Optical Coherence Tomography Reveals Longitudinal Changes in Retinal Damage Under Different Treatments for Neuromyelitis Optica Spectrum Disorder

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Background: Progressive retinal neuroaxonal damage after acute optic neuritis may occur in neuromyelitis optica spectrum disorder (NMOSD). However, it is unclear if treatments used to prevent attacks influence neurodegeneration.

Objectives: We aimed to investigate retinal damage in patients treated with disease-modifying drugs in a longitudinal study.

Methods: We retrospectively included 50 patients with aquaporin 4-antibody-seropositive NMOSD. Peripapillary retinal nerve fiber layer (pRNFL) thickness, macular ganglion cell complex (mGCC) thickness, total macular volume (TMV), and optic disc measures were acquired by spectral domain optical coherence tomography in patients treated with tocilizumab, rituximab, and azathioprine.

Results: Longitudinally, in eyes with a history of ON (NMOSDON+), we observed annual thinning of mGCC [tocilizumab: −1.77 (−3.44, −0.09) µm, p = 0.041; rituximab: −2.03 (−3.58, −0.48) µm, p = 0.017; azathioprine: −1.79 (−2.22, −1.37) µm, p < 0.001], and pRNFL [tocilizumab: −2.07 (−0.75, −3.39) µm, p = 0.005; rituximab: −2.18 (−0.36, −4.00) µm, p = 0.023; azathioprine: −2.37 (−0.98, −3.75) µm, p = 0.003], reduced TMV [tocilizumab: −0.12 (−0.22, −0.01) mm³, p = 0.028; rituximab: −0.15 (−0.21, −0.08) mm³, p = 0.001; azathioprine: −0.12 (−0.20, −0.04) mm³, p = 0.006], and increased cup area [tocilizumab: 0.08 (−0.01, 0.16) mm², p = 0.010; rituximab: 0.07 (0.01, 0.12) mm², p = 0.019; azathioprine: 0.14 (0.02, 0.26) mm², p = 0.023]. However, we detected no significant differences in annual changes in mGCC, pRNFL, TMV, and cup area between patients with tocilizumab, rituximab, and azathioprine in NMOSDON+ eyes. NMOSDON− eyes did not display mGCC or pRNFL thinning in patients treated with tocilizumab and rituximab. Intriguingly, we observed significant thinning of mGCC in patients treated with azathioprine compared with tocilizumab [−0.84 (−1.50, −0.18) µm vs. −0.19 (−0.87, 0.48) µm, p = 0.012] and rituximab [−0.84 (−1.50, −0.18) µm vs. −0.07 (−1.25, −2.51) µm, p = 0.015] in NMOSDON− eyes.

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INTRODUCTION

Neuromyelitis optica spectrum disorder (NMOSD) is a debilitating humoral-mediated autoimmune disease, which is typically characterized by attacks of acute optic neuritis (ON) and longitudinally extensive transverse myelitis (1). Inflammation of the optic nerve and impaired axonal transport are associated with severe visual dysfunction during acute ON. Retinal ganglion cell and axonal loss after acute ON in NMOSD have been detected extensively by the potential use of quantitative optical coherence tomography (OCT). Neuronal thinning was observed in the ganglion cell layer in ON-affected eyes even within 3 and 6 months. Only minimal retinal nerve fiber layer thickness (RNFL) loss was observed after this period (2, 3).

Recent studies indicate that retinal ganglion cell loss may persist and be independent of ON attacks in patients with NMOSD with seropositive anti-aquaporin 4 antibody (AQP4-IgG) over a 2-year follow-up (4–7). Progressive ganglion cell layer thinning may be partly due to retrograde neuroaxonal degeneration from lesions or primary retinopathy (8, 9). In addition, different from ON in patients with multiple sclerosis (MS), patients with NMOSD exhibit greater reductions in RNFL thickness and average macular volume (10–12). Several molecular pathways have been identified as potential therapeutic targets to induce remyelination after ON in patients with MS (13). However, to date, few therapies for NMOSD improve remyelination directly. Empirical treatments including azathioprine and B cell-depleting treatments have been extensively used to prevent ON attacks (14, 15). Recently, IL-6 receptor monoclonal antibody satralizumab and tocilizumab have demonstrated a reduced risk of attacks in NMOSD (16, 17). However, it remains unclear whether these drugs can prevent or delay neurodegeneration in eyes with and without a history of ON in patients with NMOSD.

In this study, we aimed to investigate longitudinal changes of OCT measures and compare them in patients with NMOSD under different treatments, including tocilizumab, rituximab, and azathioprine.

MATERIALS AND METHODS

NMOSD Patients and Healthy Controls

One hundred and twenty-one patients with NMOSD from the research database of the Department of Neurology, Tianjin Medical University General Hospital, were screened. Inclusion criteria included the following: (1) a confirmed diagnosis of AQP4-IgG seropositive NMOSD according to the 2015 international consensus criteria (18); (2) complete longitudinal clinical and OCT imaging data with a minimum follow-up of 1 year; and (3) age between 18 and 75 years at baseline.

