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Retinal degeneration in MOG antibody Associated Disease (MOGAD): a longitudinal OCT study

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Introduction

Myelin Oligodendrocyte Glycoprotein Antibody Associated Disease (MOGAD)

Myelin oligodendrocyte glycoprotein (MOG) is a component of Central Nervous System (CNS) myelin and is expressed on the outer lamella of the myelin sheath. In humans MOG is thought to be involved in completion and maintenance of the myelin sheath and in cell–cell communication [Johns et al.]. Moreover, it is well established as an antigenic target in the experimental autoimmune encephalomyelitis mouse model [Lassmann et al]. The emergence of protein conformation-dependent assays for the detection of anti-MOG antibodies has revealed distinct clinical phenotypes in children and adults with CNS demyelination [Cobo Calvo et al; Hacohen et al]. Thus, MOG antibody-associated disease (MOGAD) is now considered a distinct disorder.

MOGAD pathogenesis

Even if the trigger for anti-MOG antibody production is unknown, the autoimmune induction is thought to occur in the peripheral immune system. Post-infection autoimmunity could be a probable trigger, but no disease-specific pathogens have been identified. Post-infectious autoimmunity could occur through different mechanisms: molecular mimicry, bystander activation, epitope spreading, B-cell receptor mediated co-capture of antigens, and polyclonal activation of B cells. In addition to anti-MOG antibodies and anti-MOG antibody-producing cells, antigen-specific T follicular helper cells are also probably involved: indeed, T follicular helper cells are required for differentiation of B cells into plasma cells producing anti-MOG antibodies. These actors cross the blood-brain-barrier in order to interact with their autoantigen, thus mediating the pathogenic effect. Anti-MOG antibodies might enter the CNS when the blood–brain barrier is damaged after binding to Fc receptors and release from endothelial cells. Then, they bind MOG expressed in the myelin sheath leading to myelin injury and subsequent demyelination [Kaneko et al; Spadaro et al, 2018]. Moreover, anti-MOG antibodies and plasma cells enhance activation of MOG-specific CD4 T cells and macrophages in the CNS [Spadaro et al, 2016], thus increasing proinflammatory cytokines (IL-6, IL-17, G-CSF, and TNFα) and B cell cytokines and chemokines (BAFF, APRIL, CXCL13 and CCL19) [Marignier et al].

Clinical and paraclinical features

MOGAD accounts for approximately 1.2–6.5% of all demyelinating syndromes in adults [Cobo Calvo et al, 2019]. It can occur in all decades of life, with a slight predominance in women and a median age of onset in the early to mid-thirties [Kezuka et al]. The most common presenting feature is optic neuritis (ON), occurring in 54–61% of patients, followed by myelitis and acute disseminated encephalomyelitis (ADEM) [Thomas et al, Banwell et al]. A relapsing course has been reported in 44–83% of patients

[Cobo Calvo et al, 2018] and more commonly involves the optic nerve [Jurynczyk et al]. MOG-positive ON is frequently bilateral and associated with optic nerve head swelling [Ramanathan et al, Biotti et al]. The impact of relapses on disability is variable: some studies report no difference between monophasic and relapsing disease courses [Cobo Calvo et al, 2018] whilst others report worsening disability associated with higher relapse frequency [Jurynczyk et al]. In case-based series, residual disability develops in 50–80% of patients [Mariotto et al, Ramanathan et al] with transverse myelitis at onset being the most significant predictor of long-term disability [Jurynczyk et al]. Cases of seizures have also been reported, leading to a new clinic-radiological sub-entity, characterized by fluid attenuated inversion recovery (FLAIR) imaging showing hyperintense cortical lesions, in MOG associated Encephalitis with Seizures (FLAMES) [Jain et al].

Brain MRI scans are abnormal in approximately 45% of patients at onset [Cobo Calvo, 2018], with percentages increasing later in the course of the disease up to 77% of patients [Mariotto et al]. The majority have bilateral lesions at onset and around one-third have sub-tentorial lesions, predominantly in the brainstem. Typically, lesions are few and appear as “fluffy”, i.e., poorly demarcated hyperintensities on T2-weighted images. Dawson’s fingers, U- or S-shaped lesions and ovoid lesions adjacent to the body of lateral ventricles are found less commonly [Jurynczyk et al, 2017]. When compared to MS-associated ON or AQP4-positive NMOSD associated ON, the MRI appearance of the optic nerve in MOG-associated ON is more oedematous and shows extensive inflammatory lesions, usually sparing chiasm and optic tracts [Biotti et al]. Thalamic and pontine lesions are more common in MOG-AD compared to AQP4-positive disease [Cobo Calvo, 2018]. In children, bilateral thalamic lesions at onset are frequent and can be found in about 60% of patients. Compared to AQP4-positive patients, cerebellar peduncle lesions are only found in MOG-positive children.
Just over half of MOG-AD patients have T2 hyperintense lesions in the spinal cord, with most lesions being short and predominantly occurring in the cervical or thoracic region.

Considering laboratoristic findings, cerebro-spinal-fluid [CSF] pleocytosis occurs in 44–85% of patients [Mariotto et al, Cobo Calvo et al, 2019] and is more common in children [Ramanathan et al, 2018]. Positive oligoclonal bands are unusual, occurring in only 6–17%, [Mariotto et al, Cobo Calvo et al, 2019 Jurynczyk M et al] and CSF protein is raised in around a third of cases [Ramanathan et al].

Treatment

For the treatment of MOG antibody positive ON or myelitis intravenous methylprednisolone has been reported to be effective. Plasma exchange is often used as a second-line therapy, following steroid resistance, with complete recovery in 40% of cases [Jarius et al]. Long-term treatments with prednisolone, intravenous immunoglobulins, rituximab, mycophenolate mofetil, methotrexate or

azathioprine have all been reported to reduce annualized relapse rate [Ramanathan et al, 2018, Jarius et al]. First-line injectable multiple sclerosis treatments (interferon-beta and glatiramer acetate) were shown to be ineffective in preventing relapses in both adults and children with relapsing MOGAD, with no change in annual relapse rate [Marignier 2021, Hacohen et al, 2018]. Treatment with natalizumab has been shown to be ineffective in preventing relapses, whereas treatment with ofatumumab was able to reduce annualised relapse rate [Jarius et al]. Therapeutic management of MOGAD is summarized in figure below.

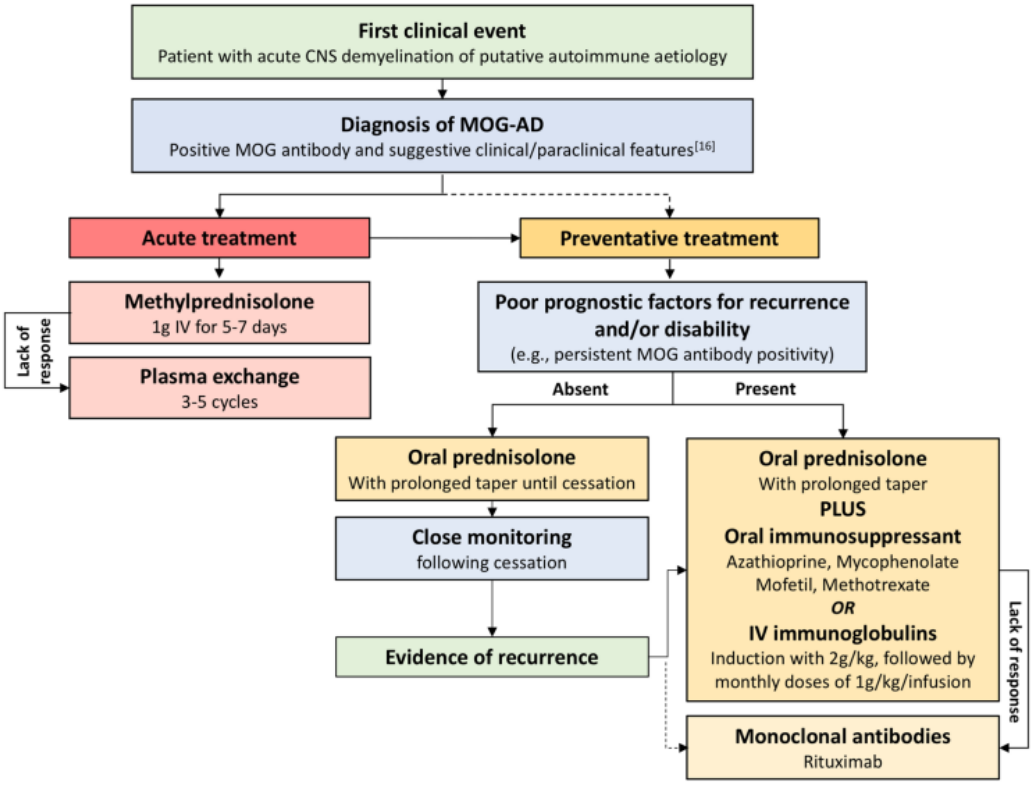

Figure 1-Management of MOG-AD [Thomas et al]

Prognostic meaning of MOG ab titres

Whether MOG ab titres are associated with a specific clinical phenotype or are useful to predict future relapses and clinical outcome is still unclear. Cobo Calvo et al in a study published in 2019 found that patients with a severe visual or motor disability showed higher MOG ab titres, while MOG ab titres did not seem to be related with a specific clinical phenotype at onset. Moreover, in this cohort, an association between MOG-Ab titres at the first episode and the risk of relapse has not been found, and the usefulness of titres at the first episode to predict visual or motor outcome was limited [Cobo Calvo et al, 2019]. Epstein et al, in a multicentric American cohort, did not observe a correlation between initial MOG-Ab titres and relapse risk, thus hypothesizing that titres may not completely predict disease activity [Epstein

et al]. More recently, a multicentric Italian group aimed to assess the clinical relevance of quantitative and longitudinal MOG-IgG testing in a large population of pediatric and adult patients with MOGAD. According to this study, that analyzed more than three hundred serum samples, MOG ab titres tend to be higher during attacks compared to remission disease phases. However, relapses occurred also with low-titres, and increasing titres were found in patients with clinical stability. Moreover, any association between titres and clinical presentation, attack severity or response to treatment has been found [Gastaldi et al]. Taken together, these data suggest that MOG ab titres dynamics still have to be interpreted carefully, and further studies are needed in order to assess their prognostic meaning.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disorder that affects the central nervous system (CNS) and the most common non-traumatic CNS disease that causes permanent disability in young adults [Battaglia et al]. According to the WHO/ MSIF MS Atlas, this disease affects 2.3 million people worldwide, with more than 600,000 cases in Europe. Mean prevalence rates are higher in northern countries. This pathologic condition occurs mostly between the ages of 20 and 50 years and affects women more than men [Khan et al].

MS pathogenesis

Inflammation of the white and grey matter tissues in the CNS due to focal immune cell and cytokine infiltration is the incipient cause of damage in MS. Many studies have suggested that T helper (Th) cells' intervention and adaptive immune responses (initiated by interaction between antigen presenting cells -APCs- and T lymphocytes) play an important role in the initiation and progression of MS [Ghandi et al; Kasper et al]. Pathogen-associated molecules simultaneously bind to toll-like receptors on antigen presenting cells and production of specific cytokines that include interleukin (IL)-12, IL-23 and IL-4 begins. These cytokines induce CD4+ T cell differentiation into Th1, Th2, or Th17 phenotypes which have ability to release special cytokines, such as interferon gamma (IFNγ) or type II interferon and tumor necrosis factor alpha (TNF-α), that promote inflammation by suppressing Th2 differentiation. In addition to the above-mentioned cells, CD8+ T cells can be found in MS lesions [Kouchaki et al]. These cells, via production of cytolytic proteins such as perforin, mediate suppression and inactivation of CD4+ T cells. Moreover, CD8+ T cells increase vascular permeability, glial cells destruction and trigger oligodendrocyte death. In addition to CNS inflammation, due to oligodendrocyte death, the myelin repair process is also impaired [Kasper et al].

B cells affect MS development and progression by targeting autoantigens. In addition, humoral antibodies are reported to lead to tissue injury when they bind to brain cells and interfere with complement factor functions. Studies have revealed that oligoclonal immunoglobulins (Ig) persist in the cerebrospinal fluid in approximately 90% of patients, further supporting the idea that B cells contribute to MS pathogenesis [Disanto et al]. The pathogenic action of the CNS-based antibodies is usually characterized by enhanced inflammatory demyelination and blood-brain barrier disruption. Antigen-activated B cells in the body can facilitate MS development by acting as potent antigen-presenting cells (APCs). Furthermore, B cells usually act as a source of antibody-generating plasma cells to contribute to MS development and progression. B cells can produce IL-6 and aid the process of T helper-17 cell differentiation. Furthermore, they prevent the production of regulatory T cells and increase the secretion of many inflammatory factors such as IL-6, TNF, lymphotoxin alfa (LT-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [Li et al].

Environmental factors and genetic susceptibility

Environmental factors, including exposure to viral and bacterial agents such as Epstein Barr virus (EBV), human herpes virus type 6, and mycoplasma pneumoniae [Fujinami et al], in addition to smoking [O'Gorman et al], vitamin deficiency [Speer et al], diet [Bäärnhielm et al, 2014], and exposure to UV radiation [Sloka et al] are associated with the onset of MS.

Currently, evidence suggests that smoking, due to nitric oxide (NO) and carbon monoxide (CO) production, plays an important role in MS. Moreover, vitamin deficiency (especially vitamins D and B12) are considered risk factors for MS. Low-term sunlight exposure has been identified as a potential risk factor for MS. The results of a previous study have demonstrated a reverse association among exposure to ultraviolet radiation and the incidence of MS [Sloka et al]. In justifying this relationship, it can be said that sun light is a principal source of vitamin D3 and via induction of T regulatory cells and anti-inflammatory cytokines such as IL-10 and TNF-α, it may have immunomodulatory effects in MS [Bäärnhielm et al, 2012].

According to previous reports, diet could be an environmental factor involved in MS. Studies reported a significant negative association between MS risk and high fish intake, a positive significant association between high animal fat-based caloric intake and MS risk, a non-significant lower risk between incidence of MS and a higher intake of linoleic acid, and a positive significant association between obesity in adolescent girls and MS risk [Langer-Gould et al].

Studies show that the risk of MS in family members of a patient depends on the amount of genetic information they share [Sadovnick et al, 1996; Sadovnick et al, 1999]. Thus, the risk rate in monozygotic twins that have 100% genetic similarity is approximately 25%. In all individuals who have 50% genetic similarities such as dizygotic twins and first-degree relatives, this risk is 2-5% [Oksenberg et al]. It has been shown that in the human leukocyte antigen (HLA) region of chromosome 6 exists a group of genes associated with an increased risk of MS. Within this region HLA-DR2+, HLA-DQ6, DQA 0102 and DQB1 0602, HLA-DRB1, DR15, DRB1*1501, and DRB1*1503 are genes susceptible to the onset of MS. In addition to these alleles, IL-7 and IL-2 receptor alpha are other sensitive genes associated with MS [Gregory et al]. Unlike the aforementioned genes, HLA-C554 and HLADRB1*11 have protective effects [Compston et al; Lemus et al].

Clinical presentation and diagnosis

The clinical symptoms and signs of MS are variable and may result from involvement of sensory, motor, visual, and brainstem pathways. Patients with MS usually present with relapsing remitting episodes of new or recurrent neurological symptoms, such as optic neuritis, incomplete myelitis, or brainstem syndrome. A variable proportion of patients with relapsing remitting MS (25–40%) develop secondary progressive disease over time with progressive accumulation of disability with infrequent or no relapses. Primary progressive MS (seen in approximately 10–15% patients) is defined by progressive

accumulation of disability from the onset with no or minor relapses and typically presents in patients with an older age at onset and involves a higher proportion of men. Both primary and secondary progressive MS share some clinical and imaging features and are now considered to be part of a progressive disease spectrum [Jacques et al].

