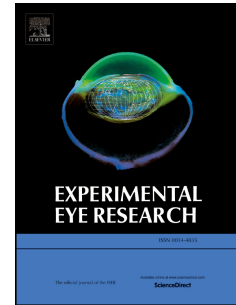


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Ex-Vivo Experimental Validation of Biomechanically-Corrected Intraocular Pressure Measurements on Human Eyes Using the CorVis ST

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Abstract

The purpose of this study was to assess the validity of the Corvis ST (Oculus; Wetzlar, Germany) biomechanical correction algorithm (bIOP) in determining intraocular pressure (IOP) using experiments on ex-vivo human eyes. Five ex-vivo human ocular globes (age 69 ± 3 years) were obtained and tested within 3-5 days post mortem. Using a custom-built inflation rig, the internal pressure of the eyes was controlled mechanically and measured using the CorVis ST (CVS-IOP). The CVS-IOP measurements were then corrected to produce bIOP, which was developed for being less affected by variations in corneal biomechanical parameters, including tissue thickness and material properties. True IOP (IOP_t) was defined as the pressure inside of the globe as monitored using a fixed pressure transducer. Statistical analyses were performed to assess the accuracy of both CVS-IOP and bIOP, and their correlation with corneal thickness. While no significant differences were found between bIOP and IOP_t (0.3 ± 1.6 mmHg, $P= 0.989$) using ANOVA and Bonferroni Post-Hoc test, the differences between CVS-IOP and IOP_t were significant (7.5 ± 3.2 mmHg, $P< 0.001$). Similarly, bIOP exhibited no significant correlation with central corneal thickness ($p= 0.756$), whereas CVS-IOP was significantly correlated with the thickness ($p< 0.001$). The bIOP correction has been successful in providing close estimates of true IOP in ex-vivo tests conducted on human donor eye globes, and in reducing association with the cornea's thickness.

1. Introduction

The evaluation of intraocular pressure (IOP) is a fundamental part of eye examination and is essential for the screening and treatment of pathologies such as glaucoma and ocular hypertension. The association between elevated intraocular pressure (IOP) and glaucoma development and progression has since been confirmed, making IOP the main modifiable risk factor for glaucoma, and establishing IOP measurement as an essential part of glaucoma risk-profiling and management (Stamper, 2011). Recent evidence estimates a rise in the risk of progression in patients with already established glaucoma of 11% for every 1 mmHg increase in IOP (Bengtsson et al., 2007). For this reason, an accurate IOP estimate is highly desirable.

The reference standard in IOP measurement is the Goldmann Applanation Tonometer (GAT), which applanates a central area of the cornea with a 3.06mm diameter and assumes that at this point the externally-applied pressure equals the IOP (Goldmann, 1955). This operating principle of the GAT, makes the device susceptible to the natural variations in corneal stiffness, caused by variations in tissue thickness and biomechanics from average levels, and introduces inaccuracies in IOP measurements (Ehlers et al., 1975; Herndon et al., 1997). Based on these findings, several attempts were made to create IOP estimates that corrected for biomechanics including, most notably, the Dynamic Contour Tonometer (DCT, Swiss Microtechnology AG, Port, Switzerland) (Kanngiesser et al., 2005), the Ocular Response Analyzer (ORA Reichert Ophthalmic Instruments, Depew, NY) (Montard et al., 2007) with its Corneal Compensated IOP (IOP_{cc}) estimates, and more recently the CorVis ST (Hong et al., 2013) (OCULUS Optikgeräte GmbH; Wetzlar, Germany) through its biomechanically-corrected IOP (bIOP) measurements (Joda et al., 2016; Vinciguerra et al., 2016). The effectiveness of these estimates has been assessed in clinical studies, primarily through evidence of reduced association between IOP measurements and the cornea's stiffness parameters, such as central corneal thickness (CCT) and age (Doyle and Lachkar, 2005; Kniestedt et al., 2005a). Other studies compared IOP measurements using tonometry

with those obtained through manometry in in-vivo eyes, but the number of these studies and the number of patients involved were limited for practical and ethical reasons (Eisenberg et al., 1998; Whitacre et al., 1993).

