratory nature of the proteomic component, differentially expressed proteins (DEPs) were highlighted based on FDR-adjusted p-value (adj.P.Val) < 0.05 and $|\log_2 \text{fold change} (\log_2FC)| \geq 0.3$, with the understanding that these findings are hypothesis-generating rather than confirmatory.

Results were visualised in GraphPad Prism using volcano plots in which each point represents a protein, the x-axis denotes $\log_2FC$, and the y-axis denotes $-\log_{10}(\text{p-value})$; proteins meeting both fold-change and p-value criteria were visually emphasised as candidate DEPs.

3.5.6.2 Integration with Imaging and Cognitive Data

Selected proteins of interest, particularly those with known or plausible vascular, endothelial, inflammatory, or neurodegenerative roles, were prioritised for descriptive comparison with imaging and cognitive outcomes in the Results chapter. Formal correlation modelling between individual protein intensities and MRI or cognitive measures was not undertaken in this thesis; instead, proteomic findings were treated as hypothesis-generating and used to identify candidates for future targeted validation.

Proteomic analyses were restricted to participants with complete serum samples at all three timepoints. No imputation was applied for missing protein intensities; proteins with highly sporadic detection were excluded from downstream analyses.

Classic BBB-related candidate proteins, including glial fibrillary acidic protein (GFAP), vascular endothelial growth factor (VEGF), and several other well-established injury markers, were not reliably quantified above background in these datasets. This was attributed to their low circulating concentrations relative to the broader serum proteome in this older, non-acute cohort. Consequently, the proteomic component was treated as a complementary, exploratory modality, principally to identify broader patterns and candidates for future targeted assays, rather than to deliver definitive biomarker endpoints.

3.5.7 Handling of Missing Data

Overall data completeness was high across visits and modalities. One participant had a medical contraindication to MRI and did not undergo MRI at any timepoint, reducing the maximum MRI sample to 17. For PV visits, which included cognitive assessment, ophthalmic assessment, and blood sampling (for the participant not undergoing MRI), sample sizes were: T0 = 18/18, T1 = 16/18, and T2 = 18/18. For MRI, sample sizes were: T0 = 17/18, T1 = 15/18, and T2 = 17/18, reflecting the global MRI contraindication in one participant and the fact that two participants were unable to complete the T1 visit but returned for T2.

For longitudinal analyses, participants were included whenever they had valid data for the timepoints being compared. Pairwise tests (e.g., T0 vs T1, T0 vs T2) used all participants with data at both timepoints. For repeated-measures analyses with three levels of time (T0, T1, T2), mixed-effects procedures that can accommodate occasional missing values were used, so that participants who missed a single visit could still contribute their available data. Correlation analyses used pairwise-available data, so the number of participants contributing to individual coefficients may vary within the same matrix.

No statistical imputation methods (e.g., mean imputation or multiple imputation) were applied; instead, statistical tests were chosen to work with the observed, occasionally unbalanced data structure without introducing additional model-based assumptions. This conservative strategy preserves the integrity of the observed data while acknowledging reduced precision where sample sizes are smaller.

3.6 Ethics and Governance

3.6.1 Ethical and Regulatory Considerations

All recruitment occurred through Progressive Vision Clinics, where patients were informed about the study and provided with a PIL containing the research team's contact details. All potential participants received a follow-up phone call 48 to 72 hours after the information meeting to allow time to consider participation. All questions were addressed prior to signing the consent.

Written informed consent was obtained from each participant and co-signed by the researcher before any study procedures were initiated. The PIL and consent form were written in plain English and clearly defined the study rationale, procedures, potential risks, and expected benefits. Participants were informed that they could withdraw from the study at any stage without needing to provide a reason and could request

that their data be deleted. They were assured that withdrawal would not affect their current or future clinical care.

Participants were advised that participation was voluntary, undertaken merely for research purposes, and not expected to provide any clinical benefit. No harm was anticipated as a result of participation. Temporary discomfort, bruising, or localised bleeding could occur during intravenous cannulation. The neuropsychological assessment could be tiring or trigger anxiety, and participants were informed that they could take rest breaks or discontinue testing at any point. Participants retained the right to decline any component of the study without impact on their standard care.

The handling of incidental findings was addressed during the consent process. Participants were informed that clinically significant findings such as cognitive impairment at T2, ophthalmic abnormalities requiring intervention, or MRI abnormalities, including mass lesions or acute cerebrovascular events, would be disclosed. Appropriate referral pathways were in place through PV and SJH, and general practitioners were to be notified. Suspected neurodegenerative cognitive decline at T2 was to trigger a referral to the cognitive neurology–memory service at SJH. Ophthalmic findings were to be reviewed by the PV lead optometrist and ophthalmologist, and MRI findings were to be managed in conjunction with the Neurology Service at SJH. Continued verbal willingness to participate was confirmed at each study visit.

No adverse events log was required for this study. No adverse events occurred that required reporting to the ethics committee. Funding for the study was provided through a Campbell Neurovascular Genetics Laboratory grant awarded by Science Foundation Ireland.

All protocol amendments were submitted to and approved by the Ethics Committee before implementation. Participants were informed that they could receive their own clinical findings only in the case of clinically relevant incidental abnormalities. Participants were also offered the option of receiving a copy of the final research publication upon completion of the study.

3.6.2 Data Protection, Governance, and Confidentiality

Following consent, all participant data were pseudonymised using a unique study code assigned to case report forms and all digital datasets. Paper records were stored in locked facilities within the Neurology Department at SJH. Electronic data were stored in an encrypted, access-restricted database maintained by authorised study personnel. MRI data, ophthalmic images, and laboratory results were stored under the same pseudonymised coding system.

Under GDPR governance, SJH acted as the Data Controller for the coded study data, and TCD acted as the Data Processor. Only the listed study investigators served as Data Handlers.

Serum samples were processed and stored in the Genetics Department at TCD in temperature-controlled, access-restricted laboratory facilities. All samples were labelled with pseudonymised identifiers only. Access to retrieval keys and laboratory servers was restricted to the principal investigator and designated laboratory staff. Digital data within the Neurovascular Genetics Laboratory were stored on password-protected institutional servers with continuous backup, RAID-supported redundancy, and version control. Secondary, secure synchronisation occurred at TCD's Westland Row site. In the event of data transfer from PV or SJH, datasets were encrypted and transported using password-protected external drives.

All data generated or collected at PV and SJH were pseudonymised, encrypted, and stored in locked rooms accessible only to authorised personnel.

3.6.3 Archiving and Retention

Upon completion of analyses, all coded data were archived in anonymised form on secure internal servers within the Genetics Department at TCD. Data will be archived for 84 months (7 years) for the purpose of comparison with future research findings. Only the listed investigators retained access to the archived dataset. Archived data remained protected through restricted proximity-card access, secure server infrastructure, real-time backup, redundancy systems, and automated error monitoring. At the end of the archiving period (31 December 2029), all data will be scheduled for deletion. Paper documents will be shredded, and electronic data will be permanently destroyed via secure overwriting or physical destruction. Data deletion will be undertaken by the listed investigators.

CHAPTER 4: RESULTS PART I: PARTICIPANT DEMOGRAPHICS AND CATARACT-RELATED VISUAL FUNCTION

4.1 Participant Demographic and Clinical Characteristics

4.1.1 Overview

Baseline demographic and clinical characteristics were summarised descriptively. Categorical variables (sex, handedness, education level, marital status, living arrangements, comorbidities, smoking and alcohol status, and family history of relevant neurological disease) are reported as absolute frequencies and percentages. Continuous variables (age and weekly frequency of hobbies or exercise) are presented as means and standard deviations (SD). Given the small sample size ($N = 18$) and the descriptive intent of this section, no inferential statistical tests were applied. These data provide context for subsequent analyses and allow comparison with previously studied cohorts in ageing, sensory impairment, and neurovascular research.

Participants' baseline demographic and clinical characteristics are presented in Table 4.1. A total of 18 participants were included in the baseline cohort. The mean age was 72.9 years (SD 5.8), with all individuals falling within the older-adult range targeted for the study.

4.1.2 Sex, Origin, and Handedness

Of the 18 participants, 11 (61.1%) were female and 7 (38.9%) male. The sample was predominantly of Irish origin, with 16 participants (88.9%) identifying as Irish and 2 (11.1%) as non-Irish (one English and one Finnish). Handedness was primarily right-dominant, with 15 individuals (83.3%) reporting right-handedness, and 3 (16.7%) left-handedness.

4.1.3 Education and Occupational Status

Educational attainment was generally high, although heterogeneous. Two participants (11.1%) reported only first-level education, 6 (33.3%) had completed second-level education, and 10 (55.6%) reported third-level education. At the time of enrolment, 3 individuals (16.7%) were still actively working, and 15 (83.3%) were retired. Among those retired ($N = 15$), 2 (13.3%) had retired within the preceding five years, 1 (6.7%) had retired between five and ten years earlier, and 12 (80.0%) had retired more than ten years before baseline assessment.

4.1.4 Marital and Cohabitation Status

With respect to family and living circumstances, most participants were married (13 participants, 72.2%). The remainder were single (2 participants, 11.1%), widowed (2 participants, 11.1%), or divorced (1 participant, 5.6%). Cohabitation patterns showed that 4 participants (22.2%) lived alone, 9 (50.0%) lived with one other person, and 5 (27.8%) resided with more than one other person.

Table 4.1. Participants' Demographic and Clinical Characterisation. (N=18)

Variable	Value
Age (y), mean $\pm$ SD	72.9 (5.8)
Sex, N (%)	
Female	11 (61.1%)
Male	7 (38.9%)
Origin, N (%)	
Irish	16 (88.9%)
Non-Irish	2 (11.1%)
Handedness, N (%)	
Right	15 (83.3%)
Left	3 (16.7%)
Education, N (%)	
First Level	2 (11.1%)
Second Level	6 (33.3%)
Third Level	10 (55.6%)
Occupation, N (%)	
Working	3 (16.7%)
Retired	15 (83.3%)
Years since retirement, N (%)	
< 5 years	2 (13.3%)
5–10 years	1 (6.7%)
> 10 years	12 (80.0%)
Marital Status, N (%)	
Married	13 (72.2%)
Single	2 (11.1%)
Divorced	1 (5.6%)
Widow(er)	2 (11.1%)
Cohabitation Status, N (%)	
Living alone	4 (22.2%)
Living with 1 person	9 (50.0%)
Living with >1 person	5 (27.8%)
Lifestyle / Leisure Indicators	
Participation in leisure / social activities, N (%)	
Yes	17 (94.4%)
No	1 (5.6%)
Frequency of hobbies (days/week), mean $\pm$ SD	3.7 (2.9)
Frequency of sports/ exercise (days/Week), mean $\pm$ SD	3.7 (3.0)
Comorbidities, N (%)	
Hypertension	9 (50.0%)
Diabetes	2 (11.1%)
Dyslipidaemia	9 (50.0%)
Thyroid disorder	5 (27.8%)
Heart disease	6 (33.3%)
Stroke	1 (5.6%)
Epilepsy	0 (0.0%)
Depression	0 (0.0%)
Anxiety	1 (5.6%)
Other psychiatric conditions	0 (0.0%)
Traumatic brain injury	1 (5.6%)
Hearing problem	3 (16.7%)
Use of hearing aids (among those with hearing problems)	1 (33.3%)
Sleep disorders	4 (22.2%)
Habits	
Smoking status, N (%)	
Active	1 (5.6%)
Number of cigarettes per day, median	20.0
Ex-Smoker	4 (22.2%)
No	13 (72.2%)
Alcohol Use, N (%)	
Yes	14 (77.8%)
Alcohol Consumption (units/week), N (%)	
≤ 2	3 (21.4%)
>2 and ≤ 14	8 (57.2%)
>14	3 (21.4%)
No	4 (22.2%)
Family History of Neurological Disease, N (%)	
Yes	10 (55.6%)
No	8 (44.4%)
Developed New Medical Conditions During Follow-Up, N (%)	
Yes	5 (27.8%)
No	13 (72.2%)

4.1.5 Lifestyle, Social Engagement, and Physical Activity

Engagement in leisure or social activities was almost universal. Seventeen participants (94.4%) reported participation in leisure or social activities, with only 1 participant (5.6%) reporting no such engagement. The mean frequency of participation in hobbies was 3.7 days per week (SD 2.9). The mean frequency of participation in sports or physical exercise was also 3.7 days per week (SD 3.0).

4.1.6 Medical Comorbidities

Medical comorbidities were common in this older cohort. Hypertension and dyslipidaemia were each reported by 9 participants (50.0%). Heart disease was present in 6 participants (33.3%), and thyroid disorders in 5 (27.8%). Diabetes was documented in 2 participants (11.1%). Less frequent conditions included prior stroke, where 1 participant (5.6%) reported a history of a transient ischaemic attack over five years before enrolment. A history of traumatic brain injury (TBI) was present in 1 participant (5.6%), and anxiety was also present in 1 participant (5.6%). Sleep disorders were reported by 4 participants (22.2%). No participant reported a history of epilepsy, depression, or other psychiatric disorders.

4.1.7 Sensory Comorbidities

Hearing problems were reported by 3 participants (16.7%). Among these, only 1 participant (33.3% of those with hearing problems) reported using hearing aids.

4.1.8 Smoking and Alcohol Use

During the follow-up (T0 to T2), 1 participant (5.6%) was an active smoker, with a median reported consumption of 20 cigarettes per day. Four participants (22.2%) identified as ex-smokers, and 13 (72.2%) reported never smoking. Alcohol consumption was reported by 14 participants (77.8%). Among those who consumed alcohol, 3 (21.4%) reported intake of up to 2 units per week, 8 (57.2%) reported more than 2 and up to 14 units per week, and 3 (21.4%) reported more than 14 units per week. Four participants (22.2%) reported no alcohol intake.

4.1.9 Family History of Neurological Diseases

A family history of neurological disease was present in 10 participants (55.6%) and absent in 8 (44.4%). Among those with a positive family history, 3 participants (30.0%) reported a first-degree relative with Parkinson disease, and the remaining 7 (70.0%) reported a first-degree relative with late-onset Alzheimer disease (AD).

4.1.10 Medical Events During Follow-up

Across the study period (T0 to T2), 5 participants (27.8%) developed at least one new medical condition, whereas 13 (72.2%) did not report any new diagnoses during follow-up. Among those with newly diagnosed conditions, 1 participant (20.0%) was diagnosed with AD mild dementia, 1 (20.0%) with non-metastatic breast cancer and commenced oral chemotherapy, and 3 (60.0%) with ischaemic heart disease.

4.2 Cataract Type and Severity at Baseline (T0)

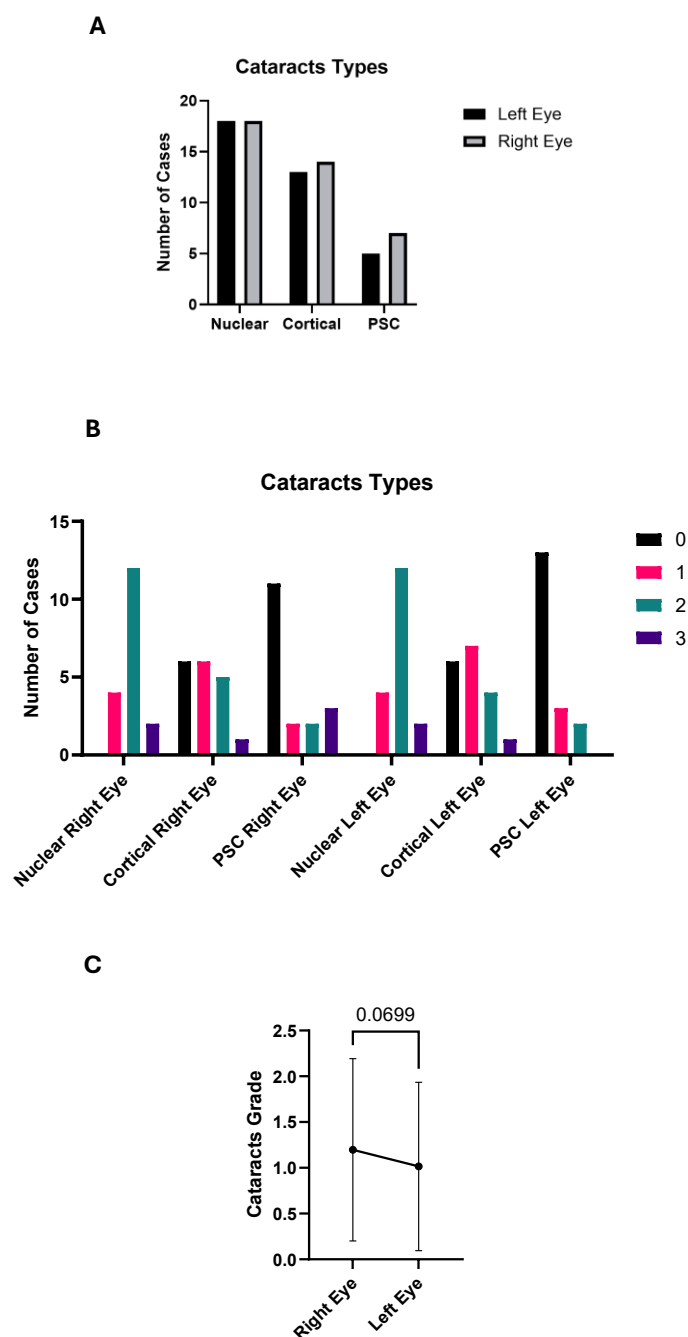


Figure 4.1. Cataract type and severity by eye. (A) Distribution of cataract types in right and left eyes (B) LOCS II severity grades by cataract type and eye (C) Composite cataract severity grade in right versus left eyes. PSC: posterior subcapsular.

4.2.1 Analytic Approach

Cataract type and severity were assessed in both eyes of all participants using the Lens Opacities Classification System II (LOCS II). For each eye (N = 36), nuclear, cortical, and posterior subcapsular (PSC) opacities were graded. Although cortical cataracts can be graded up to 4 in LOCS II, none of the eyes in

this cohort reached that grade, so grades 0–3 are shown in all summaries and figures. This produced a total of 108 graded cataract components. As LOCS II grades are ordinal and non-normally distributed, analyses relied primarily on descriptive statistics; only the comparison of composite cataract severity between eyes used inferential testing, via the Wilcoxon matched-pairs signed-rank test, to account for the paired and non-parametric nature of the data.

4.2.2 Frequency of Cataract Types by Eye

Nuclear cataract was present in all eyes (18 left; 18 right), representing 36 lenses (100%) with nuclear change. Cortical cataracts were less frequent, affecting 13 left eyes (72.2%) and 14 right eyes (77.8%). PSC change was the least common subtype, present in 5 left eyes (27.8%) and 7 right eyes (38.9%). Across all cataract components recorded, nuclear cataracts comprised 52.6% of left-eye cataract types and 48.7% of right-eye types. Cortical cataracts accounted for 34.2% left-eye and 34.15% of right-eye components. PSC accounted for 13.16% of left-eye and 17.07% of right-eye components. Therefore, nuclear change represented approximately half of all cataract components in both eyes, cortical change roughly one-third, and PSC less than one-fifth.

4.2.3 Distribution of LOCS II Grades by Type and Eye

Because each eye contributed separate LOCS II grades for nuclear, cortical, and PSC opacities, subtype frequencies are not mutually exclusive. Percentages are therefore reported per eye (denominator $n = 18$ per eye), and totals across subtypes should not be interpreted as summing to 100%. A total of 108 graded entries were available across the three LOCS II categories (Grades 0–3). The overall distribution was grade 0, 36/108 (33.3%); grade 1, 27/108 (25.0%); grade 2, 37/108 (34.3%); and grade 3, 8/108 (7.4%). The majority of cataract components fell within Grades 1–2, representing mild to moderate severity.

Nuclear Cataracts

Nuclear cataracts demonstrated a highly symmetrical severity distribution between eyes:

- Right eye: grade 1 = 4/18 (22.2%), grade 2 = 12/18 (66.7%), grade 3 = 2/18 (11.1%),
- Left eye: grade 1 = 4/18 (22.2%), grade 2 = 12/18 (66.7%), grade 3 = 2/18 (11.1%).

All eyes exhibited nuclear opacity in the lenses (36/36, 100%), with grade 2 as the dominant, accounting for two-thirds of all nuclear cataracts.

Cortical Cataracts

Cortical cataracts showed more heterogeneity in severity and were absent in approximately one-third of lenses:

- Right eye: grade 0 = 6/18 (33.3%), grade 1 = 6/18 (33.3%), grade 2 = 5/18 (27.8%), grade 3 = 1/18 (5.6%).
- Left eye: grade 0 = 6/18 (33.3%), grade 1 = 7/18 (38.9%), grade 2 = 4/18 (22.2%), grade 3 = 1/18 (5.6%).

Across eyes, grade 1 was the most frequent severity level (13/36, 36.1%), followed by grade 2 (9/36, 25.0%), while severe cortical cataracts (grade 3) were rare (2/36, 5.6%).

PSC Cataracts

PSC opacities were the least frequent subtype and showed the greatest asymmetry between eyes:

- Right eye: grade 0 = 11/18 (61.1%), grade 1 = 2/18 (11.1%), grade 2 = 2/18 (11.1%), grade 3 = 3/18 (16.7%).
- Left eye: grade 0 = 13/18 (72.2%), grade 1 = 3/18 (16.7%), grade 2 = 2/18 (11.1%), grade 3 = 0.

PSC was absent in most lenses (24/36, 66.7%). When present, PSC was predominantly mild–moderate (grades 1–2). Severe PSC (Grade 3) occurred only in right eyes (3/18 eyes, 16.7%) and was not observed in the left eye.

4.2.4 Comparison of Cataract Severity Across Eyes

A composite measure of overall cataract grade (averaging grades across nuclear, cortical, and PSC for each eye) showed a slightly higher mean cataract grade in the right eye compared with the left eye, with overlapping standard deviations. While not statistically significant, the difference approached the threshold for significance ($p = 0.0699$), indicating a trend toward greater cumulative lens opacity in right eyes.

4.3 Visual Acuity Before and After Cataract Surgery

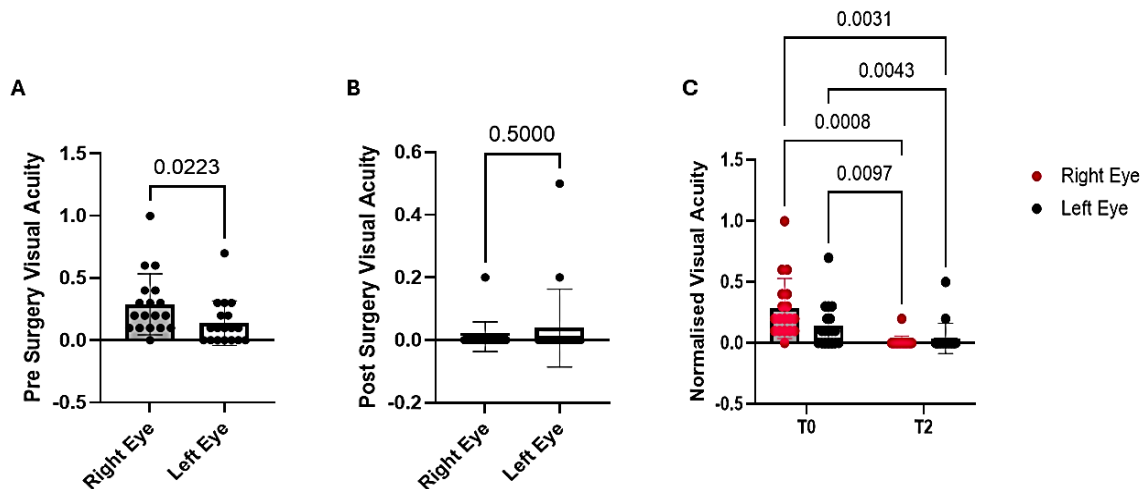


Figure 4.2. Best-corrected visual acuity (LogMAR units) before and after bilateral cataract surgery. (A) Pre-surgery (T0) visual acuity. (B) Post-surgery (T2) visual acuity. (C) Longitudinal change in visual acuity from T0 to T2 in right and left eyes.

4.3.1 Analytic Approach

Visual acuity (VA) analyses were conducted at the eye level using best-corrected logarithm of the minimum angle of resolution (logMAR) values, with higher logMAR values indicating worse vision. Because of the small sample size ($n = 18$) and the limited power of formal normality tests, longitudinal comparisons were performed using a non-parametric paired analysis (Wilcoxon matched-pairs signed-rank test). Inter-eye concordance was assessed using Spearman correlation, and Spearman correlations between eyes are reported with one-tailed p-values (directional hypothesis: positive concordance).

4.3.2 Baseline Pre-surgery (T0) Visual Acuity

At T0, there was a clear asymmetry between eyes. LogMAR VA in the right eye was more impaired and more variable than in the left eye, with several participants showing marked reductions in acuity in the right eye on the baseline plot, including outliers approaching 1.0 logMAR. In contrast, left-eye acuity clustered closer to 0, with a narrower spread.

A paired comparison confirmed that right-eye VA was significantly worse than left-eye VA at baseline (Wilcoxon matched-pairs signed-rank test, $p = 0.0223$). The median paired difference was -0.10 logMAR (left minus right), indicating that, on average, the right eye was 0.10 logMAR poorer than the left. The correlation between right- and left-eye VA at T0 was weak (Spearman $r_s = 0.19$, one-tailed $p = 0.229$), suggesting that, in this sample, early cataract-related visual loss did not progress in a tightly synchronised manner between the two eyes.

4.3.3 Post-surgery (T2) Visual Acuity

By the final visit, best-corrected VA had normalised in both eyes, with the majority of values clustering tightly around 0 logMAR on the post-operative plot. Only a small number of mild outliers remained, but there was no systematic difference between eyes. Consistent with this pattern, no significant difference was observed between right- and left-eye VA at T2 (Wilcoxon matched-pairs signed-rank test, $p = 0.5000$; median paired difference 0.00 logMAR).

In contrast to the baseline findings, the correlation between eyes was strong post-operatively (Spearman $r_s = 0.73$, one-tailed $p = 0.0003$), indicating that after surgery, right and left eyes converged towards a similar, high level of best-corrected VA.

4.3.4 Change in Visual Acuity from T0 to T2

Longitudinal comparisons demonstrated a robust improvement in best-corrected logMAR VA from baseline (T0) to final follow-up (T2) in both eyes. On the combined T0–T2 plot, right-eye values moved from a dispersed distribution centred above 0 at T0 to a tight cluster at or near 0 by T2. Left-eye values showed a similar but slightly smaller shift, moving from mildly elevated baseline values to near-normal acuity at T2.

Within-eye analyses (using Wilcoxon matched-pairs tests) showed that VA improved significantly from T0 to T2 in each eye (both p values ≤ 0.01). When eyes were considered together in a pooled analysis of all eye-level values, the overall improvement in VA from T0 to T2 was also significant (Wilcoxon matched-pairs signed-rank test, $p \approx 0.003$). Hence, across the cohort of 18 participants, bilateral cataract surgery produced a consistent, clinically meaningful gain in best-corrected VA, eliminating the pre-operative asymmetry between eyes and bringing most eyes to near-normal logMAR values by the final follow-up.

4.4 Correlation Between Baseline Visual Acuity and Cataract Severity (T0)

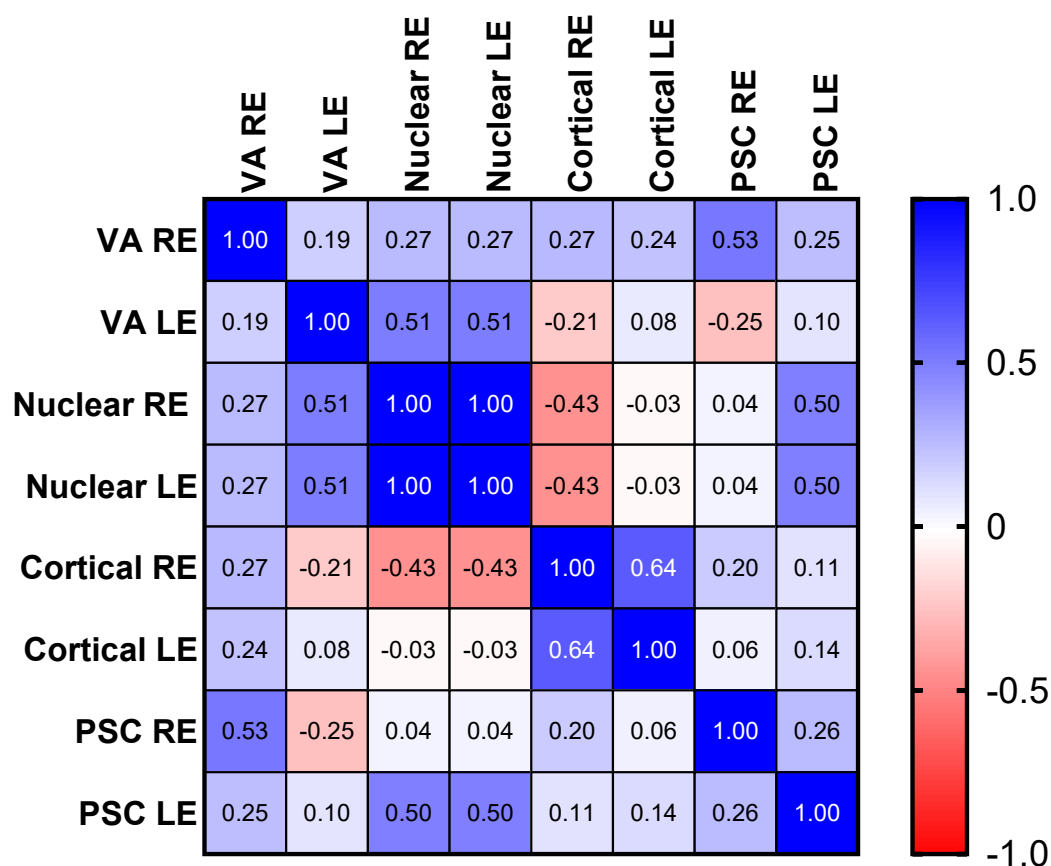


Figure 4.3. Correlation matrix of baseline visual acuity and cataract severity by eye (T0). VA: visual acuity. RE: right eye. LE: left eye. PSC: posterior subcapsular.

