Changes in spectral parameters of corneal pulse following canaloplasty

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Abstract

Purpose To ascertain whether changes in the spectral content of the corneal pulse (CP) signal, measured in vivo in primary open-angle glaucoma (POAG) patients, indirectly reflect changes in corneal biomechanics after canaloplasty.

Methods Fifteen eyes of 15 POAG patients who underwent canaloplasty combined with phacoemulsification were enrolled. Standard ophthalmic examinations were conducted before washout, pre-operatively, at days 1, 7, and 1, 3, 6, and 12 months after surgery. Non-contact measurements of the CP signal were performed at pre-washout, pre-operatively, and at 3, 6, and 12 months post-operatively. Then, amplitudes of the CP first five harmonics associated with the heart rate were estimated. Temporal changes of all considered parameters were tested at a Bonferroni-adjusted significance level set to 0.005.

Results A decrease in the amplitude of the first harmonic and an increase in the normalized amplitude of the third harmonic (A_{CP3n}) of the CP signal were noticed between the pre-washout and the pre-operative stages ($p = 0.003$ and $p = 0.004$, respectively). This corresponds to an increase in median intraocular pressure (IOP) values by 6.0 mmHg ($p = 0.0045$). After surgery, A_{CP3n} reached the highest value at 3 months post-operatively, compared with pre-washout level ($p = 0.0045$).

Conclusions Alterations in corneoscleral stiffness caused by surgery are reflected in changes in the A_{CP3n} value. Hence, post-operative corneal biomechanics could be monitored indirectly by this supporting indicator that can be used to estimate the time at which measures of IOP are no longer biased by the changed cornea boundary conditions caused by canaloplasty.

Clinical Trials Registration NCT02908633

Keywords Corneal biomechanics · Glaucoma surgery · Spectral analysis · Ocular pulse

Introduction

Canaloplasty is a modern and safer alternative to traditional surgeries used for surgical treatment of open-angle glaucoma [1]. The advantage of canaloplasty over filtering surgeries is the lack of subconjunctival bleb and hence no need for antimetabolites, rare post-operative complications, and uncomplicated follow-up [2]. The purpose of canaloplasty is

to restore natural trabeculocanalicular outflow pathways by catheterizing and viscodilating the entire length of Schlemm's canal as well as placing a 10-0 Prolene suture with the use of flexible microcatheter [3]. Canaloplasty is characterized by a high short- and long-term safety profile and high efficiency in the sustained intraocular pressure (IOP) reduction in adult patients with open-angle glaucoma [3–5]. Although proved to be safer than trabeculectomy, which is still regarded as the gold standard, canaloplasty has less pronounced IOP-lowering potential [6, 7].

Static evaluation of IOP is usually the standard parameter for monitoring long-term efficacy of canaloplasty [8–10]. However, it is well known that IOP undergoes cyclic fluctuations due to eye dynamics [11]. Also, the biomechanical properties of ocular tissues affect the IOP measurement [12]. Recently, more attention has been paid to finding parameters complementary to IOP for better assessing the outcome of glaucoma surgery, particularly the parameters describing the

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aforementioned dynamic nature of the eye. Rekas and colleagues [13] were first to report that 24-h fluctuations pattern of corneoscleral limbus area (CSLA), measured with the Sensimed Triggerfish contact lens sensor in glaucoma patients, can be clinically useful for assessing mid-term efficacy of canaloplasty. Those findings emphasize the significance of characteristics of the eye dynamics in monitoring glaucomatous eyes after surgeries aiming at IOP reduction. CSLA changes, reflecting the ocular pulse (OP), undergo not only diurnal processes related to the circadian rhythm but also those linked to the heart rhythm. The dynamics of CSLA and OP characteristics may have a diagnostic potential that is worth exploring.

OP is a highly complex phenomenon reflecting the dynamic behavior of the eye. It manifests itself in ocular volume changes according to the heart rhythm and includes signals of natural pulsatile variations in IOP [11, 14, 15], ocular blood flow [16, 17], aqueous humor secretion and outflow [18, 19], and movements of anterior [14, 20–22] and posterior eye tissues [23–26]. Some of OP signals have found their origins in the systemic circulation [11, 14–16, 27], and their parameters have been shown to be correlated with cardiovascular activity [28–31]. Further, OP signals have also been considered in studying pathological conditions of the eye, such as glaucoma, [24, 32, 33], underlining the role of ocular biomechanical properties in disease processes.

