Ganglion Cell Layer and Inner Plexiform Layer as Predictors of Vision Recovery in Ethambutol-Induced Optic Neuropathy: A Longitudinal OCT Analysis

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Purpose. To describe changes in the retina during ethambutol-induced optic neuropathy (EON) progression, and determine the retinal layer thickness as a predictive factor for vision recovery after stoppage of ethambutol (EMB) in EON.

Methods. A total of 42 eyes in 21 patients with EON underwent Spectralis optical coherence tomography after the onset of symptoms. Peripapillary and macular retinal nerve fiber layer as well as ganglion cell layer plus inner plexus layer (GCIPL) thickness was measured using Early Treatment Diabetic Retinopathy Study (ETDRS) circles. Data on best-corrected visual acuity at first visit and 1, 3, 6, and 12 months after stoppage of drug were collected. The longitudinal change of each retinal layer was described, and the association between retinal thickness at the first visit and the degree of vision recovery at each visit was analyzed.

Results. In inner temporal GCIPL, a 10-μm-thickness loss in the initial OCT was associated with a 0.5 decrease in the amount of logMAR visual acuity recovery at 12 months ($P < 0.001$, 95% confidence interval [CI]: 0.2–0.7), and a 10-μm-thickness reduction between follow-up visits was associated with a 0.5 decrease in the amount of logMAR visual acuity recovery ($P < 0.001$, 95% CI: 0.3–0.7).

Conclusions. We described a structure–function relationship between the retinal changes that occur in early EON and vision recovery. Temporal GCIPL could be used to predict vision recovery at 12 months after stoppage of EMB. Careful evaluation for GCIPL damage is required for visual prognosis in early EON.

Keywords: ethambutol, optic neuropathy, visual acuity, recovery, ganglion cell layer

Ethambutol (EMB), the first-line drug used to treat mycobacterium tuberculosis, is a common cause of toxic optic neuropathy. EMB-induced optic neuropathy (EON) has been reported in 1% to 5% of all patients, accounting for 100,000 new cases per year.[1–3] EON is characterized by dyschromatopsia and gradual visual loss of bilateral eyes. Since a delayed diagnosis of EON carries the risk of irreversible visual loss, early detection of EON and early stoppage of EMB are required.[4] Unfortunately, there are no obvious predisposing or risk factors to contribute to the poor visual gain after stoppage of EMB.[5–7]

Despite the importance of early detection, early-stage EON (within 6 months of the onset of visual deterioration) does not present remarkable findings in a fundus examination.[8] Many trials have investigated structural changes to particular retinal layers to allow early detection of EON.[9–13] Evidence has emerged that the structural changes occur around the time of onset of symptoms. Han and associates[10] prospectively enrolled 1 EON patient (2.7%) from among 37 patients taking EMB and reported early thickening of peripapillary retinal nerve fiber layer (cpRNFL) and thinning of the ganglion cell layer plus inner plexus layer (GCIPL) at the onset of symptoms.[10] The previous research work has not yet demonstrated whether these early structural changes are associated with visual prognosis or not.

If there is a significant relationship between these structural changes and visual prognosis, this may provide invaluable information on the therapeutic window in which to prevent further structural damage to avoid irreversible visual loss. Therefore, we aimed to quantify the retinal thickness changes of cpRNFL, macular RNFL (mRNFL), and GCIPL after stoppage of EMB in EON, and to establish a relationship between any structural changes and the amount of vision recovery.

Materials and Methods

Subjects

This hospital-based retrospective observational study was a single-center study performed from 2009 to 2016 in accordance with the tenets of the Declaration of Helsinki. This study was approved by the institutional review board of Samsung Medical Center. Patient records were anonymized and de-identified prior to analysis. All patients diagnosed with EON were reviewed.

Patients were eligible for inclusion if they were receiving EMB doses for the treatment of pulmonary tuberculosis. Inclusion criteria were as follows: slowly progressive visual loss accompanied by dyschromatopsia after taking EMB, taking EMB for more than 2 months, normal appearance of the optic disc at the first visit, and absence of symptoms associated with optic neuritis, such as pain on ocular movement. All patients who reported any suspicious visual symptoms were referred

immediately to the neuro-ophthalmology clinic and told to stop taking EMB. All subjects underwent Spectralis optical coherence tomography (OCT; Heidelberg Engineering, Heidelberg, Germany) after the onset of symptoms (visit 1) and at a follow-up visit (visit 2). We considered the retinal thickness measurements from visit 1 to be representative data. Only high-quality (Q values > 25) scans were included. The change in retinal thickness between visits 1 and 2 was used to evaluate a possible association with vision recovery. All study participants underwent a detailed ophthalmologic examination, including assessment of refraction, best-corrected visual acuity (BCVA), color vision, relative afferent pupillary defect, slit-lamp examination, tonometry, and a fundus examination. Exclusion criteria included the presence of concurrent retinal diseases, any history of glaucoma, and ocular surgery except for cataract surgery.

