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Assessing Optic Nerve Involvement in Multiple Sclerosis Using Optical Coherence Tomography and Magnetic Resonance Imaging

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ABSTRACT

Background: Optic nerve involvement in Multiple Sclerosis (MS) can be detected with Optical Coherence Tomography (OCT) based on intereye difference (IED). We aimed to assess optic nerve involvement in a large real-world MS cohort applying the 2024 McDonald IED cut off criteria, and to investigate OCT–MRI concordance.

Methods: OCT measurements of peripapillary-retinal-nerve-fiber-layer (pRNFL) and ganglion-cell-inner-plexiform-layer (GCIPL) thickness were analyzed in 740 MS patients. Clinical histories of optic neuritis (ON) were reviewed. Three-dimensional double inversion recovery MRI sequences for optic nerve assessment were available for 240 patients.

Results: The IED cutoff (GCIPL $\geq 4\,\mu m$ or pRNFL $\geq 6\,\mu m$) demonstrated 83% sensitivity and 55% specificity for identifying prior ON. In the MRI subgroup, it yielded 67% sensitivity and 83% specificity for detecting asymptomatic optic nerve lesions. MRI identified lesions in 46% of patients without a prior history of ON, whereas OCT-IED was positive in 40%, resulting in an overall OCT–MRI concordance of 77%. Among asymptomatic patients ($n = 164$), 25 were OCT-negative/MRI-positive (11 with bilateral optic nerve involvement) and 15 were OCT-positive/MRI-negative, mostly with isolated GCIPL or pRNFL asymmetry.

Conclusions: The 2024 McDonald IED cutoff demonstrates high sensitivity for prior ON but reduced sensitivity for subclinical optic nerve involvement. MRI appears slightly more sensitive, with both modalities providing complementary information on optic nerve pathology.

1 | Introduction

The optic nerve has been recognized as the fifth typical region for the demonstration of lesion dissemination in space (DIS) within the newly revised 2024 McDonald Multiple Sclerosis (MS) diagnostic criteria [1, 2]. Optic nerve MRI and optical coherence tomography (OCT), together with visual evoked potentials (VEPs), are considered structural paraclinical tools capable of detecting optic nerve lesions in addition to neuro-ophthalmological examinations [3]. Optic nerve MRI is recommended when MS-related Optic Neuritis (ON) is suspected, due to its high sensitivity in detecting symptomatic lesions [4], as demonstrating a typical demyelinating lesion in the optic nerve confirms the clinical suspicion [5]. Importantly, such a lesion can also serve as evidence

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of one anatomical location required for DIS, even in individuals without a clinical history of ON. Similarly, OCT can detect both post–ON retinal thinning and subclinical optic nerve involvement in patients without previous clinical ON [3], based on inter-eye differences (IED) of ≥6 μm in pRNFL and ≥4 μm in GCIPL thickness [1].

Different IED cutoff values have been investigated [6–11] to optimize the diagnostic accuracy of OCT in detecting optic nerve lesions. Most studies exploring reliable thresholds have tested the role of IED in identifying clinically manifest ON rather than asymptomatic optic nerve involvement. However, OCT would provide its greatest value when it is able to detect asymptomatic optic nerve involvement. Post-mortem evidence indicates that demyelinating plaques within the optic nerves are found in nearly all individuals with MS, irrespective of a history of prior ON [12, 13]. In vivo, retinal asymmetry has been observed in approximately 30% of patients without a clinical history of ON [6], and MRI studies have further reported asymptomatic optic nerve lesions in up to 50% of asymptomatic eyes [9, 14, 15].

Research investigating the relationship between asymptomatic MRI-detected optic nerve lesions and OCT-derived IED in patients without prior ON remains scarce, leaving the clinical applicability of the proposed cutoff values in asymptomatic patients poorly defined. To address this gap, our study aims to evaluate optic nerve involvement according to 2024 McDonald IED cutoff, in a large real-world MS cohort and to investigate OCT–MRI concordance in a subgroup of patients with available MRI, describing potential discrepancies between OCT and MRI findings.

2 | Methods

2.1 | Study Population

We retrospectively analyzed data from a prospective longitudinal cohort of 785 MS patients [16] who underwent structural spectral-domain OCT. For all patients, a detailed clinical history of multiple sclerosis–related optic neuritis (MS-ON) was retrieved from medical records. The diagnosis of MS-ON was based on clinical features and, when available, supported by paraclinical tests, most commonly VEPs [5]. In cases where information in the medical charts was limited (e.g., unreported laterality or atypical findings), patients were re-contacted to verify whether their symptoms were consistent with MS-ON. OCT and MRI data analyzed in the present study were not used to establish the diagnosis of MS-ON. Exclusion criteria were major ophthalmologic comorbidities (including iatrogenic optic neuropathy, diabetic retinopathy, or uncontrolled hypertension) and refractive errors >±6 diopters. The study was approved by the local ethics committee (ID 10346).

2.2 | Optical Coherence Tomography

Spectral-domain OCT (Spectralis, Heidelberg Engineering) imaging of undilated eyes was performed in a dark, enclosed room by a trained operator. The following scan protocols were used:

- **Peripapillary RNFL**: 360° circular scan located at 3.4 mm from the center of the optic nerve head; peripapillary measurements were averaged from 100 images and macular estimations from 15 ART.
- **Macular scans:** 25 horizontal B-scans within a 6-mm diameter area centered on the fovea. Regional macular thickness was divided into four quadrants according to the ETDRS grid (superior, temporal, inferior, nasal). The GCIPL value was calculated as the sum of the mean GCL and IPL thickness in the four quadrants.