The DCT, ORA and CorVis ST adopted different approaches to the biomechanical correction of IOP measurements. The DCT used a tonometer tip with a concave front (rather than a flat front as in GAT) in order to reduce the cornea's deformation required during the measurement process, and hence reduce interference of corneal biomechanics in the IOP measurements. In contrast, the ORA – a non-contact tonometry technique – produced the cornea-corrected IOP (IOP_{cc}) estimate. That estimate relied on a correction algorithm based on the cornea's two applanation pressures and constants, which were determined empirically using clinical data (obtained pre- and post-LASIK), in an attempt to reduce association with CCT, the main corneal stiffness parameter (Luce, 2005).

On the other hand, the CorVis ST – another non-contact tonometer – traced deformation of both the cornea's anterior and posterior surfaces under dynamic, external air pressure using a high speed Scheimpflug camera. This information enabled accurate determination of corneal thickness profile and curvature, both of which are important biomechanical parameters. It also allowed reliable representation of corneal behaviour in numerical modelling, which was then used to derive a bIOP algorithm to produce IOP estimates that were intended to be much less dependent on corneal biomechanics than the uncorrected CVS-IOP (Joda et al., 2016; Vinciguerra et al., 2016).

This study was designed to determine the effectiveness of the CorVis ST bIOP algorithm in eliminating, or significantly reducing, the effect of biomechanics parameter variation on IOP estimates using ex-vivo human eye globes, in which the IOP was controlled and then measured with the CorVis ST.

2. Material and Methods

2.1. Specimen Preparation

Five ex-vivo human ocular globes (age 69 ± 3 years) were obtained from the Fondazione Banca degli Occhi del Veneto Onlus, Venice, Italy, and tested within 3-5 days post mortem. Ethical approval to use the specimens in research was obtained by the eye bank in accordance with the Declaration of Helsinki. The central corneal thickness (CCT) was measured using a DGH 55 Pachmate pachymeter (DGH Technology, Exton, USA). After removing the extraocular tissues, a G14 needle was inserted through the posterior pole, glued around the insertion point to prevent leakage, and used to remove the vitreous. The inside of the globe was washed with Phosphate Buffered Saline (PBS, P4417, Sigma-Aldrich, Darmstadt, Germany) a few times until a smooth movement of fluid was achieved through the needle and a syringe connected to it. The eye was then injected with a 10% Dextran solution (Sigma-Aldrich, Darmstadt, Germany) to prevent swelling during the experiment, before fitting it inside the test rig. Throughout these steps, the eye was kept moist using Everclear; a viscous tear film supplement (Melleson Pharma, Breda, Netherlands) to prevent drying.

2.2. Test Setup

A custom-built inflation rig was used in the study to control the IOP in ex-vivo eye globes, and measure it with the CorVis ST, Figures 1 and 2. The rig included a support mechanism for the eye to allow it to sit in its natural position with the cornea horizontally facing the CorVis ST while preventing both vertical and horizontal rigid-body motion. Inside the horizontal support, a skin-safe, soft silicone rubber padding (Ecoflex® Series, Smooth-On, Pennsylvania, USA.) was placed to simulate the effect of fatty tissue around the eye.

The needle that had been inserted through the posterior pole was connected to a 4mm diameter tube attached to a syringe pump, which was controlled using bespoke LabVIEW software. The pressure applied through the syringe pump on the inside of the globe was

monitored using an FDW pressure transducer (RDP Electronics, Wolverhampton, UK) fixed at the same horizontal level as the centre of the eye to avoid pressure head differences. The readings of the pressure transducer were assumed to represent the true IOP (IOPt) acting on the eye globe, and since the internal globe contents including the vitreous and crystalline lens had been removed, IOPt represented the pressure acting on all the cornea's and sclera's internal surface.