Spearman correlations were calculated to examine associations between baseline best-corrected VA and LOCS II grades for nuclear, cortical, and PSC cataracts in each eye (N = 18).

In the right eye, baseline VA showed weak positive correlations with nuclear cataract grade ($r_s = 0.27$) and cortical grade ($r_s = 0.27$), and a stronger correlation with PSC grade ($r_s = 0.53$). In the left eye, baseline VA correlated positively with nuclear grade ($r_s = 0.51$), weakly with cortical grade ($r_s = 0.08$), and with PSC grade ($r_s = 0.10$). Overall, these patterns indicate that greater cataract severity was associated with poorer baseline VA, with the relationship most pronounced for nuclear cataracts in the left eye and for PSC cataracts in the right eye.

Nuclear cataract grades were strongly intercorrelated between eyes ($r_s = 1.00$), suggesting that nuclear cataract severity tended to progress bilaterally in a similar pattern within this cohort. Cortical cataracts showed moderate bilateral correspondence ($r_s = 0.64$), whereas PSC opacities showed weaker symmetry ($r_s = 0.26$), indicating greater inter-ocular variability for this subtype. Negative correlations were observed between cortical and nuclear cataract grades in both eyes ($r_s = -0.43$), suggesting that higher nuclear

severity did not necessarily co-occur with greater cortical severity, consistent with differing pathological pathways for these subtypes. Negative cross-eye associations, such as between left-eye VA and right-eye PSC ($r_s = -0.25$), are also evident on the heatmap and likely reflect small-sample variability rather than a stable pattern. Full coefficients are presented in Figure 4.3

Taken together, the heatmap suggests that cataract severity, particularly for nuclear and PSC types, is meaningfully associated with baseline VA, in line with previous literature. Variability across subtypes and between eyes indicates that no single cataract component fully accounted for VA impairment in this sample; instead, visual loss likely reflects the combined effects of multiple cataract subtypes.

4.5 Summary of Findings

This chapter shows that the cohort consisted of older adults living independently in the community, with generally high levels of educational attainment, and preserved social and physical engagement. Vascular risk factors such as hypertension and dyslipidaemia were common, and a family history of late-onset neurodegenerative disease (Parkinson disease or Alzheimer disease) was present in more than half of participants.

From an ophthalmic perspective, all lenses demonstrated nuclear cataract change, with additional contributions from cortical and PSC subtypes. Nuclear cataracts were bilaterally symmetrical and predominantly of mild–moderate severity, whereas PSC changes were less frequent and more asymmetric, occurring more often in the right eye.

Baseline best-corrected VA was clearly impaired and asymmetric, with worse and more variable acuity in the right eye. Following bilateral cataract surgery, VA normalised in both eyes, and the pre-operative asymmetry disappeared, with strong post-operative inter-eye coupling. Correlation analyses confirmed that greater cataract severity, particularly nuclear and PSC, was associated with poorer baseline VA, although no single subtype fully accounted for the observed visual loss.

These findings establish that the study cohort was characterised by clinically meaningful cataract-related visual impairment on a background of typical age-related comorbidity, and that bilateral cataract surgery produced robust and symmetric restoration of visual acuity. This provides the necessary visual and clinical context for interpreting the longitudinal cognitive, blood–brain barrier, structural MRI, and proteomic results presented in subsequent chapters.

CHAPTER 5: RESULTS PART II: COGNITIVE TRAJECTORIES IN OLDER ADULTS UNDERGOING CATARACT SURGERY.

5.1 Baseline Cognitive Profile (T0)

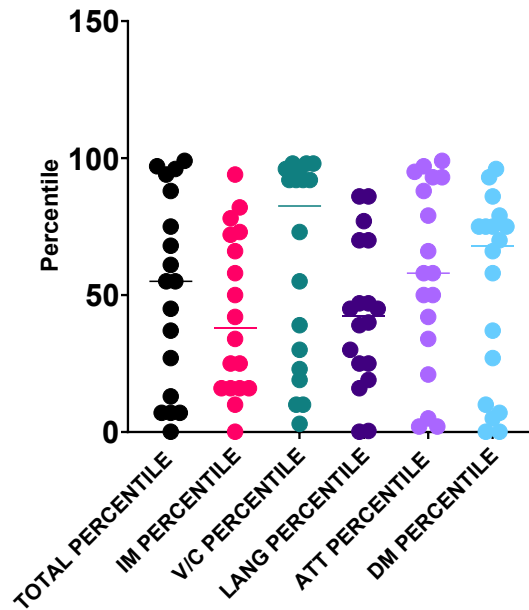


Figure 5.1. Baseline RBANS-U percentile scores across cognitive domains (T0).

IM: Immediate memory. V/C: Visuospatial/Constructional. Lang: Language. Att: Attention. DM: Delayed memory.

5.1.1 Analytic Approach

Baseline cognitive performance was assessed using the Repeatable Battery for the Assessment of Neuropsychological Status – Update (RBANS-U) age-adjusted percentile scores for the Total Scale and five domain indices: immediate memory (IM), visuospatial/constructional (V/C), language, attention, and delayed memory (DM). Distributions were examined with normality tests (D’Agostino–Pearson, Anderson–Darling, Shapiro–Wilk, Kolmogorov–Smirnov), and domain differences were assessed using one-way repeated-measures ANOVA with Tukey’s multiple comparisons test.

5.1.2 Distribution of RBANS-U Percentile Scores

At T0, group mean percentiles were: Total 51.73, IM 42.95, V/C 62.00, Language 42.64, Attention 57.33, and DM 51.90. Overall, the cohort performed within an average range across domains, with numerically higher scores in the V/C and attention indices and slightly lower scores in IM and language (Figure 5.1).

5.1.3 Comparison Between RBANS-U Domains at Baseline

Mean differences between domains were modest, and none reached statistical significance after adjustment for multiple comparisons (all adjusted $p > 0.16$). For example, the difference between Total

and IM percentiles was 8.78 (95% CI –8.06 to 25.62, $p = 0.57$), and between Total and V/C percentiles was –10.27 (95% CI –26.05 to 5.51, $p = 0.34$). Comparisons such as IM versus V/C (–19.05, $p = 0.29$) and Language versus Attention (–14.69, $p = 0.41$) were non-significant either. Post hoc Tukey comparisons did not identify any statistically significant differences between domains.

Taken together, these results indicate that the baseline cognitive profile of the cohort was relatively balanced across domains at the group level, with no focal pattern of impairment or sparing emerging when domains were compared directly.

5.1.4 Individuals with Low Baseline Cognitive Performance

Despite the broadly normative group means, there was marked inter-individual variability. Seven of the 18 participants (38.9%) scored at or below the 10th percentile in at least one RBANS-U domain at T0, fulfilling criteria for mild cognitive impairment (MCI) (reviewed in Chapter 1, part 2). Of these, 3 participants (42.9%) showed an isolated low score in a single domain, whereas 4 participants (57.1%) had scores at or below the 10th percentile in two or more domains, indicating a more global pattern of cognitive weakness.

5.2 Longitudinal Cognitive Changes from T0 to T2

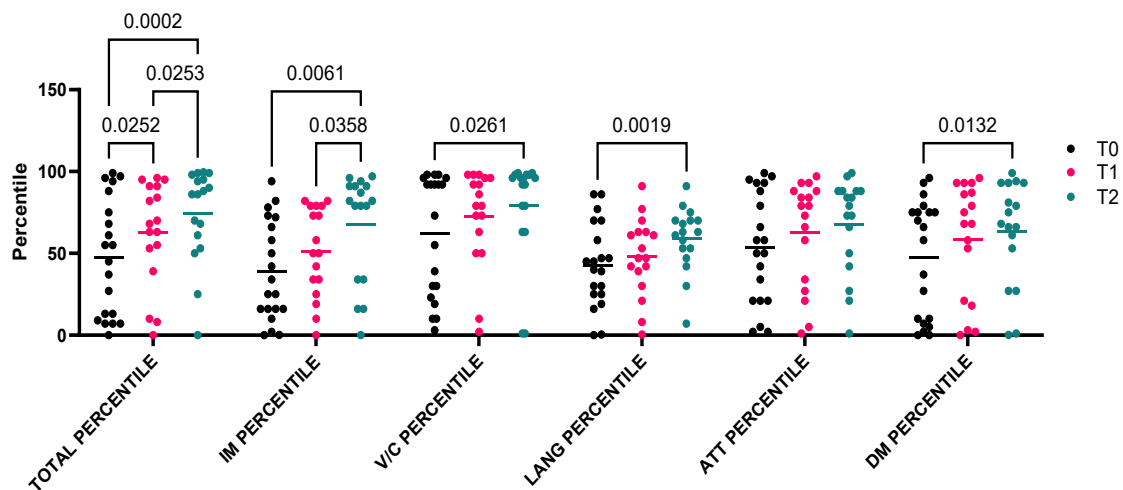


Figure 5.2. Longitudinal RBANS-U domain percentile scores from T0 to T2.

IM: Immediate memory. V/C: Visuospatial/Constructional. Lang: Language. Att: Attention. DM: Delayed memory.

5.2.1 Analytic Approach

Longitudinal change in RBANS-U age-adjusted percentile scores was evaluated using a linear mixed-effects model (LMM). Where an overall time effect was observed, post hoc pairwise contrasts between timepoints (T0 vs T1, T0 vs T2, T1 vs T2) were performed with multiplicity adjustment.

5.2.2 RBANS-U Total Scale Percentile Trajectories

Group mean RBANS-U Total percentile increased from 51.73 at T0 to 62.54 at T1 and 74.22 at T2. Pairwise comparisons showed a significant improvement from T0 to T1 (mean difference -13.59 , 95% CI -25.56 to -1.62 , $p = 0.0252$) and a larger improvement from T0 to T2 (-25.09 , 95% CI -37.08 to -13.11 , $p = 0.0002$). There was also a significant gain between T1 and T2 (-12.48 , 95% CI -23.46 to -1.49 , $p = 0.0253$). Overall, the Total Scale Index showed a progressive increase across the three timepoints, with significant stepwise improvements from T0 to T2.

5.2.3 Domain-level Trajectories

Mean percentile scores for each domain increased over time:

- **Immediate Memory:** 39.95 at T0, 51.12 at T1, 67.54 at T2.
 - T0 vs T1: not significant (-11.18 , $p = 0.2521$).
 - T0 vs T2: significant improvement (-25.47 , 95% CI -43.60 to -7.34 , $p = 0.0061$).
 - T1 vs T2: significant improvement (-17.44 , 95% CI -33.77 to -1.10 , $p = 0.0358$).
- **Visuospatial/Constructional:** 59.88 at T0, 72.65 at T1, 79.18 at T2.
 - T0 vs T1: not significant (-12.76 , $p = 0.2061$).
 - T0 vs T2: significant improvement (-19.29 , 95% CI -36.39 to -2.19 , $p = 0.0261$).
 - T1 vs T2: not significant (-6.81 , $p = 0.3114$).
- **Language:** 41.03 at T0, 47.96 at T1, 58.82 at T2.
 - T0 vs T1: not significant (-6.94 , $p = 0.3767$).
 - T0 vs T2: significant improvement (-18.74 , 95% CI -30.28 to -7.19 , $p = 0.0019$).
 - T1 vs T2: did not reach significance after correction (-11.41 , $p = 0.0575$).
- **Attention:** 55.12 at T0, 62.94 at T1, 67.53 at T2.
 - T0 vs T1: not significant (-7.82 , $p = 0.2122$).
 - T0 vs T2: did not reach significance (-12.29 , $p = 0.0563$).
 - T1 vs T2: not significant (-5.44 , $p = 0.5148$).
- **Delayed Memory:** 49.48 at T0, 58.42 at T1, 63.31 at T2.
 - T0 vs T1: not significant (-8.94 , $p = 0.4988$).
 - T0 vs T2: significant improvement (-13.41 , 95% CI -24.06 to -2.76 , $p = 0.0132$).
 - T1 vs T2: not significant (-5.38 , $p = 0.7418$).

In summary, IM, V/C, language, and DM showed significant gains between T0 and T2, with IM also improving significantly between T1 and T2. Attention showed numerically higher scores over time but did not reach statistical significance after correction.

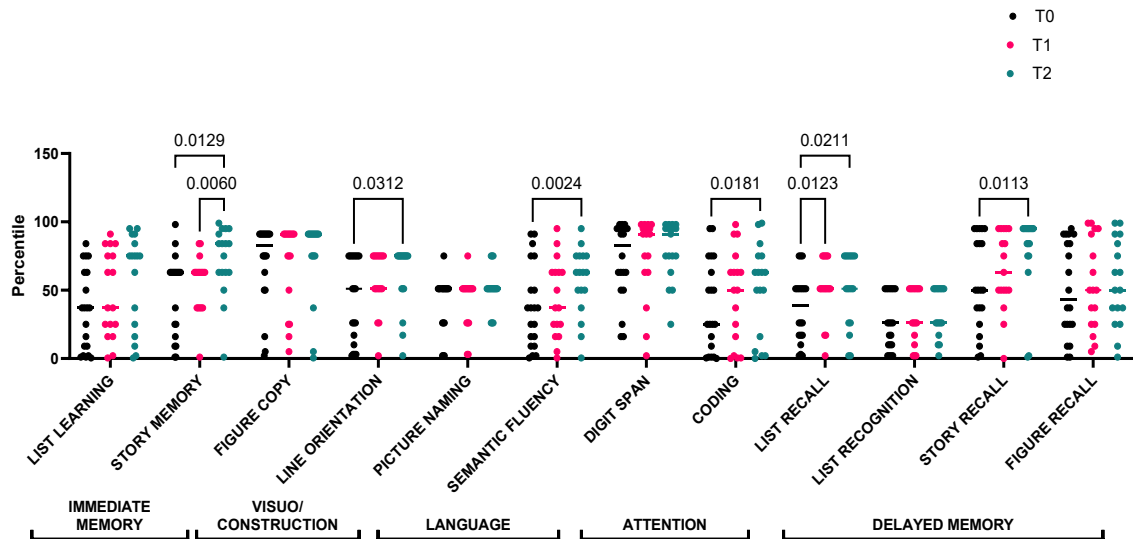


Figure 5.3. RBANS-U subtest percentile scores at T0, T1, and T2, grouped by parent domain.

5.2.4 Subtest-level Exploratory Patterns

Figure 5.3 shows longitudinal trajectories for the 12 RBANS-U subtests across T0, T1, and T2. Mean percentile scores increased over time for most subtests, but statistically significant changes after Bonferroni correction were concentrated in a subset of measures.

- **Immediate Memory domain subtests**

- *List Learning*: mean percentiles increased from 36.55 (T0) to 47.20 (T1) and 57.85 (T2), but none of the pairwise contrasts reached significance (all adjusted $p \geq 0.0878$).
- *Story Memory*: mean scores grew from 49.35 (T0) to 54.88 (T1) and 72.12 (T2). There was a significant improvement from T0 to T2 (-20.71 , 95% CI -37.36 to -4.05 , $p = 0.0129$) and from T1 to T2 (-17.56 , 95% CI -30.24 to -4.89 , $p = 0.0060$), with no significant change between T0 and T1.

- **Visuospatial/Constructional domain subtests**

- *Figure Copy*: mean percentiles increased modestly from 66.06 at T0, to 68.53 at T1, and 73.67 at T2, with no significant pairwise differences (all adjusted $p > 0.88$).
- *Line Orientation*: mean rose from 43.59 (T0) to 56.47 (T1) and 60.18 (T2). The T0–T1 contrast showed a trend (-12.88 , $p = 0.0655$), and the T0–T2 comparison reached significance (-16.59 , 95% CI -31.87 to -1.31 , $p = 0.0312$). T1–T2 differences were not significant.

- **Language domain subtests**

- *Picture Naming*: mean percentiles increased from 42.29 (T0) to 42.35 (T1) and 50.82 (T2); no comparison was statistically significant (all adjusted $p \geq 0.17$).
- *Semantic Fluency*: mean increased from 35.96 (T0) to 43.67 (T1) and 56.44 (T2). The T0–T2 comparison showed a significant improvement (-20.47 , 95% CI -33.73 to -7.21 , $p = 0.0024$), whereas T0–T1 and T1–T2 comparisons were not significant.

- **Attention domain subtests**

- *Digit Span*: mean percentiles were high at baseline (71.82 at T0) and remained relatively stable over time (75.35 at T1, 79.47 at T2), with no significant pairwise differences (all adjusted $p > 0.84$).
- *Coding*: mean scores increased from 38.59 (T0) to 46.35 (T1) and 50.48 (T2). The T0–T2 comparison was significant (-11.88 , 95% CI -21.92 to -1.84 , $p = 0.0181$), whereas T0–T1 showed a near-threshold approach (-7.75 , $p = 0.0533$) and T1–T2 was not significant.

- **Delayed Memory domain subtests**

- *List Recall*: mean rose from 38.24 (T0) to 51.18 (T1) and 50.18 (T2). Both T0–T1 and T0–T2 comparisons were significant (-12.94 , 95% CI -23.27 to -2.61 , $p = 0.0123$; and -11.94 , 95% CI -22.27 to -1.61 , $p = 0.0211$, respectively), whereas T1–T2 was not ($p > 0.9999$).
- *List Recognition*: mean scores increased slightly (27.24 at T0, 32.06 at T1, 33.94 at T2), but no contrasts reached significance (all adjusted $p \geq 0.31$).
- *Story Recall*: means increased from 57.53 (T0) to 64.30 (T1) and 77.82 (T2). The T0–T2 comparison showed a significant improvement (-20.29 , 95% CI -36.32 to -4.27 , $p = 0.0113$), while T0–T1 and T1–T2 were not significant after correction.
- *Figure Recall*: mean percentiles remained stable (52.00 at T0, 54.18 at T1, 55.06 at T2), with no significant differences between timepoints (all adjusted $p > 0.99$).

Overall, subtest-level analyses indicate that longitudinal gains were most evident in Story Memory, Line Orientation, Semantic Fluency, Coding, List Recall, and Story Recall, whereas other subtests showed smaller or non-significant changes over the 9-month follow-up.

5.3 Individuals with Low Baseline Cognitive Performance: Subgroup Trajectories

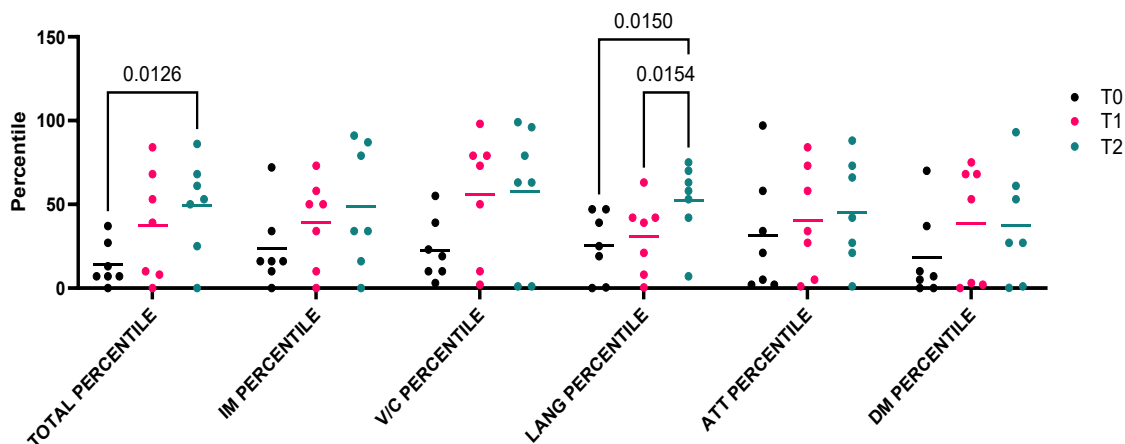


Figure 5.4. Longitudinal RBANS-U domain percentile scores in individuals with low baseline cognitive performance (T0 to T2). N=7.

IM: Immediate memory. V/C: Visuospatial/Constructional. Lang: Language. Att: Attention. DM: Delayed memory.

5.3.1 Analytic Approach

Longitudinal RBANS-U performance was examined in the subgroup of participants who showed low baseline cognitive performance, which, as described previously in this chapter, is defined as scoring at or below the 10th percentile in at least one RBANS-U domain at T0 ($n = 7$). Age-adjusted percentile scores for the RBANS-U Total Scale and five domain indices were analysed across the three timepoints. Within each domain, one-way repeated-measures ANOVA was performed with Tukey's post hoc tests ($\alpha = 0.05$) to assess pairwise differences between timepoints.

5.3.2 RBANS-U Domain Scores in Individuals with Low Baseline Cognitive Performance

Although representing over a third of the research population ($n=7$, 38.9%), the subgroup that met MCI criteria on testing was small, clinically heterogeneous, and included one participant who was diagnosed with Alzheimer disease (AD) mild dementia during follow-up and another who underwent active oral chemotherapy for non-metastatic breast cancer. Within this context, consistent patterns appeared across several domains.

- **RBANS-U Total Percentile**

RBANS-U Total percentile scores showed a significant increase between T0 and T2 (mean difference – 35.00, 95% CI –60.20 to –9.81, $p = 0.0126$), indicating higher overall performance 9 months after surgery compared with baseline. The comparison between T0 and T1 did not reach statistical significance ($p = 0.0660$), nor did the comparison between T1 and T2 ($p = 0.4260$), suggesting that the largest change in Total percentile occurred over the full T0–T2 interval rather than immediately after surgery.

- **Immediate Memory**

IM percentile scores did not change significantly across timepoints. None of the pairwise comparisons (T0 vs T1, T0 vs T2, T1 vs T2) reached statistical significance (all $p > 0.20$), although numerical increases from T0 to both T1 and T2 were observable. Confidence intervals were wide, reflecting variability within this small subgroup.

- **Visuospatial/Constructional**

No statistically significant differences were detected either for the V/C index (all $p > 0.07$). Both the T0–T1 and T0–T2 comparisons showed numerical improvements, but the associated confidence intervals crossed zero and did not meet the predefined significance threshold.

- **Language**

Language performance showed a clearer pattern of improvement within the subgroup. The T0–T2 comparison was significant (mean difference –27.21, 95% CI –47.59 to –6.84, $p = 0.0150$), as well as the T1–T2 comparison (mean difference –21.80, 95% CI –38.22 to –5.38, $p = 0.0154$). The T0–T1 contrast was not significant ($p = 0.4230$), indicating that higher language percentiles were evident at 9 months relative to both baseline and the 3-month assessment.

- **Attention**

Attention scores remained statistically stable over time. Pairwise comparisons between T0, T1, and T2 did not reach significance (all $p > 0.16$), although small numerical increases were observed across the follow-up period.

- **Delayed Memory**

No significant pairwise differences were found for DM percentiles across timepoints (all $p > 0.07$). The T0–T2 comparison generated a $p = 0.0707$, close to but still above the predefined significance threshold of 0.05, with wide confidence intervals reflecting considerable variability in this small group.

To summarize, in individuals with low baseline cognitive performance, the most consistent postoperative improvements were observed in RBANS-U Total and language percentiles, both of which were significantly higher at T2 compared with T0. Other domains (IM, V/C, attention, DM) showed numerical increases over time but did not reach statistical significance, reflecting the combination of small sample size and clinical heterogeneity within this subgroup.

5.3.3 Course of Individuals with Low Baseline Cognitive Performance: Resolution and Persistence of Low-Range Cognitive Scores

By design of the subgroup analysis, all seven individuals had at least one RBANS-U index at or below the 10th percentile at T0, with a median of three domains at this level (range 1–5). By T2, four participants (57.1%) no longer showed low-range performance on any RBANS-U index, whereas three (42.9%) continued to have at least one domain at or below the 10th percentile. In terms of burden of impairment, five participants (71.4%) had fewer low-range domains at T2 than at baseline, one (14.3%) remained stable with a single low domain, and one (14.3%) showed an increase, with very low scores persisting across most domains at follow-up.

5.4 Mood and Anxiety Measures

5.4.1 Analytic Approach

Depressive symptoms were assessed using the Beck Depression Inventory-II (BDI-II), and anxiety was assessed using the State–Trait Anxiety Inventory, Form Y, State subscale (STAI-Y1) and Trait subscale (STAI-Y2) at the three timepoints. For each measure, differences between timepoints were evaluated using a linear mixed-effects model (LMM). Where an overall time effect was observed, post hoc pairwise contrasts between timepoints (T0 vs T1, T0 vs T2, T1 vs T2) were performed with multiplicity adjustment.

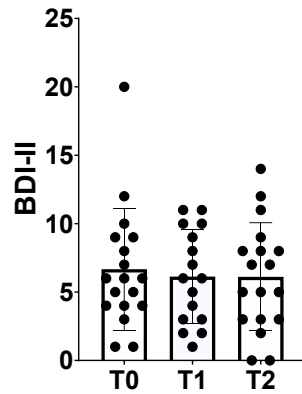


Figure 5.5. Beck Depression Inventory-II (BDI-II) scores at T0, T1, and T2.

5.4.2 Depressive Symptoms (BDI-II)

BDI-II total scores remained stable across all three assessments (Figure 5.5). No significant differences were detected between T0 and T1 (mean difference 0.53, 95% CI -1.43 to 2.49 , adjusted $p = 0.7677$), between T0 and T2 (mean difference 0.53, 95% CI -1.41 to 2.47 , adjusted $p = 0.7652$), or between T1 and T2 (mean difference -0.06 , 95% CI -1.61 to 1.48 , adjusted $p = 0.9939$).

When scores were categorised using standard BDI-II severity ranges (0–10 minimal; 11–16 mild; 17–20 borderline; 21–30 moderate; 31–40 severe; >40 extreme), the majority of participants at each timepoint fell within the minimal range. At T0, 88.9% of the participants were in the minimal range, 5.6% in the mild range, and 5.6% in the borderline range. At T1, 87.5% of the participants scored in the minimal range and 12.5% in the mild range, with no borderline or higher scores. At T2, 83.3% of the participants were in the minimal range and 16.7% in the mild range, again with no scores in the borderline, moderate, severe, or extreme ranges. Overall, there was no systematic shift in the distribution of severity categories between T0, T1, and T2.

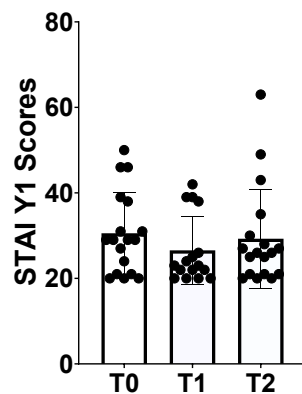


Figure 5.6. State–Trait Anxiety Inventory, State subscale (STAI-Y1) scores at T0, T1, and T2.

5.4.3 State Anxiety (STAI-Y1)

State anxiety (STAI-Y1) scores also showed no statistically significant change over time (Figure 5.6). The T0–T1 comparison approached but did not reach significance (mean difference 4.18, 95% CI –0.57 to 8.92, adjusted $p = 0.0891$). No significant differences were observed between T0 and T2 (mean difference 1.00, adjusted $p = 0.8728$) or between T1 and T2 (mean difference –3.50, adjusted $p = 0.1767$). All three timepoints were classified into a single group (A) in the compact letter display.

Using conventional STAI severity bands (20–37 low/no anxiety; 38–44 moderate; 45–80 high), most scores at each assessment fell within the low range. At T0, approximately 72% of participants were in the low range, 11% in the moderate range, and 17% in the high range. At T1, around 75% scored in the low range and 25% in the moderate range, with no scores in the high range. At T2, about 83% were in the low range, 6% in the moderate range, and 11% in the high range. Across the three assessments, only a small minority of observations fell into the moderate or high anxiety bands, and there was no clear time-specific shift in the distribution of severity categories.

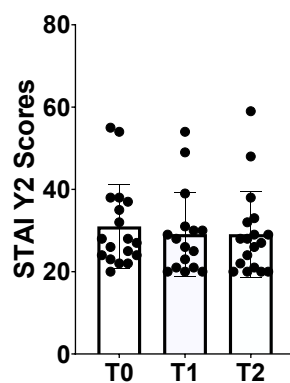


Figure 5.7. State–Trait Anxiety Inventory, Trait subscale (STAI-Y2) scores at T0, T1, and T2.

