• Clinical Research •

# Compatibility of 24-2C central extra test points with OCT and OCTA parameters in early-stage glaucoma

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## Abstract

● **AIM:** To facilitate structure-function analysis by evaluating the compatibility of newly added 10 extra test points in early-stage glaucoma cases with optical coherence tomography (OCT) and OCT-angiography (OCTA) glaucoma parameters in clinical practice.

● **METHODS:** Cases diagnosed with primary open angle glaucoma underwent Humphrey 24-2C visual field (VF) test, OCT, and OCTA tests on the same day. The arithmetic average of the central 10 extra points in the 24-2C VF was calculated, and its compatibility with OCT and OCTA parameters was assessed. Patients with low signal strength OCT and OCTA images, high fixation losses in VF, intraocular pressure of 25 mm Hg and above, systemic vascular diseases [diabetes mellitus (DM), hypertension (HT)] were excluded from the study.

● **RESULTS:** Totally 68 early-stage glaucoma cases (68 right eyes, 37 males) were included with the mean age of 64.52±8.89y. In the Spearman correlation test, all OCT and OCTA parameters except inside disc vascular density showed a positive correlation with the mean of additional VF test points ($P$<0.05 for all parameters, $P$=0.114 for inside disc vascular density). In multiple linear regression analysis, superior hemifield vessel density and whole image vessel density percentages were found to be significantly associated with additional points ($P$=0.029, 0.039, respectively).

● **CONCLUSION:** It is known that specific regions of the macula may be sensitive to early-stage glaucomatous damage. This suggests that in early-stage glaucoma, attention should be paid to central points in addition to peripheral findings on VF. The compatibility of the mean of 24-2C SITA-Faster VF central extra points with the defined OCTA parameters may demonstrate the value of OCTA in diagnosis and follow-up in early stages for clinics without access to the new software VF devices.

● **KEYWORDS:** glaucoma; optical coherence tomography; optical coherence tomography angiography; visual field; preperimetric glaucoma

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## INTRODUCTION

Preperimetric glaucoma (PPG) is defined as observable glaucomatous alterations in the optic disc and heightened susceptibility to damage in the retinal nerve fiber layer (RNFL), even though standard automated perimetry does not yet reveal any visual field (VF) defects[1]. In early stages of the disease, glaucomatous alterations in the VF typically initiate in the arcuate region and subsequently advance towards the central visual field (CVF) area as the condition advances. Conversely, certain individuals with glaucoma may experience loss in the CVF even during the initial phases of the disease[2].

At present, the 24-2 VF test persists as the benchmark for evaluating VF in glaucoma patients, encompassing the CVF, despite the potential for overlooking subtle CVF abnormalities due to the relatively wide spacing between test points within the central 10 degrees VF region[3]. Incorporating 10-2 testing into a perimetry examination to central vision defects would nearly double the duration of testing, which might not be practical in a clinical setting. To tackle this challenge, central test points from the 10-2 test grid have been integrated into the 24-2 test, leading to the development of a modified 24-C testing grid. The 24-2C protocol integrates the 10 most frequently abnormal central test points from the 10-2 grid into the standard 24-2 test grid. This integration allows for both peripheral and central VF testing in a single examination. The use of the new Swedish Interactive Thresholding Algorithm (SITA) Faster testing algorithm enhances test efficiency, resulting in improved test duration[4].

Optical coherence tomography (OCT) stands out as one of the most widely utilized imaging techniques for diagnosing glaucoma. It furnishes a consistent and quantitative evaluation of structural impairment in glaucoma, a condition that frequently manifests before functional alterations become discernible *via* perimetry. In more recent times, optical coherence tomography angiography (OCTA), an advancement of OCT technology, has emerged as a tool for assessing the perfusion of various retinal layers. There is evidence that OCTA may improve the detection of glaucoma in the early stages[5-6].

Although OCT and OCTA devices are available in many centers for glaucoma monitoring in clinical practice, there is currently no 24-2C SITA Faster test available to detect early-stage glaucomatous VF losses. The aim of this study is to facilitate structure-function analysis by evaluating the compatibility of newly added 10 additional test points in early-stage glaucoma cases with OCT and OCTA glaucoma parameters in clinical practice.

