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Comparison of Corneal Endothelial Parameters Measured by Three Different Specular Microscopy Devices in Healthy Subject: A Comparative Clinical Study

Üç Farklı Speküler Mikroskopi Cihazı ile Ölçülen Korneal Endotel Parametrelerinin Karşılaştırılması: Karşılaştırmalı Klinik Çalışma

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ABSTRACT Objective: The present study aimed to compare the Nidek CEM-530 (Nidek Co., Japan), Konan CellChek XL (Konan Medical, Hyogo, Japan), and Topcon SP-3000P (Topcon Corporation, Tokyo, Japan) noncontact specular microscopes (NCSM) in terms of endothelial cell density (ECD), morphological endothelial cell parameters, and central corneal thickness (CCT), in healthy subjects. **Material and Methods:** Right eyes of 49 patients were included in this study. ECD, CCT, average cell area, coefficient of variation, standard deviation of cell area, cell hexagonality ratio were evaluated with Nidek CEM-530, Topcon SP-3000P and The CellChek XL NCSMs. **Results:** The mean age was 36.2±9.6 years. The mean ECD was 2809±24.1, 2626±50.4, and 2588±32.1 in measurements with Konan CellCheck XL, Topcon SP-3000P, and Nidek CEM-530 NCSMs, respectively. The difference was statistically significant between Konan CellCheck XL with Nidek CEM-530 and Topcon SP-3000P ($p<0.001$ for both). The hexagonality ratio results between these 3 devices were statistically significant in all paired comparisons ($p<0.001$, for all). The mean CCT was 534.4±5.0, 553.9±5.6, 567.5±5.3 in measurements with Nidek CEM-530, Konan CellCheck XL, and Topcon SP-3000P, respectively. CCT values obtained with Topcon SP-3000P were found to be higher than with other devices ($p<0.001$, for both). **Conclusion:** Among the 3 devices, the highest ECD values were obtained with Konan CellCheck XL and the highest CCT values were obtained with Topcon SP-3000P. On account of the discrepancies in endothelial parameters tested in our study, we do not advise using these devices interchangeably.

Keywords: Noncontact specular microscope; endothelial cell density; hexagonality cell ratio; central corneal thickness

ÖZET Amaç: Bu çalışmada, sağlıklı kişilerde Nidek CEM-530 (Nidek Co., Japonya), Konan CellChek XL (Konan Medical, Hyogo, Japonya) ve Topcon SP-3000P (Topcon Corporation, Tokyo, Japonya) temassız speküler mikroskopların [noncontact specular microscopes (NCSM)] endotel hücre yoğunluğu (EHY), morfolojik endotel hücre parametreleri ve santral kornea kalınlığı (SKK) açısından karşılaştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Bu çalışmaya, 49 hastanın sağ gözü dâhil edildi. EHY, SKK, ortalama hücre alanı, varyasyon katsayısı, hücre alanının standart sapması, hekzagonal hücre oranı, Nidek CEM-530, Topcon SP-3000P ve CellChek XL temassız speküler mikroskop cihazları incelendi. **Bulgular:** Ortalama yaş 36,2±9,6 yıl idi. Konan CellCheck XL, Topcon SP-3000P ve Nidek CEM-530 NCSM'lerle yapılan ölçümlerde ortalama EHY sırasıyla 2809±24,1, 2626±50,4 ve 2588±32,1 idi. Konan CellCheck XL ile Nidek CEM-530 ve Topcon SP-3000P ölçümleri arasındaki fark istatistiksel olarak anlamlıydı (her ikisi için $p<0,001$). Bu 3 cihaz arasındaki hekzagonal hücre oranı sonuçları, tüm ikili karşılaştırmalarda istatistiksel olarak anlamlıydı (tümü için $p<0,001$). Nidek CEM-530, Konan CellCheck XL ve Topcon SP-3000P ölçümlerinde ortalama SKK sırasıyla 534,4±5,0, 553,9±5,6, 567,5±5,3 idi. Topcon SP-3000P ile elde edilen SKK değerleri diğer cihazlara göre daha yüksek bulundu (her ikisi için $p<0,001$). **Sonuç:** Üç cihaz arasında en yüksek EHY değerleri Konan CellCheck XL ile, en yüksek SKK değerleri ise Topcon SP-3000P ile elde edildi. Çalışmamızda test edilen endotelyal parametrelerdeki farklılıklar nedeniyle bu cihazların birbirinin yerine kullanılmasını önermiyoruz.