Segmentation was performed automatically using Heidelberg Eye Explorer software version 6.0.9.0. All scans were reviewed for quality by an experienced ophthalmologist, following OSCAR-IB quality control criteria [17]. Data were reported in accordance with the APOSTEL recommendations [18]. A total of 740/785 subjects met quality control criteria for pRNFL measurements and 708/785 for GCIPL measurements.

2.3 | MRI Protocol and Optic Nerve Evaluation

A total of 710 patients underwent brain MRI on a 3 T scanner (MAGNETOM Prisma, Siemens Healthineers) using a 64-channel head and neck coil. Of these, 178 patients had MRI performed more than 3 months apart from OCT and were therefore excluded from the analysis. Among the remaining 532 patients, 260 had 3D sagittal double inversion recovery (DIR) sequences available for optic nerve assessment (TR 5500 ms, inversion times 2500 and 450 ms, T2 preparation time 125 ms, TE 270 ms, spatial resolution $1.2\times1.2\times1.2\,mm^3$) together with 3D fluid-attenuated inversion recovery (FLAIR) sequences (TR 6000 ms, TE 356 ms, voxel size $0.4\times0.4\times1\,mm^3$). Optic nerve analysis was independently performed by two blinded raters (A.G. and G.B.) on 3D DIR [19] and 3D FLAIR sequences. Lesions were defined as hyperintense areas visible in at least three consecutive coronal DIR slices [20–22]. When necessary, 3D-FLAIR images were used as supportive sequences. Because the chiasmatic region is highly susceptible to artifacts [23, 24] and could not be reliably evaluated in all patients of our cohort, only the intraorbital, intracanalicular, and prechiasmatic segments were analyzed. Inter-rater reliability for optic nerve lesion detection, assessed on an eye-based analysis, showed Cohen's $\kappa=0.83$ (95% CI: 0.77–0.89). Discrepancies were resolved by consensus after joint image review. MRI scans with poor quality, significant artifacts, or insufficient optic nerve visualization were excluded (7.7%). Illustrative cases of combined OCT and MRI assessments are represented in Figure 1.

2.4 | Statistical Analysis

Statistical analyses were conducted in R (version 4.4.3). We evaluated the proposed 2024 McDonald criteria threshold for IED, as well as individual pRNFL, GCIPL thresholds and a combined pRNFL and GCIPL threshold. For each threshold, diagnostic performance in identifying a previous history of ON was assessed in terms of sensitivity, specificity, accuracy, balanced accuracy, and precision. Within the subgroup of patients with available MRI, ROC curve analyses were

