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RESEARCH ARTICLE

CORNEAL DENSITOMETRY IN HEALTHY PATIENTS MEASURED WITH SCHEIMPFLUG PENTACAM® CAMERA

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ABSTRACT

Purpose: To evaluate the normal values of corneal densitometry of healthy subjects in order to assess a normative database.
Methods: This retrospective non-randomized single-center was conducted on healthy subjects who performed a topographic examination before refractive surgery and showed no corneal or other ocular pathology. A slit-lamp examination was carried out before Scheimpflug topography scanning to exclude any corneal opacity. Parametric paired Student t-test and non-parametric Kruskal-Wallis test with Bonferroni correction was performed.
Results: 311 healthy patients with a mean age of 39.4 +/- 15.7 [11; 82] years at the time of the inclusion were recruited. Mean total densitometry was 18.8 +/- 3.2 [13.2; 33.7] and a statistical difference was found between each layer and corneal rings. Graph distribution of the average corneal densitometry represents a gentle slope with a mild peak in the anterior stroma. Anterior densitometry was significantly higher than central and posterior densitometry (25.2+/- 4.4 [17.3; 46.2] vs 16.6+/- 3.1 [11.5; 31.2], p<0.0001 and 14.8 +/- 2.8 [9.6; 27.8], p<0.0001 respectively). Strong correlation was calculated between age and corneal densitometry, with a significant increase in corneal density with age (p<0.0001).
Conclusion: We propose in this study Scheimpflug database of normal corneal densitometry. This can provide a useful objective measure of corneal transparency and may help routine clinical practice.

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INTRODUCTION

Within the last decades, many hypotheses for corneal transparency have been made, from refractive index homogeneity (Schweigger-Seidel, 1896), through Maurice's (Maurice, 1957) regular organization in fibrils with interference destructive phenomenon, reinforced by Hart and Farrell (Hart, 1969) and Benedek (Benedek, 1971). Spaces between fibril, minor to light wavelength allows light transmission (Farrell, 2000). Thus, any disturbance in corneal architecture, like infection, traumatism or edema can affect corneal transparency by increasing light-scattering (Rawe, 1994). Transparency is directly correlated with corneal density (Wu, 2014). Grading corneal transparency is subjective and vulnerable to inconsistencies over time and between observer.

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The Pentacam® camera (Oculus, Inc., Wetzlar, Germany) uses rotating Scheimpflug non-contact camera to acquire cross sectional images of the entire anterior segment, corneal and lens densitometry (No authors, 2003). Densitometry is described as backscattered light. It has been first proposed for cataract grading (Kirkwood, 2009), but Scheimpflug photography can be used as an objective measurement of corneal transparency. Corneal densitometry has been used to assess and quantify haze due to refractive surgery (Cennamo, 2011 and Takacs, 2011), cross linking (Gutierrez, 2012 and Greenstein, 2010) or as an aid in the diagnosis of infectious keratitis or during the healing process (Otri, 2012). The purpose of the present study was to evaluate the normal values of corneal densitometry of healthy subjects in order to assess a normative database.

MATERIALS AND METHODS

This retrospective non-randomized single-center was conducted in a universitary ophthalmology department setting.

This study evaluated the corneal densitometry data on healthy subjects between February 2011 and March 2013. All patients were informed and gave their consent to the study in accordance to the Helsinki declaration. Institutional review board was obtained.

Patients

Healthy subjects were defined as patients who performed a topographic examination before refractive surgery and showed no corneal or other ocular pathology nor previous ocular surgery.

A slit-lamp examination was carried out before Pentacam® scanning (Pentacam®, Oculus Optikgeräte GmbH, Inc., Wetzlar, Germany) to exclude any corneal opacity.

