Reproducibility of Retinal Nerve Fiber Layer Thickness Measures Using Eye Tracking in Children With Nonglaucomatous Optic Neuropathy

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• PURPOSE: **To determine the intra- and intervisit reproducibility of circumpapillary retinal nerve fiber layer (RNFL) thickness measures using eye tracking–assisted spectral-domain optical coherence tomography (SD OCT) in children with nonglaucomatous optic neuropathy.**

• DESIGN: **Prospective longitudinal study.**

• METHODS: **Circumpapillary RNFL thickness measures were acquired with SD OCT using the eye-tracking feature at 2 separate study visits. Children with normal and abnormal vision (visual acuity ≥0.2 logMAR above normal and/or visual field loss) who demonstrated clinical and radiographic stability were enrolled. Intra- and intervisit reproducibility was calculated for the global average and 9 anatomic sectors by calculating the coefficient of variation and intraclass correlation coefficient.**

• RESULTS: **Forty-two subjects (median age 8.6 years, range 3.9–18.2 years) met inclusion criteria and contributed 62 study eyes. Both the abnormal and normal vision cohort demonstrated the lowest intravisit coefficient of variation for the global RNFL thickness. Intervisit reproducibility remained good for those with normal and abnormal vision, although small but statistically significant increases in the coefficient of variation were observed for multiple anatomic sectors in both cohorts. The magnitude of visual acuity loss was significantly associated with the global (ß = 0.026, $P < .01$) and temporal sector coefficient of variation (ß = 0.099, $P < .01$).**

• CONCLUSION: **SD OCT with eye tracking demonstrates highly reproducible RNFL thickness measures. Subjects with vision loss demonstrate greater intra- and intervisit variability than those with normal vision. (Am J Ophthalmol 2015;159:71–77. © 2015 by Elsevier Inc. All rights reserved.)**

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OPTICAL COHERENCE TOMOGRAPHY (OCT) HAS become an invaluable and objective tool in the management of optic neuropathies.[1–7] The evolution from time-domain OCT to spectral-domain OCT (SD OCT) not only has improved image resolution and acquisition time, but now includes mechanisms to improve test-retest reliability.[8–10] Specifically, some current-generation SD OCT devices provide an eye tracking feature that uses a confocal scanning laser ophthalmoscope or line scanning ophthalmoscope to accommodate for eye movements and that improves the accuracy of subsequently acquired images to be captured in the same anatomic location.[11–15] The within- and between-visit reproducibility of circumpapillary retinal nerve fiber layer (RNFL) thickness measures have been reported most frequently using the Spectralis OCT (Heidelberg Engineering, Inc, Heidelberg, Germany) and using the eye tracking feature.[11,13–15]

Most studies describing the reproducibility of circumpapillary RNFL measures have examined adult subjects with glaucoma[11,13] and/or healthy controls.[11–15] Only 1 study has reported the intravisit reproducibility of circumpapillary RNFL thickness measures for children with glaucoma using the Spectralis with eye tracking.[11] While the data presented to date is helpful when examining healthy subjects and adults with glaucoma, it is unclear whether reproducibility measures are comparable in children with optic neuropathies other than glaucoma, since the mechanism and pattern of RNFL loss differ.

The ability of SD OCT to monitor longitudinal circumpapillary RNFL changes in glaucoma and nonglaucomatous optic neuropathies could be especially beneficial in children, since their functional assessment of vision (ie, visual acuity and visual field) relies heavily on their cooperation and cognitive abilities.[16] The goal of the current study is to determine the intra- and intervisit reproducibility of RNFL thickness measurements using eye tracking in children with nonglaucomatous optic neuropathies.

METHODS

• SUBJECTS: Children being cared for in the Neuro-Ophthalmology clinic at Children's National Medical

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Center who underwent SD OCT imaging were eligible for study enrollment. Informed consent was obtained from the parent/legal guardian before study enrollment. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board at Children's National Medical Center. All data collected were Health Insurance Portability and Accountability Act compliant.

