Analysis of Impact of Humidity and Temperature on Excimer Laser Ablation of Polyethylene Terephthalate, Polymethylmethacrylate, and Porcine Corneal Tissue

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Background and Objectives: To analyze the impact of humidity and temperature on excimer laser ablation of polyethylene terephthalate (PET), polymethylmethacrylate (PMMA) and porcine corneal tissue, and an ablation model to compensate for the temperature and humidity changes on ablation efficiency.

Study Design/Materials and Methods: The study was conducted using an AMARIS 1050RS (Schwind eye-tech-solutions) placed inside a climate chamber at ACTS. Ablations were performed on PET, PMMA, and porcine cornea. The impact of a wide range of temperature (~18°C to ~30°C) and relative humidity (~25% to ~80%) on laser ablation outcomes was tested using nine climate test settings. For porcine eyes, change in defocus was calculated from the difference of post-ablation to pre-ablation average keratometry readings. Laser scanning deflectometry was performed to measure refractive change achieved in PMMA. Multiple linear regression was performed using the least square method with predictive factors: temperature, relative humidity, time stamp. Influence of climate settings was modeled for pulse energy, pulse fluence, ablation efficiency on PMMA and porcine cornea tissue.

Results: Temperature changes did not affect laser pulse energy, pulse fluence (PET), and ablation efficiency (on PMMA or porcine corneal tissue) significantly. Changes in relative humidity were critical and significantly affected laser pulse energy, high fluence and low fluence. The opposite trend was observed between the ablation performance on PMMA and porcine cornea.

Conclusions: The proposed well-fitting multi-linear model can be utilized for compensation of temperature and humidity changes on ablation efficiency. Based on this model, a working window for optimum operation has been found (temperature 18°C to 28°C and relative humidity 25% to 65%) for a maximum deviation of ±2.5% in ablation efficiency in PMMA and porcine corneal tissue. Lasers Surg. Med. © 2019 The Authors. *Lasers in Surgery and Medicine* Published by Wiley Periodicals, Inc.

Key words: impact of humidity and temperature; excimer laser ablation; PMMA; cornea; PET; refractive surgery

INTRODUCTION

Laser-based refractive surgery techniques incorporate sophisticated calculations and compensation of several variable factors involved in the entire process, with the global aim of optimizing surgery outcomes in terms of visual acuity, contrast sensitivity, and night vision. It involves application of laser pulses on the corneal tissue, with the procedures being performed in Operation

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Theaters, where a temperature and humidity controlled environment is maintained (~20°C and ~60% relative humidity [1]). However, despite the climatic controls, the varying degree of corneal hydration acts as one of the contributing factors that may affect excimer laser corneal ablation rates and clinical outcomes [2,3]. Means like air conditioners, temperature and humidity monitors are available and used in the clinics, to generate measurement data that is used to react if the room conditions vary largely. Luger et al. [4] analyzed the effect of seasonal changes in residual refraction in 5740 consecutive treatments, one year after corneal refractive surgery using the SCHWIND AMARIS laser system. In their results, treatments performed in spring and summer showed relative undercorrections of the SE (−0.04D), whereas treatments performed in winter showed relative overcorrections of the SE (+0.10D).

Dehydration of the cornea begins as soon as the blinking is prevented (e.g., with use of lid speculum) [5]. Changing corneal hydration affects laser ablation efficiency, which could influence the accuracy of correction [1,2]. Online optical coherence pachymetry reveals that ambient temperature and humidity levels intraoperatively do not influence the outcome. Yet, basic structural characteristics of patients along with both change in refractive index and corneal shrinkage (because of corneal dehydration) are associated with differences in pachymetry during the procedure [6]. In another study, dehydration of the human cornea after insertion of a lid speculum showed corneal thinning with a rate of 0.19 μm/s. [7]

As demonstrated in bovine eye experiments, significant changes in corneal hydration are realized under different drying conditions and treatment methodologies [8], and significant dehydration of the cornea before ablation might lead to relative overcorrections of myopia [9].

