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Comparative intraocular pressure measurements using three different rebound tonometers through in an ex vivo analysis and clinical trials in canine eyes

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Abstract

Objective: To evaluate the clinical relevance of intraocular pressure (IOP) measured with three different rebound tonometers in an ex vivo analysis and clinical trials in dogs.

Animals and procedures: Ex vivo analysis and clinical trials were performed separately. For the ex vivo analysis, eight enucleated eyes were obtained from four Beagle dogs. IOP values measured with TONOVET® (TV-IOP), TONOVET-Plus® (TVP-IOP), and SW-500® (SW-IOP) were compared with manometric IOPs. For clinical trials, each tonometer was evaluated separately, depending on whether TVP-IOP was higher or lower than 14 mm Hg. One-way repeatedmeasures analysis of variance, simple linear regression analysis, and Bland-Altman plots were used for statistical analyses.

Results: In ex vivo analysis, TV-IOP and TVP-IOP were not significantly different from manometric IOP. However, SW-IOP underestimated IOP compared to manometry. Higher discrepancy was observed in TV-IOP and SW-IOP with an increase in manometric IOP. In clinical trials, no significant difference was observed between TV-IOP (9.73 ± 2.92) and TVP-IOP (11.36 ± 2.23) when TVP-IOP was lower than 14 mm Hg, but SW-IOP (8.70 ± 3.03) was significantly lower than TVP-IOP. TV-IOP (15.96 ± 6.47) and SW-IOP (13.09 ± 3.72) were significantly lower than TVP-IOP (20.08 ± 6.60) when the IOP was higher than 14 mm Hg of TVP-IOP.

Conclusions: This study demonstrates that the TONOVET® and TONOVET-Plus® provide a useful approach for ex vivo analysis. In clinical trials, results of TV-IOP and SW-IOP were significantly lower than of TVP-IOP when IOP was higher than 14 mm Hg of TVP-IOP. The characteristics of rebound tonometers should be considered in clinical settings.

KEYWORDS

cornea, direct manometer, dogs, glaucoma, intraocular pressure, rebound tonometer

1 | INTRODUCTION

Measurement of intraocular pressure (IOP) is essential for the diagnosis and monitoring of ocular diseases.[1] A direct manometer is the most accurate device to measure IOP.[2] However, this technique is invasive and hence inappropriate for clinical use. In clinical settings, noninvasive tonometers such as indentation tonometer, applanation tonometer, and rebound tonometer have been commonly used.[3-5]

A rebound tonometer allows IOP measurement using a light aluminum probe, which is electro-magnetically propelled against the corneal surface.[6] This tonometer offers several advantages over others and could be used without topical anesthesia, as the procedure is not painful.[7] Furthermore, the diameter of the probe head is small, allowing selective IOP measurement on the normal cornea of patients with focal corneal lesions.[8] Replaceable tips may prevent cross-infection among patients.[6] For these reasons, the rebound tonometer is becoming popular in veterinary ophthalmology, and the accuracy of this method has been widely studied.[1,9-11]

TONOVET® (Icare Finland Oy, Helsinki, Finland) is a widely used tonometer; however, new rebound tonometers such as TONOVET-Plus® (Icare Finland Oy, Helsinki, Finland) and SW-500® (Pioway, Nanjing, China) have been recently introduced in veterinary ophthalmology. These newly introduced tonometers need to be calibrated for each species.[12-15] Different species have distinct anatomical structures, including tear film, corneal thickness, curvature, and rigidity,[14,16-18] which could affect tonometry readings. To our knowledge, differences between readings from the TONOVET-Plus®, TONOVET®, and SW-500® have not been determined as these devices have been rarely studied in veterinary ophthalmology. Therefore, we aimed to compare the IOP readings measured with the three different rebound tonometers, TONOVET® (TV-IOP), TONOVET-Plus® (TVP-IOP), and SW-500® (SW-IOP), in an ex vivo analysis and clinical trials.

2 | MATERIALS AND METHODS

2.1 | Ex vivo analysis

2.1.1 | Preparation of enucleated canine eyes

Eight enucleated eyes were obtained from four adult Beagle dogs (5-7 years old, 6-10 kg). The beagles were euthanized for reasons unrelated to this study. All eyes were determined to normal by routine ophthalmic examinations before euthanasia. Each enucleated eye was stored in 0.9% normal saline at room temperature, and all IOPs were measured within 1 hours after enucleation. Each enucleated eye was fixed on a polystyrene bed using pins with the cornea facing horizontally. This study was approved by the guidelines of the Institutional Animal Care and Use Committee of Seoul National University (SNU-190218-1).