## PARTICIPANTS AND METHODS

**Ethical Approval** The study obtained ethics approval from the Human Research Ethics Committee of the Muğla Sıtkı Koçman University (Number:65/ Date:04.06.2024). The research adhered to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all individual participants included.

**Patients and Design** In this cross-sectional, retrospective study 68 cases of early-stage glaucoma were included, all of which involved the right eye. Patients who visited Glaucoma Department of Muğla Research and Training Hospital between April 2019 and December 2023 were involved in study.

For inclusion in the study, all glaucoma subjects were required to have open angle glaucoma regardless of their intraocular pressure (IOP) level, medication usage and meet the following additional criteria: a best-corrected visual acuity of ≥20/40; a spherical equivalent refractive error between -5.0 and +3.0 diopters (D); cylinder correction within ±3 D; and normal anterior chamber and open angle on slit-lamp and gonioscopic examinations, respectively. Primary early-stage glaucoma was defined by the presence of glaucomatous optic nerve head (ONH) changes (*e.g.*, vertical cup-to-disc ratio >0.7; asymmetry in the vertical cup-to-disc ratio between eyes exceeding 0.2, not attributable to optic disc size; focal or generalized neuroretinal rim thinning; optic disc hemorrhage; or RNFL defects on OCT). Early-stage pre-perimetric and perimetric glaucomas according to the Hoddap-Parish-Anderson staging were included. Patients with non-glaucomatous optic nerve pathologies, inflammatory eye diseases, history of ocular surgery, systemic vascular diseases [diabetes mellitus (DM), hypertension (HT)], and

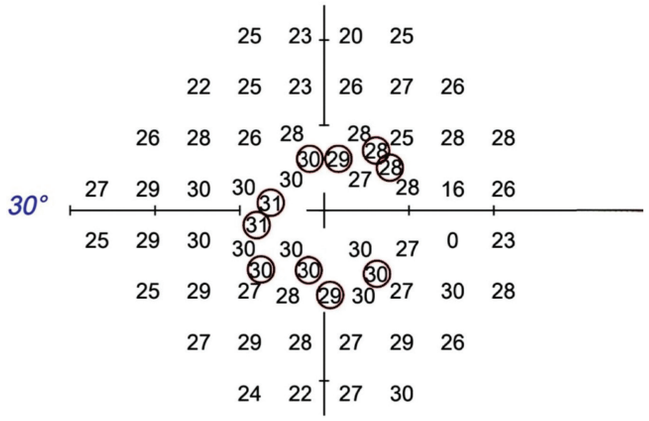



**Figure 1 The schematic view of included additional points in 24-2C visual field print.**

environmental opacities affecting image quality were excluded from the study. All subjects were well-controlled early-stage primary open angle glaucoma and IOP of 25 mm Hg or higher are also excluded from the study since this exclusion criterion was implemented to eliminate the additional variability caused by high IOP, thereby allowing for a more focused analysis of the relationship between vascular, structural changes, and the CVF. The arithmetic mean of the central 10 additional points in the 24-2C VF was calculated (Figure 1), the relationship and compatibility with OCT and OCTA parameters [RNFL, ganglion cell complex (GCC) thickness, whole image vascular density (VD), inside disc VD, peripapillary VD, superior and inferior hemi VD] were assessed.

All cases underwent Humphrey 24-2C VF testing (Carl Zeiss Meditec, 740i, Dublin, CA, USA), OCT, and OCTA (AngioVue Software Version 2017.1.0.134, Optovue, Fremont, CA, USA) tests on the same day. A 4.5 mm×4.5 mm-diameter peripapillary OCTA scan centered on the optic disk was used. Capillary density was measured from the internal limiting membrane to the posterior magin of the RNFL, including the radial branching peripapillary vessels. The software version 2017.1.0.134 automatically set an annular zone around the optic disk (750 μm width with an inner edge adjusted to the optic disk margin), giving a sector capillary density map. The whole enface image VD was measured over the entire 4.5×4.5 fields, and the peripapillary capillary density was calculated within a 750-mm-wide elliptical annulus extending from outward from the optic disk border. The capillary density percentages were automatically calculated as the proportion of the area with flowing blood vessels, defined by pixels with decorrelation values above the split-spectrum amplitude-decorrelation angiography (SSADA) threshold level.