5.4.4 Trait Anxiety (STAI-Y2)

Trait anxiety (STAI-Y2) remained consistent over time, with no significant differences between T0 and T1 (mean difference 1.82, 95% CI –1.05 to 4.69, adjusted $p = 0.2584$), between T0 and T2 (mean difference 2.00, 95% CI –0.88 to 4.89, adjusted $p = 0.2048$), or between T1 and T2 (mean difference 0.00, 95% CI –1.82 to 1.82, adjusted $p > 0.9999$).

Using the same STAI cut-offs, most participants consistently scored in the low trait anxiety range (20–37). At T0, approximately 78% of valid observations fell in the low range, 11% in the moderate range (38–44), and 11% in the high range (≥ 45). At T1, around 81% of scores were in the low range, 6% in the moderate range, and 13% in the high range. At T2, about 83% of participants scored in the low range, 6% in the moderate range, and 11% in the high range. Occasional values in the high range were rare and did not show any systematic accumulation at a particular timepoint, and the proportions in each severity band were broadly similar across all three assessments.

5.5 RBANS-U Total Scores in Relation to Mood and Anxiety (BDI-II, STAI-Y1, STAI-Y2)

5.5.1 Analytic Approach

To examine whether mood or anxiety severity was associated with cognitive performance, RBANS-U Total percentile scores (pooled across T0, T1, and T2) were compared between participants classified as higher versus lower scorers on the BDI-II, STAI-Y1, and STAI-Y2. Given the non-normal distribution of the affective measures, group differences in RBANS-U Total were evaluated using Mann–Whitney U tests.

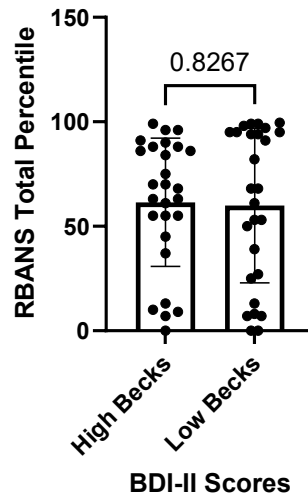


Figure 5.8. RBANS-U Total Percentiles by BDI-II Symptom Level (High vs Low Becks).

5.5.2 RBANS-U Total vs BDI-II Severity

RBANS-U Total scores did not differ between participants with higher versus lower BDI-II scores (Figure 5.8). The Mann–Whitney test showed no significant group difference ($U = 351.5$, $p = 0.8267$). Median RBANS-U Total percentiles were identical in both groups (median 68.0), and the Hodges–Lehmann estimate of the median difference was minimal (2.0 points). Within the minimal-to-mild range of depressive symptoms observed in this cohort, there was no evidence that BDI-II severity was associated with variation in overall cognitive performance.

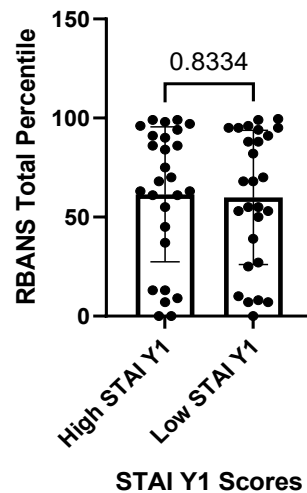


Figure 5.9. RBANS-U Total Percentile Scores in High vs Low STAI-Y1 (State Anxiety) Groups.

5.5.3 RBANS-U Total vs STAI-Y1 (State Anxiety)

A similar pattern was observed for state anxiety (STAI-Y1; Figure 5.9). The comparison between higher and lower STAI-Y1 scorers showed no statistically significant difference in RBANS-U Total scores ($U = 352$, $p = 0.8334$). Both groups again displayed identical median RBANS-U Total percentiles (68.0), with a negligible Hodges–Lehmann estimate (-2.0 points). These findings suggest that short-term fluctuations in state anxiety were not meaningfully related to overall RBANS-U performance in this sample.

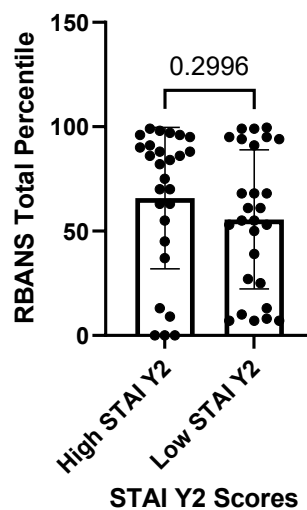


Figure 5.10. RBANS-U Total Percentile Scores in High vs Low STAI-Y2 (Trait Anxiety) Groups.

5.5.4 RBANS-U Total vs STAI-Y2 (Trait Anxiety)

RBANS-U Total scores also did not differ significantly between higher and lower trait anxiety (STAI-Y2) groups ($U = 304$, $p = 0.2996$). Although the group medians diverged numerically (82.0 in the higher-anxiety group vs 55.0 in the lower-anxiety group), variability was considerable, and the Hodges–Lehmann estimate of the median difference (–8.0 points) did not reach statistical significance. In this cohort, trait anxiety levels therefore did not show a reliable association with overall cognitive performance.

Overall, across all three affective measures, there was no evidence that differences in depressive symptoms, state anxiety, or trait anxiety were linked to differences in RBANS-U Total scores.

5.6 Preoperative Visual–Cognitive Correlations (RBANS-U Total Percentile)

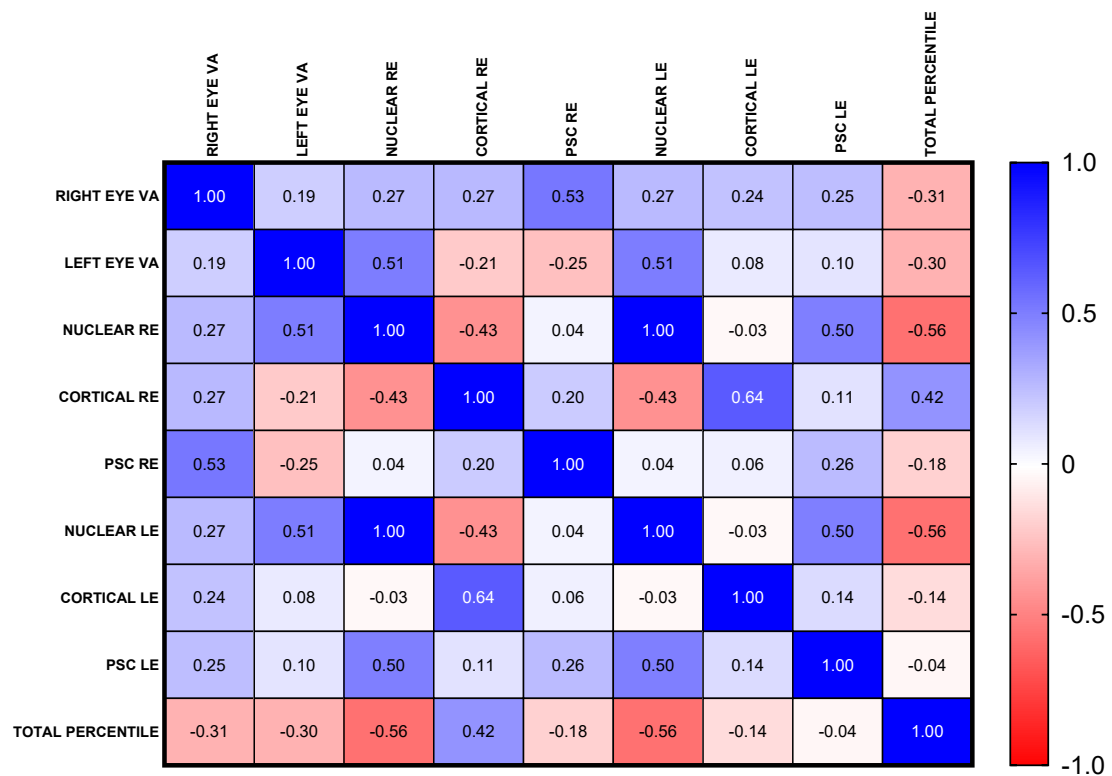


Figure 5.11. Baseline correlations between RBANS-U Total percentile and eye-level visual parameters (T0). VA: visual acuity. RE: right eye. LE: left eye. PSC: posterior subcapsular.

Associations between baseline global cognition and visual status were examined using Pearson correlation coefficients between RBANS-U Total percentile scores and eye-level visual measures at T0: nuclear, cortical, and posterior subcapsular (PSC) cataract grades in each eye, and best-corrected visual acuity (VA). Results are summarised in a correlation heatmap (Figure 5.11), in which point colour encodes the direction and magnitude of r .

Of note, although both eyes were analysed separately, nuclear cataract grading values were identical for both eyes in this cohort, and consequently right- and left-eye nuclear cataract correlations (and p -values) are identical.

Nuclear cataract grading in the right and left eyes showed the largest effect sizes, with negative correlations ($r = -0.56$). These coefficients suggest a moderate inverse trend, where greater nuclear opacity tended to accompany lower RBANS-U Total scores, and this association was statistically significant ($p = 0.0156$). Given the exploratory nature of these correlations and the number of visual variables assessed, this finding is interpreted with appropriate caution.

Correlations involving cortical and PSC cataracts and IOP were smaller in magnitude. Cortical severity showed mixed-direction associations with RBANS-U Total (right eye $r = 0.42$, $p = 0.0806$; left eye $r = -0.14$, $p = 0.5839$), and PSC grades produced similarly small effects (right eye $r = -0.18$, $p = 0.4728$; left eye $r = -0.04$, $p = 0.8888$), indicating no clear relationship between global cognition and these cataract subtypes at baseline.

Best-corrected VA in both eyes demonstrated small-to-moderate negative correlations with RBANS-U Total percentile (right eye $r = -0.31$, $p = 0.2170$; left eye $r = -0.30$, $p = 0.2205$). These inverse coefficients indicate that poorer acuity tended to accompany lower global cognitive performance, but these associations did not reach statistical significance.

Overall, the heatmap indicates that, within this sample, baseline variation in visual parameters was not consistently associated with global cognitive performance as indexed by RBANS-U Total percentile. Nuclear cataract severity showed the clearest inverse relationship and was the only association to reach statistical significance at T0, while VA demonstrated a directionally similar inverse trend that did not reach statistical significance, plausibly reflecting limited statistical power given the modest sample size; cortical and PSC cataract grades showed smaller and less consistent associations.

5.7 Post-operative Visual–Cognitive Correlations (RBANS-U Total Percentile)

At the post-operative follow-up (T2), Spearman rank correlations were calculated between RBANS-U Total percentile scores and best-corrected VA in the right and left eyes. Correlation coefficients (ρ) and corresponding p values are summarised in a correlation heatmap (Figure 5.12).

No statistically significant associations were observed between post-operative global cognitive performance and VA at T2. The right eye VA showed a small negative correlation with RBANS-U Total percentile ($\rho = -0.20$, $p = 0.588$), indicating a non-significant trend whereby poorer acuity tended to accompany lower global cognitive performance. The left eye VA demonstrated a small positive correlation ($\rho = 0.12$, $p = 0.651$), again non-significant.

Overall, RBANS-U Total percentile scores at T2 did not show a statistically reliable monotonic association with concurrent post-operative VA in either eye. As expected, post-operative VA values were strongly correlated between eyes ($\rho = 0.73$, $p = 0.0006$), supporting internal consistency of the visual acuity measurements at T2.

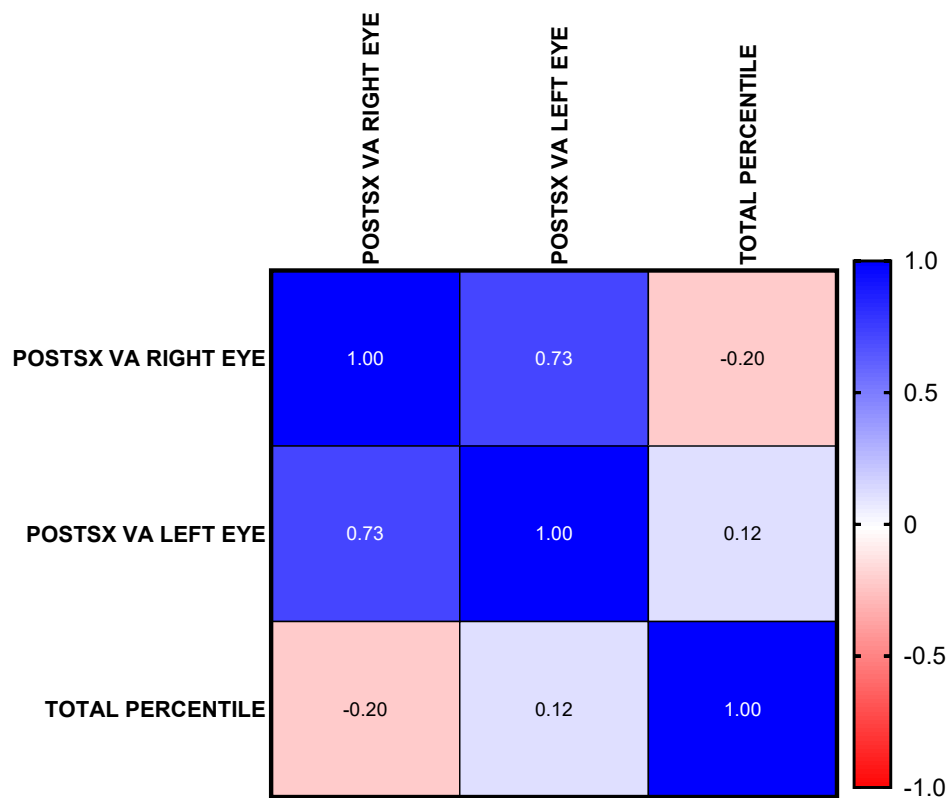


Figure 5.12. Post-operative correlations between RBANS-U Total percentile and best-corrected visual acuity at T2.

5.8 Summary of Findings

Across the full cohort, baseline RBANS-U performance was largely within the average range, with no preferential sparing or impairment of specific domains when compared directly. However, more than one-third of participants showed at least one domain at or below the 10th percentile at T0, highlighting substantial inter-individual variability.

Following bilateral cataract surgery, RBANS-U Total percentile scores increased progressively from T0 to T2, with significant improvements evident at each step. Domain-level and subtest analyses showed that improvements were most robust for immediate and delayed memory, visuospatial/constructional abilities, language, and selected attention measures (notably Coding), with particularly consistent changes in Story Memory, List Recall, Story Recall, Semantic Fluency, and Line Orientation.

In the subgroup of individuals with low baseline cognitive performance, RBANS-U Total and language percentiles improved significantly by T2, and most participants moved out of the low-range category on at least some domains. However, a minority showed persistent or worsening low-range scores, underlining ongoing heterogeneity and the influence of co-existing medical and neurodegenerative conditions.

Mood and anxiety indices remained stable and predominantly in the minimal or low ranges throughout follow-up. There were no significant associations between BDI-II, STAI-Y1, or STAI-Y2 scores and RBANS-U Total performance, suggesting that the observed cognitive trajectories are unlikely to be explained by changes in depressive symptoms or anxiety.

Baseline correlations between RBANS-U Total percentile and visual parameters at T0 identified a statistically significant inverse association with nuclear cataract severity, while VA showed a directionally similar but non-significant inverse trend. At T2, RBANS-U Total percentile was not significantly related to concurrent post-operative VA in either eye, despite strong inter-eye concordance of VA at follow-up. Taken together, these patterns are consistent with the possibility that baseline visual degradation beyond high-contrast acuity (e.g., contrast sensitivity, glare disability, colour discrimination, or higher-order optical disturbances, which were not directly measured) may have contributed to reduced cognitive performance at baseline, and that cognitive changes observed over time may reflect a broader set of visual improvements than acuity alone.

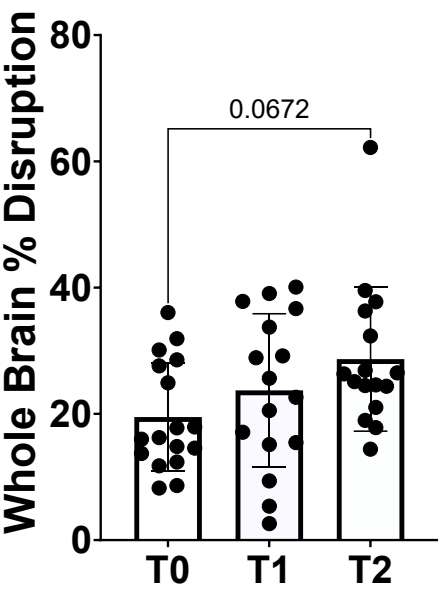


Figure 6.1. Whole-brain Blood-brain Barrier disruption (%) across the perioperative timepoints.

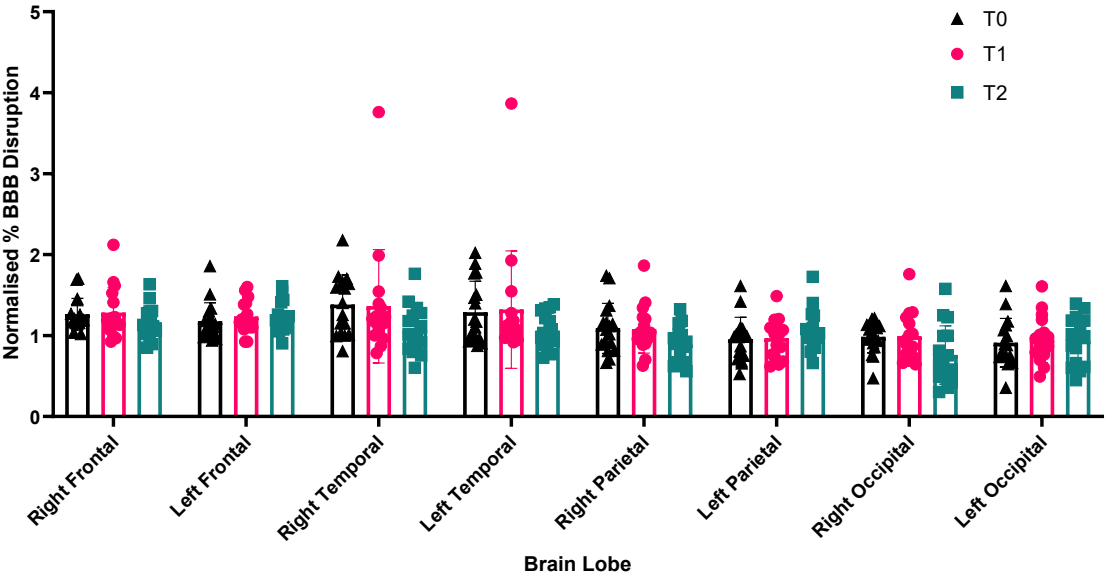


Figure 6.2. Lobar BBB disruption normalised to whole-brain disruption at each timepoint (T0–T2).

6.1. Whole-Brain Blood–Brain Barrier Disruption (T0–T2)

6.1.1. Analytic Approach

Whole-brain blood–brain barrier (BBB) disruption was quantified at T0 (pre-surgery), T1 (3 months post-surgery), and T2 (9 months post-surgery) as the percentage (%) of suprathreshold voxels. Distributional checks indicated acceptable normality at T0–T1 across most tests, but T2 deviated from normality on multiple indices. Given the longitudinal structure and minor timepoint-specific missingness, change across timepoints was evaluated using a mixed-effects model (restricted maximum likelihood, REML) with timepoint as a fixed effect and participant as a random effect, followed by Tukey-adjusted pairwise comparisons (T0 vs T1, T0 vs T2, T1 vs T2). Statistical significance was set at $p < 0.05$.

6.1.2 Whole-Brain BBB Disruption Across Timepoints

Whole-brain BBB disruption did not differ significantly across timepoints (Figure 6.1). The mean whole-brain disruption increased numerically over follow-up (T0: 18.82%; T1: 23.71%; T2: 28.95%), but none of the Tukey-adjusted pairwise comparisons reached statistical significance. The comparison between T0 and T1 showed a mean difference of -4.89% (95% CI -14.18 to 4.40 , $p = 0.3824$), and T1 vs T2 showed a mean difference of -3.83% (95% CI -15.22 to 7.55 , $p = 0.6604$). The contrast between T0 and T2 approached significance (mean difference -8.89% , 95% CI -18.35 to 0.58 , $p = 0.0672$) but remained above the predefined threshold.

6.2. Lobar BBB Disruption Normalised to Whole-Brain Values (T0–T2)

6.2.1 Analytic Approach

To evaluate whether any lobes showed disproportionate BBB disruption relative to global change, lobar BBB disruption was normalised to whole-brain disruption at the corresponding timepoint. For each participant and timepoint, normalised lobar disruption = (lobar BBB disruption) $\div$ (whole-brain BBB disruption), generating a unitless ratio. Values > 1 indicate proportionally greater disruption in that lobe relative to the whole-brain average at that timepoint, while values < 1 indicate proportionally lower disruption. For each lobe (right/left frontal, temporal, parietal, occipital), timepoint effects were assessed using the same longitudinal framework, with Tukey-adjusted pairwise comparisons within each lobe.

6.2.2. Normalised Lobar BBB Disruption Across Timepoints

Across all eight lobes, there were no statistically significant differences in normalised BBB disruption between timepoints (Figure 6.2). In every lobe, all three Tukey-adjusted pairwise comparisons produced confidence intervals crossing zero with adjusted p -values exceeding 0.05. Several lobes showed numerically higher normalised disruption at T2, most notably the temporal lobes. In the right temporal lobe, the T0 vs T2 contrast yielded a mean difference of 0.3040 (95% CI -0.003070 to 0.6111 , adjusted $p = 0.0525$), and the left temporal lobe showed a comparable non-significant pattern (mean difference 0.2466, 95% CI -0.02437 to 0.5177 , adjusted $p = 0.0772$). Similar non-significant increases were observed in the right parietal (T0 vs T2 mean difference 0.2128, 95% CI -0.02902 to 0.4547 , adjusted $p = 0.0889$) and right occipital lobes (T0 vs T2 mean difference 0.2145, 95% CI -0.03906 to 0.4680 , adjusted $p =$

0.1041). All remaining lobes demonstrated smaller mean differences without a consistent directional pattern.

Overall, normalised lobar analyses provided no evidence of a statistically significant perioperative change in BBB disruption relative to whole-brain values, and no lobe demonstrated a significant reduction in normalised BBB disruption over follow-up.

6.3. Whole-Brain Ktrans (BBB Permeability) Across the Perioperative Period (T0–T2)

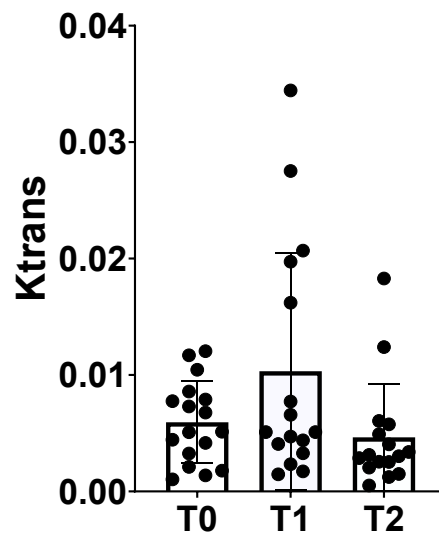


Figure 6.3. Whole-brain Ktrans across the perioperative timepoints (T0, T1 and T2).

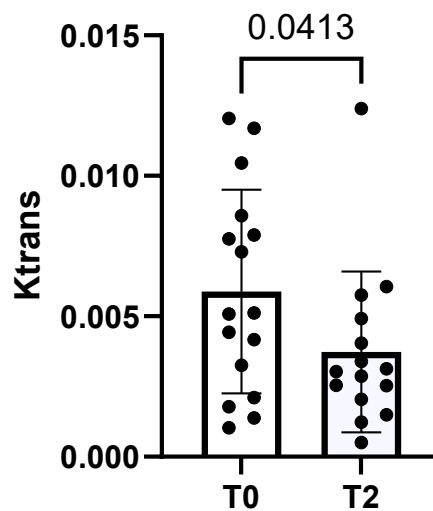


Figure 6.4. Paired whole-brain Ktrans change from T0 to T2 (Wilcoxon matched-pairs signed-rank test).

6.3.1. Analytic Approach

Whole-brain Ktrans (volume transfer constant; dynamic contrast-enhanced MRI (DCE-MRI)–derived BBB permeability parameter) was quantified at T0, T1 and T2. Normality testing produced mixed results, with T0 and T1 meeting most criteria, whereas T2 failed multiple tests, indicating deviation from normality at T2. Whole-brain Ktrans values were presented across all three timepoints to characterise the perioperative trajectory, and paired change from T0 to T2 was assessed using a Wilcoxon matched-pairs signed-rank test (two-tailed) to evaluate longer-term within-participant change.

6.3.2. Whole-Brain Ktrans Across Timepoints

Whole-brain Ktrans values across T0, T1 and T2 are shown in Figure 6.3. Median Ktrans increased numerically from T0 to T1 and declined again at T2; however, the distribution at T1 showed marked inter-individual variability, including several high values, which limited the interpretability of the three-timepoint trajectory as a single coherent pattern. So, to evaluate longer-term BBB permeability while minimising the influence of short-term postoperative variability at T1, paired comparison of T0 vs T2 was performed (Figure 6.4). This analysis demonstrated a statistically significant reduction in whole-brain Ktrans between T0 and T2 ($p = 0.0413$, two-tailed), with a median paired difference ($T2 - T0$) of -0.001567 . Collectively, these findings indicate that, despite substantial heterogeneity at 3 months, whole-brain BBB permeability as indexed by Ktrans was lower at 9 months post-surgery compared with pre-surgical levels.

6.4. Qualitative Spatial Patterns of BBB Leakage on Voxelwise Ktrans Maps (T0–T2)

6.4.1. Analytic Approach

To complement whole-brain quantitative Ktrans analyses, voxelwise Ktrans maps were visually inspected in a subset of participants with available paired imaging at **T0 and T2**. Because regional Ktrans summary values could not be extracted numerically due to pipeline restrictions, assessment of spatial patterns was qualitative and focused on the presence, distribution, and apparent extent of focal permeability signal at each timepoint.

6.4.2. Qualitative Spatial Patterns Across Timepoints

Voxelwise maps for illustrative participants are shown in Figure 6.5. At T0, visible BBB permeability signal was most commonly distributed posteriorly, with predominant involvement of occipital cortices and patchy extension into posterior temporal regions in some cases. This posterior-predominant pattern was identified in 11 of 17 participants with available baseline Ktrans maps. By T2, these focal areas of elevated Ktrans were reduced or no longer visually apparent in the majority of inspected participants. This pattern is directionally consistent with the quantitative whole-brain finding of reduced Ktrans from T0 to T2 ($p = 0.0413$), suggesting that the observed global decrease may be driven, at least in part, by attenuation of posterior-predominant permeability signal over follow-up.

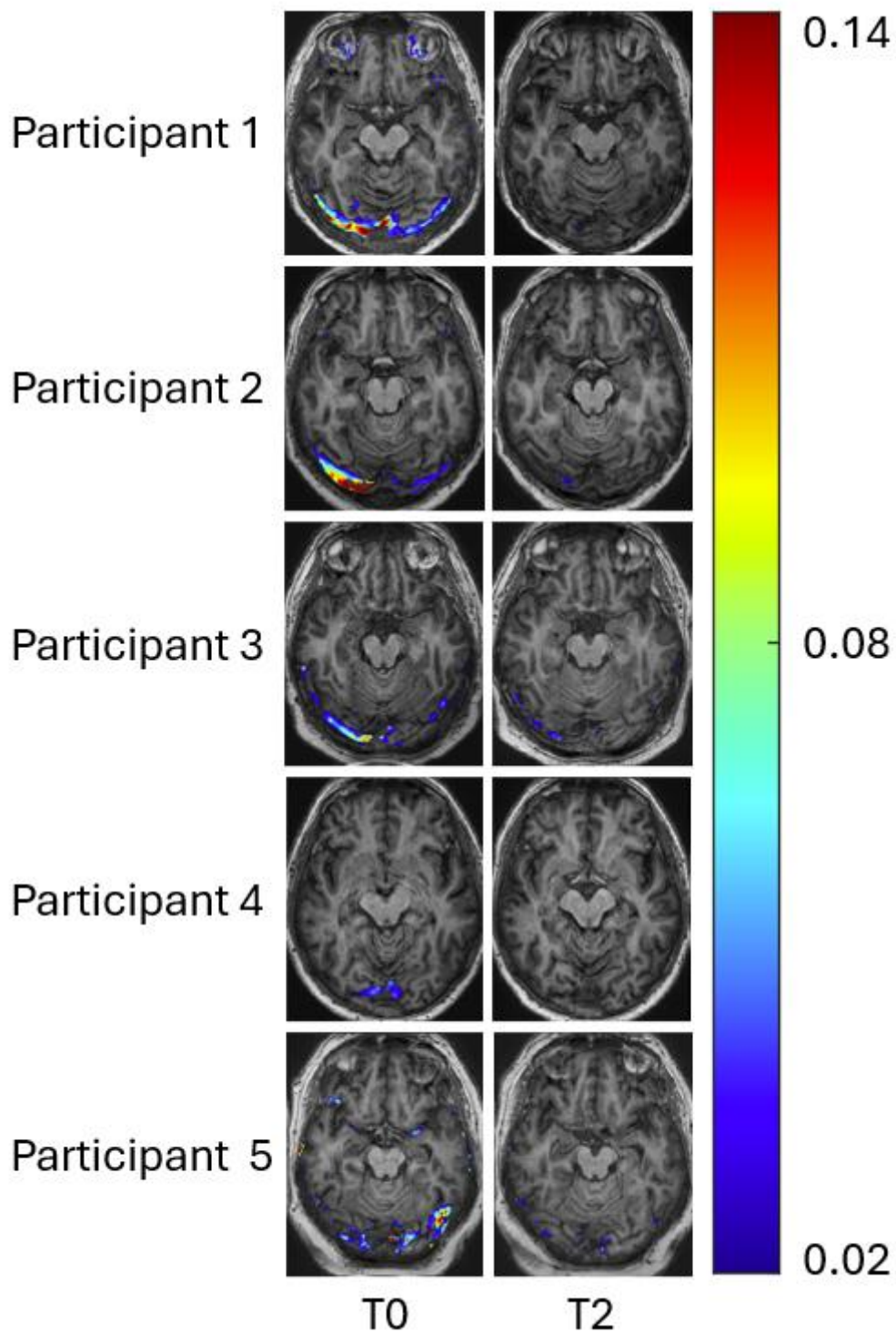


Figure 6.5. Representative voxelwise Ktrans maps at T0 and T2 illustrating spatial patterns of permeability signal. N=11. The colour scale indicates the magnitude of voxelwise Ktrans (BBB permeability), with cooler colours (blue/purple) representing lower permeability values and warmer colours (green/yellow/red) representing higher permeability values; values correspond to the numeric range shown on the colour bar.