In the current study, we focus particularly on the corneal pulse (CP), which is an OP manifestation representing a superposition of longitudinal eye movements and slight semi-periodic corneal surface expansion [14, 20, 21]. The CP is closely related to both cardiovascular activity [30, 31] and corneal biomechanics [34]. The CP signal can be acquired in a simple way, without disturbing the natural movements of the cornea, using a non-invasive and non-contact ultrasonic technique [21, 22, 35]. This method has recently been used to measure corneal pulsation in elderly subjects [22] and glaucoma patients [36]. As a result, the morphology of the CP signal has been shown to change with age [22] and in glaucoma [36], pointing at potential higher ocular stiffness in those patients. Spectral analysis of OP signals has proved to be useful for differentiating glaucoma patients from healthy subjects [37, 38]. Hence, further investigation of the CP characteristic in frequency domain could provide more insight into natural corneal expansion and ocular rigidity, and offer independent and supportive parameters to standard ophthalmological ocular parameters with clinical utility in glaucoma.

The purpose of this study was to ascertain whether changes in spectral parameters of the CP signal, acquired *in vivo*, provide indirect information on corneal biomechanics after canaloplasty.

Materials and methods

Study design

Fifteen Caucasian patients with uncontrolled mild to moderate primary open-angle glaucoma (POAG) and cataract were enrolled in this study. All patients underwent canaloplasty combined with phacoemulsification. Additionally, a reference age-matched group of 15 healthy subjects was included.

At the time of qualification, all participants underwent past and current general medical as well as ophthalmic history review, including pharmacological treatment and surgical interventions. Exclusion criteria were narrow-angle or secondary glaucoma, neovascular disease, uveitis, peripheral anterior synechiae, any corneal abnormalities, history of angle recession or trauma, previous antiglaucoma surgery or laser therapy, and high myopia (spherical equivalent greater than 6D). Patients with a previous history of IOP greater than 30 mmHg were also excluded, based on the observation made in an animal *ex vivo* study that distal aqueous outflow pathway may be irreversibly collapsed in such individuals [39]. Only one eye per patient was eligible.

The study was approved by the Bioethics Committee of the Military Institute of Medicine in Warsaw (decision no. 67/WIM/2015) and adhered to the tenets of the Declaration of Helsinki. Before any measurements, the purpose of the study and the study protocol were explained to the patients, who were also informed about the surgical alternatives. After declaring participation in the study for at least 12 months, all participants signed a consent form. The trial was registered at clinicaltrials.gov before recruitment (registration no. NCT02908633).

Pre-operative protocol and surgical technique

Patients qualified for combined cataract and glaucoma surgery were scheduled for a washout period of 4 weeks before surgical intervention. Before washout, all patients underwent baseline ophthalmic examination including best-corrected visual acuity (BCVA), IOP measurement with Goldmann tonometry (GAT), gonioscopy, slit-lamp, and fundus examination. Additionally, ultrasound pachymetry (DGH-550 Pachette 2, DGH Technology, Inc., Exton, PA, USA) and optical biometry (IOL-Master, Carl Zeiss Meditec AG, Jena, Germany) were performed. At the pre-operative stage, GAT was obtained and only patients with post-washout IOP of 18 mmHg or higher were scheduled for surgery. Monocular visual field (VF) testing was performed with the 24-2 algorithm of the Humphrey Field Analyzer (Carl Zeiss Meditec AG, Germany) prior to surgery.