2.1.2 | IOP measurements

All IOP measurements were carried out at the central cornea by one examiner (JS). Each fixed eye was frequently moistened with 0.9% normal saline. The anterior chamber was cannulated with 26 G needles through the peripheral cornea, 1 mm from the limbus, at 3 and 9 o'clock position. Each needle was secured and was prevented from pullout by applying one drop of a tissue adhesive (Vetbond®, 3M). During the experiment, leakage was not observed. Corneal curvature was unaffected by needle insertion. One needle was connected to a pressure transducer, while the other was connected to a polyethylene tube. The pressure transducer was connected to a monitor (GE Healthcare, Helsinki, Finland), which showed real-time IOP. The polyethylene tube was hung on a height-adjustable pole to modulate IOP. The IOP values were measured by three rebound tonometers at a tube height from 5 to 80 mm Hg. All IOP measurements were obtained using instruments in the same order by the same examiner (JS) in a consistent environment. The "d" mode of the TONOVET® and TONOVET-Plus® was used for every measurement. Triplicate values of the rebound tonometer were averaged and recorded. Only values with standard deviation (SD) of 5% or lower were recorded. The IOP was measured at 5 mm Hg intervals from 5 to 40 mm Hg and 10 mm Hg intervals from 40 to 80 mm Hg. The pressure transducer was calibrated using a mercury manometer before measurement.

2.1.3 | Clinical trials

In total, 106 eyes of 53 client-owned dogs were evaluated in this study. All dogs were presented at the Seoul National University Veterinary Medical Teaching Hospital for various ophthalmic diseases. Approval of each owner was obtained prior to the examination. The study was conformed to the statement of the Association of research in Vision and Ophthalmology (ARVO) for the Use of Animals in Ophthalmic and Vision Research. The IOP values were measured with TONOVET®, TONOVET-Plus®, and SW-500® in a fixed sequence. The tonometer was held perpendicular to the cornea. Each measurement was performed when the animal was stable enough to minimize any effect on IOPs. The animals were restrained in a sitting position. The IOP readings were not recorded if the animal was tensed

or needed excess restraint. Exclusion criteria for this study consisted of phthisis bulbi, previous intraocular prosthesis surgery, and distinct corneal morphologic changes (eg, pigmentation, vascularization, edema, scarring, corneal deposit, corneal ulcer, and others) that could reduce the reliability of measurement and make comparative measurement impossible on the same eye. Dogs with glaucoma were contained in the study if the patient's cornea did not manifest morphologic changes.

2.2 | Statistical analyses

For ex vivo analysis, IOPs measured with three rebound tonometers were compared to manometric IOP. Measured IOPs were expressed as mean ± SD. One-way repeated measures analysis of variances (ANOVA) and post hoc Tukey's honestly significant difference (HSD) were performed to compare the mean values of rebound tonometer IOPs and manometric IOP. Correlations between each rebound tonometer and direct manometer were detected with a simple linear regression analysis.

In clinical trials, TV-IOP and SW-IOP were compared to TVP-IOP. According to whether TVP-IOP was higher than 14 mm Hg (≥14 mm Hg) or lower than 14 mm Hg (<14 mm Hg), the three tonometers were assessed separately. ANOVA and post hoc Tukey's HSD were used to compare the mean IOPs between the rebound tonometers. A simple linear regression analysis was carried out to calculate correlations between TV-IOP and TVP-IOP and between SW-IOP and TVP-IOP. Bland-Altman plot was constructed to evaluate bias (mean difference) and limit of agreement (LoA) between TV-IOP and TVP-IOP and between SW-IOP and TVP-IOP.