6.5. Ktrans Change in Participants with Occipital-Predominant Leakage Pattern at Baseline (T0–T2)

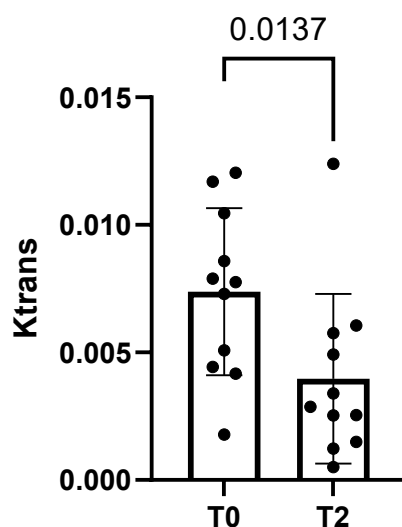


Figure 6.6. Paired Ktrans change from T0 to T2 in the occipital-predominant baseline leakage subgroup. N=11

6.5.1. Analytic Approach

A focused analysis was performed in the subgroup of participants whose baseline voxelwise maps demonstrated a predominantly occipital-predominant permeability pattern and who had paired values available at T0 and T2 (N=11). Given non-normality at T2, within-participant change from T0 to T2 was assessed using a paired Wilcoxon matched-pairs signed-rank test (two-tailed).

6.5.2. Ktrans Change in the Occipital-Predominant Subgroup

In this subgroup, Ktrans decreased from T0 to T2 in all participants except one, indicating a consistent directional shift over follow-up (Figure 6.6). The median paired difference was -0.00298 ($T2 - T0$). This change was statistically significant (Wilcoxon $W = 6.0$, $p = 0.0137$), supporting a reduction in BBB permeability over time in participants with visually apparent occipital-predominant baseline permeability signal. These quantitative findings align with the qualitative map observations, in which the posterior focal permeability signal present at baseline was markedly reduced by 9 months in most cases.

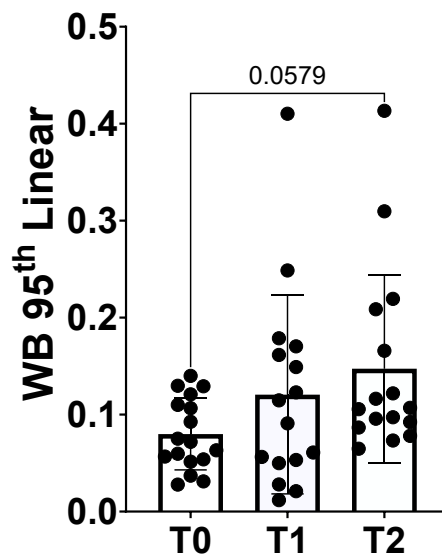


Figure 6.7. Whole-brain 95th percentile linear leakage (WB 95th Linear) across the perioperative timepoints (T0–T2).

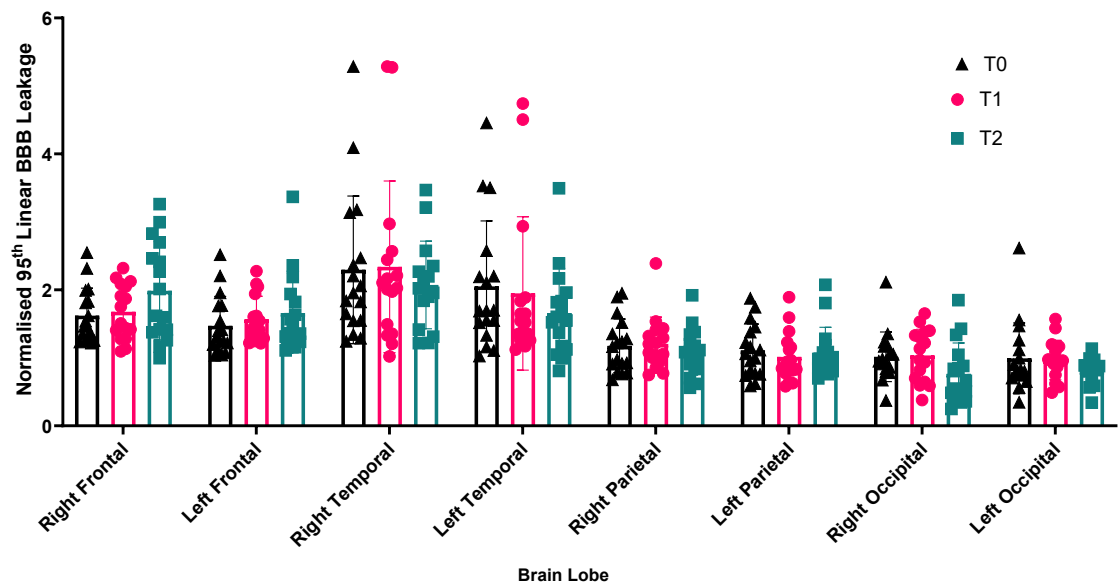


Figure 6.8. Normalised lobar 95th percentile linear leakage (WB 95th Linear) across timepoints (T0–T2).

6.6. Whole-Brain 95th Percentile Linear Leakage (WB 95th Linear) (T0–T2)

6.6.1. Analytic Approach

Whole-brain 95th percentile linear leakage (WB 95th Linear) was evaluated at T0, T1, and T2. WB 95th Linear represents the upper tail (95th percentile) of voxelwise linear permeability values, capturing focal high-leakage voxels instead of average whole-brain permeability, and is complementary to whole-brain % BBB disruption and whole-brain Ktrans. Change across timepoints was assessed using a repeated-measures mixed-effects model (restricted maximum likelihood, REML) with timepoint as a fixed effect and participant as a random effect, followed by Tukey-adjusted pairwise comparisons (T0 vs T1, T0 vs T2, T1 vs T2).

6.6.2. Whole-Brain WB 95th Linear Across Timepoints

WB 95th Linear did not differ significantly across timepoints (Figure 6.7). Although the metric increased numerically over follow-up (T0 mean approximately 0.078–0.081, T1 mean approximately 0.121–0.128, T2 mean approximately 0.147–0.149, with minor variation according to timepoint-specific missing data and available paired observations), none of the Tukey-adjusted contrasts reached statistical significance.

The comparison between T0 and T1 showed a mean difference of -0.04260 (95% CI -0.1139 to 0.02867 , adjusted $p = 0.2958$), and T1 vs T2 showed a mean difference of -0.02116 (95% CI -0.09646 to 0.05414 , adjusted $p = 0.7470$). The contrast between T0 and T2 approached significance (mean difference -0.06580 , 95% CI -0.1337 to 0.002049 , adjusted $p = 0.0579$), but remained above the predefined threshold. Overall, these data suggest a possible upward shift in the most extreme BBB leakage values between T0 and T2, but this did not reach statistical significance and should be interpreted cautiously, given variability and modest sample size.

6.7. Lobar WB 95th Linear Leakage Normalised to Whole-Brain Values (T0–T2)

6.7.1. Analytic Approach

To assess whether any lobes demonstrated disproportionate change in the most extreme BBB leakage values relative to global change, lobar WB 95th Linear leakage was normalised to the corresponding whole-brain WB 95th Linear value at each timepoint. For each participant and timepoint, normalised lobar WB 95th Linear = (lobar WB 95th Linear) $\div$ (whole-brain WB 95th Linear), producing a unitless ratio. Values > 1 indicate that the lobe contributes proportionally higher high-leakage voxels relative to the whole-brain reference at that timepoint, whereas values < 1 indicate proportionally lower contribution. For each lobe, timepoint effects were assessed using the same longitudinal framework with Tukey-adjusted pairwise comparisons within lobes.

6.7.2. Normalised Lobar WB 95th Linear Across Timepoints

Across all eight lobes, there were no statistically significant differences in normalised lobar WB 95th Linear between timepoints (Figure 6.8). In each lobe, all three Tukey-adjusted pairwise comparisons produced confidence intervals crossing zero with adjusted p -values above 0.05. Numerically, some lobes showed

higher normalised values at T2, but without a consistent directional pattern across the brain. For example, the right frontal lobe showed a non-significant increase from T0 to T2 (mean difference -0.3401 , 95% CI -0.8074 to 0.1272 , adjusted $p = 0.1757$), whereas the left temporal lobe showed a non-significant decrease from T0 to T2 (mean difference 0.3911 , 95% CI -0.3828 to 1.165 , adjusted $p = 0.4101$). Non-significant differences were also observed in posterior lobes, including the right occipital lobe (T0 vs T2 mean difference 0.2541 , 95% CI -0.1284 to 0.6366 , adjusted $p = 0.2283$) and left occipital lobe (T0 vs T2 mean difference 0.2081 , 95% CI -0.1609 to 0.5772 , adjusted $p = 0.3349$). Overall, normalised lobar analyses provide no evidence of a statistically significant perioperative change in lobar WB 95th Linear leakage relative to the whole-brain value.

6.8. Associations Between Pre-Surgical Visual Parameters and BBB Integrity Metrics (T0)

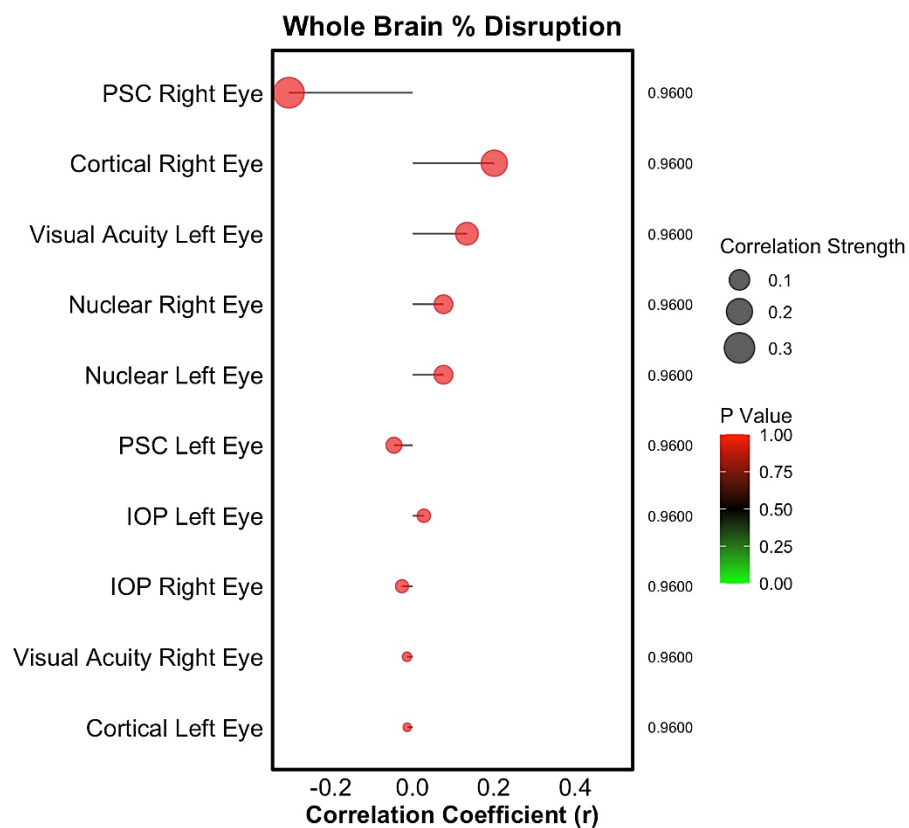


Figure 6.9. Associations between pre-surgical ocular parameters and whole-brain % BBB disruption (T0).
PSC: Posterior subcapsular. IOP: intraocular pressure

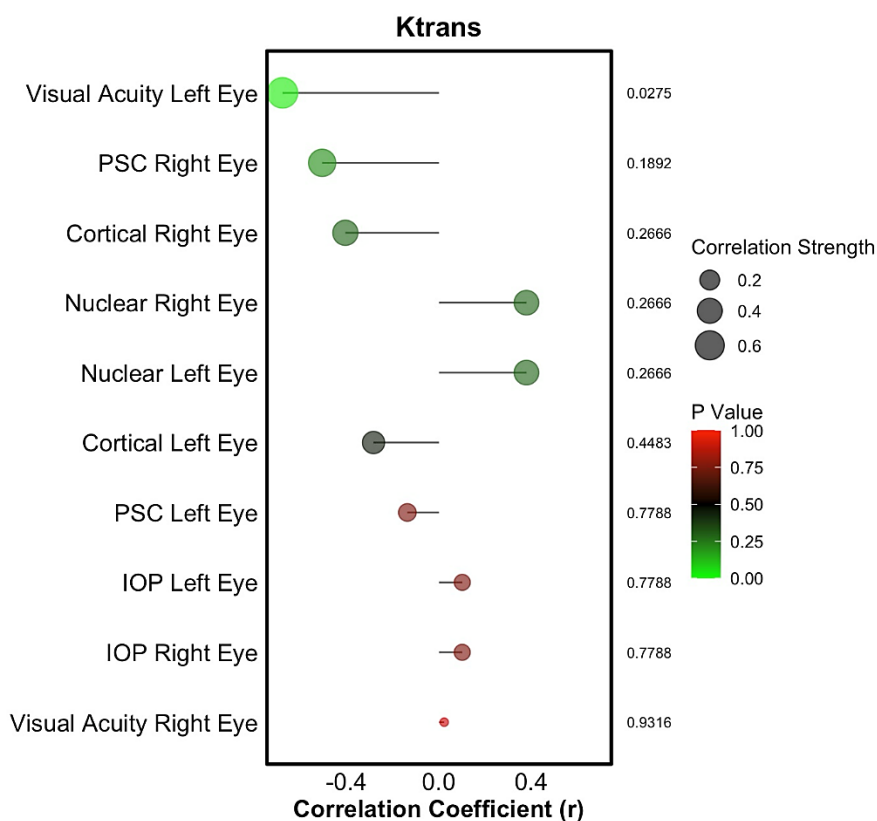


Figure 6.10. Associations between pre-surgical ocular parameters and whole-brain Ktrans (T0). PSC: Posterior subcapsular. IOP: intraocular pressure

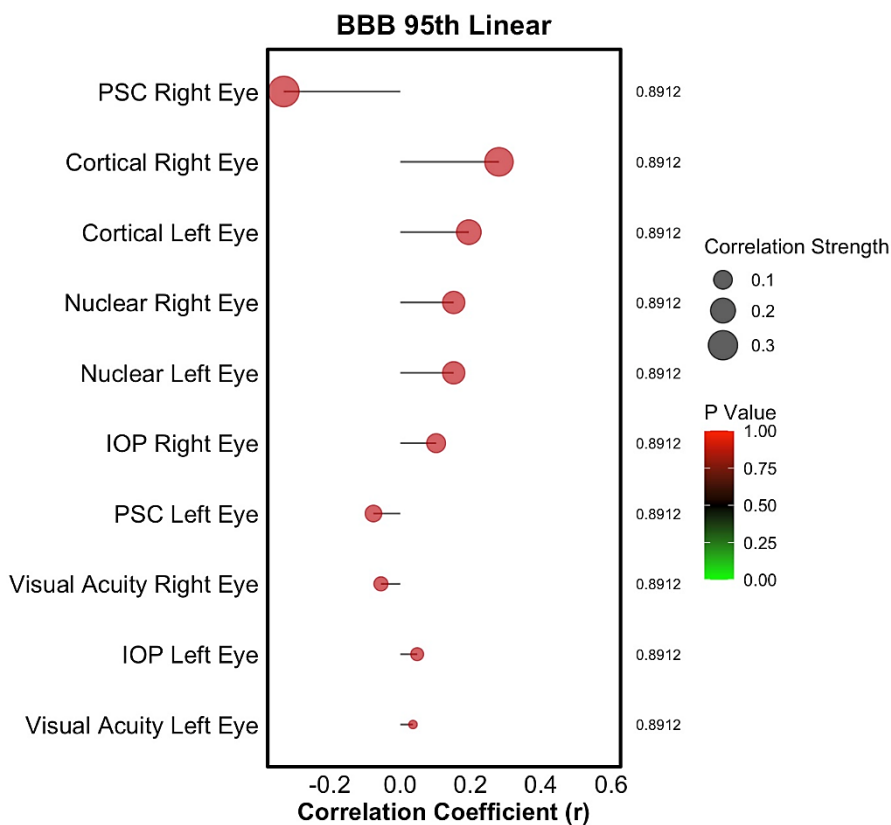


Figure 6.11. Associations between pre-surgical ocular parameters and whole-brain WB 95th Linear leakage (T0). PSC: Posterior subcapsular. IOP: intraocular pressure

6.8.1. Analytic Approach

Bivariate correlation analyses were performed at T0 to examine associations between ocular parameters: visual acuity (VA) and cataract type/severity grading (nuclear, cortical, and posterior subcapsular (PSC) cataract for each eye), and global BBB indices (whole-brain % BBB disruption, whole-brain Ktrans, and WB 95th Linear). Results are summarised using forest plots showing correlation coefficients (r) and associated p values for each visual–BBB pair (Figures 6.9–6.11). Given the number of correlations tested, findings were interpreted as exploratory.

Of note, for these correlation analyses, the forest plots were generated using VA expressed as a Snellen fraction quotient (decimal acuity). In this scale, higher values (approaching 1.0) indicate better VA, whereas lower values (approaching 0) indicate worse acuity. Accordingly, negative correlation coefficients indicate that better acuity is associated with lower BBB permeability (or lower BBB disruption indices). In contrast, positive coefficients indicate that better acuity is associated with higher permeability. Additionally, intraocular pressure (IOP) was included in the correlation plots for completeness but was not a prespecified primary variable and will not be interpreted further.

6.8.2. Associations Across BBB Metrics

6.8.2.1 Whole-brain % BBB disruption (T0)

No statistically significant correlations were observed between any pre-surgical ocular parameters and whole-brain % BBB disruption (Figure 6.9). All correlations were weak (approximately $|r| \leq 0.30$) and non-significant (all $p > 0.05$) with no consistent pattern across eyes or cataract subtypes.

6.8.2.2 Whole-brain Ktrans (T0)

In contrast to % disruption, whole-brain Ktrans demonstrated one nominally statistically significant association (Figure 6.10). Left-eye VA showed a moderate-to-strong negative correlation with Ktrans ($r = -0.55$, $p = 0.0275$), suggesting that worse VA was associated with higher BBB permeability (higher Ktrans). Other ocular parameters showed non-significant trends only, including moderate positive correlations for some cataract (e.g., PSC and cortical in the right eye and nuclear bilaterally), but with p values remaining well above 0.05. Given the exploratory nature of these analyses and the absence of a consistent pattern across eyes and BBB indices, this association requires cautious interpretation and is considered further in the Discussion.

6.8.2.3 WB 95th Linear (T0)

No statistically significant correlations emerged between ocular parameters and WB 95th Linear (Figure 6.11). Correlations were weak and non-significant (all $p > 0.05$), and VA associations were negligible. Overall, the upper-tail leakage metric did not show evidence of a meaningful relationship with baseline visual system status.

Across the three BBB indices, pre-surgical ocular parameters showed no consistent association with whole-brain % BBB disruption or WB 95th Linear, whereas Ktrans identified a single left-eye VA association that reached nominal significance. Taken together, these findings suggest that any visual–BBB relationship at baseline, if present, may be more detectable using Ktrans, while global summary indices (% disruption and WB 95th Linear) appear largely insensitive to baseline ocular disease burden in this cohort.

6.9. Correlations Between Post-Operative Visual Acuity and BBB Integrity Metrics (T2)

6.9.1. Analytic Approach

At T2 (9 months post-surgery), bivariate correlation analyses were performed to examine associations between VA in each eye and three global indices of BBB integrity: whole-brain % BBB disruption, whole-brain Ktrans, and WB 95th Linear. Forest plots summarise correlation coefficients (r) and associated p values for each VA–BBB pairing (Figures 6.12–6.14). Like in subsection 8.8, for these correlation analyses, the forest plots were generated using VA expressed as a Snellen fraction quotient (decimal acuity). Again, IOP was included in the correlation plots for completeness but was not prespecified as a primary variable and will not be interpreted further.

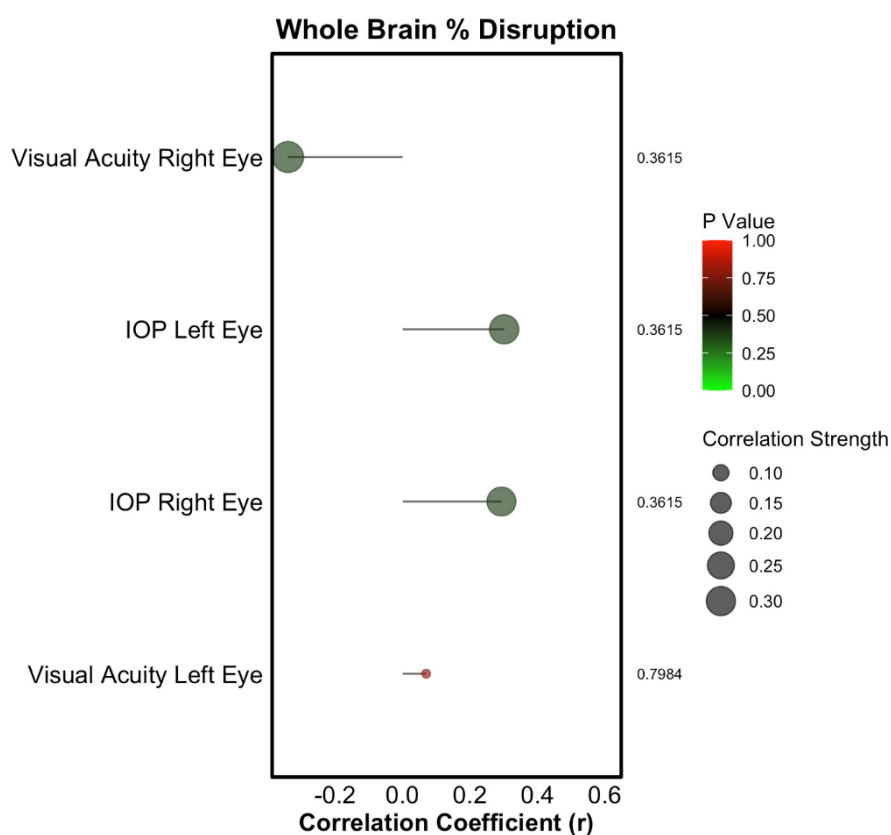


Figure 6.12. Associations between post-surgical visual acuity and whole-brain % BBB disruption (T2).
IOP: intraocular pressure

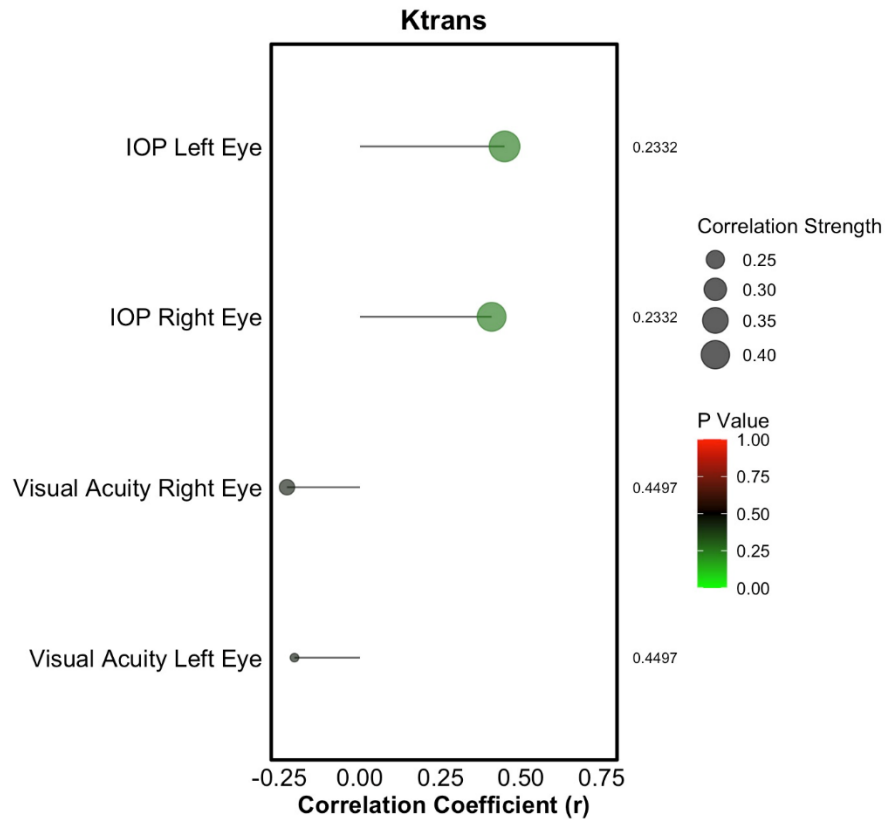


Figure 6.13. Associations between post-surgical visual acuity and whole-brain Ktrans (T2).
IOP: intraocular pressure

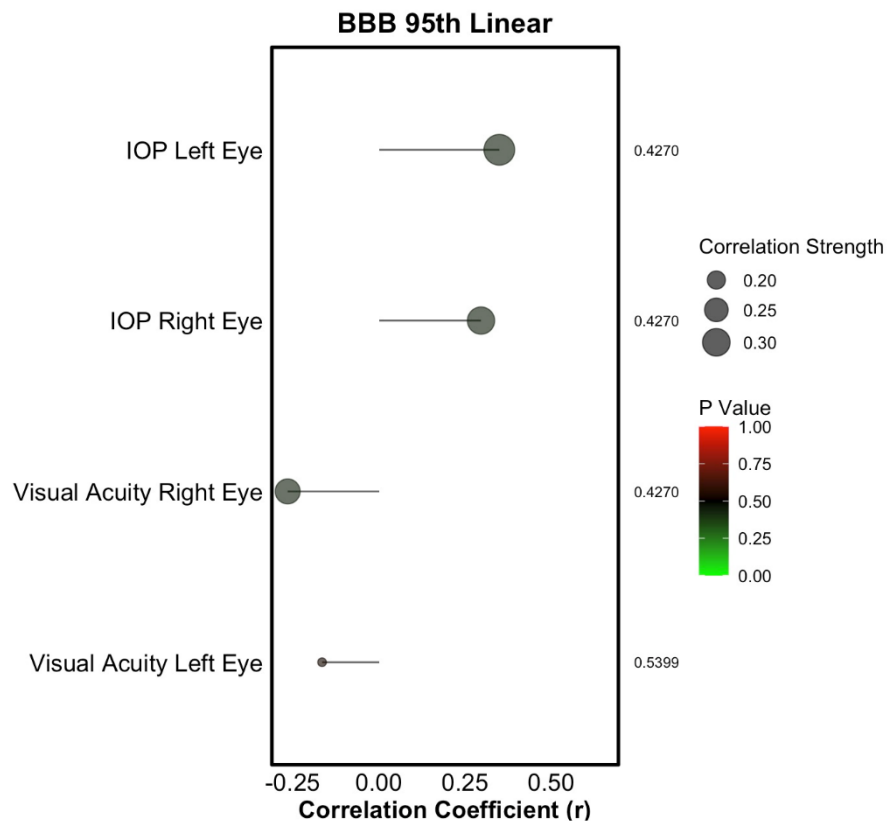


Figure 6.14. Associations between post-surgical visual acuity and whole-brain WB 95th Linear leakage (T2). IOP: intraocular pressure

6.9.2. Visual Acuity–BBB Associations at T2

6.9.2.1. Whole-brain % BBB disruption (T2)

VA showed no statistically significant correlation with whole-brain % BBB disruption at T2 (Figure 6.12). Right-eye VA demonstrated a small negative association with whole-brain % disruption ($r = -0.25$, $p = 0.3615$), indicating a trend where better right-eye acuity (higher Snellen quotient) was associated with lower BBB disruption. Left-eye VA showed a near-zero association ($r = 0.05$, $p = 0.7984$). Overall, postoperative VA did not show a statistically reliable relationship with whole-brain % BBB disruption at 9 months.