Conclusions: This study demonstrated that retinal ganglion cell loss is independent of ON attacks in NMOSD. Tocilizumab and rituximab may delay mGCC thinning in NMOSDON+ eyes compared with azathioprine.

Keywords: neuromyelitis optica spectrum disorder, retinal ganglion cells, azathioprine, tocilizumab, rituximab

Visual Acuity Measurements

To assess vision precisely, low-contrast letter acuity was measured using retro-illuminated 2.5% Sloan letter chart (Precision Vision, La Salle, IL, USA) with best refractive correction at 2.52 m. The maximal number of letters visible on this chart was 70. Best-corrected high-contrast logMAR visual acuity was measured using retro-illuminated Early Treatment Diabetic Retinopathy Study chart (Precision Vision, La Salle, IL, USA) at 2.52 m. When no letters could be correctly identified, a score of 1.7 was assigned (19).

Spectral-Domain Optical Coherence Tomography

High-resolution spectral-domain optical coherence tomography (SD-OCT) images were acquired at baseline and the last follow-up using identical protocols (RTVUE100-2, Optovue Inc., Fremont, CA, USA). We used the Auto buttons for the first measurement for each eye to fix scanning. After the first scan was optimized and captured, the system stored the settings for each eye and the same settings were used for all subsequent visits. The OCT Image acquisition rate was 26,000 A-scans/s with 1024 A-scan for each frame. The in-tissue optical resolution was 5 µm in depth and 15 µm in beam spot size. We used ONH and GCC (ganglion cell complex) scan patterns for measurements. The ONH mode was used to get several pieces of important data.
disc morphology information, including disc and cup areas, C/D ratio, and RNFL 3.45 thickness map. The ONH protocol used 12 radial line scans (each composed of 12 × 455 A-scans) with 3.4-mm length and 13 concentric rings (each composed of 3 × 965 A-scans). Software available on this machine automatically detects the center of the optic disc and provides the mean RNFL around a circumpapillary circle with a 3.45-mm radius from the center of the optic disc. The GCC mode was used to get mGCC thickness. The GCC protocol adopted 1 horizontal line (1 × 933 A-scans) with 7 mm scan length, followed by 15 vertical lines (each composed of 15 × 933 A-scans) with 7 mm scan length and 0.5 mm interval, centered 1 mm temporal to fovea. An auto average model was set to calculating the average of repeated B-scans to help reduce the possibility of test–retest variability.

In the workstation of the OCT instrument, the macular GCC (mGCC) indicates the combination of macular RNFL, ganglion cell layer, and inner plexiform layer. We also measured the focal loss volume (FLV) and global loss volume (GLV). FLV is a parameter that provides a quantitative determination of the amount of significant GCC loss. It is the total sum of significant GCC loss (in volume) divided by the map area. GLV is the sum of the pixels where the Fractional Deviation map value is <0 and then divided by the total area to give a percent loss of GCC thickness.

The peripapillary RNFL (pRNFL) was measured with activated eye tracker using the innermost 3.45-mm ring of a star-and-ring scan around the center of the disc. Total macular volume (TMV) was calculated as a 6-mm-diameter cylinder around the fovea from a macular volume scan. The fovea thickness (FT) was defined as the mean thickness of the 1-mm-diameter cylinder around the fovea from the same scan. Optic disc measurements included optic disc area, cup area, rim area, cup volume, rim volume, and nerve head volume. We adhered to the APOSTEL criteria when reporting OCT results (20). An example of the scanning procedure in one eye of a patient with NMOSD is shown in Supplementary Figures 1–3.

One experienced rater carefully checked all scans for sufficient quality and segmentation errors and corrected them when necessary. The OSCAR-IB quality control criteria were used to report OCT data quality and exclusions (21). Seven eyes were excluded because of insufficient data quality and severe ONs that rendered patients unable to cooperate with the scanning.

Intervention of the Patients With NMOSD

Patients with tocilizumab received infusions at the dose of 8 mg/kg/month and patients with azathioprine received oral treatment at the dose of 2–3 mg/kg per day. Patients with rituximab received periodical treatment according to the proportion of B cells in peripheral blood mononuclear cells (PBMCs), as previously published (22). Briefly, the percentage of circulating CD19+ B cells in the PBMCs was determined. The doses of rituximab were administered to the patients according to the CD19+ B cell counts: (1) 0.5–1.5% of PBMCs, 100 mg of rituximab was administered intravenously once; (2) 1.5–5.0% of PBMCs, 100 mg of rituximab was administered intravenously for 2 consecutive days; (3) 5.0–15.0% of PBMCs, 100 mg of rituximab was administered intravenously for 3 consecutive days; and (4) >15.0% of PBMCs, 100 mg of rituximab was administered intravenously for 4 consecutive days. The percentage of CD19+ B cells was monitored at 12-week intervals. The indicated dose reinfusion regimen was based on CD19+ B-cell repopulation, when the circulating CD19+ B-cell proportion >0.5% of PBMCs.