Subjects determined to have the following nonglaucomatous optic neuropathies were eligible for enrollment: low-grade gliomas intrinsic to the visual pathway (ie, optic pathway gliomas), extrinsic tumors involving the anterior visual pathway (ie, craniopharyngioma, prolactinoma), and demyelinating disease. Healthy children with normal examinations and children with neurofibromatosis type 1 who did not have an optic pathway glioma were eligible to contribute a single eye to the healthy/unaffected cohort, since previous work has shown that RNFL thickness does not differ between these groups.[17] Subjects were included if they met all of the following criteria: (1) 2 separate study visits within 12 months; (2) clinically stable disease without radiographic (ie, tumor growth, new/worsening signal characteristics, or new/worsening contrast enhancement) or clinical progression (ie, >0.1 logMAR worsening in visual acuity or progressive visual field loss); (3) absence of examination findings suggestive of glaucoma; (4) 3 or more acceptable circumpapillary RNFL scans acquired at the initial study visit and a minimum of 1 scan at the follow-up visit; (5) SD OCT scan signal strength >20 db; and (6) acquisition of the entire image volume without appreciable movement or acquisition artifact. Subjects who did not complete a second study visit or experienced clinical progression were excluded from the study. Subjects with tumors that could potentially cause vision loss in 1 or both eyes (ie, optic chiasm or optic tract lesions) who demonstrated clinical progression in only 1 eye were excluded.

Clinical and demographic characteristics were abstracted from the subject's clinical record. Subjects were classified as having abnormal vision is their visual acuity was ≥0.2 logMAR above normal for age or had visual field loss. The magnitude of visual acuity loss was calculated by subtracting the current logMAR from the normal logMAR for age. When able to cooperate, visual field loss was determined by automated or kinetic perimetry.

• **IMAGE ACQUISITION AND ANALYSIS:** All images were acquired with the Spectralis SD OCT (Heidelberg Engineering GmbH, Heidelberg, Germany) using the "Nsite Analytics" (version 5.6.3.0) and "TruTrack" eye tracking. Once the patient fixates on an internal or external target, allowing the optic nerve to be visualized on the infrared image, this feature permits the operator to "freeze" the image and then manually align the 3.5 mm circular scan over the optic nerve head. If the subject blinks, stops fixating, or moves out of focus, the OCT acquisition will pause until proper alignment is again achieved. RNFL measures were acquired in high-speed mode (768 A-scans) with an automatic real-time (ART) setting of 16. RNFL thickness was measured in 9 sectors: temporal-superior, temporal, temporal-inferior, inferior, nasal-inferior, nasal, nasal-superior, superior, and papillomacular bundle. The global average of all 4 quadrants was also calculated. Scans and their segmentation were reviewed by 1 investigator (R.D.R.) who was masked to all clinical information. Each scan was inspected for segmentation errors and, when necessary, was manually adjusted using the manufacturer-supplied segmentation software. After the first study visit, the scan with the highest-quality score, absent of image artifacts, was chosen as the reference scan for the second-visit acquisitions.

• **STATISTICS:** Standard descriptive statistics were used to summarize clinical and demographic characteristics. Coefficient of variation and intraclass correlation coefficient (2-way mixed-effects model) were calculated for the intra- and intervisit analysis. The coefficient of variation, calculated as the standard deviation divided by the mean, represents the variability of a measure. A lower coefficient of variation value represents less variance, suggestive of more consistent measurements, whereas a higher coefficient of variation value represents greater variance among measurements. The intraclass correlation coefficient, another quantitative assessment of reproducibility, calculates the extent of congruence between individuals within a group. A higher intraclass correlation coefficient value represents greater reproducibility. Since this calculation is specific to the study population, intraclass correlation coefficient values cannot be compared across studies. Wilcoxon rank-sum test was used to compare the RNFL thickness, coefficient of variation, and image quality between subjects with and without vision loss. Paired *t* test using the Bonferroni adjustment for multiple comparisons (significance adjusted to $P < .005$) was used to compare the intervisit change of RNFL thickness and coefficient of variation within each cohort. Linear regression was used to evaluate the unadjusted and adjusted associations of age and tumor location (intrinsic vs extrinsic tumor) on coefficient of variation measures from the 4 anatomic quadrants (superior, nasal, inferior, temporal) and global average. Since only 1 eligible subject had demyelinating disease, that subject was included in the extrinsic tumor cohort. For subjects with normal vision in both eyes, 1 eye was selected for inclusion using a random number generator. Subjects with abnormal vision in both eyes could contribute 2 eyes, but a generalized estimating equation (GEE) was used instead of standard linear regression to account for the intereye correlation. The GEE model evaluated the unadjusted and adjusted associations of age, magnitude of visual acuity loss, and tumor location (intrinsic tumor vs others) on coefficient of variation measures in the abnormal vision cohort. Subjects with 1 eye

with abnormal vision and 1 eye with normal vision could contribute to both vision cohorts.