6.9.2.2. Whole-brain Ktrans (T2)

No statistically significant correlations were observed between VA and whole-brain Ktrans at T2 (Figure 6.13). Both left-eye and right-eye VA showed small negative associations with Ktrans ($r = -0.20$ and -0.25 , both $p > 0.05$), suggesting that better acuity may be associated with slightly lower BBB permeability; however, effect sizes were modest and did not reach statistical significance.

6.9.2.3. WB 95th Linear (T2)

Similarly, VA did not correlate significantly with WB 95th Linear at T2 (Figure 6.14). Right-eye VA again showed a small negative association ($r = -0.25$, $p = 0.4270$), while left-eye VA demonstrated a weak negative association ($r = -0.15$, $p = 0.5399$). These findings do not support a robust relationship between postoperative VA and the upper-tail BBB leakage metric at 9 months.

Overall, at T2, VA did not show statistically significant associations with any of the three global BBB indices. Across measures, there was a consistent but weak negative direction for right-eye VA (better right-eye acuity tending to accompany lower BBB leakage/permeability), but effect sizes were small, and p values remained above the predefined threshold. Taken together, these data suggest that postoperative visual acuity alone is unlikely to be a major determinant of inter-individual variability in BBB integrity metrics at 9 months in this cohort.

6.10. Baseline Associations Between Visual Measures, BBB Leakage Indices, and RBANS-U Performance (T0).

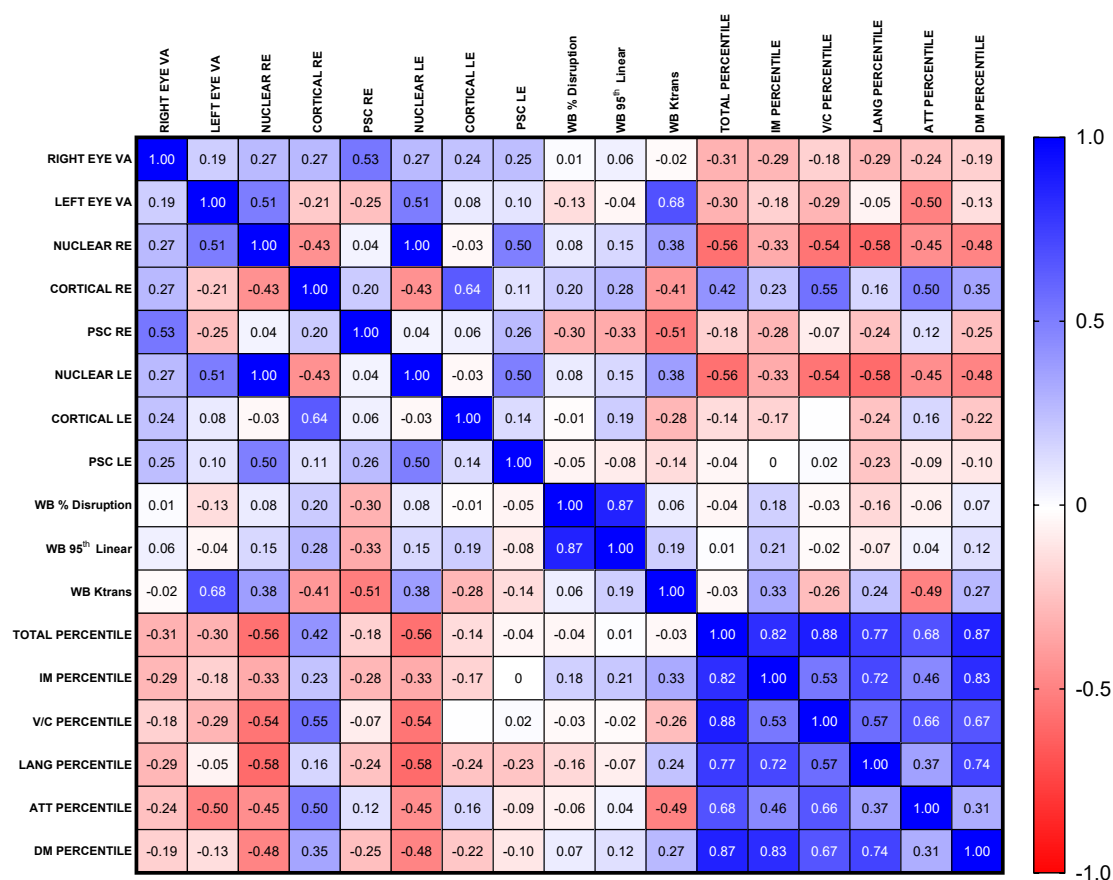


Figure 6.15. Baseline correlation matrix of visual acuity, cataract grading, whole-brain BBB leakage indices, and RBANS-U domain percentiles (T0). VA: visual acuity. RE: right eye. LE: left eye. PSC: posterior subcapsular. WB: whole brain. IM: Immediate memory. V/C: Visuospatial/Constructional. Lang: Language. Att: Attention. DM: Delayed memory.

6.10.1. Analytic Approach

At T0 (pre-surgery), an exploratory bivariate correlation matrix was generated to characterise cross-sectional relationships between baseline ocular measures (best-corrected VA (expressed in logMAR units) in each eye, and cataract grading by type and eye (nuclear, cortical, PSC)), whole-brain BBB indices (whole-brain % BBB disruption, whole-brain Ktrans, and WB 95th Linear), and cognitive performance (Repeatable Battery for the Assessment of Neuropsychological Status-Update (RBANS-U) percentiles: immediate memory, visuospatial/constructional, language, attention, and delayed memory). Correlation coefficients (r) are presented as a heatmap (Figure 6.15), with colour and intensity reflecting the direction and magnitude of association. Findings are interpreted descriptively, given the modest sample size and the number of inter-relationships examined.

Cataract grading was recorded for both eyes; however, nuclear cataract severity was graded identically in the right and left eyes across this cohort, resulting in perfect inter-eye concordance for nuclear grade in the correlation matrix and effectively reflecting a shared baseline nuclear cataract burden.

6.10.2. Correlation Patterns at Baseline (T0)

6.10.2.1. Internal Structure Of RBANS-U Percentiles

Total RBANS-U percentile correlated strongly with all domain percentiles (r approximately 0.68–0.88), including visuospatial/constructional ($r = 0.88$), delayed memory ($r = 0.87$), language ($r = 0.77$), immediate memory ($r = 0.82$), and attention ($r = 0.68$). Domain-to-domain correlations were also substantial (e.g., immediate memory with delayed memory ($r = 0.83$); visuospatial/constructional with attention ($r = 0.66$), supporting internal coherence of the RBANS-U percentile metrics at baseline.

6.10.2.2. Visual Acuity in Each Eye: Relationships with Cognition and BBB Indices

Because VA is expressed in logMAR, higher VA values indicate worse vision. Consistent with this, VA showed predominantly negative associations with RBANS-U percentiles, indicating that worse baseline VA tended to co-occur with lower baseline cognitive performance.

Right-eye VA showed small-to-moderate negative correlations with RBANS-U performance: Total percentile ($r = -0.31$), Immediate Memory ($r = -0.29$), Language ($r = -0.29$), Attention ($r = -0.24$), Visuospatial/Constructional ($r = -0.18$), and Delayed Memory ($r = -0.19$). Left-eye VA showed a similar pattern in several domains, with the most notable association for attention ($r = -0.50$). Correlations with Total percentile ($r = -0.30$) and Visuospatial/Constructional ($r = -0.29$) were also negative, while associations with Language ($r = -0.05$) and Delayed Memory ($r = -0.13$) were weaker.

With respect to BBB indices, VA demonstrated a marked asymmetry across eyes: Left-eye VA and Ktrans were positively correlated ($r = 0.68$), indicating that worse left-eye vision (higher logMAR) was associated with higher whole-brain Ktrans (greater BBB permeability). Right-eye VA and Ktrans were essentially uncorrelated ($r = -0.02$).

Associations between VA and the threshold/intensity BBB metrics were small: right-eye VA with whole-brain % disruption ($r = 0.01$) and with WB 95th Linear ($r = 0.06$); left-eye VA with whole-brain % disruption ($r = -0.13$) and with WB 95th Linear ($r = -0.04$). Taken together, baseline VA relates more clearly to BBB permeability when BBB integrity is indexed by Ktrans (particularly for the left eye), whereas relationships with whole-brain % disruption and WB 95th Linear are minimal.

6.10.2.3. Cataract Grading: Ocular Inter-Relationships and Associations With VA

Several expected relationships between cataract phenotype and VA were observed: Nuclear grade correlated positively with VA (greater nuclear burden associated with worse vision): nuclear with right-eye VA $r = 0.27$ and nuclear with left-eye VA $r = 0.51$. Cortical grading was strongly correlated between eyes (r

= 0.64), consistent with bilateral similarity for cortical changes, whereas PSC grading showed only a modest inter-eye correlation ($r = 0.26$). PSC in the right eye showed a relatively strong positive association with right-eye VA ($r = 0.53$), again in the expected direction given logMAR scaling (greater PSC burden co-occurring with worse VA).

Nuclear and cortical grades were inversely related ($r = -0.43$), which is relevant when interpreting their opposing correlation patterns with cognition and BBB metrics.

6.10.2.4. Cataract Grading and RBANS-U Performance

The most consistent cataract–cognition pattern at baseline involved nuclear grading, which showed moderate-to-strong negative correlations with RBANS-U performance across several domains:

- Nuclear grade with Total percentile $r = -0.56$
- Nuclear grade with Language $r = -0.58$
- Nuclear grade with Visuospatial/Constructional $r = -0.54$
- Nuclear grade with Attention $r = -0.45$
- Nuclear grade with Delayed Memory $r = -0.48$
- Nuclear grade with Immediate Memory $r = -0.33$

This suggests that greater nuclear cataract burden co-occurred with lower baseline cognitive performance across multiple RBANS-U domains, most prominently Language and Visuospatial/Constructional.

In contrast, cortical grading (right eye) showed positive correlations with RBANS-U percentiles (e.g., Total $r = 0.42$, Visuospatial/Constructional $r = 0.55$, Attention $r = 0.50$, Delayed Memory $r = 0.35$). Given the inverse relationship between nuclear and cortical grading ($r = -0.43$), these positive cortical–cognition correlations are most plausibly attributable to cataract phenotype/collinearity effects in a small cohort and should be interpreted cautiously.

PSC showed generally smaller and less consistent correlations with RBANS-U percentiles. For example, PSC (right eye) was weakly negative with several domains (e.g., Immediate Memory $r = -0.28$, Language $r = -0.24$, Delayed Memory $r = -0.25$), but these effects were not as coherent or strong as those seen for nuclear grading.

6.10.2.5. Relationships Among BBB Indices and Their Links with Cognition and Cataract Phenotype

The BBB indices are separated into two clear patterns: Whole-brain % disruption and WB 95th Linear were strongly interrelated ($r = 0.87$), while Ktrans correlated only weakly with whole-brain % disruption ($r = 0.06$) and weakly to modestly with WB 95th Linear ($r = 0.19$), supporting the idea that Ktrans captures a partially distinct physiological component of BBB permeability relative to the threshold-based metrics.

BBB–cognition associations were most notable for Ktrans and the RBANS-U attention domain ($r = -0.49$), suggesting that higher BBB permeability was associated with poorer attention performance at baseline. Ktrans also showed a smaller negative association with visuospatial/constructional ($r = -0.26$), and a small positive correlation with Immediate Memory ($r = 0.33$) and Delayed Memory ($r = 0.27$), indicating that BBB–cognition relationships in this dataset are not uniformly negative across all domains.

For BBB indices, correlations with cognition were generally small (e.g., whole-brain % disruption with Total ($r = -0.04$); WB 95th Linear with Total ($r = 0.01$)), suggesting that baseline cognition relates more detectably to BBB integrity when indexed by Ktrans rather than by % disruption or WB 95th Linear.

Cataract–BBB associations varied by BBB metric and cataract phenotype. Nuclear grade showed a moderate positive correlation with Ktrans ($r = 0.38$). Cortical grade (right eye) correlated negatively with Ktrans ($r = -0.41$). PSC (right eye) correlated negatively with Ktrans ($r = -0.51$) and also negatively with whole-brain % disruption ($r = -0.30$) and WB 95th Linear ($r = -0.33$), indicating heterogeneity across cataract phenotypes in their direction of association with BBB metrics.

6.10.3. Integrated baseline interpretation

At baseline (T0), the correlation matrix supports three key patterns:

1. Vision–cognition coupling at baseline: Worse VA generally co-occurs with lower RBANS-U performance, with the most prominent association being left-eye VA and Attention ($r = -0.50$).
2. Cataract phenotype matters: Nuclear cataract burden demonstrates the most consistent negative relationship with RBANS-U performance across domains. Other cataract types show less coherent patterns and appear influenced by inter-relationships between cataract phenotypes.
3. Ktrans is the BBB metric most strongly linked to baseline clinical variables: Ktrans behaves distinctly from the threshold-based BBB indices and shows a clear association with left-eye VA ($r = 0.68$), and a domain-specific association with RBANS-U Attention ($r = -0.49$). In contrast, whole-brain % disruption and WB 95th Linear are highly correlated with each other but show limited relationships with cognition and ocular measures at baseline.

6.11. Post-Surgical Associations Between Visual Acuity, BBB Leakage Indices, and RBANS-U Performance (T1).

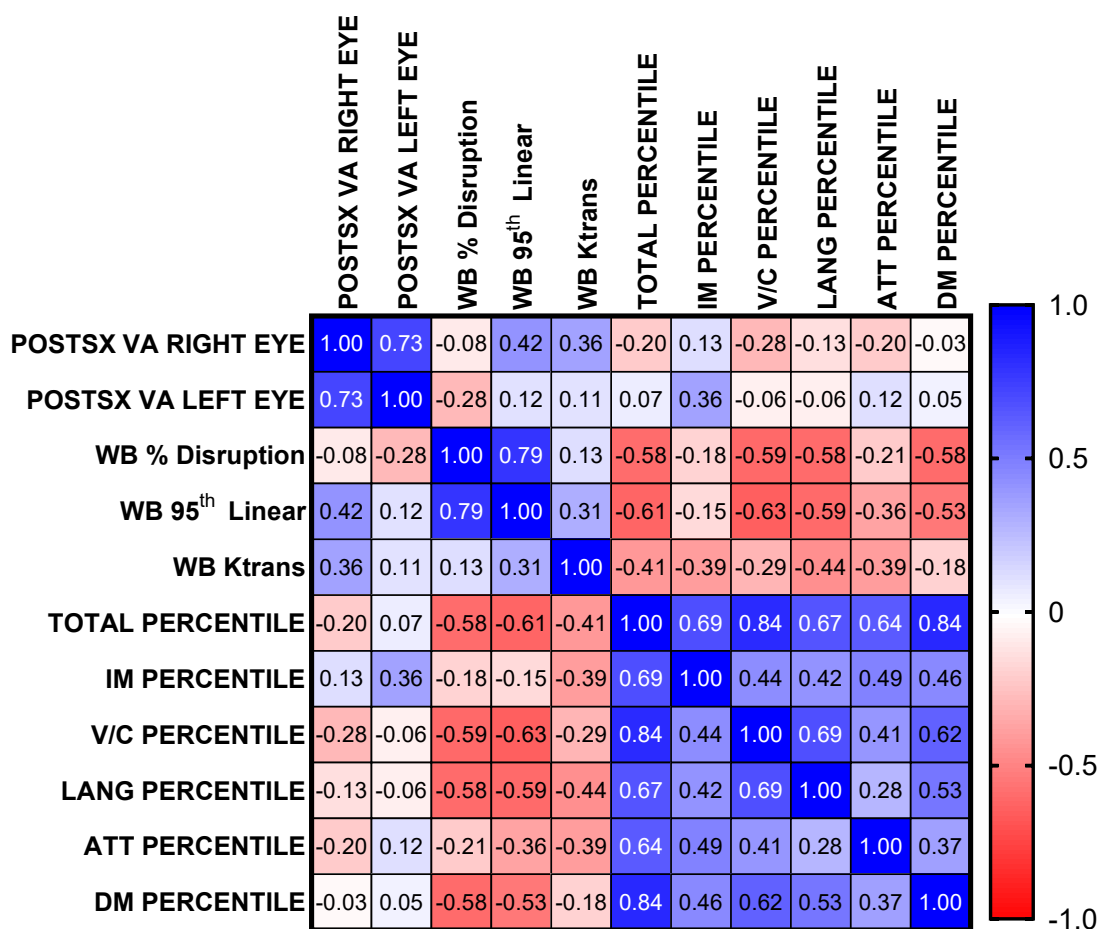


Figure 6.16. Post-surgical correlation matrix of visual acuity, whole-brain BBB leakage indices, and RBANS-U domain percentiles (T1). VA: visual acuity. RE: right eye. LE: left eye. WB: whole brain. IM: Immediate memory. V/C: Visuospatial/Constructional. Lang: Language. Att: Attention. DM: Delayed memory.

6.11.1. Analytic Approach

At T1 (3 months post-surgery), exploratory bivariate correlation analyses were performed to characterise cross-sectional relationships among post-surgical visual acuity (expressed in logMAR units), BBB indices, and RBANS-U percentile scores. Correlation coefficients (r) were computed using a consistent bivariate framework and summarised visually in a correlation matrix heatmap (Figure 6.16). Given the exploratory nature of the matrix and multiple comparisons, interpretation focused on correlation direction and magnitude rather than statistical significance

6.11.2. Correlation Patterns at T1 (3 Months Post-Surgery)

6.11.2.1. Internal Structure Of RBANS-U Percentiles at T1

RBANS-U percentiles remained strongly intercorrelated at T1, consistent with the expected shared variance across cognitive domains. Total RBANS-U percentile correlated strongly with Visuospatial/Constructional ($r = 0.84$), Delayed Memory ($r = 0.84$), Immediate Memory ($r = 0.69$), Language ($r = 0.67$), and Attention ($r = 0.64$). Domain-to-domain correlations were also substantial (e.g., Visuospatial/Constructional with Language ($r = 0.69$); Immediate Memory with Language ($r = 0.42$); Attention with Delayed Memory ($r = 0.37$)), supporting internal coherence of the RBANS-U percentile metrics at this timepoint.

6.11.2.2. Relationships Among BBB Indices at T1

At T1, whole-brain % disruption and WB 95th Linear were again closely aligned, showing a strong positive correlation ($r = 0.79$), indicating that higher global leakage burden tended to co-occur with higher extreme-value leakage intensity. In contrast, Ktrans showed weaker coupling with the other BBB metrics: correlations were small-to-modest with whole-brain % disruption ($r = 0.13$) and modest with WB 95th Linear ($r = 0.31$). This, again, is directionally consistent with Ktrans reflecting a partially distinct permeability construct compared with the threshold-based indices.

6.11.2.3. Post-Surgical VA: Inter-Eye Concordance and Associations with BBB Indices

Post-surgical VA showed strong inter-eye concordance: right- and left-eye VA correlated highly ($r = 0.73$), which is consistent with a broadly symmetric post-surgical visual function within individuals.

Associations between post-surgical VA and BBB indices were more evident for the right eye than the left one. Right-eye VA showed a moderate positive correlation with WB 95th Linear ($r = 0.42$) and a moderate positive correlation with Ktrans ($r = 0.36$), while being basically unrelated to whole-brain % disruption ($r = -0.08$). Interpreted in logMAR terms, these directions suggest that worse right-eye VA tended to co-occur with more intense BBB leakage intensity and higher Ktrans at T1. Left-eye VA showed weak relationships with BBB indices overall, as it correlated negatively with whole-brain % disruption ($r = -0.28$) and only weakly with WB 95th Linear ($r = 0.12$) and Ktrans ($r = 0.11$). The left-eye pattern is therefore smaller in magnitude and less consistent across BBB indices.

Taken together, these findings suggest that at T1, any visual–BBB coupling is modest and appears metric- and eye-dependent, with the clearest pattern being a right-eye association with WB 95th Linear and Ktrans, rather than with % disruption.

6.11.2.4. BBB Indices And RBANS-U Performance at T1

At T1, all three BBB metrics demonstrated predominantly negative associations with RBANS-U percentiles, with the strongest and most consistent effects seen for the threshold-based measures (whole-brain % disruption and WB 95th Linear).

Whole-brain % BBB disruption showed moderate negative correlations with several RBANS-U outcomes: total percentile ($r = -0.58$), visuospatial/constructional ($r = -0.59$), language ($r = -0.58$), and delayed memory ($r = -0.58$). Associations with immediate memory were smaller ($r = -0.18$) and with attention were weak ($r = -0.21$).

WB 95th Linear demonstrated a similar but slightly stronger pattern across multiple cognitive domains: total percentile ($r = -0.61$), visuospatial/constructional ($r = -0.63$), language ($r = -0.59$), and delayed memory ($r = -0.53$). Associations were smaller for immediate memory ($r = -0.15$) and mild, but more pronounced for attention ($r = -0.36$).

Ktrans showed negative associations of smaller magnitude than the threshold-based indices, but still directionally coherent across several domains: total percentile ($r = -0.41$), language ($r = -0.44$), immediate memory ($r = -0.39$), and attention ($r = -0.39$). Smaller associations were observed for visuospatial/constructional ($r = -0.29$) and delayed memory ($r = -0.18$).

Overall, the T1 matrix suggests that higher BBB leakage burden, particularly when indexed by % disruption and WB 95th Linear, tended to co-occur with lower RBANS-U performance across multiple domains at 3 months post-surgery, with the most prominent negative associations involving total, visuospatial/constructional, language, and delayed memory percentiles.

6.11.2.5. Post-Surgical VA And RBANS-U Performance at T1

Associations between post-surgical VA and RBANS-U percentiles at T1 were generally small to moderate and less coherent than the BBB–RBANS-U relationships. Right-eye VA showed negative correlations with total percentile ($r = -0.20$), visuospatial/constructional ($r = -0.28$), language ($r = -0.13$), and attention ($r = -0.20$), consistent with worse VA tending to accompany lower cognitive performance, although effect sizes were modest. Left-eye VA showed weak or mixed associations with cognition (i.e., total percentile ($r = 0.07$); immediate memory ($r = 0.36$); attention ($r = 0.12$)). In logMAR terms, the direction of the left-eye VA association with immediate memory is not in the expected direction and may reflect sampling variability, timepoint-specific noise, or residual heterogeneity in post-surgical visual function at T1.

Across eyes, VA–cognition coupling at T1 appears weaker and less internally consistent than the BBB–cognition patterns observed in this matrix.

6.11.3. Integrated Interpretation at T1

At 3 months post-surgery, the correlation matrix shows three relevant features:

1. RBANS-U percentiles remain strongly interrelated, supporting internal coherence of the cognitive metrics at T1.

2. Whole-brain % disruption and WB 95th Linear remain tightly coupled, whereas Ktrans is more distinct, paralleling the pattern observed at baseline.
3. Most notably, BBB leakage indices, particularly % disruption and WB 95th Linear, show broadly negative associations with RBANS-U performance across multiple domains, suggesting that individuals with higher BBB leakage burden at T1 tended to have lower concomitant cognitive percentile scores.

The pattern for post-surgical VA is comparatively modest and less consistent across eyes and domains, with a clearer signal for right-eye VA associations with WB 95th Linear and Ktrans than for left-eye VA. As this is an exploratory correlation analysis at a time point, characterized by greater postoperative physiological variability, these relationships are best viewed as descriptive of the T1 cross-sectional structure and as informing targeted interpretation alongside the longitudinal trajectories of the BBB and RBANS-U.

6.12. Post-Surgical Associations Between Visual Acuity, BBB Leakage Indices, and RBANS-U Performance (T2)

6.12.1. Analytic Approach

At T2 (9 months post-surgery), bivariate correlations were used to characterise cross-sectional relationships between post-surgical best-corrected VA (logMAR), whole-brain BBB leakage indices, and RBANS-U percentile outcomes. Results are summarised in a correlation heatmap (Figure 6.17), in which colour encodes the direction and magnitude of the correlation coefficient. Given the exploratory purpose and the number of pairwise comparisons, interpretation emphasised effect size and directional consistency across related measures.

6.12.2. Correlation Patterns at T2 (9 months post-surgery)

6.12.2.1. Internal Structure Of RBANS-U Percentiles at T2

RBANS-U percentiles remained strongly intercorrelated at T2, consistent with the expected shared variance across cognitive domains. Total RBANS-U percentile correlated strongly with delayed memory ($r = 0.78$), immediate memory ($r = 0.69$), visuospatial/constructional ($r = 0.68$), attention ($r = 0.68$), and language ($r = 0.53$). Domain-to-domain associations were also moderate to strong (i.e., language with attention ($r = 0.49$); immediate memory with delayed memory ($r = 0.54$); visuospatial/constructional with attention ($r = 0.44$), supporting the internal coherence of the RBANS-U percentile metrics at this time point.

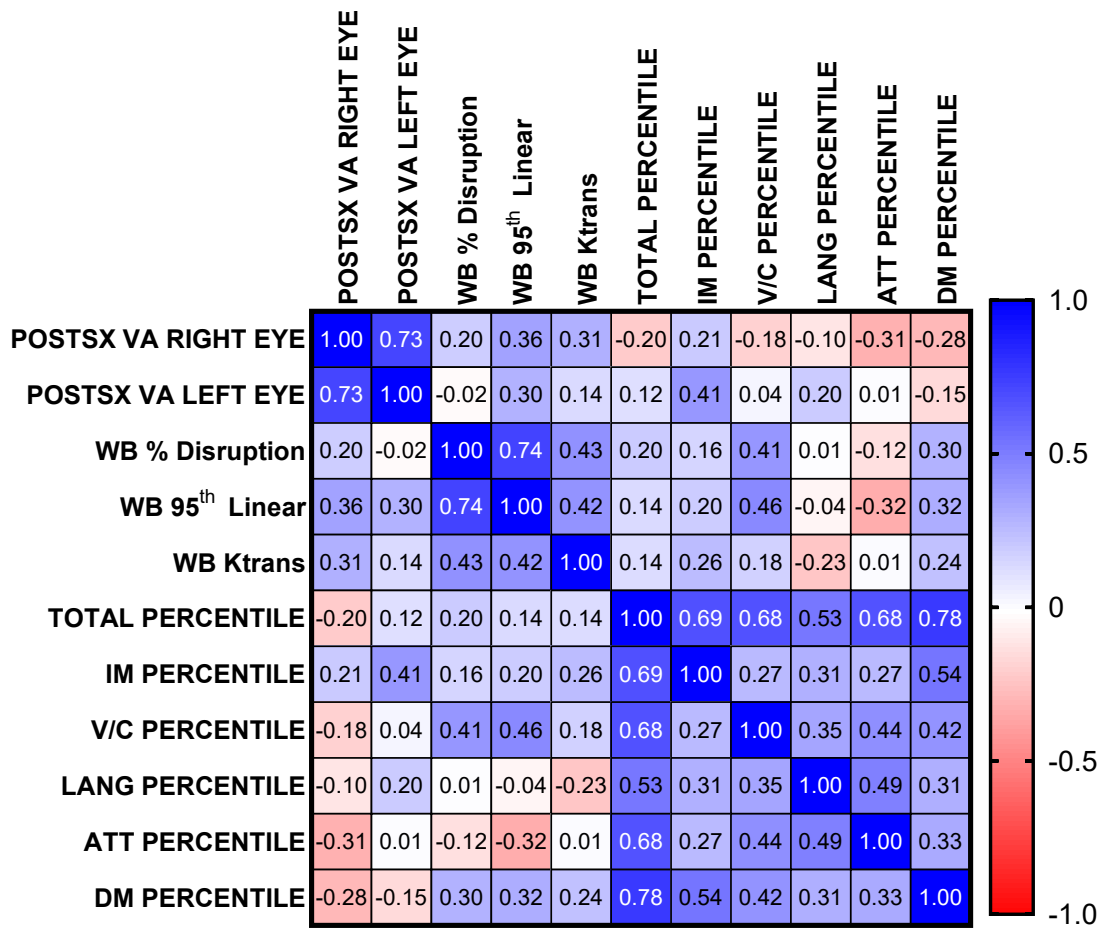


Figure 6.17. Post-surgical correlation matrix of visual acuity, whole-brain BBB leakage indices, and RBANS-U percentile performance (T2). VA: visual acuity. RE: right eye. LE: left eye. WB: whole brain. IM: Immediate memory. V/C: Visuospatial/Constructional. Lang: Language. Att: Attention. DM: Delayed memory.

6.12.2.2. Relationships Among BBB Indices at T2

At T2, BBB measures again showed a close association: whole-brain % disruption correlated strongly with WB 95th Linear ($r = 0.74$), indicating that greater global leakage burden tended to co-occur with higher values in the most permeable voxels. Ktrans showed moderate positive associations with both whole-brain % disruption ($r = 0.43$) and WB 95th Linear ($r = 0.42$), suggesting a tighter coupling between Ktrans and the threshold-based indices at T2 than what was observed at T1.