Statistical Analysis

Group differences between NMOSD patients and HCs were tested by $\chi^2$ test for sex and Wilcoxon rank-sum test for age. The primary outcome was the change in mGCC over the follow-ups; secondary outcomes were changes in pRNFL, FT, TMV, and visual acuity. Cross-sectional differences of OCT values and visual acuity among all groups were analyzed pairwise. Annual loss was estimated for each eye as change from baseline at last visit divided by the follow-up time (years). Taking inter-eye correlation into account, we performed a generalized estimating equation (GEE) analysis with changes of retinal index as the dependent variable and treatments as predictors. This model tested the interaction between predictors. Comparisons of mGCC, pRNFL, TMV, and FT between different treatment groups were performed by GEE. The Wald $p$-value, regression coefficient ($\beta$), and 95% confidence interval (CI) are reported. All tests and graphical representations were performed with SPSS v23.0 (IBM SPSS, Armonk, NY, USA) in accordance with APOSTEL. Statistical significance was established at $p < 0.05$.

RESULTS

Cohort Description and Follow-Up

Among the 121 patients with NMOSD screened, data for 50 patients with NMOSD with a mean follow-up time of 1.18 (1.01) years fulfilled the inclusion criteria: 20 patients received tocilizumab treatment, 18 patients received azathioprine treatment, and 12 patients received rituximab treatment. Of all patients with NMOSD, 45 experienced unilateral ON, and 5 patients experienced bilateral ON. Thus, 55 eyes experienced a history of ON (NMOSDON+) and 45 eyes had no history of ON (NMOSDON–). Visual acuity and Expanded Disability Status Scale assessment at baseline are available in Table 1. Sex, age, follow-up time, disease duration, ARR, EDSS, eyes with a history of ON, ON episodes per ON eye, time since last ON, LogMAR visual acuity, and 2.5% low-contrast letter acuity were comparable between the patients treated with tocilizumab, rituximab, and azathioprine ($p > 0.05$).

Baseline Characteristics of Visual Acuity and OCT Measures

At baseline, the LogMAR visual acuity of NMOSDON+ eyes (0.76, IQR 0.73–0.78) was significantly lower than that of HCs eyes (0.10, IQR 0–0.15, $p = 0.037$). The 2.5% low-contrast letter acuity of NMOSDON+ eyes (6.9, IQR 6.6–7.1) was also significantly lower than that of HCs eyes (23.8, IQR 22.8–24.6, $p < 0.001$). The LogMAR visual acuity of NMOSDON– eyes (0.18, IQR 0.16–0.19) did not differ significantly from that of HCs eyes ($p = 0.438$). The 2.5% low-contrast letter acuity of NMOSDON– eyes (23.5, 23.6, IQR 23.0–24.2) was lower than that of HCs eyes (24.8, IQR 24.0–25.0, $p = 0.037$).
TABLE 1 | Demographic characteristics of NMOSD patients and HCs.

|                | Tocilizumab | Rituximab | Azathioprine | HCs  |
|----------------|-------------|-----------|--------------|------|
| Subject (N)    | 20          | 12        | 18           | 10   |
| Sex (female in %) | 20 (100)   | 11 (91.67)| 18 (100)     | 9 (90)|
| Age at baseline (years, mean ± SD) | 41.35 ± 17.52 | 53.25 ± 13.09 | 41.28 ± 14.61 | 44.43 ± 12.90 |
| Follow-up time [days, median (IQR)] | 403.5 (307.5–617.3) | 390 (263.3–552.5) | 438.50 (173.5–619.5) | 412.0 (323.5–667.5) |
| Disease duration at baseline (years, mean ± SD) | 5.85 ± 2.81 | 5.78 ± 2.53 | 5.61 ± 2.58 | / |
| Time on current treatment [months, median (IQR)] | 8.68 (7.3–10.5) | 8.45 (6.3–11.5) | 8.89 (6.3–9.33) | / |
| ARR before baseline [mean ± SD] | 1.18 ± 0.97 | 1.23 ± 1.11 | 1.65 ± 1.37 | / |
| EDSS at baseline [median (IQR)] | 3.5 (2.3–6.5) | 3.8 (2.5–6.5) | 3.5 (3.3–7.0) | / |
| Eyes with a history of ON (N, %) | 22 (55.0%) | 12 (50.0%) | 21 (58.3%) | / |
| ON episodes per ON eye (mean ± SD) | 1.0 ± 0.3 | 1.0 ± 0.4 | 1.1 ± 0.3 | / |
| Tine since last ON [months, median (IQR)] | 8.2 (6.3–9.7) | 8.7 (7.3–9.8) | 8.3 (6.5–9.3) | / |
| LogMAR visual acuity of NMOSDON+ eyes (median, IQR) | 0.72 (0.71–0.76) | 0.69 (0.68–0.71) | 0.73 (0.70–0.75) | 0.10 (0–0.15) |
| 2.5% low-contrast letter acuity of NMOSDON+ eyes (median, IQR) | 6.8 (6.3–6.9) | 6.7 (6.5–7.0) | 7.1 (6.9–7.3) | 23.6 (22.8–24.3) |
| LogMAR visual acuity of NMOSDON− eyes (median, IQR) | 0.17 (0.14–0.19) | 0.16 (0.16–0.19) | 0.18 (0.17–0.20) | / |
| 2.5% low-contrast letter acuity of NMOSDON− eyes (median, IQR) | 23.5 (22.3–25.0) | 23.5 (22.1–25.3) | 24.3 (23.3–25.7) | / |

ARR, annualized relapsing rate; EDSS, the Kurtzke Expanded Disability Status Scale; HCs, healthy controls; NMOSD, neuromyelitis optica spectrum disorder; ON, optic neuritis; SD, standard deviation. The patients between tocilizumab, rituximab, and azathioprine groups are comparable in all the parameters (p > 0.05).