Surgical technique

Surgical procedures have been performed under retrobulbar anesthesia (2% xylocaine and 0.5% bupivacaine) by one surgeon (M. R.). First, a fornix-based conjunctival flap was dissected to expose sclera. This was followed by creating scleral flaps, as described previously by Lewis et al. [40], and Bellucci and Morseli [41]. A 5.0 mm × 5.0 mm, one-third scleral thickness, parabolic in shape, the superficial flap was extended forward into the clear cornea and a 1.0-mm smaller, in size, the deep flap was dissected to expose trabeculo-Descemet's membrane (TDM) and reach Schlemm's canal. At this stage, 2.2 mm clear corneal phacoemulsification was performed using the Infiniti Vision System (Alcon Surgical, Fort Worth, TX) followed by an IOL implantation. After completing the TDM, the inner wall of Schlemm's canal was removed and the deeper scleral flap was excised. Catheterization around the entire circumference of the canal was carried out with a standard canaloplasty set (iTrack from Ellex Medical Lasers Pty Ltd., Adelaide, Australia). Once the distal tip was exposed, a 10-0 polypropylene suture was tied to it and pulled into the canal. With careful withdrawing of microcatheter under control of an illuminating beacon tip, viscoelastic was injected every 2 h. After viscodilation, the tip was exposed at the ostium and the suture was cut off and knotted under tension in order to distend the trabecular meshwork inward. After the excision of the deep flat, the superficial flap was sutured in a watertight manner over intrascleral space, which resulted in creating a scleral lake. The conjunctival flap was fixed to the corneal limbus.

Post-operative protocol

Post-operative examinations were performed at days 1 and 7, and 1, 3, 6, and 12 months post-operatively, and whenever it seemed clinically necessary. It included BCVA testing, GAT, slit-lamp biomicroscopy for anterior segment assessment, gonioscopy with angle grading, and indirect funduscopy. Pachymetry and optic biometry measurements were taken at 3, 6, and 12 months. At all follow-up examinations, patients were reviewed for the number and type of antiglaucoma medications; adverse events were also recorded.

Two definitions of success were used: complete and qualified. Complete surgical success was defined as IOP ≤ 18 mmHg with no antiglaucoma medications, and qualified success was defined as IOP ≤ 18 mmHg with or without medications. A procedure was considered to be a failure when IOP > 18 mmHg with or without glaucoma medication, or when further glaucoma surgery was required.

Corneal pulse measurement

Additionally, at pre-washout, on the day of surgery (pre-op), and at 3, 6, and 12 months after surgery, non-contact measurements of the CP signal were performed by using an ultrasonic transducer [35, 42] that was placed in a specially constructed holder mounted on a slit-lamp. To minimize the movements of the patient's head, a belt was used that strapped the head to the ophthalmic headrest frame. The single CP recording took 10 s and was repeated five times for the eye classified to canaloplasty. Synchronously with the CP signal, the blood pressure (BP) signal was measured using a CNAP Monitor 500 (CNSystems Medizintechnik GmbH, Graz, Austria).

Data analysis

Estimation of spectral parameters of the CP and BP signals was performed in a custom program written in MATLAB (MathWorks, Inc., Natick, MA, USA). First, raw CP and BP signals were preprocessed numerically, including linear trend removal and band-pass filtering in the range from 0.6 to 20 Hz to eliminate the frequencies related to respiration modulation (below 0.6 Hz) and those not related to the fundamental frequencies of the heart activity. Then, for each patient and at each visit, the first three pairs (CP, BP) of signals that were not affected by eye blink artifacts or substantial head movements in the CP signal, as evaluated by an expert from the signal trend, were selected for further analysis. For each BP signal, the mean arterial pressure (MAP) and systolic (SBP) and diastolic (DBP) blood pressures were computed. Then, the representative mean values of MAP, SBP, and DBP were calculated across the three selected repetitions of BP signals for each patient and at each visit.

Signals spectra were computed using the Fourier transform. Since a 10-s-long BP signal can be assumed stationary (spectral content not changing in time) [30], its amplitude spectrum was used to estimate the frequency f_{BP1} of the first harmonic (the fundamental frequency) associated with the heart rate. Then, the corresponding first harmonic, f_{CP1} , was determined in the CP spectrum in the range of $f_{BP1} \pm 0.2$ Hz. For f_{CP1} and its four subsequent harmonics, amplitudes A_{CP1} , A_{CP2} , A_{CP3} , A_{CP4} , and A_{CP5} were calculated. Next, for each CP recording, amplitudes of its second to fifth harmonics were normalized to the amplitude of the first harmonic, giving A_{CP2n} , A_{CP3n} , A_{CP4n} , and A_{CP5n} , where the subscript n denotes normalization. Finally, the representative mean values of A_{CP1} , A_{CP2n} , A_{CP3n} , A_{CP4n} , and A_{CP5n} were calculated across the three selected repetitions of the CP signals for each patient and at each visit.

For each patient's visit, the mean values from the three repeated measurements of IOP were calculated. For pre-washout as well as post-operatively, analogous means were calculated also for CCT, ACD, and AL. Visual acuity in

Snellen equivalents was converted to logMAR units (logarithm of minimum angle of resolution).

