Title of study

MODULATION OF NEURONAL CONNETTIVITY ALONG THE VISUAL PATHWAYS IN PATIENTS AFFECTED BY GLAUCOMA THROUG TREATMENT WITH CITICOLINE ORAL SOLUTION: MULTIMODAL MORPHO-FUNCIONAL STUDY.

GENERAL INDEX

GENERAL INFORMATIONS

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1. INTRODUCTION

1.1 Basic Information

Open angle glaucoma (OAG) is a chronic neurodegenerative disease characterized by the progressive loss of retinal ganglion cells and structural changes of the optic nerve head (1). It is the second leading cause of blindness in the world. The most common form, primary open-angle glaucoma, has as its main risk factor the increase in intraocular pressure for which the first therapeutic approach is represented by hypotonizing topical drugs. However, given that more than one third of patients under good blood pressure control delay, but do not stop the progression of visual impairment, other non-pressure dependent mechanisms are thought to be involved (2). In particular, following the primary insult, of a hyperbaric nature, apoptosis of the neuron is triggered, which interferes with the normal blood supply at the level of the capillary district of this structure, and the regular axonal transport is compromised, both antegrade and retrograde, of metabolites and neurotrophins essential for the survival of the ganglion cell (3). Then follows the secondary insult linked to the mechanisms of local excitation toxicity due to the hyperstimulation of NMDA receptors by the glutamate released by the cells in apoptosis (4). In fact, when present in excessive concentrations in the extracellular space, glutamate hyperstimulates the NMDA receptors on the surface of the surrounding neurons, which determine the opening of channels for Ca ++. The overflow of Ca ++ ions into the cell represents the trigger of the biochemical cascade that will lead to apoptosis of the neuron itself, configuring a mechanism capable of self-feeding even in the absence of the primary insult (5). Another key step in the mechanism of cell damage during apoptosis is represented by the hyperactivation of phospholipase A2, an enzyme capable of destabilizing and disrupting the cell membrane through the catabolism of its main constituent, the phospholipid phosphatidyl-choline (6).

Glaucomatous visual impairment can be assessed through various computerized perimetry techniques such as standard achromatic perimetry and Fundus Automated Perimetry (7).

The morphological abnormalities of the retinal ganglion cells (RGCs) that give rise to the optic nerve and the optic nerve fibers (RNFL) can be detected through the use of Optical Coherence Tomography (OCT) (8).

Current functional evaluation techniques allow to quantify in a specific and differentiated way the dysfunctions of the various elements that form the optical pathway. In particular, through the Pattern electroretinogram (PERG) or the PhNR it is possible to quantify the functionality of the RGCs, while the nerve conduction along the optical pathways is objectivable through the recording of the Visual Evoked Potentials (VEP). Through the simultaneous recording of PERG and PEV it is possible to derive an electrophysiological index of post-retinal nerve conduction dysfunction: Retino-Cortical Time (RCT) (9).

By evaluating the RCT, it was hypothesized that in glaucoma the perimetric deficit is the result of two types of dysfunction: one at the level of the RGCs and the other at the level of the post-retinal optic pathway (10). The second dysfunction would be due to a reduction in nerve input from the RGCs on the cells of the Lateral Geniculate Nucleus (NGL) with a consequent reduction in bioelectric activity at the level of the occipital cerebral cortex (10).

Therafore mentioned post-retinal dysfunctions were supported by scientific evidence obtained thanks to the use of modern neuro-imaging techniques.

In fact, in recent years, neuro-imaging studies have shown in glaucoma patients a neuronal loss in the various structures that form the optical pathways and in particular at the level of the NGL (11-14), of the optic tract (12) and of the visual cortex. (13-16). Not only the structure, but also the functional connectivity of cortical neurocognitive networks is altered in glaucomatous patients, and

this aspect is not exclusive to visual networks, but also affects attentional and executive ones (17-18). This is to underline that a dysfunction of the visual system inevitably affects several other cognitive areas of the person.

In the current state of knowledge, it is not known whether in glaucomatous disease there is a relationship between electrophysiologically detected post-retinal nerve conduction dysfunction (10) and structural and functional changes in the NGL, optic tract or visual cortex (11- 16) and to what extent both the functional and structural changes of the post-retinal optical pathways are related to the morpho-functional modifications of the RGCs.

Since 1998, various studies have shown that in glaucoma patients treatment with Citicoline is able to induce a neuropotentiation action with a consequent improvement in the functionality of RGCs (19-22) and a neuroprotection action with a consequent reduction in loss of RGCs and their nerve fibers (23, 24).

Furthermore, based on a neuromodulatory action, it was observed that treatment with citicoline administered intramuscularly or in oral suspension for a period of 2 months induced an improvement in nerve conduction along the post-retinal optic pathways indicated by a reduction of the RCT (19, 22).

Such multifactorial mechanisms of action may explain the reduction in the progression of perimetric glaucomatous deficit (24, 25).

In the current state of knowledge, it does not appear to have been studied whether the improvement in RCT detected after treatment with citicoline administered intramuscularly or in oral suspension (19, 22) can also be obtained with treatment with citicoline administered in oral solution.

Furthermore, it does not appear to have been evaluated whether there are structural and functional correlates [detectable with magnetic resonance methods in the various structures that form the optical pathways and in particular at the level of the NGL (11-14), of the optic tract (12) and of the cortex visual (13-16)] with the well-documented improvements in nerve conduction along the optical pathways obtained in glaucoma patients after treatment with Citicoline (19-22).

1.2 Citicoline mechanism of action

From the data available in the literature, it is known that the exogenous administration of citicoline causes an increase in endogenous phosphatidylcholine synthesis and thus accelerates the repair of the previously damaged cell membranes (26).

The cell membrane dysfunction or degeneration induces the release of cytotoxic substances (lyticenzymes, excitotoxic amino acids, etc.) which induce an extension of the area of damage or activate the mechanisms that lead to apotosis (for example, an increase in the concentration of intracellular ceramide, a compound produced by catabolism of sphingolipids, is a potent proapoptotic agent).

In particular, in the etiology of vascular diseases (for example in stroke) a loss of phospholipid components is found resulting from an alteration in their metabolism, which leads to irreversible damage to the cell membranes of neurons. Biochemically a loss of phosphatidylcholine is observed, which is degraded during the ischemic processes into fatty acids with the production of reactive oxygen species (ROS).

