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Neurodegenerative analysis with fMRI for cognitive impairment in glaucoma disease

Settore scientifico-disciplinare: **MED/48**

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1. Abstract

Glaucoma is a group of progressive optic neuropathies resulting from a gradual degeneration of retinal ganglion cells and their axons, leading to a distinctive appearance of the optic disc and a concomitant pattern of visual loss which affects the quality of life. It is a multifactorial disease process, and its pathogenesis is incompletely understood. Although much attention is focused on the role of intraocular pressure (IOP), other factors such as abnormal ocular blood flow, abnormal structural susceptibility of the lamina cribrosa, low intracranial pressure, autoimmunity, and mitochondrial dysfunction may also be involved.

Dementia and glaucoma are both neurodegenerative conditions characterized by neuronal loss leading to cognitive and visual dysfunction, respectively. A variety of evidence exists linking the two diseases including structural signs, specifically degenerative changes within ganglion cells.

The aim of this study is to employ improved analysis methods functional MRI to study changes in functional brain connectivity and evaluated alteration of functional brain networks in glaucomatous patients, examine the association between neurocognitive status, fMRI parameters and other diagnostic test.

The integration of neurocognitive assessments along with fMRI data and other diagnostic tests could provide valuable insights into the mechanistic links between aging, optic nerve degeneration, and brain health.

Understanding these connections could potentially lead to improved diagnostic and therapeutic strategies for both glaucoma and dementia.

1.1. Glaucoma disease

1.1.1. Epidemiology

It is estimated that glaucoma affects approximately 70 million people worldwide, with at least 6.8 million being bilaterally blind. Vision loss caused by glaucoma is irreversible and is the second leading cause of blindness globally.

Among various types of glaucoma, primary open-angle glaucoma is the most common form. However, the extent of the problem is likely broader than these numbers suggest, and a substantial proportion of individuals remain undiagnosed or inadequately treated due to the absence of symptoms until the advanced stage. The number of people suspected of having glaucoma, usually those with elevated intraocular pressure (ocular hypertension) or asymmetric appearance of the optic disc, far exceeds those diagnosed with the disease.

With the growing number and proportion of older persons in the population, it is projected that 111.8 million people will have glaucoma in 2040.[1]

1.1.2. Pathogenesis

Increased intraocular pressure (IOP) is the primary risk factor for the development of glaucoma, despite the existence of forms characterized by normal IOP. Intraocular tone is determined by the balance between the rate of aqueous humor production and outflow. The normal range is between 10

and 20 mmHg, with the average value generally around 15 mmHg ± 2.5. Circadian fluctuations can be recorded, with variations of 3-5 mmHg, based on heart rate, blood pressure, respiration, and time of day (morning IOP tends to be higher, at least partially due to decreased production at night).
The increase in IOP is associated with the progressive appearance of permanent damage to the optic nerve fibers, accompanied by characteristic changes in the optic disc (referred to as "glaucomatous neuropathy") and visual function.

Two theories, which essentially coexist, have been formulated to explain the different mechanisms responsible for structural alterations: the *mechanical theory* and the *vascular theory*. According to the mechanical theory, IOP exerts a compressive action on the lamina cribrosa at the level of the scleral canal, affecting the nerve fibers passing through this level, resulting in mechanical stress. The vascular theory, on the other hand, asserts that the cause of these alterations is attributable to ischemia: when IOP exceeds the pressure of the arterial vessels, they are compressed, leading to inadequate perfusion of the optic nerve head; this is also associated with an imbalance in vascular autoregulation mechanisms.

A common point can be identified in both pathways: both mechanisms cause a reduction in axoplasmic transport, interfering with the supply of nutrients and removal of metabolic products, depriving nerve endings of neuronal growth factors.[3] Additionally, oxidative stress and immunomediated damage occur.

Retinal ganglion cells respond to these damage mechanisms by initiating apoptotic processes, explaining why, despite well-conducted hypotensive therapy, patients may still experience progressive damage.

As mentioned earlier, the laminar region is the primary site of insult. Biomechanically, the lamina cribrosa (LC) represents a locus minoris resistentiae: its thickness is approximately 1/3 of that of the sclera at the level of the scleral canal. This is because it is tasked with conflicting functions: on the one hand, it must provide structural support to the optic nerve (resisting mechanical deformation related to IOP and local deformation), and on the other hand, it must allow the exit of fibers. The LC is somewhat protected from mechanical tension by the circumpapillary ring of collagen and elastin fibers located in the immediate peripapillary sclera, which serves as reinforcement against excessive expansion of the scleral canal and the consequent inward or outward displacement of the LC. While advancement or retrocession is generally minimal, overall mechanical deformation in the LC can be quite substantial: even small expansion of the scleral canal will radially stretch the LC and impart significant tensile stress.

LC cells and astrocytes can detect this tension through integrin receptors, which directly link their cytoskeletons to the adjacent fibrillar extracellular matrix, making them highly sensitive to changes in local biomechanical tension.[4] It is interesting to note that pores located at the upper and lower poles appear larger, providing less structural support to axons running at this level, which may explain why these bundles are more susceptible to damage.

1.1.3. Glaucomatous Neuropathy

Glaucomatous optic neuropathy is characterized by the slow degeneration of retinal ganglion cells and their axons, associated with the progressive appearance of typical alterations involving the optic nerve head, peripapillary area, and neuroretina.

The optic nerve consists of over a million afferent nerve fibers, originating from the convergence of ganglion cell axons that form a 90° angle at the optic disc before exiting the eyeball. At its point of emergence, the retina and choroid abruptly cease; at this level, the lamina cribrosa is present a fibroelastic perforated diaphragm crossed by bundles of optic nerve fibers (which become myelinated from this point onward) and serves as their supportive tissue. The optic nerve head represents the intraocular portion of the nerve and is confined within the walls of the eyeball (1 mm); it has an oval shape, with a vertical diameter of about 1.5-1.7 mm, approximately 9% larger than the horizontal diameter.

Physiologically, the optic disc appears rosy with sharp margins and a more or less deep central excavation. Its unique anatomical structure, where nerve fibers bend at right angles and extend posteriorly through the scleral canal and lamina cribrosa pores towards the brain, makes it more vulnerable to pressure or ischemic stimuli. The most striking modification in glaucoma is the increase in the central excavation of the optic disc, a direct consequence of the progressive rarefaction of nerve fibers due to apoptotic phenomena.

To distinguish a glaucomatous eye from a normal one, the ratio between the central excavation and the optic disc (cup/disc ratio or C/D) is used. It is considered normal if around 1/3; if it exceeds 1/2, it is necessary to determine whether this is due to the presence of an anatomically large optic disc or if the ratio is genuinely increased. In the former case, it is an anatomical variant (distinguishable because the neuroretinal margin will be well-preserved); in the latter, a diagnosis of glaucoma can be made, even in the absence of visual field defects.