Anahtar Kelimeler: Temassız speküler mikroskopi; endotel hücre yoğunluğu; hekzagonal hücre oranı; santral kornea kalınlığı

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A specular microscope is a diagnostic tool that allows the non-invasive, *in vivo* quantitative, and morphological evaluation of the corneal endothelial layer and it has a wide range of applications in clinical practice.[1] Corneal endothelial assessment is needed in case of routine follow-up after corneal transplant surgery, evaluation of corneal endothelial layer health status in corneal diseases or those before intraocular surgery, and also for research purpose to investigate the safety and efficacy of new procedures, devices, and pharmaceutical intervention on the endothelium.[2-5]

With the specular microscopy, besides the endothelial cell density (ECD), other parameters that help the assessment of the corneal endothelium status like pleomorphism (percentage of hexagonal cells), polymegathism (variation in cell shape), and central corneal thickness (CCT) can also be evaluated.

There are a lot of models of specular microscopes in the market. Repeated measurements with the specular microscope are usually necessary to follow changes in the endothelial layer. The devices in the clinics may change over time or patients may have to be examined with different devices in different clinics. So, it is important to know the compatibility of different specular microscopes.

The present study aimed to compare the Nidek CEM-530 (Nidek Co., Japan), Konan Cell Chek XL (Konan Medical, Hyogo, Japan), and Topcon SP-3000P (Topcon Corporation, Tokyo, Japan) noncontact specular microscopes (NCSMs) in terms of ECD, morphological endothelial cell parameters, and CCT in healthy subjects.

MATERIAL AND METHODS

This observational, prospective, cross-sectional study was done in accordance with the 1964 Helsinki Declaration ethical standards following the approval of the Istanbul Medipol University Ethics Committee (date: December 25, 2019, no: 10840098-604.01.01-E.66752). A total of 49 eyes of 49 healthy volunteers aged between 18-60 years were included in the study. Informed consent was obtained from all participants.

Subjects with any history of intraocular surgery or trauma, astigmatism greater than 2 diopters, contact lens usage (within 2 weeks), ocular inflammation, any history of corneal disease, irregular astigmatism, best-corrected visual acuity (BCVA) less than 20/25 (with Snellen chart) were excluded from the study. Also, subjects with any systemic disease and history of drug usage that could disrupt the corneal structure were not included in the study.

A complete ophthalmologic examination including BCVA, intraocular pressure measurement with non-contact tonometer, slit-lamp biomicroscopic evaluation and posterior segment evaluation was performed in all subjects before measurements with specular microscopes.

Measurements were done between a daytime of 10 am and 12 am in order not to be affected by daytime variation in CCT. The measurements were performed with Nidek CEM-530 (Nidek Co., Japan), Topcon SP-3000P (Topcon Corporation, Tokyo, Japan) and the Konan CellChek XL (Konan Medical, Hyogo, Japan) NCSMs in a variable order. Measurements were taken from both eyes but results of the right eyes were taken into account for statistical analysis.

Average cell area, standard deviation (SD) of cell area, ECD, coefficient of variation (CV), cell hexagonality ratio, and CCT were evaluated with NCSMs.

Before each measurement, it was ensured that the patient's chin and forehead were properly placed on the devices. First, patients were asked to blink their eyes to get a smooth tear film and then look at the central fixation target.

Three measurements were taken from all eyes and the highest quality images were selected for measurements. Measurements for all 3 devices are performed according to the manufacturer's instructions.

Topcon SP-3000P device automatically takes 15 pictures for each measurement and pictures are sorted by the image quality. Automatic cell detection and counting are done according to the manufacturer's software on the highest quality image selected by the inspector.

The CEM-530 NCSM captures an endothelial image measuring 0.1 mm^2. This NCSM takes a total of 16 endothelial photographs with each scan, and these are automatically sorted according to image quality. The most appropriate image according to the decision of the examiner was selected for automated cell detection with the manufacturer's software.