Data on sex, age, duration of taking EMB, BCVA at first visit, presence of visual recovery, BCVA at 1, 3, 6, and 12 months after stoppage of EMB, and color vision examined by Hardy-Rand-Rittler (HRR) test were collected from the electronic records of each patient. Corrected visual acuities were transformed to a logarithmic scale (logMAR) for statistical analysis. The amount of visual acuity recovery was calculated as the difference between the first BCVA logMAR and the follow-up BCVA logMAR at each visit.

Image Acquisition and Retinal Layer Thickness Measurement

One experienced technician (Y.L.) performed imaging on all subjects with disc and retinal Spectralis OCT (software version 1.6.4.0; Heidelberg Engineering). A 360° 3.4-mm-diameter peripapillary circle scan centered on the optic disc was performed for RNFL assessment, and all OCT scans were performed with spectral-domain (SD)-OCT that provided 40,000 A-scans/second with 7-μm optical and 3.5-μm digital axial resolution. Horizontal and vertical SD-OCT scans of the fovea were obtained for all subjects. All OCT images were converted to grayscale for better visualization and accurate analysis. The thickness of each retinal layer was measured automatically using the SD-OCT software. The thickness values for the inner locations (inner superior, inner nasal, inner temporal, and inner inferior) were measured in the Early Treatment Diabetic Retinopathy Study (ETDRS) central circular 1000-μm-diameter area. Thicknesses of the outer areas (outer superior, outer nasal, outer temporal, and outer inferior) were measured in the ETDRS circular 3000-μm-diameter area. Using this method, we acquired the thicknesses of the cpRNFL, mRNFL at the outer locations, and GCIPL at the inner and outer locations. Main outcomes were grouped into two categories: each retinal layer thickness measurement at visit 1 (initial) and difference in each thickness measurement between visits 1 and 2.

Statistical Analysis

Statistical analyses were performed using SAS version 9.4 software (SAS Institute, Cary, NC, USA). We used the generalized estimating equation (GEE) with Bonferroni's correction to assess the associations between retinal thickness measurements and clinical factors. We described the relationships between thickness as a function of time with scatter plots fitted with a locally weighted regression smoothed nonparametric regression line. Both categories of OCT measurements were used for the analysis. Age, sex, laterality of EON, and timing of OCT after the onset of symptoms were adjusted. A P value < 0.05 was considered significant. All data were presented as mean ± standard deviation.

Results

We enrolled a total of 42 eyes in 21 patients, 9 males and 12 females (Table). The mean age was 59 ± 12 years (range, 33–77 years). The mean time between the onset of symptoms and the first SD-OCT scan was 0.5 ± 0.5 months (range, 0–1.2 months). Duration of taking EMB was 5.7 ± 2.9 months (range, 2–12 months), and the mean dosage of EMB was 17.8 ± 2.3 mg/kg (range, 15.0–22.8 mg/kg). No patient had prior history of EON. The symptoms at the first visit were gradual decrease in visual acuity in 15 (72%) patients, visual field constriction in 3 (14%) patients, and dyschromatopsia in 3 (14%) patients. These symptoms developed during taking EMB in all patients.

BCVA was 0.83 ± 0.43 logMAR at the first visit and 0.99 ± 0.42 logMAR 1 month, 0.82 ± 0.52 logMAR 3 months, 0.69 ± 0.42 logMAR 6 months, and 0.38 ± 0.54 logMAR 12 months after stoppage of EMB. There was a significant difference in BCVA between the first visit and 1 month, between 1 and 3 months, between 3 and 6 months, and between 6 and 12 months (paired t-test, $P = 0.014$, 0.026, 0.037, and < 0.0001, respectively).

The mean interval between the initial and follow-up OCT was 6.2 ± 0.6 months (range, 5.5–7.0 months). The mean thickness of cpRNFL at the temporal, nasal, and inferior locations, inner GCIPL at the temporal, nasal, and inferior locations, and outer GCIPL at the superior and inferior locations was significantly thinner in the follow-up OCT than in the initial OCT (GEE with Bonferroni's correction: $P = 0.012$, 0.014, 0.001, 0.003, 0.002, 0.048, 0.009, and 0.041, respectively) (Fig. 1). The longitudinal change in each thickness measurement is presented in Figure 2.