Experimental studies suggest that the administration of citicoline induces a reactivation of the anabolism of the phospholipids with reduced degradation of phosphatidylcholine and thus reduced

formation of ROS. In some animal models a reduction was also observed of the infarcted area volume after treatment with Citicoline.

Therefore the therapeutic approach with citicoline allows the maintaining and/or restoring of the integrity of the cell membranes of neurons subjected to ischemic insults, such as can occur in some of the many forms of glaucomatous syndrome.

1.3. Neuro-functional considerations on treatment with Citicoline systemically and safety and tolerability in humans

The fundamental element for cell function and survival is the ability to maintain an internal environment different to the external one. This function is ensured by the cell membrane, a discriminating barrier that selects and regulates the passage of the substances inside the cell and controls the difference in electrical potential.

Also, most of the biochemical reactions occur close to the cell membrane and in its context surface receptors are located allowing a dialogue of the cell with the surrounding cells and the rest of the organism.

In fact, the cell membrane:

· is the site of receptors and synapses that govern communications with neighbouring cells and the rest of the organism,

· maintains and controls the difference in electrical potential, which is necessary to allow propagation of the nerve impulse,

· handles most enzymatic reactions, at the mitochondrial level, the inner membrane is the site of the main energy production processes.

A lesion of the cell membrane leads to three important events:

1. some ions normally concentrated in the extracellular environment, in particular Ca^{++},and other potentially harmful molecules, can enter uncontrollably into the cytoplasm, altering the vital processes of the cell
2. the ability to communicate through surface receptors is lost;
3. the intracytoplasmic content, rich in lytic enzymes and toxic mediators, can flow outside of the cell resulting in an extension of the damage to the contiguous cells. Therefore, an alteration of the phospholipids turnover compromises the validity of the membrane protection systems. Furthermore, as a result of activating the particular lytic enzymes, the phospholipases, the catabolism of the membrane phospholipids is accelerated and, if the resynthesis mechanism is insufficient, toxic molecules accumulate, such as ceramide, which can activate the metabolic pathways that lead to programmed cell death (apoptosis).To maintain the perfect functionality of the cells, therefore, the cell membrane needs to stay structurally intact. This is absolutely necessary in the case of cells without replicative capacity like the RGC, the loss of which results in irreversible functional impairment.

The cell membrane is composed of a double layer of phospholipids. Phospholipids are continuously exposed to chemical and physical insults which damage the molecular structure: to protect itself from this, the cell possesses an enzyme system that catabolises the damaged phospholipids and reuses the constituent elements (phosphocholine, glycerol and fatty acids), in order to synthesise them again.

An alteration of the phospholipids turnover compromises the validity of the membrane protection systems and puts the specific cell functionality at risk. In the case of peripheral neurons such as RGC, the peculiar length of their axonal endings makes these cells particularly sensitive to any alteration in the cell membrane protection systems. In fact, precisely by virtue of the enormous

development of the membrane surfaces, due to the length of their axons, these cells are more susceptible than others to the harmful effects caused by a phospholipid synthesis slowdown.

The clinical conditions where a slowdown of the phospholipid metabolism occurs are those that present when the neural tissue undergoes a type of traumatic, ischemic or degenerative process. In glaucoma and in NAION a pathological situation is configurated in which one or more of these 3 elements can contribute to the development of damage to the optic nerve. Both glaucoma and NAION are neurotticopathies where alterations of the retinal structures are observed (22, 27) and retinal structures (28).

An improvement of the VA, the VEP and contrast sensitivity after treatment with Citicoline was also observed in partially sighted patients (29-31). These studies suggested that the effects observed were attributable to the activity of dopamine-like effect of Citicoline (32, 33).

This pharmacological property, together with three other fundamental properties of this molecule [Neuroenhancement, neuroprotection and neurorestoration (34)] could explain the improvement of the morpho-functional condition of RGCs (35, 36) and of nerve conduction along the optical pathways (VEP with reduction of latency times) observed in glaucoma patients (19-22, 37).

In addition to the evaluation of retinal function, post-retinal nerve conduction was examined in glaucoma patients by measuring the RCT (38). The reduction in RCT, observed after treatment with Citicoline (19), can be attributed to an improvement in retinal function with consequent improvement of nerve conduction along the optical pathways and relative increase in bioelectric activity in those cells in which the cortical potential has its origin.

In addition to inducing an improvement in retinal bioelectric activity, Citicoline also produces neuroprotective effects, understood as a reduction in the progression of nerve fiber loss. This emerges from studies carried out in patients with ischemic optic neuritis treated with citicoline in oral solution (23) or from the results of a randomized clinical trial in which glaucoma patients were treated for 3 years with eye drops containing citicoline (24).

Therefore, these effects of Citicoline on the morpho-functional condition of RGCs and on the improvement of post-retinal nerve conduction, may explain the improvement of the visual field observed both in the first pioneering studies (20, 24, 25, 39, 40) and in studies. more recent where improvement in perimetric indices (Mean Deviation-MD of Humphrey perimetry) are significantly correlated with an increase in PERG and VEP parameters (20, 41), it is likely that the previously mentioned sources of cortical improvement may also be suggested. to explain the improvement.

In conclusion, everything reported suggests that Citicoline has a significant action in improving retinal and cortical bioelectric responses and in reducing the loss of peripillary nerve fibers in patients suffering from glaucomatous or ischemic optic nerve pathologies with consequent decrease in progression of perimeter damage.

To further advantage of this therapy, it should be emphasized that in no clinical study carried out in humans (19, 23, 25, 30, 31, 37, 42) are reported significant adverse reactions both ocular and systemic.

1.4 Rationale of the study

Considering that:

1) the visual field loss in glaucoma patients is attributable to a dysfunction of the RGCs associated with post-retinal nerve conduction delay (10),

2) the neuroradiological evaluations have highlighted changes in the nervous structures (NGL, optic tract, visual cortex) that form the optical pathways (11-18),

3) Citicoline possesses neuroenhancement and neuroprotection properties (34-36), with consequent improvement of nerve conduction along the optic pathways, reduction of the rate of progression of glaucomatous damage and maintenance of peripapillary nerve fibers (36),

we think that these assumptions constitute the rationale for carrying out a randomized clinical study that evaluates the effects of citicoline oral solution on the nerve conduction along the post-retinal optic pathways in glaucoma patients (information obtained through the registration of PERGs and VEPs and the calculation of the RCT) and if these effects have a morphological and functional correlation on the nervous structures that form the optical pathways (NGL, optic tract, visual cortex) (information obtained through the acquisition of structural and functional magnetic resonance).