Statistical analyses were performed using SPSS 25 (SPSS Inc) and MedCalc 14.8.1.0 program (MedCalc Software). Correlation coefficient (R^2) values higher than 0.700 indicated strong correlations, and $P < .05$ was considered statistically significant.[19]

TABLE 1 The intraocular pressure (IOP) values measured by TVP, TV, and SW at each manometric IOP reading in ex vivo analysis (n = 8)

Manometric IOP (mm Hg)	TVP-IOP	TV-IOP	SW-IOP
5	7.00 ± 1.14*	3.20 ± 0.72	3.45 ± 0.88
10	11.33 ± 1.16	7.25 ± 1.22	7.50 ± 1.02
15	15.41 ± 1.86	11.50 ± 2.18	10.79 ± 1.25
20	20.33 ± 2.49	15.12 ± 2.15	13.04 ± 1.08
25	25.79 ± 3.69	20.12 ± 2.57	14.20 ± 1.06
30	31.37 ± 3.26	24.91 ± 2.22	18.25 ± 2.19
35	35.66 ± 2.54	30.04 ± 2.23	22.37 ± 3.00
40	40.54 ± 2.26	35.58 ± 2.78	26.37 ± 2.76
50	51.54 ± 4.23	45.25 ± 2.90	33.04 ± 2.47
60	60.95 ± 2.97	54.75 ± 3.35	37.83 ± 3.76
70	72.41 ± 5.71	62.54 ± 2.26	42.79 ± 3.13
80	84.95 ± 6.61	70.20 ± 3.63	46.95 ± 2.40

Abbreviations: TV, TONOVET®; TVP, TONOVET-Plus®; SW, SW-500®.
*Mean ± SD (mm Hg).

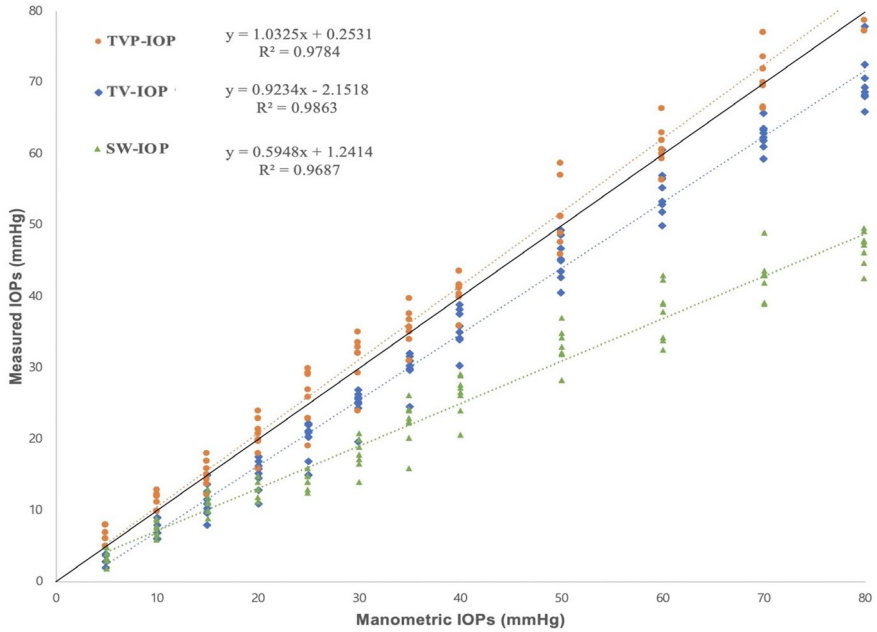

FIGURE 1 Correlation between intraocular pressures by using direct manometer and each of TONOVET-Plus® (●), TONOVET® (♦), and SW-500® (▲) in an ex vivo analysis. The solid black line represents the ideal regression line (manometric IOPs)

TABLE 2 The intraocular pressure (IOP) measured by TV, TVP, and SW in clinical trials (n = 106)

	Lower than 14 mm Hg of TVP-IOP			Higher than 14 mm Hg of TVP-IOP		
Tonometer	**Mean ± SD**	***P* value**	**Mean differences**	**Mean ± SD**	***P* value**	**Mean differences**
TVP-IOP	11.36 ± 2.23	-	-	20.08 ± 6.60	-	-
TV-IOP	9.73 ± 2.92	$P = .298$	1.63	15.96 ± 6.47	*	4.12
SW-IOP	8.72 ± 3.03	$P = .048$*	2.64	13.09 ± 3.72	*	6.99

Abbreviatrions: TV, TONOVET®; TVP, TONOVET-Plus®; SW, SW-500®.

*Significant difference between the two groups. *P* values were generated by the ANOVA test with post hoc analysis and adjusted by Tukey HSD in comparison with TVP.