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the null hypothesis of data normality and there were no reasons to reject it for demographic data, IOP, ocular biometric, and blood pressure parameters for POAG patients at the pre-washout stage and for healthy subjects ($p > \alpha = 0.05$, α being the significance level). Therefore, an independent-samples t test was used to compare those parameters between POAG patients and the healthy group.

Averaged spectral parameters, IOP values, ocular parameters, and blood pressure parameters for POAG patients after the pre-washout stage generally did not follow a normal distribution. Hence, temporal changes of the abovementioned parameters were tested using the Wilcoxon signed rank test with Bonferroni corrected $\alpha = 0.005$. Temporal analyses did not include the healthy group, which was only used as a reference for the pre-washout stage of the POAG group.

Partial correlations, with time set as a control variable, were applied between the CP signal parameters, IOP and blood pressure parameters, separately for data before (pre-operative

stage) and after surgery (post-operative stage). The calculations were performed in SPSS 22.0 (SPSS, Inc., Chicago, IL, USA).

Results

Demographic data and ocular and blood pressure parameters for POAG patients and healthy subjects are summarized in Table 1 with the corresponding results of t test. Note that no statistically significant differences were found between POAG patients and the healthy reference group in all considered parameters.

In the time period between the pre-washout and the pre-op stage—across which the cornea demonstrates natural (that is, without surgical incision) biomechanical changes with the IOP increase—the median value of IOP increased by 6 mmHg (Wilcoxon test, $p < 0.001$) (see Tables 2, 3, and Fig. 1). At 3 months after canaloplasty—when changes in corneal biomechanics governed by different boundary conditions occur—the median value of IOP decreased statistically significantly by 9 mmHg with respect to the pre-op stage ($p < 0.001$) and then remained at almost the same level up to 12 months post-operatively (see Fig. 1). The complete or

Table 1 Patients' demographics and the considered set of parameters for the POAG group at the pre-washout stage and a reference healthy group with the results of the independent-samples t test

Data	POAG group Mean $\pm$ SD (range)	Healthy group	t test
Age (years)	73 $\pm$ 8 (59–87)	69 $\pm$ 4 (64–75)	$t(28) = 1.671$, $p = 0.106$
Sex (female/male)	9/6	9/6	–
Eye (right/left)	3/12	4/11	–
IOP (mmHg)	16.9 $\pm$ 2.7 (12.0–21.0)	15.5 $\pm$ 1.6 (13.0–18.0)	$t(28) = 1.769$, $p = 0.088$
CCT (μ m)	531 $\pm$ 33 (481–584)	526 $\pm$ 33 (466–574)	$t(28) = 0.368$, $p = 0.715$
ACD (mm)	3.13 $\pm$ 0.39 (2.53–3.90)	2.90 $\pm$ 0.35 (2.22–3.48)	$t(28) = 1.746$, $p = 0.092$
AL (mm)	23.50 $\pm$ 1.14 (21.76–25.83)	22.88 $\pm$ 0.84 (21.54–24.32)	$t(28) = 1.695$, $p = 0.101$
MD (dB)	– 5.29 $\pm$ 3.05 (– 0.91 to – 13.29)	–	–
PSD (dB)	5.27 $\pm$ 2.72 (1.25–9.19)	–	–
SBP (mmHg)	152 $\pm$ 22 (128–183)	141 $\pm$ 19 (102–166)	$t(23) = 1.426$, $p = 0.167$
DBP (mmHg)	82 $\pm$ 8 (73–96)	77 $\pm$ 7 (64–85)	$t(23) = 1.811$, $p = 0.083$
MAP (mmHg)	108 $\pm$ 9 (91–120)	102 $\pm$ 12 (79–116)	$t(23) = 1.465$, $p = 0.156$

IOP intraocular pressure, CCT central cornea thickness, ACD anterior chamber depth, AL axial length, MD visual field mean deviation, PSD visual field pattern standard deviation, SBP systolic blood pressure, DBP diastolic blood pressure, MAP mean arterial pressure, SD standard deviation

Table 2 Number of medications, IOP, ocular, and blood pressure parameters at specific times before and after surgery for a group of 15 patients undergoing canaloplasty. All subjects completed the whole course of the study