RESULTS

FORTY-TWO SUBJECTS MET INCLUSION CRITERIA AND contributed 62 study eyes. Fifteen subjects contributed both eyes to the abnormal vision cohort while 4 subjects contributed 1 eye to the abnormal vision cohort and 1 eye to the normal vision cohort. Twenty subjects contributed 1 eye to the normal vision cohort. Table 1 lists the demographic and clinical characteristics for subjects with and without vision loss. Both groups had a similar interval between study visits.

There was no statistical difference in scan quality between vision loss cohorts (Wilcoxon, $P > .05$). None of the sectors demonstrated a statistical change in RNFL thickness between visit 1 and visit 2 (Table 2).

TABLE 1. Demographic and Clinical Characteristics of Children With and Without Vision Loss Undergoing Spectral-Domain Optical Coherence Tomography Using Eye Tracking

	Abnormal Vision (N = 22)	Normal Vision (N = 24)
Age, y (mean/median)	10.7/10.2	8.5/8.2
Range	(4.9–18.2)	(3.9–14.4)
Female sex, n (%)	14 (64)	16 (67)
Race, n (%)		
White/Caucasian	18 (82)	19 (79)
Black/African American	4 (18)	4 (17)
Asian	0 (0)	0 (0)
Multiple races	0 (0)	1 (04)
Ethnicity, n (%)		
Non-Hispanic	19 (86)	22 (92)
Hispanic	3 (14)	2 (8)
Time (mo) between visits (mean/median)	4.2/2.8	4.2/3.3
Range	(1.1–10.4)	(1.3–9.3)
Diagnosis, n (%)		
NF1 with OPG	6 (27)	9
Sporadic OPG	11 (50)	6
Craniopharyngioma	2 (9)	–
Prolactinoma	1 (5)	–
Langerhans cell histiocytosis	1 (5)	–
Demyelination	1 (5)	–
Healthy/unaffected	–	9
Category of vision loss, (eyes, n)		
Abnormal visual acuity/normal visual field	5	–
Abnormal visual field/normal visual acuity	20	–
Abnormal visual acuity and visual field	12	–

NF1 = neurofibromatosis type 1; OPG = optic pathway glioma.

• **INTRAVISIT REPRODUCIBILITY:** For the intravisit analysis, both the abnormal and normal vision cohort demonstrated the lowest intraclass correlation coefficient (.961 and .992, respectively) and the highest coefficient of variation (6.2% and 2.2%, respectively) in the papillomacular bundle (Table 3). Similarly, the abnormal and normal vision cohort demonstrated the lowest coefficient of variation (1.5% and 0.7%, respectively) for the global thickness. The normal vision cohort demonstrated statistically lower coefficient of variation measures compared to the abnormal vision cohort in the global, temporal, temporal-inferior, and nasal sectors (Table 3).

• **INTERVISIT REPRODUCIBILITY:** Both the abnormal and normal vision cohorts demonstrated lower intraclass correlation coefficient and slightly higher coefficient of variation values in the intervisit analysis (Table 4). In the abnormal vision cohort, the lowest coefficient of variation was again demonstrated in the global measure (2.3%) and was highest in the papillomacular bundle (6.6%). For the normal vision cohort, the global measure also had the lowest coefficient of variation (1.3%), but the nasal and nasal-superior sectors (3.5% and 3.6%) demonstrated the highest coefficient of variation. The normal vision cohort demonstrated statistically lower coefficient of variation measures compared to the abnormal vision cohort in the global, temporal, temporal-inferior, nasal-inferior, nasal, and papillomacular bundle sectors (Table 4).