6.12.2.3. Post-Surgical VA: Inter-Eye Concordance and Associations with BBB Indices At T2

Post-surgical VA remained highly concordant between eyes (right vs left $r = 0.73$), consistent with broadly symmetric post-operative acuity within individuals. Associations between VA and BBB indices were modest and more evident for WB 95th Linear and Ktrans than for whole-brain % disruption. Right-eye VA showed small-to-moderate positive correlations with WB 95th Linear ($r = 0.36$) and Ktrans ($r = 0.31$), and a smaller positive correlation with whole-brain % disruption ($r = 0.20$). These directions indicate that worse right-eye VA tended to co-occur with higher WB 95th Linear values and higher Ktrans at T2.

Left-eye VA showed a similar but weaker pattern overall: a moderate positive correlation with WB 95th Linear ($r = 0.30$), a small positive correlation with Ktrans ($r = 0.14$), and a near-zero association with whole-brain % disruption ($r = -0.02$). Overall, any relationship between VA and BBB measures at T2 was small, and more evident for WB 95th Linear and Ktrans than for whole-brain % disruption.

6.12.2.4. BBB Indices And RBANS-U Performance at T2

At T2, BBB–cognition relationships were more mixed in direction than at T1 and were not uniformly negative across domains. Whole-brain % disruption showed small positive correlations with total percentile ($r = 0.20$) and delayed memory ($r = 0.30$), and a moderate positive association with visuospatial/constructional ($r = 0.41$), while correlations with language were near zero ($r = 0.01$) and with attention were small and negative ($r = -0.12$). WB 95th Linear showed a similar pattern, including a moderate positive association with visuospatial/constructional ($r = 0.46$) and delayed memory ($r = 0.32$), but a moderate negative association with attention ($r = -0.32$). Ktrans demonstrated generally small correlations with cognition (e.g., total $r = 0.14$; immediate memory $r = 0.26$; delayed memory $r = 0.24$), with the largest magnitude association being a moderate negative correlation with language ($r = -0.23$). Overall, by T2, the BBB–RBANS-U correlation structure appears less coherent and less consistently directional than at T1, in keeping with increased heterogeneity and/or reduced variance in key measures later in follow-up.

6.12.2.5. Post-Surgical VA And RBANS-U Performance at T2

Correlations between post-surgical VA and RBANS-U percentiles at T2 were generally small to moderate and not consistent across eyes and domains. Right-eye VA showed modest negative correlations with total percentile ($r = -0.20$), attention ($r = -0.31$), and delayed memory ($r = -0.28$), consistent with worse VA tending to accompany lower cognitive percentile performance; however, other domain associations were smaller (e.g., language $r = -0.10$). Left-eye VA showed weaker and mixed-direction correlations (i.e., total $r = 0.12$; immediate memory $r = 0.41$; delayed memory $r = -0.15$), without a consistent pattern across domains. Taken together, post-surgical VA at T2 does not show a stable or internally consistent cross-sectional relationship with RBANS-U performance in this cohort.

6.12.3. Integrated interpretation at T2

At 9 months post-surgery, RBANS-U percentiles remained strongly interrelated, supporting continued internal coherence of the cognitive metrics. BBB indices also remained correlated, with whole-brain % disruption and WB 95th Linear again closely aligned and Ktrans moderately coupled to both. In contrast to T1, correlations between BBB indices and RBANS-U performance at T2 were less uniform in direction and differed across cognitive domains and BBB measures, with mixed positive and negative associations rather than a broadly consistent negative pattern.

Post-surgical VA showed strong inter-eye concordance but only modest associations with BBB indices and an inconsistent pattern of correlations with RBANS-U percentiles. Although VA-cognition relationships were weak or absent at T2, it remains plausible that cognitive change over follow-up was influenced by broader aspects of visual restoration not captured by high-contrast acuity alone, which were not directly assessed in this protocol and may be particularly relevant to performance on visually mediated cognitive tasks.

6.13. Summary of Findings

Across T0–T2, threshold-based BBB disruption indices showed no statistically significant longitudinal change: whole-brain % BBB disruption increased numerically but remained non-significant, and WB 95th Linear demonstrated a non-significant upward trend that approached but did not reach the predefined threshold. In contrast, whole-brain Ktrans showed a statistically significant reduction between T0 and T2 in paired analyses, despite marked inter-individual heterogeneity at T1. Qualitative voxelwise Ktrans map inspection was directionally consistent with this reduction, with a posterior-predominant baseline leakage pattern observed in 11 of 17 baseline maps and reduced visual permeability signal in most inspected participants at T2. In the occipital-predominant subgroup (N = 11), T0–T2 Ktrans reduction was statistically significant.

Exploratory correlation analyses indicated that pre-surgical ocular parameters did not show consistent associations with whole-brain % BBB disruption or WB 95th Linear, while Ktrans identified a nominally significant association with left-eye VA at baseline. Cross-sectional correlation matrices suggested that BBB–cognition coupling was most evident at T1 (predominantly negative associations between BBB indices and RBANS-U percentiles), whereas T2 associations were more mixed and less internally consistent. Across post-operative timepoints, VA showed strong inter-eye concordance but only modest, variable associations with BBB indices and an inconsistent relationship with RBANS-U performance.

CHAPTER 7: RESULTS IV: STRUCTURAL BRAIN PARAMETERS, BRAIN AGEING RATE, AND PROTEOMIC MARKERS

7. 1. Total Brain, Grey Matter, and White Matter Volumes (T0–T2)

7.1.1 Analytic Approach

Total brain, grey matter, and white matter tissue volumes were expressed as a percentage of total intracranial volume (% TIV) and evaluated at T0 (pre-surgery), T1 (3 months post-surgery), and T2 (9 months post-surgery). Longitudinal change across timepoints was then examined using a mixed-effects model, with timepoint as a fixed effect and participant as a random effect, followed by Tukey-adjusted pairwise comparisons (T0 vs T1, T0 vs T2, T1 vs T2).

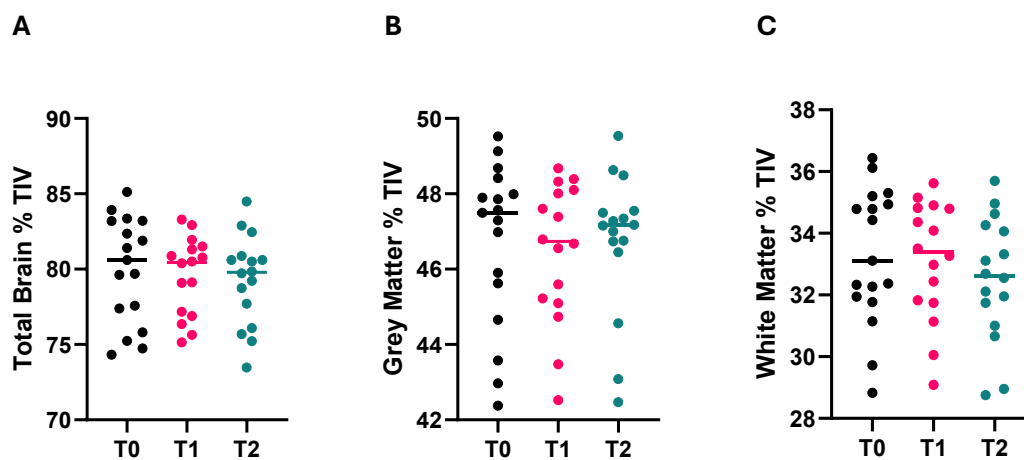


Figure 7.1. Total Brain (A), Grey Matter (B), and White Matter (C) volumes expressed as % total intracranial volume (% TIV) across T0, T1, and T2.

7.1.2 Results Across Timepoints

Across the 9-month follow-up, all %TIV tissue compartments remained stable with no statistically significant change over time (Figure 7.1).

For total brain %TIV, none of the Tukey-adjusted contrasts reached significance (T0 vs T1 mean difference = 0.430%, 95% CI -0.911 to 1.771, $p = 0.6891$; T0 vs T2 mean difference = 0.506%, 95% CI -1.160 to 2.173, $p = 0.7149$; T1 vs T2 mean difference = 0.210%, 95% CI -1.321 to 1.740, $p = 0.9321$). Mean total brain %TIV was stable across assessments (approximately 79–80%), and minor differences in reported means reflect the slightly different participant subsets contributing to each paired contrast.

For grey matter %TIV, volumes were similarly unchanged (T0 vs T1 mean difference = 0.218%, 95% CI -0.496 to 0.932, $p = 0.7134$; T0 vs T2 mean difference = -0.110%, 95% CI -1.071 to 0.852, $p = 0.9530$; T1

vs T2 mean difference = -0.209% , 95% CI -1.296 to 0.878 , $p = 0.8707$), with mean values remaining around 46–47% across assessments.

For white matter %TIV, no significant differences were detected (T0 vs T1 mean difference = 0.212% , 95% CI -0.692 to 1.117 , $p = 0.8172$; T0 vs T2 mean difference = 0.616% , 95% CI -0.311 to 1.543 , $p = 0.2281$; T1 vs T2 mean difference = 0.419% , 95% CI -0.459 to 1.297 , $p = 0.4459$). Mean values remained closely grouped (approximately 32.5–33.3%).

In all three analyses, no longitudinal volumetric change was detected for total brain, grey matter, or white matter % TIV from T0 to T2.

7.2. Lobar Brain Volumes Across T0–T2

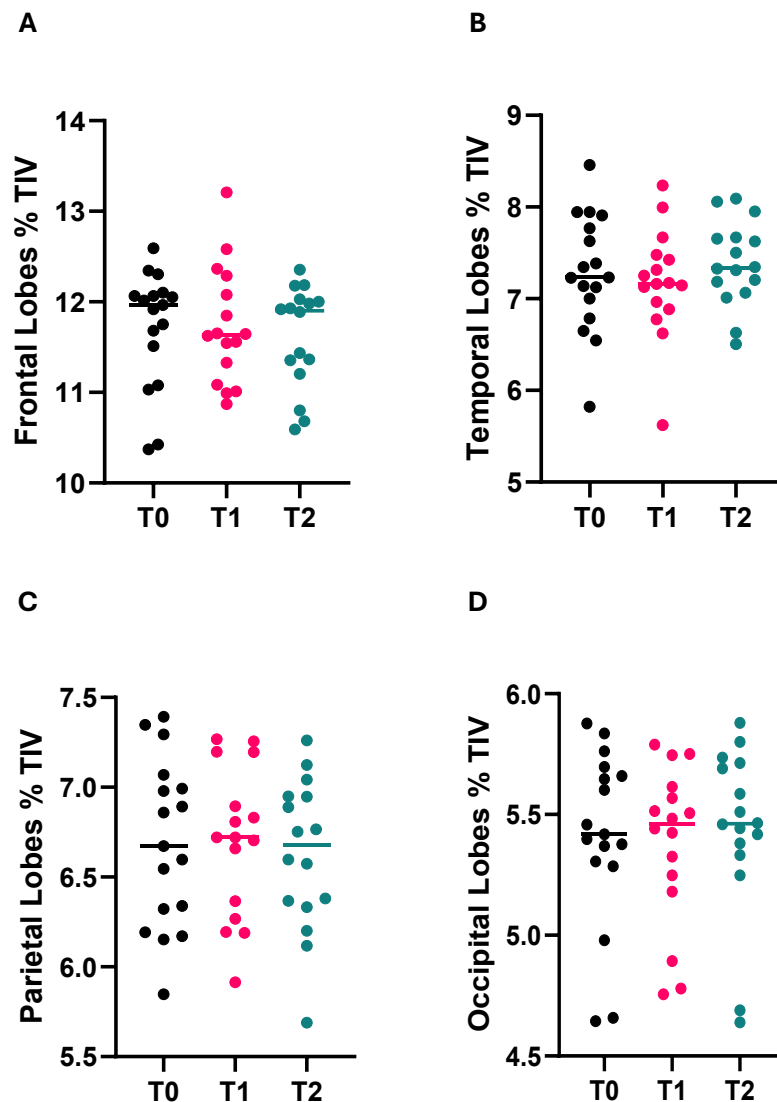


Figure 7.2. Frontal (A), Temporal (B), Parietal (C), and Occipital (D) Lobar Volumes (% TIV) Across T0, T1, and T2.

7.2.1 Analytic Approach

Frontal, temporal, parietal, and occipital lobe tissue volumes were expressed as a % TIV and evaluated at T0, T1, and T2. As with the global volumetric analyses, distributional checks were performed, and longitudinal change across timepoints was examined using a mixed-effects model. Tukey-adjusted pairwise comparisons were then used to test T0 vs T1, T0 vs T2, and T1 vs T2 within each lobe.

7.2.2 Results Across Timepoints

Across the 9-month follow-up, no statistically significant longitudinal change was detected in frontal, temporal, parietal, or occipital lobe volumes expressed as % TIV (Figure 7.2). For all lobes, Tukey-adjusted confidence intervals for each contrast crossed zero, and adjusted p-values exceeded 0.05.

For the frontal lobes % TIV, no contrasts were significant (T0 vs T1 mean difference = -0.0285 , 95% CI -0.3439 to 0.2869 , $p = 0.9701$; T0 vs T2 mean difference = 0.0851 , 95% CI -0.2645 to 0.4347 , $p = 0.8049$; T1 vs T2 mean difference = 0.1290 , 95% CI -0.1723 to 0.4304 , $p = 0.5176$). Mean frontal % TIV values were closely grouped (approximately 11.6–11.7%), with only small numerical fluctuations across timepoints.

For the temporal lobes % TIV, no contrasts reached statistical significance (T0 vs T1 mean difference = 0.0906 , 95% CI -0.1346 to 0.3158 , $p = 0.5615$; T0 vs T2 mean difference = -0.1244 , 95% CI -0.4465 to 0.1978 , $p = 0.5864$; T1 vs T2 mean difference = -0.1706 , 95% CI -0.5048 to 0.1636 , $p = 0.3996$). Mean temporal % TIV values remained stable overall (approximately 7.2–7.4%).

For the parietal lobes % TIV, all contrasts were non-significant (T0 vs T1 mean difference = 0.0181 , 95% CI -0.0934 to 0.1295 , $p = 0.9075$; T0 vs T2 mean difference = 0.0425 , 95% CI -0.0779 to 0.1630 , $p = 0.6378$; T1 vs T2 mean difference = 0.0375 , 95% CI -0.1000 to 0.1751 , $p = 0.7594$). Mean parietal % TIV values were tightly clustered (approximately 6.6–6.7%).

For the occipital lobes % TIV, no significant differences were detected (T0 vs T1 mean difference = 0.0418 , 95% CI -0.0650 to 0.1485 , $p = 0.5787$; T0 vs T2 mean difference = -0.0559 , 95% CI -0.1486 to 0.0369 , $p = 0.2906$; T1 vs T2 mean difference = -0.0861 , 95% CI -0.2196 to 0.0474 , $p = 0.2439$). Mean occipital % TIV values remained stable (approximately 5.36–5.45%).

In summary, all lobe volumes remained stable across T0, T1, and T2, with no evidence of longitudinal volumetric change over the 9-month follow-up. This mirrors the global tissue findings in Section 7.1 and indicates that, within this cohort, there were no detectable macrostructural shifts at either whole-brain or lobar levels across the perioperative period.

7.3. Lobar Cortical Thickness Across T0–T2

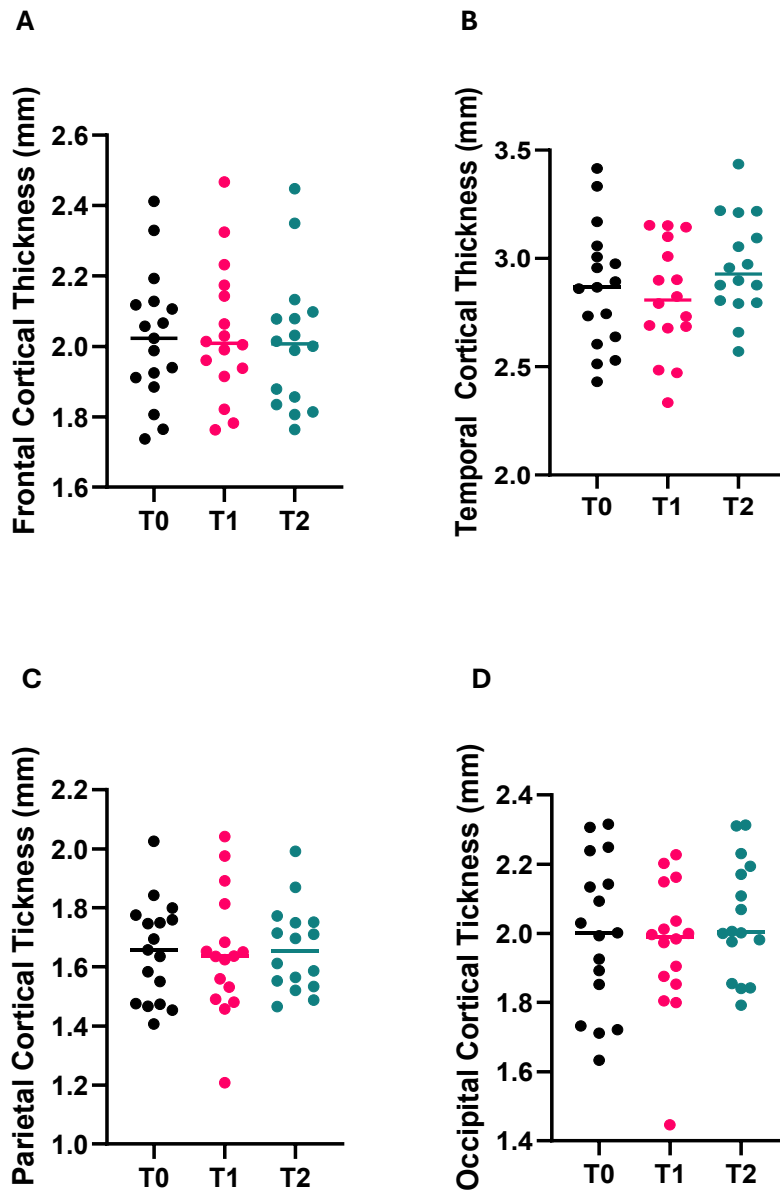


Figure 7.3. Lobar cortical thickness across T0, T1, and T2. Frontal (A), Temporal (B), Parietal (C), and Occipital (D) Lobes.

7.3.1. Analytic Approach

Cortical thickness was evaluated at T0, T1, and T2 for frontal, temporal, parietal, and occipital lobes (in mm). Since VolBrain’s vol2Brain cortical thickness pipeline provides lobar/regional outputs rather than a single whole-cortex scalar, a total cortical thickness measure was not available for analysis. Longitudinal change across timepoints was examined using a mixed-effects model, followed by Tukey-adjusted pairwise comparisons (T0 vs T1, T0 vs T2, T1 vs T2).

7.3.2. Results Across Timepoints

Across all lobes, no statistically significant differences in cortical thickness were detected between T0, T1, and T2 (Figure 7.3).

Frontal lobe cortical thickness remained stable across timepoints. None of the Tukey-adjusted contrasts reached significance (T0 vs T1 mean difference = -0.018 mm, 95% CI -0.109 to 0.073 , $p = 0.8647$; T0 vs T2 mean difference = 0.006 mm, 95% CI -0.059 to 0.071 , $p = 0.9664$; T1 vs T2 mean difference = 0.035 mm, 95% CI -0.036 to 0.105 , $p = 0.4212$). Mean values were closely clustered (T0 = 2.021 mm; T1 = 2.039 mm; T2 = 2.011 mm).

Temporal lobe cortical thickness similarly showed no significant timepoint effects, although there was a non-significant tendency for higher mean thickness at T2 relative to T0/T1. Pairwise contrasts were non-significant (T0 vs T1 mean difference = 0.039 mm, 95% CI -0.110 to 0.187 , $p = 0.7788$; T0 vs T2 mean difference = -0.107 mm, 95% CI -0.241 to 0.026 , $p = 0.1270$; T1 vs T2 mean difference = -0.120 mm, 95% CI -0.263 to 0.022 , $p = 0.1034$). Mean values were T0 = 2.855 mm; T1 = 2.816 mm; T2 = 2.965 mm.

Parietal lobe cortical thickness was highly stable across follow-up, with minimal differences and uniformly non-significant contrasts (T0 vs T1 mean difference = 0.012 mm, 95% CI -0.073 to 0.096 , $p = 0.9337$; T0 vs T2 mean difference = -0.015 mm, 95% CI -0.091 to 0.061 , $p = 0.8658$; T1 vs T2 mean difference = -0.022 mm, 95% CI -0.139 to 0.095 , $p = 0.8742$). Mean values were T0 = 1.657 mm; T1 = 1.646 mm; T2 = 1.661 mm.

Occipital lobe cortical thickness also showed no significant timepoint effects, with a non-significant pattern suggesting slightly higher mean thickness at T2. All contrasts remained non-significant (T0 vs T1 mean difference = 0.032 mm, 95% CI -0.066 to 0.131 , $p = 0.6774$; T0 vs T2 mean difference = -0.065 mm, 95% CI -0.153 to 0.024 , $p = 0.1731$; T1 vs T2 mean difference = -0.088 mm, 95% CI -0.204 to 0.027 , $p = 0.1474$). Mean values were T0 = 1.996 mm; T1 = 1.964 mm; T2 = 2.043 mm.

Overall, across frontal, temporal, parietal, and occipital lobes, cortical thickness remained stable from baseline through 9 months post-surgery, with no statistically significant longitudinal changes detected and only small non-significant fluctuations in mean thickness across timepoints.

7.4. Rate of Brain Ageing (T0–T2)

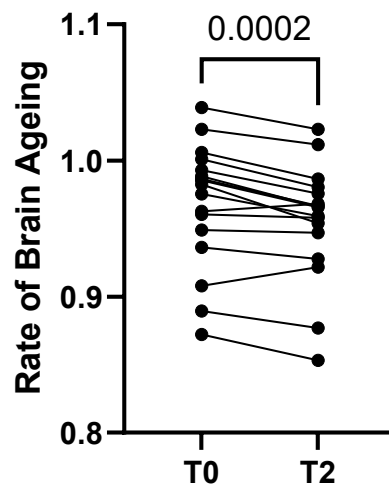


Figure 7.4. Rate of Brain Ageing Index (BrainAGE/Chronological Age) at T0 and T2.

7.4.1. Analytic Approach

To assess change in biological brain ageing over follow-up, brain age estimates were derived at T0 and T2 using the VolBrain BrainAGE pipeline, which generates a predicted biological brain age from structural MRI features. A Rate of Brain Ageing index was calculated for each timepoint as predicted brain age ÷ chronological age, so that values > 1.0 indicate a biologically “older-appearing” brain relative to age, and values < 1.0 indicate a comparatively “younger-appearing” brain structure.

Distributional checks indicated that both T0 and T2 Rate of Brain Ageing values met normality assumptions (all $p > 0.05$). Accordingly, within-participant change from T0 to T2 was assessed using a two-tailed paired-samples t-test.

7.4.2. Results Across Timepoints

Across the cohort that underwent MRI ($N=17$), the Rate of Brain Ageing index showed a statistically significant reduction between baseline and 9 months post-surgery (Figure 7.4). The paired t-test confirmed a robust within-subject decrease ($t(16) = 4.814$, $p = 0.0002$).

The mean paired difference ($T2 - T0$) was -0.01278 (SD of differences 0.01095, SEM 0.00266), with a 95% confidence interval of -0.01841 to -0.007154 . This corresponds to an approximate 1.3% reduction in the ageing-rate ratio across follow-up. In practical terms, participants predicted brain age, relative to chronological age, shifted downward from T0 to T2, indicating that the brain-age estimate moved closer to (or below) chronological age expectations at T2.

Effect size estimates indicated a large within-subject effect (partial $\eta^2 = 0.592$). Pairing was highly effective, with a very strong correlation between T0 and T2 values ($r = 0.971$, $p < 0.0001$), indicating that individuals' relative ranking on this measure was stable while the group as a whole shifted downward.

Overall, these results indicate a group-level reduction in the Rate of Brain Ageing index from T0 to T2, reflecting a statistically reliable downward shift in predicted brain age relative to chronological age over the perioperative follow-up interval.

7.5 Serum Proteomic Profiles (T0–T1–T2)

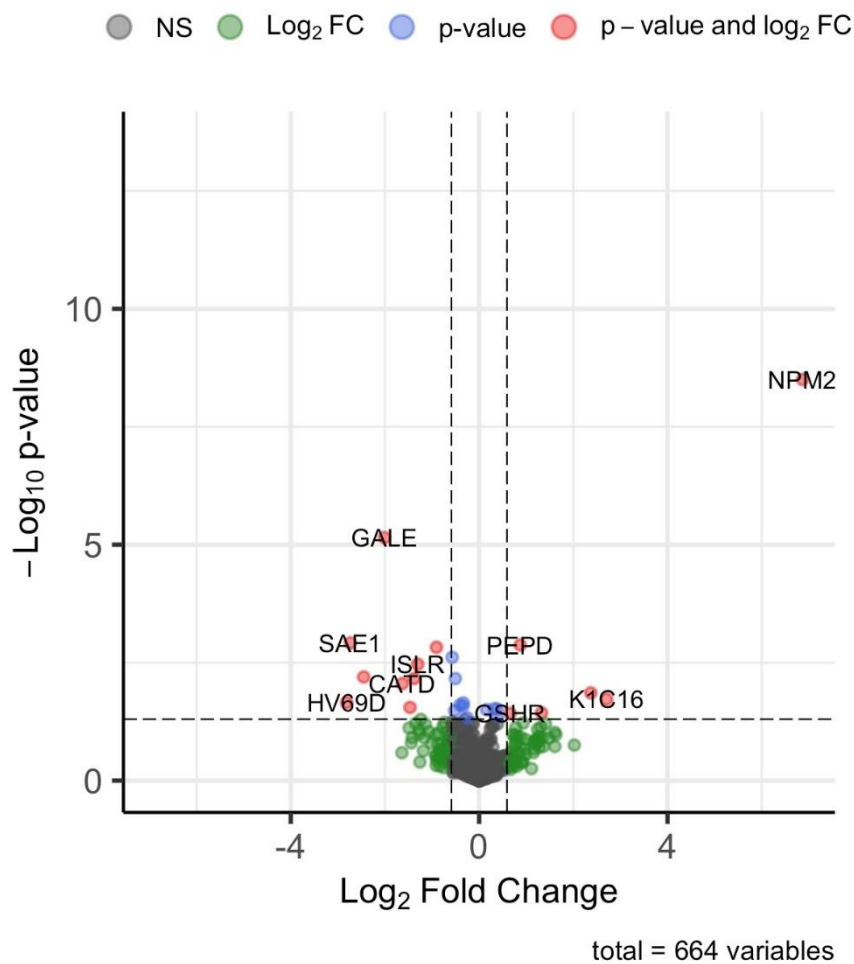


Figure 7.5. Volcano plot of serum proteomic changes after cataract surgery at 3 months (T1 vs T0). Each point represents one quantified protein ($n = 664$). The horizontal line indicates $p = 0.05$, and the vertical lines denote $|\log_2 \text{fold change}| = 0.3$ thresholds. Red markers highlight proteins meeting both criteria. For inferential reporting in Sections 9.5.2–9.5.4, significance was defined using FDR-adjusted p -values (adj.P.Val) from Limma.

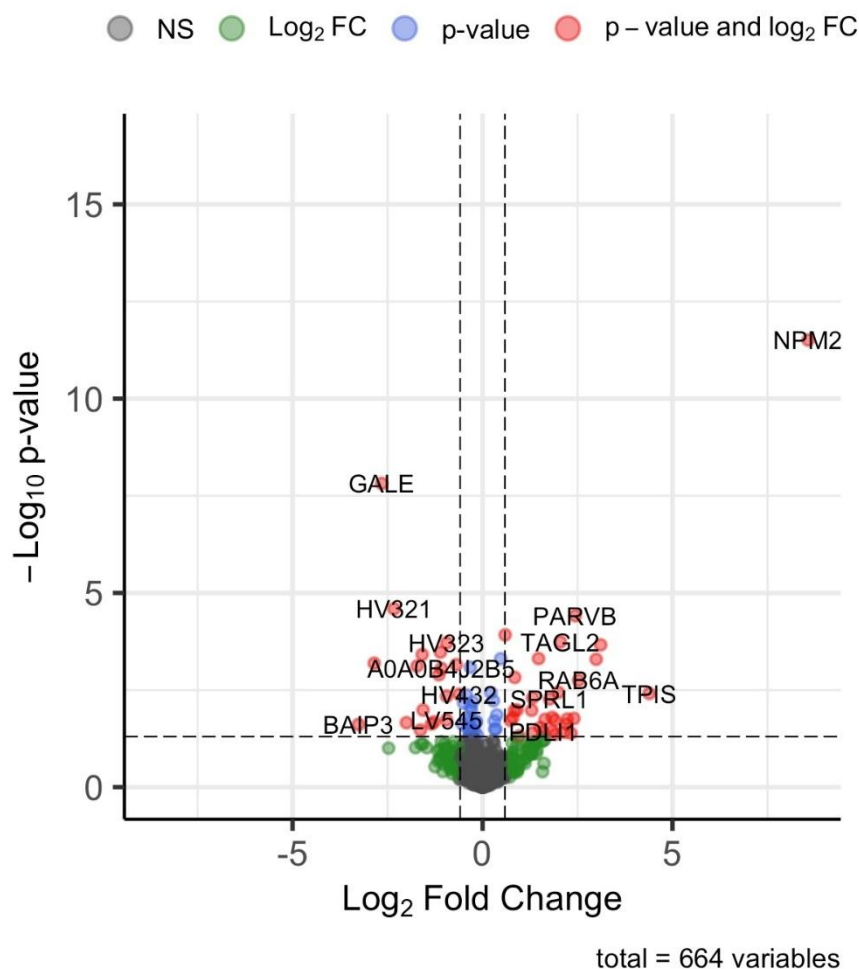


Figure 7.6. Volcano plot of serum proteomic changes after cataract surgery at 9 months (T2 vs T0). Each point represents one quantified protein ($n = 664$). The horizontal line indicates $p = 0.05$, and the vertical lines denote $|\log_2 \text{fold change}| = 0.3$ thresholds. Red markers highlight proteins meeting both criteria. For inferential reporting in Sections 9.5.2–9.5.4, significance was defined using FDR-adjusted p -values (adj.P.Val) from Limma.