Time	Medications (<i>n</i>) Median (range)	IOP (mmHg) Median, IQR (range)	CCT (μ m)	ACD (mm)	AL (mm)	BCVA (logMAR)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
Natural corneal biomechanics									
Pre-washout	2 (1–3)	17, 4 (12–21)	524, 56 (481–584)	3.21, 0.67 (2.53–3.90)	23.24, 1.45 (21.76–25.83)	0.30, 0.25 (0.05–0.60)	151, 43 (128–183)	81, 12 (73–96)	108, 16 (91–120)
Pre-op	0	23, 4 (18–29)	–	–	–	–	144, 20 (121–157)	83, 14 (68–111)	103, 8 (94–130)
Changing corneal biomechanics									
3 m post-op	0	14, 6 (5–20)	523, 55 (483–583)	4.09, 0.91 (2.53–5.19)	22.99, 1.44 (21.56–25.81)	0.10, 0.10 (0.00–0.30)	145, 28 (107–172)	76, 25 (41–97)	108, 15 (68–117)
6 m post-op	0	13, 2 (10–21)	530, 49 (498–605)	4.47, 0.82 (2.38–5.33)	23.04, 1.48 (21.56–25.85)	0.00, 0.23 (0.00–0.30)	162, 41 (103–187)	80, 27 (59–128)	112, 24 (74–148)
12 m post-op	0	13, 6 (10–20)	533, 58 (499–601)	4.32, 0.56 (2.38–5.21)	23.04, 1.53 (21.52–25.87)	0.05, 0.15 (0.00–0.52)	141, 31 (118–165)	81, 26 (48–93)	102, 9 (85–122)

IQR interquartile range, *n* number of medications

qualified success rate evaluated at 12-month post-op was reached in thirteen out of fifteen patients (~ 86%).

Before washout, patients were taking three groups of topical medications: β -blockers (60% of patients), prostaglandin analogues (PGAs) (94%), and carbonic anhydrase inhibitors (40%), in various combinations. Six out of fifteen patients were on PGA monotherapy (latanoprost). Three patients took PGA-based fixed combination alone, whereas three patients took the same fixed combination with an adjunctive anhydrase inhibitor concomitantly. One patient used the combination of carbonic anhydrase inhibitors and β -blocker alone, whereas two patients used this combination with an unfixed combination of PGA. The median number of medications before washout was two (range one to three). At 12-month follow-up, no patient required antiglaucoma medication.

From all the ocular parameters measured, CCT, ACD, and BCVA changed significantly after 12 months post-operatively in relation to the pre-washout stage (Wilcoxon test, $p < 0.001$, $p = 0.003$, and $p = 0.002$, respectively).

Correlation between IOP and DBP ($p = 0.046$, $R^2 = 0.176$) was found only before surgery, which can be associated with the washout of glaucoma medications.

Complications

No intra-operative complications were noted. Successful 360° catheterization with the placement of a tension suture was achieved in all cases. Majority of early post-operative complications included hyphema, defined as layered blood level in anterior chamber measured after at least 1 h in an upright position, or microhyphema, defined as erythrocytes in the anterior chamber without layered blood (see Table 4). In all of these cases, the blood resolved within 1 week. No transient IOP elevation was noted. In one case, post-operative macular edema was diagnosed causing BCVA decrease—this resolved after topical non-steroid anti-inflammatory drugs and subtenon injection of methylprednisolone acetate.

Spectral parameters of the CP signal

At the pre-washout stage, there were no statistically significant differences in A_{CP1} and A_{CP3n} between POAG patients and the healthy reference group ($t(26) = 1.037$, $p = 0.309$, and $t(26) = 0.587$, $p = 0.562$, respectively). Statistically significant differences were observed only in A_{CP1} and A_{CP3n} . Specifically, a decrease in A_{CP1} and an increase in A_{CP3n} were observed between the pre-washout and the pre-operative stage ($p = 0.003$; $p = 0.004$, respectively) (see Figs. 2a and b). This corresponds to an increase in IOP values between those stages (see Fig. 1).