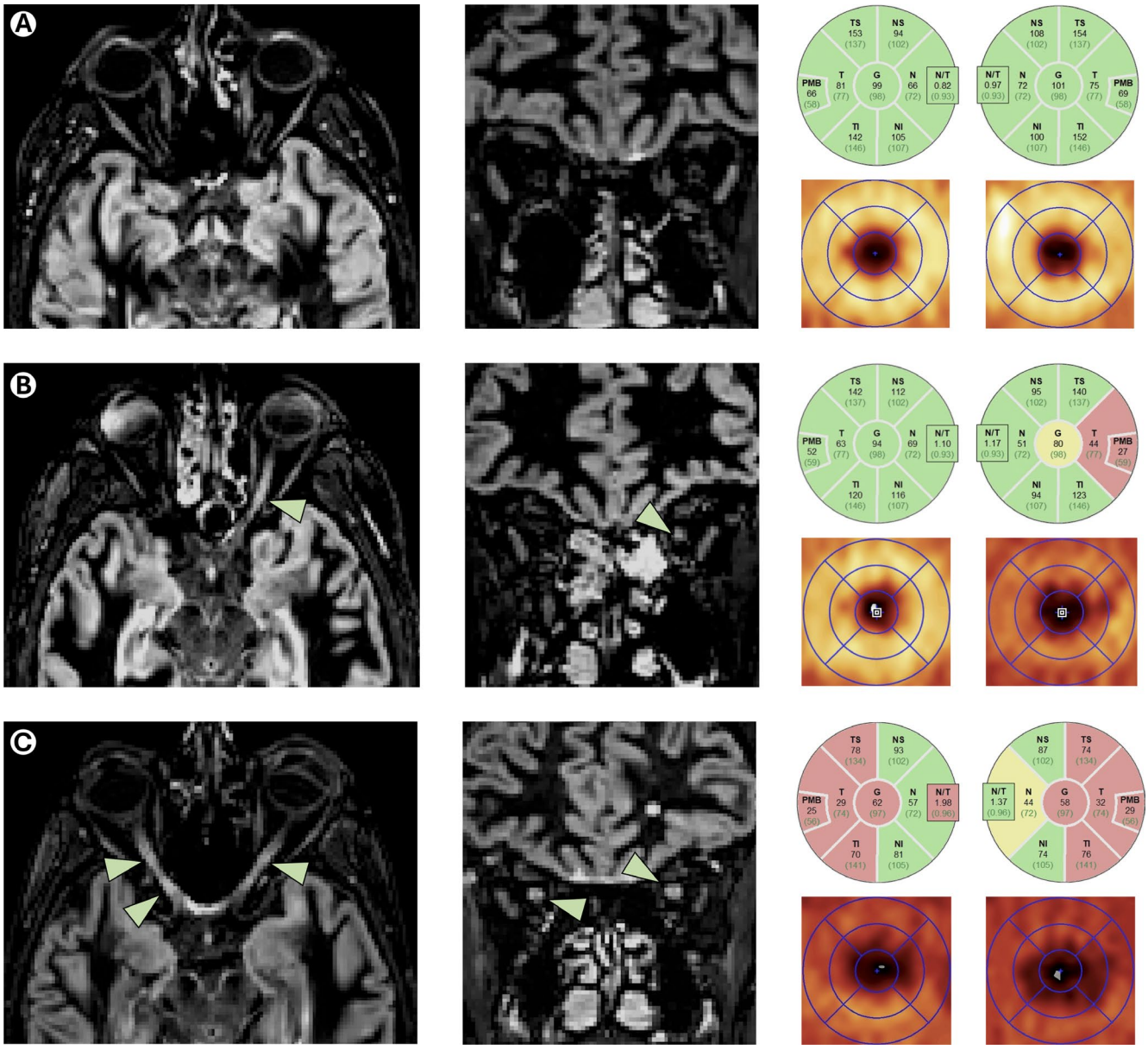

FIGURE 1 | Optic Nerve MRI with corresponding OCT images. Selected coronal and axial brain DIR MR images of the optic nerve with corresponding OCT images showing pRNFL thickness maps (upper row) and macular ganglion cell layer (GCL) thickness maps (lower row) exported from Heidelberg Spectralis reports. In the pRNFL thickness maps red indicates values below the 1st percentile, yellow below the 5th percentile, green between the 5th and 95th percentiles, and blue above the 95th percentile compared with age-matched healthy controls. (A) Selected DIR MR images from a **patient without optic nerve visible lesions**: OCT shows preserved retinal thickness in both peripapillary and macular regions; (B) Selected DIR MR images from a **patient with left intraorbital optic nerve lesion** (green arrows): OCT shows retinal asymmetry with peripapillary and macular thinning on the left side; (C) Selected DIR MR images from a **patient with bilateral optic nerve lesions** (green arrows): OCT shows bilateral retinal thinning in both peripapillary and macular regions.

performed to calculate the area under the curve (AUC) and to derive data-driven optimal cut-off values based on Youden's index. The same analyses as in the whole cohort were performed to assess the diagnostic performance in identifying optic nerve lesion. Finally, we calculated mean pRNFL and GCIPL thicknesses in symptomatic eyes, asymptomatic eyes with an optic nerve lesion at MRI and in asymptomatic eyes without evidence of optic nerve lesion and compared them using ANOVA.

3 | Results

3.1 | Study Cohort

We analyzed 2 cohorts of patients. The first cohort consisted of all patients with available OCT ($n = 740$), while the second cohort was composed of patients with concomitant OCT and MRI scans for optic nerve assessment ($n = 240$). Characteristics of the study cohorts are shown in Table 1 and Figure 2.

TABLE 1 | Demographics and clinical characteristics of study participants.

Characteristic	OCT cohort (740)	MRI-OCT cohort (240)
Age, year, mean (±SD)	46.5 (±12.6)	42.9 (±11.9)
Sex, female (%)	470 (64)	148 (62)
Phenotype, *n* (%): RR, SP, PP	549 (74), 100 (14), 91 (12)	193 (81), 34 (14), 13 (5)
Disease Duration, year, mean (±SD)	15 (9.4)	13.9 (9,9)
EDSS, median (range)	2 (0–7)	2 (0–7)
History of Acute Optic Neuritis (ON)		
Non-ON (%)	540 (73)	164 (68)
Unilateral ON (%)	160 (22)	52 (22)
Bilateral ON (%)	40 (5)	24 (10)

Abbreviations: Bilateral ON, acute optic neuritis involving both eyes over the course of the disease; EDSS, Expanded disability status scale; MRI, magnetic resonance imaging; Non-ON, absence of acute optic neuritis; OCT, optical coherence tomography; PP, primary progressive; RR, Relapsing remitting; SP, secondary progressive; Unilateral ON, acute unilateral optic neuritis.

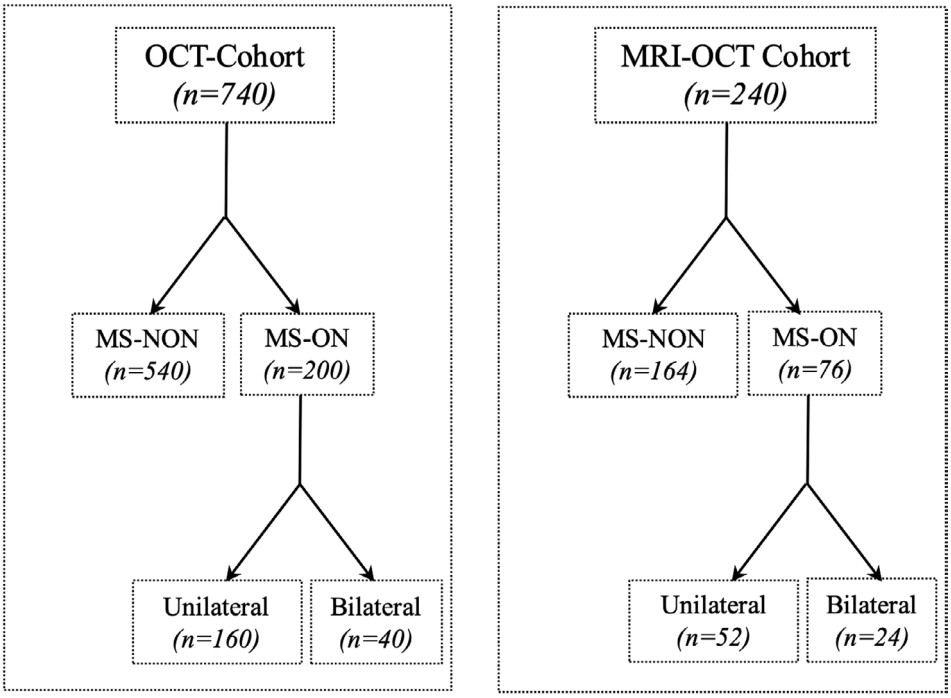

FIGURE 2 | Study cohorts. OCT, optical coherence tomography; MRI, magnetic resonance imaging; MS-ON, patients with reported history of optic neuritis; MS-NON, patients without a history of optic neuritis; Unilateral, acute unilateral optic neuritis; Bilateral, acute optic neuritis involving both eyes over the course of the disease.