There is no single diagnostic test for MS and the diagnosis is usually based on the clinical presentation, supported by neuroimaging, CSF analysis and evoked potential studies. There have been several proposed diagnostic criteria incorporating the clinical and ancillary data, the most commonly used one is the McDonald criteria initially proposed in 2001, revised in 2005 and most recently in 2011 and 2017. The basic concept behind these criteria is demonstration of dissemination in time (DIT) and space using clinical and/or MRI data. Both criteria DIS and DIT have to be fulfilled either by clinical disease course with relapses and different neurological symptoms or by magnetic resonance imaging (MRI) demonstrating inflammatory lesions in different regions and different activity stages. Since oligoclonal bands are considered an independent risk factor for further clinical episodes in patients with clinically isolated syndrome [Gandhi et al; Kasper et al] the presence of oligoclonal bands in cerebrospinal fluid can also be used as substitute for DIT.

Treatment

Nine classes of disease-modifying therapies (DMTs), with varying mechanisms of action and routes of administration, are available for relapsing-remitting MS and secondary progressive MS with activity. These drugs include interferons, glatiramer acetate, teriflunomide, sphingosine 1-phosphate receptor modulators, fumarates, cladribine, and monoclonal antibodies (alemtuzumab, natalizumab, ofatumumab). One additional DMT, ocrelizumab, is approved for primary progressive MS. These DMTs reduce clinical relapses and MRI lesions (new T2 lesions, gadolinium-enhancing lesions). Efficacy rates of current DMTs, defined by reduction in annualized relapse rates compared with placebo or active comparators, range from 29%-68%. Adverse effects include infections, bradycardia, heart blocks, macular edema, infusion reactions, injection-site reactions, and secondary autoimmune adverse effects, such as autoimmune thyroid disease [McGinley et al].

Figure 2 summarizes clinical and paraclinical features of MOGAD and MS.

Characteristics	MOG-AD	MS [41]
Age of onset	Early to mid-30s	Around 30
Sex	Slight predominance in women	More common in women
Clinical phenotype	Commonly ON at onset (better visual field outcomes compared to AQP4-positive ON); other presentations include myelitis, ADEM and ADEM-like events	ON, usually with good recovery; other neurological systems involved
Disease course	Monophasic or relapsing	Relapsing or progressive
Type of relapses	Commonly ON (more than in NMO)	Any, with relapse phenotype predicted by previous relapse phenotypes [42]
MRI brain	Abnormal in 45–77%; fluffy T2 hyperintense lesions; few lesions (e.g., ≤3); bilateral lesions at onset (about 50% of cases); Dawson's fingers and U- or S-shape lesions uncommon; thalamic and pontine lesions more common compared to NMO; more oedematous and extensive inflammatory lesions in the optic nerve, sparing chiasm and optic tracts; in children, cerebellar peduncle lesions	Always abnormal; presence of Dawson's fingers, subcortical S-shaped or U-fibre lesions [44]
MRI spinal cord	Abnormal in about 50% of cases; lesions more commonly short; in children, LETM more common	Lesions more commonly short [44]
CSF	Pleocytosis variable; OCBs uncommon	Commonly < 50 WCC/mm^3; OCBs in up to 95% of RRMS patients

Figure 2 – Clinical, radiological and laboratoristic features in MOGAD and MS [Wynford-Thomas R et al]

Optical coherence tomography (OCT)

OCT is a valuable tool for monitoring many neuro-ophthalmological and neurological conditions, including multiple sclerosis and MOGAD. It makes in situ imaging of tissue microstructure possible with a resolution approaching that of light microscopy histology. OCT provides cross-sectional and volumetric images of areas of interest by acquiring either the echo time delay or frequency information of back-reflected light. Differences in the optical properties of biological tissues allow the recognition of layered structures. The speed of light makes it impossible to analyze the acquired information directly, since it would be in the order of femtoseconds, thus OCT systems use the optical technique known as interferometry. Low-coherence interferometry enables analysis of this information and the creation of a depth-resolved reflectivity profile of the scanned tissue by matching the light profiles from the scanning and reference arms. Utilization of light provides OCT technology the ability to obtain images in a non-contact fashion and to achieve resolutions of 1–15 μm [Grzybowski, Barboni]. Light is highly absorbed or scattered in most biological tissues, and therefore the use of this technology is limited only to locations that are optically accessible or that can be imaged using devices such as endoscopes or catheters.

OCT technique can be classified into two groups: time-domain (TD) and Fourier (or frequency) domain (FD). FD can be further classified into spectral-domain (SD) and swept-source (SS) techniques. In TD-OCT, a broad-bandwidth laser or a low-coherence superluminescent diode light source projects light that is then divided into two arms by a partially reflecting mirror (beam splitter). In the first arm light is projected toward the sampling location, while in the second arm light is projected toward a moving reference mirror at a known position. The backscattered light from both sites travel back to a detector and recombine to form an interference pattern, which is sensed by the interferometer. The interference is only observed when both the sample and the reference arm light beams travel the same distance [Hee et al]. Changing the position of the reference mirror allows the machine to sequentially acquire information from different depths in the tissue sample. A cross-sectional image, also known as a B-scan, is generated by performing fast, subsequent axial scans (A-scans) at different transverse positions. Each axial scan represents the echo time delay of back-reflected light from the tissue and gives a profile of the tissue's dimensions along the optical beam. SD-OCT is similar in principle, but the data acquisition varies slightly from TD-OCT. The main difference is the use of light frequency information instead of time delay data to determine the spatial location of reflected light. SD technology utilizes the Fourier transformation of the reflected light frequencies to encode distances within tissue microstructure [Fercher et al]. Instead of a moving reference mirror, the mirror is stationary and the interference signal is split into its frequency components using a diffraction grating. The signal is simultaneously detected by a charge-coupled device (CCD). The CCD has an array of photodetectors that are each sensitive to a range of specific frequencies [Wojtkowski et al, Fujimoto et al]. SD technology allows the acquisition of information from all points along each A-scan simultaneously, substantially increasing scan speed

[Kim et al, Ahlers et al]. The substantial increase in scanning speed allows for the acquisition of three-dimensional (3D) data sets, which is done by combining rapidly acquired subsequent cross-sectional scans. The wide bandwidth of the light source also enables a substantial enhancement in axial resolution up to 1 μm [Drexler et al, Lim et al] and an improved signal-to-noise ratio [Leitgeb et al].

OCT measurements

The visual pathway is made up of 4-neuron chains that convey visual information from the retina to the primary visual cortex. Photoreceptor cells, bipolar cells and retinal ganglion cells (RGCs) represent the first, second and third order neurons of the anterior visual pathway; however, the retina includes two additional neural types that modulate the activity of the other retinal neurons, namely amacrine and horizontal cells. This network is important to modulate the signals to the cortex. The axons from RGCs constitute the retinal nerve fiber layer (RNFL), the optic nerve, the chiasm and the optic tracts; and ultimately, they form synapses with neurons of the lateral geniculate nucleus (LGN). Axons from the RGCs of the macula are small (parvoganglion cells) and they travel along the papillomacular bundle of the RNFL to the temporal quadrant of the optic disk. In addition to the LGN, the posterior visual pathway contains the optic radiations and the primary visual cortex. The LGN is part of the thalamus and it modulates visual information before projecting it to the visual cortex. Compared to the retina, there are 300 to 400-fold more neurons in the visual cortex and LGN [Martínez-Lapiscina et al]

The ganglion cell is the first order neuron in the visual pathway, with the cell body located in the retinal GCL (Ganglion Cell Layer) while the retinal nerve fiber layer (RNFL) represents the axon of these neurons that leaves the eye and enters the brain through the optic nerve, where it synapses at the lateral geniculate body. Figure 3 shows a microscopic cross-sectional view through the optic nerve (A) and an OCT retinal image in a healthy individual (B).

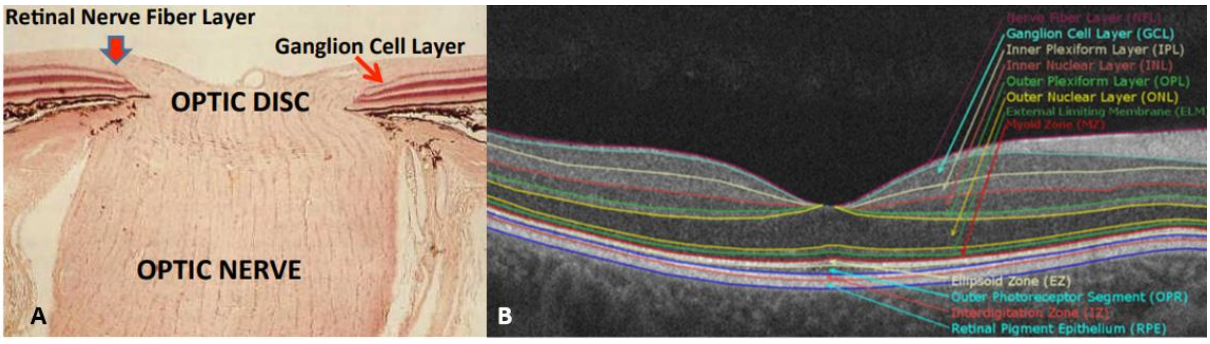

Figure 3 – A: microscopic cross-sectional view of the optic nerve. **B**: OCT retinal image with its 12 layers for a typical healthy person in macular region of retina [Eladawi et al]

SD-OCT measurement of circumpapillary RNFL thickness can be extracted from a circle centered on the Optic Nerve Head (ONH) with a diameter of 3.4 mm. The limitation of this approach is that the sampling of the tissue is performed only along the circle, and therefore any misplacement of the circle during repetitive scanning will result in increased measurement variability. Figure 4 shows Cirrus OCT RNFL printout.

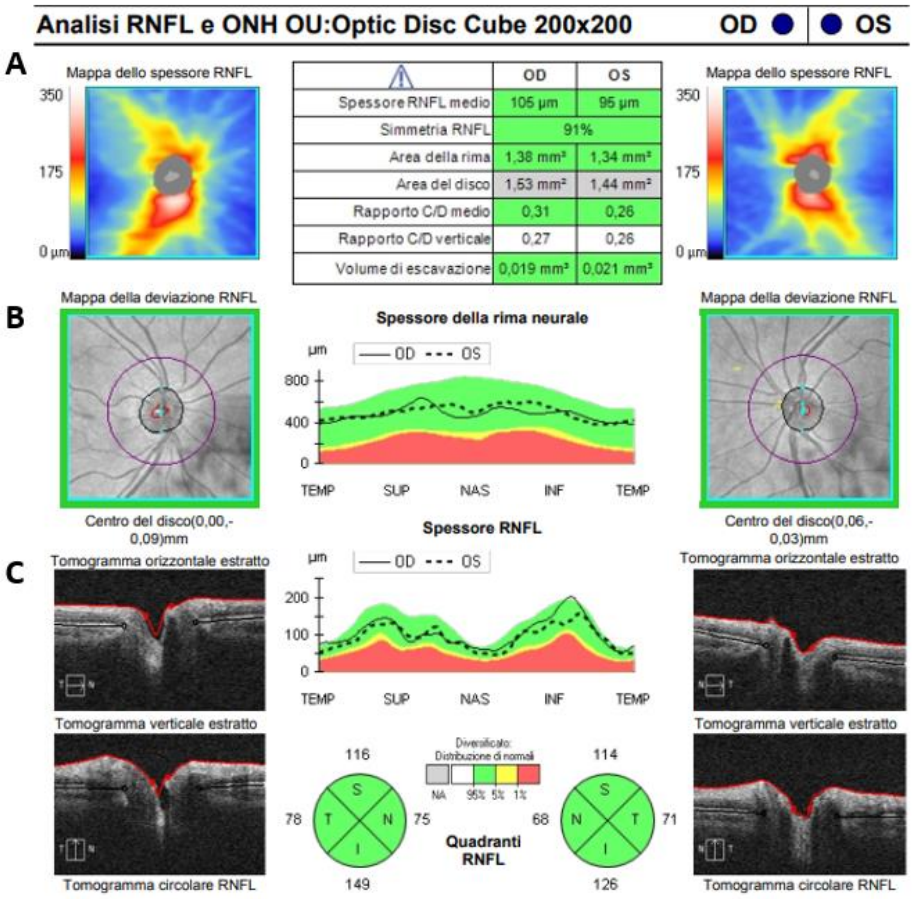

Figure 4 – Cirrus OCT RNFL printout showing: **A**: thickness map; **B**: deviation map; **C**: cross-sections.

The high resolution of SD-OCT allows the acquisition of reproducible segmentation and the analysis of individual macular layers that are of particular diagnostic interest: in this way, it is possible to extract the information from an ellipse centered on the fovea providing measurements that includes retinal Ganglion Cell Layer (GCL-figure 5).

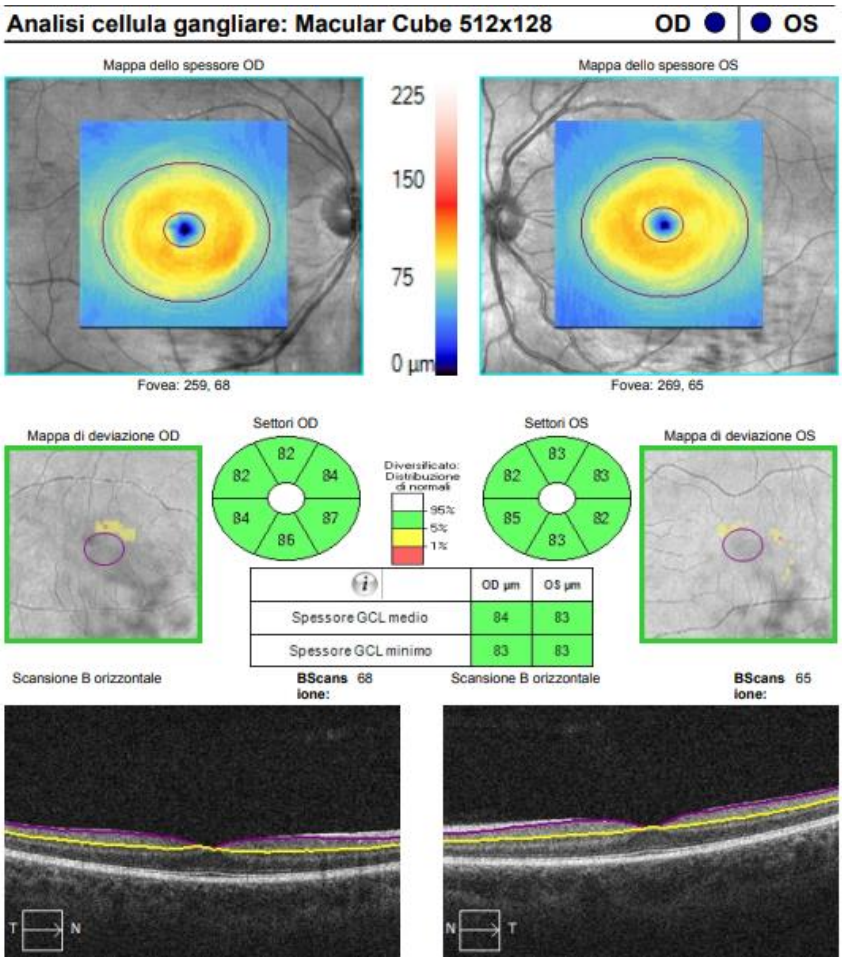

Figure 5 – Cirrus OCT GCL printout: the ganglion cell analysis.