After surgery, when corneal biomechanics differs from that of the pre-operative stage, A_{CP3n} reaches the highest value at 3 months post-operatively in relation to the pre-

Table 3 Results of temporal changes of IOP, ocular, and blood pressure parameters. Differences were assessed using the Wilcoxon signed rank test with the Bonferroni correction (pre-washout vs pre-op, 3 m, 6 m, and 12 m post-op). Italicized values indicate $p < 0.005$

	Time	IOP (mmHg) <i>p</i> value	CCT (μ m)	ACD (mm)	AL (mm)	BCVA (logMAR)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
Natural corneal biomechanics	Pre-op	< 0.001	–	–	–	–	0.203	0.799	0.285
Changing corneal biomechanics	3 m post-op	0.017	0.220	0.003	0.010	0.001	0.441	0.038	0.173
	6 m post-op	0.008	0.025	0.004	0.010	< 0.001	0.953	0.515	0.441
	12 m post-op	0.018	< 0.001	0.003	0.010	0.002	0.441	0.314	0.678

washout ($p = 0.0045$) and then significantly decreases at 12 months ($p = 0.0045$) (see Fig. 2b). Since nominal levels of A_{CP1} and A_{CP3n} values for healthy subjects are less known, those values are additionally shown in Figs. 2a and b.

Correlation analysis with time used as a control variable showed that A_{CP1} and IOP values before canaloplasty were weakly correlated ($R^2 = 0.174$); however, this result was statistically significant ($p = 0.024$).

Discussion

Numerous earlier works widely investigated the amplitudes of OP signals, pointing at their role in the estimation of ocular rigidity [24, 32] and in the differentiation of glaucomatous eyes [33, 43]. Also, spectral analysis of OP signals has shown to be a powerful tool for investigating the relationship between OP and cardiovascular activity in healthy individuals [21, 30], differentiating ocular hemodynamics and biomechanical properties in healthy subjects from those in glaucoma patients [25], and discriminating glaucomatous eyes from healthy eyes [37, 38, 44]. This study extends those developments to glaucoma patients undergoing canaloplasty.

To the best of our knowledge, this is the first study presenting spectral changes of CP signals in POAG patients treated with canaloplasty. It is revealed that the information about corneal biomechanics is indirectly carried by the spectral content of the CP signal. Hence, it has the potential to differentiate corneal biomechanics in the pre- and post-operative stages. Unlike the IOP measurement, the non-contact and non-invasive registration of the CP signal, on its own, is independent of the ocular biomechanical properties.

In this study, statistically significant differences in the amplitude of the first CP harmonic, A_{CP1} , and the normalized amplitude of the third CP harmonic, A_{CP3n} , have been observed between pre-washout and pre-op, the two stages between which the cornea demonstrates natural (that is, without surgical incision) biomechanical changes with IOP increase. Namely, the greater the IOP difference between pre-washout and pre-op stages, the smaller the A_{CP1} and the higher the A_{CP3n} . Our results for A_{CP1} recorded in humans are in agreement with the earlier *in vivo* animal study of Rogala et al. [34], where the acute increase in IOP in rabbit eyes was reflected in a decrease in the power of the first CP harmonic. It is known that elevated IOP increases ocular rigidity [45] and that higher ocular stiffness related to the IOP elevation is expressed in

Fig. 1 Boxplots of IOP at consecutive measurement stages. * $p < 0.001$

Table 4 Ocular-related postsurgical complications

Complication	Number of patients (%)
Hyphema	6 (40.0)
Inflammatory exudates	2 (13.3)
Macular edema	1 (6.7)
IOP ≥ 30 mmHg	none
Blood in vitreous	1 (6.7)

higher mechanical resistance of cornea [46]. We have demonstrated that changes in the measured IOP during the course of study alter the spectral content of the CP signal, as indicated by A_{CP1} and A_{CP3n} values. However, the relationships between the IOP and the CP signal harmonics are not directly proportional, suggesting that the latter carries additional to IOP information on the ocular biomechanics.

It is important to note that our findings showed unchanged CCT values at the 3-month post-op after surgery, when IOP rapidly decreases and A_{CP3n} achieves the highest value. Therefore, we conclude that CCT has no impact on the spectral content of the CP signal. Hence, to evaluate the efficacy of canaloplasty, other biomechanical properties of cornea underlying its stiffness and biomechanically corrected IOP should be considered.