IOPt was controlled to vary between values that covered the natural variation in IOP seen in ophthalmic practice; 10, 15, 20, 25 and 30 mmHg. These variations were introduced through movement of a stepper motor connected to the syringe pump. After reaching each IOPt level, the eye was allowed to stabilise for 60 seconds before measuring IOP using the CorVis ST, which provided an uncorrected measurement (CVS-IOP) and a biomechanically-corrected measurement (bIOP). CorVis ST measurements, which included CCT, were taken at each IOPt level until at least three readings of acceptable quality were achieved. Acceptable quality was in reference to the CorVis ST built-in standards in assessing a reading, and as part of this assessment, the device could trace and record fully the deformation profiles of the cornea during the application cycle of air puff. At least 120 seconds were allowed between successive CorVis measurements at the same IOPt that should enable the cornea to recover fully from the distortion caused by previous air puffs.

2.3. Statistical Analysis

Statistical analyses were performed on IBM SPSS version 24. The three acceptable CorVis ST readings of bIOP and CVS-IOP were averaged and compared with the true IOP (IOPt) measured with the pressure transducer. After a normality analysis, the one-way ANOVA and Bonferroni Post-Hoc analysis were performed to compare the mean differences followed by Pearson correlations used to assess the association of both bIOP and CVS-IOP with CCT and IOPt. p-values of less than 0.05 were considered indicative of statistical significance.

3. RESULTS

Both uncorrected IOP values (CVS-IOP) and biomechanically-corrected values (bIOP) are presented in Table 1 along with the corresponding true IOP (IOP_t) applied by the syringe pump system. The eye donor's age and the mean and standard deviation of CCT obtained at each pressure level are also included.

The ANOVA test between the three normally distributed groups of bIOP, CVS-IOP and IOP_t showed significant differences ($p < 0.001$) and allowed for Bonferroni post-hoc test to be performed. The average difference for all specimens and all IOP_t levels between CVS-IOP and IOP_t was 7.5 ± 3.2 (1.9 to 13.0) mmHg, while it was 0.3 ± 1.6 (-2.9 to 2.4) mmHg between bIOP and IOP_t. While the difference between CVS-IOP and IOP_t was significant ($p < 0.001$), the difference between bIOP and IOP_t was not significant ($P = 0.989$). The error in CVS-IOP (CVS-IOP – IOP_t) decreased significantly, in percentage values, with higher IOP_t ($p < 0.001$). However, while there was also a reduction in CVS-IOP error, in absolute values, with higher IOP_t, the association of the reduction in error with IOP_t was not significant ($p = 0.617$). On the other hand, no correlation was found for bIOP errors with IOP_t in either percentage ($p = 0.756$) or values ($p = 0.617$). Further, the CVS-IOP error increased significantly with higher CCT (0.0196 mmHg/um, $p < 0.001$), unlike the errors in bIOP, which were smaller and not correlated with CCT (-0.002 mmHg/um, $p = 0.482$).

4. Discussion

Measurement of intraocular pressure (IOP) is of great clinical importance for a number of applications including the management and risk profiling of glaucoma. Several methods have been developed to provide estimates of IOP, most of which rely on a simple concept involving the application of a mechanical force – usually on the cornea – and correlating the

resistance to deformation under this force to the value of IOP. While this technique is simple to apply, it introduces inaccuracies that are difficult to eliminate. It has long been realised that corneal stiffness, which is influenced by the tissue's thickness and material biomechanics, also influences the resistance to deformation under the applied force, and hence can cause changes in the IOP measurement. The difficulty to separate the effects of IOP and tissue biomechanics on the IOP measurement has been the subject of numerous research studies and has not been entirely possible to date. (Brown and Congdon, 2006)

The CorVis ST, a relatively new non-contact tonometer, aims to address this challenge. It acquires detailed cross-sectional profiles of the cornea's anterior and posterior surfaces recorded during the application of the external, dynamic air pressure. This information, along with measurements of thickness over the central corneal region, was used in an earlier study to distinguish between the effects of IOP and corneal biomechanics on corneal deformation, and hence provide an estimate of IOP (bIOP) that is designed to be less dependent on the corneal biomechanical parameters including CCT and age. (Joda et al., 2016; Vinciguerra et al., 2016)

In this study, we are providing an assessment of the bIOP estimates using a direct experimental technique, in which the IOP is controlled in ex-vivo human eyes followed by measuring the pressure using the CorVis ST and providing bIOP estimates. The technique showed that while uncorrected IOP estimates exhibited inaccuracies, and appeared to be influenced by corneal biomechanics, bIOP values were significantly more accurate and closer to the values of true IOP.