Exclusion criteria were

- Pachymetry less than 490 microns, keratometry higher than 47.8 diopter[15]and irregular or asymmetric astigmatismin order to exclude any subclinical keratoconus
- History of eye disease
- Corneal opacity
- Eye drops use
- Any systemic conditions

Scheimpflug imaging

A Pentacam® imaging was performed in all patients. This non-invasive system uses a rotating Scheimpflug camera, allowing anterior segment imaging from cornea to lens. A 475 nm wavelength LED is used. This system measures corneal topography and corneal density maps (Cornea Densito® software) with a resolution of 1.4 Megapixels (Rajeev, 2009). Densitometry, intended as light-scattering, is calculated on the 12 corneal millimeters, on 4 concentric rings centered on the corneal apex. Zones ranges from apex to 2 mm, then 2 to 6 mm, 6 to 10 mm and finally from 10 to 12 mm. Cornea is divided in three layers: 120 anterior microns, central layer and 60 posterior microns. Total layer gives the average density over the complete thickness. Corneal density is expressed on a gray scale from 0 (maximal transparency, minimal light-scattering) to 100 (minimal transparency, maximum light-scattering) (Gray Scale Unit = GSU). Acceptable maps had at least 10.0 mm of corneal coverage with no extrapolated data in the central 9.0 mm zone. In this study, we collected the following data from all Pentacam examination: central pachymetry, mean keratometry, and corneal density.

Statistical analyses

Statistical analyses were performed using the XLstat-Pro 2015 software package (2015.1.02 Version, Addinsoft, Inc., Brooklyn, NY, USA).

Table 1. Characteristics of the population

	Eyes (n)	622 (311 right eyes, 311 lefteyes)
Total	Age (years +/- SD) [min; max]	39.4 +/- 15.7 [11; 82]
	Gender (men vs women)	260 (41.8%) vs 362 (58.2%)
	Mean keratometry (D+/- SD) [min; max]	43.5 +/- 1.6 [36.6; 47.8]
	Mean pachymetry (µ +/- SD) [min; max]	543.5 +/- 31.1 [490; 642]
11-20 years	Eyes (n)	50 (26 right eyes, 26 lefteyes)
	Age (years +/- SD) [min; max]	17.9 +/- 2.3 [11; 20]
	Gender (men vs women)	26 (50%) vs 26 (50%)
	Mean keratometry (D+/- SD) [min; max]	44.2 +/- 1.2 [42.2; 47.8]
	Mean pachymetry (µ +/- SD) [min; max]	534.1 +/- 28.2 [491; 588]
21-30 years	Eyes (n)	194
	Age (years +/- SD) [min; max]	25.7 +/- 2.7 [21; 31]
	Gender (men vs women)	74 (38.1%) vs 120 (61.9%)
	Mean keratometry (D+/- SD) [min; max]	43.5 +/- 1.5 [40.2; 47]
	Mean pachymetry (µ +/- SD) [min; max]	541.5 +/- 33.5 [490; 642]
31-40 years	Eyes (n)	108 (54 right eyes, 54 lefteyes)
	Age (years +/- SD) [min; max]	34.8 +/- 2.9 [31; 40]
	Gender (men vs women)	46 (42.6%) vs 62(57.4%)
	Mean keratometry (D+/- SD) [min; max]	43.4 +/- 1.6 [39.4; 46.7]
	Mean pachymetry (µ +/- SD) [min; max]	546.1 +/- 34.2 [491; 622]
41-50 years	Eyes (n)	104 (52 right eyes, 52 lefteyes)
	Age (years +/- SD) [min; max]	46.8 +/- 2.6 [41; 50]
	Gender (men vs women)	38 (36.5%) vs 66 (63.5%)
	Mean keratometry (D+/- SD) [min; max]	43.2 +/- 1.9 [36.6; 46.5]
	Mean pachymetry (µ +/- SD) [min; max]	543.8 +/- 32.7 [492; 613]
51-60 years	Eyes (n)	120 (60 right eyes, 60 lefteyes)
	Age (years +/- SD) [min; max]	54.6 +/- 2.8 [51; 60]
	Gender (men vs women)	58 (48.3%) vs 62 (51.7%)
	Mean keratometry (D+/- SD) [min; max]	43.3 +/- 1.6 [39.4; 47.3]
	Mean pachymetry (µ +/- SD) [min; max]	544.8 +/- 24.4 [500; 609]
61- 70 years	Eyes (n)	36 (18 right eyes, 18 lefteyes)
	Age (years +/- SD) [min; max]	64.6+/- 2.6 [61; 68]
	Gender (men vs women)	18 (50%) vs 18 (50%)
	Mean keratometry (D+/- SD) [min; max]	43.2 +/- 1.2 [41; 46.1]
	Mean pachymetry (µ +/- SD) [min; max]	542.1 +/- 25.8 [498; 598]
>70 years	Eyes (n)	26 (13 right eyes, 13 lefteyes)
	Age (years +/- SD) [min; max]	74.2 +/- 3.3 [71; 82]
	Gender (men vs women)	8 (30.8%) vs 18 (69.2%)
	Mean keratometry (D+/- SD) [min; max]	43.8 +/- 1.1 [40.9, 44.8]
	Mean pachymetry (µ +/- SD) [min; max]	559.9 +/- 31.8 [510; 621]