Over the last years of OCT research, the measurement of retinal layer thicknesses was harmonized by standardized acquisition, analysis and nomenclature in line with consensus criteria as OSCAR-IB, APOSTEL, IN-OCT [Oertel et al]

Retinal degeneration in multiple sclerosis

Many studies have shown that both RNFL and GCL are statistically significantly reduced in patients with multiple sclerosis, both with and without prior optic neuritis [Petzold et al; Britze et al, 2017]. The pathological mechanism behind the reduction in RNFL and GCL thickness in MS patients is still debated. In patients with prior ON, the most likely explanation is loss of axons due to retrograde degeneration after ON. In patients who have not had clinical ON, several different mechanisms have been proposed: loss of GCL and RNFL thickness could be caused by retrograde degeneration after a mild, subclinical optic neuritis [Britze et al]. Another theory suggests that OCT alterations could be due to primary degeneration of the GCL neurons due to MS. Other studies suggested that lesions in the optic radiation via trans-synaptic degeneration could lead to loss of retinal ganglion cells and their axons.

According to this, an Italian study found an inverse correlation between the thickness of the temporal RNFL and the ipsilateral optic radiation white matter lesion load, and between the nasal RNFL and the contralateral optic radiation white matter lesion load [Puthenparampil et al].

Moreover, many studies indicate that the temporal development, severity and distribution of changes in the GCIPL and RNFL may help differentiate between MS and some differential diagnoses. For instance, in neuromyelitis optica spectrum disorder, the reduction in GCIPL and RNFL thickness is usually much more severe than in MS. Furthermore, the distribution of RNFL loss tends to involve the temporal quadrant in MS, whereas it is more diffusely distributed in neuromyelitis optica spectrum disorder [Schneider et al]. Many studies have found statistically significant reductions in the GCL thickness in eyes of MS patients without prior ON, which may reflect subclinical structural damage. Some have suggested that this could help identify patients with ON who are at risk of developing MS. Coric et al. investigated whether the inter-eye percentage difference could be useful in distinguishing MS patients from healthy controls. They found that the inter-eye percentage difference of the GCIPL could distinguish healthy controls and patients with MS without history of optic neuritis [Coric et al].
Oberwahrenbrock et al observed that, among a large cohort of MS patients without prior optic neuritis, RNFL thickness was lower in patients with secondary progressive MS compared with patients with relapsing-remitting MS. Moreover, according to this study, total macular volume was reduced in progressive MS compared to the relapsing-remitting form, but this difference disappeared when authors corrected for EDSS score [Oberwahrenbrock et al]. Another study observed lower GCIPL and RNFL values in SPMS compared to RRMS. However, after correcting for disease duration this was only significant with regards to the GCIPL, thus suggesting that GPL could be more reliable than RNFL in assessing neuronal degeneration [Saidha et al]. A Sweden study correlated the GCIPL and RNFL thickness with Visual Evoked Potentials (VEP) latency, and VEP P100 latency with EDSS. Moreover, linear regression showed an association between GCIPL thickness and EDSS [Eklund et al]. Vidal-Jordana et al confirmed that OCT and MRI volumetric measures correlate with disability, supporting the use of OCT as a biomarker of neurodegeneration in MS [Vidal-Jordana et al]. Considering cognitive impairment, an Italian study [Frau et al] did not find association between RNFL thickness and cognitive tests, hypothesizing that RNFL and cognitive impairment may represent different aspects of neurodegeneration in MS.

Retinal degeneration in MOGAD

Differently from MS, in MOGAD optic neuritis the pathology of afferent visual system damages mediated by MOG-Ab is less well understood. Acute ON in MOGAD is often bilateral and localized in the anterior optic nerve inducing severe and characteristic retinal oedema [Vicini et al]. Initially covered by the oedema, the neuroaxonal layers of the retina degenerate significantly during the following

months. These losses accumulate with each additional ON episode, which occur frequently in MOGAD [Zhao et al, Lerch et al]. Therefore, although a single episode does not often lead to disastrous damage, the highly recurrent ON attacks accumulate with RNFL and GCIPL loss [Akaishi et al, Stiebel-Kalish et al]. In comparison with MS, MOGAD patients are described as undergoing more severe retinal neurodegeneration after ON; however, a final consensus on this topic has not been reached [Bartels et al]. Havla et al. found that ON eyes of MOG-antibody positive patients show reduced pRNFL thickness of the temporo-inferior and temporo-superior quadrants and reduced macular RNFL thickness in comparison to MS-ON [Havla et al]. In contrast, in comparison to MS-ON, Martinez et al. found no significant differences in the thickness of different retinal layers in six eyes with MOG-ON [Martinez-Lapiscina, 2016]. More recently, Sotirchos et al. found a decrease in thickness of the macular ganglion cell and inner plexiform layer (IPL) of MOG-ON as compared to MS-ON [Sotirchos et al]. A small case series of three patients with MOG-ON reported preferential thinning of pRNFL of some quadrants [Jelcic et al], which is in contrast to the thinning of the temporal pRNFL quadrant typically seen in MS optic neuritis [Schneider et al].

Finally, a longitudinal OCT study investigated MOGAD patients for potential progressive or covert damage in the retina in the absence of new clinical optic neuritis. In this study authors did not detect progressive CGIP thinning during follow-up. Moreover, a longitudinal pRNFL reduction was observed in patients with non-ipsilateral ON attacks within 6 months before baseline, maybe due to a remission of pRNFL edema [Oertel et al, 2019; Akaishi et al, 2021].

Altogether, these studies have to be interpreted with caution due to limited sample sizes, potentially explaining the conflicting findings. Moreover, no study has so far analysed the potential correlation between MOG ab titres and OCT parameters.

Aims of the study

Since MOG ab titres prognostic meaning and OCT characteristics in MOGAD are still poorly understood, in this study we analysed longitudinal dynamics of MOG antibodies and GCL and RNFL thicknesses in our cohort of MOGAD patients. Moreover, we compared GCL and RNFL thicknesses and dynamics between MOGAD patients and a cohort of RRMS patients.

Primary outcomes:

- To correlate MOG ab titres at baseline and MOG ab titre dynamics with:
    - Clinical course of disease
    - Recurrence of optic neuritis
    - GCL and RNFL thicknesses at baseline and follow-up
    - GCL and RNFL thinning rates in eyes with and without optic neuritis

- To correlate GCL and RNFL thicknesses at baseline and follow-up and GCL and RFNL thinning rates with
    - relapse occurrence
    - disability progression

Secondary outcomes:

- To compare GCL and RNFL thicknesses at baseline and follow-up in patients with MOGAD and RRMS
- To compare GCL and RNFL thinning rates in patients with MOGAD and RRMS
- To compare GCL and RNFL thicknesses at baseline and follow-up in eyes with optic neuritis in MOGAD and RRMS patients
- To compare GCL and RNFL thinning rates in eyes with optic neuritis in MOGAD and RRMS patients
- To compare GCL and RNFL thicknesses at baseline and follow-up in eyes without optic neuritis in MOGAD and RRMS patients
- To compare GCL and RNFL thinning rates in eyes without optic neuritis in MOGAD and RRMS patients

Materials and methods

The study presented here is a prospective and retrospective monocentric study.

Prospective study

MOGAD cohort

Inclusion criteria

- Age >/=18 years
- Willing to provide an informed consent
- MOG IgG seropositivity
- At least one of the following:
    - Optic neuritis (both mono and bilateral)
    - Transverse myelitis (both mono-multimeric)
    - Brain lesions (single or multiple) suggestive for an acute demyelinating inflammatory event

Exclusion criteria

- Ophthalmological comorbidities other than optic neuritis.

RRMS cohort

Inclusion criteria

- Age >/=18 years
- Willing to provide an informed consent
- MOG IgG seronegativity
- Fulfilment of 2017 McDonalds criteria for relapsing remitting Multiple Sclerosis

Exclusion criteria

- Ophthalmological comorbidities other than optic neuritis
- Previous therapy with sphingosine 1-phosphate receptor modulators

Patients underwent periodical (every six months) clinical follow-up, laboratory testing for MOG ab titres (if positive) and OCT scans.

Retrospective study

Clinical records of patients admitted to Mondino IRCCS Foundation (Pavia) between January 2020 and January 2022 were screened for patients fulfilling the inclusion criteria. Clinical and serological data

were collected as described for the prospective cohort at the beginning of the inflammatory disorder and at last follow-up. Longitudinal data regarding antibody titres, if available, were collected. Patients without sufficient clinical and paraclinical data were excluded from the study.

Optical Coherence Tomography

OCT was performed with Cirrus Zeiss 5000, following APOSTEL guidelines [Cruz-Herranz et al]. For every patient data about RNFL and GCL thicknesses at baseline and follow-up were collected. RNFL and GCL annualized variation rate was calculated in μm/year with the following formula: (Δ RNFL/months between baseline OCT and follow-up OCT) *12; or (Δ GCL/months between baseline OCT and follow-up OCT) *12.

Laboratory methods

MOG antibodies were tested using a live cell-based assay. HEK293T cells are cultured on 12 mm coverslips until 80% confluent, and then transfected with a plasmid encoding for the protein of interest using lipofectamine (untagged MOG full length, kind gift of Dr. Patrick Waters; EGFP-tagged MOG full length, kind gift of Dr. Markus Reindl). After 24 hours, cells are incubated with patients' serum (1:20 dilution) for 1 hour at room temperature, and afterwards with an anti-human IgG fluorescent secondary antibody. Results are assessed using a fluorescence microscope. Samples are screened at 1:20 using an Fc-specific anti human IgG. Positive samples are then titred up to their endpoint and, in parallel, tested for the presence of IgG1 antibodies. Patients are considered MOG positive if they have MOG IgG1 abs and/or MOG IgG abs titring >/=1:640.
MOG variation rate was calculated via the following formula: (Δ titre/months between baseline and FU) *12.

Statistical analysis

SPSS program (version 24) was used to perform statistical analysis. To assess the association of two categorical variables the chi-square test was used. The independent-samples t-test was used to determine the difference between the means of two independent groups on a continuous dependent variable.
To determine the strength of a linear relationship between continuous variables and dichotomous variables a point-biserial correlation was used. To determine the strength of a linear relationship between two continuous variables a Pearson correlation was used. P-value was considered significative if < 0.05.
The candidate participated to data collection and analysis and interpretation of results. This study was approved by Ethics Committee.

Results

The MOGAD cohort

Our MOGAD cohort was made up of 16 patients with a F/M ratio of 10/6. Mean age of the cohort was 38 years (SD 10). Clinical characteristics of disease onset are described in figure 6.

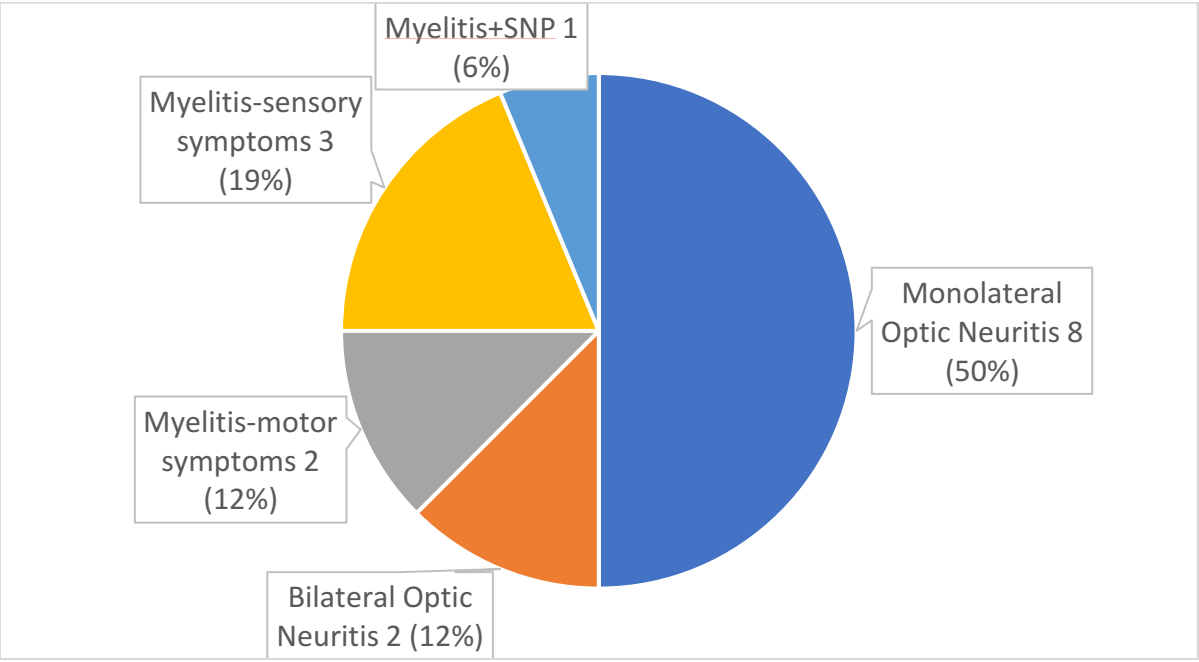

Figure 6– Clinical onset in MOGAD cohort

Eight/16 patients showed a relapsing form of disease, and in 5/8 patients the relapse occurred in the same district of the first clinical event: (1 monolateral ON, 1 bilateral ON, 1 with periferic involvement and 2 myelitis). Mean EDSS at onset was 1.8 (SD 1.3). Baseline MOG ab titres were collected at mean 1.5 years since clinical onset (SD 2.8).

At disease onset, 15/16 patients received steroids as acute therapy, and one patient underwent plasma-exchange as a second line acute therapy. Only one patient did not receive steroids as acute therapy after disease onset. Ten patients took maintenance oral steroids for mean 7 months after disease onset (SD 6).

Nine/16 patients started chronic immunosuppression after mean 41 months since disease onset. Among these patients, eight were relapsing, and one patient showed a monophasic course.
In 4 patients azathioprine (AZT) was used as first line treatment. In one patient AZT was discontinued because of gastroenteric side effects: after a clinical relapse the patient underwent cyclophosphamide (CPX) for two years and then started methotrexate (MTX). Five patients underwent antiCD20 therapy.

15/16 patients underwent periodical titration of MOG ab titres (every 8 mean months, SD 6.7). Figure 7 and figure 8 show longitudinal dynamics of MOG ab titres in 8 relapsing and 7 monophasic patients, respectively.