These acquisitions will allow us to assess how much post-retinal nerve conduction and the structural integrity of the optical pathways are influenced by changes in the morpho-functional state of the RGCs.

2. OBJECTIVES AND AIMS OF THE STUDY

1) Primary Objective: to evaluate whether treatment with **Citicoline in oral solution** can produce an improvement of the post-retinal nerve conduction, altered base in patients with OAG.
2) Secondary objective: to evaluate in patients with OAG whether the possible changes in post-retinal nerve conduction induced by treatment with Citicoline in oral solution (information obtained through electrophysiological recordings) are associated or not with **morphological and functional variations of the nervous structures that form the optical pathways** (NGL, optic tract, visual cortex, information obtained through the acquisition of structural and functional magnetic resonance imaging) and whether both conditions can be related to the morpho-functional variations of the RGCs and of the visual field (CV).

3. PARTICIPATING SITES

- IRCCS - Fondazione Bietti, Rome
- Department of Sensory Organs, Sapienza University of Rome,
- Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, Rome

4. STUDY POPULATION

Sixty patients affected by bilateral OAG, between the ages of 20 and 70, will be enrolled in the stydy within a maximum time frame of 12 months (see point 9.1 "sample size").

4.1 Inclusion criteria:

- Age between 20 and 70 years old
- Diagnosis of glaucoma. Glaucoma is defined as: glaucomatous damage of the CV (Humphrey 24-2 standard SITA with mean deviation between -6 and -25 dB) and glaucomatous appearance of the optic nerve
- Visual acuity not less than 5/10

- Eye pressure below 21 mmHg with the use of ocular hypotonizing drugs including sympathomimetics, beta-blockers, prostaglandins, beta-adrenergics, carbonic anhydrase inhibitors. Such drugs can be used both alone and in combination with each other.
- Documented post-retinal nerve conduction delay through simultaneous recording of VEP and PERG showing an increase in RCT

4.2 Exclusion criteria:

- Ocular surgery in the 3 months preceding the study, including surgery for cataracts in the previous three months.
- Cataract or maculopathy
- Argon laser trabeculoplasty (ALT) within the previous 6 months
- Known hypersensitivity to the study product
- Secondary causes of ocular hypertension, including systemic or topical use of steroids
- Positive history of ocular or systemic diseases that could preclude enrollment in the study in the opinion of the investigators
- Changes in systemic therapies that could compromise intraocular pressure values (beta-blockers, alpha and beta adrenergics, calcium inhibitors, ACE inhibitors) in the 30 days prior to enrollment
- Ongoing therapy with vasoactive cerebral drugs, neurotrophic, lutein, zeaxanthin, retinal, acid, docosahexaenoic, Ubiquinone and / or its derivatives, Citicoline and / or its derivatives (possible previous treatment with Ubiquinone, L-Carnitine, Citicoline and / or its derivatives must have been suspended at least 6 months prior to inclusion in the study)
- Pregnancy, breastfeeding
- Diabetes
- Systemic lupus erythematosus, rheumatoid arthritis, connectivitis
- Concomitant use of anticoagulants and lithium

5. EXPERIMENTAL DESIGN

5.1 Experimental plan

A prospective, multicentre, randomized, blinded, masked study that involves the enrollment of a minimum of 60 (see sample size in paragraph 9 "Statistical Analysis") affected by OAG.
Patients selected according to the inclusion / exclusion criteria, after signing the informed consent, will be randomized into two groups:

a) In a group of patients with OAG, **Citicoline in oral solution (10 ml / day, Neurotidine®)** will be administered for 12 months (Citicoline Treated Group, TC Group)

b) in another group of patients with OAG will be **administered Placebo** (Containing all excipients of Neurotidine ®) (10 ml / day) for 12 months (Placebo Treated Group, TP Group)

Randomization will be done by dividing the selected patients into two groups based on similar characteristics of: age, perimetric defect and, mainly, RCT values.

Patients will be assigned to each group by an investigator not involved in functional and structural

testing.

The key will be opened only at the end of the treatment in order to evaluate the first effects.

5.2 Schematic diagram of the experimental plan

The schematic diagram of the study is shown below.

	Screening	**Baseline**		
Visit	**T0**	**T1**	**T2**	**T3**
Months		**± 7-15 days after T0**	**6 months after T0**	**12 months after T0**
Time window			**± 10 days**	**± 10 days**
Informed consent	✔			
Demographic data	✔	✔		
Medical records	✔	✔		
Visual Field	✔	✔	✔	✔
PERG	✔	✔	✔	✔
PEV	✔	✔	✔	✔
OCT	✔	✔	✔	✔
Concomitant diseases	✔	✔	✔	✔
Previous treatments	✔	✔		
Concomitant treatments	✔	✔	✔	✔
Complete Ophthalmological examination: visual acuity anterior and posterior biomicroscopy ophthalmoscopy intraocular pressure	✔	✔	✔	✔
Magnetic Resonance		✔		✔
Inclusion/exclusion	✔	✔	✔	✔
Randomization		✔		
Compliance			✔	✔
Delivery of food Supplement or Placebo		✔	✔	
Incidents			✔	✔

5.3 Trial term and visits

The study will last a total of 24 months, of which 12 for the selection and enrollment of patients and 12 months of follow-up from the last patient enrolled. During this period, patients will undergo 4 visits:
• T0 screening visit
• baseline visit T1
• visit at 6 months T2
• visit at 12 months T3

5.4 Planned visits

The visits will be performed at T0 (screening), at T1 (baseline, 7-15 days after T0), after 6 months (T2) and after 12 months (T3) of follow-up not knowing which group the subject belongs to.

After screening (T0), enrolled patients will perform a baseline visit (T1) during which they will be randomized to one of the two groups. The visits will take place 6 and 12 months after the baseline visit.
A complete eye examination will be performed at T0, T1, T2 and T3 including the following assessments: corrected visual acuity, biomicroscopy, indirect ophthalmoscopy, intraocular pressure measurement with Goldmann applanation tonometer, CV test, recording of PERGs and VEPs, imaging of the optic nerve and retinal nerve fiber layer by OCT. Neuroradiological evaluation will also be performed at T1 and T3.