For quantitative values, mean comparisons were performed by a parametric paired Student t-test. For sub-group analysis, a non-parametric Mann-Whitney test was used, because of the samples size. The data had a normal distribution. A p value <0.05 was considered statistically significant. Pearson's test was used for correlation between quantitative values. To compare ordinal values, non-parametric Kruskal-Wallis test with Bonferroni correction was performed.

RESULTS

622eyes from 311 healthy patients were included, with a mean age of 39.4 +/- 15.7 [11; 82] years at the time of the inclusion. Population features are summarized in Table I.

respectively). Peripheral densitometry (10-12 mm)is significantly higher than 0-2mm and 2-6mm densitometry (24.8+/- 6.5 [9.2; 50.2] vs 17.8+/-1.5 [9.4; 34.3], p< 0.0001 and 16.3 +/- 1.5 [13.2; 25.1], p<0.0001). No statistical relationship was found between gender and the mean densitometry measurements (p=0.341). Corneal densitometry, age, central corneal thickness, mean keratometry and Pearson's correlations are summarized in table II. There was no correlation between densitometry, pachymetry and K-readings. Pachymetry was weakly correlated to corneal densitometry in central layer (R= 0.094, p= 0.028), total densitometry (R= 0.116, p=0.007) and age (R=0.102, p=0.017), with a significant p value. A strong statistical correlation was found between each layer corneal densitometry and age (p<0.0001) (Figure 2).

Table 2. Corneal densitometry, central corneal thickness (CCT), mean keratometry (Km), age and Pearson's correlations. Bold text means significant p-values

		Avg	*Min*	*Max*	*SD*	*Pearson's correlation (p-value)*		
						Age	*CCT*	*Km*
Corneal densitometry	*Anterior (120µm)*	25.6	17.3	46.2	4.4	0.296(<0.0001)	0.058 (0.172)	0.032 (0.458)
	Central layer	16.6	11.5	31.2	3.1	0.720 (<0.0001)	0.094 (0.028)	-0.002 (0.970)
	Posterior (60 µm)	14.8	9.6	27.8	2.8	0.765(<0.0001)	0.066 (0.121)	0.032 (0.461)
	Total	18.9	13.2	33.7	3.2	0.725(<0.0001)	0.116 (0.007)	-0.016 (0.701)
	Age	39.4	11	82	15.7	1(0)	0.102(0.017)	-0.083 (0.050)
	CCT	543.5	490	642	31.1	0.102 (0.017)	1(0)	-0.032 (0.450)
	Km	43.5	36.6	47.8	1.6	-0.083(0.05)	-0.032(0.450)	1(0)