For the five ex-vivo eye globes employed in this study, for which age varied little between 67 to 76 years, and CCT varied between 458 to 880 μm , IOP was maintained at specific values between 10 and 30 mmHg in 5 mmHg increments. At each true IOP (IOP_t) level, at least three acceptable-quality IOP readings by the CorVis ST were obtained, along with estimates of bIOP. While the uncorrected CVS-IOP measurements resulted in large and significant errors (7.5 ± 3.2 mmHg, $p < 0.001$), bIOP was closer to IOP_t with small and non-significant

errors of (0.3 ± 1.6 mmHg, $p= 0.989$). Further, the CVS-IOP errors were significantly correlated with, and possibly caused by, the increases in CCT beyond average values ($p< 0.001$). This observation was not repeated with bIOP where the small errors were not correlated with CCT ($p= 0.482$) (McCafferty et al., 2017).

These results, although based on a limited number of human globes, are promising when compared to previous studies, in which either Perkins or Goldmann tonometers were employed and exhibited significant errors ranging from a mean of 1.7 ± 1.8 to 5.2 ± 1.6 mmHg (Kniestedt et al., 2004; McCafferty et al., 2017; Riva et al., 2012). Conversely, when considering DCT readings, the published literature shows inconsistent results with reports showing minimal differences compared to true IOP [0.58 ± 0.70 mmHg¹⁸ or 0.50 mmHg (95% CI= $0.40-0.60$) (Kniestedt et al., 2005b)] and another showing higher error values (2.3 ± 2.4 mmHg). (Riva et al., 2012) On the other hand, to the authors knowledge there is no published work on the evaluation of ORA IOPcc versus true IOP.

The lack of comparison of bIOP with GAT, DCT and IOPcc could be considered a limitation of the present study. A further limitation of this study has been the difficulty in obtaining human donor eyes with a wider range of age, which would have allowed consideration of the effects of age, and subsequently tissue stiffness, on both uncorrected and corrected IOP estimates. Further, although the specimens were tested soon post-mortem and kept hydrated during shipping, preparation and testing phases, it is possible that they experienced changes in mechanical properties from their in-vivo state, which in turn could have affected especially the uncorrected CVS-IOP measurements. This was particularly evident in specimen S4 where there was a growth of >60 microns in CCT over the last stage of the test, likely due to swelling. Another limitation was caused by the lack of availability of donor keratoconic eyes, which meant that the effect of geometry and biomechanical abnormalities could not be studied. Furthermore, while the study demonstrated lack of correlation of bIOP with CCT across measurements at different IOPt levels, it was not

possible to repeat measurements at the same IOPt while tissue hydration was allowed to vary – causing changes in CCT – due to the long time this process would take.

In conclusion, our study provides further evidence of the capability of the bIOP algorithm in providing a close estimate of the true IOP in the range of 10 to 30 mmHg in healthy eyes and the reduction in the association with the cornea's stiffness parameters, most notably the thickness. Based on this and the previously published studies, the bIOP may provide a possible solution to the long-standing challenge of offering IOP estimates that are significantly less affected by corneal biomechanics than other, commonly used, tonometry methods.