3.2 | OCT-Cohort: IED For Detecting a History of Clinical ON

200/740 patients (27%) had a history of previous ON (MS-ON), while the remaining 540 patients had no history of ON (MS-NON). The diagnostic performance of the recently proposed 2024 McDonald IED cut-off (GCIPL ≥4 μm or pRNFL ≥6 μm), along with the pRNFL IED only (pRNFL ≥6 μm), the GCIPL IED only (GCIPL ≥4 μm), and the combined pRNFL and GCIPL IED (GCIPL ≥4 μm and pRNFL ≥6 μm) is shown in Table 2. The 2024 McDonald cut-off "GCIPL ≥4 μm or pRNFL ≥6 μm" IED maximized sensitivity (83%), with a specificity of 55% and an accuracy of 62%. The "GCIPL ≥4 μm and pRNFL ≥6 μm" IED maximized specificity (84%).

3.3 | MRI-OCT Cohort: IED For Detecting Optic Nerve Involvement at MRI

Diagnostic performance for different OCT IED cutoff in identifying optic nerve involvement at MRI in the whole MRI-OCT group (*n* = 240) and in MRI-OCT MS-NON (*n* = 164) is shown in Table 3. In particular, the 2024 McDonald IED cut-off (GCIPL ≥4 μm or pRNFL ≥6 μm) yielded 67% sensitivity and 83%

specificity for detecting subjects with asymptomatic optic nerve lesions at MRI.

Among patients with MRI evidence of optic nerve involvement ($n = 143$), 107 (75%) were identified with retinal asymmetry according to the 2024 McDonald criteria for IED, whereas 36 did not show significant IED. Of these, the majority, $n = 25$ (70%), had no history of ON: 11 had bilateral optic nerve lesions with symmetric bilateral retinal thinning and 14 had unilateral optic nerve lesions (10 intraorbital and 4 intracanalicular). Among MRI-negative patients ($n = 97$), 78 (80%) did not have retinal asymmetry, while 19 showed an IED above cut-off. Among these, 15 had no history of ON, most showing isolated IED in either GCIPL ($n = 7$) or pRNFL ($n = 5$).

Overall, 185/240 patients (77%) showed concordance between OCT and MRI in identifying optic nerve involvement. Concordance was higher in MS-ON patients (90%). Figure 3 shows the MRI and OCT findings in the MRI-OCT according to the presence of a previous ON. In MS-ON patients, MRI detected optic nerve lesions in 68/76 (90%), with OCT-IED positive in 57/68 (84%). In MS-NON patients, MRI detected optic nerve lesions in 75/164 (46%); of those, OCT-IED was positive in 50/75 (67%).

Considering the eyes rather than the subjects, in this cohort we analyzed 380 asymptomatic eyes and 100 symptomatic ones. MRI detected optic nerve lesions in 115/380 (30%) of asymptomatic eyes and in 91/100 of symptomatic eyes (with 8 patients with history of unilateral ON having no detectable MRI lesion and one patient with bilateral ON having only a single optic nerve lesion). Symptomatic eyes with optic nerve lesions had significantly lower retinal thickness compared to asymptomatic eyes with optic nerve lesions and asymptomatic eyes without optic nerve lesions, with a mean pRNFL of 72 μm (±13) vs 89 μm (±13) vs 98 μm (±11) respectively ($F = 93$, $p < 0.001$), and a corresponding mean GCIPL of 57 μm (±13) vs 73 μm (±12) vs 84 μm (±10) ($F = 115$, $p < 0.001$).

TABLE 2 | Performance of different IED thresholds in identifying a ON event in OCT cohort.

	OCT cohort (740)				
Threshold	**Sens[a]**	**Spec[b]**	**Acc[c]**	**BAcc[d]**	**Prec[e]**
GCIPL ≥ 4	75%	68%	70%	72%	47%
pRNFL ≥ 6	66%	73%	71%	70%	48%
GCIPL ≥ 4 or pRNFL ≥ 6	83%	55%	62%	69%	41%
GCIPL ≥ 4 & pRNFL ≥ 6	57%	84%	77%	71%	60%

Abbreviations: GCIPL, ganglion cell + inner plexiform layer; OCT, optical coherence tomography; pRNFL, peripapillary retinal nerve fiber layer.
[a]Sensitivity: probability of detecting an IED at or above the cutoff in patients with a history of ON.
[b]Specificity: probability of detecting an IED below the cutoff in patients without a history of ON.
[c]Accuracy: proportion of correctly classified patients (both with and without a history of ON) over the total population.
[d]Balanced Accuracy: mean of sensitivity and specificity, providing an equal weight to both measures regardless of class prevalence.
[e]Precision: probability that a patient with an IED at or above the cutoff truly has a history of ON.

3.4 | MRI-OCT Discrepancies in Patients Without a History of ON

Collectively, 40 MS-NON patients showed discordant findings between MRI and OCT: 15 subjects had negative optic nerve MRI but significant IED according to the 2024 McDonald threshold, whereas 25 subjects had optic nerve lesions at MRI but were IED negative. Representative cases are reported in Figure 4.