At the end of the T1 visit, the patient will be given 4 bottles containing 500 ml Neurotidine ® or Placebo and will be given the relative instructions for administration. The regimen will be 1 administration of 10 ml once a day for 6 months. Each bottle can be used for 50 days. At the end of the 6 month period the patient will be asked to hand over the used bottles.

After 6 months ± 10 days (T2), patients will be given an additional 4 bottles containing Neurotidine ® or Placebo and will be given instructions for their administration. The regimen will be 1 administration of 10 ml once a day for 6 months. Each bottle can be used for 50 days. Again at the end of this 6 month period the patient will be asked to hand over the used bottles.

5.5 Outcomes

The nerve conduction along the post-retinal optical pathways will be determined through the evaluation of the RCT through the simultaneous recording of PERG and PEV, the function of the RCGs will be evaluated through the recording of the PERG, the morphological evaluation of the RGCs will be carried out through the OCT evaluations and the morphological and functional evaluation of the nerve structures that form the optical pathways will be performed by means of Cerebral Magnetic Resonance (MRI).

The primary objective is the assessment of changes in the RCT in patients treated with citicoline in oral solution compared to changes in the RCT assessed in patients treated with placebo.
The secondary objectives will be to evaluate whether the changes in RCT in patients treated with Citicoline in oral solution are associated or not with **morphological and functional changes in the nerve structures that form the optical pathways** (NGL, optic tract, visual cortex) and if both conditions may be related to the morpho-functional variations of the RGCs and of the visual field.

5.5.1. Psychophysical Evaluations

a) Visual Field (VF)

Static perimetry will be performed for VF measurements (Humphrey, 24-2 and 10-2 SITA Standard Program, with fixation losses, false positive and false negative rates, which should be <20%). The main indices of Humphrey perimetry are: Mean Deviation (MD) and Pattern Standard Deviation (PSD) and Mean Sensitivity (MS). The MD establishes the average of the defect obtained in all the points tested, considers the increasing dispersion of sensitivity values with respect to the data obtained in normal subjects based on the eccentricities and therefore can represent an index of the severity of the global damage. PSD indicates the homogeneity of the distribution of the visual field defect and thus provides information on localized damage. MS is the average of the sensitivity values measured at each probed point.

5.5.2. Electrophysiological Evaluations

a) Pattern electroretinogram (PERG)

The PERG shows the bioelectric activity of the innermost layers of the retina (ganglion cells, their fibres). PERG response is characterised by a series of waves with three successive peaks, with polarity negative, positive and negative respectively. In normal people, these peaks have the following implicit times: 35, 50 and 95 msec (N35, P50 and N95). An increase in the N35, P50 and N95 times and a decrease in amplitude N35-P50 and P50-N95 was found in glaucoma patients. These abnormalities in PERG are significantly related to visual field defects and to a reduction in the thickness of the layer of nerve fibres.
Also, PERG has shown a high clinical capacity (sensitivity: 100%, specificity: 100%) to detect malfunctions of the ganglion cells in glaucoma patients (43).

b) Visual evoked potentials (PEV)

VEPs reflect the bioelectric activity of the visual cortex in response to visual stimulation and thus evaluate the function of the visual pathway as a whole. VEP response is characterised by a series of waves with three successive peaks, with polarity negative, positive and negative respectively. In normal people, these peaks have the following times: 75, 100 and 145 msec (N75, P100 and N145). An increase in the N75, P100 and N145 times and a decrease in amplitude N75- P100 and P100-N145 was found in glaucoma patients. These VEP abnormalities are significantly correlated with visual field defects. Also, VEP has shown a high clinical capacity (sensitivity: 100%, specificity: 100%) to detect a delay in the neural conduction along the visual pathways in patients with glaucoma (43).

Comparing VEP and PERG times allows for the obtaining a neural conduction index in the postretinal visual pathways. In fact, the difference between the VEP P100 and P50 times is known as "Retino-cortical Time (RCT)".

5.5.3. Morphological Evaluation

Optical coherence tomography (OCT)

OCT is a non-invasive imaging technique designed to quantitatively evaluate, in cross section, the macular retinal layers, the peripapillary retinal nerve fiber layer, where the ganglion cell axons reside, and the optic nerve head, in vivo. The most recent generation of OCT available on the market is equipped with Spectral Domain (SD-OCT) technology, characterized by higher axial resolution

and faster scanning speed than the previous Time Domain technology, leading to less susceptibility to artifacts eye movement and improved reproducibility of measurements.
Furthermore, with SD-OCT it is possible to obtain the segmentation of the internal retinal layers (ganglion cell layer and internal plexiform layer) from the total macular thickness, thus measuring the layers where the cell bodies and the dendrites of the ganglion cells reside, primarily affected by glaucomatous disease.

5.5.4. Neuroradiological Evaluation

MRI with a diffusion tensor allows you to study the diffusivity of water molecules along the various directions of the axes, in order to calculate the fractional diffusivity, the average, axial and radial diffusivity. With this acquisition technique we will evaluate the microstructural integrity of the thalami in its entirety and of the lateral geniculate ganglion. Furthermore, we will evaluate the structural integrity of the optical sections.

Voxel-based morphometry allows us to analyze the volume of cortical gray matter, white matter and CSF. This technique will be used to verify the presence of variations in neuronal density in the various cortical areas, linked or not to the visual system.

Functional MR at rest is an MRI technique that allows to study the low frequency oscillations of the BOLD signal of the cortical gray related to the "rest" task. The functional assumption is that if the BOLD signal of a certain number of cortical areas fluctuates coherently with each other, then they form a functional resting-state network (RSN). We will use this acquisition technique in order to study the connectivity strength of the various RSNs linked or not to the visual system. In addition, we will use a "seed-based" approach, using the thalamus in its entirety and the NGL as areas of interest in order to verify their functional connectivity with the various cortical networks.

5.6 Efficacy parameters

1) For the evaluation of the RCT, the difference of the latency time P100 of the PEV and the latency time P50 of the PERG will be considered.

2) For the assessment of the visual field (HFA 24-2 and 10-2), the following will be considered: MS, MD and PSD.