Correlation Between Thickness Measurements and Vision Recovery

A multivariable analysis between visit 1 retinal layer thicknesses and visual recovery showed that as the thickness of the temporal inner GCIPL increased, the degree of visual acuity improvement 12 months after discontinuation of EMB increased significantly ($P < 0.001$). In the inner GCIPL a 10-μm-thickness loss was associated with a 0.5 decrease in the amount of logMAR visual acuity recovery at 12 months (95% confidence interval [CI]: 0.2–0.7). We found no significant correlation with the amount of visual acuity recovery at 1, 3, and 6 months. A scatter plot of the relationship between GCIPL measurements and the amount of visual acuity recovery is presented in Figure 3.

A multivariable analysis between the change in thickness measurements between visits 1 and 2 and visual recovery showed that as the reduction in temporal inner GCIPL and superior cpRNFL increased between visits 1 and 2, the degree of visual acuity improvement 12 months after discontinuation of EMB decreased significantly ($P < 0.001$ and 0.09, respectively). In the inner GCIPL, a 10-μm-thickness reduction was associated with a 0.5 decrease in the amount of logMAR visual acuity recovery (95% CI: 0.3–0.7); in the superior cpRNFL, a 10-μm-thickness reduction was associated with a 0.5 decrease in the amount of logMAR visual acuity recovery at 12 months (95% CI: 0.2–0.8).

Other Clinical Factors Correlated With Visual Recovery

Two other clinical factors, duration of medication (interval between starting EMB and the first visual symptoms) and initial BCVA, were significantly correlated with visual acuity recovery. As duration of medication use increased, the degree of visual acuity recovery decreased 3, 6, and 12 months after stoppage

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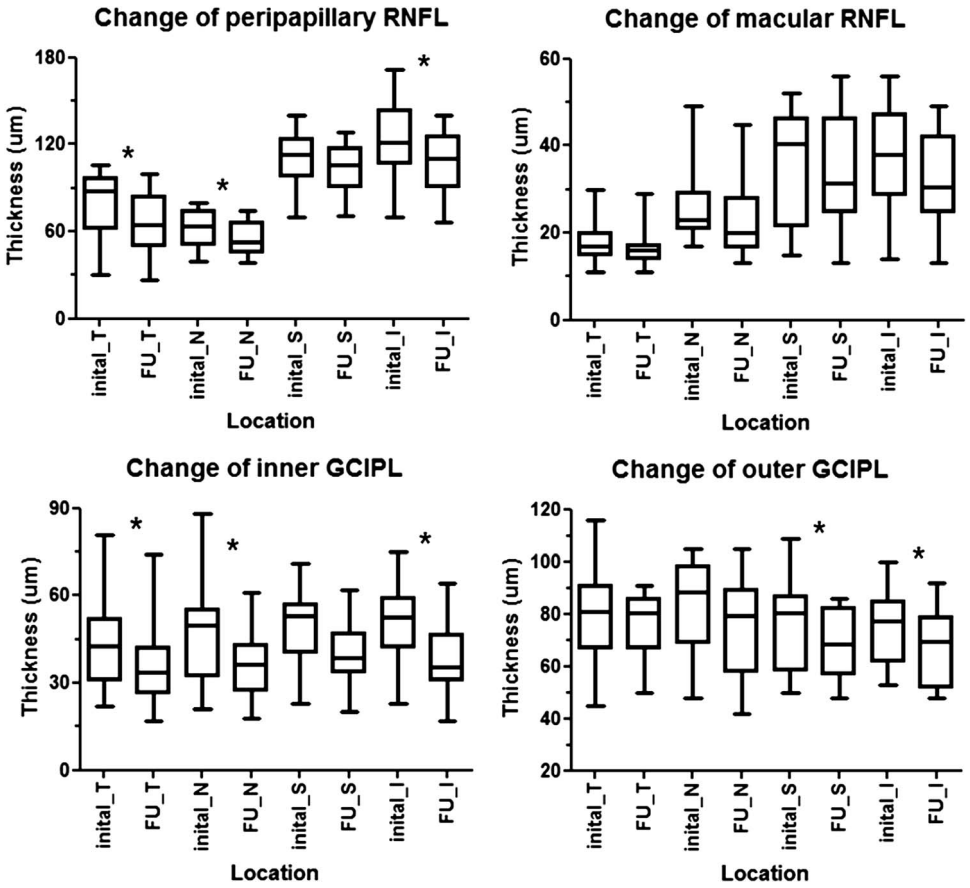