Within the MRI-negative/OCT-positive group:

- 3 patients had "GCIPL ≥4 μm and pRNFL ≥6 μm" IED, 2 with nasal peripapillary RNFL thinning associated with myopic or tilted optic disc profile and one with temporal peripapillary RNFL thinning (Figure 4, Case 7). All subjects exhibited concentric macular GCIPL thinning, in one case with a myopic macular profile (Figure 4, Case 5).
- 5 patients had isolated peripapillary RNFL thinning: 2 with nasal thinning secondary to myopic profile, 2 with

TABLE 3 | Performance of different IED thresholds in identifying optic nerve lesions at MRI in the MRI-OCT cohort and in MRI-OCT MS-NON subgroup.

	MRI-OCT cohort (240)					*MRI-OCT MS-NON cohort (164)*				
Threshold	**Sens[a]**	**Spec[b]**	**Acc[c]**	**BAcc[d]**	**Prec[e]**	**Sens[a]**	**Spec[b]**	**Acc[c]**	**BAcc[d]**	**Prec[e]**
GCIPL ≥ 4	66%	86%	74%	76%	87%	55%	89%	73%	72%	80%
pRNFL ≥ 6	62%	89%	73%	75%	89%	53%	91%	74%	72%	83%
GCIPL ≥ 4 or pRNFL ≥ 6	75%	80%	77%	78%	85%	67%	83%	76%	75%	77%
GCIPL ≥ 4 & pRNFL ≥ 6	54%	94%	70%	74%	93%	41%	97%	71%	69%	91%

Abbreviations: GCIPL, ganglion cell + inner plexiform layer; MRI, magnetic resonance imaging; MS-NON, patients without a history of optic neuritis; OCT, optical coherence tomography; pRNFL, peripapillary retinal nerve fiber layer.
[a]Sensitivity: probability of detecting an IED at or above the cutoff in patients' optic nerve lesions.
[b]Specificity: probability of detecting an IED below the cutoff in patients without optic nerve lesion.
[c]Accuracy: proportion of correctly classified patients (both with and without optic nerve lesion) over the total population.
[d]Balanced Accuracy: mean of sensitivity and specificity, providing an equal weight to both measures regardless of class prevalence.
[e]Precision: probability that a patient with an IED at or above the cutoff truly has optic nerve lesion.