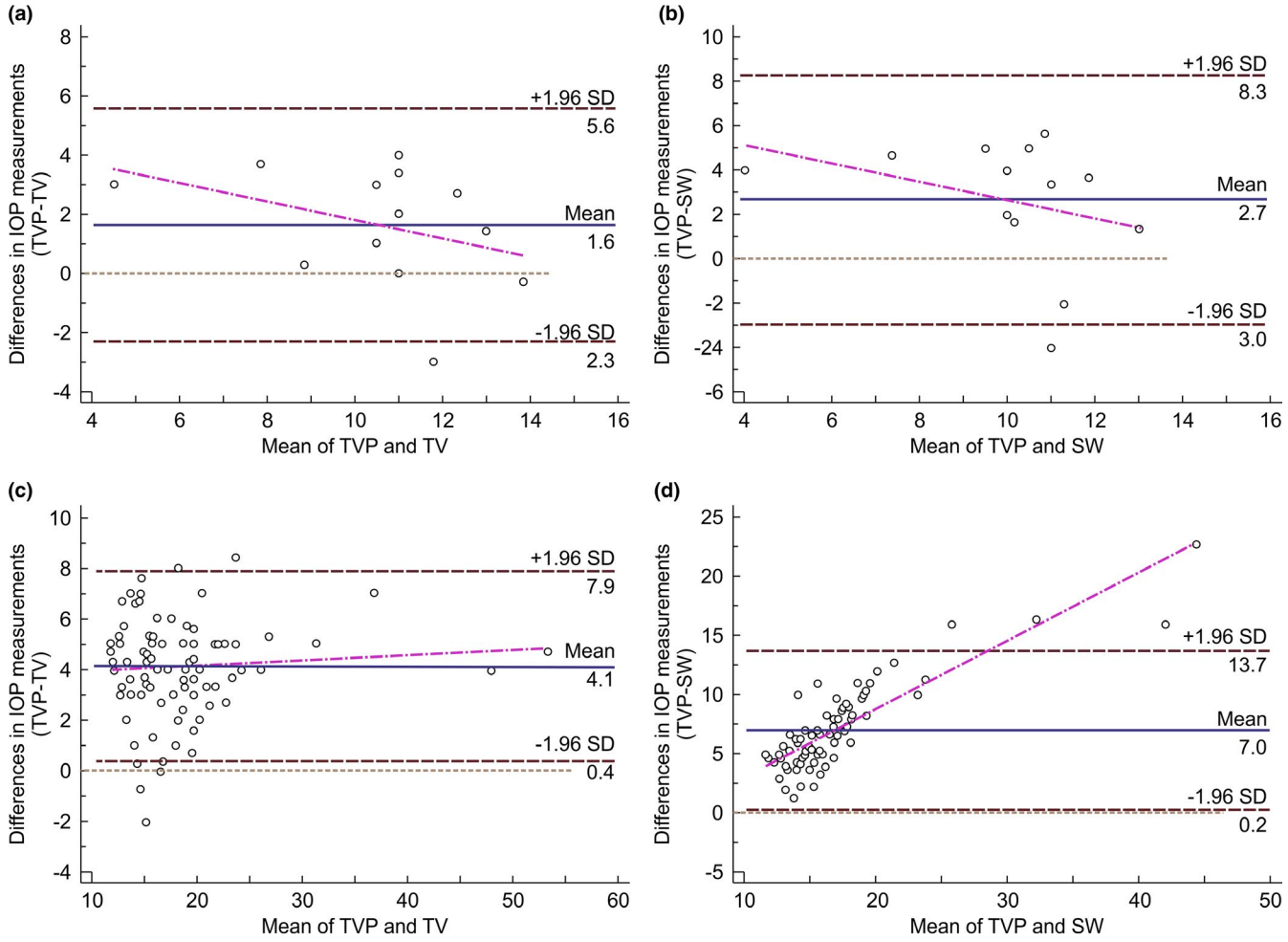

FIGURE 2 Bland-Altman results of clinical trials. Bland-Altman plots demonstrating the bias in intraocular pressure (IOP) values between TONOVET® and TONOVET-Plus® (A, C) and SW-500® and TONOVET-Plus® (B, D). The dash-dotted line in the center represents the regression line. The dotted line indicates the line of equality. The dashed lines on the side represent the upper and lower limits of agreements (LoAs)

3 | RESULTS

3.1 | Ex vivo analysis

The recorded IOPs for all three rebound tonometers at a fixed manometric IOP were shown in Table 1. Data from the ANOVA showed no significant difference between TV-IOP ($P = .36$) as well as TVP-IOP ($P = .96$) and manometric IOP. However, SW-IOP ($P = .001$) was significantly lower than the manometric IOP.