TABLE 2 | Baseline visual acuity and OCT measures NMOSD eyes and HCs eyes.

|                | EyesON+ | EyesON− | HCs   |
|----------------|---------|---------|-------|
| LogMAR visual acuity | 0.76 (0.73–0.78) | 0.18 (0.16–0.19) | 0.10 (0–0.15) |
| 2.5% low-contrast letter acuity | 6.9 (6.6–7.1) | 23.5 (22.5–24.5) | 23.8 (22.8–24.6) |
| mGCC thickness (µm) | 66.91 (8.23) | 91.92 (5.22) | 97.53 (2.32) |
| Superior | 79.11 (16.33) | 91.15 (6.13) | 94.35 (4.21) |
| Inferior | 69.45 (9.97) | 90.91 (5.72) | 94.37 (3.67) |
| FLV (%) | 6.83 (4.54) | 1.16 (1.63) | 0.45 (0.49) |
| GLV (%) | 25.19 (9.66) | 6.69 (4.62) | 4.59 (1.83) |
| Global pRNFL (µm) | 71.88 (12.94) | 110.50 (6.94) | 112.40 (8.10) |
| Macular |                |         |       |
| TMV (mm²) | 6.30 (0.30) | 6.93 (0.27) | 7.23 (0.37) |
| FT (µm) | 227.57 (13.13) | 240.47 (13.01) | 252.73 (18.58) |
| Optic disc |                |         |       |
| Disc area (mm²) | 2.36 (0.51) | 2.14 (0.33) | 2.25 (0.38) |
| Cup area (mm²) | 1.19 (0.67) | 0.62 (0.31) | 0.61 (0.37) |
| Cup volume (mm³) | 0.24 (0.17) | 0.10 (0.07) | 0.10 (0.09) |
| Rims area (mm²) | 1.18 (0.44) | 1.52 (0.28) | 1.70 (0.34) |
| Rim volume (mm³) | 0.08 (0.07) | 0.18 (0.07) | 0.23 (0.12) |
| Nerve head volume (mm³) | 0.19 (0.12) | 0.35 (0.13) | 0.44 (0.23) |

Visual acuity was shown using median (IQR), and other data were shown using mean (SD). FLV, fovea thickness; FT, fovea thickness; GLV, global loss volume; HCs, healthy controls; mGCC, macular ganglion cell complex; pRNFL, peripapillary retinal nerve fiber layer thickness; TMV, total macular volume.

IQR 22.5–24.5) was not lower than that of HCs eyes (p = 0.983) (Table 2).

We detected significant differences in OCT measures between patients with NMOSD and HCs (Table 2). In patients with NMOSD at baseline, the mGCC thickness in both NMOSDON+ eyes (66.91 ± 8.23 µm, p < 0.001) and NMOSDON− eyes (91.92 ± 3.52 µm, p < 0.001) was significantly lower than that of HCs eyes (97.53 ± 2.32 µm). The mGCC thickness in NMOSDON+ eyes was also lower than that of NMOSDON− eyes (mean difference 25.00 ± 1.64 µm, p < 0.001). Similarly, both the superior hemisphere mGCC thickness (79.11 ± 16.33 µm) and inferior hemisphere mGCC thickness (69.45 ± 9.79 µm) of NMOSDON+ eyes were significantly lower than those of HCs eyes (94.35 ± 4.21 µm, p = 0.043; 94.37 ± 3.67, p < 0.001, respectively) and NMOSDON− eyes (91.15 ± 6.13 µm, p = 0.012; 90.91 ± 5.72 µm, p < 0.001, respectively). Also,
the thickness in the superior hemisphere (91.15 ± 6.13 μm, p = 0.043) and inferior hemisphere (90.91 ± 5.72 μm, p = 0.036) of NMOSDON− eyes was significantly lower than that in HC eyes (94.35 ± 4.21 μm). The FLV% in NMOSDON+ eyes (6.83 ± 4.54) was significantly higher than that in NMOSDON− eyes (1.16 ± 1.63, p < 0.001) and in HC eyes (0.45 ± 0.49, p < 0.001); however, FLV% did not differ between NMOSDON− eyes and HC eyes. The GLV% in NMOSDON+ eyes (25.19 ± 9.66) was significantly higher than that in NMOSDON− eyes (6.69 ± 4.62, p < 0.001) and HC eyes (4.59 ± 1.85, p < 0.001), and there was no difference in the GLV% between NMOSDON− eyes and HC eyes.

Besides, pRNFL thickness was significantly reduced in NMOSDON+ eyes (71.88 ± 12.94 μm, p < 0.001) compared to HC eyes (112.40 ± 8.10 μm), but there was no significant reduction in NMOSDON− eyes (110.50 ± 6.94 μm, p = 0.391).