With refractive surgery procedures being performed worldwide, laser systems need to operate in different geographical locations with highly variable climatic characteristics. Although, Operation Theater conditions are relatively standardized, local climatic conditions may still influence the working environment of the laser system itself. These factors are highly important for both the laser ablation rates and hence post-ablation outcomes. Previous attempts have analyzed these factors but at a single test location and under controlled climatic conditions that may vary minutely compared with the standard climatic conditions at different geographic locations. Plastic models like polymethylmethacrylate (PMMA) are readily used for calibration of the laser system, but PMMA ablation may also be equally affected by the variations in temperature and humidity [10].

Our aim with this work is to quantitatively analyze the impact of a wide range of temperature (~18°C to ~30°C) and humidity (~25% to ~80% relative humidity) conditions on excimer laser ablation polyethylene terephthalate (PET), PMMA, and *ex-vivo* porcine cornea. Furthermore, the objective is to develop and propose an ablation model that can be utilized for compensation of temperature and humidity changes on ablation efficiency. As a secondary aim, we examine whether PMMA calibration may compensate for the variations in the climatic conditions, to limit their possible influence in clinical outcomes.

MATERIAL AND METHODS

All the equipment used for the tests, including the SCHWIND AMARIS 1050RS (Schwind eye-tech-solutions, Kleinostheim, Germany) were placed inside a climate chamber (Weiss Umwelttechnik GmbH, type "315"/SD 40… 120DU – S' at ACTS, Magna in Sailauf, Germany). The various tests were conducted at different climate (temperature and relative humidity) settings. The porcine eyes were procured from a local slaughterhouse in the early morning on each day, and transported to the test location. The pigs were euthanized less than 4 hours before starting the tests; however, they were euthanized for the commercial purposes of the slaughterhouse and were not sacrificed for the tests mentioned in this work. The porcine eyes were stored in glucose solution (1 L saline solution + 19 ml of DMEM Low glucose solution from PAA Laboratories GmbH [Cölbe, Germany]) at room temperature (~23°C), during transportation. At the test location, the eyes were stored in refrigeration, until being ablated with an excimer laser. The eyes showing unwanted characteristics like tears and abrasions in the epithelium, non-transparent cornea and stale smell were removed from the cohort.

A schematic representation of the workflow involving various steps followed in the methods is presented in Figure 1.

The various steps shown in the workflow (Fig. 1) are elaborated as follows.

Climate Chamber Setting (Constitutes "a" From the Workflow)

The temperature and relative humidity in the climate chamber was set to the base setting of temperature ~24 ± 1°C and relative humidity (RH) ~45 ± 2% respectively. This setting represented the normal room conditions.

Energy Measurement (Constitutes "b" From the Workflow)

The single laser pulse energy was measured with a moving laser spot (using coherent EnergyMAX J-25MUV-193 [Coherent Inc., Santa Clara, CA]) and the values were recorded. The energy sensor was placed in the open, under the laser beam (close to the laser focus).

Material Ablation (Constitutes "c to d" From the Workflow)

Ablations were performed on PET and PMMA material of standard and controlled thickness and shapes (PET thickness = 96 μm, PMMA thickness = 2 mm, with square windows of 12 mm size, including a pupil of 8 mm diameter). The ablations were performed to measure the fluence of a single laser pulse, and to estimate the efficiency of laser ablation on material of known ablation properties [11]. Fluence was measured for two energy settings

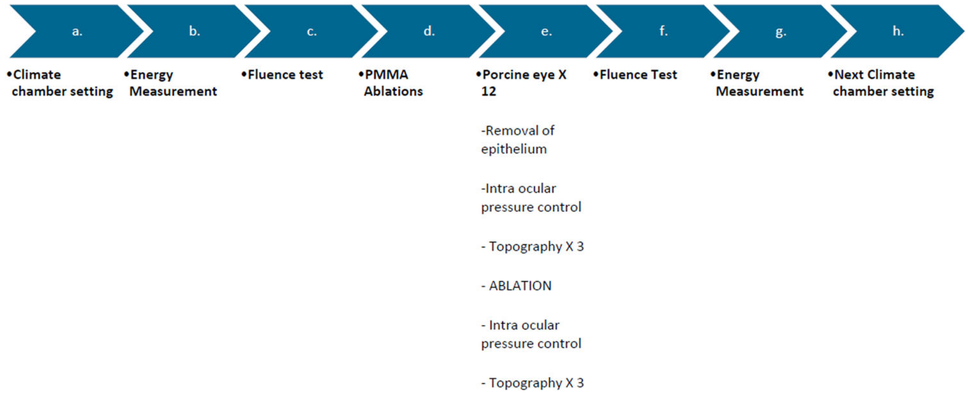

Fig. 1. A schematic representation of the workflow involving various steps followed in the methods, with an aim to analyze the impact of humidity and temperature on excimer laser ablation of polymethylmethacrylate (PMMA), polyethylene terephthalate (PET), and porcine corneal tissue.