FIGURE 1. The change in mean thickness measurements of peripapillary retinal nerve fiber layer (cpRNFL), macular RNFL, and ganglion cell layer plus inner plexus layer (GCIPL) between initial and follow-up visits in patients with ethambutol-induced optic neuropathy (*asterisks*: $P < 0.05$).

of EMB (all $P < 0.001$). Also, the lower the initial BCVA was at the first visit, the greater the visual acuity improvement was 12 months after stoppage of EMB ($P < 0.001$). This study has an 80% or greater power to evaluate the difference in temporal GCIPL thickness between visits 1 and 2.

DISCUSSION

Many trials have been done to evaluate structural changes to the disc and retina in early-stage EON because OCT clearly demonstrates the structural loss of particular retinal layers, despite a near-normal appearance of the fundus. Early GCIPL loss, which refers to primary axonal loss in some optic neuropathies, was previously reported.[14,15] Balducci et al.[14] detected damage to the GCIPL starting 6 weeks before visual loss in Leber's hereditary optic neuropathy (LHON). Viera et al.[15] reported a 34% to 40% decrease in the thickness of the retinal ganglion cell layer (compared with a control group) in eight patients with EMB-induced and nutritional optic neuropathy.[15]

In the present longitudinal analysis, we also observed initial cpRNFL swelling within 3 months after symptom onset. Initial decrease of GCIPL, reflecting early axonal damage caused primarily by EMB toxicity, was more remarkable in inner locations than in outer locations. The results were similar to those in a previous study reporting early swelling of cpRNFL and an early decrease in the perifoveal GCIPL.[10] The amount of reduction in the cpRNFL during follow-up was much greater than in the GCIPL (visit 1 versus visit 2). Despite greater reduction in cpRNFL, GCIPL values are preferable to cpRNFL or mRNFL values due to earlier expression of structural damage compared to cpRNFL for detecting early neuronal loss caused by EON, whereas during the progression of EON, structural changes to the cpRNFL should also be carefully observed.

Our most important finding was that the amount of reduction in temporal GCIPL thickness negatively correlated with the degree of vision recovery. Some studies previously reported a relationship between visual acuity and GCIPL thickness that was related to the pathophysiology of each optic neuropathy. Kim et al.[16] demonstrated that thinning of the superior and inferior GCIPL was significantly correlated with visual acuity in severe glaucoma, and Moster et al.[17] reported that a decrease in GCIPL thickness was associated with worsening vision in the acute phase of LHON.[17] In addition to those optic neuropathies, we found a significant effect of GCIPL thickness on the potential for visual recovery in patients with EON. In our results, as the thickness of the remaining GCIPL increased and the damage to the GCIPL decreased during 6 months of follow-up, the possibility of visual acuity recovery before permanent retinal ganglion cell death increased. That result suggests the importance of preventing early axonal damage and further deterioration, particularly before ganglion cell death, for long-term visual recovery. This finding could be helpful to other researchers investigating therapeutic targets and devising a cure for EON in the future.

In a previous study of EON, Zoumalan et al.[11] observed 79% of mean loss in temporal cpRNFL thickness, and the proportion of nerve fiber loss was greater in temporal cpRNFL thickness than in nasal, inferior, and superior cpRNFL thickness in subjects with acute and persistent vision loss. In relation to visual acuity, those authors emphasized the