In addition, TMV in NMOSDON+ eyes (6.30 ± 0.30 mm³) was significantly reduced compared with that in NMOSDON− eyes (6.93 ± 0.27 mm³, p < 0.001) and HC eyes (7.23 ± 0.37 mm³, p < 0.001). TMV in NMOSDON− eyes was also lower than in HC eyes (p = 0.005). Both NMOSDON+ eyes (227.57 ± 13.13 μm, p < 0.001) and NMOSDON− eyes (240.47 ± 13.01 μm, p = 0.018) showed lower FT than HC eyes (252.73 ± 18.58 μm).

Although, disc area in NMOSDON+ eyes (2.36 ± 0.51 mm²) was significantly greater than that in NMOSDON− eyes (2.14 ± 0.33 mm², p = 0.018), it was only slightly, but not significantly, increased in HC eyes (2.25 ± 0.38 mm², p = 0.484). NMOSDON+ eyes displayed significantly increased cup area (1.19 ± 0.67 mm²) compared to NMOSDON− eyes (0.62 ± 0.31 mm², p < 0.001) and HC eyes (0.61 ± 0.37 mm², p = 0.001). Correspondingly, cup volume in NMOSDON+ eyes (0.24 ± 0.17 mm³) also increased compared with NMOSDON− eyes (0.10 ± 0.07 mm³, p < 0.001) and HC eyes (0.10 ± 0.09 mm³, p = 0.006). However, NMOSDON+ eyes demonstrated significantly decreased rim area (1.18 ± 0.44 mm²), rim volume (0.08 ± 0.07 mm³), and nerve head volume (0.19 ± 0.12 mm³) compared to NMOSDON− eyes (1.52 ± 0.28 mm², p < 0.001; 0.18 ± 0.07 mm³, p < 0.001; 0.35 ± 0.13 mm³, p < 0.001) and HC eyes (1.70 ± 0.34 mm², p = 0.001; 0.23 ± 0.12 mm³, p = 0.001; 0.44 ± 0.23 mm³, p = 0.002). Although, NMOSDON− eyes demonstrated a reduction in rim area, rim volume, and nerve head volume, there were no significant differences between NMOSDON− eyes and HC eyes.

All OCT measures were collected at baseline for the three groups of medications (tocilizumab, rituximab, and azathioprine) and showed no significant differences, in either NMOSDON+ or NMOSDON− eyes (Figures 1, 2 and Supplementary Table 1).

**Visual Acuity Changes During Follow-Ups**

We did not detect any changes in LogMAR visual acuity or low-contrast letter acuity either in either NMOSDON+ eyes or in NMOSDON− eyes.

**Changes of OCT Measures in NMOSDON+ Eyes During Follow-Ups**

Longitudinally, we identified mGCC annual thinning in NMOSDON+ eyes in the tocilizumab group (annual thinning −1.77 [−3.44, −0.09] μm, p = 0.041), the rituximab group (annual thinning −2.03 [−3.58, −0.48] μm, p = 0.017), and the azathioprine group (annual thinning −1.79 [−2.22, −1.37] μm, p < 0.001), compared with the baseline for each subgroup. This effect remained significant for each group relative to HCs. We also observed a thinning of pRNFL in NMOSDON+ eyes compared with the baseline among the three groups, respectively: tocilizumab group (annual thinning −2.07 [−0.75, −3.39] μm, p = 0.005); rituximab group (annual thinning −2.18 [−0.36, −4.00] μm, p = 0.023); azathioprine group (annual thinning −2.37 [−0.98, −3.75] μm, p = 0.003). Significant thinning of pRNFL for each group against HCs was observed. Compared with the baseline in each group, all patients had significant annual TMV loss in NMOSDON+ eyes: tocilizumab (annual loss −0.12 [−0.22, −0.01] μm, p = 0.028), azathioprine (annual loss −0.12 [−0.20, −0.04] μm, p = 0.006), and rituximab (annual loss −0.15 [−0.21, −0.08] μm, p = 0.001) (Table 3).

We observed no significant differences in annual thinning of pRNFL or mGCC, or annual loss of TMV among the three groups (Wald p > 0.05). However, the annual loss of pRNFL, mGCC, and TMV in the three groups was significantly greater than that in the HC groups, respectively (Wald p < 0.05). Compared to HCs, the variation coefficient (β) and 95% CIs of the tocilizumab group were −1.53 (−2.79, −0.27; pRNFL), −1.29 (−2.71, 0.13; mGCC), and −1.03 (−2.01, −0.05; TMV). In the rituximab group, β and 95% CIs were −1.646 (−3.269, −0.024; pRNFL), −2.053 (−3.324, −0.782; mGCC), and −0.133 (−0.203, −0.063; TMV). In the azathioprine group, β and 95% CIs were −1.828 (−3.078, −0.577; pRNFL), −1.881 (−2.279, −1.484; mGCC), and −0.108 (−0.183, −0.033; TMV) (Figure 3).