Changes in corneal biomechanics caused by the surgical incision and tensioning suture in the Schlemm's canal (SC) in canaloplasty are the contributing factors further increasing the A_{CP3n} value to that achieved after the washout. The tensioning suture stiffens the limbus area changing the boundary conditions for the cornea and, subsequently, results in a damped low-frequency component (A_{CP1}) and an amplified high-frequency component (A_{CP3n}) of the recorded CP signal. As it was shown in the earlier *in vivo* study with rabbits [34], biomechanical properties of the stiffer cornea (a higher Young's modulus) are reflected in a higher power of the third CP harmonic (around 3 Hz) at normal IOP. In a way, the cornea could be viewed as a mechanical high-pass filter with gain depending on the IOP and cornea boundary conditions. Hence, the spectral parameters of the CP signal could be

valuable factors to indirectly assess post-operative alterations in corneoscleral stiffness caused by surgeries aiming at IOP reduction.

Many studies reported that increased IOP caused a reduction in the pulsatile ocular blood flow (POBF) [46–48]. Since there is a strong correlation between the CP and cardiovascular activity signal parameters [14, 21, 30, 31], indicating similarity of their signal spectra [21, 26, 30], the CP signal parameters contain also information about the cardiovascular activity, apart from biomechanical properties of the cornea. In this study, POBF has not been measured directly, but the continuous blood pressure (BP) signal was registered synchronously with the CP signal. At the consecutive measurement stages, mean, systolic, and diastolic values of the BP signal were not correlated with the CP parameters, meaning no direct effect of systemic blood pressure on the CP characteristic. This outcome supports the postulate that variations in spectral parameters of the CP signal measured after canaloplasty are likely due to changes in corneal biomechanics.

Canaloplasty offers a very high safety profile and greater IOP reduction when combined with cataract surgery [49]. Our study confirms the earlier results of Rekas et al. [53] and Zhang et al. [54] that phacocanaloplasty decreases IOP effectively up to 12 months after surgery. Most frequent complications were hyphema or microhyphema, commonly reported in canaloplasty literature, which are considered to be a positive prognostic indicator of surgery effectiveness [50]. According to Grieshaber et al. [50], hyphema, as a result of blood reflux due to pressure gradient reversion, is to be a proof of distal aqueous outflow pathway patency. We did not record any case of persistent hypotony after surgery or choroidal or retinal detachment. No bleb-associated complications like blebitis or endophthalmitis were present. The absence of conjunctival bleb made the follow-up quicker and less demanding for patients, since no additional procedures were required.

According to the literature, dorzolamide can induce corneal edema in human eyes with borderline endothelial function [51] or corneal guttata [52], but it does not change CCT in healthy individuals. Also, the influence of using topical dorzolamide on endothelial pump function was shown in the

Fig. 2 Boxplots of A_{CP1} (a) and A_{CP3n} (b) of the CP signal at consecutive measurement stages

animal study of Teus et al. [53], where it was proved to reduce the negative intrastromal corneal pressure. Topical β -blockers have been associated with a reversible increase in CCT [54]. Prostaglandin F2 α (PG) analogues, on the other hand, affect corneal hysteresis, which reflects some corneal viscoelastic properties [55, 56], corneal deformation amplitude [57], and also cause a decrease in CTT with no correlation between the magnitude of CCT reduction and the reduction in IOP [58]. This effect on CCT is probably due to alteration of the corneal stromal structure by the PG F-receptor mediated synthesis of metalloproteinases [59–61] and reduction in collagen synthesis. Topical application of latanoprost also stimulates fibroblasts-mediated collagen gel contraction; thus, CCT might be affected by changes in corneal shape, as suggested by Liu et al. [62]. In clinical practice, interactions between IOP-lowering medication and the CCT are of special interest because of the risk of improper estimation of the GAT-derived IOP readings during glaucoma patients observation. In the current study, CCT increased slightly ($\sim 2\%$), however significantly, after 12 months post-operatively in relation to the pre-washout stage, which can be an effect of surgical intervention on the corneal structure and biomechanics. On the other hand, vascular effects are the other aspect of the antiglaucoma drug action on the eye dynamics. β -blockers have been shown to reduce ocular blood flow, in particular choroidal and optic disc pulsatile blood flow [63–66]. However, in the case of other classes of drugs, contradictory results have been obtained, which could be also explained by using different measurement techniques and different treatment regimens [63, 67, 68]. No effect of antiglaucoma medications on blood flow velocities in either the central retinal or the ophthalmic artery has been observed [63, 69–73]. Furthermore, cardiovascular system can be provoked by β -blockers [74]; however, there was no measurable effect on systemic hemodynamics in healthy subjects [63, 69]. In our study, blood pressure parameters of glaucoma patients did not differ from those of healthy subjects at the pre-washout stage, and even after washout of antiglaucoma medications, which emphasizes the lack of systemic effects. Because no systemic hemodynamic changes have been observed in our study, increased IOP should theoretically result in a decreased ocular perfusion pressure, which cannot be excluded as contributing to potential changes of choroidal blood flow. Since variations of the CP characteristic are correlated with the ocular perfusion pressure and fundus pulsation, being a valid index of pulsatile choroidal perfusion [75], drug-induced changes of choroidal blood flow may be reflected in the CP characteristics as well. Based on the aforementioned studies and our own results reported here, we conclude that the increased mechanical resistance of the ocular surface after the period of washout from antiglaucoma medications, manifesting also in changes in the CP characteristic, could be caused by the increase in the IOP as well as the drug-modified cornea surface, biomechanics, and choroidal vasculature.