Moreover, with the progressive depletion of nerve fibers, lamina pores become more exposed, and in advanced stages, they can be clinically visualized. Another typical alteration involves the neuroretinal margin (the intrapapillary equivalent of retinal and optic nerve nerve fibers)[5]; physiologically, it appears clear and well-defined, with an orange-pink color. The neuroretinal margin correlates with the optic disc area: the larger the area, the thicker the margin. Similarly, as the size of the optic disc increases, the number of nerve fibers and the number and total area of lamina cribrosa pores increase proportionally. Due to high interindividual variability in the population, there is significant overlap between patients with early damage and normal individuals (even though the neuroretinal margin is not markedly altered in the latter). To increase diagnostic power, the disc can be divided into multiple sectors. Typically, the thickest margin is usually inferior, followed by superior and then nasal and temporal regions (ISNT rule: if the thickness follows the order indicated by the acronym, there is no glaucoma)[6]. Conversely, glaucomatous discs undergo a gradual irregular thinning of the margin and/or the appearance of notches. These

changes progressively affect different sectors of the neuroretinal margin depending on the stage of the disease: initially involving the inferotemporal and superotemporal disc regions, later, in intermediate stages, the most affected area is the horizontal temporal sector, and in very advanced glaucoma, there is thinning of the entire retinal margin. Typically, nasal quadrants are affected last. This sequence of papillary sectors (inferotemporal, superotemporal, horizontal temporal, nasal-inferior, nasal-superior) correlates with the progression of defects detectable in perimetry.[7]

As the disease worsens, vascular peduncle nasalization and bending of arterial vessels can be observed.[8] It is important to assess the emergence point of the central vascular trunk because the distance from it appears to influence axonal loss and seems to correlate, in part, with local susceptibility to neuroretinal margin thinning. The greater the distance, the more pronounced the reduction in thickness and, consequently, the scotoma appreciable in the corresponding quadrant on perimetry. Additionally, there is a widespread thinning of retinal vessels, a common alteration in other neuropathies.

The presence of optic disc hemorrhages (with "flame" or "splinter" morphology) is considered one of the most important factors related to glaucoma progression. Their frequency increases with the progression of the disease until the middle-advanced stage, then decreases in the very advanced stage. They are associated with localized defects in the nerve fiber layer, notches in the neuroretinal margin, and circumscribed perimetric loss. In

early forms, they are usually located in the inferior or superior temporal disc portions.[4,5]

Finally, the analysis of the retinal nerve fiber layer (RNFL) is crucial, as its alterations precede the development of optic disc and visual field changes. Normally, during fundus examination, eight regions where these fibers are visualized differently can be identified. They are more visible in the lower temporal sector, followed by the upper temporal, lower nasal, and finally upper nasal sectors; they are less appreciable in the upper, lower, horizontal temporal, and horizontal nasal sectors. Visibility decreases with age, correlating with fiber depletion related to aging (at birth, the optic nerve consists of approximately 1.4 million fibers, progressively lost at a rate of about 4,000-5,000 fibers per year). However, the physiological decrease in ganglion cells does not correlate with perimetric changes, as there is some reserve and overlap between the receptive fields of cells conveying stimuli from the same retinal area. The retinal nerve fiber layer can exhibit two types of defects: localized defects and diffuse defects (larger with indistinct borders). The former consist of wedge-shaped alterations directed towards the optic disc or in contact with its margin. In glaucoma, their frequency significantly increases from the early stage to the middle-advanced stage, then decreases in the very advanced stage, where individual defects are no longer distinguishable due to a pronounced reduction of nerve fibers in all disc sectors. They do not represent a specific sign, as they can also be found in cases of optic nerve atrophy due to other causes (such as drusen, chorioretinal scars from toxoplasma, ischemic retinopathies, and some optic neuritis). However, since they are almost never present in normal eyes, they are almost always an indicator of pathology. They predominantly affect the

lower temporal sector, followed by the upper temporal sector, while rarely appearing in the nasal regions, probably because the RNFL is less visible and thinner in these areas. A 40% loss of nerve fibers is required for the manifestation of the scotomatous area in the visual field. Therefore, a thorough analysis and careful evaluation of damage to these fibers can anticipate perimetric changes by years.[5]

1.1.4. Diagnosis

Glaucoma is associated with sometimes subtle symptoms and strong interindividual variability, leading to patients not seeking an ophthalmologist, resulting in a delay in diagnosis. While there is evidence that the progression of neuropathy can be preventable through the control of intraocular pressure (IOP), there are currently no means for functional recovery once the damage is established; it is irreversible.

Glaucoma has traditionally been defined by Von Graefe, who described its eponymous triad consisting of elevated intraocular pressure (>21 mmHg), optic nerve pathology, and visual field deficits. The diagnosis is typically made when these three abnormalities are present. However, subsequent studies have shown that nerve damage can precede the onset of functional deficits by years. It is now clear that, although elevated IOP is a major risk factor for glaucomatous neuropathy, it is not necessarily high in all eyes with this pathological alteration. Some authors argue that the presence of optic nerve pathology or typical visual field defects is sufficient for the diagnosis of open-angle glaucoma.[9,10]

Therefore, it is necessary to proceed with the assessment of the patient's overall clinical picture and the careful analysis of the ocular fundus with a slit lamp, integrating the information obtained with instrumental examinations to study the functional and structural defects precisely.
In particular, the following are utilized:
- measurement of intraocular pressure using tonometry
- evaluation of the optic nerve head through a fundus examination
- examination of the anatomy of the irido-sclero-corneal angle through gonioscopy
- functional damage with computerized or kinetic perimetry
- estimation of the state of nerve fibers and ganglion cells using tomography

1.1.4.1 Tonometry and Pachymetry

Tonometry estimates the pressure inside the eye. Various instruments exist, but the most commonly used is the Goldmann applanation tonometer, which calculates intraocular pressure (IOP) based on the mechanical force required to flatten a small circular area in the central cornea. It is important to note that corneal rigidity is influenced by its thickness and curvature, factors not considered by tonometers currently in use. To address this, these parameters can be obtained through other investigations, such as pachymetry for thickness and corneal topography using a Scheimpflug Camera for curvature.

1.1.4.2 Ocular Fundus

Examination of the ocular fundus allows visualization of the retina and optic nerve head. It is performed using a slit lamp. The following characteristics should be evaluated:

- Size, shape, and color of the optic disc
- Configuration of the disc margin
- Optic disc excavation
- Cup/disc ratio
- Visibility of the lamina cribrosa
- Vascular anomalies
- Peripapillary chorioretinal atrophy

1.1.4.3 Visual Field Study

From a clinical perspective, glaucomatous pathology is associated with the development of progressive visual field defects, which can be documented through computerized perimetry. This involves studying the perception of visible space by an immobile eye in the primary position. Since perimetric damage is always a consequence of damage to nerve fibers, it allows correlating visual deficit with the loss of ganglion cells. However, it should be considered that papillary and fiber defects precede perimetric alterations by 4-5 years.

The test is subjective and has significant individual variability, so it is recommended to perform it at least three times to obtain a realistic visual

field model. During the examination, the patient sits in front of the instrument monitor, fixing their gaze on a central point on the screen. A series of light signals are then sent, and the subject is asked to press a button whenever they see them. By recording the differential light sensitivity (the difference between the stimulus and the background) at various points in the visual field, perimetry allows testing retinal sensitivity at predetermined points. The data obtained are analyzed by specific software through a complex series of operations, and the results are provided both numerically and graphically.