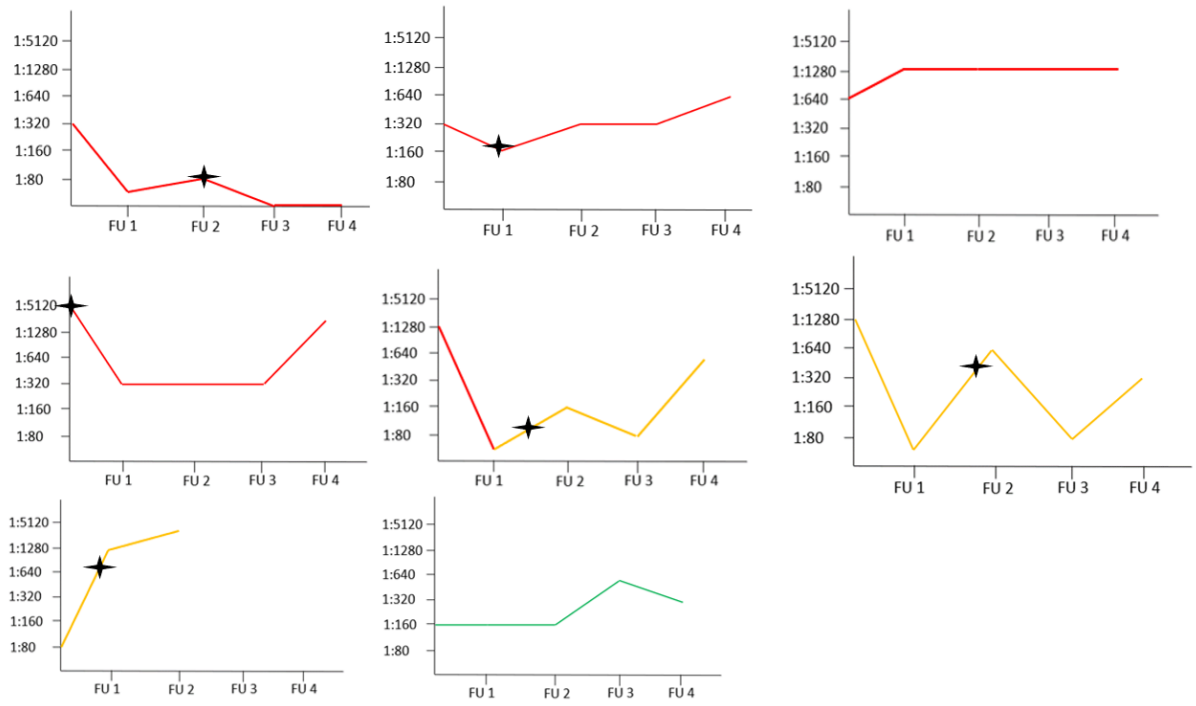

Figure 7 – Longitudinal dynamics of MOGab titres in relapsing patients. Colours represent immunosuppressive therapy. Red: antiCD20. Yellow: AZA. Green: MTX. ✦ =clinical relapse. Two patients underwent clinical relapse before being tested for MOG ab titres.

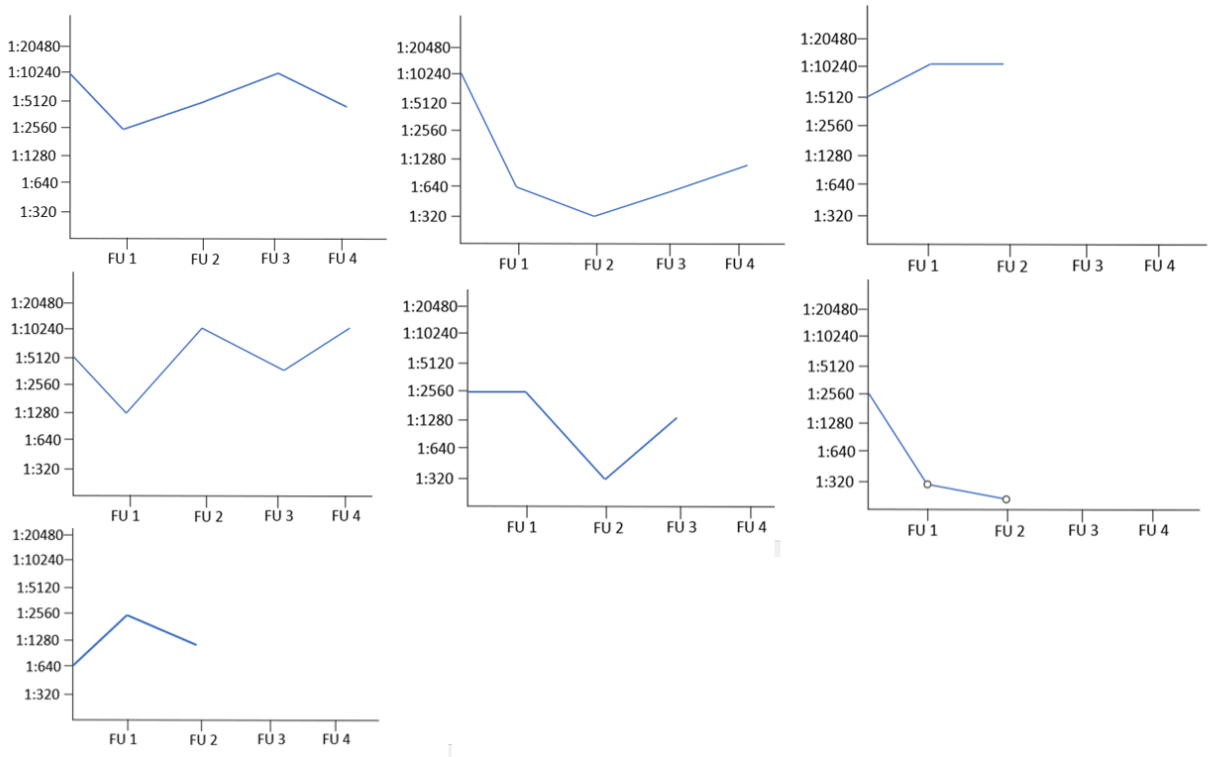

Figure 8 – Longitudinal dynamics of MOGab titres in monophasic patients. None of these patients underwent chronic immunosuppressive treatment.

As shown in figure 7 and 8, dynamics of MOG ab titres vary between patients, without recognizing a common trend among the two groups. However, considering relapsing patients, in 5 cases clinical relapse occurred in concomitance with an increase in MOGab titres.

Considering MOG ab titres dynamics, we divided our cohort in two groups: the group of patients who experienced, at the end of follow up, a reduction of MOG ab titres ("Reduced" group) and the group of patients who experienced an increase in MOG ab titres ("Increased" group). As shown in figure below,

the proportion of the two groups are similar despite different clinical course ("monophasic" and "relapsing").

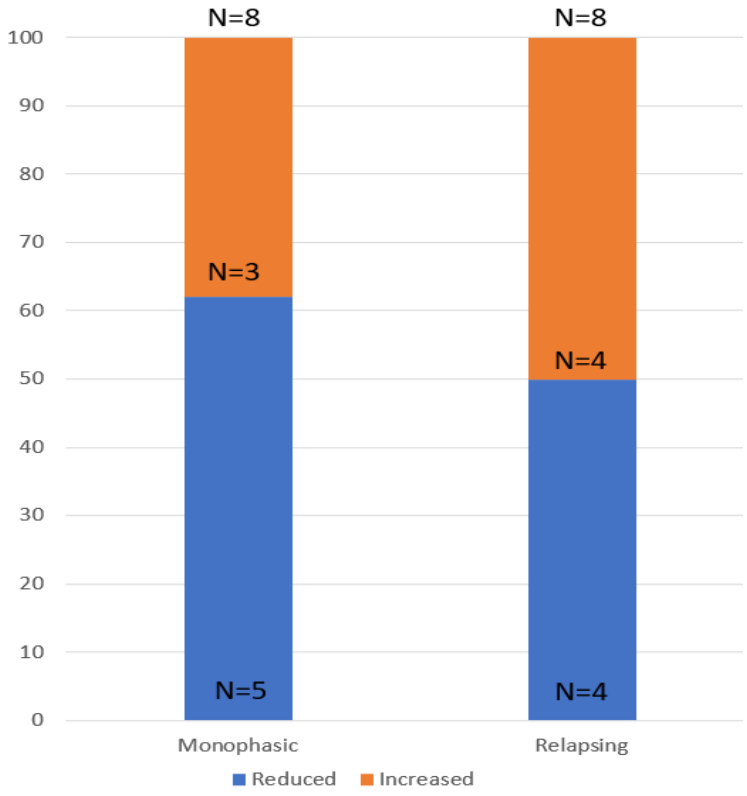

Figure 9 – Proportion of MOGab "reduced" and "increased" groups according to different clinical course of disease. Chi square test showed no significative difference (p=0.6) between the two groups

This result was confirmed considering MOG ab titres' annualized variation rate: performing a point-biserial analysis, we did not find any significant correlation between the ab titre variation rate and the different clinical course of disease (point-biserial correlation coefficient=0.06, p-value=0.8).

MOG antibody titres and OCT thicknesses

All MOGAD patients underwent a baseline OCT, performed at 3 mean years (SD 3) since disease onset. Considering the whole MOGAD cohort, we did not find a significant correlation between MOG antibody titres at baseline and mean GCL (Pearson correlation coefficient=0.16, p-value=0.5) and RNFL (Pearson correlation coefficient=0.2, p-value=0.3) thicknesses, as shown in figure 10.

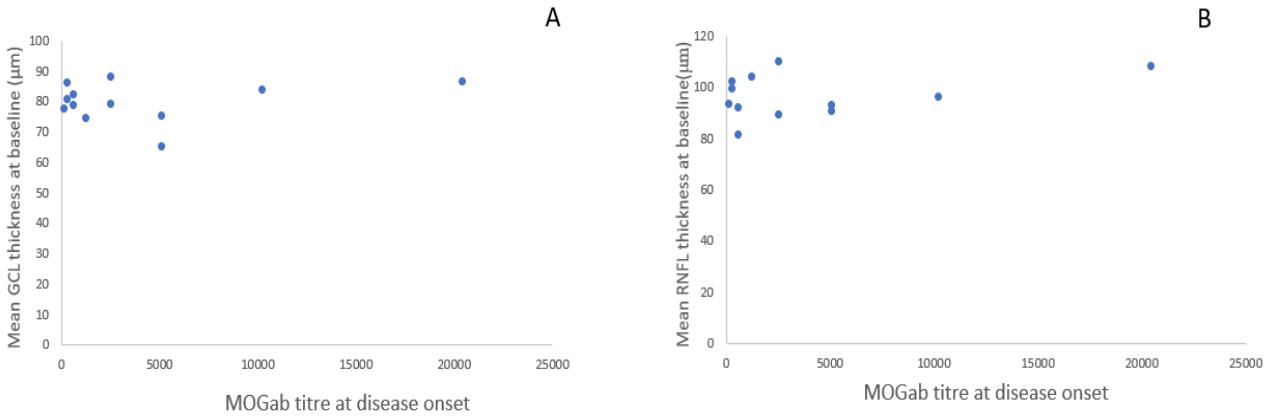

Figure 10 – Scatterplots showing mean GCL (A) and RNFL (B) thicknesses according to MOG ab titres at disease onset.

A follow up OCT was obtained in 12/16 patients, at 11 mean months (SD 5) since the baseline one. For all patients we assessed annualized mean GCL and RNFL variation rates. Performing a Pearson correlation analysis, we did not find a significant association between baseline MOG ab titres and OCT thicknesses variation rates (Pearson correlation coefficient=-0,1, p-value=0.68 considering GCL variation rate; Pearson correlation coefficient=-0.1, p-value=0.7 considering RNFL variation rate). Similarly, considering MOG ab titres' annualized variation rate, we did not find a strong correlation with GCL and RNFL thinning rates (Pearson correlation coefficient=-0.09, p-value=0.76 considering GCL variation rate; Pearson correlation coefficient=-0.3, p-value=0.24 considering RNFL variation rate).

MOGAD-ON + and MOGAD-ON - groups

Our MOGAD cohort was divided into two groups: patients who experienced optic neuritis (MOGAD-ON+) and patients who did not experience optic neuritis (MOGAD-ON-). The group MOGAD-ON+ was made up of 11 patients: 9 with history of monolateral ON and 2 with history of bilateral ON. The remaining 5 patients belonged to the MOGAD-ON- group.

Mean MOG antibody titres at baseline were similar in the MOGAD-ON+ and MOGAD-ON- groups (1:4320 and 1:3149, respectively, p=0.697). We did not find a significative correlation between MOG ab titre at baseline and the occurrence of optic neuritis (point-biserial correlation coefficient =-0.1, p=0.69).

Moreover, mean GCL and RNFL thickness at baseline OCT were similar between the two groups, even if these thicknesses were lower in MOG-ON+, as shown in table below.

	MOG-ON+	MOG-ON-	P-value
GCL thickness, mean μm (SD)	76 (7.5)	83 (3.8)	0.1
RNFL thickness, mean μm (SD)	90 (11)	101 (5)	0.4

Figure 11– A student T test showed no significant differences between OCT measurements in MOG-ON+ and MOG-ON- groups. However, patients with history of optic neuritis had lower GCL and RNFL thicknesses.

Figure below shows longitudinal OCT thicknesses dynamics in MOGAD-ON- group.

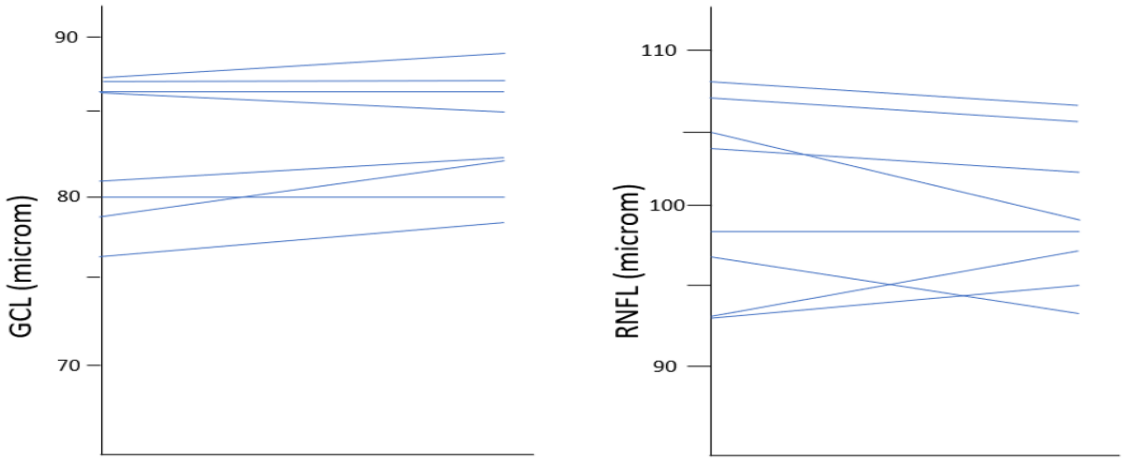

Figure 12 – Longitudinal dynamics of GCL and RNFL thicknesses among eight eyes of patients without history of optic neuritis (MOGAD-ON-; two eyes lost at follow-up)

Considering the group MOGAD-ON+ (9 patients with monolateral ON and 2 patients with bilateral ON), we described longitudinal dynamics of retinal thicknesses among eyes affected by ON (figure 13) and among eyes not affected by inflammatory event (figure 14).