When discussing our findings, some limitations and other aspects of the presented study should be considered. For instance, a relatively small number of patients were enrolled in the study. Hence, we performed a post-hoc estimation of statistical power. Our analysis, based on the assumption of Gaussianity and a significance level of 5%, was conducted at a statistical power of 90%. For a sample size of fifteen POAG patients included in this study, a decrease of 1.6 μm in the mean value of A_{CP1} can be differentiated, which translates to a change of 25%. Similarly, we can differentiate an increase of 0.32 in the mean value of A_{CP3n} , which is a 50% change. Detecting 10% differences in those values would require 94 and 419 patients, respectively. In that sense, our study has a pilot character. Another limitation is the lack of a control group after cataract surgery. However, it can be assumed that the amount of any bias resulting from phacoemulsification with canaloplasty in POAG patients is likely to be the same for all patients and should not affect greatly the main results achieved in the study.

Another limitation of our study is the lack of measuring suture tension during surgery. Such measurement could have added valuable information to the change of the corneal stiffness. Intra-operative non-contact monitoring of canaloplasty procedure and measuring suture tension is challenging, because there is no method to directly measure suture tension during surgery. Recently, intra-operative optical coherence tomography (iOCT) has been reported as a promising non-contact real-time imaging tool during canaloplasty for monitoring the correct implantation and position of the suture into SC. Also, measurements of changes in anterior chamber angle of the steepening of SC enabled assessing suture tensioning indirectly [76]. Once iOCT is not available, suture tension during surgery can be clinically subjectively evaluated by observing the indentation of the trabeculo-Descemet's membrane [77], which has been done, in this study, by the operating surgeon (M. R.). The surgeon has pulled both thread ends synchronously and equally towards each other and perpendicularly to the trabecular meshwork in accordance with “closing a sling” in order to avoid cheese-wiring [78]. It can be assumed that for each patient, during surgery, the suture was knotted under similar tension in order to distend the trabecular meshwork optimally, which in consequence likely results in similar corneal stiffness. Knowledge about the degree of suture tensioning may be helpful in assessing the success of canaloplasty. Also, the amount of SC distention can be assessed post-operatively using anterior imaging techniques, such as high-resolution ultrasound biomicroscopy [4, 40, 79–82] and anterior segment optical coherence tomography [77, 81, 83].

The consequence of the medication washout may also have an impact on the CP parameters, since some topical antiglaucoma medications have been found to modify corneal structure [51, 84] and biomechanics [55–57] as well as

choroidal circulations [63, 65, 66]. However, the effect of the specific medications taken by patients on the CP signal characteristics could not be evaluated because of the applied fixed-combination drug therapy and limited sample size.

Summarizing, based on our findings with the animal model, which showed an increase in the power of the third CP harmonic with increasing stiffness of the cornea estimated by Young's modulus [34], we conclude that changes in A_{CP3n} could reflect alterations in corneoscleral stiffness caused by the surgery. In this sense, A_{CP3n} could be viewed as an indirect supporting indicator for monitoring post-operative corneal biomechanical behavior and for estimating the time at which measures of IOP are no longer biased by the changed cornea boundary conditions caused by canaloplasty.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Bioethics Committee of the Military Institute of Medicine in Warsaw and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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