7.5.1 Analytic Approach

Serum proteomics analysis was performed using label-free liquid chromatography–tandem mass spectrometry (LC–MS/MS), quantifying 664 proteins across samples collected at T0, T1, and T2. $\log_2$ -transformed intensities were analysed in R using Limma (Linear Models for Microarray Data) to test differential abundance (T1 vs T0; T2 vs T0) with empirical Bayes–moderated statistics. Statistical significance was assessed at a false discovery rate (FDR)–adjusted p -value (Benjamini–Hochberg; adj.P.Val) < 0.05 . Fold-changes were expressed on the $\log_2$ scale, where positive values represent higher protein abundance post-surgery relative to baseline. Volcano plots were generated for each contrast, displaying $\log_2$ fold-change on the x-axis and $-\log_{10} p$ -value on the y-axis. Differential abundance was defined using FDR-adjusted p -values (adj.P.Val) from limma together with a minimum effect-size threshold ($|\log_2 \text{FC}| \geq 0.3$).

Table 7.1. Differentially Abundant Plasma Proteins After Cataract Surgery (T1 vs T0 and T2 vs T0), Grouped by Functional Category.

Functional group	Protein	Protein name	T1 vs T0	T2 vs T0	Function
Chromatin / nucleosome handling	NPM2	Nucleoplasmin-2	↑	↑	Histone chaperone involved in chromatin/nucleosome assembly and chromatin organisation.
Carbohydrate metabolism	GALE	UDP-glucose 4-epimerase	↓	↓	UDP-sugar epimerase supporting galactose/glucose interconversion and glycan metabolism.
Actin cytoskeleton / adhesion	PARVB	Parvin beta	—	↑	Actin-binding focal-adhesion protein; cytoskeletal organisation and cell adhesion signalling.
	COTL1	Coactosin-like protein 1	—	↑	F-actin binding protein; implicated in cytoskeletal regulation.
	TAGLN2	Transgelin-2	—	↑	Actin-associated protein contributing to actin filament organisation/remodelling.
	CAP1	Actin filament-associated protein 1	—	↑	Regulates actin filament dynamics and cell morphology pathways.
	ABRACL	ABRA C-terminal-like protein	—	↑	Reported modulator of actin dynamics and cell motility (mainly cell-model evidence).
ECM / vascular—platelet-associated	MMRN1	Multimerin-1	—	↑	Platelet/vascular-associated ECM protein; carrier/binding partner for platelet factor V.
ECM / vascular structure	MMRN2	Multimerin-2	—	↓	Vascular ECM-associated protein implicated in blood-vessel integrity.
ECM / structural matrix	COMP	Cartilage oligomeric matrix protein	—	↑	Extracellular matrix glycoprotein supporting structural matrix interactions.
ECM / stromal marker	ISLR	Immunoglobulin superfamily containing leucine-rich repeat	—	↓	Stromal/ECM-associated protein discussed in fibroblast biology and matrix remodelling.
Proline/collagen-related peptide	PEPD	Xaa—Pro dipeptidase	—	↑	Hydrolyses proline-containing dipeptides; supports proline recycling and proline-rich peptide turnover.
Post-translational modification	SAE1	SUMO-activating enzyme subunit 1	—	↓	E1 enzyme subunit initiating SUMO activation for downstream protein SUMOylation.
Immune/secretory	ADA2	Adenosine deaminase 2	—	↓	Extracellular adenosine metabolism; linked to immune/monocyte biology.
	SRGN	Serglycin	—	↓	Secretory granule proteoglycan involved in storage/packaging of immune mediators.
Metabolic secreted protein	AZGP1	Zinc-alpha-2-glycoprotein	—	↓	Secreted protein associated with lipid mobilisation/metabolic signalling.
Immunoglobulin V-region entries	IGHV3-21 (HV321)	Immunoglobulin heavy variable 3-21	—	↓	Heavy-chain variable-region entry contributing to antigen-binding diversity; best interpreted as repertoire-related peptide signal.
	IGHV3-23 (HV323)	Immunoglobulin heavy variable 3-23	—	↓	

	IGHV1-18 (HV118)	Immunoglobulin heavy variable 1-18	—	↓	
	IGHV3OR16-9 (A0A0B4J2B5)	Immunoglobulin heavy variable 3/OR16-9	—	↓	

7.5.2 Proteomic Changes at 3 Months (T1 vs T0)

At 3 months post-surgery, the global proteomic pattern indicated relative stability, with most quantified proteins clustering close to $\log_2$ fold change = 0 and low $-\log_{10}$ p-values (Figure 7.5). Using the combined criteria (adj.P.Val < 0.05 and $|\log_2$ fold-change| $\geq$ 0.3), 2 of 664 proteins (0.3%) met thresholds.

Nucleoplasmin-2 (NPM2) showed a marked increase at T1 relative to baseline ($\log_2$ fold change = +6.87; adj.P.Val = 2.34×10^{-6}). In contrast, UDP-glucose 4-epimerase (GALE) showed a significant decrease ($\log_2$ fold change = -2.01; adj.P.Val = 0.00234). No other proteins met the adjusted significance criterion at this timepoint under the prespecified thresholds.

7.5.3 Proteomic Changes at 9 Months (T2 vs T0)

By 9 months post-surgery, the overall profile remained stable, with most proteins still clustering near $\log_2$ fold change = 0 (Figure 7.6). However, a larger subset met combined thresholds at T2: 20 of 664 proteins (3.0%) met criteria (adj.P.Val < 0.05 and $|\log_2$ fold-change| $\geq$ 0.3).

NPM2 again showed a highly significant increase at T2 ($\log_2$ fold change = +8.57; adj.P.Val = 2.06×10^{-9}), while GALE again showed a highly significant decrease ($\log_2$ fold change = -2.65; adj.P.Val = 5.06×10^{-6}), indicating consistent directionality across both timepoints.

Additional proteins that met thresholds at T2 included upregulated proteins: parvin beta (PARVB; $\log_2$ fold change = +2.44; adj.P.Val = 0.00643), coactosin-like protein 1 (COTL1; +3.11; 0.0180), transgelin-2 (TAGLN2; +2.04; 0.0180), actin filament-associated protein CAP1 (CAP1; +2.99; 0.0261), ABRACL (ABRACL; +1.47; 0.0261), multimerin-1 (MMRN1; +0.595; 0.0160), cartilage oligomeric matrix protein (COMP; +0.478; 0.0261), and Xaa-Pro dipeptidase (PEPD; +0.847; 0.0498).

Downregulated proteins meeting thresholds at T2 included SUMO-activating enzyme subunit 1 (SAE1; SUMO = small ubiquitin-like modifier; -2.85; 0.0306), immunoglobulin superfamily containing leucine-rich repeat (ISLR; -1.59; 0.0255), multimerin-2 (MMRN2; -1.11; 0.0246), adenosine deaminase 2 (ADA2; -1.72; 0.0316), serglycin (SRGN; -1.15; 0.0445), zinc-alpha-2-glycoprotein (AZGP1; -0.325; 0.0319), and multiple immunoglobulin heavy variable entries (including IGHV3-21 (HV321; -2.33; 0.00568), IGHV3-23 (HV323; -0.942; 0.0180), IGHV1-18 (HV118; -0.694; 0.0311), and IGHV3OR16-9 (A0A0B4J2B5; -1.09; 0.0322)).

7.5.4 Interpretation of the Proteomic Signature

Across both comparisons, the serum proteome was dominated by overall stability when inference was restricted to false discovery rate (FDR)–adjusted significance and a minimum effect-size threshold. At 3 months, only 2/664 proteins (0.3%) met combined criteria, whereas at 9 months 20/664 proteins (3.0%) met criteria, suggesting that postoperative proteomic shifts were selective rather than global.

Two proteins showed consistent directionality across both postoperative timepoints after multiple-testing correction: NPM2 was increased at both timepoints, while GALE was decreased at T1 and T2.

At T2, the broader set of significant proteins included modest increases in several cytoskeletal and adhesion-associated proteins (PARVB, CAP1, TAGLN2, COTL1, and ABRACL), alongside increases in selected extracellular matrix and platelet/vascular-associated proteins (MMRN and COMP). Concurrent decreases were observed in proteins linked to extracellular matrix/vascular structure (MMRN1 and ISLR), immune/secretory components (SRGN and ADA2), a metabolic protein (AZGP1), and several immunoglobulin heavy variable region entries (including IGHV3-21 (HV321), IGHV3-23 (HV323), IGHV1-18 (HV118), and IGHV3OR16-9 (A0A0B4J2B5)).

Overall, the proteins meeting FDR-adjusted significance at T2 clustered into a small number of functional groups, principally cytoskeletal organisation, extracellular matrix/vascular-associated components, and immune-related proteins, without suggesting a widespread acute-phase or systemic inflammatory serum response.

Importantly, within the proteins quantified in this LC–MS/MS panel, none of the “standard” circulating blood–brain barrier (BBB) serum marker candidates (outlined in Chapter 1) met the combined thresholds ($\text{adj.P.Val} < 0.05$ and $|\log_2 \text{fold-change}| \geq 0.3$). Therefore, no proteomic evidence of a change in standard BBB serum markers was identified.

Given that several of the differentially abundant proteins are predominantly intracellular or represent immunoglobulin heavy-chain variable-region peptides, these results are most appropriately framed as modest alterations within a small subset of circulating protein signals, rather than as a definitive mechanistic indicator of systemic inflammation or BBB remodelling. In the context of the neurovascular and cognitive findings presented earlier, the proteomic results are consistent with selective peripheral changes associated with the postoperative phenotype but do not demonstrate a direct causal link to BBB permeability or brain-ageing trajectories.

7.6 Summary of Findings

Across follow-up, structural brain measures obtained from structural MRI remained stable at both whole-brain and lobar levels. Total brain, grey matter, and white matter volumes expressed as % TIV showed no statistically significant change across timepoints, and frontal, temporal, parietal, and occipital lobe

volumes similarly remained stable. Lobar cortical thickness showed no statistically significant longitudinal differences, with values remaining tightly clustered across T0, T1, and T2. The lack of detectable change in volumetric measures or cortical thickness over the follow-up interval is noteworthy in the light of what has been published on age-related decline rates; however, this finding has to be interpreted cautiously given the sample size, follow-up duration, and potential measurement variability, with mechanistic interpretation deferred to the Discussion.

In contrast, the Rate of Brain Ageing index showed a statistically significant group-level reduction from T0 to T2 (N = 17), indicating a downward shift in predicted brain age relative to chronological age over follow-up.

Serum proteomics similarly indicated predominant stability under FDR-controlled inference. At 3 months, only 2/664 proteins met combined thresholds, with NPM2 increased and GALE decreased. At 9 months, 20/664 proteins met combined thresholds, again including NPM2 (increased) and GALE (decreased), along with a small set of additional proteins with modest, directionally mixed changes.

CHAPTER 8: DISCUSSION, CONCLUSIONS, LIMITATIONS, AND FUTURE DIRECTIONS.

8.1 Introduction

The primary aim of this thesis is to examine the impact of cataract-related visual impairment and subsequent visual restoration following surgery on cognition and neurovascular integrity in older adults. Although visual impairment is increasingly recognised as a modifiable risk factor for cognitive decline and dementia, the biological pathways linking sensory deprivation and restoration to brain health remain incompletely characterised. Cataract surgery provides a clinically common and scalable model of partial sensory deprivation followed by sensory restoration, offering a practical framework in which to evaluate whether improved visual input is associated with measurable changes in cognitive performance and blood–brain barrier (BBB)–related physiology over time.

The central working hypothesis of this project is that partial sensory deprivation due to cataract-related visual impairment negatively influences the cognitive profile of older adults, even in the absence of manifest cognitive complaints, and that this may be accompanied by changes in BBB permeability. In parallel, restoration of best-corrected vision after bilateral cataract surgery is expected to be associated with measurable improvement or stabilisation in cognition and BBB-related measures, rather than continued deterioration over time.

To test this hypothesis, three key study aims are defined. First, baseline cataract severity is characterised, and its cross-sectional associations with cognition, MRI-derived BBB permeability, structural indices, and brain-age estimates are examined at baseline (T0). Second, cognitive trajectories following bilateral cataract surgery are characterised by tracking Repeatable Battery for the Assessment of Neuropsychological Status-Updated (RBANS-U) global and domain scores across T0, 3 months (T1), and 9 months post-intervention (T2), with separate evaluation of the low baseline cognitive performance subgroup and concurrent monitoring of mood and anxiety (Beck Depression Inventory–II (BDI-II) and State–Trait Anxiety Inventory (STAI) forms Y1 (state anxiety) and Y2 (trait anxiety)). Third, longitudinal changes in BBB metrics, structural brain measures, brain-age indices, and circulating proteomics are evaluated, and their relationships with visual and cognitive trajectories are explored to inform mechanistic interpretation of sensory restoration over time.

In this chapter, I summarise the principal findings of the thesis and discuss them in relation to the aforementioned literature reviewed on sensory impairment, cognitive ageing, and neurovascular dysfunction. Project limitations are highlighted. Finally, I outline future directions required to strengthen causal inference and to clarify the subgroups in whom any cognitive or neurovascular benefit of sensory restoration may be most plausible.

8.2 Demographic and Clinical Profile of the Study Cohort

The cohort comprises community-dwelling older adults with a mean age of 72.9 years (range 60–85 years), placing the sample directly within the life stage in which both cataract prevalence and age-related neurovascular vulnerability accelerate. This age window is also the period in which subtle cognitive consequences of sensory impairment become most detectable, while many individuals remain functionally independent and capable of participating in detailed longitudinal phenotyping. Accordingly, the cohort is well positioned to test the study's central framework: whether partial sensory deprivation from cataracts is associated with cognitive and BBB-related signatures, and whether restoration of best-corrected vision modifies these trajectories.

The demographic profile suggests a relatively health-literate and cognitively resilient group. Educational attainment is generally high, with more than half of participants having completed third-level education. This is relevant because higher education is a proxy for cognitive reserve, which can buffer against clinically apparent cognitive difficulties despite emerging neurovascular ageing. In practical terms, higher reserve may attenuate baseline cognitive effects of cataract-related visual deprivation and may also constrain the magnitude of postoperative cognitive change, increasing the likelihood that any improvements are subtle, domain-specific, or best detected through sensitive longitudinal measures. At the same time, a higher-education profile reduces heterogeneity due to educational disparities and strengthens the interpretability of within-cohort cognitive comparisons.

Behavioural enrichment is also prominent. Nearly all participants report engagement in leisure or social activities, and average participation in hobbies and exercise occurs on approximately 3–4 days per week. In sensory–cognitive models of ageing, sustained social and physical engagement is associated with slower cognitive decline and may support frontal and hippocampal efficiency through reduced allostatic stress and maintenance of cognitive stimulation (Lin et al., 2014; Lin et al., 2018). This level of engagement represents a potentially protective background factor and provides additional context for interpreting cognitive trajectories: the cohort enters the study with substantial behavioural reserve, which may limit large absolute cognitive gains after surgery, but also means that small changes may still be meaningful against a relatively optimised baseline.

Medical comorbidity reflects the expected burden of vascular risk in later life and is directly relevant to BBB-focused outcomes. Hypertension and dyslipidaemia are each present in half of the participants, with additional cardiac disease in one-third and thyroid disorders in approximately one-quarter. These conditions are plausible contributors to inter-individual variability in BBB permeability and cognitive performance at baseline. Hypertension and dyslipidaemia, in particular, are mechanistically linked to endothelial dysfunction and microvascular vulnerability and therefore provide an ecologically valid clinical context for examining BBB-related indices. Importantly, these vascular comorbidities do not appear to change uniformly across follow-up, reducing the likelihood that systematic worsening of vascular health is

the principal driver of any longitudinal BBB signal at the group level, while still allowing meaningful baseline heterogeneity.

Psychiatric comorbidity is minimal in this cohort, with no reported depression and only one participant reporting anxiety at baseline. This is noteworthy in a cohort of this age and strengthens interpretability, as affective symptoms can lower cognitive test performance and complicate attribution of longitudinal cognitive change. In the context of this thesis, the low prevalence of affective diagnoses strengthens the specificity of cognitive findings by reducing a major non-neurological source of variance in RBANS-U performance. Sensory comorbidity beyond vision is present but not dominant: hearing problems are reported by a minority (16.7%), and hearing-aid use is limited. This supports the interpretation that the cohort primarily reflects a visual deprivation/restoration model rather than a dual-sensory impairment cohort, which would be expected to amplify cognitive load and complicate attribution of cognitive outcomes to visual change alone.

Lifestyle factors also suggest a relatively low exposure to additional systemic stressors. Smoking prevalence is very low, and alcohol use is largely within low-to-moderate ranges for most participants. While these behaviours are not primary variables in the study, their distribution and likely stability reduce the probability that longitudinal cognitive or BBB-related changes are driven by major behavioural shifts. A family history of neurological disease was reported by more than half of participants; while late-onset Alzheimer's disease and Parkinson's disease are typically sporadic, this information provides clinical background and helps contextualise perceived risk and health awareness within the cohort.

Finally, new medical diagnoses occur in a minority of participants during follow-up (27.8%), including ischaemic heart disease, breast cancer requiring treatment, and one diagnosis of Alzheimer's disease mild dementia. These events reinforce the clinical heterogeneity inherent to ageing cohorts and underscore why interpretation of longitudinal patterns is appropriately group-level rather than anchored to isolated individual changes. They also emphasise the need for cautious mechanistic interpretation when a small cohort is followed during a period in which health status can change naturally over time.

Taken together, the cohort represents an older, medically typical for this age group, and behaviourally engaged population with relatively high educational attainment, preserved independence, and common vascular risk factors, alongside minimal psychiatric confounding and limited dual-sensory impairment. This combination is well suited to detecting subtle sensory–cognitive–neurovascular relationships, while also implying that cognitive reserve and high engagement may reduce effect sizes and limit generalisability to lower-reserve or more medically complex populations. This demographic context should therefore be acknowledged as both a strength, supporting a cleaner interpretation of sensory restoration effects, and a limitation, in that ceiling effects and cohort selectivity may reduce the magnitude of detectable change.

8.3 Cataract-Related Visual Function and Surgical Restoration

From an ophthalmic perspective, the baseline cataract phenotype is characterised by universal nuclear change, with additional cortical involvement in approximately three-quarters of eyes and posterior subcapsular cataract (PSC) in a smaller proportion. Overall severity is predominantly mild to moderate, which is consistent with timely diagnosis and intervention within the Irish healthcare context, where cataracts are typically managed before progression to advanced, sight-threatening grades.

Nuclear opacities are predominantly mild–moderate (grade 2 most common), whereas PSC shows the greatest asymmetry, occurring more frequently and more severely in the right eye (including grade 3 PSC only in right eyes). This distribution aligns closely with the baseline visual acuity (VA) findings: at T0, best-corrected visual acuity (VA) is significantly worse in the right eye than in the left. The correlation heatmap supports a clinically coherent relationship between greater lens opacity and poorer VA, with the strongest associations observed for nuclear grading in the left eye and PSC in the right eye. This pattern is consistent with established clinical expectations, as nuclear and PSC subtypes can meaningfully impair distance VA, and PSC may disproportionately affect visual function relative to its frequency. The observed inverse association between nuclear and cortical grades ($r_s = -0.43$) suggests subtype heterogeneity and potential collinearity; accordingly, subsequent associations between cataract subtypes and cognitive or neuroimaging outcomes should be interpreted cautiously, particularly given the small cohort size.

A key methodological consideration is that nuclear cataract grading values are recorded identically for both eyes in the source dataset. This likely contributes to the apparent perfect inter-eye nuclear correlation ($r_s = 1.00$) and limits inference regarding true biological symmetry for nuclear severity within this cohort. In contrast, cortical and PSC measures demonstrate more plausible inter-ocular variability, supporting the broader conclusion that cataract phenotype is heterogeneous across participants and between eyes, particularly for PSC.

Most importantly for the thesis hypothesis, bilateral cataract surgery is followed by robust restoration of best-corrected vision: by T2, VA normalises in both eyes, the pre-operative inter-eye asymmetry resolves, and inter-eye concordance becomes strong. This confirms a clinically meaningful reversal of partial visual deprivation and strengthens the interpretation of subsequent longitudinal analyses by anchoring them to a clear, quantifiable improvement in sensory input. Accordingly, this study meets its key prerequisite, substantial and symmetric postoperative visual restoration, thereby providing a valid basis on which to evaluate whether cognitive and neurovascular trajectories track with this sensory change over time.

8.4 Cognitive Outcomes Across the Peri-Operative Period

This thesis demonstrates a clear and internally coherent pattern of cognitive change following bilateral cataract surgery, while also delineating substantial baseline heterogeneity that is clinically meaningful in our ageing cohort. At T0, RBANS-U performance is located within the average range at the group level

(Total percentile in the low 50s), and direct comparisons between RBANS-U domains do not show statistically significant differences after correction for multiple comparisons. Importantly, this group-level “average” profile is not synonymous with uniformly normal cognition, as 7 participants (38.9%) scored at or below the 10th percentile in at least one RBANS-U domain at baseline, and more than half of these individuals have low-range performance in two or more domains. On standard neuropsychological criteria, and in the absence of functional decline to explain the deficit pattern, this low-performance subset would fulfil criteria for objective cognitive impairment compatible with mild cognitive impairment (MCI) “on paper”. The baseline results, therefore, establish that the cohort contains a spectrum of cognitive status at study entry, ranging from clearly intact to objectively vulnerable, which is a crucial prerequisite for interpreting who may benefit most from sensory restoration.

Across the full cohort, RBANS-U Total percentile increases progressively from T0 to T1 and again to T2, with statistically significant stepwise improvements across all pairwise contrasts. This is not a subtle drift: the mean Total percentile rises from approximately the 50th percentile at baseline to the mid-70s by nine months, indicating a clinically interpretable upward shift in age-adjusted performance. Domain trajectories indicate that improvement is distributed but not uniform. By T2, immediate memory, visuospatial/constructional, language, and delayed memory show statistically significant gains, whereas attention increases numerically but does not meet the multiple-comparison-adjusted significance threshold. From a neurological perspective, this profile is important because it does not suggest that change is confined to a single RBANS-U index, nor is it restricted to domains where performance could be expected to improve simply because the tasks are visually presented. Instead, the pattern is more consistent with broader change across memory- and language-weighted functions, likely to reflect downstream encoding, retrieval, and lexical access processes rather than improved perception alone.

The timing of the cognitive change is also informative. Improvement in RBANS-U Total percentile is not restricted to the early postoperative interval; instead, performance increases significantly from T0 to T1 and shows an additional significant gain from T1 to T2, indicating a continued upward trajectory across the 12 to 18 months of follow-up (from T0 to T2). Several domain-level effects follow a similar pattern, with the clearest and most consistent gains evident at T2, and immediate memory showing a significant improvement between T1 and T2. Although the observational design does not allow causal inference, the stepwise pattern is not consistent with a purely short-term perioperative effect and instead suggests that the cognitive changes accumulate over months rather than appearing only immediately after surgery.

The exploratory subtest analysis usefully clarifies which components drive the domain-level findings. Statistically significant improvement concentrates on story memory, story recall, list recall, semantic fluency, line orientation, and coding. Conversely, several subtests show stability across timepoints, including figure copy, figure recall, digit span, list recognition, and picture naming. This selectivity supports the argument that change is not merely global inflation of scores, as it is preferentially expressed in tasks that rely on effortful learning and retrieval, speeded or efficiency-weighted output, and visually

demanding discrimination (line orientation in particular). In a neurological interpretation, this profile is consistent with improvement being more evident in retrieval-demanding memory and generative language (semantic fluency), rather than in recognition memory or relatively constrained naming, and with greater sensitivity in measures that can be affected by both perceptual quality and processing efficiency (coding, line orientation). A practical point should be made explicitly: the RBANS-U includes visually presented and timed tasks, so improved vision after surgery can enhance test performance by making materials easier to perceive and respond to. This does not nullify the findings, but it means the observed changes may reflect both improved perceptual access and genuine changes in cognitive efficiency. On the other hand, several of the statistically improved subtests (e.g., story memory, story recall, and list recall) are aurally presented rather than visually mediated, reinforcing that the observed gains are unlikely to be attributable solely to enhanced perceptual access and are compatible with broader improvements in cognitive performance.

By definition, all seven participants in the Low Baseline Cognitive Performance subgroup fulfil objective criteria compatible with MCI at T0 (≤ 10 th percentile in at least one RBANS-U domain, without functional decline). By T2, four of seven no longer meet this “on paper” MCI criteria, as none of their RBANS-U domains remain at or below the 10th percentile. This pattern is compatible with the view that the most measurable cognitive benefit from sensory intervention may occur when impairment is still at a prodromal or early stage. Conversely, those who remain in the low-performance range at follow-up may reflect a higher underlying cognitive burden, including the effects of evolving neurodegeneration and/or systemic illness during the study period. This degree of heterogeneity is expected in a real-world ageing cohort and supports a cautious, group-level interpretation rather than inference from individual cases. It should also be stated explicitly that individual-level trajectory analysis is not performed; therefore, the reported gains reflect group and subgroup averages rather than individual response patterns.

Taken together, these subgroup findings also inform clinical interpretation regarding timing. The data are most compatible with larger measurable gains when cognitive vulnerability is present but not yet dominated by fixed neurodegenerative burden, while remaining exploratory given sample size. At the same time, the results should not be used to imply that visual restoration is less important once cognitive impairment is established. Even where substantial cognitive improvement is unlikely, optimising vision remains clinically meaningful for maintaining function, reducing disability related to sensory loss, and supporting engagement in daily activities that underpin independence and cognitive resilience.

Affective measures serve as an important contextual comparator when interpreting the cognitive findings. BDI-II and both STAI measures remain stable across T0–T2, with the vast majority of scores in minimal/low ranges and no systematic shift in severity categories. Moreover, RBANS-U Total results do not differ between higher and lower affective symptom groups on BDI-II, STAI-Y1, or STAI-Y2. This convergent evidence supports a firm but appropriately bounded conclusion: that cognitive improvements observed

over follow-up, particularly those consolidating at T2, are unlikely to be explained primarily by improvements in mood or reduced test anxiety.

The baseline visual–cognitive correlation findings add specificity to the cognitive narrative. At T0, RBANS-U Total shows a statistically significant inverse association with nuclear cataract severity ($r = -0.56$, $p = 0.0156$), while correlations with best-corrected VA are directionally similar but non-significant ($r \approx -0.30$). This pattern suggests that, within the available visual metrics, lens opacity burden (nuclear grade) tracks global cognitive performance more clearly than logMAR visual acuity at baseline. This is clinically plausible because acuity is an incomplete proxy for the functional burden imposed by cataracts.

At T2, RBANS-U Total is not significantly associated with postoperative VA in either eye. This is not necessarily contradictory; after surgery, VA values cluster near normal, so there is little variability for correlations to detect, and logMAR becomes a weak discriminator of cognitive differences. The strong inter-eye VA correlation at T2 supports measurement consistency and also indicates that postoperative acuity is relatively homogeneous, further limiting the ability to observe VA–VA-cognition associations at follow-up. In addition, the study does not capture other postoperative visual functions that may be more relevant to cognitive performance, such as contrast sensitivity, glare disability/light scatter, colour perception, or real-world visual function, so any cognitive change may relate to broader improvements in visual quality that are not reflected in logMAR VA alone.

Taken together, the baseline and postoperative correlation results support a specific and defensible synthesis: cognitive change over time is not simply a linear function of high-contrast visual acuity. Any relationship between visual status and cognition is more likely to be mediated by broader aspects of visual quality and perceptual efficiency than by VA alone, particularly at baseline, and becomes harder to detect once acuity normalises.

From a neuropsychological standpoint, it is important to be explicit about what the RBANS-U can and cannot resolve. While executive processes are likely recruited indirectly across several tasks (i.e., strategic encoding during learning, speeded performance, and set-shifting demands in Coding, and controlled retrieval in Semantic Fluency), the battery does not include dedicated executive-function measures that would allow clear separation of inhibition, set-shifting, planning, or higher-order working memory manipulation. Accordingly, although the observed gains in learning, Coding, and Semantic Fluency are compatible with improved processing efficiency and retrieval dynamics, postoperative change cannot be confidently attributed to specific executive constructs. This limitation should be carried forward when interpreting cognitive mechanisms and when relating cognitive trajectories to the neurovascular outcomes in later sections.