Physiologically, a visual field appears round, of light gray color, extending for about 70° inferiorly, 50° superiorly, 90° temporally, and 60° nasally. There is a negative absolute scotomatous area between 10° and 20° temporally, known as Mariotte's blind spot, corresponding to the area of projection of the optic nerve head in the visual field.

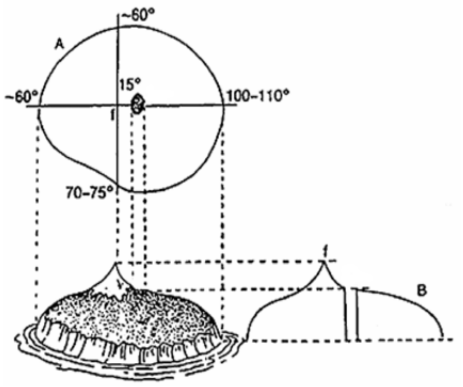


Figure 1: Traquair's Island

The apparatus can also compare patient data with those of the normal population, adjusting for age. Results are provided through differential

maps, indicating the variation from normal average for each point (negative values indicate below-average sensitivity, positive values above-average), and through indices such as:

- VFI (Visual Field Index), measuring the overall function of the patient's visual field as a percentage (the normal value adjusted for age is 100%)
- MD (Mean Deviation), providing an indication of the patient's overall sensitivity; values between -2 and +2 dB are considered normal
- PSD (Pattern Standard Deviation, for Humphrey perimeters), expressing variability within the visual field
- SF (short-term fluctuation), calculated by testing the threshold of certain points multiple times, indicating visual field instability; alterations may indicate poor concentration or fatigue and tend to increase with age and in glaucoma
- CPSD (Corrected Pattern Standard Deviation), similar to PSD but corrected for SF. [11]

Glaucomatous damage initially involves fibers from the temporal hemiretina, which, in an arcuate arrangement, extend toward the upper and lower poles of the optic disc. This is associated with the early appearance of paracentral visual field defects within 30°, predominantly nasally (the so-called *nasal step of Ronne*). These defects may progress and merge to form a single scotoma surrounding the fixation point, known as the *arcuate scotoma of Bjerrum*. Another possible initial sign is the deformation, typically vertical, of the blind spot, forming a slightly curved defect called the *Seidel scotoma*; however, changes in the blind spot should not be

considered a sign of glaucoma, as they are relatively common in myopic or elderly patients.

As the pathology progresses, scotomas widen, and the visual field narrows further, reaching a preterminal stage where only a very small central island of residual light perception remains, giving the impression of a tubularized visual field (second-to-last stage of Malbran). Visual acuity is fully preserved until shortly before blindness. Less typical of glaucomatous pathology are generalized defects, where threshold reduction is homogeneous (as in the case of cataracts); however, they can be associated with localized defects to constitute mixed defects, which are sometimes encountered.

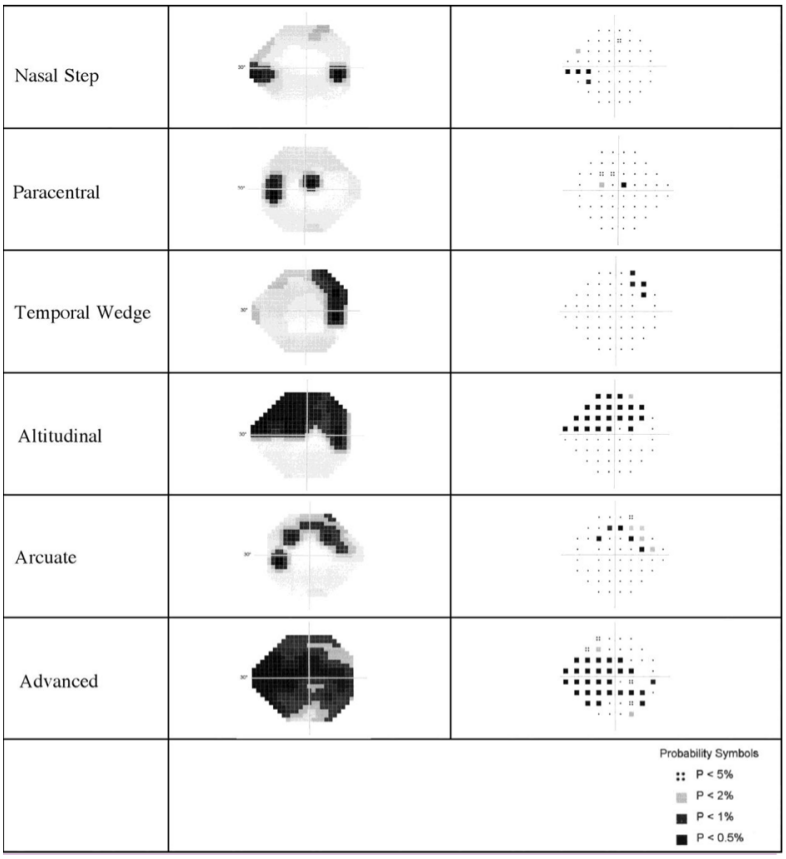


Figure 2: Patterns of glaucomatous visual field defects

The severity of functional damage can be assessed by observing indices such as MD and PSD. For the staging of the deficit, guidelines suggest using the Glaucoma Staging System 2 (GSS2), which utilizes MD and CPSD indices arranged on a Cartesian graph. The graph intervals decrease from left to right and from top to bottom to emphasize lower values, improving the definition of early defects. MD is a nonspecific index of damage, altered especially in the presence of generalized depressions or large and deep localized defects. Conversely, CPSD indicates the heterogeneity of the defect and is altered in the presence of localized defects. The intersection of the two values on the

graph simultaneously indicates the type of defect (generalized, top right; localized, bottom left; mixed, center) and its severity, divided into the following stages:

- Stage 0 = completely normal visual field
- Borderline stage = very mild defects, often statistically insignificant
- Stage 1 = mild defects (such as small paracentral relative scotomas)
- Stage 2 = moderate defects (such as nasal step, scotomas of limited extent, etc.)
- Stage 3 = confirmed defects (such as absolute arcuate scotomas)
- Stage 4 = highly advanced and absolute defects, involving at least 2 quadrants
- Stage 5 = sub-terminal defects, with residual sensitivity islands.