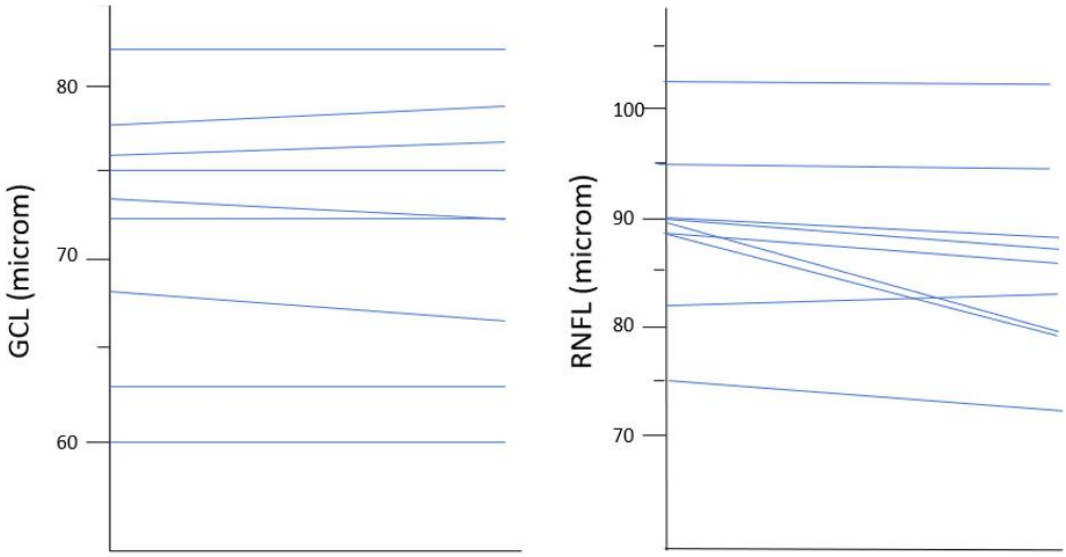

Figure 13 – Patients with history of optic neuritis (MOGAD-ON+): longitudinal dynamics of GCL and RNFL among 9 eyes affected by optic neuritis (4 eyes lost at FU)

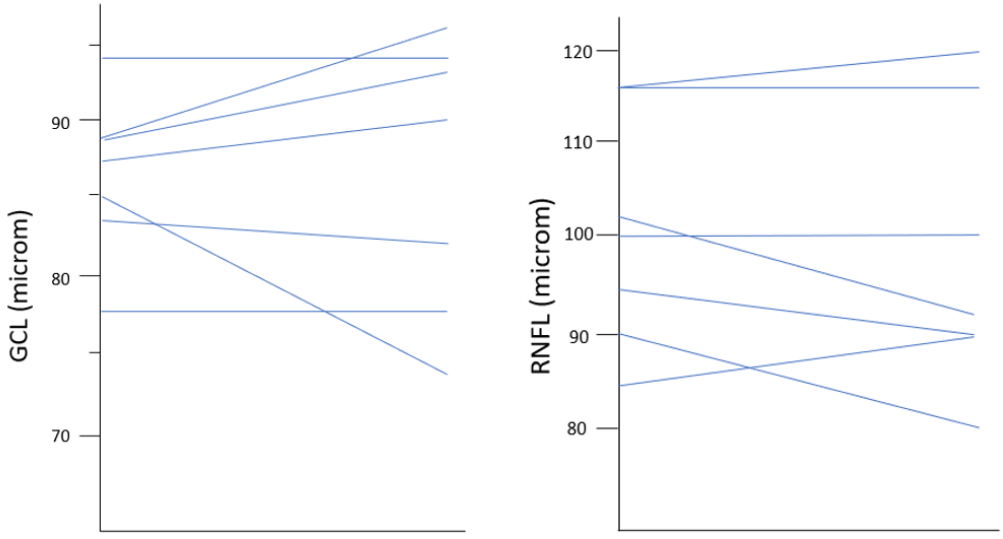

Figure 14 – Patients with history of optic neuritis (MOGAD-ON+): longitudinal dynamics of GCL and RNFL among 7 eyes not affected by optic neuritis (2 eyes lost at FU)

Performing a point-biserial analysis, we did not find significant differences in GCL and RNFL variation rates when comparing MOG ON+ and MOG ON- groups (point-biserial coefficient=-0.2, p-value=0.19

for GCL variation rate; point-biserial coefficient=-0.2, p-value=0.52 when considering RNFL variation rate).

We analysed the correlation between MOG ab titres and GCL and RNFL annualized variation rates in the two subgroups of eyes with and without history of optic neuritis. Considering 9 eyes with history of optic neuritis, we did not find a strong correlation between MOG ab titres at disease onset and GCL and RNFL thinning rate, as shown in figure 15 and 16 below.

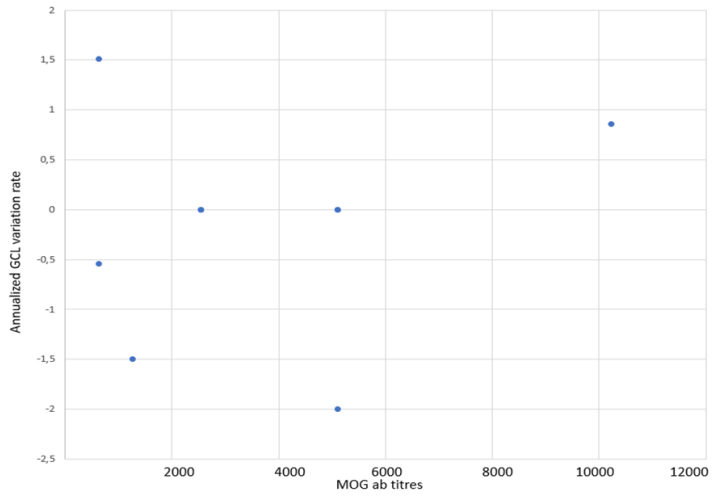

Figure 15 – Patients with history of optic neuritis (MOGAD-ON+): we did not find a strong correlation between MOG ab titres at disease onset and annualized GCL variation rate (Pearson correlation coefficient = 0,05, p-value=0.3)

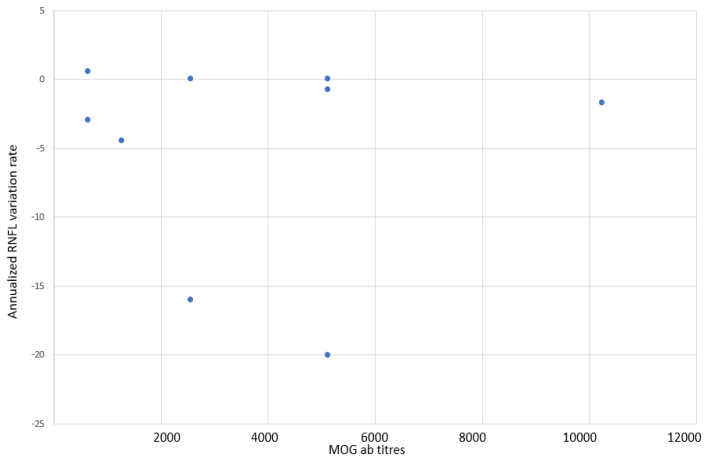

Figure 16 – Patients with history of optic neuritis (MOGAD-ON+): we did not find a strong correlation between MOG ab titres at disease onset and annualized RNFL variation rate (Pearson correlation coefficient=0.06, p-value=0.19)

These findings also applied when assessing correlation between MOG ab titres' annualized variation

rate and GCL and RNFL thinning rates. In fact, Pearson analysis did not show a significant correlation between these variables (Pearson correlation coefficient=-0.04, p-value=0.23 considering GCL variation rate; Pearson correlation coefficient=-0.03, p-value=0.36 considering RNFL variation rate).

Similarly, considering eight eyes without optic neuritis, we did not find a strong Pearson correlation between MOG ab titres at disease onset and GCL and RNFL variation rate (for GCL variation rate: Pearson correlation coefficient= -0.3, p-value=0.3; for RNFL variation rate: Pearson correlation coefficient=0.03, p-value=0.9), nor between MOG ab titres variation rate and GCL and RNFL thinning rates (Pearson correlation coefficient=-0.3, p-value=0.38 considering GCL variation rate; Pearson correlation coefficient=0.3, p-value=0.39 considering RNFL variation rate).

Disease course and retinal degeneration

Considering the subgroup of 12 patients with a follow-up OCT, we compared OCT thicknesses variation rates in monophasic and relapsing patients. As shown in figure below, we found a strong positive association between annualized GCL variation rate and the relapsing course of disease (Pearson correlation coefficient=0.7, p-value=0.01 – figure 17). We did not find a similar result considering annualized RNFL variation rates (Pearson correlation coefficient=0.3, p-value=0.2).

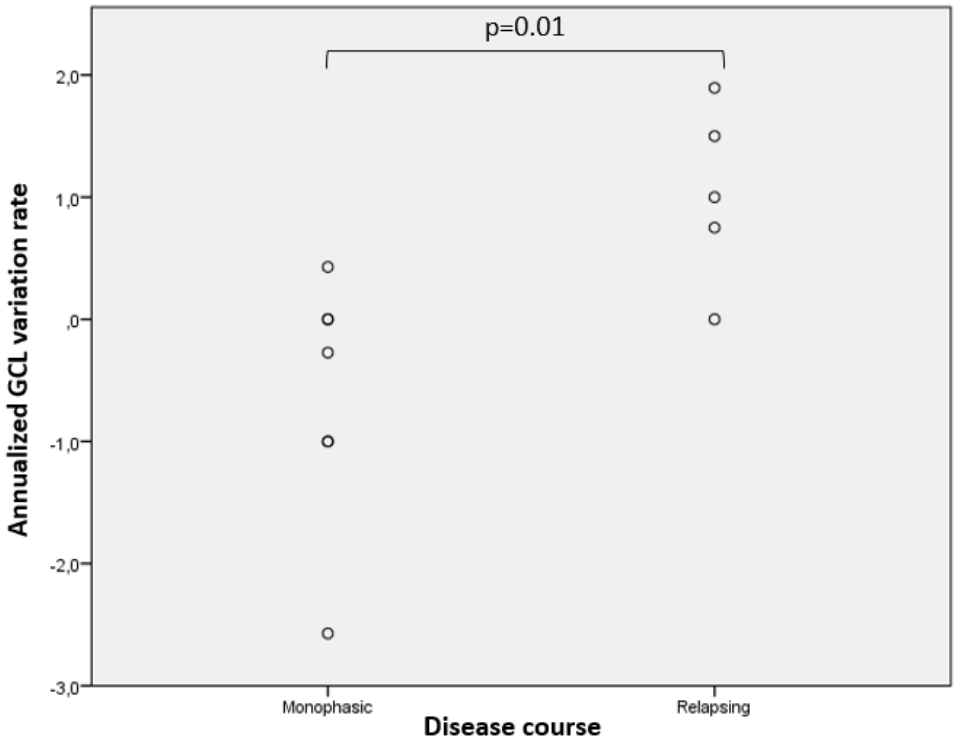

Figure 17 – Scatterplots showing mean GCL variation rate (μm/yr) according to disease course.

This result also applies when splitting our cohort into MOG-ON+ and MOG-ON- subgroups. Indeed, performing a point-biserial analysis of GCL variation rates among the group of patients with optic neuritis, we found a positive trend of correlation (even if not statistically significant) in patients with the relapsing form of disease (point-biserial correlation=0.68, p-value=0.06). Considering the group of patients without optic neuritis, we found a slight positive trend of correlation of GCL thinning rate in the relapsing group, even if this data did not reach statistical significance (point-biserial correlation coefficient =0.4, p-value=0.37). Figure below shows mean GCL variation rates according to different disease course in patients with (A) and without (B) optic neuritis.

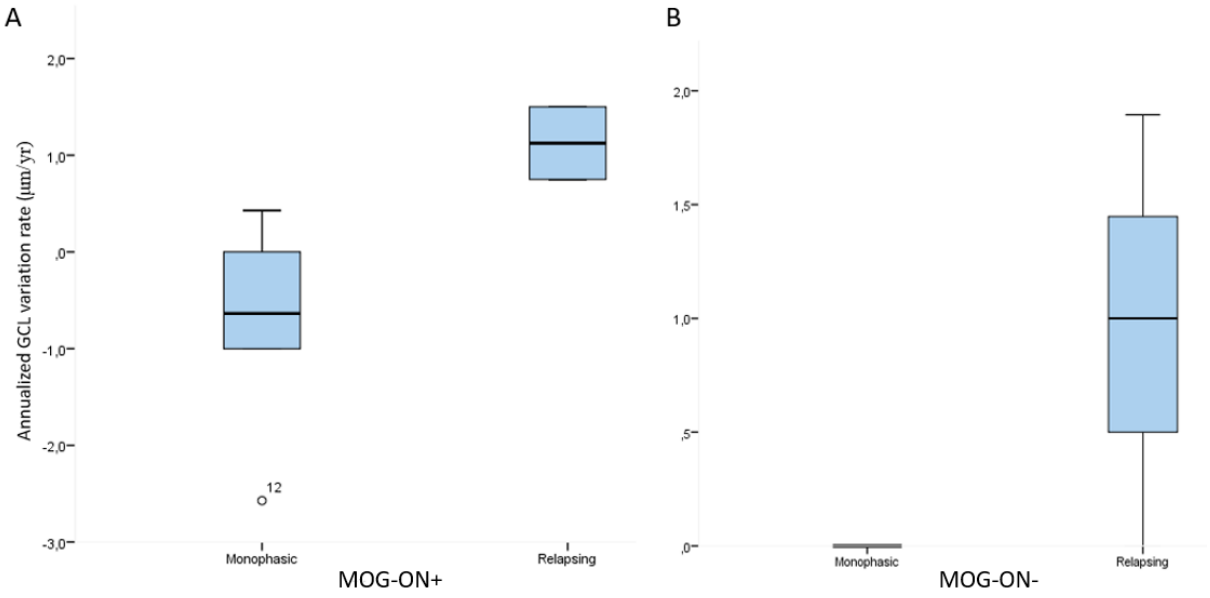

Figure 18 – Mean GCL annualized variation rate (µm/yr) according to disease course in patients with (A) and without (B) optic neuritis. A: mean GCL variation rate in monophasic=-0.7 (SD 1), in relapsing=1.1 (SD 0.5), p-value=0.06. B: mean GCL variation rate in monophasic=0, in relapsing=0.9 (SD 0.9), p-value=0.4

Disability progression and retinal degeneration

In our cohort mean EDSS at clinical onset was 1.8 (SD 1.3). At clinical follow-up performed at mean 44 months (SD 42) since disease onset, 7 patients (43%) experienced EDSS improvement, in 7 patients (43%) EDSS was stable, and the remaining 2 patients (14%) underwent EDSS worsening. EDSS change at the end of follow-up among the MOGAD cohort is described in figure.

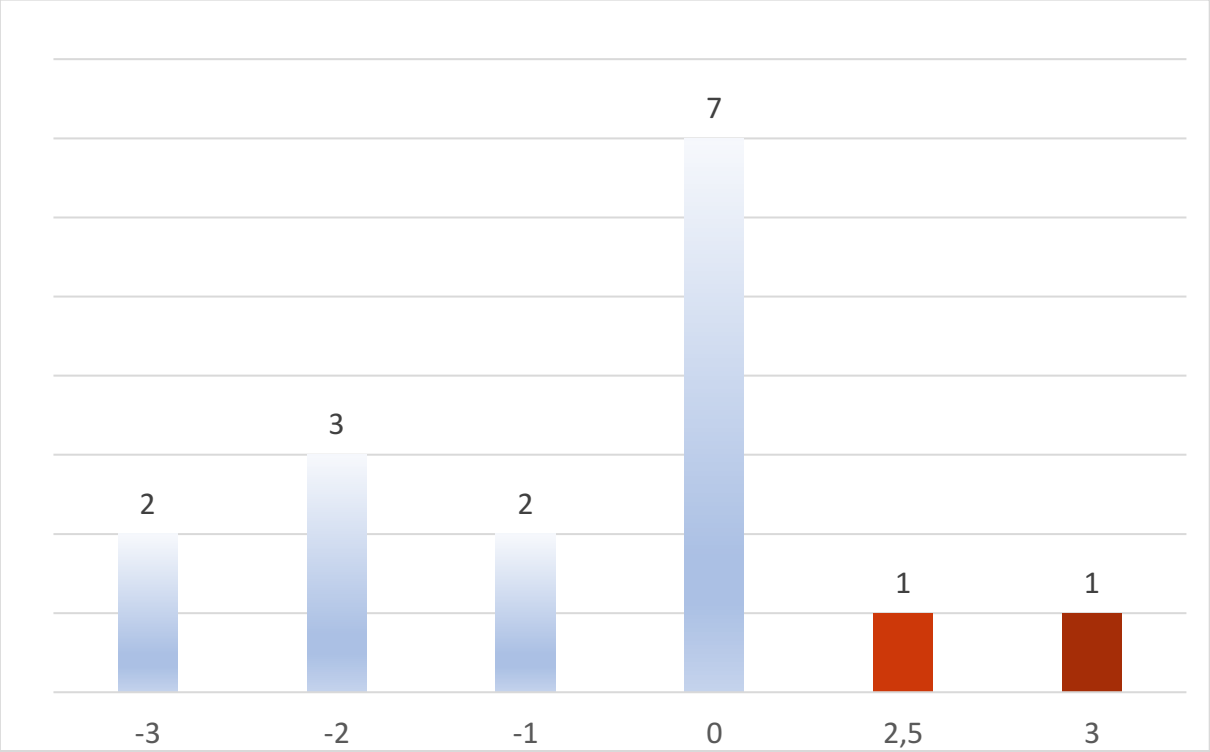

Figure 19– EDSS change among the MOG-AD cohort at follow-up.