Both the abnormal and normal vision cohorts demonstrated an increase in coefficient of variation measures from visit 1 to visit 2 in all sectors. In the abnormal vision cohort, the coefficient of variation increase reached statistical significance for the global, temporal-superior, nasal-inferior, nasal-superior, and superior sectors ($P < .005$, for all comparisons). The normal vision cohort also demonstrated an increase in coefficient of variation from visit 1 to visit 2 in all sectors and reached statistical significance for the global, temporal-superior, temporal, inferior, and nasal sectors ($P < .005$, for all comparisons).

• **REGRESSION ANALYSIS:** The linear regression model demonstrated that the magnitude of visual acuity loss (ie, logMAR difference from normal) was the primary predictor of global coefficient of variation ($P = .009$), whereas the other clinical variables (ie, patient age and tumor location) did not significantly contribute to the model; therefore the abnormal and normal vision cohorts were analyzed separately.

Both age and tumor location (intrinsic tumor vs extrinsic tumor) failed to demonstrate a statistically significant association with global or sector coefficient of variation in the GEE regression model for abnormal vision cohort; therefore both variables were excluded from the adjusted analysis. The magnitude of visual acuity loss

TABLE 2. Circumpapillary Retinal Nerve Fiber Layer Thickness Measures Using Eye Tracking With Spectral-Domain Optical Coherence Tomography in Children With Optic Neuropathy

	Abnormal Vision (N = 37)[a]		Normal Vision (N = 25)[a]	
Sector[b]	Visit 1	Visit 2	Visit 1	Visit 2
G	63 ± 16.7	64 ± 17.5	97 ± 14.9	97 ± 13.8
TS	92 ± 24.9	93 ± 25.4	137 ± 25.8	138 ± 24.1
T	41 ± 14.4	41 ± 15.6	67 ± 13.3	66 ± 13.0
TI	93 ± 36.1	94 ± 36.9	143 ± 23.7	143 ± 22.1
I	83 ± 25.2	85 ± 26.2	124 ± 20.4	125 ± 19.7
NI	74 ± 22.9	75 ± 24.3	106 ± 21.1	108 ± 22.2
N	46 ± 20.7	47 ± 21.1	73 ± 19.4	73 ± 19.1
NS	74 ± 31.2	76 ± 31.9	109 ± 26.5	109 ± 25.5
S	83 ± 23.1	85 ± 23.8	123 ± 21.5	123 ± 19.4
PMB	32 ± 16.6	32 ± 17.8	49 ± 9.6	49 ± 9.5

G = global average; I = inferior; N = nasal; NI = nasal-inferior; NS = nasal-superior; PMB = papillomacular bundle; S = superior; T = temporal; TI = temporal-inferior; TS = temporal-superior.
[a]Study eyes.
[b]In μm.

TABLE 3. Intravisit Intraclass Correlation Coefficient and Coefficient of Variation for Circumpapillary Retinal Nerve Fiber Layer Thickness in Children Using Spectral-Domain Optical Coherence Tomography

	Abnormal Vision (N = 37)[a]		Normal Vision (N = 25)[a]		
Sector	ICC (95% CI)	CV (%)	ICC (95% CI)	CV (%)	*P* Value
G	.998 (.99, .99)	1.5	.998 (.99, .99)	0.7	.04
TS	.994 (.98, .99)	2.4	.995 (.99, .99)	1.4	N/S
T	.984 (.97, .99)	3.1	.997 (.99, .99)	1.2	.03
TI	.998 (.99, .99)	2.5	.998 (.99, .99)	1.0	.01
I	.996 (.99, .99)	2.0	.998 (.99, .99)	1.0	N/S
NI	.998 (.99, .99)	2.1	.997 (.99, .99)	1.4	N/S
N	.997 (.99, .99)	3.7	.997 (.99, .99)	1.4	<.01
NS	.997 (.99, .99)	3.1	.995 (.99, .99)	2.0	N/S
S	.997 (.99, .99)	1.9	.995 (.99, .99)	1.4	N/S
PMB	.961 (.93, .97)	6.2	.992 (.98, .99)	2.2	N/S

CI = confidence interval; CV = coefficient of variation; G = global average; I = inferior; ICC = intraclass correlation coefficient; N = nasal; NI = nasal-inferior; NS = nasal-superior; N/S = not significant; PMB = papillomacular bundle; S = superior; T = temporal; TI = temporal-inferior; TS = temporal-superior.
[a]Study eyes.