8.5 Blood–Brain Barrier Permeability Outcomes Across the Peri-Operative Period

Across the perioperative period, the BBB results are best understood by separating the threshold-based “burden/extent” measures (whole-brain % disruption) from an “upper-tail intensity” measure (whole-brain 95th percentile linear leakage (WB 95th Linear)) and an absolute permeability parameter (whole-brain Ktrans (volume transfer constant)). This is not a merely semantic distinction, as whole-brain % disruption primarily reflects how widespread suprathreshold leakage signal is, whereas WB 95th Linear reflects the intensity within the most permeable voxels (95th percentile) rather than the spatial spread of abnormality.

At the whole-brain level, % BBB disruption increased numerically from T0 (18.82%) to T1 (23.71%) and T2 (28.95%) but did not reach statistical significance at any interval after Tukey correction. The T0–T2 contrast approached significance ($p = 0.0672$), yet remained above threshold, and confidence intervals overlapped zero. In parallel, the normalised lobar disruption ratios (lobar/whole-brain) showed no significant perioperative redistribution, indicating that any numerical increases were broadly proportional to the global signal rather than driven by a clearly disproportionate lobar shift. That said, the temporal lobes carried the largest non-significant numerical signal (right temporal T0–T2 $p = 0.0525$; left temporal $p = 0.0772$), which is directionally consistent with regional vulnerability in ageing cohorts but must remain explicitly qualified as borderline and non-significant within this sample.

WB 95th Linear behaved similarly to % disruption: it rose numerically from T0 to T2 and approached but did not meet significance (T0–T2 adjusted $p = 0.0579$). Taken together, the two threshold-based indices support a conservative conclusion: there is no statistically robust evidence that suprathreshold leakage burden or upper-tail “hot voxel” intensity changed across T0–T2. At the group level, BBB measures appear stable, with small numerical increases that could simply reflect normal ageing and sample variability in this modest cohort.

In contrast to the threshold-based indices, whole-brain Ktrans shows a clearer, longer-term signal. Although the three-timepoint pattern is difficult to summarise because T1 is highly variable, the paired analysis shows a statistically significant reduction from T0 to T2 (Wilcoxon $p = 0.0413$). This difference across metrics reflects what each measure is most sensitive to: % disruption and WB 95th Linear (extent of suprathreshold leakage and upper-tail intensity, respectively) do not change significantly, whereas Ktrans can detect a small shift in average permeability that may not be large enough to move many voxels below a threshold or alter the extreme tail. At the same time, the non-significant upward drift in the threshold-based indices is compatible with background ageing-related variability in older adults. Overall, the results support relative stability in threshold-based leakage indices alongside a modest longer-term reduction in Ktrans, consistent with subtle physiological recalibration rather than a major redistribution of BBB leakage.

The qualitative voxelwise map review supports the paired Ktrans finding. At baseline, 11 maps show a posterior-predominant pattern (mainly occipital, with patchy posterior temporal extension). By T2, this focal signal is reduced or no longer visible in most cases. Because regional Ktrans values could not be extracted, these observations remain qualitative, but their direction is consistent with the quantitative result of lower whole-brain Ktrans at T2. This is reinforced by the occipital-predominant subgroup Ktrans analysis, in which Ktrans decreased from T0 to T2 in all but one participant, and this reduction was statistically significant (Wilcoxon $p = 0.0137$).

These findings show a topographical agreement between the baseline map phenotype and the direction of longitudinal change, in that the leakage signal is most often posterior at T0 and is visually attenuated by T2, alongside the paired reduction in whole-brain Ktrans. This alignment makes it reasonable to consider a *possible* link between sensory restoration and posterior neurovascular physiology: improved visual input after cataract surgery could increase physiological activation of occipital and posterior associative networks, promote more efficient neurovascular coupling, and, through activity-dependent signalling within the neurovascular unit, support partial tightening of BBB permeability, reflected as lower Ktrans at follow-up. However, this should be understood only as a plausible explanation, not as evidence of region-specific BBB “recovery”, because regional Ktrans values could not be quantified.

The targeted forest-plot correlations indicate that pre-surgical ocular parameters were not consistently associated with whole-brain % disruption or WB 95th Linear at T0. The principal exception was whole-brain Ktrans, where left-eye VA showed a nominally significant association ($r = -0.55$, $p = 0.0275$), implying that worse acuity tracked with higher permeability in that analysis framework. This should be understood explicitly as exploratory, particularly given multiple correlations and the absence of a concordant effect across eyes and BBB indices.

At T2, VA did not correlate significantly with any BBB metric. This is expected rather than contradictory: after surgery, VA values converge, and the range narrows, so VA has limited variability to explain differences between participants in BBB measures. The strong inter-eye concordance at follow-up supports measurement consistency and also indicates that postoperative VA was relatively homogeneous across the cohort.

Crucially, VA is only one dimension of visual function. A limitation that should be stated plainly here is that the protocol did not capture several cataract-relevant visual domains, contrast sensitivity, glare disability/light scatter, colour transmission, higher-order optical distortions, and low-luminance performance, which may change substantially after surgery and may be more behaviourally relevant than high-contrast acuity for downstream cognitive expression. Therefore, a weak VA–BBB coupling at T2 does not exclude a relationship between broader sensory restoration and neurovascular physiology; it indicates that VA alone is an insufficient proxy for “total visual improvement” in mechanistic correlations.

The correlation matrices add a clinically useful layer, as they show how BBB indices, cognition, and vision co-vary at each timepoint. At baseline, BBB–cognition associations were most evident for *Ktrans* and attention ($r = -0.49$), whereas the threshold-based indices showed minimal links with RBANS-U Total. At T1, however, the cross-sectional structure became striking: whole-brain % disruption and WB 95th Linear showed moderate-to-strong negative associations with Total, visuospatial/constructional, language, and delayed memory percentiles (approximately $r \approx -0.58$ to -0.63), with *Ktrans* showing a smaller but still coherent negative pattern across several domains. This pattern is noteworthy, but it must be handled carefully: it is a timepoint-specific, exploratory, cross-sectional coupling at the period where postoperative physiological and behavioural variability is greatest, and it cannot establish directionality. A more appropriate statement is that, at 3 months, higher BBB leakage indices were associated with lower concurrent cognitive percentiles, with the clearest negative relationships observed for the threshold-based measures.

By T2, the BBB–RBANS-U correlation structure was mixed in direction and less internally consistent, and VA–cognition coupling remained weak. This shift is compatible with several non-exclusive explanations: reduced variance in postoperative acuity; heterogeneity in longer-term medical evolution; attenuation of early postoperative physiological dispersion; and sampling limitations that make moderate correlations unstable across timepoints. Overall, BBB–cognition associations varied by timepoint: the clearest, most consistently negative correlation structure was observed at T1, whereas at T2 the pattern was mixed in direction and less internally consistent, limiting interpretability.

Based on the observed data, the BBB findings support a cautious but meaningful conclusion: threshold-based BBB indices (% disruption; WB 95th Linear) show no statistically significant T0–T2 change, compatible with relative stability (and possibly attenuation of an expected age-related rise, which cannot be proven in this design). In parallel, whole-brain *Ktrans* shows a small but statistically significant reduction from T0 to T2, reinforced by qualitative posterior-predominant map patterns and by a significant reduction in the occipital-predominant subgroup. These findings do not establish causality, and they do not support the claim that cataract surgery “improves the BBB.” However, they provide a coherent neurovascular context for interpreting the cognitive trajectories: cognitive gains occurred without evidence of a clear group-level deterioration in BBB indices, and the BBB signal that emerged was metric-dependent, with the clearest longer-interval finding being a small but statistically significant reduction in whole-brain *Ktrans* from T0 to T2.

8.6 Structural MRI and Brain Ageing Rate Across T0–T2

Across the peri-operative follow-up, the conventional morphometric outputs are remarkably stable. Total brain, grey matter, and white matter volumes expressed as percentage of total intracranial volume (% TIV) showed no statistically significant change, and the same was true for lobar %TIV volumes (frontal, temporal, parietal, occipital), with all Tukey-adjusted contrasts non-significant. Lobar cortical thickness also showed no significant timepoint effects, with only small, non-significant fluctuations. Overall, the

study does not show detectable macrostructural change at the level of tissue volumes or lobar cortical thickness over follow-up.

Set against the ageing literature, “no detectable change” is itself informative but must be interpreted carefully. Normative longitudinal MRI studies often report annualised percentage brain volume change (PBVC/year), meaning the percentage change in total brain volume per year. In healthy ageing, whole-brain volume loss becomes more negative with age, averaging around -0.4% per year across adulthood and approximately -0.5% per year in the 80s (Fujita et al., 2023). Over a 12-to-18-month interval (T0 to T2), this corresponds to an expected whole-brain reduction of roughly $0.4\text{--}0.8\%$, which translates into a small absolute shift when expressed as %TIV (in this cohort, total brain is $\sim 79\text{--}80\%$ TIV, so the expected change is approximately $0.3\text{--}0.6$ percentage points). In this dataset, total brain %TIV showed no significant longitudinal change, and the T0 vs T2 contrast estimated a small mean increase ($+0.506$ percentage points), but with wide uncertainty (95% CI -1.160 to $+2.173$). Practically, this indicates that a typical age-related decline over 12–18 months is small enough to be plausibly obscured by segmentation variability, inter-individual physiological heterogeneity, and modest sample size. The most appropriate interpretation is macrostructural stability over follow-up and no evidence of accelerated atrophy, while not excluding small age-expected volume loss.

In contrast, the brain-age result stands out because it does show a statistically reliable shift despite stable volumes and thickness. The VolBrain BrainAGE-derived Rate of Brain Ageing (predicted brain age $\div$ chronological age) shows a clear group-level decrease from T0 to T2 (paired t-test $p = 0.0002$). The mean paired change is -0.01278 , equating to an approximate 1.3% reduction in the ageing-rate ratio. The very strong T0–T2 correlation ($r = 0.971$) indicates that participants largely retain their relative ranking on this measure, while the cohort as a whole shifts downward. Taken together with the absence of detectable change in bulk %TIV volumes and lobar cortical thickness, the significant downward shift in the BrainAGE-derived ageing-rate ratio is most consistent with sensitivity differences between metrics: the brain-age model may detect small, distributed shifts in structural signatures across the brain that remain below the detection threshold of bulk tissue volumes and lobar thickness measures over 12–18 months.

Mechanistically, the most sensible interpretation at this stage is metric sensitivity rather than overt morphology change: the cohort shows stable %TIV volumes and lobar thickness, alongside a measurable shift in a composite brain-age model. This can be framed as suggesting that the peri-operative period is associated with a subtle, distributed change in structural “age-likeness” that is not expressed as detectable volumetric loss or cortical thinning over 9 months in this sample. Importantly, mechanistic attribution should remain constrained: brain-age models are probabilistic and integrate multiple structural features, and the study design does not allow claims of “rejuvenation” or direct causality from surgery. The appropriate emphasis here is that the brain-age signal moves in a favourable direction without concomitant evidence of macrostructural deterioration in the standard morphometric outputs during follow-up.

Finally, interpretation should explicitly retain the study's analytic scope. These are group-level results; individual trajectory modelling is not performed, and isolated single-participant outcomes are not interpreted (including the participant diagnosed with Alzheimer's disease). This matters clinically because modifiability of neurodegenerative trajectories is less plausibly inferred in established disease without individual-level longitudinal modelling and substantially larger samples.

8.7 Circulating Proteomics Across T0–T2: Modest Shifts Without a BBB Serum-Marker Signal

Using the predefined thresholds (false discovery rate (FDR)-adjusted $p < 0.05$ and $|\log_2 \text{fold-change}| \geq 0.3$), the serum proteome is largely stable after cataract surgery. At 3 months (T1 vs T0), only 2/664 proteins meet criteria (Nucleoplasmin-2 (NPM2) increased; UDP-glucose 4-epimerase (GALE) decreased). By 9 months (T2 vs T0), 20/664 proteins meet criteria, indicating a slightly broader but still selective longer-term signal. Overall, this supports modest, time-dependent peripheral change rather than widespread systemic activation.

The most consistent finding at both postoperative timepoints is the same direction of change in two proteins: NPM2 increases, and GALE decreases. NPM2 is described as a nuclear histone-binding chaperone involved in chromatin organisation and nucleosome dynamics, while GALE catalyses the interconversion of UDP-galactose and UDP-glucose in galactose/hexose metabolism (UniProt Consortium, n.d.). The main interpretive point is that this is a light, directionally stable signal rather than a pattern suggestive of broad inflammatory activation. However, because NPM2 is primarily an intracellular protein, its presence in plasma, especially with very large fold changes, should be interpreted cautiously, as it may reflect altered cellular release/turnover or vesicle-associated cargo rather than straightforward "upregulation" attributable to a specific tissue source.

At T2, the significant proteins cluster into a small number of biologically interpretable groupings, again without a clear acute-phase pattern. Several cytoskeletal/adhesion-linked proteins increase (actin filament-associated protein CAP1 (CAP1), coactosin-like protein 1 (COTL1), transgelin-2 (TAGLN2), parvin beta (PARVB), ABRACL), which is compatible with subtle changes in circulating cell activation state, motility/adhesion programming, or vesicle cargo composition over the longer postoperative interval (UniProt Consortium, n.d.). This remains pathway-level plausibility only since discovery liquid chromatography–tandem mass spectrometry (LC–MS/MS) does not localise the source (e.g., leukocytes vs platelets vs endothelium), so no cell-type or tissue-specific conclusions can be made.

At T2, a small set of extracellular matrix (ECM) and vascular-interface proteins also changes, but in mixed directions: multimerin-1 (MMRN1) and cartilage oligomeric matrix protein (COMP) increase, while multimerin-2 (MMRN2) and immunoglobulin superfamily containing leucine-rich repeat (ISLR) decrease (UniProt Consortium, n.d.). Overall, this looks more like a modest shift in ECM/vascular-related proteins in plasma than a sustained postoperative inflammatory response. It can be described as a peripheral signal

that sits alongside the study's vascular-ageing framework, without implying that these proteins directly drive the imaging-based BBB permeability changes.

At T2, immune/granule- and antibody-related proteins also show small, selective shifts. Serglycin (SRGN) decreases, consistent with its role in secretory granule biology in immune cells (UniProt Consortium, n.d.; Niemann, 2004; Sutton, 2016). Adenosine deaminase 2 (ADA2) also decreases; this is relevant to extracellular adenosine metabolism and is described in the literature in relation to immune/vascular inflammatory phenotypes within ADA2 biology (UniProt Consortium, n.d.; Lee, 2022). Several immunoglobulin heavy-chain variable-region identifiers (e.g., IGHV3-21, IGHV3-23, IGHV1-18, IGHV3OR16-9) decrease; biologically, these relate to antigen recognition by immunoglobulin variable domains, but in LC-MS/MS outputs, they often reflect peptide-level and repertoire/compositional effects rather than a clean readout of "total immunoglobulin" or a single immune activation pathway (UniProt Consortium, n.d.; Schroeder and Cavacini, 2010).

Two additional proteins add a metabolic/matrix-maintenance angle: zinc-alpha-2-glycoprotein (AZGP1) decreases (linked to lipid mobilisation biology) and Xaa-Pro dipeptidase (PEPD) increases (linked to proline-containing dipeptide cleavage and collagen turnover) (UniProt Consortium, n.d.; Mracek, 2010; Eni-Aganga, 2021). Overall, the most appropriate interpretation is that these findings reflect modest, longer-term shifts in peripheral protein representation, and they should be described as descriptive systemic correlates rather than evidence of a direct mechanistic pathway to BBB permeability.

Critically, within the proteins quantified in this LC-MS/MS panel, none of the standardised BBB-associated circulating serum markers discussed earlier in the thesis meet the prespecified inferential thresholds, meaning there is no proteomic evidence of a postoperative shift in canonical BBB serum-marker candidates in this cohort. This limits any claim that systemic BBB-marker dynamics mirror the imaging-derived BBB findings and supports a narrower conclusion: the circulating proteome shows selective, modest postoperative change, whereas the BBB results are best interpreted on their own terms as neuroimaging-derived neurovascular measures.

8.8 Conclusions

This thesis provides an integrated account of how cataract-related visual impairment and subsequent sensory restoration relate to cognition and neurovascular integrity in later life. In an older cohort with preserved independence, high educational attainment, and a clinically typical burden of age-associated medical comorbidity, bilateral cataract surgery produced robust and symmetric restoration of best-corrected VA by 9 months (T2), resolving the pre-operative inter-eye asymmetry and resulting in tightly clustered postoperative acuity values. Against this clearly identified sensory change, cognitive performance improved consistently over follow-up: RBANS-U Total percentile increased stepwise from T0 to T1 and again to T2, with gains most evident in immediate memory, delayed memory, language, and

visuospatial/constructional indices, and subtest patterns indicating selectivity rather than uniform score inflation.

Baseline heterogeneity was clinically meaningful. A low-performing subgroup met objective “on paper” criteria compatible with mild cognitive impairment at T0, and a majority no longer met those criteria at T2, supporting the possibility that measurable benefit is most apparent when vulnerability is present but not (yet) dominated by fixed neurodegenerative burden. Importantly, mood and anxiety remained mild and stable across timepoints, including stable STAI-Y1, making it unlikely that the observed RBANS-U gains are explained primarily by reduced test anxiety or mood change.

The blood–brain barrier (BBB) findings provide essential neurovascular context and require a metric-specific interpretation. Threshold-based indices capturing leakage extent (whole-brain % disruption) and upper-tail intensity (WB 95th Linear) showed no statistically significant change across T0–T2, supporting relative group-level stability within the resolution of this modest cohort and remaining compatible with small age-linked upward tendencies that cannot be separated from background ageing and sampling variability. In contrast, whole-brain Ktrans showed a small but statistically significant reduction from T0 to T2, despite heterogeneity at T1. Qualitative voxelwise map review added internal coherence: most inspected baseline maps showed a posterior-predominant pattern (occipital with patchy posterior temporal extension) that was visually attenuated by T2, and a focused analysis of the occipital-predominant subgroup demonstrated a largely consistent paired reduction. These results do not establish causality, nor do they support the narrative that surgery “improves the BBB.” Rather, they indicate that cognitive gains occurred without evidence of group-level worsening in BBB indices, and that the clearest BBB signal was subtle, longer-interval, and metric-dependent, with Ktrans being the most responsive measure in this dataset.

Structural MRI outcomes add a further layer. Conventional morphometrics, bulk tissue volumes expressed as %TIV (total brain, grey matter, white matter), lobar %TIV volumes, and lobar cortical thickness, remained tightly clustered with no statistically significant longitudinal change, indicating no detectable macrostructural shift over the 12–18 month (T0–T2 interval) at the level of these summary measures. When set against normative ageing trajectories, this “no detectable change” should be interpreted cautiously: expected age-related volume loss over 12–18 months is small in absolute terms and can plausibly sit within the combined noise of segmentation variability, physiological heterogeneity, and modest sample size, so stability in this context does not imply true volumetric gain, but it does argue against accelerated atrophy during follow-up. Notably, the VolBrain BrainAGE-derived Rate of Brain Ageing decreased significantly from T0 to T2, indicating a coherent group-level downward shift despite stable bulk morphometrics. This pattern supports the interpretation that the brain-age modelling may be more sensitive to subtle, distributed structural signatures than conventional volume and lobar thickness summaries over short-to-medium follow-up windows, while remaining uncertain on mechanism and avoiding any claim of “rejuvenation.”

Finally, discovery-based plasma proteomics was dominated by stability under FDR-controlled inference. Only a small fraction of proteins met combined statistical and effect-size thresholds, with a sparse but consistent core signal across timepoints (NPM2 increased; GALE decreased) and a modestly broader but still selective profile by T2. Critically, no postoperative signal was detected in canonical circulating BBB serum-marker candidates within the quantified panel. This limits any claim that systemic BBB-marker dynamics track the imaging-derived BBB findings in this cohort and supports a bounded interpretation: peripheral molecular shifts, where present, were modest and non-specific in tissue origin, while BBB conclusions should be drawn primarily from the neuroimaging-derived permeability measures.

Taken together, the thesis supports a coherent, all-encompassing message: Cataract surgery achieved clear sensory restoration and was accompanied by measurable cognitive improvement, most convincingly demonstrated at the longer interval of 9 months, while BBB and structural brain outcomes showed no evidence of group-level deterioration across follow-up. The neurovascular findings were metric-dependent, with threshold-based indices remaining stable and Ktrans demonstrating a small longer-interval reduction that aligned with posterior-predominant qualitative map patterns. In parallel, conventional morphometrics remained stable while brain-age modelling detected a favourable shift, consistent with sensitivity to subtle distributed signatures not captured by bulk volume and lobar thickness measures. Collectively, these findings support the concept that sensory restoration in later life can coincide with improved cognitive performance and stable (or subtly recalibrated) neurovascular physiology, while also delineating clear restrictions on mechanistic inference that justify replication in larger, controlled, and more comprehensively phenotyped cohorts.

8.9 Limitations

Several limitations should be stated clearly because they limit what we can conclude about mechanisms and changes over time:

First, the study design was observational and lacked a non-surgical control group, such as age-matched participants without cataract surgery or a waiting-list control. This limits causal attribution and makes it difficult to fully separate surgery-related sensory restoration effects from background ageing, regression to the mean, or practice effects from repeated cognitive testing. Although mood and anxiety measures provide contextual information, they cannot fully exclude nonspecific contributors to cognitive variability, such as fluctuating physical health, sleep, or situational factors surrounding the assessment.

Second, the cohort size was modest, and follow-up coverage varied across timepoints. This reduces statistical power, limits the precision of effect estimates, and increases vulnerability to both false-negative findings and unstable subgroup effects. While the design was predominantly longitudinal and paired, missing data across timepoints and modalities, including differential availability across clinical, neuropsychological, MRI, and proteomic datasets, restrained certain comparisons and limited the robustness of timepoint-to-timepoint inference. Analyses were therefore conducted at the group level;

isolated single-participant trajectories were not interpreted, and individual-level mechanistic conclusions, particularly in participants with established neurodegenerative disease, cannot be drawn from these data.

Third, cohort characteristics may restrict generalisability. Participants were relatively highly educated and behaviourally engaged, which may reduce observable effect sizes, introduce ceiling effects on cognitive measures, and limit extrapolation to populations with lower cognitive reserve, greater comorbidity burden, or more complex medical profiles. Recruitment from a specific service pathway and geographic context further limits external validity.

Fourth, the neuropsychological assessment imposes important interpretive limits. RBANS-U is a global cognitive battery with visually presented and timed components. Improvements following cataract surgery may therefore partly reflect enhanced perceptual access and processing efficiency rather than isolated changes in specific cognitive constructs. In addition, the protocol did not include a direct and comprehensive executive-function battery. Although executive processes were indirectly engaged by complex, multi-domain tasks, no dedicated measures were available to generate domain-specific executive scores or to resolve executive mechanisms explicitly. .

Fifth, several methodological constraints apply to the neuroimaging component. DCE-MRI-derived BBB permeability measures are sensitive to acquisition parameters, modelling assumptions, physiological state, and motion or artefact effects. Metric dependence is a core feature of the dataset: continuous Ktrans measures behaved partly independently from threshold-based indices (percentage disruption and whole-brain 95th percentile metrics), supporting a multi-metric interpretation rather than a single unified “BBB integrity” construct. Regional quantification was also limited, as regional Ktrans values could not be extracted numerically; consequently, posterior-predominant patterns of apparent improvement are supported by qualitative permeability maps and subgroup whole-brain Ktrans patterns rather than formal regional statistics. Multiple comparisons apply across correlation analyses, and isolated nominal p-values should therefore be treated as hypothesis-generating. Structural volumetry and cortical thickness estimates are likewise influenced by segmentation performance and scan–rescan variability; the absence of significant longitudinal structural change over the follow-up period should be interpreted cautiously in light of sample size, follow-up duration, and measurement variability.

Sixth, interpretation of the proteomic findings is constrained by the discovery-based, label-free LC–MS/MS approach and available sample size. Multiple testing across hundreds of proteins increases the risk of type I error, while stringent correction increases the risk of type II error for small effects. Discovery proteomics cannot localise protein source, and several significant proteins were intracellular or reflected immunoglobulin variable-region peptides, which require cautious biological interpretation. Importantly, no postoperative signal was detected in canonical circulating BBB-associated serum markers under FDR-controlled inference. This should not be interpreted as definitive evidence that systemic biology is

irrelevant, but rather as indicating that, within this dataset, standard BBB serum-marker candidates did not show detectable change.

Seventh, aspects of visual phenotyping further limit inference. Postoperative VA converged substantially across participants, reducing variance and limiting the sensitivity to explain between-subject differences in cognitive or BBB outcomes. Moreover, the protocol did not include measures of contrast sensitivity, glare disability, low-luminance vision, or other optical quality metrics that may be more informative mediators of cognitive performance after cataract surgery than high-contrast acuity alone.

Additional design and methodological factors limit interpretability, particularly regarding vascular burden, sensory confounding, imaging standardisation, and analytic approach:

The study did not incorporate formal MRI-based small-vessel disease burden scoring (i.e., the Fazekas scale), which is highly relevant in an older cohort with vascular risk factors and may confound both BBB permeability measures and cognitive outcomes.

In parallel, the absence of an age- and sex-matched healthy control group for DCE-MRI limits the ability to contextualise observed BBB metrics against normative ageing distributions derived under identical acquisition and processing conditions, restricting interpretation to within-cohort longitudinal change rather than deviation from expected age-related permeability profiles.

The protocol did not include a formal audiometric assessment. Given the recognised interactions among sensory impairment, cognitive performance, and potentially neurovascular physiology, unmeasured hearing loss is a plausible confounder that may contribute to variability in both cognitive and BBB-related outcomes.

The cohort selection also introduced a potential inclusion bias: recruitment from a predominantly private healthcare pathway, with shorter waiting times for surgical intervention, may select for individuals with different health-seeking behaviours, comorbidity profiles, or disease trajectories compared to publicly managed populations, thus limiting broader generalisability.

Finally, structural MRI analyses relied on a cloud-based automated segmentation platform (volBrain), which, while standardised and efficient, provides limited quality-control outputs, limits user-level parameter adjustment, and introduces uncertainty regarding segmentation accuracy relative to more flexible, locally implemented and extensively validated open-source pipelines. This methodological choice may influence volumetric and cortical thickness estimates and should be considered when interpreting the stability of structural measures over time.

Collectively, these limitations support cautious interpretation of the magnitude and underlying mechanisms of the observed changes. They highlight the need for replication in larger, well-characterised cohorts with appropriate control groups and longer follow-up, alongside more comprehensive assessment of vascular burden and sensory function, a broader and more targeted neuropsychological coverage (including direct executive-function measures), richer visual and auditory phenotyping, improved normative benchmarking of BBB metrics, and complementary validation of imaging and molecular findings using robust, flexible analytic pipelines.

8.10 Future Directions

Future work should build on the feasibility and directionality observed here while using designs that better support causal inference and clearer mechanistic interpretation. Larger longitudinal cohorts with a priori power calculations, improved retention strategies, and more consistent completion across cognitive, MRI, ophthalmic, and proteomic timepoints would increase precision and reduce instability in subgroup findings. Including an appropriate comparator group, such as age-matched non-surgical participants or a waiting-list/delayed-surgery control, would help separate sensory restoration effects from ageing-related change, regression to the mean, and repeated-testing effects.

Cognitive phenotyping should be broadened beyond global screening batteries. Adding dedicated executive-function measures (set-shifting, inhibition, planning, and working memory manipulation), alongside processing-speed tasks, would help clarify whether gains in measures such as Coding and Semantic Fluency reflect executive control, perceptual efficiency, or broader domain-general mechanisms. Including ecologically valid outcomes (instrumental activities of daily living, real-world functional performance, and patient-reported cognitive change) would also strengthen clinical interpretation and relevance.

Visual phenotyping should be expanded to better characterise “sensory restoration” as an exposure. Future protocols should include contrast sensitivity, glare disability, low-luminance performance, and patient-reported visual function, and model cataract subtype and severity where possible. These measures are likely to capture meaningful postoperative visual change more comprehensively than high-contrast VA alone and may improve mechanistic modelling of brain and cognitive outcomes. Incorporating a hearing assessment would further help disentangle sensory contributions and reduce confounding from dual-sensory impairment.

For BBB and neurovascular mechanisms, future imaging pipelines should enable quantified regional K_{trans} extraction to test the posterior-predominant hypothesis directly and to determine whether occipital and posterior temporal patterns are reproducible. DCE-MRI protocols would also benefit from standardised acquisition and modelling, rigorous motion and quality-control reporting, and the planned use of complementary permeability metrics to address known differences between threshold-based indices and continuous measures.

The molecular component should progress from discovery to validation. Candidate protein signals should be confirmed using immunoassays or targeted mass spectrometry panels, with careful control of pre-analytical variables and replication in independent cohorts. Given the absence of postoperative change in canonical circulating BBB marker candidates under FDR-controlled inference in this dataset, future work should also consider alternative biomarker classes, multi-omic approaches, and sampling schedules better matched to expected systemic response kinetics.

Finally, the next step is to use more integrated analytic approaches. Future studies should prioritise preregistered models that jointly assess visual restoration, cognition, BBB metrics, brain structure, and brain-age measures, and circulating proteins within a single coherent framework, with explicit handling of missingness, prespecified primary outcomes, and sensitivity analyses. Together, these steps would improve generalisability, sharpen mechanistic interpretation, and clarify whether cataract surgery is associated with sustained neurocognitive and neurovascular benefits, and in which subgroups such effects are most plausible.

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