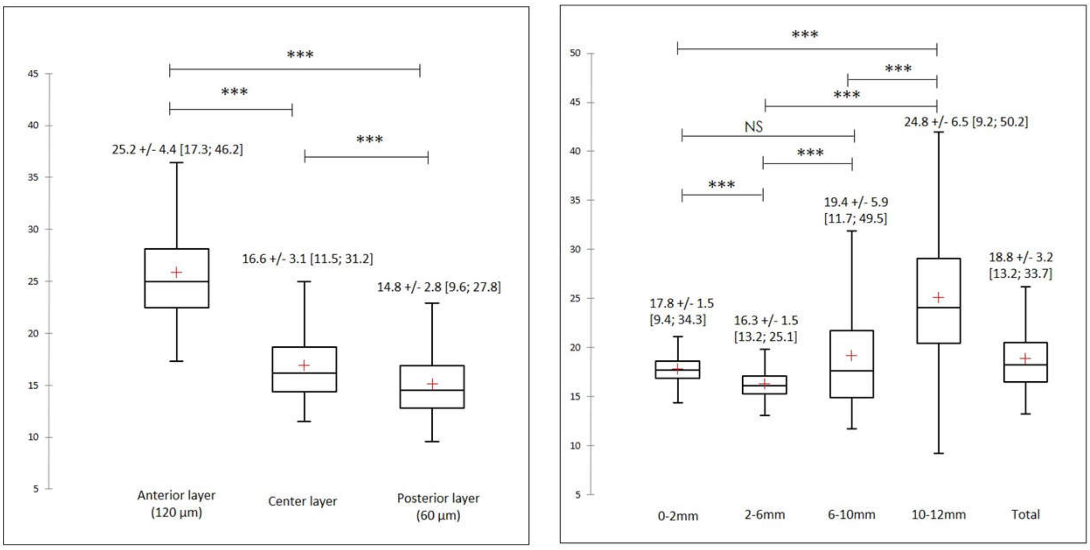

Fig. 1. Corneal densitometry in function of layers and eccentricity* shows significant results. NS means Non Significant

Healthy corneal density pattern

The graph distribution of the average corneal densitometry as a function of the radius of concentric rings around the corneal apex exhibited a gentle slope with a mild peak in the anterior cornea. This pattern was seen in all the healthy patients.

Corneal densitometry and corneal layers

Cross-sectional and anterior, middle and posterior layers mean corneal densitometry and corneal densitometry in function of eccentricity are represented in figure 1.Mean total corneal densitometry was 18.8 +/- 3.2 [13.2; 33.7]. A statistically significant difference (p< 0.05) was found between each of the corneal layers and between each ring. Corneal densitometry in anterior layer was significantly higher than in center and posterior layer (25.2+/- 4.4 [17.3; 46.2] vs 16.6+/- 3.1 [11.5; 31.2], p<0.0001 and 14.8 +/- 2.8 [9.6; 27.8], p<0.0001

Correlation between age and specific corneal layers

Correlation between age and corneal layers is illustrated in figure 2. We could calculate the theoretical value of corneal densitometry of each layer from the subject age with the following equations, from Figure 2:

- Cross-sectional densitometry = 0.1503 x age + 12.936
- Anterior densitometry = 0.1793 x age + 18.185
- Central densitometry = 0.1438 x age + 10.895
- Posterior densitometry = 0.1353 x age + 9.4841

Correlation between the two eyes

Mean and Standard Deviation Values of Corneal Densitometry in right (OD) and left eyes (OS) are shown in table III. Strong positive statistical correlation was found between the two eyes. Right and left eyes were both included because, even if there were highly correlated, values were not exactly the same, and excluding one eye would impaired results.