(ie, logMAR difference from normal) was significantly associated with the global (ß = 0.026, $P < .01$) and temporal sector coefficient of variation (ß = 0.099, $P < .01$).

The normal vision cohort failed to demonstrate a significant association between tumor location (intrinsic tumor vs healthy/unaffected) and coefficient of variation

TABLE 4. Intervisit Intraclass Correlation Coefficient and Coefficient of Variation for Circumpapillary Retinal Nerve Fiber Layer Thickness in Children Using Spectral-Domain Optical Coherence Tomography

	Abnormal Vision (N = 37)[a]		Normal Vision (N = 25)[a]		
Sector	ICC (95% CI)	CV (%)	ICC (95% CI)	CV (%)	*P* Value
G	.995 (.99, .99)	2.3	.991 (.98, .99)	1.3	.01
TS	.991 (.98, .99)	3.5	.985 (.96, .99)	2.4	N/S
T	.994 (.99, .99)	4.0	.987 (.97, .99)	2.2	<.01
TI	.995 (.99, .99)	3.3	.982 (.95, .99)	1.6	<.01
I	.993 (.98, .99)	2.8	.987 (.97, .99)	1.7	N/S
NI	.990 (.98, .99)	4.2	.987 (.97, .99)	2.5	.01
N	.992 (.98, .99)	5.2	.968 (.92, .98)	3.5	.03
NS	.991 (.98, .99)	4.5	.993 (.98, .99)	3.6	N/S
S	.990 (.98, .99)	3.0	.988 (.97, .99)	2.7	N/S
PMB	.996 (.99, .99)	6.6	.976 (.94, .98)	3.2	.01

CI = confidence interval; CV = coefficient of variation; G = global average; I = inferior; ICC = intraclass correlation coefficient; N = nasal; NI = nasal-inferior; NS = nasal-superior; N/S = not significant; PMB = papillomacular bundle; S = superior; T = temporal; TI = temporal-inferior; TS = temporal-superior.
[a]Study eyes.

measures in both unadjusted and adjusted analysis and was subsequently excluded. Older age was significantly associated with a higher coefficient of variation in the global (ß = 0.001, $P = .004$) and superior sector (ß = 0.003, $P = .01$) but not in the nasal, inferior, and temporal sectors ($P > .05$ for all comparisons).

DISCUSSION

CURRENT-GENERATION SD OCT DEVICES ARE NOW ABLE TO provide a highly reproducible, objective measurement (ie, circumpapillary RNFL) of the structural integrity of the anterior visual pathway.[9–11,13–15,18–22] The addition of eye tracking software can now accommodate for eye movements and provide accurate image alignment during subsequent image acquisition, and thus is believed to improve reproducibility. We report good intra- and intervisit reproducibility in children with and without vision loss using the eye tracking technology.

In our study, the intra- and intervisit coefficient of variation results demonstrated small but statistically significant differences between subjects with and without vision loss in clinically important regions such as the global, temporal, and temporal-inferior sectors. While all coefficient of variation results were below 5% in these regions for both cohorts, indicating good reproducibility, understanding the influence of vision loss on intra- and intervisit variability is important. In the abnormal vision group, the regression analysis revealed that subjects with greater visual acuity

loss demonstrated higher coefficient of variation values, indicative of more variability, in the global and temporal sectors. The papillomacular bundle measures demonstrated the highest coefficient of variation for almost all comparisons, likely owing to its being the thinnest section, thereby making it more susceptible to differences in segmentation and movement artifacts. Slight changes in the papillomacular bundle thickness may cause large deviations in its coefficient of variation values. Therefore, clinicians should be cautious when interpreting relatively small changes in papillomacular bundle thickness.