CellChek XL specular microscopy captures an area of 0.1 mm^2 in size. The image is acquired automatically after auto-alignment. The automatic analysis method outlines the endothelial cells and calculates the cell density, cell size, and hexagonality ratio.

STATISTICAL ANALYSIS

SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Descriptive statistics are presented as mean±SD. Inter-group comparisons were investigated by using the student t-test or Mann-Whitney U test. The Pearson correlation coefficient (for normally distributed data) and the Spearman rank test (for non-normally distributed data) were used to assess the correlation between instruments.

Bland-Altman plots were used to analyze the agreement among different devices, with 95% limits of agreement calculated as mean difference± (1.96xSD). A p value below 0.05 was considered to be statistically significant.

RESULTS

The right eyes of 49 healthy subjects were included in the study. 67% of the subjects were female (16 male, 33 female). Patients' age ranged from 24-58 years with a median of 36.2 years.

The average ECD, morphological endothelial cell parameters, and CCT values obtained with by Nidek CEM-530, Konan CellCheck XL, and Topcon SP-3000P NCSM devices were summarized in Table 1.

ECD

The mean ECD results were given in Table 1. Among the 3 devices, the highest ECD values were obtained with Konan CellCheck XL device. The difference was statistically significant between Konan CellCheck XL with Nidek CEM-530 and Topcon SP-3000P ($p<0.001$ for both). There was no significant difference between Nidek CEM-530 and Topcon SP-3000P ($p=0.404$) (Table 2).

There was a high rate of positive correlation between the measurements of all 3 devices. The highest correlation was between Nidek CEM-530 and Topcon SP-3000P ($r=0.907$, $p<0.001$) (Table 2).

The agreement of 3 devices in terms of ECD is shown with Bland-Altman plots in Figure 1.

HEXAGONALITY RATIO

The hexagonality ratio between the 3 devices was obtained from the highest to the lowest in the Nidek CEM-530, Topcon SP-3000P, and Konan CellCheck XL, respectively. Measurements from the 3 devices differed greatly from each other. Differences were statistically significant in all paired comparisons ($p<0.001$, for all) (Table 2).

In correlation analysis, a significant correlation was found only between Nidek CEM-530 and Konan CellCheck XL ($r=0.506$, $p<0.001$) (Table 2).

TABLE 1: CD, CV, average cell area, SD of cell area, hexagonality ratio, and CCT measurements between three different devices.

	Nidek CEM-530	Konan CellCheck XL	Topcon SP-3000P
CD (cell/mm^2)	2588.5±32.1	2809.2±24.1	2626.9±50.4
CV	29.5±0.6	30.7±0.6	34.6±0.7
Average cell area (µm^2)	389.5±5	357.3±3.2	386.6±7.9
SD of cell area	107.9±2.8	110.0±2.4	131.8±3.7
Hexagonality ratio (%)	66.8±0.6	44.7±1.0	53.0±1.4
CCT (µm)	534.4±5.0	553.9±5.6	567.5±5.3

CD: Cell density; CV: Coefficient of variation; SD: Standart deviation; CCT: Central corneal thickness.

TABLE 2: CD, CV, average cell area, SD of cell area, hexagonality ratio, and CCT measurements between three different devices.

	Nidek-Konan	Nidek-Topcon	Konan-Topcon
ECD			
p value	<0.001	0.404	<0.001
Correlation (r, p)	r=0.774, p<0.001	r=0.907, p<0.001	r=0.773, p<0.001
CV			
p value	0.219	<0.001	<0.001
Correlation (r, p)	r=0.440, p=0.002	r=0.659, p<0.001	r=0.443, p=0.001
Average cell area			
p value	<0.001	1	<0.001
Correlation (r, p)	r=0.773, p<0.001	r=0.888, p<0.001	r=0.792, p<0.001
SD of cell area			
p value	1	<0.001	<0.001
Correlation (r, p)	r=0.565, p<0.001	r=0.587, p<0.001	r=0.435, p=0.002
Hexagonality ratio			
p value	<0.001	<0.001	<0.001
Correlation (r, p)	r=0.506, p<0.001	r=0.102, p=0.487	r=0.238, p=0.100
CCT			
p value	<0.001	<0.001	<0.001
Correlation (r, p)	r=0.908, p<0.001	r=0.927 p<0.001	r=0.884, p<0.001

CD: Cell density; ECD: Endothelial cell density; CV: Coefficient of variation; SD: Standart deviation; CCT: Central corneal thickness.