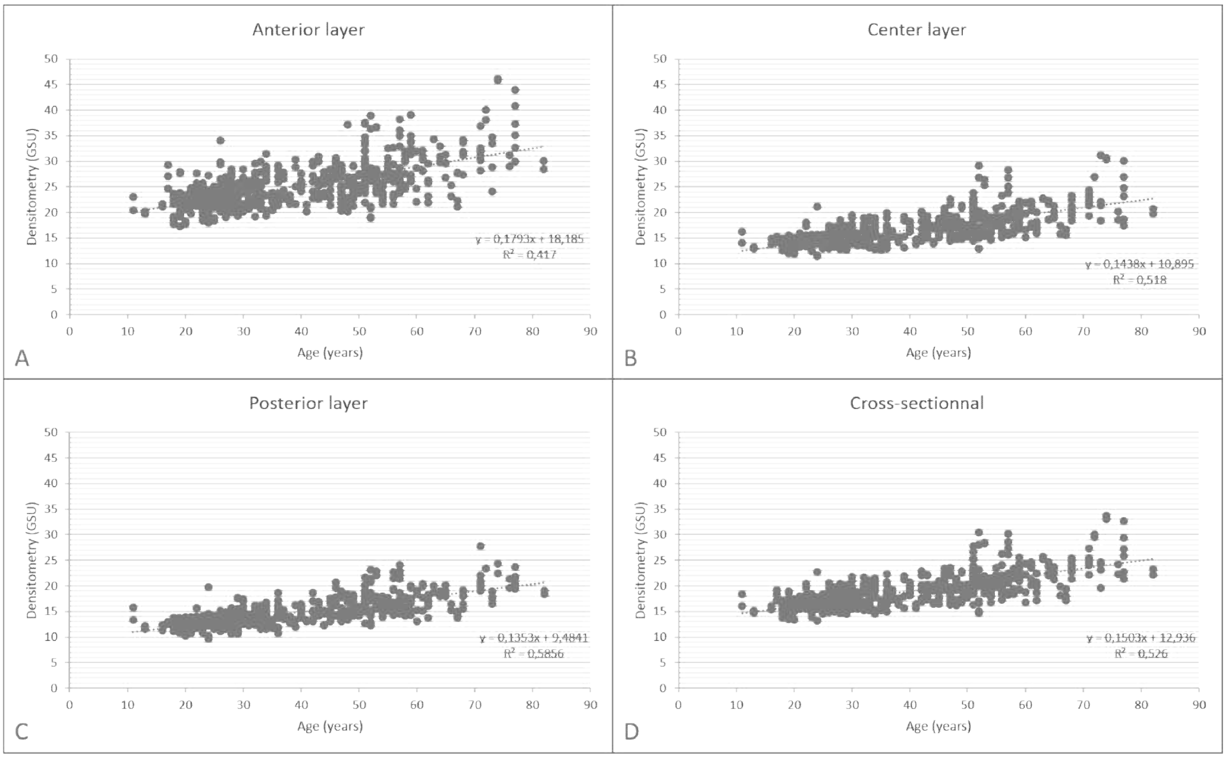

Fig. 2. Correlation between densitometry (in Grey Scale Unit (GSU)) in each layer and age (years). (a) Anterior layer. (b) Central layer. (c) Posterior layer. (d) Cross-sectional

Table 3. Mean and standard deviation values of corneal densitometry in right (OD) and left eyes (OS) and Pearson correlation between the two eyes of a same patient. Bold text shows significant p-values

		0-2mm	2-6mm	6-10mm	10-12mm	Total
Ant	OD	24.8 +/- 2.7	22.4 +/- 2.4	24.7 +/- 7.6	31.9 +/- 9.8	25.1 +/- 4.4
	OS	24.0 +/- 2.4	22.5 +/- 2.2	24.7 +/- 7.3	33.3 +/- 10.2	25.4 +/- 4.3
	Correlation	0.728	0.848	0.945	0.779	0.895
	p-values	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Cent	OD	15.3 +/- 1.3	13.9 +/- 1.5	17.1 +/- 5.9	21.7 +/- 5.6	16.5 +/- 3.1
	OS	15.3 +/- 1.3	14.0 +/- 1.4	17.2 +/- 5.7	22.2 +/- 5.8	16.6 +/- 3.1
	Correlation	0.756	0.843	0.961	0.819	0.927
	p-values	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Post	OD	13.4 +/- 1.4	12.4 +/- 1.4	15.6 +/- 4.6	19.5 +/- 5.3	14.8 +/- 2.8
	OS	13.3 +/- 1.4	12.4 +/- 1.3	15.5 +/- 4.5	20.0 +/- 5.6	14.8 +/- 2.8
	Correlation	0.679	0.828	0.951	0.869	0.913
	p-values	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
Total	OD	17.8 +/- 1.6	16.3 +/- 1.6	19.1 +/- 5.9	24.4 +/- 6.4	18.8 +/- 3.3
	OS	17.8 +/- 1.5	16.3 +/- 1.5	19.2 +/- 5.7	25.2 +/- 6.6	18.9 +/- 3.2
	Correlation	0.738	0.864	0.956	0.816	0.929
	p-values	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001

DISCUSSION

Any disturbance in corneal architecture from nanometric to micrometric scale, can affect corneal transparency and corneal refractive power by increasing light-scattering. Spaces between fibril, minor to light wavelength permits light transmission (Cox, 1970 and Feuk, 1971). Measuring light-scattering in the three spatial directions can bring precious information on corneal architecture (Reinstein, 2005). Several methods have been developed in recent years to objectively measure postoperative stromal scars using ultrasounds (Reinstein, 2005), confocal microscopy (Buhren, 2012), van den Berg straylight meter (Jinabhai, 2012), modified slit lamp called “scatterrometer” (Patel, 2007), or OCT (Wang, 2004). Newly developed techniques are based on measuring the intensity of back-scattered light and anterior eye segment analysis systems based on the Scheimpflug technology (Rajeev, 2006; Soya, 2002).