Compared with baseline and HCs, NMOSDON+ eyes revealed longitudinal optic disc cup area enlargement in the three groups of patients (annual increase 0.08 [−0.01, 0.16] mm² in the tocilizumab group, 0.07 [0.01, 0.12] mm² in the rituximab group, and 0.14 [0.02, 0.26] mm² in the azathioprine group). In addition, NMOSDON+ eyes also displayed an annual reduction of rim volume and increased cup volume in the three groups (p < 0.05). Post-hoc analysis did not reveal any differences in changes in disc cup area, cup volume, or rim volume, among the three groups. There were no significant changes in disc area, rim area, or nerve head volume in the three groups of patients with NMOSD, compared to HCs.

**Changes of OCT Measures in NMOSDON− Eyes During Follow-Ups**

NMOSDON− eyes did not display mGCC and pRNFL thinning, or TMV loss compared with baseline in patients with tocilizumab and rituximab (p > 0.05). However, we observed significant loss of pRNFL (−5.34 [−2.87, −7.82] μm, p < 0.001), mGCC (−0.84 [−1.50, −0.18] μm, p = 0.017), and TMV (−0.09 [−0.14, −0.04] mm², p = 0.003) compared with baseline in patients treated with azathioprine (Table 4).

When we compared the pRNFL, mGCC, and TMV annual changes in the three treatment groups with those in the HC group, no significant differences were observed in the tocilizumab or the rituximab group. However, the NMOSDON− eyes treated with azathioprine displayed significantly reduced
FIGURE 1 | Baseline SD-OCT measures in NMOSDON+ eyes in patients with NMOSD. Mean thickness of the macular GCC (A), RNFL (B), FT (C), and TMV (D) in eyes with a history of ON were measured by SD-OCT at baseline. GCC, ganglion cell complex; RNFL, retinal nerve fiber layer thickness; FT, fovea thickness; TMV, total macular volume; TCZ, tocilizumab, n = 19; RTX, rituximab, n = 12; AZA, azathioprine, n = 18; HC, healthy control, n = 20. **p < 0.01, ***p < 0.001. NS, not significant.

FIGURE 2 | Baseline SD-OCT measures in NMOSD− eyes in patients with NMOSD. Mean thickness of the macular GCC (A), RNFL (B), FT (C), and TMV (D) in eyes without a history of ON were measured by SD-OCT at baseline. GCC, ganglion cell complex; RNFL, retinal nerve fiber layer thickness; FT, fovea thickness; TMV, total macular volume; TCZ, tocilizumab, n = 18; RTX, rituximab, n = 11; AZA, azathioprine, n = 15; HC, healthy control, n = 20. *p < 0.05. NS, not significant.
TABLE 3 | Mean changes in the eyes with a history of ON in SD-OCT measures in patients with NMOSD and HCs.

|                          | Tocilizumab | Rituximab | Azathioprine | HCs |
|--------------------------|-------------|------------|--------------|-----|
| Eyes ON+                 |             |            |              |     |
| mGCC thickness (µm)      |             |            |              |     |
| Superior                 | −1.77 (−3.44, −0.09) | 0.041 | −2.03 (−3.58, −0.48) | 0.017 | −1.79 (−2.22, −1.37) | <0.001 | 0.00 (−0.31, 0.32) | 0.980 |
| Inferior                 | −0.37 (−7.32, 2.68) | 0.706 | 0.33 (−4.59, 1.98) | 0.569 | −0.84 (−3.01, 3.01) | 0.578 | 1.40 (−0.51, 2.01) | 0.151 |
| FLV (%)                  | −0.66 (−2.96, 0.94) | 0.992 | −6.15 (−6.65, −4.34) | 0.001 | 1.20 (−2.40, 4.80) | 0.471 | 0.01 (−0.26, 0.27) | 0.965 |
| GLV (%)                  | 1.15 (−1.90, 4.19) | 0.435 | 0.91 (−0.78, 2.60) | 0.261 | 0.06 (−1.38, 1.50) | 0.928 | −0.16 (−0.80, 0.48) | 0.587 |
| Global pRNFL (µm)        | −2.07 (−0.75, −3.39) | 0.005 | −2.18 (−0.36, 4.00) | 0.023 | −2.37 (−0.98, −3.75) | <0.001 | 0.23 (−1.69, 2.19) | 0.796 |
| Macular                  |             |            |              |     |
| Disc area (mm²)          | 0.00 (−0.01, 0.01) | 0.706 | 0.01 (−0.01, 0.03) | 0.266 | 0.00 (−0.03, 0.01) | 0.777 | −0.01 (−0.02, 0.01) | 0.391 |
| Cup area (mm²)           | 0.08 (−0.01, 0.16) | 0.010 | 0.07 (0.01, 0.12) | 0.019 | 0.14 (0.02, 0.26) | 0.023 | 0.03 (−0.01, 0.07) | 0.129 |
| Cup volume (mm³)         | 0.03 (0.00, 0.06) | 0.038 | 0.05 (0.016, 0.09) | 0.006 | 0.02 (−0.00, 0.05) | 0.020 | 0.01 (−0.03, 0.05) | 0.588 |
| Rim area (mm²)           | −0.10 (−0.21, 0.02) | 0.103 | −0.07 (−0.12, 0.02) | 0.050 | −0.14 (−0.27, −0.02) | 0.024 | −0.05 (−0.11, 0.01) | 0.087 |
| Rim volume (mm³)         | −0.01 (−0.02, 0.000) | 0.031 | −0.01 (−0.02, 0.000) | 0.028 | −0.01 (−0.02, 0.00) | 0.041 | −0.01 (−0.02, 0.00) | 0.055 |
| Nerve head volume (mm³)  | −0.01 (−0.06, 0.02) | 0.247 | −0.02 (−0.06, 0.00) | 0.050 | −0.02 (−0.06, 0.00) | 0.050 | −0.01 (−0.06, 0.02) | 0.068 |