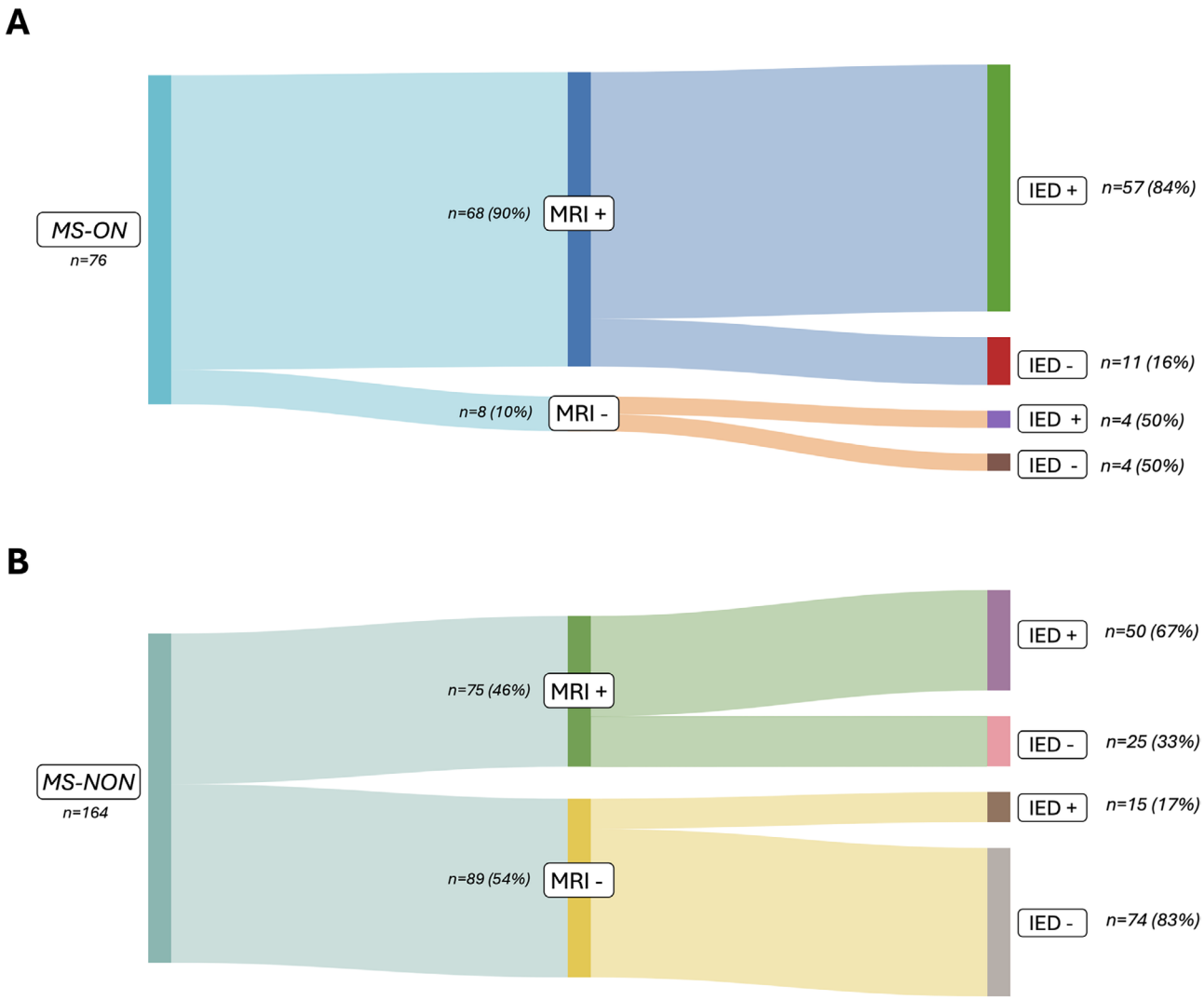

FIGURE 3 | Performance of the revised McDonald OCT cut-offs in detecting optic nerve involvement compared to MRI-detected optic nerve lesions. MS-ON: Patients with reported history of optic neuritis ($n = 76$); MS-NON: Patients without a history of optic neuritis ($n = 164$); MRI+: Presence of at least one MRI-detected optic nerve lesion; MRI−: Absence of MRI-detected optic nerve; IED+: Retinal asymmetry, according to the revised McDonald criteria; IED−: Absence of retinal asymmetry.

temporal thinning, and one with all values within normal limits (Figure 4, Case 8).

- 7 patients had isolated GCIPL thinning: 5 with concentric macular thinning, one with bilateral temporal hemimacular thinning suggestive of chiasmatic involvement, and one with temporal hemimacular ganglion cell loss in the thinner eye, combined with nasal hemimacular ganglion cell loss in the fellow eye, suggestive of retrochiasmatic involvement. (Figure 4, Case 6).

Within the MRI-positive/OCT-negative group:

- 10 subjects had unilateral intraorbital lesions, which were associated in 6 cases with mild ipsilateral temporal peripapillary RNFL thinning (Figure 4, Case 1 and Case 2), in 3 cases with normal OCT parameters (Figure 4, Case 3), and in one case with marked ipsilateral temporal peripapillary thinning with diffuse macular GCIPL loss, together with nasal hemimacular thinning in the affected eye and temporal hemimacular thinning in the fellow eye.
- 4 subjects had intracanalicular lesions, which in 1 case were associated with mild ipsilateral temporal peripapillary RNFL thinning and in 3 cases with normal OCT values.
- 11 patients had bilateral optic nerve involvement: 6 patients showed bilateral peripapillary RNFL thinning associated with diffuse macular GCIPL loss (Figure 4, Case 4), 1 had isolated bilateral temporal peripapillary RNFL thinning with preserved macular GCIPL thickness, 2 showed isolated bilateral macular GCIPL thinning with preserved peripapillary RNFL thickness, and 2 reported OCT values within normal limits in both peripapillary and macular layers.

4 | Discussion

In this study we evaluated the performance of the IED cut-off proposed in the 2024 McDonald criteria in identifying optic nerve involvement in a large cohort of people with MS and investigated the combined role of OCT and MRI in patients without a history of ON.