VF showing a learning curve (*i.e.*, subsequent VF assessments displaying sustained enhancement relative to initial assessments) were also disregarded until outcomes reached stability. VF were additionally assessed for lid and rim

anomalies, lack of focus, and signs of abnormal VF outcomes not linked to glaucoma-related conditions (*e.g.*, neuro-ophthalmological pathologies). Low image quality in sections of OCT and OCTA (<Q8) were excluded.

**Sample Size and Power Analysis** The sample size for this study was determined using an a priori power analysis with G*Power 3.1.9.7 software. For a Spearman correlation analysis, assuming a medium effect size ($r$=0.4), a 5% Type I error probability ($\alpha$=0.05), and 80% power (1-$\beta$=0.80), the calculated minimum required sample size was 47. The inclusion of 68 patients in the current study indicates that sufficient statistical power was achieved under these assumptions.

**Statistical Analysis** SPSS, version 22.0 (SPSS, Inc., Chicago, IL, USA), a statistical software, was used for the statistical analysis. Descriptive statistics were recorded as mean±standard deviation (SD). The distribution of continuous variables was evaluated using histograms and the Shapiro-Wilk $W$ normality test. Comparison of data with a normal distribution was conducted using the Pearson correlation test, while data not fitting a normal distribution were analyzed using the Spearman correlation test. Differences between parameters were tested using multiple linear regression analysis. A significance level of $P$<0.05 was considered statistically significant.

## RESULTS

Upon examining the demographic characteristics of the 68 patients (68 right eyes) included in our study, it was determined that 31 patients (45.6%) were female and 37 patients (54.4%) were male. The mean age of the patients was recorded as 64.52±8.89y, while the mean IOP adjusted for central corneal thickness during measurements was 15.88±3.68 mm Hg. The mean deviation (MD) of the VF test was -1.17±2.20 dB, the pattern standard deviation (PSD) was 4.79±18.68 dB, and the VF index was 96.82%±3.12%. Mean spherical equivalent was -1.85±2.50 D, and the vertical cup-to-disc ratio was 0.62±0.15.

According to the Spearman correlation test results, all OCT and OCTA parameters, with the exception of inside disc VD, showed a positive correlation with the mean of the VF extra test points ($P$<0.05 for all parameters, $P$=0.114 for inside disc VD). This suggests that RNFL, GCC, and various VD measurements are associated with changes in CVF sensitivity in early-stage glaucoma. The highest correlation was observed in total image VD and inferior hemifield VD (both $P$<0.001). This finding indicates that these specific vascular parameters have strong potential in reflecting CVF changes (Table 1).

Multiple linear regression analysis revealed that total image VD percentages ($P$=0.039) and superior hemifield VDs ($P$=0.029) were significantly associated with the extra points. These results suggest that these OCTA parameters may be independent predictors of changes in CVF sensitivity. Among the OCTA parameters, peripapillary and inferior hemifield VDs were identified as the least correlated parameters with CVF extra points ($P$=0.888 and $P$=0.586, respectively). This may suggest that these specific vascular regions are less effective in reflecting CVF changes (Table 2).

**Table 1 Correlation of mean central visual field extra points and OCT, OCTA parameters**

| Parameters | Mean±SD | $r$ | $P$ |
|---|---|---|---|
| Retinal nerve fiber length (μm) | 90.36±1.68 | 0.406 | 0.001 |
| Ganglion cell complex (μm) | 90.25±1.41 | 0.284 | 0.019 |
| Whole image vascular density (%) | 53.56±0.68 | 0.504 | <0.001 |
| Inside disc vascular density (%) | 57.77±0.86 | 0.194 | 0.114 |
| Peripapillary vascular density (%) | 55.66±0.80 | 0.426 | <0.001 |
| Superior hemifield vascular density (%) | 55.95±0.80 | 0.394 | 0.001 |
| Inferior hemifield vascular density (%) | 55.03±0.78 | 0.467 | <0.001 |
| Central visual field extra points (dB) | 31.02±0.25 | | |

$r$: Spearman correlation coefficient; OCT: Optical coherence tomography; OCTA: Optical coherence tomography angiography; SD: Standard deviation.