3) The following parameters will be recorded for each SD-OCT imaging session:
• the average thickness of the layer of the peripapillary retinal nerve fibers and the thickness divided by sectors.
• the average thickness of the neural rim and the thickness divided by sectors
• the average thickness of the ganglion cell layer and the internal plexiform layer (GCL / IPL) and the thickness divided by sectors

4) For each diffusion-weighted MRI acquisition, the following parameters of the thalami and LGN and of the optical tracts will be acquired: fractional anisotropy (FA), average diffusivity (MD, axial (AD) and radial (RD).
For each acquisition of morphometry based on the voxel, the neuronal density of all cortical areas of the entire brain will be acquired.
For functional MRI at rest, the connectivity strength (Z-score) of all the independent cortical networks (linked to vision and not, independently) will be analyzed, as well as the degree of functional

connectivity between the thalami and the LGN and RSNs detected in the previous independent analysis.

6. ASSIGNMENT AND MASKING

Patients with OAG will be selected from a large cohort of already known diseases and followed at our facility for at least 12 months.
Only patients who meet the inclusion criteria will be selected. Patients will undergo a baseline screening visit during which conditions corresponding to the inclusion criteria will be assessed and the study will be proposed.
Only after the signing of the informed consent, a single investigator ("enrolling physician"), not involved in the clinical and instrumental evaluations, will decide whether to assign the selected patient to one of two groups: treated-Citicoline or treated-Placebo.
The assignment to one or the other group will take place in sequential order of presentation of the patient (with a 1: 1 probability of joining one or the other group) in order to constitute two homogeneous groups in number.
The aforementioned assignment will not be known to the patient and will not be known to the "enrolling physician".
Consequently, during the entire duration of the study (both at the baseline visit and at the 6 and 12 month visit), all evaluations foreseen by the study will be carried out by investigators who will not know whether the patient being examined belongs to the Citicoline-treated group or treated-Placebo.

7. TREATMENT OF SUBJECTS

The active form of the food for special medical purposes under study will be produced in accordance with the Good Manufacturing Standards, GMP (Good Manufacturing Practice) and labeled according to the applicable regulatory provisions.

7.1 Dosage, posology and route of administration

During the randomized treatment period, patients will take:

a) Neurotidine ® at a dosage of 10 ml / day.

b) Placebo at a dosage of 10 ml / day.

7.2 Composition of the treatment in use

The food intended for special medical purposes Neurotidine Ofta® under study is produced by Omikron Italia, srl.
Each bottle of Neurotidine will contain:
water; fructose; Citicoline (500 mg per 10 ml); acidity regulators: sodium citrate, sodium hydroxide; preservative: potassium sorbate; dye: riboflavine.

Each bottle of Placebo, the solution of which will be indistinguishable from the active product in terms of appearance and flavor, will contain:
water; fructose; acidity regulators: sodium citrate, sodium hydroxide; preservative: potassium sorbate; dye: riboflavin.

7.3 Packing of integrator supplement

The samples of Neurotidine® or of Placebo required for the conducting of the study will be provided free of charge by Omikron Italy.

7.4 Preservation method for Nurotidine® or Placebo

The Investigator must store the samples of Neurotidine® or of Placebo at appropriate temperature and humidity conditions as indicated on the label, in the Institution's internal Pharmacy or in his/her department in a locked room, accessible only to persons authorised to withdraw the samples.

7.5 Drug accountability

The Investigator and the Pharmacy will be responsible for receiving, storing and consigning the food supplement and the Placebo to patients; the Investigator will keep an inventory of the amounts stored and of those returned by individual patients.

7.6 Preservation of materials

The vials of Neurotidine® or of Placebo will be sent to the Pharmacy of the Study Centre and must be stored at the Pharmacy of the Study Centre in a locked area, with access limited to the personnel
involved only, out of reach of children, at appropriate temperature and humidity conditions and kept in the boxes provided until used to protect it from exposure to light.

7.7 Concomitant Pharmacological Treatments

Information on concomitant pharmacological treatments includes information on the treatments
administered at the time of being included in the study (recorded in the "Ongoing systemic therapies" section of the CRF).
Any pharmacological treatment taken for diseases unrelated to that of the study and considered necessary for the patient's health which does not interfere with the food supplement of the study, may be administered as deemed fit by the Investigator and the GP.
All treatments should be recorded in the CRF, indicating the dosage and date of administration as required.
In addition, if, due to the onset of symptoms and/or signs after commencing administration of the food supplement, the administration of any type of medication should become necessary, this should be reported in the "Ongoing systemic therapies" section of the CRF.

7.8 Admitted Therapies

During the experimental treatment period, any medications considered essential for the treatment of any concomitant diseases may be administered.
Any changes in dosage and/or new concomitant therapies will only be introduced if strictly necessary, and must be reported on the Patient's Medical Record and the study CRF.

7.9 Not-admitted therapies

The administration of medications that may interfere with the assessment of oral Citicoline supplements or which could alter the evaluation of efficacy parameters, and in particular medications with potential effects on retinal or macular function or nerve conduction along the

optic pathways, such as:

- cerebral vasoactives
- neurotrophics
- lutein, zeaxanthin, retinals
- docosahexaenoic acid
- ubiquinone and/or its derivatives, may not be administered.

Any previous treatment with Ubiquinone, L-Carnitine, Citicoline and/or its derivatives in other formulations and/or dosages must have been discontinued at least 6 months prior to inclusion in the study.

7.10 Compliance with treatment

The importance of regularly assuming what is assigned by the "recruiting physician" (Neurotidine® or Placebo) must be emphasized by the Investigator to the patient, who, based on the signature of the informed consent, must also be instructed to return, during the follow-up visits following the delivery date, according to the timing of the study, the unused and / or surplus samples (bottles).
In this way, the Investigator will be able to fill in, both during the control visits and at the end of the trial, the section of the CRF in which compliance with the prescription is recorded, calculate the compliance with the treatment and provide for the conservation of the material returned in a place accessible only to authorized persons.
At T2 and T3 compliance will be assessed by counting the quantity of product returned by the patient because it is not used and calculating the ratio between the number of quantities taken and the quantity that should have been taken according to the dosage schedule provided by the protocol.
Based on compliance, all patients who have taken at least 80% of the prescribed dosage regimen will be considered compliant with the treatment.