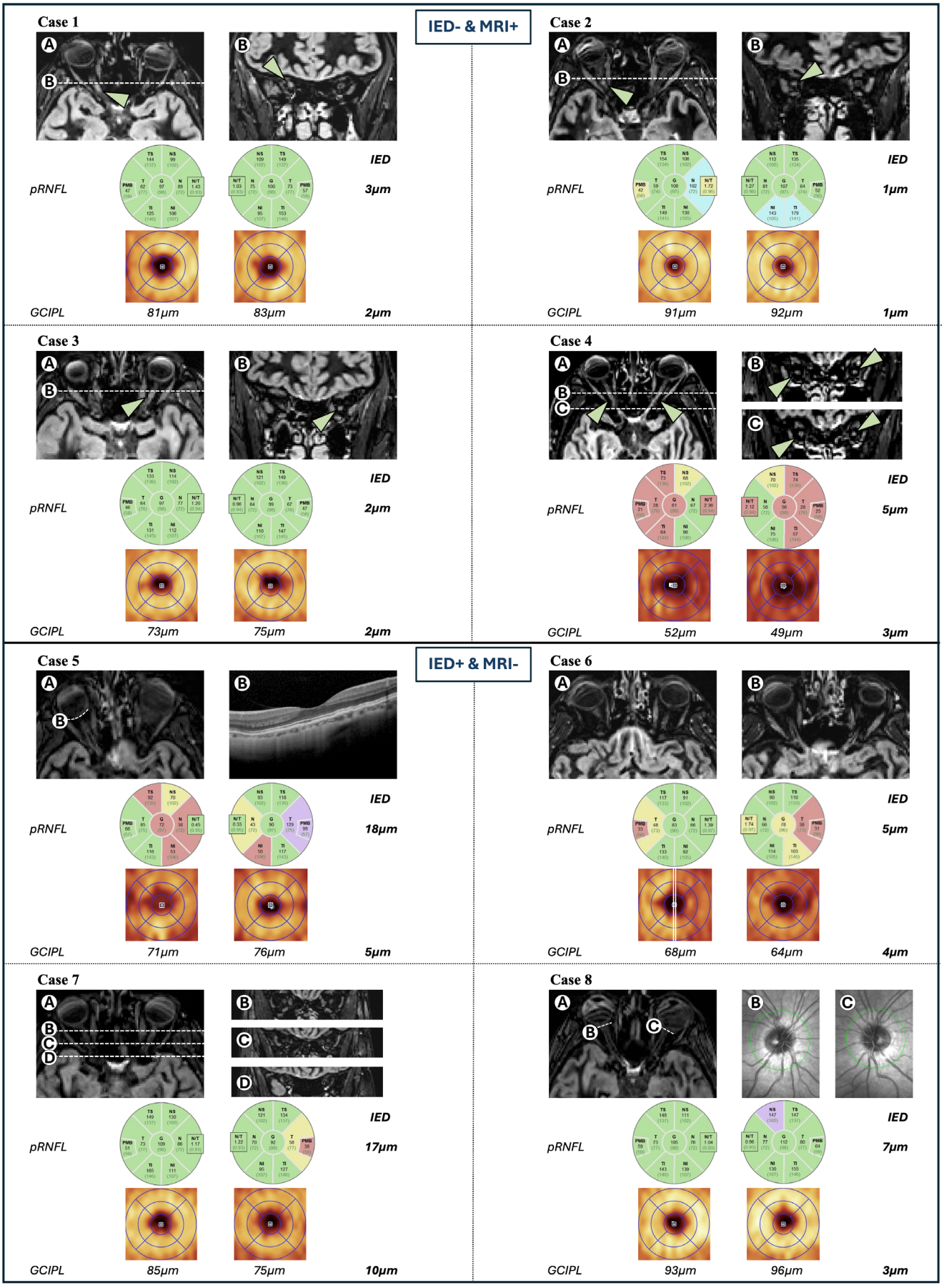

FIGURE 4 | Legend on next page.

FIGURE 4 | Discordance in MRI–OCT Evaluation of the optic nerve. This figure illustrates eight representative asymptomatic patients with discordant findings between MRI and OCT, in optic nerve involvement assessment, according to the revised McDonald criteria. **Cases 1–4** represent patients with absence of OCT inter eye difference (IED) despite the presence of DIR MR-visible optic nerve lesion. **Cases 5–8** show patients presenting OCT-IED in the absence of DIR MR-visible optic nerve lesion. Axial brain DIR MR reconstructions are shown for all cases, cropped to emphasize the optic nerves. For cases 1–4, additional coronal DIR sections highlight the lesion site along the affected nerve (green arrows). In case 5, panel B displays the macular OCT scan of the right eye (Heidelberg Spectralis). In case 7, additional coronal DIR sections highlight the absence of optic nerve lesions. In case 8, panels B and C include infrared fundus images of both optic discs (Heidelberg Spectralis). Case 1: Right anterior optic nerve lesion. OCT showed subthreshold peripapillary and macular thinning (pRNFL IED = 3 μm; GCIPL IED = 2 μm) with a pattern consistent with mild structural involvement. Case 2: Right anterior optic nerve lesion. OCT presented minimal IED values of 1 μm for both pRNFL and GCIPL, with subtle papillomacular bundle involvement. Case 3: Left anterior optic nerve lesion. OCT values were within normal limits with no detectable retinal thinning. Case 4: Bilateral optic nerve lesions. OCT showed bilateral retinal thinning resulting in absent retinal asymmetry (pRNFL IED = 5 μm; GCIPL IED = 3 μm). Case 5: OCT showed nasal pRNFL thinning (IED = 18 μm) and GCIPL IED = 5 μm without a defined topographic pattern; right macula displayed a typical myopic profile. Case 6: OCT showed bilateral temporal pRNFL thinning (IED = 5 μm) and GCIPL IED = 4 μm, with bilateral hemimacular atrophy, suggestive of retrochiasmatic involvement. Case 7: OCT showed temporal pRNFL thinning (IED = 17 μm) and concentric GCIPL thinning (IED = 10 μm), consistent with the typical optic nerve lesion pattern, despite normal MRI findings. Case 8: OCT showed pRNFL IED = 7 μm without a specific thinning pattern, with normal retinal thickness and no morphological alterations on infrared fundus images.

We found that the recently proposed IED cutoff (GCIPL ≥ 4 μm or pRNFL ≥ 6 μm) showed high sensitivity (83%) for identifying a history of ON, with a specificity of 55% and an overall accuracy of 62%. This IED cutoff was intentionally designed to best balance sensitivity and specificity, aiming to minimize the risk of missing one anatomical location required for DIS and thereby delaying MS diagnosis. Regarding the modest specificity, it is essential to emphasize that OCT should not be used as a stand-alone criterion, but rather as a "paraclinical adjunct to careful and thorough neuro-ophthalmological examinations". Interestingly, in line with a recent study [25], combined IED definition (as GCIPL ≥ 4 μm and pRNFL ≥ 6 μm) demonstrated higher specificity, albeit with significantly lower sensitivity, in identifying optic nerve involvement. Retinal thinning does not solely reflect axonal damage determined by optic nerve lesions, as it is influenced by several factors, including age, disease-related factors (disease duration, phenotype, brain atrophy, retrochiasmatic involvement), and systemic comorbidities [26–29]. In real-world clinical settings, where these factors may affect threshold accuracy, either multivariable adjustment may be needed or stricter cutoffs may be adopted to enhance specificity.

When we analyzed the performance of IED cutoff in identifying subclinical optic nerve lesions rather than prior ON, we found a lower sensitivity (67%), with slightly higher specificity (83%). Nearly one third of patients with MRI evidence of optic nerve involvement were not identified by the 2024 McDonald IED cutoff; notably, most of these had no prior history of optic nerve. Our results are in line with previous studies [6, 9, 14, 15] which suggested a higher sensitivity of MRI compared to OCT in detecting asymptomatic optic nerve involvement. Asymptomatic optic nerve lesions on MRI were associated with only modest pRNFL thinning compared to ON (89 vs 72 μm), producing mild IEDs that fell below the proposed thresholds. Because pRNFL and GCIPL specifically reflect optic nerve axonal integrity, whereas MRI hyperintensities may indicate either demyelination, inflammation, or axonal loss, it is plausible that asymptomatic optic nerve lesions without pathological OCT IED represent less destructive lesions with relative axonal preservation and/or limited myelin loss. As MRI appears more sensitive than OCT and remains a mandatory paraclinical test in the initial diagnostic work-up of MS, our findings suggest that MRI should be considered the first-line tool for assessing optic nerve involvement. OCT may serve as a valuable adjunct in cases where MRI findings are negative, equivocal, or of suboptimal quality, when additional evaluation is needed to support the diagnosis.