We divided our cohort into two groups, according to the overall MOG-ab titres trend at the end of follow-up (the "increased" and "reduced" groups) and analysed proportion of the two groups according to EDSS variation at follow-up: we did not find significative differences ($p = 0.199$) in the longitudinal dynamics of MOG antibody titres in relation with disability progression (figure 20). However, all patients experiencing EDSS progression showed increased MOG ab titres at follow-up.

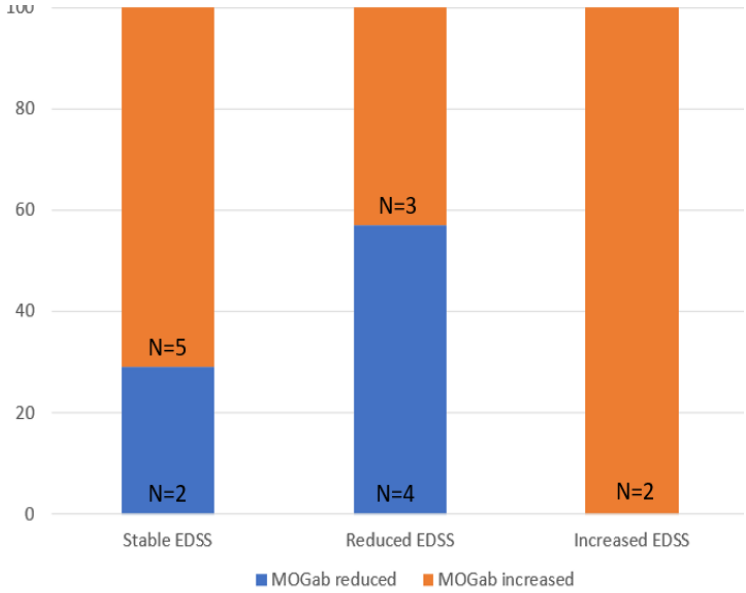

Figure 20- Proportion of MOGab titres "reduced" and "increased" groups according to disability progression.

Considering OCT parameters at baseline, mean GCL and RNFL thicknesses were lower in the group of patients experiencing disability progression at the end of follow-up (74 µm -SD 1.3-and 79 µm-SD 2-, respectively) in comparison with patients with stable or reduced EDSS value (86 µm-SD 1.5- and 95 µm -SD1.9-, respectively), even if this analysis did not reach a statistical significance (p-value=0.09 and 0.08, respectively).

We then considered OCT parameters dynamics and performed a point biserial correlation between annualized GCL variation rate and disability progression. According to this analysis, annualized CGL variation rate was significantly higher in patients with disability progression at the end of follow-up (point biserial coefficient: 0.6, p-value=0.03). Moreover, we observed that mean GCL variation rate was significantly higher in patients with disability progression, as shown in figure below. This result did not apply to annualized RNFL variation rate, even if a positive trend of association was observed (point biserial coefficient: 0.4, p-value=0.19).

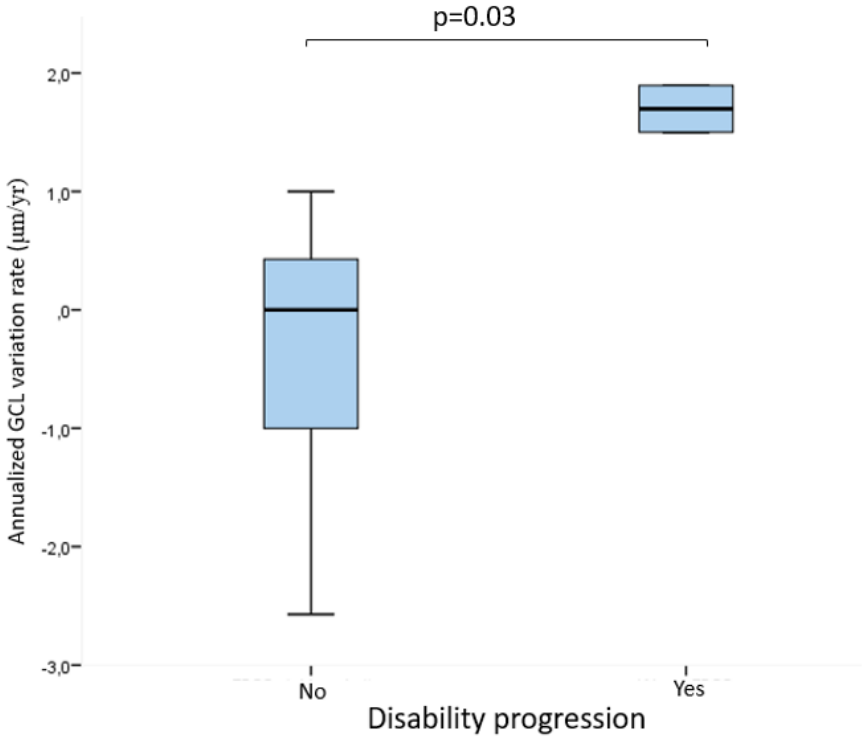

Figure 21- Boxplot showing mean GCL variation rate in patients with (1.7 µm/yr, SD 0.2) and without (-0.2 µm/yr, SD 1) disability progression.

The relapsing-remitting multiple sclerosis (RRMS) cohort

Our MS cohort was made up 21 patients (F/M: 19/2) with a mean age of 34 years (SD 7.9). 52% of patients (N=11) experienced monolateral ON at disease onset. Among the other 10 patients: one experienced myelitis with motor symptoms, 5 patients experienced myelitis with sensitive onset, 2 had a troncoencephalic onset. One patient underwent motor symptoms and one sensory symptoms not due to myelitis.

The distribution of disease-modifying treatments (DMT) among this cohort is shown in figure.

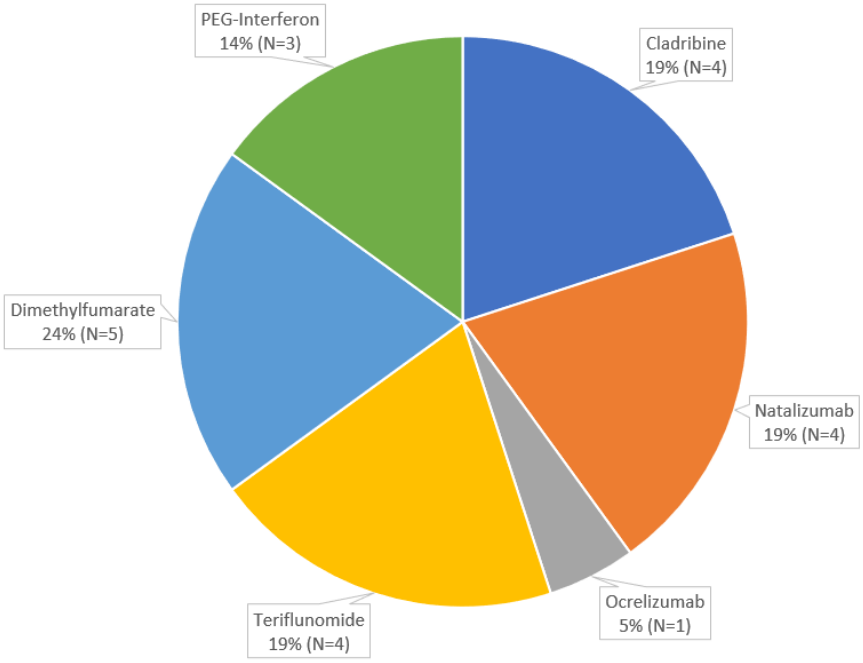

Figure 22- Distribution of DMTs among the RRMS cohort (21 patients)

At the end of mean 40 months of clinical follow-up (SD 45), 13 patients (62%) experienced a clinical relapse.

All patients underwent a baseline OCT at 24 mean months (SD 2.7) since disease onset, and 17 patients (80%) underwent a follow-up OCT performed at mean 11 months (SD 4) since the first one.

RRMS- ON+ and RRMS-ON- groups

The RRMS-ON+ group was made up of 11 patients, all experiencing monolateral optic neuritis. For 8/11 patients a follow-up OCT was available, at mean 11 months (SD 4.3) since the first one. Longitudinal dynamics of GCL and RNFL thicknesses in 8 eyes affected by ON and 8 eyes not affected are shown in figures below.

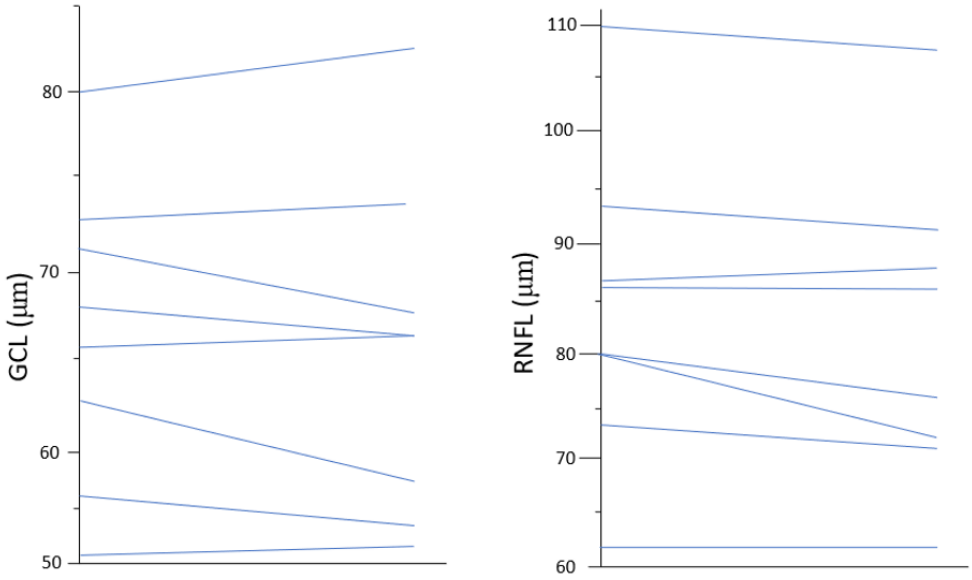

Figure 23- Longitudinal dynamics of GCL and RNFL thicknesses among 8 eyes with history of optic neuritis (RRMS-ON+ group).

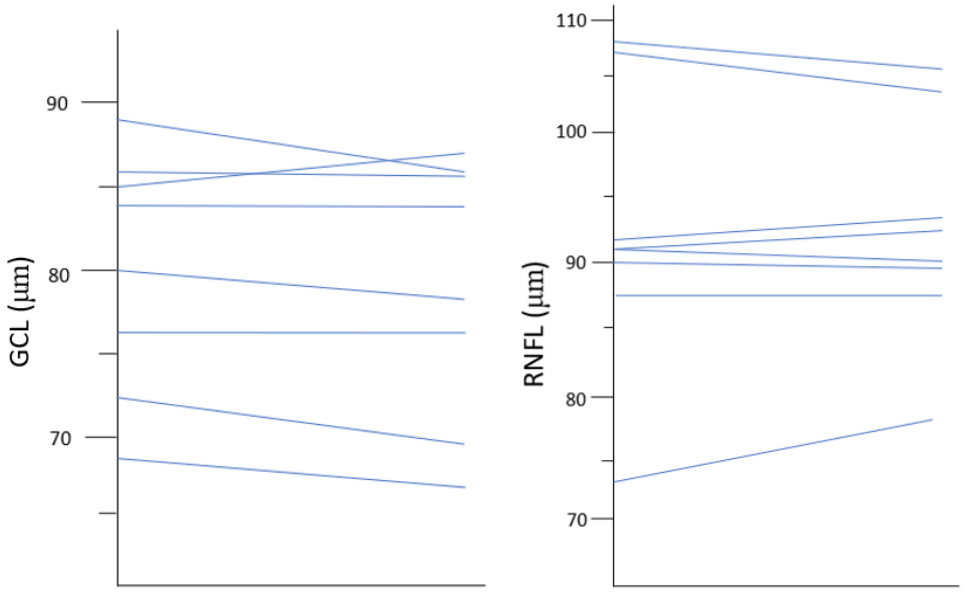

Figure 24- Longitudinal dynamics of GCL and RNFL thicknesses among 8 eyes without history of optic neuritis (RRMS-ON+ group).

Among our RRMS cohort, 10 patients did not experience optic neuritis during follow-up. Among them, 6 patients underwent a follow-up OCT at mean 11 months since the first one. Longitudinal dynamics of GCL and RNFL thicknesses among 12 eyes not affected by ON is shown in figure below.

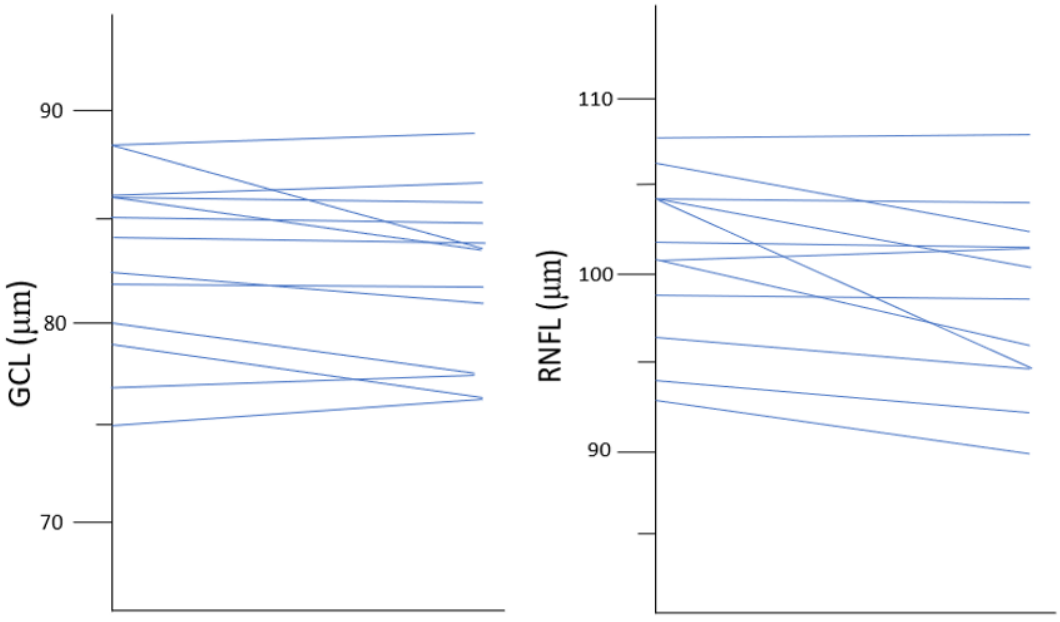

Figure 25- Longitudinal dynamics of GCL and RNFL thicknesses among 12 eyes without history of optic neuritis (RRMS-ON- group).