7.11 Suspension/interruption of treatment

The food supplement may be discontinued spontaneously by the patient at any time he/she deems appropriate or upon decision of the Investigator (the patient will be considered “drop-out”) should his/her clinical conditions require. Any discontinuation must be fully documented in the CRF by the Investigator.
Treatment may be discontinued prematurely for the following reasons:

· refusal of the patient to continue treatment;
· the occurrence of an Incident that may interfere with the patient's assessment or cause the continuation of the study to be considered inappropriate;
· logistical variations that make it impossible for the patient to participate in the study.

It will be the Investigator's responsibility to follow-up patients after discontinuation for an appropriate period of time (30 days) to assess their clinical conditions and/or verify the occurrence of Incidents even after the end of the trial therapy.
It is also recommended that these patients carry out the check-ups envisaged for the end of the study, since they will equally be considered for the intention-to-treat analysis.
For each of the patients who drops out of the trial, all available documentation will however be collected, where clinically possible, until they leave the study.

8. ASSESSMENT OF SAFETY AND TOLERABILITY

8.1 Safety and Tolerability

Neurotidine®, used in the present study, is a Citicoline-based food supplement. The tolerability of Citicoline by the human body is well known since it is an endogenous substance, and has so far been administered intravenously, intramuscularly, orally and topically (26). No adverse events attributable to the administration of such Citicoline-based supplement have been reported to date.
The only warnings are not to take Neurotidine® during pregnancy and lactation, as the possible effects on the fetus and/or newborn are not fully known.
For this reason, pregnant or breast-feeding women cannot participate in the study; at the same time, women of childbearing age must agree to the use of adequate contraception methods throughout the duration of treatment with Citicoline envisaged by the study; for the safety of patients it should be emphasised that scientifically accepted birth control methods do not provide absolute protection: some women have become pregnant even with the regular use of these types of methods. However, despite our experience and that of others (19, 23, 25, 30, 31, 37, 40) leading us to assume that no patient should suffer adverse events, throughout the duration of the study, particular attention will be paid to patients' descriptions of their general conditions (e.g. increase/decrease in systemic blood pressure, gastric pyrosis, skin rashes, etc.) The safety and tolerability of the food supplement will be evaluated at the end of the study,analysing all the information collected.
All this information will be recorded on the appropriate CRF, at the times and in the manner described in the dedicated sections of this protocol.

8.2 Incidents or Near-Incidents

Clinical tolerability to treatment will be assessed by recording the occurrence of incidents or near-Incidents reported by the Patient or observed by the Investigator. This registration will be made from the moment the patient signs the informed consent.
All Incidents occurring during the clinical trial must be reported on the CRF.

8.2.1 Definitions

a) Incident

Also taking into account the European guideline on vigilance "incident" means the condition in which any malfunction or deterioration in the characteristics and/or performance, as well as any inadequacy in the labelling or the instructions for use of a food supplement has led, directly or indirectly, to the death of a patient or user or to a serious deterioration in their state of health. Serious deterioration of state of health means: a life-threatening illness or injury; an impairment of a bodily function or injury to a body structure; a condition that requires medical or surgical intervention to

prevent an impairment of a bodily function or injury to a body structure; a condition that causes hospitalization or the extension of hospitalization.

b) Near-Incident

A near-incident means: (a) the condition in which any malfunction or deterioration in the characteristics or performance, as well as any inadequacy in the labelling or instructions for use of a food supplement, could have caused, directly or indirectly, if the food supplement had been used, a serious deterioration of the state of health or death of the patient or user;
(b) the condition in which any malfunction or deterioration in the characteristics or performance, as well as any inadequacy in the labelling or instructions for use of a food supplement, could have caused, during use or as a result of use, a serious deterioration in the state of health or death of the patient or a user, had the health care professional not intervened.

8.2.2 Incident or near-incident reporting

Investigators involved in the clinical trial are required to complete the standard section for gathering information on Incidents or near-Incidents in the CRF of the study.

a) Methods of communicating the Incident or Near-Incident

Upon the occurrence of an incident or near-incident, the Investigator must complete the CRF page for reporting all Incidents and near-incidents and the appropriate form (Incident or near-incident report by health care professionals to the Ministry of Health) relative to the reporting of Incidents caused by taking food supplements.
This Form, accompanied by a duly compiled submission form, must be sent by fax (064424800) within 24 hours of knowledge of the incident to the Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation, which will directly inform the persons responsible for monitoring medical devices of the Ministry of Health.

b) Further Investigations

The Investigator and the other persons responsible for Patient health must conduct any further investigation of Incidents or Near-Incidents, based on their clinical judgement of the likely causal factors.

c) Regulatory aspects

The Bietti-IRCCS Foundation is responsible for reporting to the competent Health Authorities any information relating to the safety of its medications. It is therefore essential for the Investigator to promptly report any Incident or Near-Incident, in order to allow it to comply with suchrequirements. These responsibilities are accepted by the Investigator as per the conditions set out in this protocol.

d) Instructions for Compiling the Forms Provided

For the compilation of the CRF page as well as the incident or near-incident report by health care professionals to the Ministry of Health, the Investigator should refer to the instructions provided to him/her together with such forms.

8.2.3 Overdose

Cases of overdose (accidental or intentional) leading to Incidents or near- incidents should be managed according to emergency procedures. This includes reports of food supplement intake for suicidal purposes with consequent overdose of such food supplement. Reports of overdose not associated with incidents should also be reported to the **Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation,** (although not requiring notification to Regulatory Authorities), in order to obtain information on symptoms, corrective treatment and the outcome of such an overdose.

9. STATISTICAL ANALYSIS

9.1 Sample size

As already highlighted, the main measure on which we relied for the calculation of the sample size is the RCT.

Our aim is to evaluate the value of the RCT parameter in the group treated with Neurotidine®, compared to that of the group treated with placebo.

We consider the following RCT values: at baseline 70.21 ± 9.12 ms and at the end of treatment 63.3 ± 8.15 ms (19), with an α error of 5% and a power of 80%.

We obtain 27 participants to which we add a drop-out equal to 10%, so the final value is 30 for each group.

9.2 General methodology

The data of each group, obtained from the PEV, PERG, CV, OCT and RM measurements will be represented by their mean and standard deviation, after having verified their normal distribution by means of the Anderson-Darling and Kolmogorov-Smirnov tests.
For each group, any variations in the measurements indicated above will be evaluated by means of a t-test for paired data.