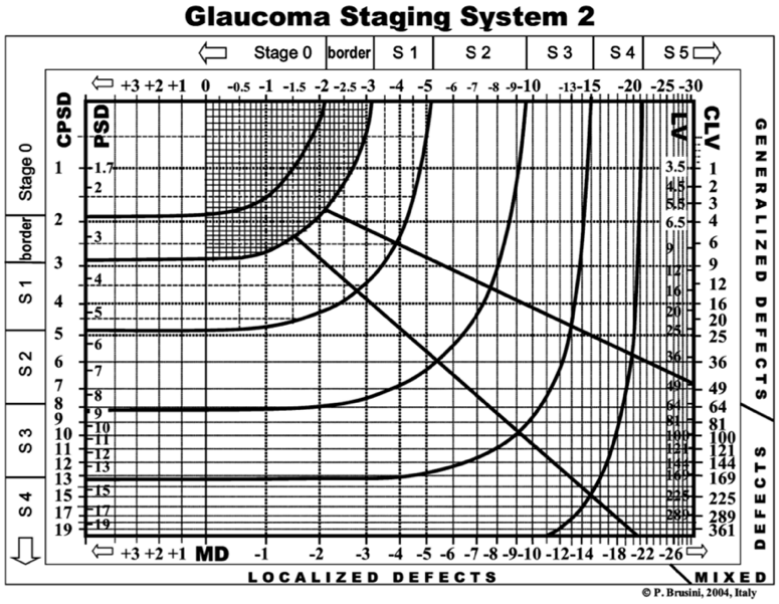


Figure 3: Glaucoma Staging System 2

The graph also includes PSD values, to be used if the corresponding corrected indices are not available or in case of high SF values (where CPSD equals 0).[12]

1.1.4.4 Optical coherence tomography (OCT)

The advent of optical coherence tomography (OCT) has revolutionized the ability to monitor the anatomic structures affected by glaucoma. OCT is a noninvasive imaging technique that relies on the use of Michelson interferometry to decode the interference patterns of light reflected from intraocular tissues. It allows quantitative measurement of the optic nerve, the retinal ganglion cell axon layer (known as the retinal nerve fiber layer), and the layer of the retinal ganglion cell bodies (Fig. 4). Structural changes such as retinal nerve fiber layer thinning typically occur before the

development of functional losses detectable by conventional visual field testing. [13]

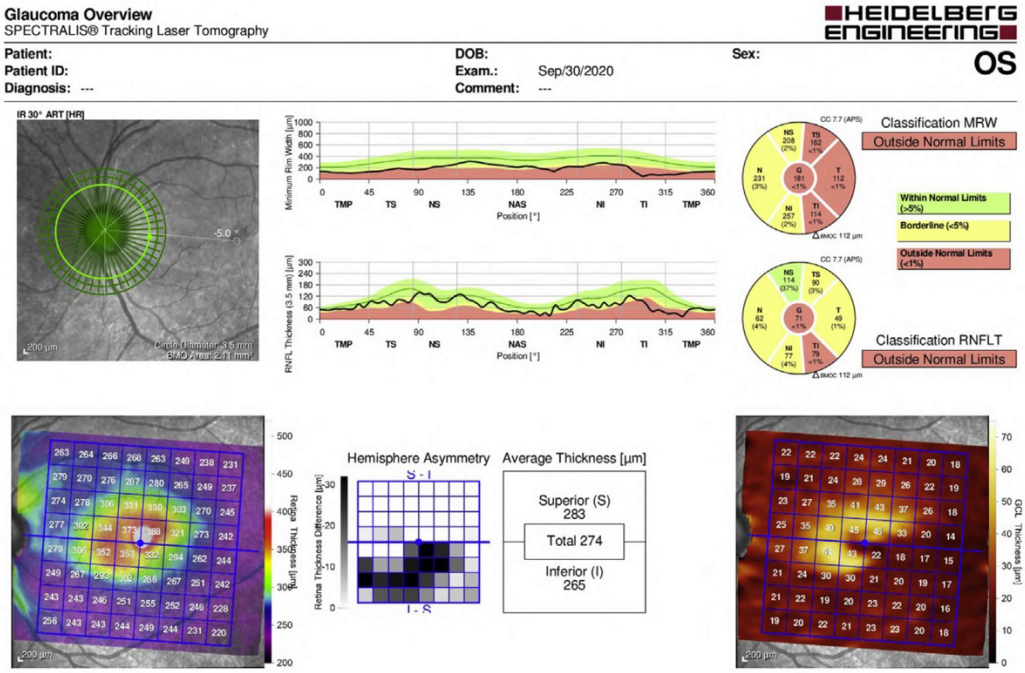


Figura 4: Peripapillar Retinal Nerve Fiber Layers OCT

1.2. Cognitive impairment

Dementia refers to a group of neurodegenerative conditions in which cognitive and/or behavioral symptoms interfere with an individual's ability to function, representing a decline from prior levels of functioning.[14] Alzheimer's disease, the predominant cause of dementia, results in cognitive and behavioral decline due to neuronal cell loss in the brain. Conversely, glaucoma, though primarily affecting the eyes, also shares characteristics of neurodegeneration, leading to optic nerve damage and visual field impairment due to retinal ganglion cell death. Despite their differing primary locations, both conditions exemplify the complexities of neurodegenerative processes and emphasize the need for comprehensive understanding and management approaches.[15]

1.2.1. Mini-Mental State Examination (MMSE)

The Mini-Mental Status Examination (MMSE) is a brief cognitive screening instrument frequently used to evaluate and monitor patients with Alzheimer's disease and other cognitive impairment. It was introduced in 1975 by Folstein, is consisting of eleven questions and evaluates six areas of cognitive function: orientation, attention, immediate recall, short-term recall, language, and the ability to follow simple verbal and written commands (Folstein et al., 1975).[16]

In a 2021 review, a table was presented wherein each cognitive domain evaluated with MMSE has corresponding brain areas responsible for that function, aiming to achieve a better understanding of the relationships

between cognitive function and the brain regions responsible for those specific functions.[17]

The MMSE total score ranged from 0–30, with lower scores indicating poorer cognitive ability. Patients with a score of ≤ 23 points were classified as having cognitive impairment[18,19]. Using the MMSE total score, any score ≥28 points indicated normal cognition, and scores <28 points indicated MCI (24–27 points) with moderate (10–23 points) or severe (≤9 points) cognitive impairment. A score of ≤9 points was considered to be almost diagnostic of dementia [18-20].