Disability progression and retinal degeneration

Among our cohort of 21 RRMS patients, mean EDSS at clinical onset was 1 (SD 0.1). At clinical follow-up, obtained at 40 mean months since disease onset, 15 patients (71%) showed stable EDSS value, while the remaining 6 patients (29%) underwent disability progression of 1 mean point (SD 0.6).

Mean RNFL and GCL thicknesses at baseline were not correlated with EDSS variation at follow-up (Pearson correlation -0.3, p=0.1 for RNFL; Pearson correlation -0.3, p=0.1 for GCL). Neither annualized RNFL and GCL variation rates were correlated with EDSS variation in our cohort (Pearson correlation 0.3, p=0.2 and Pearson correlation 0.1, p=0.6, respectively).

MOGAD cohort versus RRMS cohort

Considering baseline characteristics of the cohorts, we did not find statistical differences between the two groups, except for sex and baseline EDSS, as shown in table below.

	RRMS (n=21)	MOGAD (n=16)	P value
Females, N(%)	19 (90.5)	10 (62.5)	0.04
Age, mean ys (SD)	34 (8)	38 (10)	0.29
Baseline EDSS, mean (SD)	1 (0.1)	1.8 (1.3)	0.007
Disease onset, N (SD) monolateral ON bilateral ON myelitis sensory symptoms motor symptoms myelitis + periferic symptoms troncoencephalic symptoms	 11 (52) 0 (0) 6 (28.5) 1 (5) 1 (5) 0 (0) 2 (9.5)	 8 (50) 2 (12.5) 5 (31) 0 (0) 0 (0) 1 (6.5) 0 (0)	0.35
Disease course-relapsing, N (%)	8 (38)	8 (50)	0.46
Clinical follow-up, mean months (SD)	40 (45)	49 (40)	0.9
OCT follow-up, mean months (SD)	32 (3.6)	36 (3.1)	0.9

Figure 26- Baseline characteristics of RRMS and MOGAD cohort.

Comparing RRMS and MOGAD cohort, we did not find significant differences between mean GCL and RNFL thicknesses at baseline and at follow-up: mean GCL thickness was 77 µm (SD 8) in RRMS and 78 µm (SD 7) in MOGAD (p-value=0.6). Mean RNFL thickness was 92 µm (SD 12) in RRMS patients and 93 µm (SD 11) among MOGAD cohort (p-value=0.8). Moreover, considering annualized GCL and RNFL variation rates we did not find strong correlation between retinal degeneration and type of disease (point biserial correlation = 0.2, p=0.16 considering annualized GCL variation rate; point biserial correlation = -0.04, p=0.83 considering annualized RNFL variation rate).

Patients with history of optic neuritis

Affected eyes

We considered 11 eyes with optic neuritis belonging to RRMS cohort, and 13 eyes belonging to the MOGAD cohort (9 eyes belonging to 9 patients with monolateral optic neuritis and 4 eyes belonging to 2 patients with bilateral optic neuritis). As observed in the whole cohort, we did not find significative differences between mean baseline OCT thicknesses among the two groups, neither at baseline nor at follow-up.

Figure below shows GCL and RNFL annualized variation rates in the RRMS and MOGAD groups. Comparison was performed thorough a point biserial analysis and showed no significative differences between GCL and RNFL thinning rates in the two groups (p=0.38 and p=0.85, respectively).

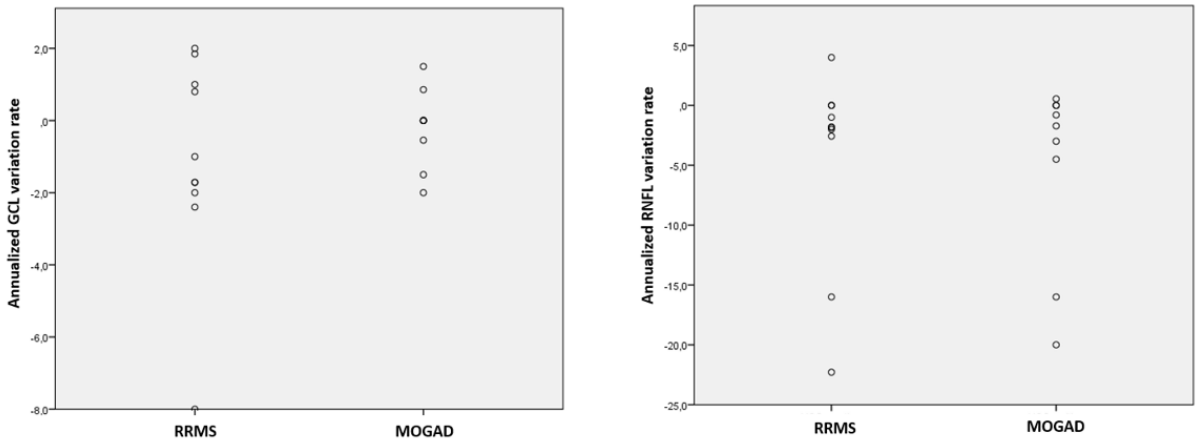

Figure 27- Scatterplots showing annualized GCL and RNFL variation rates (μm/yr) in RRMS and MOGAD groups considering eyes with optic neuritis

Patients without history of optic neuritis

Considering 15 patients without history of optic neuritis (10 RRMS and 5 MOGAD), a student t-test showed that mean GCL and RNFL thicknesses at baseline were similar between the two groups: (mean GCL 82 µm in both groups, p=0.77; mean RNFL 98 µm in RRMS and 101 µm in MOGAD; p=0.57). This result also applied considering mean GCL and RNFL thicknesses at follow-up and the annualized GCL (figure 27) and RNFL variation rates in the two groups. However, we found a weak positive trend (point biserial correlation coefficient=0.4) of correlation considering GCL thinning rate in MOGAD patients compared with RRMS patients.

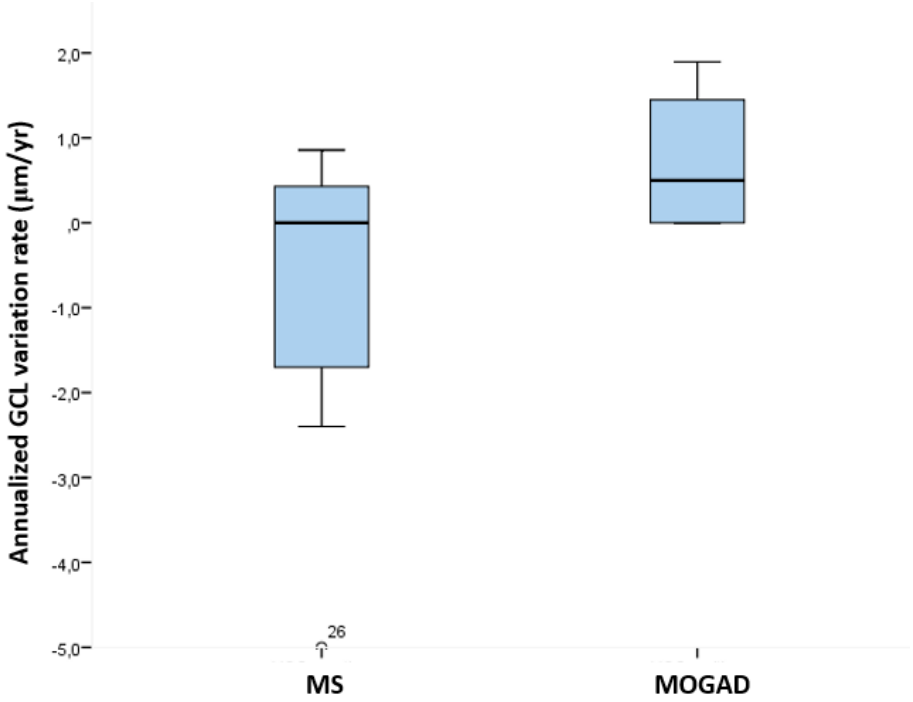

Figure 28- Boxplot showing mean GCL annualized variation rates in patients with MS and MOGAD. MS=-0.9 (SD2). MOGAD=0.7(SD 0.9); p-value=0.2

Eyes without history of optic neuritis

The group of eyes without history of optic neuritis was made up of 54 eyes: 31 (57%) belonged to RRMS patients, the remaining 23 (43%) belonged to the MOGAD group. A follow-up OCT was available for 41 patients (24 RRMS and 17 MOGAD) at mean 10 months since the first one (SD 4.6) Baseline and follow up mean GCL and RNFL thicknesses are similar in the two groups, as showed in figure below.

A

	RRMS	MOGAD	p-value
Mean GCL thickness, µm (SD)	81 (7.1)	89 (7.8)	0.4
Mean RNFL thickness, µm (SD)	97 (10)	96 (10)	0.9

B

	RRMS	MOGAD	p-value
Mean GCL thickness, µm (SD)	81 (5.8)	82 (8)	0.6
Mean RNFL thickness, µm (SD)	96 (7.6)	98 (10)	0.3

Figure 29- Considering the group of unaffected eyes**,** mean GCL and RNFL thicknesses at baseline (A) and at follow-up (B) are similar between RRMS and MOGAD.

When considering retinal degeneration at follow-up, we found a significant higher annualized GCL variation rate in the group of MOGAD patients (figure 30). However, this result did not apply when considering RNFL variation rate and did not associate with MOGab titres annualized variation rate (Pearson correlation coefficient=-0.1, p-value=0.5).

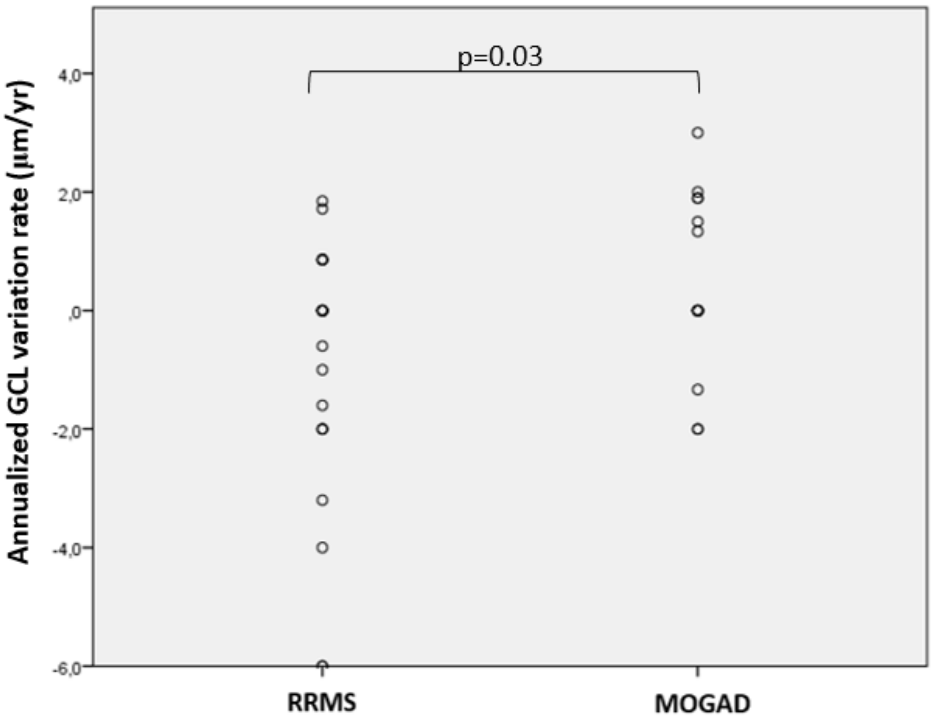

Figure 30- Annualized GCL variation rate in RRMS and MOGAD patients (point biserial correlation coefficient=0.4, p value=0.03)

Fellow Eyes

We then considered 24 fellow eyes (11 RRMS and 13 MOGAD) and compared OCT thicknesses among RRMS and MOGAD cohort. Mean GCL and RNFL thicknesses at baseline were similar in the two groups (mean GCL: 79 -SD 9- µm in RRMS vs 78-SD 19-µm in MOGAD, p-value=0.8; mean RNFL: 94 µm-SD 12-in RRMS vs 91 µm-SD 91-in MOGAD, p-value=0.6).

Similarly, considering 19 eyes with available follow-up OCT (10 RRMS and 9 MOGAD), we did not find significative differences between GCL and RNFL thicknesses at follow-up (mean GCL 80-SD 7-µm in RRMS vs 81 µm-SD 19-in MOGAD, p-value=0.7; mean RNFL 95 µm-SD 9-in RRMS and 96-SD13-µm in MOGAD, p-value=0.7). These results also applied when considering mean GCL and RNFL variation rates (mean GCL variation rate: 0.8 µm/yr-SD 2- in RRMS and 0.05 µm/yr (SD 1) in MOGAD, p-value=0.3; mean RNFL variation rate: 0.05 µm/yr (SD 3) in RRMS and 0.33 µm/yr (SD 7) in MOGAD, p-value=0.1). Similarly, a point biserial correlation analysis did not show significative correlations between GCL and RNFL thinning rates and type of disease (point-biserial correlation coefficient=0.2, p-value=0.35 considering GCL variation rate; point-biserial correlation coefficient=0.3, p-value=0.1 considering RNFL variation rate).

Unaffected eyes

Considering 30 eyes belonging to patients without history of optic neuritis (20 RRMS and 10 MOGAD), mean GCL and RNFL thicknesses were similar between groups at baseline and at follow-up. Indeed, at baseline mean GCL thickness was 82 µm in both groups (p-value=0.9); and RNFL thickness was 98 (SD 7) µm in RRMS and 101 (SD 5) µm in MOGAD (p-value=0.3). At follow-up (available for 22 eyes, 14 RRMS and 8 MOGAD), mean GCL was 82 µm (SD 4) in RRMS and 83 (SD 3) µm in MOGAD (p-value=0.4). However, considering GCL variation rate, it was significantly higher in MOGAD patients, as shown in figure below.