Furthermore, the parameters of the electrophysiological, morphological and MRI measurements related to the two groups will be compared pre and post treatment, by means of ANOVA with correction of multiple comparisons based on Tukey's method.
The functional electrophysiological parameters (PEV and PERG) will be correlated by Pearson's test with the morphological OCT ones.

Finally, the possible relationship between the PEV, PERG and functional RM parameters of connection of the networks will be assessed using Pearson's test.
This analysis will be repeated respectively between the PEV and PERG parameters and the RM parameters in diffusion for each area indicated in paragraph 5.6.
The p-value of less than 5% is considered significant for any previously exposed test.

10. EXPECTED RESULTS AND NEUROFUNCTIONAL CONSIDERATIONS

10.1 Expected results and their impact in clinical practice.

> As for the **primary objective, the expected result is that Citicoline in oral solutionion can improve post-retinal nerve conduction**.

As regards the **secondary objectives**, the expected result consists in the possibility of detecting that any improvements in post-retinal nerve conduction after treatment with Citicoline in oral solution may or may not be associated with changes in the structure and function of the nerve stations that form the pathways. optical (assessed by MRI) depending on or independently of the morpho-functional condition of the RGCs and of the CV.

10.2 Neurofunctional considerations

Whatever the result obtained, the neurodegeneration model we use (OAG), will be able to provide important information on the neuroenhancement and neuroprotection potential of Citicoline, ie on the therapeutic opportunity to increase the morpho-functional condition of the optic pathways. These results may open up new neurophysiopathological considerations regarding the degenerative processes that may be present, with different etiologies, in glaucomatous pathology.

11. DIRECT ACCESS TO ORIGINAL DOCUMENTS

The Investigator/Institution must allow national and foreign Regulatory Authorities and personnel designated by the Independent Ethics Committee direct access, and verification thereof, to all original study documentation, including Informed Consent forms signed by the subjects in the study or their Legally Recognized Representatives and hospital records and/or outpatient records. Those who have direct access to such documentation must take all reasonable precautions to keep the identity of the subjects confidential in compliance with applicable regulatory provisions.

12. QUALITY CONTROL AND ASSURANCE PROCEDURES

12.1 Data collection or Case Report Form

The Case Report Form (CRF) is the hard copy document that is managed by the Investigator Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation.

a) Presentation of the CRF

The CRF consists of numbered pages which show in sequence the information required at each visit/assessment of the Patient.

Each individual page of the CRF will be identified by the centre no., Patient initials, Patient screening number, randomization number, trial time and date of the visit.

b) Instructions for compiling the CRF

The CRF must be compiled in Italian, with legible handwriting, in capital letters, using a black ink ballpoint pen. Each individual page must be compiled immediately after the assessments to which it refers, and must be signed by the Responsible Investigator or other qualified person duly authorized, immediately after checking the data transcribed. CRFs should remain available for monitoring and for any audit and/or inspection visits.

If a determination is not available, the corresponding space for its registration must not be left blank, but the wording NA (“Not Available") must be reported; in the case of tests not performed, the corresponding space for registration must not be left blank, but the wording NP ("Not Performed") must be written.

Any correction must be made cancelling the wrong data by crossing it out and entering the correct data near it. Deleted or corrected data should not be erased, masked or corrected, but should remain legible. The correction made must be initialled and dated by the Investigator or by a qualified person designated by him, possibly justifying with a comment, if necessary, the appropriateness of the same. The copy of each page will remain at the Study Centre as documentation of the clinical case. It will be the responsibility of the Investigator to report on the appropriate form the normal ranges of laboratory parameters provided by the analysis laboratory.

12.2 Clinical Monitoring

The clinical monitoring activities of the study will be entrusted to the **Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation,** Monitoring activities will be conducted in accordance with the Guidelines of the European Union of Good Clinical Practice adopted by Ministerial Decree of 15 July 1997.

12.3 Audits

The Investigator/Institution must allow audits to be carried out as an integral part of the quality assurance system. The Audit is an independent inspection, separate from the monitoring of the study activities and documents to verify whether the relevant study activities have been conducted and whether the data has been recorded, analysed and transmitted in accordance with the protocol, the GCP, SOPs and applicable regulatory provisions.

12.4 Inspections

The Investigator/Institution must allow national and foreign Regulatory Authorities to carry out Inspections.
The Inspection, by one or more Regulatory Authorities, shall consist of the official review of documents, facilities, records and any other resources considered by such authorities as related to the clinical trial.

13. CLINICAL DATA MANAGEMENT

The management of clinical data is entrusted to the **Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation.**
All statistical analyses will be carried out by personnel of the Bietti Foundation using the SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

14. ETHICS CONSIDERATIONS

All the parties involved in the study agree and will make sure that this trial is conducted in accordance with the ethical principles deriving from the Declaration of Helsinki, Good Clinical Practice (GCP) guidelines and applicable regulatory provisions.

14.1 Ethical Authorisations

A clinical study may only be started after written approval from the Independent Ethics Committee (IEC), which the Study Centre reports to. The Investigator must then receive the positive opinion of the IEC of the facility where the trial will be conducted before starting to recruit subjects for the study. The Investigator must provide the IEC with all the documents required for the approval application.
A copy of the IEC approval must be available at the Scientific Directorate of the Bietti Foundation before commencing the study.This study must be submitted for the authorisation of the Local Regulatory and Health Authorities in accordance with the rules and laws currently in force. The study may not commence without
first obtaining a copy of the study authorisation document from the Local Regulatory and Health

Authorities, as required by the applicable regulatory provisions. This documentation must be available prior to providing the Citicoline supplement samples for the study and starting recruitment.

14.2 Informed Consent

Before commencing the study, the Written Information and the Informed Consent form to be provided to subjects must be submitted for review and approval by the Independent Ethics Committee, together with the protocol.
Informed Consent must be requested, obtained and documented by the Investigator in compliance with applicable regulatory provisions, GCP and ethical principles deriving from the Helsinki Declaration.
For details on the procedure for requesting and obtaining Informed Consent, please refer to ICHGCP E6, paragraphs 4.3.3, 4.3.4, 4.8.2, 4.8.3, 4.8.4, 4.8.5, 4.8.6, 4.8.7, 4.8.8, 4.8.11, 8.3.2, 8.3.11, and the procedure for requesting Informed Consent in special situations the following paragraphs also 4.8.9, 4.8.12, 4.8.13, 4.8.14, 4.8.15.