TEST / FUNCTION (EVALUATED BY MMSE)		CORRESPONDING BRAIN AREA	DEMENTIA DIFFERENTIAL
Orientation to time		Temporal, frontal	Impaired relatively late in the course of dementia
Orientation to place		Temporal, frontal	Impaired relatively late in the course of dementia
Orientation to person		SELF: medial prefrontal and posterior cingulate cortex OTHERS: prosopagnosia: Fusiform gyrus (*occipitotemporal gyrus) in temporal lobe*	Impaired very late in the course of dementia
Immediate recall (sec) impairment		Wernicke, Broca, Arcuate fasciculus	FTD>AD
Delayed recall (2-3 min) impairment		Hippocampus, medial temporal lobe (high density of NFT and NP) which disconnects hippocampus from cortex	AD>FTD AD-rapid rate of forgetting for 10 min.
Attention	Spelling	Prefrontal; Frontal dorsolateral Inferior parietal Cingulate gyrus	AD>FTD
	Calculation	Prefrontal; Frontal dorsolateral Left parietal Cingulate gyrus	AD>FTD>VASC
	Perseveration, inability to shift attention/tasks	Frontal lobe	FTD>AD
Language	Naming	Left temporal; parietal	VASC>AD>FTD
	Repetition	Wernicke, Broca, Fasciculus arcuatus	VASC>AD>FTD
	3-step command	Temporal; Frontal; Premotor	Variable
	Reading and comprehension	Left parietal Temporal	Variable
	Writing	Left parietal	Variable
Copy design (asymmetry, distortion, loss of gestalt)		Right parietal (construct, gestalt) Basal ganglia with projections to prefrontal cortex	DLB=DPD>VASC>AD>FTD VASC: Visuospatial impairment > then delayed recall (2-3 min)
Abstract thinking	Proverb interpretation Similarities Conceptualization	Frontal, prefrontal	FTD>AD
Trail making test A		Right-sided lesions Frontal; Parietal	FTD>AD
Trail making test B		Left-sided lesions Frontal; Parietal	FTD>AD
Verbal fluency		Frontal, prefrontal	FTD>AD
Right-left orientation		Left parietal	VASC >AD
Clock drawing test	Comprehension	Temporal lobe	AD>Vascular
	Planning, sequencing, organizing	Frontal lobe	
	Constructional ability, gestalt, spatial relationships, attention to the left side	Right (non-dominant) parietal	
	Praxis: ability to execute learned functions, writing	Left (dominant) parietal lobe	
	Visual processing	Occipital lobe	

Note: Table abbreviations: AD - Dementia of Alzheimer's type; FTD - Frontal-Temporal Dementia; VASC - Vascular Dementia; DLB - Lowy-Body Dementia; DPD - Parkinson's Disease related Dementia. Sign > means "more impaired"

Tabella 1 correspondence of cognitive functions evaluated by MMSE to specific brain areas. (Khachiyants, N ,2012)

1.2.2. Functional Magnetic Resonance Imaging (fRMI)

Functional magnetic resonance imaging (fMRI) has risen as the primary method for non-invasively examining brain function in humans. Despite its relatively recent inception in the early 1990s, fMRI now holds a pivotal role across various domains such as psychology, cognitive science, and neuroscience.

fMRI entails scrutinizing minute fluctuations in the blood oxygen level-dependent (BOLD) signal within the brain, either during task performance (task-based fMRI) or while at rest (resting state fMRI, RS-fMRI). This BOLD signal serves as an indirect marker of changes in neuronal activity, relying on the magnetic properties of hemoglobin. Oxygenated hemoglobin (oxy-Hb), being diamagnetic, minimally affects the MRI's magnetic field, while deoxygenated hemoglobin (deoxy-Hb), with paramagnetic attributes, alters the field proportionate to the oxygen released to neuronal cells. Consequently, heightened brain activity prompts increased cerebral blood flow (CBF) and glucose consumption, surpassing oxygen utilization. This leads to a reduction in deoxy-Hb in active regions, thereby elevating the BOLD signal. Conversely, decreased brain activity elicits the opposite effect. T2-weighted MRI sequences are employed to detect deoxy-Hb's paramagnetic impact.[21]

Functional connectivity (FC) within fMRI enables the assessment of synchronized neuronal activity between anatomically distant brain areas. FC involves examining concurrent fluctuations in the BOLD signal across

various brain regions, indicating functional connections. These fluctuations, occurring at very low frequencies (<0.1 Hz), have been observed throughout the brain.

RS-fMRI delves into synchronous activations among spatially distinct regions during rest, revealing *resting-state networks* (RSNs). Prominent RSNs encompass the default mode network (DMN), sensory-motor network, right and left lateral networks, salience network, ventral stream network, task-positive network, primary, medial, and lateral visual networks, as well as the auditory network.

Default mode network (DMN)

The DMN is a network of brain regions that are active when an individual is not focused on the external environment and instead is engaged in internal modes of cognition, such as daydreaming, remembering past experiences, envisioning the future, or considering the perspectives of others.[22]
Bancker et al., in 2008, synthesizes the main characteristics of these areas in order to emphasize the importance of the DMN in understanding brain function, both in normal cognition and in the context of mental disorders. The key word of this paper are:

anatomical Definition: the DMN is described as a specific, anatomically defined brain system that shows preferential activity during internally focused tasks;

functional roles: DMN is implicated in various cognitive functions, including autobiographical memory retrieval, envisioning the future, and understanding others' perspectives;

subsystem: DMN is understood to comprise multiple interacting subsystems, such as the medial temporal lobe subsystem, which provides information from past experiences, and the medial prefrontal subsystem, which aids in the flexible use of this information in constructing self-relevant mental simulations.

Integration Nodes: Important nodes of integration within the DMN include the posterior cingulate cortex, where the various subsystems converge.

Adaptive Roles: the DMN is proposed to play adaptive roles in using past experiences to plan, navigate social interactions, and optimize moments when individuals are not externally engaged.
The relevance of the DMN in understanding various mental disorders, such as autism, schizophrenia, and Alzheimer's disease, suggesting that dysfunctions in the DMN may contribute to the pathophysiology of these conditions.

This synthesis underscores the significance of the DMN in understanding the brain's functioning, both in normal cognition and in the context of mental disorders. It highlights the intricate interplay between brain regions involved in internal cognition and suggests avenues for further research into the neural basis of complex human behaviors and experiences.

REGION	ABREV	INCLUDED BRAIN AREAS
Ventral medial prefrontal cortex	vMPFC	24, 10 m/10 r/10 p, 32ac
Posterior cingulate/retosplenial cortex	PCC/Rsp	29/30, 23/31
Inferior parietal lobule	IPL	39, 40
Lateral temporal cortex†	LTC	21
Dorsal medial prefrontal cortex	dMPFC	24, 32ac, 10p, 9
Hippocampal formation††	HF+	Hippocampus proper,EC, PH

Tabella 2: Core regions associated with the brain's default network (Buckner RL, 2008)

2. Clinical research

Elevated intraocular pressure (IOP) is a well-established risk factor in the pathogenesis of glaucoma, and reducing IOP remains the primary treatment option for this condition. However, many glaucoma patients continue to experience vision loss despite successful medical or surgical interventions. Consequently, our efforts are dedicated to identifying new markers for controlling glaucoma progression.

In addition to the focus on identifying new markers for controlling glaucoma progression, there is growing interest in exploring potential biomarkers that may also indicate cognitive decline, such as alterations in the default mode network (DMN). Research has already demonstrated that the DMN, a brain network associated with introspection, self-referential thoughts, and memory consolidation, can be altered in primary open-angle glaucoma (POAG) patients. This raises intriguing questions regarding the potential interplay between glaucoma severity, neurocognitive deterioration, and DMN alterations. Therefore, our study aims to investigate the association between the severity of POAG, the degree of neurocognitive decline, and alterations in resting-state functional magnetic resonance imaging (RS-fMRI) patterns within the DMN.

2.1. Materials and methods

2.1.1. Study population

Thirty patients diagnosed with primary open-angle glaucoma (POAG) were enrolled in this study. They were recruited from Eye Clinic of Trieste between 2021 and 2022.