Simple linear regression analysis revealed the strong R^2 value between each of the three tonometer measurements and manometric IOP (TVP: $R^2 = 0.9784$,TV: $R^2 = .9863$, SW: $R^2 = .9687$, $P < .001$). Regression equations were calculated

based on the measured values of three rebound tonometers and the direct manometer as follows: TVP-IOP = 1.0325 × manometric IOP + 0.2531; TV-IOP = 0.9234 × manometric IOP − 2.1518; and SW-IOP = 0.5948 × manometric IOP + 1.2414 (Figure 1).

3.1.1 | Clinical trials

The mean ± SD of IOP across all patients was 19.01 ± 6.86 with TONOVET-Plus®, 15.20 ± 6.47 mm Hg with TONOVET®, and 12.55 ± 3.91 with SW-500®. The measured IOPs with TONOVET® ranged from 3 to 51 mm Hg (median: 14.0), while those with TONOVET-Plus® ranged from 6 to 55.7 mm Hg (median: 18.0). The IOPs measured with SW-500® were in the range of 2-34 mm Hg (median: 12.3). In clinical trials, three rebound tonometers were evaluated separately, according to the range of TVP-IOP being either higher (n = 93) or lower (n = 13) than 14 mm Hg (Table 2). Under this criterion, the tonometers from each range showed a different tendency.

When TVP-IOP was lower than 14 mm Hg, the mean ± SD was 11.36 ± 2.23 mm Hg (range: 6.0 to 13.7, median: 12.0), 9.73 ± 2.92 mm Hg (range: 3.0 to 14.0, median: 10.0), and 8.72 ± 3.03 mm Hg (range: 2.0 to 13.0, median: 9.0) for TVP-IOP, TV-IOP, and SW-IOP, respectively. No significant difference was observed between TV-IOP and TVP-IOP ($P = .29$). However, the value reported for SW-IOP was significantly lower than that reported for TVP-IOP ($P = .048$). The mean difference between TV-IOP and TVP-IOP was 1.6 mm Hg (LoA, −2.3 to 5.6 mm Hg, Figure 2A), and that between SW-IOP and TVP-IOP was 2.7 mm Hg (LoA, −3.0 to 8.3 mm Hg, Figure 2B). Ten of 13 (77%) TV-IOPs and four of 13 (30%) SW-IOPs were within 3 mm Hg of TVP-IOP.

When TVP-IOP was higher than 14 mm Hg, the mean ± SD of TVP-IOP, TV-IOP, and SW-IOP were 20.08 ± 6.60 mm Hg (range: 14.0-55.7, median: 18.3), 15.96 ± 6.47 mm Hg (range: 9.0-51.0, median: 15.0), and 13.09 ± 3.72 mm Hg (range: 9-34.0, median: 13.0), respectively. Both TV-IOP and SW-IOP were significantly lower than TVP-IOP. The mean difference between TV-IOP and TVP-IOP was 4.1 mm Hg (LoA, 0.4-7.9 mm Hg, Figure 2C), while that between SW-IOP and TVP-IOP was 7.0 mm Hg (LoA, 0.2-13.7 mm Hg, Figure 2D). Twenty-five of 93 (26%) TV-IOP and seven of 93 (7%) SW-IOP were within 3 mm Hg of TVP-IOP.

A simple linear regression analysis revealed the strong correlation between TV-IOP and TVP-IOP ($R^2 = .9077$) and SW-IOP and TVP-IOP ($R^2 = .8333$). Regression equations calculated by comparing TV-IOP and SW-IOP to TVP-IOP were as follows: TV-IOP = 0.8992 × TVP-IOP – 1.8951 and SW-IOP = 0.5209 × TVP-IOP + 2.649 (Figure 3).