The Pentacam® system provides quantification of light scattering with densitometries values from 0 (maximal transparency) to 100 (maximal clouding). Validity and repeatability measurements of the Pentacam® system have been prouved (Shankar, 2008). Herein, we report our results about 311 healthy patients with a mean age of 39.4 +/- 15.7 [11; 82] years at the time of the inclusion. Mean total densitometry was 18.8 +/- 3.2 [13.2; 33.7] and a statistical difference was found between each layer and corneal rings. Graph distribution of the average corneal densitometry represents a gentle slope with a mild peak in the anterior stroma. Anterior densitometry was significantly higher than central and posterior densitometry, corresponding to the epithelium and bowman layer light-scattering. A loss of specularly reflected light can explain this difference, mainly because the Scheimpflug system illuminate perpendicularly the cornea and detect light-scattering from an angle of 45°, as it is evident from Boettner’s (Boettner, 1967) data that the density coefficient strongly depends on the scatter angle. Peripheral densitometry was significantly higher than 0-6 mm densitometry, which is consistent with clinical observations and the peripheral increase of pachymetry. However, the standard deviation in the 6-10mm and 10-12 mm annuli were larger than in the 0-2 and 2-6mm. This can be explained by difference of fixation and by inter-individual variability of corneal diameter (Cakmak, 2012). Strong correlation was calculated between age and corneal densitometry, with a significant increase in corneal density with age. In the earliest report of corneal backscatter assessments, Olsen (Olsen, 1982), concluded that there was a significant increase in corneal density with age. This theory was reinforced by Lerman (Lerman, 1984), who demonstrated the decreased of corneal transparency with age.

Quite the opposite, van de Kraatset al (van de Kraats, 2007), and van den Berg et al (van den Berg, 1994), assumed that aging of the optical density of the cornea is absent because they did not found correlation with age in their study on eight patients. They did not measure directly light-scattering but deduced ocular density from light transmittance. Their explanation of this results is that spectral transmittance function of the cornea seems to be dominated by forward light-scattering, which interferes with direct transmittance measurements. Our result is easily understanding by the fact that corneal transparency decreases with age due to development of age-related corneal degenerations such as crocodile shagreen, cornea farinata and so on, which may not be detected of the early stage of the disease. In NíDhubhghaill (NíDhubhghaill, 2014) study, 445 healthy participants were recruited with a mean age of 48.0+/- 15.3 years. Mean corneal densitometry over the 12 mm diameter was 19.74 +/- 3.89 GSU. This result was a little bit higher than our study, perhaps because the mean patient age was higher, reinforcing our conviction that density increases with age. Densitometry values were lowest in the central zone (16.76 +/- 1.87 GSU) and highest in the periphery (27.36 +/- 7.47 GSU). The anterior layer displayed the highest densitometry of 25.81 +/- 5.14 GSU, which was significantly higher than that of both central ($p < 0.001$) and posterior layers ($p < 0.001$). NíDhubhghaill also found a strong correlation with age. These results are consistent with ours. Koh et al (Hillenaar, 2011) described patterns of corneal scatter in patients undergoing Penetrating Keratoplasty (PK), Deep Anterior Lamellar Keratoplasty (DALK), Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) and 29 patients in control group. Normal pattern was defined as a gentle slope with a mild peak in the anterior cornea. This pattern was seen in all patients in control group and in 75% of PK group. The same pattern was found in our study. Hillenaar (Hillenaar, 2011) reported backscatter profiles using In Vivo Confocal Microscopy (IVCM). In this study, sex and time of measurement significantly affect corneal backscatter. From the age of 50 years, increase in the anterior stroma light back-scatter was found. These results are contrary to ours, because of the method of acquisition of corneal backscatter, which in IVCM requires corneal contact and therefore the scattering angle measurement is different from Scheimpflug's (Koh, 2012). In conclusion, we propose in this study Scheimpflug database of normal corneal densitometry with innovative equations giving attempting normal densitometry in function of age, in a mixed population Mediterranean and Caucasian. These results are consistent with previous results (Otri, 2012 and NíDhubhghaill, 2014) and confirmed them. This program of the Pentacam® can provide a useful objective measure of corneal transparency and may help routine clinical practice, such as refractive post-operative follow-up or early detection or corneal graft rejection or corneal diseases.

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Conflict of Interest: None

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