Inclusion Criteria

Participants were considered eligible for inclusion in the study if they met the following criteria:

- primary open-angle glaucoma, aged 18 years or older, and provided informed consent to participate in the study.

- Elevated Intraocular Pressure (IOP) > 21 mmHg or under treatment patients with intraocular pressure greater than 21 mmHg at the time of diagnosis

- Visual Field (VF) glaucomatous defects: evidence of glaucomatous defects in the visual field as assessed by automated perimetry.

- Anatomic optic disc glaucomatous damage: Presence of characteristic optic disc changes consistent with glaucomatous damage, such as cupping, notching, or thinning of the neuroretinal rim, as determined by ophthalmoscopic examination.

- Best Corrected Visual Acuity (BCVA) > 0.1 logMAR

- Clear Dioptric Media
- Adherence to Therapy

Exclusion Criteria

- Patients were excluded if they had any of the following conditions:
- secondary glaucoma
- history of ocular trauma or surgery
- significant comorbidities affecting cognitive function (such as dementia or Alzheimer's disease),
- contraindications for MRI scans

Data on demographic characteristics, clinical history, ophthalmologic examination findings, and neurocognitive assessments were collected for each participant.

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

2.1.2. Glaucoma state assesment

The ophthalmologic examination included measurement of the visual acuity using the Snellen Chart, refraction, biomicroscopic examination, gonioscopy, measurement of the intraocular pressure (IOP) via Goldman applanation tonometry, fundoscopy and visual field testing with Swedish Interactive Threshold Algorithm SITA strategy, program 24-2, on the

Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, a standard method for the examination of the 308 central visual field. In this type of measurement, the subject is facing a white illuminated sphere, on which points of light with varying intensities are briefly flashed. Subjects respond when they perceive the flash. The sensitivity at each location in the visual field is determined by changing the intensity of the flash on subsequent presentations. Each eye is measured independently so that one eye is covered while the other is tested. This test provides visual field indices such as: Mean Deviation (MD), providing an indication of the degree of the generalized loss in the visual field; Pattern Standard Deviation (PSD), which is a summary measure of the average deviation of individual visual field sensitivity values from the normal slope after correcting for any overall sensitivity differences.

Patients were stratified into two groups based on the MD

- *mild group*: patients with a MD higher than -12 dB
- *severe group*: patients with a MD equal to or less than -12 dB.

This stratification allows for the categorization of patients according to the severity of their visual field defects, facilitating comparative analyses between groups in subsequent analyses.

2.1.3. Cognitive assessment

2.1.3.1 Mini-Mental State Examination (MMSE)

The Mini-Mental State Examination (MMSE) is a widely used screening tool for cognitive impairment.

The MMSE was administered to each of the 30 patients individually by a trained healthcare professional. The examination consists of 11 questions, covering various cognitive domains, including orientation, memory, attention, calculation, language, and visuospatial skills. Each correct answer receives one point, with a maximum score of 30.
The time taken to complete the examination was recorded for each patient.

- Orientation (10 points): patients were asked about the current date (month, day, year), location (city, state, country), and current season.

- Registration 3 points: the examiner read three unrelated words, asking the patient to repeat them immediately. The patient received one point for each correctly repeated word.

- Attention and calculation (5 points): Patients were asked to count backward from 100 by sevens or spell "CARNE" backward. One point was awarded for each correct response.

- Recall (3 points): after a brief delay, patients were asked to recall the three words previously mentioned in the registration section. One point was awarded for each correctly recalled word.

- Language (9 points): this section included naming objects, repeating a phrase, following a three-step command, writing a sentence, and copying a complex figure.

- Visuospatial Skills (1 point): patients were asked to copy a specific geometric figure.

2.1.3.2 MRI data acquisition and data preprocessing

All participants in the study underwent MRI investigation using the same 3.0 T equipment (Ingenia, Philips Medical Systems, Best, The Netherlands). All participants were instructed to remain relaxed, with their eyes closed, trying to stay awake without falling asleep. All study participants underwent a high-resolution 3D T1-weighted anatomical sequence and a resting-state functional MRI (rs-fMRI) sequence. The parameters used for these sequences are as follows: for the high-resolution 3D T1-weighted sequence, TR=8.2 ms, TE=3.8 ms, flip angle=8°, FOV=256x256x180 mm, matrix=256x222, with a total of 180 slices and a duration of 05:47 minutes; resting-state functional imaging was obtained using a 2D T2*-weighted echo planar imaging (EPI) sequence with TR=2000 ms, TE=30 ms, flip angle=90°, FOV=224x224x112 mm, matrix=112x109, with a total of 25 slices and a duration of 07:26 minutes.

The rs-fMRI images were analyzed using Philips' "IntelliSpace Portal" image processing software version 9.0. For each subject, the T2* images were corrected for motion artifacts, realigned, and co-registered with structural images, specifically with the 3D T1 anatomical sequence.