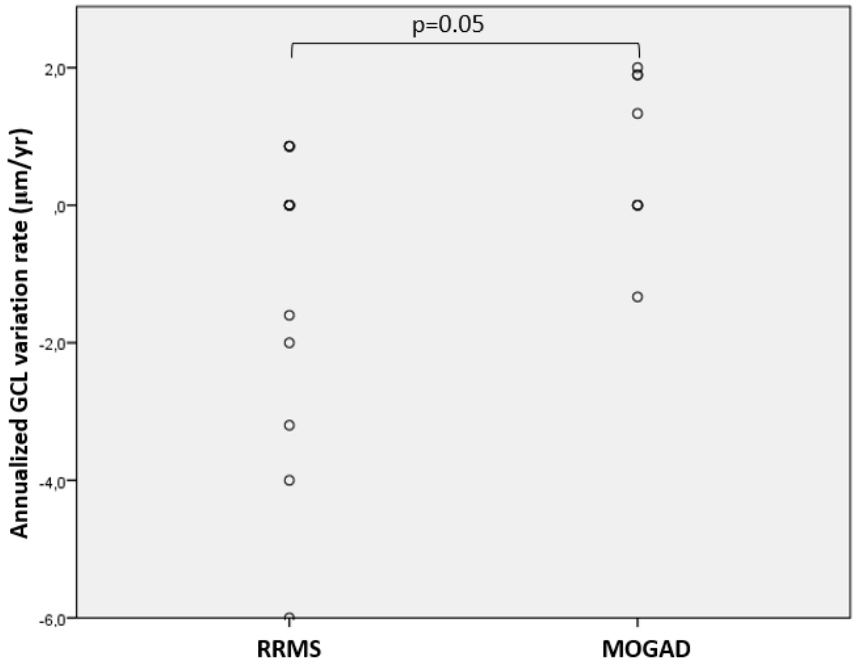

Figure 31- Annualized GCL variation rate in RRMS and MOGAD patients in unaffected eyes (point biserial correlation coefficient=0.4, p value=0.05)

These results did not apply when considering RNFL variation rates (point-biserial correlation coefficient=0.2, p-value=0.2).

Discussion

In this study we analysed and compared OCT findings in a monocentric cohort of patients with acquired demyelinating diseases (MOGAD and RRMS). We tried to assess the longitudinal variation of MOG ab titres, calculated through the annualized variation rate, and to correlate it with the longitudinal modifications of OCT parameters. Whilst data about OCT thicknesses and GCL and RNFL thinning in multiple sclerosis cohorts are extensive, this field in MOG ab associated disease is still matter of debate. Indeed, in MOGAD optic neuritis the pathology of afferent visual system damages mediated by MOG-Abs is less well understood, and a final consensus regarding retinal degeneration has not been reached. One of the reasons of this uncertainty might be related to the small sample sizes of the studies published so far, thus leading to conflicting results.

The MOGAD cohort and the prognostic meaning of MOG ab titres

Demographic characteristics of our MOGAD cohort are in line with other cohorts published in literature so far. Indeed, sex, mean age, clinical onset and relapses occurrence mirror what already described by other groups [Cobo-Calvo et al; Jarius et al; Jurynczyk et al]. However, despite what has been described in Ramanathan's cohort [Ramanathan et al, 2018], in our cohort only 2 out of 16 patients experienced bilateral optic neuritis.

We longitudinally monitored MOG ab titres and described longitudinal dynamics among the relapsing and the monophasic groups of patients. Considering MOG ab titres dynamics, we did not find a common trend in the two groups, and we did not find significative differences in MOG ab titres annualized variation rate between the two groups. Moreover, when considering the overall evolution of titres at the end of follow-up (the "reduced" titre group and the "increased" titre group), we did not find significative differences among the monophasic and relapsing patients. The meaning of monitoring MOG ab titres is still matter of debate, but a growing amount of studies highlights its prognostic utility. In 2019 Olivera et al demonstrated, in a cohort of 31 MOG ab positive patients, that the risk of clinical relapse was associated with longitudinally persistent MOG-IgG seropositivity. In contrast, patients who experienced a single clinical attack became spontaneously seronegative for MOG-IgG during long-term follow-up [Oliveira et al]. Moreover, a recently published multicentre Italian study including more than three hundred serum samples of MOGAD patients showed that persistent MOG-IgG positivity and high remission titres were associated with an increased relapse risk, while onset titres did not correlate with relapses [Gastaldi et al]. Despite the fact that MOG ab titres dynamics in our cohort did not seem to be correlated with clinical course, we observed that the occurrence of clinical relapse was, in most of the cases, preceded by an increasing in MOG ab titres. This could further corroborate the concept that monitoring MOG ab titres could be useful, mostly when considering the intra-patient variability.

Moreover, an increasing in MOG ab titres could suggest us to actively monitor our patient in order to perform a prompt intervention in case of new symptoms.

MOG ab titres, retinal degeneration and disease course

In our cohort we did not find any correlation between MOG ab titres and GCL and RNFL thicknesses at baseline; moreover, considering follow-up OCT data, we did not find a significant correlation between MOG ab titres variation rate and GCL and RNFL thinning rates. Previous literature regarding OCT thicknesses dynamics is made up of several studies that analyse GCL and RNFL variations in MOGAD cohorts without considering MOG ab titres. According to these studies, retinal degeneration is considered the result of several episodes of optic neuritis [Akaishi et al, Stiebel-Kalish et al], and is partially explained by remission of retinal edema [Oertel et al]. Evidence of retinal thinning in MOGAD patients without optic neuritis has not been completely described and explained, but several studies conducted in MS patients show that a subclinical retinal degeneration can occur even in eyes without previous ON [Petzold et al; Britze et al, 2017]. In these cases, it may be due to primary degeneration of the GCL neurons, or to lesions in the optic radiation that could lead, via trans-synaptic degeneration, to loss of retinal ganglion cells and their axons [Puthenparampil et al].

When we focused on the subgroup of MOGAD relapsing patients, we found that GCL variation rate was more pronounced in comparison with monophasic patients, thus meaning that neuronal degeneration could be faster when occurs in patients with more than one inflammatory event. This result was similar, even if not statistically significant, when considering the group of patients with history of optic neuritis: we found a strong positive correlation between GCL variation rate and the relapsing course. To a lesser extent, these data also applied when considering the group of patients without history of optic neuritis. According to these data we may hypothesize that retinal degeneration in MOGAD could be independent of prior optic neuritis, but it may be accelerated by the occurrence of several inflammatory events, independently of the district in which they occur.

Comparing MOG-ON+ and MOG-ON- groups, even if GCL and RNFL thicknesses at baseline and follow-up were lower in MOG-ON+, we did not find significant differences between GCL and RNFL annualized variation rates. This data don't agree with what already published [Oertel et al]. We could explain these conflicting results considering that in literature retinal degeneration in eyes with optic neuritis has been often considered the result of remission of post-inflammatory edema. Since in our cohort mean follow-up duration was longer than in Oertel's study, we could suppose that retinal edema in our cohort was already resolved, thus explaining why retinal degeneration could be similar in the two groups.

We did not find significative correlations between MOG antibody titres at baseline and the occurrence of optic neuritis. Moreover, according to our data, MOG ab dynamics over time did not correlate with retinal degeneration in the two groups.

All these findings may indicate that, in MOGAD, retinal degeneration could be accelerated by the occurrence of repeated inflammatory episodes, independently of the affected district and of the occurrence of optic neuritis. In this context, the utility of measuring MOG ab titres is still uncertain, as it seem that it doesn't reflect the severity of retinal degeneration over time, even in eyes with history of optic neuritis. Moreover, the similarity of retinal thicknesses and retinal degeneration rates at follow-up in MOG-ON+ and MOG-ON- could be explained considering that optic neuritis in MOGAD seems to have a good functional and morphological prognosis [Garg et al; Martinez-Lapiscina et al; Oertel et al]. Despite these considerations, we have also to consider that our cohort is relatively small: further studies are needed in order to assess in larger cohorts the differences between eyes with and without optic neuritis and the power of MOG ab titres in predicting retinal degeneration.

MOG ab titres, retinal degeneration and disability progression

Seventeen per cent of patients in our MOGAD cohort underwent disability progression at the end of follow-up. We did not find significative differences in MOG ab titres when considering EDSS variation in our cohort. However, all patients experiencing disability progression showed increased titres at the end of follow-up. This could be explained by the fact that, in literature, higher MOG ab titres were found during relapses [Gastaldi et al], that are thought to be the leading cause of disability progression in MOGAD [Marignier et al]. Moreover, these data further corroborate the concept that monitoring intra-patient titres could be of prognostic utility in predicting disease course.

We observed lower GCL and RNFL baseline thicknesses in patients with disability progression, even if this data did not reach statistical significance. Moreover, GCL thinning rate was significantly higher in patients with increased EDSS at follow-up. This result was not replicated considering RNFL thinning. It is well known that, in multiple sclerosis patients [Eklund et all] retinal degeneration correlates with disability progression independently of occurrence of optic neuritis. Oberwahrenbrock et al demonstrated, among a large cohort of MS patients without prior optic neuritis, that RNFL thickness was lower in patients with secondary progressive MS compared with patients with relapsing-remitting MS, and that total macular volume was reduced in progressive MS compared to the relapsing-remitting form. However, data about retinal thinning and disability progression in MOGAD cohorts are still scarce. Considering that, in our cohort, we found higher GCL degeneration rates in relapsing patients, and taking into account that disability accumulation in MOGAD patients is often due to relapses, we

may hypothesize that retinal degeneration and disability progression in MOGAD could be, at least in part, the result of the recurrence of inflammatory episodes.

According to our results, unlike GCL, RNFL thinning rate was not associated with disability progression. This could be explained by the fact that RNFL reduction has been previously interpreted as the result of edema resolution after an inflammatory episode [Vicini et al]. Moreover, previous literature showed that RNFL could be less reliable than GCL in assessing neurodegeneration [Saidha et al].

The MOGAD cohort versus the RRMS cohort

In our study we performed a comparative analysis between our MOGAD cohort and a RRMS cohort of patients. Baseline characteristics of the two cohorts were similar, except for sex and baseline EDSS. Indeed, MOGAD patients were more likely males and slightly more disabled than RRMS patients. However, mean age of the cohorts was similar, as disease and follow-up duration. About one third of RRMS patients underwent a slight (mean 1 EDSS point) disability progression at follow-up, and we did not find a significant correlation between OCT thicknesses and dynamics and disability progression. These data differ with what already published in literature: some studies demonstrated that retinal degeneration was independent of previous optic neuritis [Petzold et al; Britze et al, 2017] thus mirroring disability progression. However, it is demonstrated that, in the progressive form of multiple sclerosis, retinal degeneration is faster when compared with the relapsing form of disease [Oberwahrenbrock et al, Saidha et al]. Thus, we could explain our data considering that the slight disability progression in our RRMS cohort could be the result of previous relapses, more than representing a neurodegenerative process independent of relapsing activity.

Eyes with history of optic neuritis

When considering eyes with history of optic neuritis, mean retinal thicknesses at baseline and at follow-up were similar between the two groups, as annualized GCL and RNFL variation rates. This result reflects what already published in literature. Indeed, Vicini et al analysed morphological characteristics in eyes affected by optic neuritis in MS and MOGAD patients. Despite a worse visual outcome at nadir in MOGAD patients, a similar thinning of the macular ganglion cell layer at follow-up was shown. Nevertheless, a significantly thinner global RNFL was found in MOGAD patients [Vicini et al]. These results were similar to what we found in our cohort; moreover, the similarities in GCL and RNFL thicknesses at follow-up may be explained by the fact that, despite a worse visual outcome at nadir, optic neuritis in MOGAD patients globally shows a good prognosis with a good visual function recovery. However, our findings regarding RNFL thicknesses were different from Vicini's study: this could be due to the different follow-up duration, that was slightly longer in our cohort, thus eliminating

the confounding effect of edema resolution. On the contrary, Havla et al supports a prominence in retinal degeneration after optic neuritis in MOGAD in comparison with MS. We have to consider although that this cohort showed a longer disease duration (97 mean months) and a higher mean EDSS (2.5), factors that could be associated with an enhanced retinal degeneration, and that could explain the differences with our results.

Eyes without history of optic neuritis

When considering eyes without optic neuritis, we found that, even if baseline and follow-up RNFL and GCL were similar, annualized GCL variation rate was higher in MOGAD patients. We divided this cohort into two groups ("fellow eyes" and "unaffected eyes") and observed that this difference was not significative considering fellow eyes, while GCL degeneration was faster in MOGAD patients when considering unaffected eyes.

These results could suggest that GCL degeneration in MOGAD and MS fellow eyes, similarly to what happens in affected eyes, could occur through similar physiopathological mechanisms. In MS it has been hypothesized that GCL thickness reduction could be caused by retrograde degeneration after a mild, subclinical optic neuritis [Britze et al]; another theory suggests that OCT alterations could be due to primary degeneration of the GCL neurons due to MS. Other studies suggested that lesions in the optic radiation via trans-synaptic degeneration could lead to loss of retinal ganglion cells and their axons [Puthenparampil et al].

Notably, we found that, in eyes without history of optic neuritis, GCL thinning rate was higher in patients with MOGAD. This result could be explained, as described above, by the accumulation of inflammatory non-ON episodes, but raises the question if, in MOGAD, a pure neurodegenerative process could occur. Since retinal ganglion cells are myelinated by oligodendrocytes when the bundle passes through the lamina cribrosa, retinal changes in MOGAD may be explained also by a retrograde degenerative process [Yao et al]. According to this theory, GCL variation rate could mirror a neurodegenerative involvement in MOGAD patients, that coexist with the accumulation of inflammatory episodes. Although there are several studies in literature that demonstrate the retinal degeneration in progressive MS [Oberwahrenbrock et al, Corci et al], thus reflecting a well-known neurodegeneration process that is independent of relapses, in MOGAD data regarding neurodegeneration are still lacking. Our data allow us to hypothesize that this neurodegenerative process could exist and perpetrate even in patients with MOGAD, thus considering that disability progression could not be caused only by clinical relapses. Further studies as warranted in order to give a better framework to neurodegeneration in MOGAD patients, not only through OCT follow-up, but also through assessment of CNS volumes with magnetic

resonance data and through longitudinal analysis of other neurodegeneration biomarkers as, for instance, neurofilaments.

Limitations of the study

Limitations of our study are the heterogeneity of MOG-IgG-seropositive patients with different clinical phenotypes in our cohort. Moreover, due to the rarity of MOG-IgG-seropositive patients in Italy, the sample size was small, leading to outliers possibly having a larger effect on the results. Additionally, our study lacks other neurophysiological tools assessing visual system (for instance, visual evoked potentials). MOGAD and RRMS cohorts differed in some baseline characteristics, as EDSS, even if baseline mean disability was low in both groups, and in preventive therapy.

Conclusions

In MOGAD patients:

1) Even if MOG ab titres dynamics do not seem to differ between monophasic and relapsing patients, we confirmed, as shown in literature, that the occurrence of a clinical relapse is often preceded by an increasing in MOG ab titres. Thus, monitoring MOG-ab titres could have a prognostic utility.
2) Retinal degeneration was higher in relapsing patients, independently of the occurrence of optic neuritis. Thus, retinal degeneration could be accelerated by inflammatory events, independently of the district in which they occur. MOG ab titres do not seem to correlate with retinal thicknesses and degeneration. In this context, monitoring MOG ab titres might not reflect the severity of retinal degeneration over time, even in eyes with history of optic neuritis.
3) All patients experiencing disability progression show increased MOG ab titres at the end of follow-up and faster GCL thinning rates. As titres increased during relapses and retinal degeneration accelerates after inflammatory episodes, these data, put together, further corroborate the concept that retinal degeneration and disability progression could be the result of recurrent relapses.

When comparing MOGAD and RRMS cohorts:

4) Retinal degeneration is similar in MOGAD and RRMS eyes with optic neuritis as in fellow eyes. Thus, we could infer that retinal degeneration, represented by GCL thinning rate, is independent of the triggering optic neuritis. Moreover, in fellow eyes, a similar physiopathological mechanism could occur, as trans-synaptic degeneration or subclinical optic neuritis.
5) In eyes of patients without optic neuritis retinal degeneration seem to be higher in MOGAD than in RRMS. This data allow us to question if, even in MOGAD, a pure neurodegenerative process could exist, thus considering that disability progression could not be caused only by clinical relapses.

All these results apply when considering GCL parameters, thus corroborating the already known concept that GCL is more reliable than RNFL in assessing neuronal degeneration.

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