available in the laser system (high fluence standard value of 1.0 mJ [440 mJ/cm^2] and low fluence standard value of 0.7 mJ [300 mJ/cm^2]). The ablations ranged from a planned ablation depth of 27 to 202 μm (in cornea, 11 to 89 μm in PMMA) and ablation zone of 6.5 mm.

Preparation of Porcine Eye (Constitutes "e" From the Workflow)

Removal of epithelium. The epithelium was removed using surgical instrument Hockey Knife and Amoils brush.

Intra ocular pressure control. Intra ocular pressure was controlled with an infusion of Fresenius freeflex® Beutel Ringerlösung (Fresenius Kabi AG, Bad Homburg, Germany). The pressure in the eye was subjectively checked by touching the corneal surface with a fingertip, by a trained tester (Biomedical Engineer), to match the intra ocular pressure commonly observed in an eye of a live human. For the purpose of these tests, it was assumed that the intra ocular pressure of the porcine eyes and human eyes is similar. After controlling the intraocular pressure, the infusion was removed from the eye.

Measurement of corneal topography. Artificial tear drops (Oculosoft Care Multi [Oculsoft, Germany]) were used to moisturize the cornea. The eye was aligned with the Keratron SCOUT topographer (Optikon2000 SPA, Rome, Italy) and corneal curvature measurements were performed. At least three measurements were performed and Maloney [12] indices were recorded in all topographic measurements.

Laser Ablation on Porcine Eyes (Constitutes "e" From the Workflow)

All the treatments were prepared using the SCHWIND CAM software in Aberration-Free treatment mode (SCHWIND eye-tech-solutions, Kleinostheim, Germany). For the entire ablation standard high (1.0mJ [440 mJ/cm^2]) and low fluence (LF 0.7 mJ [300 mJ/cm^2]) laser pulse distribution (nearly 80% of the ablation profile being created by high fluence pulses, and the rest by low fluence pulses) of the Schwind ablation profile was used [13–15]. In total, 12 eyes were tested for each climate chamber setting, as high fluence and low fluence laser pulses belong to the same treatment. The eye was mounted on a holder and positioned at the ablation plane (aligned to the corneal apex). For all the eyes, identical treatment parameters were used. These are summarized in Table 1. The repetition rate of the laser system was 1050 Hz for all the tests, however, the local frequency of ablation was limited to the "intelligent thermal effect control" frequency (normal iTEC frequency) of the AMARIS platform (i.e., system repetition rate limited to below 40 Hz local ablation frequency) [16].

After ablation, the corneal topography measurement was repeated. The porcine eye was discarded and the step "e" was repeated for the next porcine eye. In this manner, a total of 12 eyes were ablated for each climate chamber setting.

Material Ablation and Energy Measurement (Constitutes "f to g" From the Workflow)

The single laser pulse energy was measured and the PET ablations were repeated (like in steps "b" and "c" above).

Change in Climate Chamber Setting (Constitutes "h" From the Workflow)

The Climate chamber was adjusted to different test settings.

For each test setting, steps "b" to "g" were repeated.