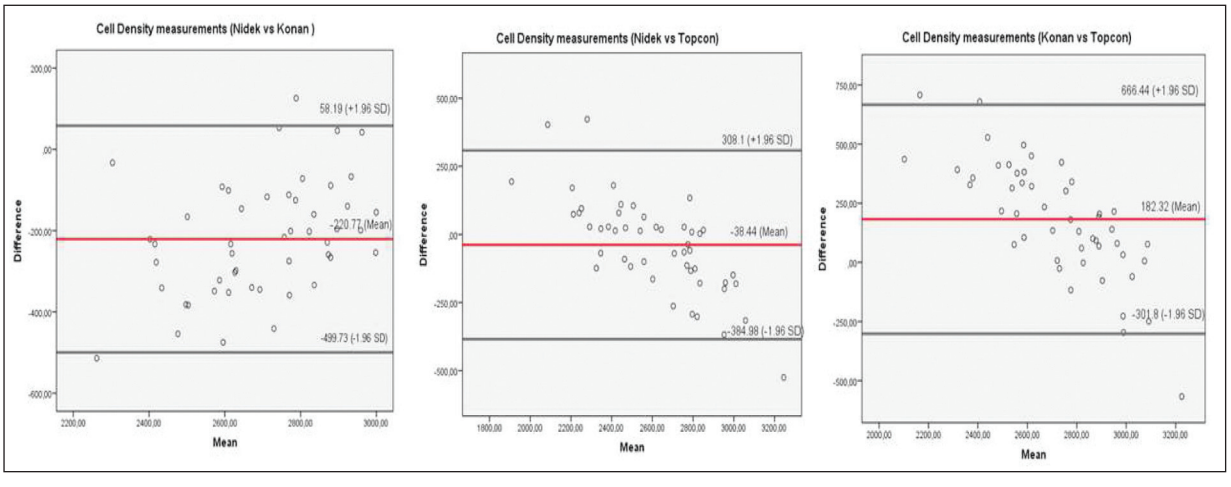

FIGURE 1: Bland Altman plots comparing ECD between Nidek CEM 530 - Konan CellCheck XL, Nidek CEM 530 - Topcon SP-3000P, Konan CellCheck XL- Topcon SP-3000P devices.

CCT

The mean CCT was found to be 534.4±5.0, 553.9±5.6, and 567.5±5.3 µm in measurements with Nidek CEM-530, Konan CellCheck XL, and Topcon SP-3000P NCSMs, respectively. CCT values obtained with Topcon SP-3000P were found to be higher than other devices. Although the measurements were well-correlated with each other, in paired comparison analysis the differences were statistically significant in each paired comparison analysis (p<0.001 for all) (Table 2).

When the mean SD of cell area and CV values were compared, a significant difference was found between Nidek CEM-530-Topcon SP-3000P and Konan CellCheck XL-Topcon SP-3000P NCSMs measurements (p<0.001 for both). Again, when we