## DISCUSSION

It is known that certain areas of the macula region may be susceptible to glaucomatous damage in early stages[7]. This has demonstrated the necessity of focusing not only on peripheral findings but also on central points in the early stages of glaucomatous VF losses[8]. In this study, the compatibility shown between the average of 24-2C SITA-Faster VF additional points and OCTA parameters such as total image and superior-hemi VDs may demonstrate the value of OCTA in diagnosis and monitoring in early stages for clinics without access to the relevant test.

The role of OCTA in early glaucoma detection has gained increasing attention in recent years, particularly regarding the temporal sequence of vascular changes relative to structural and functional alterations. Recent evidence suggests that OCTA offers a promising approach for detecting subclinical microvascular changes in glaucoma suspects before structural or functional damage becomes clinically apparent. A comprehensive scoping review by Hidalgo Ramos *et al*[1] demonstrated that VD in glaucoma suspects was significantly reduced compared to healthy controls (whole-image VD: 51.3% *vs* 55.5%, $P$<0.001), with diagnostic accuracy [the area under the receiver operating characteristic curve (AUC): 0.70–0.84] comparable to structural OCT metrics such as RNFL. Notably, microvascular asymmetry and deep-layer capillary dropout emerged as early markers that often precede VF defects, highlighting the potential of OCTA in identifying glaucomatous damage at its earliest stages. Longitudinal data from the same review revealed a significantly faster rate of VD decline in PPG (-2.23%/y) compared to healthy eyes (0.29%/y), underscoring OCTA's value in monitoring disease progression even before perimetric changes manifest[1].

**Table 2 Multiple linear regression analysis of the association between central visual field extra points average and OCT, OCTA parameters**

| Parameters | *B* (SE) | 95%CI | *P* |
|---|---|---|---|
| Retinal nerve fiber length (μm) | 0.072 (0.042) | -0.013 to 0.156 | 0.096 |
| Ganglion cell complex (μm) | -0.070 (0.046) | -0.163 to 0.023 | 0.137 |
| Whole image vascular density (%) | 0.544 (0.258) | 0.028 to 1.06 | 0.039 |
| Inside disc vascular density (%) | -0.088 (0.048) | -0.185 to 0.008 | 0.073 |
| Peripapillary vascular density (%) | -0.025 (0.177) | -0.379 to 0.329 | 0.888 |
| Superior hemifield vascular density (%) | -0.344 (0.154) | -0.652 to -0.037 | 0.029 |
| Inferior hemifield vascular density (%) | 0.093 (0.170) | -0.247 to 0.433 | 0.586 |

B: Beta coefficient; SE: Standard error; CI: Confidence interval; OCT: Optical coherence tomography; OCTA: Optical coherence tomography angiography.

The temporal relationship between vascular and structural changes in glaucoma pathophysiology remains an area of active investigation. Rao *et al*[9] emphasized the need for future longitudinal studies to evaluate whether OCTA can detect vascular changes earlier than RNFL thinning, noting that such findings could lead to a paradigm shift in glaucoma management. Their research demonstrated that lower baseline macular and peripapillary VDs were associated with faster rates of RNFL progression in mild to moderate glaucoma, and importantly, this association was independent of baseline RNFL thickness, suggesting that OCTA may offer additional prognostic information beyond traditional structural assessments[9]. Furthermore, the presence of microvascular dropout has been consistently associated with faster rates of RNFL thinning and VF progression, including CVF deterioration[9]. However, the temporal sequence of these changes appears to be complex and may vary among individuals. While some studies suggest that structural thinning may more often precede microvascular changes in early glaucoma, approximately one-third of cases demonstrate greater VD loss than GCC loss, indicating that vascular changes can also occur as a primary event[10]. As noted by recent investigators, regardless of which event occurs first, vascular abnormalities and structural changes appear to be interdependent processes in glaucoma pathogenesis[10].