Interestingly, 11 of the 25 undetected cases had bilateral optic nerve lesions on MRI, showing symmetrical retinal thinning. This underscores a limitation of IED-based definitions, which may fail to capture bilateral structural damage. Although simultaneous bilateral optic nerve involvement is extremely rare in MS and typically suggests alternative etiologies [30], sequential involvement of both optic nerves is common in MS [12, 13], and may reduce the utility of IED measures in detecting optic nerve involvement in patients with long disease duration. This issue, however, is expected to have little impact in early MS. Conversely, 15 patients showed abnormal IED despite normal-appearing optic nerves on MRI, with only 3 cases with abnormalities in both pRNFL and GCIPL IED cutoff. Possible explanations include physiological inter-eye asymmetry, unknown ophthalmological conditions, subtle segmentation or acquisition artifacts, or lesions along optic nerve and chiasma not identified without a customized optic nerve protocol. However, MRI may underestimate small or posterior optic nerve lesions, suggesting that some OCT-defined abnormalities may represent true, but radiologically occult, lesion-related axonal damage. Moreover, OCT can also reflect retrochiasmatic damage (e.g., optic tract lesions or trans-synaptic degeneration secondary to optic radiation damage), presenting as homonymous macular hemiatrophy [29]. Although rare, such cases highlight the importance of qualitative OCT assessments that focus on specific morphological signatures (e.g., temporal pRNFL thinning, concentric GCIPL loss), providing complementary information beyond simple IED metrics. Similarly, OCT morphology assessment could aid in interpreting IED-/MRI+ patients with mild but typical retinal thinning pattern, such as the selective involvement of the peripapillary bundle, which might be specifically vulnerable in ON, due to the elevated macular cells energy demand [31].

5 | Limitations

This study has several limitations. First, the inclusion of a heterogeneous cohort of MS patients with long disease duration may limit the generalizability of our findings to individuals with

shorter disease duration in whom an initial diagnosis of MS is being considered. As an example, in our cohort, GCIPL-based IED showed slightly lower specificity in identifying prior ON than previously reported [6, 7]. This likely reflects the quite long disease duration of our cohort, since longer disease duration increases the likelihood of retrochiasmal degeneration, transsynaptic ganglion cell loss, and age-related macular changes, processes that preferentially affect GCIPL rather than pRNFL. In addition, the OCT cohort included slightly older patients and a higher proportion of individuals with a primary progressive (PP) phenotype. This reflects the original study protocol, which initially focused on PP patients and was subsequently expanded to include relapsing–remitting patients after the first year of recruitment. Additionally, DIR sequences were introduced later in the study protocol and were therefore available only for patients recruited more recently, resulting in a more representative phenotype distribution within the MRI–OCT cohort.

Second, VEPs, the third paraclinical tool recognized in the recent diagnostic criteria for assessing optic nerve involvement, were not analyzed, which prevented a comprehensive characterization of the spectrum of optic nerve findings in both MS-ON and MS-NON patients. Third, chiasmatic and optic tract lesions were not evaluated due to technical constraints. Fourth, although double inversion recovery is sensitive to MS optic nerve lesions, it may be less specific and more prone to artifacts; therefore, caution is recommended when interpreting DIR hyperintensities in individuals without an appropriate clinical background. Fifth, this was a single-center study performed using a single OCT platform (Spectralis). This may limit the generalizability of the proposed IED thresholds to other centers, given the inter-device variability in pRNFL and GCIPL measurements, particularly considering the small micrometric thresholds evaluated (4–6 μm). This suggests the need for multicenter studies to better evaluate the real applicability of the proposed thresholds.

Future studies should prioritize longitudinal evaluation of discordant cases to determine whether OCT-negative/MRI-positive lesions eventually develop retinal thinning that meets IED thresholds or instead represent true non-destructive lesions with more favorable outcomes. Finally, upcoming work should incorporate morphological OCT features, such as concentric GCIPL thinning or temporal pRNFL loss, which may reveal bilateral or symmetric damage overlooked by current cut-offs and help distinguish false positives due to ophthalmologic comorbidities. Incorporating qualitative OCT analysis would help to overcome the limitations of a purely quantitative approach by relying on morphological features, in line with the MRI approach.

Author Contributions

Lapucci Caterina: writing – review and editing, data curation, investigation, visualization. **Gualco Alessandro:** conceptualization, investigation, writing – original draft, methodology, visualization, data curation. **Boffa Giacomo:** conceptualization, investigation, writing – original draft, visualization, methodology, data curation. **Cipriano Emilio:** methodology, software, formal analysis, data curation, project administration. **Razzetta Chiara:** writing – original draft, methodology, software, formal analysis, data curation, validation. **Al Qudsi Sabrina:** investigation, writing – review and editing. **Sirito Tommaso:** investigation, writing – review and editing. **Nasone Lorenza:** visualization, data curation, project administration. **Saitta Laura:** investigation, data curation, visualization. **Campi Cristina:** writing – review and editing, methodology, software, formal analysis, data curation, validation. **Iester Michele:** investigation, writing – review and editing. **Cellerino Maria:** conceptualization, investigation, writing – review and editing, data curation. **Inglese Matilde:** writing – review and editing, supervision, funding acquisition, resources. **Garbarino Sara:** writing – review and editing, methodology, software, formal analysis, data curation, validation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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