4 | DISCUSSION

Accurate measurement of IOP is essential for the diagnosis and monitoring of ocular diseases.[1] Three measurement methods, including indentation, applanation, and rebound tonometers, have been used in veterinary medicine to determine IOP. Of these, the rebound tonometer TONOVET® has been widely used among veterinarians.[6,10,20-25] Validation of this device has been reported not only for dogs but also for mice, monkeys, pigeons, birds of preys, and rabbits.[12,15,26-28] Other rebound tonometers such as TONOVET-Plus® and SW-500® have been recently used for laboratory animals.[29,30] IOP values measured with the rebound tonometer could be affected by differences in the biomechanical properties of the cornea between species[16,17,31]; therefore, studies are being conducted to verify the accuracy of these instruments in each species. Here, the comparison of the performance of the newly introduced rebound tonometers and TONOVET®

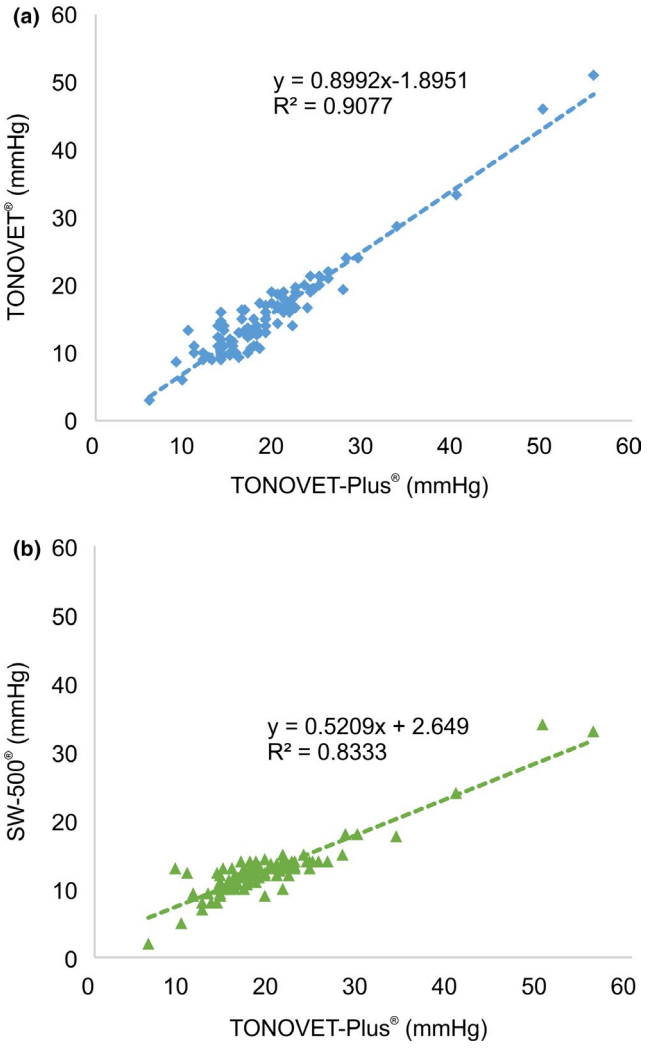

FIGURE 3 Simple linear correlation analyses of intraocular pressure measurements between TONOVET® and TONOVET-Plus® (A) and SW-500® and TONOVET-Plus® (B)

provides important information relevant to canines with ophthalmic issues.

In the present study, all the three rebound tonometers were well tolerated by 53 dogs and did not induce any discomfort and corneal lesions after measurements. Previous studies reported high levels of precision for IOP measurement using TONOVET®; however, results of the regression analysis showed that TV-IOP was lower than manometric IOP in enucleated canine eyes.[11,24,25] A study by Tofflemire et al showed that the mean difference between TV-IOP and manometric IOP was approximately 5 mm Hg.[25] In the present investigation, TV-IOP and TVP-IOP showed a strong correlation compared with the manometric IOP, with no significant difference. Although a significant difference was not achieved between TV-IOP and manometric IOP, TV-IOP was consistently lower than manometric IOP, and the discrepancy was greater with an increase in manometric IOP. This result was in agreement with those from previous reports, wherein the accuracy and reproducibility of TONOVET® reduced with an increase in manometric IOP.[10,24,32] TVP-IOP was the closest to manometric IOP among the analyzed devices and showed less discrepancy despite the increase in manometric IOP.