The observed significant association between CVF extra points and OCTA parameters in our study is likely mediated by several underlying mechanisms, including neurovascular coupling (NVC) dysfunction and the macular blood supply-function correlation. Retinal ganglion cells (RGCs) which are selectively damaged in glaucoma, have a high metabolic demand, requiring a finely tuned blood supply to sustain their activity. The NVC mechanism ensures that blood flow is precisely regulated in response to neuronal activity. However, many glaucoma patients exhibit vascular deficits, including reduced blood flow and impaired NVC, suggesting a breakdown in the neurovascular unit, the complex cellular community that regulates these interactions[11]. Pericytes, key components of this unit, are crucial for regulating NVC, and their dysfunction or loss, often seen as macular VD (MvD) in OCTA, can directly impair the retina's ability to supply oxygen and nutrients to RGCs, leading to functional deficits that manifest as VF loss. Furthermore, the strong correlation between mvD and central visual function, as demonstrated here and in other studies[12], supports the concept of a macular blood supply-function correlation mechanism, where microvascular impairment is a significant contributing factor to central functional loss. The central extra points of the 24-2C test, which sample the macula's central 10 degrees, are therefore highly sensitive to these early vascular-driven functional deficits.

Our finding that superior hemifield VD is a significant predictor aligns with the known vulnerability of the inferior macula to early glaucomatous damage, which corresponds to defects in the superior VF. The integration of vascular assessment through OCTA with traditional structural OCT measurements provides a more comprehensive evaluation of the glaucomatous disease process, potentially enabling earlier detection and more precise risk stratification for disease progression. These findings suggest that in clinical settings where 24-2C SITA Faster testing may not yet be available, OCTA parameters can serve as valuable complementary tools for detecting and monitoring CVF changes in early-stage glaucoma.

A number of recent studies have proposed that 10-2 VF tests could enhance the clinical diagnosis of glaucoma since incorrect 24-2 VF findings are frequently observed in eyes whose 24-2 VF values are within normal ranges. De Moraes *et al*[13], for example, showed that 61.5% of glaucoma eyes with 10-2 defects were classified as normal by 24-2 testing, based on a cluster criteria used to classify 10-2 and 24-2 VF deficiencies[2]. Furthermore, 52.5% of early glaucoma eyes with matching 10-2 and OCT-measured ganglion cell-inner plexiform layer faults were categorized as normal by

one or more of the metrics used to characterize 24-2 defects, according to a study by Grillo *et al*[14]. A small but statistically significant difference in glaucoma severity, specifically an MD of -0.73 dB, was reported when comparing 24-2C testing to 24-2 testing in glaucoma and glaucoma suspect eyes. However, no significant difference was found in PSD and glaucoma hemifield test indices. This difference was observed when comparing the novel 24-2C protocol, which includes additional central test points, to standard 24-2 testing. The findings indicate that 24-2C testing may be more sensitive in identifying glaucomatous loss of CVF sensitivity[15]. A recent study compared the testing time of the 24-2C SITA Faster, 24-2 SITA Standard, and 10-2 SITA Standard tests. Nishijima *et al*[2] revealed that the 24-2C SITA Faster test accurately detects mild glaucoma CVF defects, and may be more useful for evaluating mild-stage glaucoma patients.

Recent OCTA investigations have demonstrated that individuals with glaucoma had decreased vascular density or blood flow in the macular, circumpapillary, and ONH areas; moreover, peripapillary VD measures were found to be just as sensitive in diagnosing glaucoma as RNFL thickness[16-18]. Furthermore, functional loss has been shown to have a stronger correlation with circumpapillary VD (cpVD) than structural loss, indicating that VD is a more accurate measure of ganglion cell function than structural loss[19]. Studies have also shown that OCTA is a more sensitive method for detecting PPG than OCT. According to certain theories, OCT parameters become less useful as glaucoma worsens because of the baseline effect, however OCTA can track the decline in VD[20]. For this reason, OCTA is a novel, noninvasive technique that shows promise and can be helpful in the early detection, staging, and follow-up of glaucoma patients[21]. However, the role of OCTA in early-stage glaucoma has not yet been definitively established. The ability of OCTA to differentiate between PPG or glaucoma suspect eyes and healthy eyes has been the focus of several studies. Kumar *et al*[20] reported that OCTA measurements from the superior and temporal sectors showed more significant VD reduction in glaucomatous eyes and provided comparable discrimination performance, as indicated by AUC, when compared to spectral-domain OCT (SD-OCT) structural parameters for distinguishing healthy eyes from those with PPG [AUC: 0.70 (sensitivity: 69.2%; specificity: 72.9%) *vs* AUC: 0.66 (sensitivity: 77.3%; specificity: 59.7%)]. In distinguishing between glaucoma suspect and healthy eyes, Triolo *et al*[17] found that annular peripapillary VD did not surpass OCT RNFL thickness in performance (average AUC: 0.506 *vs* 0.649; superior AUC: 0.562 *vs* 0.687; inferior AUC: 0.438 *vs* 0.508; all $P>0.05$).