The differences were shown using B (lower CI, upper CI). FLV, focal loss volume; FT, fovea thickness; GLV, global loss volume; HCs, healthy controls; mGCC, macular ganglion cell complex; pRNFL, peripapillary retinal nerve fiber layer thickness; TMV, total macular volume. p-values that were <0.05 are in bold.

FIGURE 3 | Comparison of the annual changes of SD-OCT measurements in NMOSDON+ eyes in patients with NMOSD. Annual loss of the macular GCC (A), RNFL (B), FT (C), and TMV (D) in eyes with a history of ON were compared between each two groups. GCC, ganglion cell complex; RNFL, retinal nerve fiber layer thickness; FT, fovea thickness; TMV, total macular volume; TCZ, tocilizumab; RTX, rituximab; AZA, azathioprine; HC, healthy control. *p < 0.05. NS, not significant.
pRNFL (β = −4.54 [−7.165, −1.923] μm, Wald p = 0.001), mGCC thickness (β = −0.94 [−1.654, −0.230] μm, Wald p = 0.010), and TMV (β = −0.08 [−0.144, −0.013] mm³, Wald p = 0.019) compared to HC eyes.

Patients treated with tocilizumab did not show any significant differences in annual changes of pRNFL, mGCC, and TMV in NMOSD^ON−^ eyes compared to patients treated with rituximab.

Post-hoc analyses also revealed that tocilizumab and rituximab significantly reduced mGCC annual thinning (β = −1.00 [−1.779, −0.223] μm, Wald p = 0.011) and TMV (β = −0.137 [−2.46, −0.27] μm, Wald p = 0.015) compared with azathioprine. Patients treated with tocilizumab (β = −3.42 [−6.61, −0.23] μm, Wald p = 0.033) but not with rituximab treatment (β = −2.41 [−6.50, 1.67] μm, Wald p = 0.089) had less pRNFL annual thinning compared with those treated with azathioprine. The annual change in TMV did not differ significantly between the three treatment groups (Figure 4).

We did not detect significant changes in FT or any disc measures in the three groups in NMOSD^ON−^ eyes compared with baseline or HCs.

**DISCUSSION**

Using SD-OCT with intra-retinal segmentation, we investigated longitudinal retinal layer changes in AQP4-IgG seropositive NMOSD^ON+^ eyes and NMOSD^ON−^ eyes under different treatments in comparison with matched HC eyes. While patients on immunosuppression had no relapses during the follow-up period, we observed longitudinal mGCC loss in NMOSD^ON+^ eyes of AQP4-IgG seropositive NMOSD patients, independent of suppressing activity. However, for NMOSD^ON−^ eyes, only those treated with azathioprine displayed significant mGCC loss compared to those treated with tocilizumab and rituximab treatment.

Consistent with previous OCT studies (3, 4, 6, 23), we observed retinal neuroaxonal degeneration in both NMOSD^ON+^ eyes and NMOSD^ON−^ eyes at baseline, displayed by reduced mGCC and thinning of pRNFL. mGCC reduction represents ganglion cell damage and pRNFL acts as a marker of retinal axonal loss compared to those treated with tocilizumab and rituximab treatment.

Evidence on subclinical retinal damage in NMOSD are very contradictory. Some studies did not detect differences in RNFL thickness between NMOSD^ON−^ eyes and healthy eyes (11, 27, 28). However, increasing evidence showed that subclinical retinal ganglion cell neuronal and axonal loss occurred in NMOSD^ON−^ eyes (7, 29). A comparative study also reported cases with no history of clinical ON but presence of RNFL and GCC loss in NMOSD, which indicates primary neuroaxonal pathology (24). Our data supported microstructural atrophy in NMOSD^ON−^.
eyes (7). The retinal changes may be explained by AQP4-IgG-mediated pathology of astrocytes and Müller cells, regardless of ON history (24).

Previous studies revealed that neuroaxonal loss was very evident in the first 6 months in NMOSDON+ eyes, and after the acute phase, there is no clear increase in neurodegeneration compared with NMOSDON− eyes (30). In our study, NMOSDON+ eyes experienced progressive neuroaxonal degeneration over 6 months. This finding is consistent with findings of previous OCT studies also indicating that NMOSD patients display a progressive retinal thinning, regardless of disease activity (4, 5). Specifically, neuropathological findings show that AQP4 immunoreactivity on astrocytes is lost in anterograde/retrograde degeneration in the optic nerves. Similar to MS, the involvement of the anterior visual pathway in NMOSD is widespread and chronically progressive (31).