15. ADMINISTRATIVE PROCEDURES

15.1 Changes in conducting the study or planned analysis

Any change in the conducting of the study is defined as an "Amendment to the Clinical Protocol": this term means any change that is made to the experimental protocol after the final approval of the Investigator.
Amendments to the Protocol are amendments to a document which has legal weight and which as such must be approved and signed in duplicate in original by the signatories to the protocol.
All amendments must be submitted to the Ethics Committee which approved the study protocol before they can be applied.
It is the responsibility of the Investigator to submit the amendment to the relevant Independent Ethics Committee and obtain an approval document.
Written approval will be distributed and filed in the manner provided for by the study protocol.
In the event that the change concerns only changes to administrative or logistical aspects of the trial, the resulting amendment must simply be notified to the Ethics Committee.
Finally, it should be noted that where the amendment substantially alters the design of the study or the potential risks to which the Patient is exposed, each Patient must be informed and must confirm in writing his/her willingness to continue the study. A specific information and consent form will be prepared by the Investigator and approved by the Ethics Committee.

15.2 Suspension /interruption of the study

15.2.1 Complete suspension of the study

The Bietti Foundation may decide to suspend/discontinue the study in progress at the Study Centre if:

- new toxicological, pharmacological or clinical information make the rationale and experimental design of the study unacceptable;
- the rate of recruitment of Patients proves ineffective;
- the Centre does not comply with the specific requirements of the study protocol, especially

regarding the assessment of the inclusion/exclusion criteria of the Patients;
• the Centre is unable to comply with the requirements of the Good Clinical Practice guidelines (GCP-ICH, Ministerial Decree no. 122 of 28/05/98);
• the Centre is not able to apply the regulations in force.

In this case, the Bietti Foundation shall promptly inform the Investigator / Institution and Regulatory Authorities of the premature discontinuation of the study, justifying the decision taken The Investigator must also inform the relevant Ethics Committee of the Institution to which the Study Centre belongs, justifying the premature discontinuation of the study.

15.2.2 Suspension of the study by the Ethics Committee

The study may also be discontinued by the Ethics Committee of the health care facility which the Investigator of the Study Centre reports to.

15.3 Archiving

The Investigator/Institution must file the essential study documents as specified by the GCP and in accordance with applicable regulatory provisions. The Investigator/Institution must take the necessary measures to prevent accidental or premature destruction of the same. The Investigator/Institution shall keep specific essential documents for at least 2 years.

15.4 Use of information and publication of results

The Investigator acknowledges that all information not made public regarding the study product(indication, patents, chemical formula, synthesis and formulation processes, experimental data or other information) is the property of: **Unit of Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation: Scientific Director: Dr. Vincenzo Parisi** and are strictly confidential. The Investigator may only use this information for the purpose of performing the research. If the Investigator intends to disclose, even partially, the results of the study, without prejudice to reports of Incidents or near-incidents provided for by current legislation on Pharmacovigilance, s/he must notify the Coordinating Centre (Bietti-IRCCS Foundation) in advance, which must respond to the Investigator within two months from the date of receipt of the publication request.

The Coordinating Centre (Bietti-IRCCS Foundation), in agreement with the Scientific Directors of all the Hospital departments involved in the project, will use the data deriving from the clinical study in connection with the development of the constituents of the food supplement and, therefore, may transmit this information, if necessary, to other Investigators and to the competent Authorities.

15.5 Liability insurance cover

This study is covered by a valid insurance policy and third party liability, stipulated by the Coordinating Centre (Fondazione Bietti), which has undertaken to pay the sums due from the Policy holder, as civilly liable by law, as compensation (capital, interest, expenses) for any type of damage caused by medicinal products, registered or not, administered in hospitals, nursing homes and by health care professionals for clinical trials, as well as for damages caused as a result of administration for pharmacology research and trials with medications and compounds already registered, but with a dosage different from that indicated by the Manufacturers, or with medications in the study phase, as well as for all activities related or connected with such trials,such as the administration of medications and taking of blood samples from the subjects participating in the study. This guarantee is also valid:
- For liabilities which may derive to the Investigators, as a result of the trials carried out on request and/or on behalf of the Insured;

- For any liability for which the Insured is called upon to respond by law, regulation, internal rules, customs, or uses.
The following shall be excluded:
- Impairment of health or deterioration of the state of health which would have occurred even in the absence of the trial;
- Genetic impairment, consisting of DNA damage, specifically related to damage to somatocytes and germ cells (egg cells, sperm cells);
- Impairment of health resulting from voluntary non-compliance with prescriptions/instructions;
- Impairment related to the AIDS virus: HIV, all AIDS syndromes, those related to HIV ARC viruses, as well as all the consequences attributable to feared or suspected infection.

15.6 Trial financing

The promoter of the study is the Bietti Foundation IRCCS. This study is partially financially supported by an unconditional contribution from the company OMIKRON ITALIA (see attached contract) which will also provide the food supplement and placebo free of charge.

16. INVESTIGATOR'S RESPONSIBILITIES

The Investigator is aware that s/he is responsible for all actions delegated by him/her to the other members of his/her staff designated to conduct the study. Except where specifically required, the term "Investigator" used in this protocol and on the Case Report Forms refers to the Investigator or a qualified person designated by her/him who may thus perform activities related to the clinical trial and sign on his/her behalf the study documents.
The Investigator is required to conduct the study in accordance with the study protocol and in accordance with the Good Clinical Practice Standards (ICH-E6), the principles of the Helsinki Declaration (1964) and subsequent revisions and in compliance with applicable regulations.

16.1 Conservation of documentation

The Investigator / Institution will provide for the conservation of the essential documents of the study as specified by the GCP and in accordance with the applicable regulatory provisions. The Investigator will take the necessary measures to prevent accidental or premature destruction.

16.2. Direct access to original documents

The Investigator will allow the Regulatory Authorities, national and foreign, and the personnel designated by the Independent Ethics Committee direct access, and relative verification, to all the original documentation of the study, including the Informed Consent forms signed by the subjects included in the study or by the their Legally Recognized Representatives and hospital records and / or outpatient records. Those who have direct access to this documentation will take every reasonable precaution to keep the identity of the subjects confidential in compliance with the applicable regulatory provisions.

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