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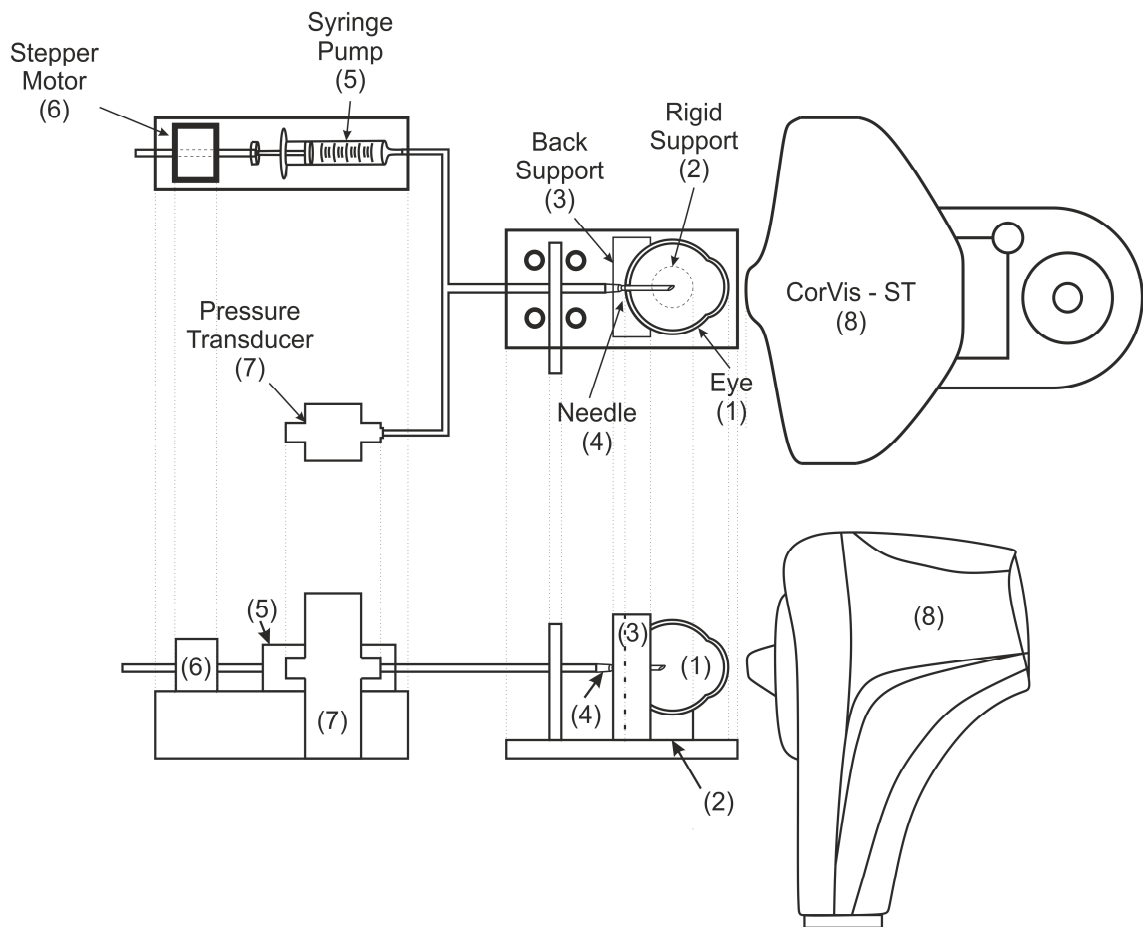


Figure 1 Schematic view of the inflation test rig showing an ex-vivo eye [1] sitting on a rigid support [2], which provided restraint against vertical motion, and a soft back support [3] that provided flexible restraint against horizontal motion. The eye has a G14 needle [4] inserted through the posterior pole and connected to a syringe pump [5] to control the intraocular pressure using a stepper motor [6]. The needle is also connected to a pressure transducer [7] to measure the pressure inside the eye. The CorVis ST [8] is used to provide estimates of IOP through the application of an air puff.



Figure 2 Test rig showing the eye sitting on a rigid support and supported from the back while being connected to a syringe pump that controls its IOP. CorVis ST is placed at a distance to enable its automatic trigger.

Table 1 IOP measurements including uncorrected CVS-IOP and biomechanically-corrected bIOP values at controlled true IOP values between 10 and 30 . Results include mean, standard deviation and range.