Although, tocilizumab and rituximab show high efficacy in reducing the risk of attacks in patients with NMOSD, neither of them can reverse axonal degeneration of mGCC and pRNFL after ON. In a case report, severe sensory impairment in a patient with NMOSD and intractable axonal neuropathy still gradually progressed during tocilizumab treatment (32). However, in an animal model of spinal cord injury pain, IL-6 signaling was predominantly elevated in reactive astrocytes and IL-6R blockade contributed to alleviating allodynia and decreasing glutamate transporter GLT-1 (33). Conflicting reports of the efficacy of tocilizumab in neurorepair warrants further confirmatory studies.

Different from previous studies, we investigated the influence of disease-modifying therapy on retinal changes. Although, we did not detect any differences in NMOSDON+ eyes when comparing patients on biological agents vs. those on azathioprine and corticosteroids, we did observe axonal loss in eyes without prior ON in azathioprine-treated patients. We postulated that chronic neurodegeneration may persist in NMOSDON− eyes, as previous research indicates progressive neurodegeneration in AQP4-expressing brain structures. Cortical neuronal loss with unique AQP4 dynamics in astrocytes was also observed in pathological processes that could reflect progressive neurodegeneration in NMOSD (34). Besides NMOSD-related retinopathy, cytostatic or cytotoxic immunosuppressants, as well as corticosteroids, might induce neuronal damage (35). Another explanation is that azathioprine may be less effective compared with rituximab or tocilizumab in deterring neuroaxonal loss. AQP4-IgG itself has also been

FIGURE 4 | Comparison of the annual changes of SD-OCT measurements in NMOSDON+ eyes in patients with NMOSD. Annual loss of the macular GCC (A), RNFL (B), FT (C), and TMV (D) in eyes with a history of ON were compared between each two groups. GCC, ganglion cell complex; RNFL, retinal nerve fiber layer thickness; FT, fovea thickness; TMV, total macular volume; TCZ, tocilizumab; RTX, rituximab; AZA, azathioprine; HC, healthy control. *p < 0.05, **p < 0.01, ***p < 0.001. NS, not significant.
associated with progressive neurodegeneration in the visual system as well as in other CNS areas (24). Compared with the azathioprine-treated patients, rituximab is more effective in preventing relapses, and could improve the symptoms (36, 37). Rituximab also decreases AQP4-IgG titers more than azathioprine (38). The TANGO study showed that tocolizumab significantly reduces the risk of relapses and AQP4-IgG compared with azathioprine (17). Therefore, second-line treatments such as rituximab and tocolizumab may be more effective than azathioprine in suppressing this AQP4-IgG related neurodegeneration.

Patients in this study displayed no significant changes in LogMAR and low-contrast letter acuity in a median 1-year follow-up. Consistent with the visual acuity, TMV and FT remained lower compared to HCs in both NMOSDON+ and NMOSDON− eyes compared to HC eyes and did not demonstrate longitudinal atrophy or improvement. This indicates that neurodegeneration is irreversible in NMOSD affected and unaffected eyes and early treatment of ON attacks is necessary, as subsequent visual acuity would not improve during the chronic remission period. Recent studies demonstrate that monoclonal antibodies (rituximab, eculizumab, inebilizumab, and satralizumab) were effective for the treatment of NMOSD (39); few studies focused on the changes of OCT measures and visual acuity. No difference was observed in changes in low-contrast letter acuity binocular score from baseline in an inebilizumab treatment group compared with placebo, though, inebilizumab significantly reduced the risk of an ON attack (40).

Earlier studies using optic disc photography revealed that pathological optic disc cupping was present in 16–37% of eyes affected by idiopathic ON (41). To the best of our knowledge, this is the first study to use OCT to determine if optic disc cupping occurs after ON in NMOSD. We found that after ON, optic disc cup area and volume were slightly enlarged in both NMOSDON+ and NMOSDON− eyes. Optic disc cupping may progress slowly during follow-ups, explained by the loss of retinal nerve fibers. However, we observed no significant changes in optic nerve head topography measures, which we interpreted as a potential flooring effect resulting from previous ON episodes.

There were several limitations in this study. The follow-up period was relatively short, and each subgroup had a small sample size. Additionally, we were not able to analyze separate layers, such as the ganglion cell-inner plexiform layer using the RTVUE100-2 OCT instrument. The GCCs and pRNFLs with a history of severe ONs that were very thin and difficult to measure by other methods were excluded. Finally, this study lacks a rescanning protocol for OCT follow-ups.

CONCLUSION

Our findings support the notion that disease-related damage occurs mainly during acute attacks. Chronic progressive retinopathy also occurs during remission. These findings suggest the importance of monitoring OCT measures to provide evidence for efficacious and safe disease-modifying therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institute Ethical Board of Tianjin Medical University General Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CZ, PZ, and CD designed the study, collected, analyzed, and interpreted the data, and drafted and revised the manuscript. RZ provided technical support for the study. PZ and CD did statistical analysis. PZ, CD, DJ, FJ, MF, and CZ collected and analyzed the data and revised the manuscript critically for intellectual content. Authors involved in drafting the text and figures were CZ and PZ. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.669567/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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