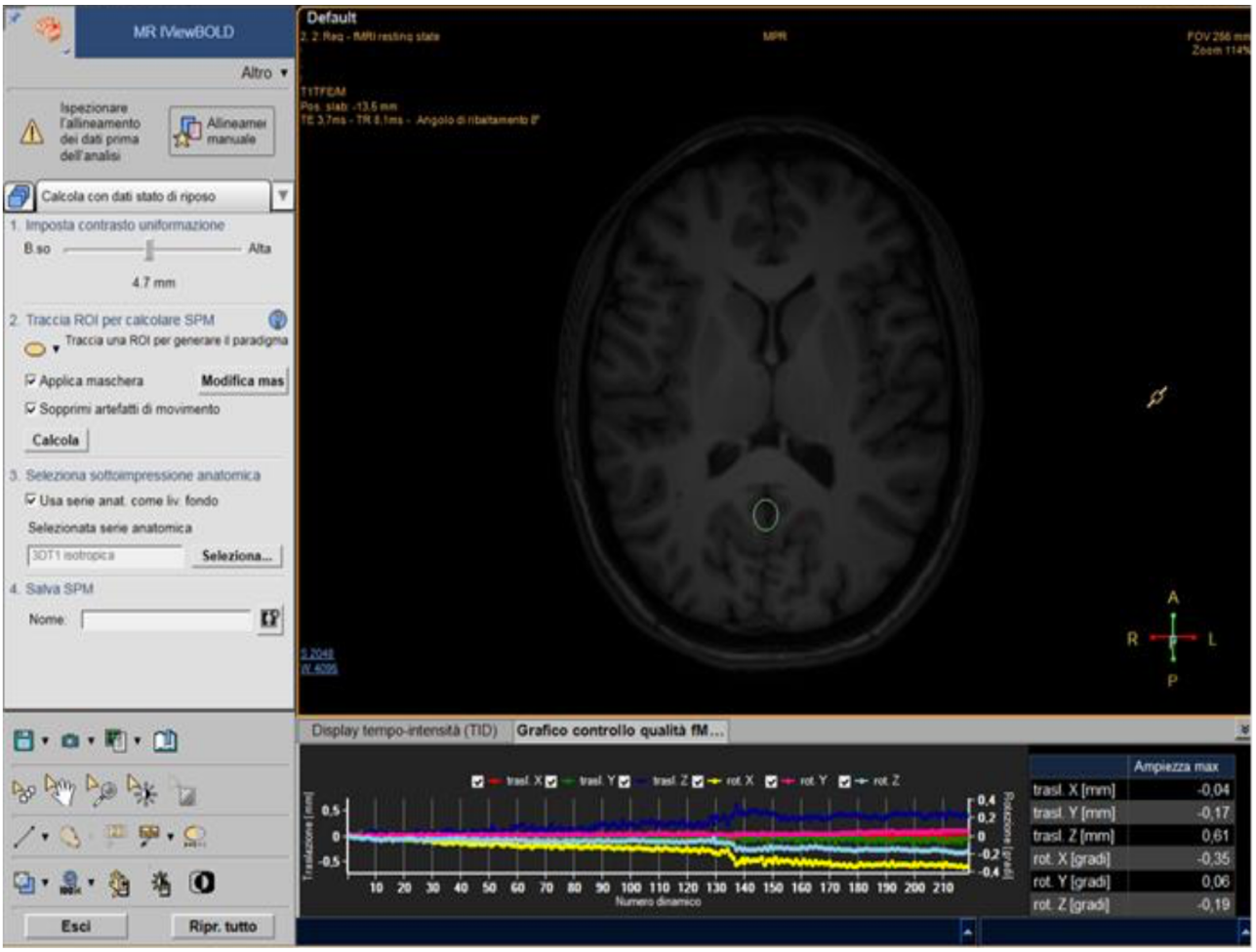


Figura 5:Screen interface of software Philips' "IntelliSpace Portal" image processing software version 9.0

To obtain the Default Mode Network (DMN), seed-based analysis was used, wherein a spherical ROI of approximately 8 mm in maximum diameter was placed in the posterior cingulate/precuneus region, a region known a priori to belong to the DMN. Once the DMN was generated, spherical ROIs were placed on the Functional Connectivity maps corresponding to the activation areas of the network, specifically in the posterior cingulate/precuneus, medial prefrontal cortex, right parietal cortex, and left parietal cortex. T-score values were then derived for each of these four regions. The t-score

indicates the statistical strength of activation of various components of functional activation, how synchronously the different activated areas oscillate. Higher or lower t-score values represent greater or lesser Functional Connectivity (FC) within the functional activation areas, particularly within the DMN.

2.1.5 Statistical analysis

The statistical analysis was performed using IBM SPSS (Statistical Package for Social Science) version 22.0. Results were reported as median and range for continuous variables, while absolute frequencies and percentages were used for categorical variables. The non-parametric Mann-Whitney test was employed for between-group analysis. Chi-square ($\chi 2$) test was u sed to determine if there is a significant relationship between two categorical variables. A significance level of $p<0.05$ was used. To assess the strength of the association between two events the odds ratio test was used.

2.2. Results

Overall 57 eyes screened, 30 eyes of 30 patients met the inclusion criteria and were included in the analysis.

Table 3 summarizes the demographic characteristics of the study population: 16 patients were males (53.3%) and 14 females (46.7%), with a mean age of 71.7 ± 5.3 years.

Table 3 Baseline demographic characteristics of the study cohort	
Characteristics	
Study cohort	30
Sex (male:female)	16:14
Mean age (y)	71.7 (SD, 5.3)
Mild subgroup analysis	
Sex (male:female)	5:7
Mean age (y)	69.4 (SD, 10.4)
Severe subgroup analysis	
Sex (male:female)	11:7
Mean age (y)	71.0 (SD, 9.7)

y: years; SD: standard deviation

According to MD evaluation, 12 patients were defined as *mild* glaucoma while 18 were *severe*. In the mild group, the mean age was 69.4 ± 10.4 years with 58.3% of them were male. The mean age of the 18 patients in severe group was 71 ± 9.7 years old, 61.1%, were male. *(table 3)*

Baseline characteristics of the two different subgroups were similar for age and gender (ANOVA $p>0.05$), constituting a homogenous study population.

The median visual field MD for the mild group was -6.3 decibels, with an interquartile range of -3.8 to -8.6 decibels. In contrast, for the severe group, the median visual field MD was significantly lower at -20.0 decibels, with an interquartile range spanning from -14.8 to -24.2 decibels. *(fig.6)*

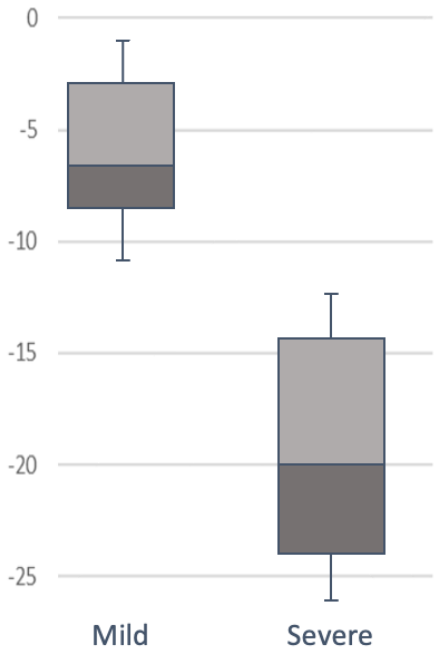


Figure 6 visual field MD in mild ande severe glaucoma group

In relation to the Mini-Mental State Examination (MMSE) the mean overall score was 27.56 ±1.12, with an average time taken to complete the examination of 7.3' ±1.51''. The overall most challenging domain for patients was attention specifically in the ability to repeat a word spelling backward. Patients, on the other hand, are all very well-oriented in time and space.

According to subgroup analysis, the MMSE score was 28.93 ±0.84 and 26.12 ±1.06 in mild and severe group, respectively. We assessed the prevalence of cognitive impairment, diagnosed when MMSE scores fell below 26. In the mild group, the prevalence was 16.1%, whereas in the

severe group, it rose to 32.9%, with a statistically significant difference between the two subgroup ($p<0.05$, $\chi 2$) test).