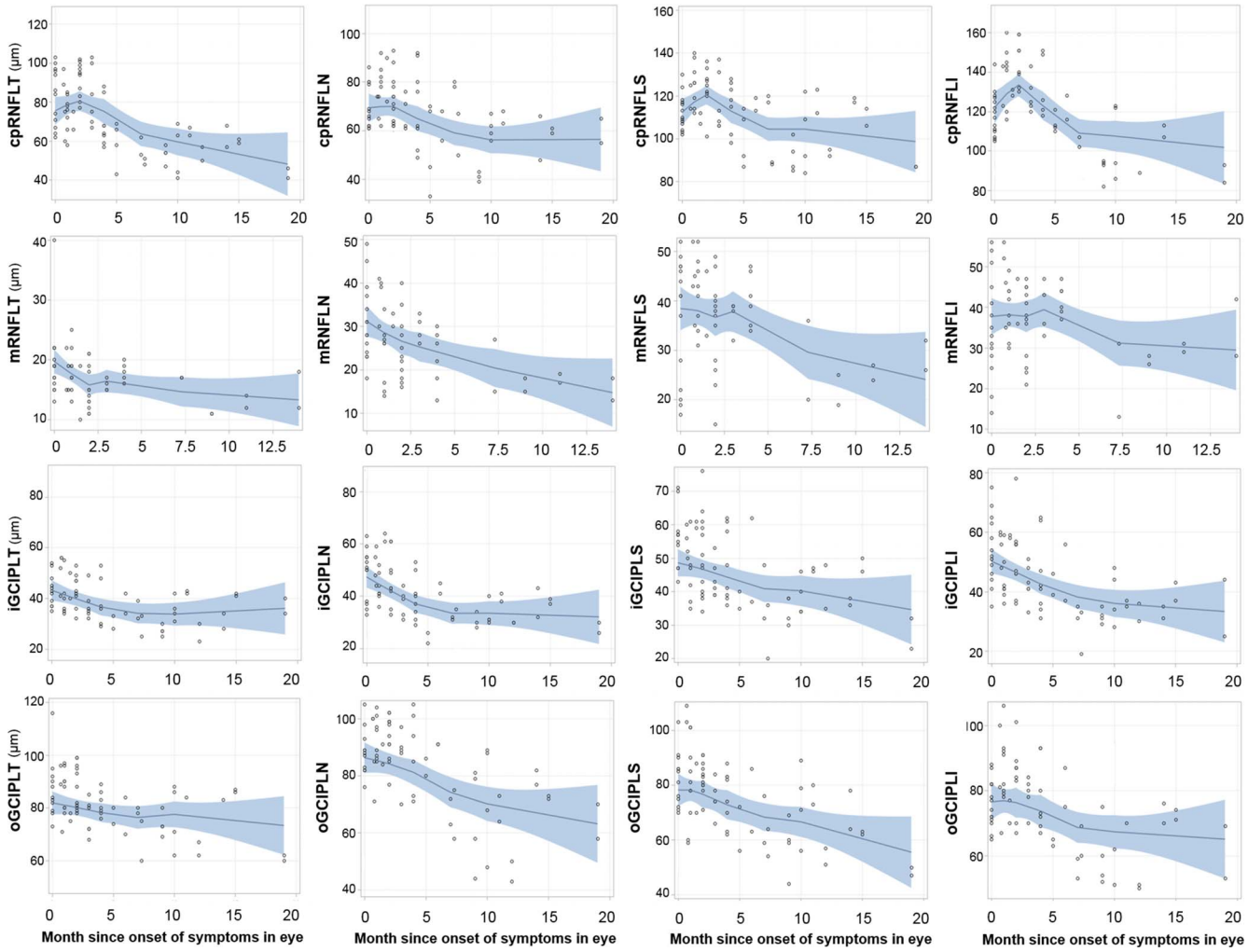

Figure 2. Peripapillary retinal nerve fiber layer (cpRNFL), macular RNFL, and ganglion cell layer plus inner plexus layer (GCIPL) thickness during ethambutol-induced optic neuropathy progression.

temporal segmental loss of the cpRNFL in EON, which is related to the papillomacular bundle. Based on their results, the nasal GCIPL could be expected to correlate with visual acuity because it is directly adjacent to the papillomacular bundle. However, contrary to our expectation, only the temporal inner GCIPL showed a significant correlation with visual recovery. This result can be explained by the severity of EON expressed in the segmental damage regarding the GCIPL. In patients with significant temporal thinning of the GCIPL, significant nasal thinning of the GCIPL was also observed. Thus, the nasal GCIPL might commonly have been damaged. Therefore, further GCIPL thinning at the temporal location could indicate more severe axonal damage and the process of global atrophy. Thus, temporal GCIPL thinning could be related to poorer visual acuity and become a reflection of the severity of EON. Moreover, any change in temporal structural loss should be carefully considered in EON since the axonal damage caused by EMB toxicity might not be limited to the papillomacular bundle. Early GCIPL thinning that is present at symptom onset commonly precedes cpRNFL changes that increase at symptom onset and subsequently normalize at 6 months[10]; we would thus recommend using the temporal GCIPL over the cpRNFL or mRNFL as an anatomic marker to predict visual acuity improvement in early-phase EON.