Our findings complement and extend recent research published on early glaucoma detection using CVF and structural parameters. Hu *et al*[22] demonstrated that macular ganglion cell-inner plexiform layer thickness (mGCIPLT) parameters were superior to circumpapillary RNFL thickness in detecting early glaucoma with CVF defects using separate 10-2 testing. Our study advances this field by demonstrating that the 24-2C SITA Faster test's central extra points show significant correlation with OCTA vascular parameters, particularly whole image VD ($P$=0.039) and superior hemifield VD ($P$=0.029). This represents a novel contribution to the literature, as no previous study in this journal has examined the relationship between 24-2C central points and OCTA parameters. The integration of central test points from the 10-2 grid into the 24-2 test grid, combined with the SITA Faster algorithm, provides a more efficient approach compared to performing separate 10-2 testing. Furthermore, our incorporation of OCTA vascular assessment adds a complementary dimension to structural OCT measurements, potentially enhancing early detection capabilities in clinical settings where 24-2C testing may not yet be available.

The reduction in RNFL thickness and its negative impact on VF tests has been demonstrated in previous studies[23]. Similarly, a decrease in GCC measurements, which assess the macular region, also negatively affects VF indices[24]. Our study, which evaluates CVF parameters, shows a positive relationship with OCT parameters. However, the results of the multiple regression analysis in our study suggest that, for the central extra points, certain OCTA parameters may be more significant than OCT parameters.

Some studies, conducted using VF tests, have shown that in normal-tension glaucoma, the progression of superior hemifield defects may be faster than that of inferior hemifield defects[25]. Consistently, in other studies, it has been reported that in glaucomatous or glaucoma-suspect cases, the region most sensitive to glaucomatous damage is the inferior region of the macula. Damage to this region corresponds to defects in the superior hemifield of the VF[26]. According to the regression analyses in our study, the sensitivities of central points in the VF were found to be strongly associated with superior hemifield VDs. This can highlight the importance of closely monitoring superior hemifield densities for early progression tracking, particularly in clinics without access to the 24-2C VF option for newly diagnosed glaucoma cases.

Among the possible limitations of the study is its retrospective nature. Additionally, it can be noted that the study did not conduct separate correlations with OCT and OCTA for the relevant extra points in the VF but rather utilized their averages. Besides, cases of Henle's fibers displacement, which could potentially cause measurement errors during GCC assessments, may have affected the relevant results. The current study may shed light on more detailed analyses to be

conducted in future studies. Another limitation of our study is its cross-sectional design. This prevents us from establishing causal links or drawing definitive conclusions about the predictive ability of the identified associations regarding future VF function. Future longitudinal studies could better elucidate the causal nature and predictive value of these relationships.

This study evaluated the compatibility between 24-2C SITA-Faster VF central extra points and OCTA parameters in early-stage open angle glaucoma. Our findings revealed a strong and significant correlation between certain OCTA parameters, particularly whole image VD and superior hemifield VD, and CVF sensitivity. This relationship once again emphasizes the importance of the macular region and the CVF in the early stages of glaucomatous damage. The parameters from OCTA demonstrate its potential as a valuable tool for the diagnosis and follow-up of early-stage glaucoma, especially in clinics without access to new software VF devices. These results reinforce the role of OCTA in clinical practice for structure-function analysis in early-stage glaucoma and enable more effective monitoring of disease progression. Future studies can focus on validating these findings in larger patient cohorts and investigating their long-term prognostic value.

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**Data Availability Statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**AI-Generated Content Disclosure:** None.

**Conflicts of Interest: Kaderli A,** None; **Camcıoğlu Ş,** None; **Tamer Kaderli S,** None; **Sül S,** None.

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