Our intra- and intervisit coefficient of variation values in children with normal vision appear to be comparable to other investigators' healthy control subjects who were imaged using the same SD OCT with eye tracking.[11,13,14,23] However, our subjects with abnormal vision demonstrated higher intra- and intervisit coefficient of variation values compared to adult and pediatric subjects with glaucoma.[11,13] The discrepancy between studies could potentially be attributable to differences in the amount of RNFL loss. In the study by Ghasia and associates,[11] their pediatric glaucoma/glaucoma suspect patients had an average global RNFL of 98 μm—much higher than that of our subjects with abnormal vision, which averaged 63 μm. The adult subjects with glaucoma in the study by Langenegger and associates[13] reported the average global RNFL thickness closer to, but still higher than, our subjects (71 vs 63 μm, respectively). Interestingly, another study reported that the magnitude of RNFL loss did not correlate with intervisit variability in adults with glaucoma, despite having a similar average global RNFL (ie, 65 μm) to that of our subjects.[23] The discrepancy between these studies and ours suggests that other clinical or technical factors can potentially influence intervisit variability.

Differences in study design may explain the variability of reproducibility results reported by investigators using the Spectralis SD OCT.[11,13,14,23] Specifically, many investigators simply have the patients reposition themselves or lean back away from the camera, restart the device, or take short breaks between scans—all of which are being performed on the same day.[13,23] When SD OCT images were acquired on different days, our subjects, as well as the pediatric healthy controls reported by Ghasia and associates,[11] demonstrated an increased coefficient of variation. Serbecic and associates[14] performed follow-up SD OCT scans using eye tracking in healthy controls on separate days and demonstrated exceptionally good coefficient of variation values (ie, less than 1%) for all quadrants except the temporal. In our opinion, a more accurate and clinically relevant assessment of SD OCT reproducibility occurs when imaging sessions occur on different days.

Imaging children as compared to adults may also influence reproducibility results. Since very few studies have examined the reproducibility of time-domain OCT or SD OCT in children,[11,24–26] it is difficult to determine which factors specific to children may contribute to the increased intervisit coefficient of variation. Surprisingly, our subjects with normal vision demonstrated a statistically significant positive correlation between coefficient of variation and age. We can only speculate as to why teenagers demonstrated higher coefficient of variation measures than younger children. The relationship between age and coefficient of variation was not significant in both the unadjusted and adjusted regression analysis of subjects with abnormal vision, suggesting that either the results in the normal vision group are erroneous or the impact of vision loss obscured the influence of age. Nonetheless, the faster acquisition speed of SD OCT and the ability of the eye tracking feature to accommodate for eye movements and co-register subsequently acquired images to a reference scan is especially helpful when imaging uncooperative children.

A number of study limitations should be considered when interpreting our results. While our inclusion criteria required subjects to demonstrate clinical and radiographic stability, it is possible that some subjects may have experienced a subclinical decline in RNFL thickness between visits, thereby influencing the reproducibility measures. Arguing against that concern is the fact that the RNFL thickness measures for both cohorts did not significantly change or show a trend downward between visits (Table 2). Secondly, we defined our abnormal vision cohort as subjects with visual acuity loss and/or visual field loss. We suspect that, similar to the magnitude of visual acuity loss, the magnitude of visual field loss also contributes to the reproducibility measures. Owing to a wide range of ages studied and the different visual field techniques used, we did not attempt to quantify the visual field loss and determine if it, too, impacts coefficient of variation and intraclass correlation coefficient values between visits. Lastly, we acquired a minimum of 3 acceptable scans at the initial visit in order to assess intravisit variability as well as choose a high-quality reference image. Only 1 acceptable scan was required during the follow-up visit, since it was being acquired and co-registered to the designated reference image from visit 1. Although the difference in number of scans acquired between visits could influence the variance measures, acquiring 1 scan at the follow-up visit is most reflective of clinical practice.

Before SD OCT results can be used to make treatment decisions, additional research must elucidate the temporal relationship between declining vision and decreasing RNFL thickness. This will require an improved understanding of disease mechanisms and the natural history of RNFL changes after injury. Until additional evidence-based studies are published, clinicians should not use RNFL thickness changes to make treatment decisions, especially in patients who are clinically stable.

In conclusion, our study demonstrated that SD OCT acquired using the eye tracking feature in young children with

nonglaucomatous optic neuropathy has excellent intervisit reproducibility of RNFL measures. When assessing longitudinal changes in RNFL, the variability between visits significantly increases in the average global and temporal quadrant, as well as in subjects with more profound visual acuity loss.

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Biosketch

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