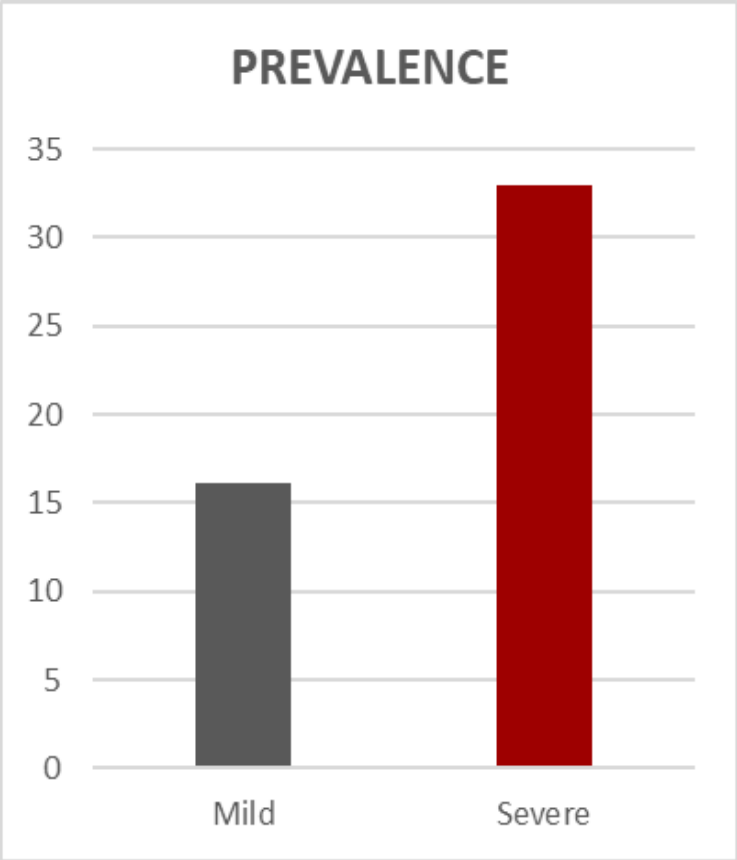
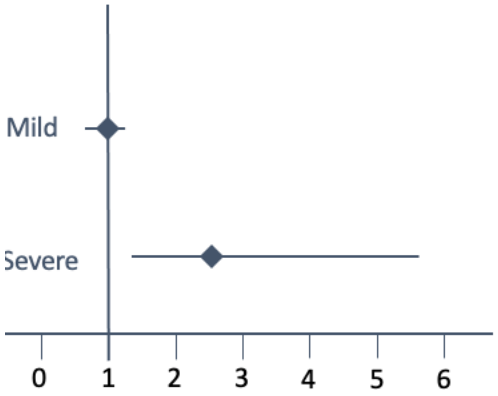


Figure 7: Prevalence of cognitive impairment in mild and severe glaucoma group

We conducted a univariable logistic regression analysis to examine a potential association between cognitive impairment in MMSE and glaucoma severity based on MD score.

For patients with mild glaucoma, no significant correlation was observed with an odds ratio of 1. In patients with severe glaucoma a substantial

statistically significant correlation with cognitive impairment was found, with an OR of 2.59 (FIG XX)



Analyzing the MMSE score of ≤ 26 with the univariable logistic regression analysis, we found that the severe glaucoma group had a significantly higher OR for cognitive impairment (OR, 2.68; 95% CI, 1.25-5.76; P=0.011).

In both study groups, severe and mild glaucoma group, utilizing seed-based analysis facilitated the visualization of the Default Mode Network (DMN), concurrently activating regions including the posterior cingulate/precuneus, medial prefrontal cortex, right parietal cortex, and left parietal cortex.

Table 4: Overall T-score Peack Voxels for different DMN analyzed area

Brain Regions	*t-score*
Posterior cingulate/precuneus	12,07 ± 0,41
Prefrontal cortex	7,77 ± 0,26
Left parietal cortex	7,70 ± 0,36
Left parietal cortex	8,11 ± 0,40

*All data are expresse as mean ± standard deviation

Table 5: T-score Peack Voxels for different DMN analyzed area in severe glaucoma subgroup

Brain Regions	*t-score*
Posterior cingulate/precuneus	9.63 ± 0.40
Prefrontal cortex	5.80 ± 0.30
Left parietal cortex	7.50 ± 0.27
Left parietal cortex	8.33 ± 0.37

*All data are expresse as mean ± standard deviation

Table 6: T-score for different DMN analyzed area in mild glaucoma subgroup

Brain Regions	*t-score*
Posterior cingulate/precuneus	13.90 ± 0.43
Prefrontal cortex	9.25 ± 0.23
Left parietal cortex	7.85 ± 0.43
Left parietal cortex	7.95 ± 0.43

*All data are expresse as mean ± standard deviation

A direct comparison between patients with mild and severe glaucoma group demonstrated a consistently lower T-score values across all DMN-activated regions. Specifically, a statistically significant diminished activation was notably observed in the posterior cingulate/precuneus and medial prefrontal cortex, ($p < 0.5$) [Table 5].

Conversely, no significant disparities in T-score values were discerned between the two groups in either the right parietal cortex ($p=0.85$) or the left parietal cortex ($p=0.71$).

2.3. Discussion

Elevated intraocular pressure (IOP) is a well-established risk factor in the pathogenesis of glaucoma, and reducing IOP remains the primary treatment option for this condition. However, despite successful medical or surgical interventions, many glaucoma patients continue to experience vision loss. Therefore, there is a pressing need to identify new markers for controlling glaucoma progression. In addition to traditional markers, there is a growing interest in exploring potential biomarkers that may indicate cognitive decline, such as alterations in the default mode network (DMN).

While its impact on vision is widely recognized, growing evidence suggests a potential association between glaucoma and cognitive decline. This raises critical questions about the overall management of patients with glaucoma and underscores the importance of early identification of cognitive decline in its early stages. Our study aimed to investigate the association between the severity of primary open-angle glaucoma (POAG), the degree of neurocognitive decline, and alterations in resting-state functional magnetic resonance imaging (RS-fMRI) patterns within the DMN.

The study population comprised thirty patients diagnosed with primary open-angle glaucoma (POAG) recruited from the Eye Clinic of Trieste between 2021 and 2022.

The glaucoma state assessment involved ophthalmologic examinations including measurement of visual acuity, biomicroscopic examination, gonioscopy, IOP measurement, fundoscopy, and visual field testing. Patients were stratified into mild and severe groups based on the degree of visual

field defects. Cognitive state assessment utilized the Mini-Mental State Examination to evaluate cognitive function, with scores indicating cognitive impairment if below 26.

The administration of the MMSE to glaucoma patients provides valuable insights into their cognitive function beyond visual impairment. The results of this study revealed a significant association between the severity of glaucoma and cognitive impairment, with the severe glaucoma group showing a higher prevalence of cognitive impairment compared to the mild glaucoma.

Understanding cognitive changes in glaucoma patients can facilitate early intervention and tailored management strategies to optimize patient care.

MRI data acquisition involved high-resolution 3D T1-weighted anatomical sequences and resting-state functional MRI (rs-fMRI) sequences. Seed-based analysis was employed to obtain the Default Mode Network (DMN), with spherical ROIs placed on functional connectivity maps corresponding to DMN activation areas.

Individuals with severe glaucoma displayed consistently lower T-score values across a specific DMN-activated regions compared to those with mild glaucoma, indicating diminished activation particularly in the posterior cingulate/precuneus and medial prefrontal cortex in our study. Functional MRI at resting-state has been pivotal in revealing structural and functional alterations in the brains of glaucoma patients.[23-26]

Despite the importance of our research in exploring correlations among DMN alterations and pathology severity, the limited sample size may hinder broader generalization. Furthermore, MRI scan complexities for both staff

and patients reduce repeatability and clinical applicability. Conversely, widespread MMSE administration could aid early cognitive decline detection, enhancing comprehensive management beyond ophthalmological concerns. Additionally, it may predict patient adherence to therapy, crucial given their management of the only modifiable risk factor, intraocular pressure.

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