Specimen	Age (years)	CCT (microns)	IOP _t (mmHg)	CVS-IOP			bIOP		
				IOP (mmHg)	CVS-IOP – IOP _t (mmHg)	% Error	IOP (mmHg)	bIOP – IOP _t (mmHg)	% Error
S1	67	465±6 (458-469)	10	14.7±0.3 (14.5-15.0)	4.7	47%	12.1±0.1 (11.9-12.2)	2.1	21%
		488±13 (476-507)	15	19.3±1.0 (18.0-20.5)	4.3	29%	16.4±0.8 (15.5-17.3)	1.4	9%
		493±2 (492-496)	20	23.5±0.0 (23.5-23.5)	3.5	18%	20.5±0.1 (20.4-20.6)	0.5	3%
		498±1 (496-499)	25	28.0±0.4 (27.5-28.5)	3.0	12%	25.1±0.6 (24.3-25.6)	0.1	0%
		487±6 (477-494)	30	31.9±1.0 (31.0-33.0)	1.9	6%	29.7±0.9 (28.7-30.7)	-0.3	-1%
S2	67	618±22 (594-639)	15	25.5±0.9 (24.5-26.5)	10.5	70%	17.3±1.2 (16.0-18.2)	2.3	15%
		619±1 (618-620)	20	30.5±0.5 (30.0-31.0)	10.5	53%	22.2±0.3 (22.0-22.6)	2.2	11%
		621±9 (613-632)	25	36.2±0.3 (36.0-36.5)	11.2	45%	27.4±0.5 (26.8-27.7)	2.4	10%
		624±2 (622-627)	30	41.3±0.6 (41.0-42.0)	11.3	38%	32.2±0.6 (31.7-32.8)	2.2	7%
S3	76	607±10 (597-618)	10	17.0±0.5 (16.5-17.5)	7.0	70%	9.5±0.9 (8.7-10.5)	-0.5	-5%
		599±18 (584-619)	15	21.8±0.8 (21.0-22.5)	6.8	45%	14.4±0.4 (14.1-14.8)	-0.6	-4%
		594±4 (590-598)	20	27.2±0.3 (27.0-27.5)	7.2	36%	19.4±0.3 (19.1-19.7)	-0.6	-3%
		603±1 (602-604)	25	31.2±1.0 (30.0-32.0)	6.2	25%	23.0±1.0 (21.9-23.7)	-2.0	-8%
S4	68	829±68 (750-870)	10	16.3±0.6 (16.0-17.0)	6.3	63%	7.1±1.2 (5.8-8.3)	-2.9	-29%
		808±3 (805-810)	15	26.9±0.5 (26.5-27.5)	11.9	79%	16.0±0.5 (15.6-16.8)	1.0	7%
		834±8 (828-840)	20	32.0±1.2 (30.0-33.0)	12.0	60%	21.0±1.1 (19.0-22.0)	1.0	5%
		808±3 (805-810)	25	38.0±1.3 (36.5-39.5)	13.0	52%	26.6±1.1 (25.2-28.0)	1.6	6%
		870±14 (860-880)	30	40.6±0.8 (40.0-41.5)	10.6	35%	29.1±0.8 (28.4-29.9)	-0.9	-3%
S5	67	553±6 (548-557)	10	15.8±1.1 (15.0-16.5)	5.8	58%	10.6±1.1 (9.8-11.4)	0.6	6%
		576±5 (572-584)	15	20.9±1.4 (19.5-22.5)	5.9	39%	14.9±1.2 (13.8-16.2)	-0.1	-1%
		582±15 (565-593)	20	27.5±0.5 (27.0-28.0)	7.5	38%	21.0±0.9 (20.3-22.0)	1.0	5%
		603±4 (599-608)	25	31.5±0.5 (31.0-32.0)	6.5	26%	24.0±0.4 (23.7-24.5)	-1.0	-4%
		605±12 (596-624)	30	35.0±1.7 (33.0-37.0)	5.0	17%	27.1±1.3 (25.4-28.1)	-2.9	-10%

Notes:

(1) IOP_t = manometric, true intraocular pressure, CCT = central corneal thickness, CVS-IOP = uncorrected intraocular pressure measurement by the CorVis ST, bIOP = biomechanically-corrected intraocular pressure measurement

(2) The pressure at 30 mmHg for S3 and the pressure at 10 mmHg for S2 is excluded from the table. These measurements could not be obtained using the device's automatic triggering mechanism despite repeated attempts.

Highlights:

- The bIOP algorithm proved to be able to compensate for variations in thickness and material stiffness.
- Application of bIOP on ex-vivo human donor globes showed no significant differences in mean values when compared to true IOP.
- CorVis IOP was found to be significantly correlated with thickness and the IOP estimates error was large in comparison to true IOP.