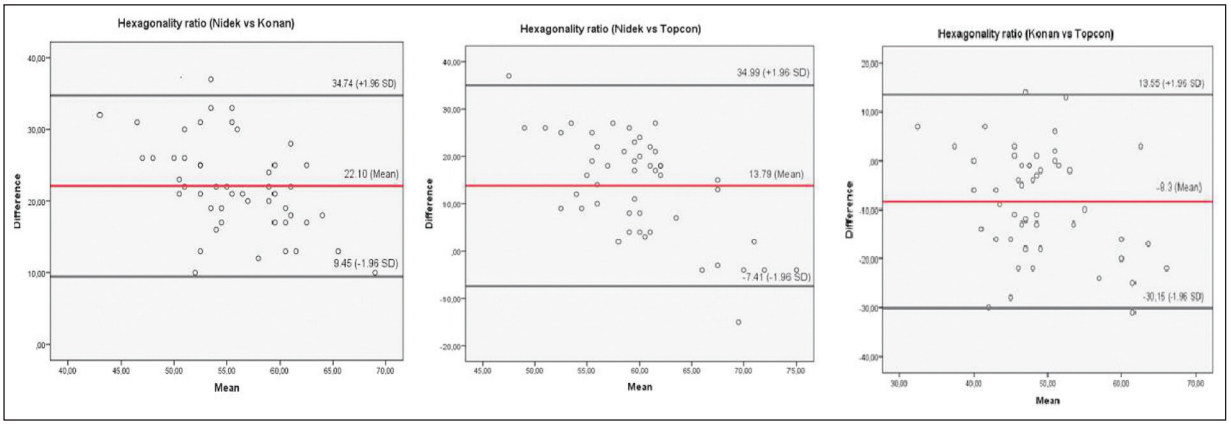

FIGURE 2: Bland Altman plots comparing the hexagonality ratio between Nidek CEM 530 - Konan CellCheck XL, Nidek CEM 530 - Topcon SP-3000P, Konan CellCheck XL- Topcon SP-3000P devices.

evaluated the average cell area measurements, results of Nidek CEM-530-Konan CellCheck XL and Konan CellCheck XL-Topcon SP-3000P were statistically significantly different ($p<0.001$ for both). The results were given in Table 2.

Finally, Figure 1 and Figure 2 show the results obtained in the Bland-Altman analysis. The highest concordance was found between Nidek CEM-530-Topcon SP-3000P in ECD measurements (Figure 1). The agreement of devices in terms of the hexagonality ratio is also presented in Figure 2.

DISCUSSION

This study was conducted to evaluate the compatibility of noncontact specular devices that we use in our daily practice to evaluate the quantitative and morphological properties of the corneal endothelium.

Various specular microscopes are available on the market, manufactured by several companies. Specular microscopy measurements are needed to repeat at certain time intervals to examine the changes in the endothelial cell layer over time. Sometimes there may be a device change in the same clinics or the patient may start follow-up in a different clinic. However, when tools are worn out and needed to be replaced, it is no longer possible to get the same model. Therefore, it is important to evaluate the compatibility of specular microscopes available on the market.

Although there are many publications in the literature comparing the endothelial cell analysis results of different NCSMs, to our knowledge, this study was the first to evaluate the compatibility of Nidek CEM-530, Topcon SP-3000P, and Konan CellCheck XL devices in the same population.

A certain level of ECD is crucial to achieve and maintain corneal transparency. Due to the lack of regeneration feature; factors that we cannot control such as genetics, race, and age and external factors such as trauma, intraocular surgery, and UV radiation cause changes in ECD over time.[6-10] When the endothelial cell count drops below a critical level, the cornea permanently loses its transparency and a cornea transplant may be the only way to save it. The critical level indicated known as the "corneal decompensation threshold" and approximately 600 to 400 cells/mm^2.[11]

In the present study, when the average ECD values were compared, it has been found that while Nidek CEM-530 and Topcon SP-3000P devices gave similar results, Konan CellCheck XL device measures ECD value significantly higher than the other 2 devices. However, there was a high rate of positive correlation between the measurements of all 3 devices. The highest correlation in terms of ECD was between Nidek CEM-530 and Topcon SP-3000P.

Similar to our study, Karaca et al. and Luft et al., showed that mean ECD given by CEM-530 was significantly lower than CellCheck XL in automated analysis.[12,13] The differences were about 250 and 380

cells per square mm, respectively. However, Luft et al., accepted the measurement with CellCheck XL in the semiautomated analysis as a gold standard method. They reported that automated analysis overestimates ECD about 400 cells/mm^2.[13] Nevertheless, we preferred automatic analysis modes for all 3 devices, because of being independent of the analyst and ease of use. On the other hand, Cakici et al., also reported that there was no significant difference between Nidek CEM-530 and Topcon SP-3000P devices in terms of ECD and also they showed a high correlation.[14]

CCT measurement is an integral part of eye examination and can be measured with several optical instruments. Accurate measurement of CCT is extremely important in the diagnosis, follow-up, and treatment of many eye diseases. It has a very important role in the selection of the surgical method to be performed in common surgeries such as refractive surgery or cataract surgery and the correct evaluation of intraocular pressure.[15-17]

CCT values received with every 3 devices were found significantly different however CCT values obtained with Topcon SP-3000P were found to be higher than Nidek CEM-530 and CellChek XL in our study.