In clinical trials, IOP readings obtained with the TONOVET® and SW-500® varied depending on if the IOP threshold was above or below 14 mm Hg of TVP-IOP. When TVP-IOP was higher than 14 mm Hg, different results among the analyzed tonometers were detected when compared to the condition of TVP-IOP lower than 14 mm Hg. When TVP-IOP was lower than 14 mm Hg, TV-IOP and TVP-IOP showed an appropriate agreement. The difference between TV-IOP and TVP-IOP was within 3 mm Hg in 77% of cases. The recommended acceptable limit under clinical settings is less than 3 mm Hg.[33,34] The Bland-Altman plot reveals good agreement between the two rebound tonometers.[35,36] In previous human studies, a Goldmann applanation tonometer was used as the "gold standard" method for evaluation of agreement and comparison between two tonometers.[7,16] The LoA between Goldmann applanation tonometer and iCare® (Tiolat Oy, Helsinki, Finland) ranged from −3.7 to 7.3 mm Hg, while that between Goldmann applanation tonometer and Tono-Pen XL® (Bio-Rad Inc) ranged from −3.0 to 8.0 mm Hg. The conclusions of the two aforementioned studies were that the Goldmann applanation tonometer and each of two other tonometers were deemed clinically useful. In the present study, the LoA between TV-IOP and TVP-IOP varied from − 2.3 to 5.6 mm Hg.[34,37] Therefore, the LoA between TONOVET® and TONOVET-Plus® was considered sufficiently narrow for the situation when IOP was lower than 14 mm Hg of TVP-IOP, and these two devices may serve as good alternatives to each other.

Contrary to the result of TVP-IOP < 14 mm Hg, TONOVET® and TONOVET-Plus® could not be interchanged when IOP was higher than 14 mm Hg of TVP-IOP. The line of equality was not within the LoA, suggesting that TV-IOP was significantly lower than TVP-IOP.[36] In addition, the difference between TV-IOP and TVP-IOP was within 3 mm Hg in 26% of cases. As it was observed from 14 mm Hg of TVP-IOP statistically significant, 14 mm Hg was set as a cut-off value. Though the value is not clinically implicated in dogs, IOP should be carefully interpreted even in normal range of IOP with TONOVET® and SW-500®.

Here, we found that SW-IOP was lower than the manometric IOP and that the LoAs between SW-IOP and TVP-IOP were too wide to meet the clinical agreement. SW-500® was initially developed for humans[38] and should be recalibrated for applications in canines. However, the correlation coefficients between SW-IOP and manometric IOP, as well as between SW-IOP and TVP-IOP, were high. Therefore, recalibration of SW-500® using the regression equations established herein could compensate for errors in IOP reading in dogs using this device.

This study has some limitations. First, the clinical study was performed in canines presented at the animal hospital with various ocular diseases, which could affect the IOP. Although exclusion criteria were applied, most animals had minor intraocular diseases or a small lesion in the cornea. In a previous study, TV-IOP showed differences depending on the corneal pathology, with a mean difference of 2.1 mm Hg.[8] TONOVET-Plus® and SW-500® have not been well investigated in canines with abnormal corneas, thereby warranting additional studies. However, the results of the present study are still relevant, since most presented animals had various ophthalmic diseases, and their IOPs were measured with rebound tonometers. Second, the patients could be stressed and tensed in unfamiliar circumstances, affecting the IOP. To minimize stress, all measurements were carried out by the same examiner with an experienced assistant, and only steadily presented values were recorded. Third, this study was conducted only in canine species, and future studies could be performed in other animal species. In particular, TV-IOP tends to be slightly higher than manometric IOP (2 to 3 mm Hg) in cats.[22,31] As "cat mode" has been added to TONOVET-Plus®, further studies would be meaningful. Fourth, the central corneal thickness (CCT) was not evaluated, although CCT could affect IOP measurements.[17,39] Park et al revealed that an increase of every 100 μm in CCT leads to a 2 mm Hg elevation in TV-IOP.[39] Therefore, the correlation between CCT and IOP readings should be evaluated with the newly introduced tonometers.

In conclusion, both TONOVET® and TONOVET-Plus® readings presented highly accurate IOP readings in ex vivo analysis. SW-500® should be recalibrated for application in dogs. Clinical results showed that when TVP-IOP was higher than 14 mm Hg, TV-IOP and SW-IOP were significantly lower than TVP-IOP. Based on the above results, TONOVET-Plus® seemed to be appropriate choice for measuring IOP in

dogs, as it provides the most accurate estimates of IOP in both normal and glaucomatous eyes.

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CONFLICT OF INTEREST

None.

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