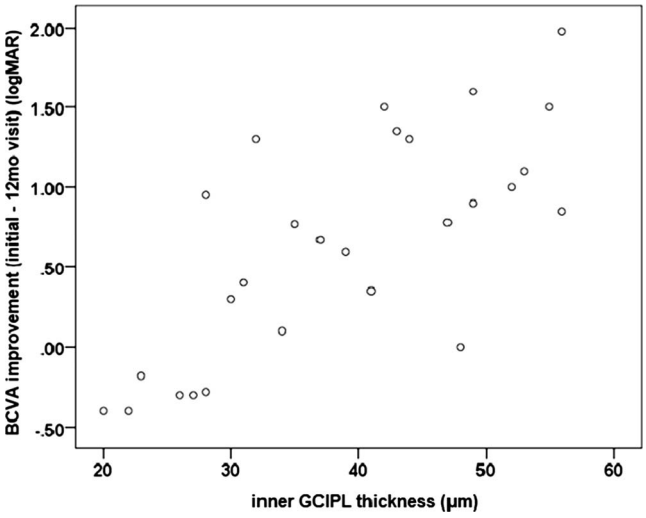

Figure 3. A scatter plot of the relationship between inner temporal GCIPL measurements and the amount of visual acuity recovery at 12 months after discontinuation of drug.

Table. The Descriptive Statistics of All Subjects

Variable	EON, $n = 21$ Mean ± SD
Age, y (range)	59 ± 12 (33–77)
Sex, M/F	9/12
Visual acuity, logMAR	
First visit	0.83 ± 0.43
1 month after EMB stoppage	0.99 ± 0.42
3 months after EMB stoppage	0.82 ± 0.52
6 months after EMB stoppage	0.69 ± 0.42
12 months after EMB stoppage	0.38 ± 0.54
Refractive error, prism diopter	−1.69 ± 2.26
Color vision, HRR/no. of test plates	0.24 ± 0.41
Number of OCT examinations, *n*	
1 exam	9
2 exams	6
3 exams	3
4 exams	3

In addition to the GCIPL thickness itself, the amount of temporal GCIPL loss during 6 months was also negatively associated with the amount of vision recovery at 12 months. To predict vision improvement, careful checkups are required not only for initial GCIPL thickness in early-stage EON, but also for the amount of temporal GCIPL change according to disease progression. In cases with considerable loss of the temporal GCIPL in early-stage EON, we recommend careful observation of the progressive loss of the temporal GCIPL because of the possibility of further visual loss and a reduced possibility of vision recovery.

The daily dosage of EMB ranged from 15 to 22.8 mg/kg in EON patients. It was consistent with that in a previous study reporting that EMB may cause optic neuropathy if the daily dosage exceeds 15 mg/kg.[18] Among other factors, duration of EMB and initial BCVA were also correlated with visual prognosis in this study. Initial visual acuity was negatively correlated with the amount of vision recovery. Similar to previous studies reporting a correlation between the duration of EMB and the risk of toxicity,[19,20] we found that the duration of EMB could affect visual prognosis. In contrast, a 10-year study reported that no obvious prognostic factors including duration of EMB and initial BCVA were found to facilitate vision recovery.[5] Hence, regarding clinical factors, further well-controlled studies are required to predict visual acuity recovery.

It is of note that EON continued to progress even after stoppage of EMB. We found that visual acuity significantly decreased within 1 month after stoppage of EMB, then gradually improved after 1 month. Similar to this result, much research work has demonstrated reversal of the toxicity induced by EMB after 1 month of stoppage of the drug, and recovery may occur as late as 1 year after discontinuation of EMB.[21] During the early progression phase after stoppage of EMB, it is very challenging to predict patients' visual prognosis. According to the results of the present study, early evaluation of GCIPL damage may provide important information to clinicians about vision recovery in this confusing early period. In addition, our results suggest that EMB toxicity may not be limited to the papillomacular bundle; thus we direct clinician attention to a careful evaluation not only of nasal GCIPL damage (regarding the papillomacular bundle), but also of temporal GCIPL damage, which may seem relatively less important.

This study has the following limitations. First, this was a retrospective, single-center study, and the sample size was relatively small. Because our results are retrospective, we could not determine the threshold of EMB dosage, and clinicians should use caution in uniform application to patients with EON. Second, long-term evaluation between clinical factors and retinal thickness could not be performed. Since the plan was "immediately stop ethambutol and regular observation" after EON diagnosis, most patients with a short follow-up were managing their tuberculosis in a hospital nearer to their hometown, thus were referred to the hospital again. Third, the relationships between retinal thickness and other visual factors including color vision and visual field defects were not analyzed. Therefore, prospective studies are needed to verify our results.

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