Karaca et al., showed that although CellChek XL measured mean CCT thicker than CEM-530, both of the devices were well correlated with Pentecam.[12] In Luft et al.'s study, CellChek XL measured mean CCT about 10 µm thicker than CEM-530 but the authors thought that this difference was not clinically significant.[13] Similarly, CellChek XL measured CCT significantly higher than Nidek CEM-530 in our study but unfortunately, we didn't have a chance to compare specular microscope devices with ultrasonic pachymeter or corneal topography. We showed that Topcon SP-3000P measured CCT values 30 µm thicker than Nidek CEM-530 and the difference was significant. Contrary to us, Cakici et al., reported that there was no significant difference in CCT measurement between Nidek CEM-530 and Topcon SP-3000P and there was also a high correlation between devices.[14]

The endothelial layer is a tight layer formed by hexagonal cells. The hexagonality ratio is; a ratio of hexagonal cells to other different cell shapes. In healthy endothelium, this ratio should ideally approach 100%. When this ratio falls below 50%, it indicates situations where healthy endothelial cells are reduced, and therefore intraocular surgeries become riskier. Therefore, it is an important parameter to evaluate the condition of the corneal endothelial layer.

In our study, we found a significant difference in terms of hexagonal ratios between the 3 devices and we obtained the rates from the highest to the lowest with Nidek CEM-530, Topcon SP-3000P, and Konan CellCheck XL, respectively. There was only a moderately significant correlation between Nidek CEM-530 and Konan CellCheck XL.

Similar to our study, it has been demonstrated that Nidek CEM-530 measures the hexagonality ratio significantly higher than CellChek XL and Topcon SP-3000P devices and no correlation was present between devices.[12-14]

There are a few limitations to this study. First, we only included healthy corneas so we don't know how these devices will work in case of any disease. Second, we didn't compare these 3 NCSMs with any other gold standard device for CCT and endothelial parameters evaluation.

Similar results were detected in terms of ECD and average cell area with Nidek and Topcon, at the same time they showed a good correlation. But results of Konan were not compatible with these 2 devices. On the other hand, the hexagonality ratio differed significantly between all 3 devices. Although there was a good correlation in CCT levels, the differences were statistically significant between devices. Among the 3 devices, the highest ECD values were obtained with Konan CellCheck XL device and the highest CCT values obtained with Topcon SP-3000P.

CONCLUSION

Our findings emphasize that we should be careful when comparing measurements of endothelial parameters obtained using different NCSM devices. Special attention should be paid to follow-up patients with the same device in clinical practice. In cases where device replacement is inevitable, evaluating

both the old and new devices in the same session and comparing the 2 devices will enable a more accurate follow-up.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Nejla Tükenmez Dikmen, Elvin Yıldız;* ***Design:*** *Elvin Yıldız,* *Ece Turan Vural;* ***Control/Supervision:*** *Elvin Yıldız, Ece Turan Vural, Şaban Şimşek;* ***Data Collection and/or Processing:*** *Nejla Tükenmez Dikmen, Funda Dikkaya;* ***Analysis and/or Interpretation:*** *Nejla Tükenmez Dikmen, Funda Dikkaya, Sevil Karaman Erdur;* ***Literature Review:*** *Nejla Tükenmez Dikmen;* ***Writing the Article:*** *Nejla Tükenmez Dikmen, Elvin Yıldız, Funda Dikkaya;* ***Critical Review:*** *Ece Turan Vural, Şaban Şimşek;* ***References and Fundings:*** *Nejla Tükenmez Dikmen;* ***Materials:*** *Nejla Tükenmez Dikmen, Elvin Yıldız.*

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