The different Climate chamber settings (test settings) are presented in Table 2. These were non-sequential to avoid/reduce hysteresis, condensation, and model bias. Each setting was realized in a quasi-adiabatic manner, taking a total 1.5 hours (1 hour for temperature/humidity change and 0.5 hour for thermal stabilization) to move from one test setting to the next. These settings were achieved with a precision of <1°C in terms of the

TABLE 1. Parameters Used for Planning the Treatment in SCHWIND CAM Software

Parameter	Value
Age	1 year
Planned refraction	–15D
Maximum ablation depth	362 µm
Keratometric readings (K1 and K2)	21D
Treatment type	LASIK Aberration free
Optical Zone	7.5 mm
Transition Zone	2.5 mm
Corneal pachymetry	750 µm
Ablation profile type	Non-wavefront guided aspheric profile

temperature and <2% in terms of the relative humidity (according to the calibration certificate, Weiss Umwelttechnik GmbH). The 3-day long test plan was designed such that in one day several ablations are performed in three different test settings. In this manner, a total of nine climate settings were tested in three days. At the end of each test day, the climatic chamber was brought back to standard base climate settings of ~24 ± 1°C temperature with ~45 ± 2% RH.

Analysis

For the purpose of analysis and presentation of results, for each test setting, all the measurements (single pulse energy, high and low fluence, efficiency on PMMA, and refractive change achieved in porcine eyes) performed before the ablation of porcine cornea are referred to as pre-ablation measurements. Similarly, all measurements performed after the ablation of porcine cornea are referred to as post-ablation measurements.

Maloney indices were analyzed in all topographic measurements. For each eye, all the measurements performed pre-ablation and post-ablation were averaged separately. The change in defocus was calculated from the difference of post-ablation to pre-ablation Average Keratometric readings.

Laser scanning deflectometry [17,18] was performed to measure the refractive change achieved in PMMA.

All the output values were normalized based on the average value of the metric. This was done to analyze the results in the form of percentage relative change. We describe the dependencies via a linear approximation. Hence, multiple linear regression was performed using the least square method with the predictive factors (input parameters): temperature, relative humidity, and a time stamp, where the latter describes the time that elapsed since the beginning of the test. In the following sections, the time stamp represents the time passed from the start point of a 3-day long test protocol.

The linear ablation model was designed to estimate the outputs based on the predictive factors. If the model could represent the obtained results in an adequate manner, the model would expand the scope of the work, beyond the nine settings tested in the climate chamber to a generic compensation model that can be applied for any room condition.

The model can be represented with the following equation:

$$\text{Output} = m1 \cdot T + m2 \cdot RH + m3 \cdot t + C$$

Here, the input parameters temperature, relative humidity, and time are represented as T, RH, and t respectively. The output means pre-ablation and post-ablation single pulse energy, high and low fluence, efficiency on PMMA, and refractive change achieved in porcine eyes. The slopes ($m1$, $m2$, and $m3$) and constant term (C) was calculated for each Output parameter. The coefficient of determination (R^2) was calculated for each Output parameter

TABLE 2. Different Temperature and Humidity Test Settings That Were Realized in a Climatic Chamber for the Purpose of the Tests that Lasted 3 Days.

Setting	Temperature	Relative humidity
1	~24 ± 1°C	~45 ± 2%
2	~18 ± 1°C	~25 ± 2%
3	~18 ± 1°C	~80 ± 2%
End of Day 1 → Return to base climate setting ~24 ± 1°C with ~45 ± 2% RH		
4	~30 ± 1°C	~80 ± 2%
5	~30 ± 1°C	~25 ± 2%
6	~24 ± 1°C	~25 ± 2%
End of Day 2 →Return to base climate setting ~24±1°C with ~45±2% RH		
7	~24 ± 1°C	~80 ± 2%
8	~30 ± 1°C	~45 ± 2%
9	~18 ± 1°C	~45 ± 2%
End of Day 3 →Return to base climate setting ~24 ± 1°C with ~45 ± 2% RH		

Each test setting was realized in a quasi-adiabatic manner, taking a total 1.5 hours (1 hour for temperature/humidity change and 0.5 hour for thermal stabilization) to move from one test setting to the next. For each test setting, several ablations were performed on plastic material and porcine eyes. At the end of each day, the climate chamber was brought back to the base setting (~24 ± 1°C with ~45 ± 2% RH).

and statistical significance of the predictive factors was evaluated, with $P < 0.05$ as the level of significance.

After ensuring the credibility of the ablation model, the influence of climate settings was modeled for single laser pulse energy, single laser pulse fluence, ablation efficiency on PMMA, and ablation efficiency on porcine cornea tissue. Based on these outcomes, a working window of climate settings was defined for an optimum operation of the laser system.

RESULTS

In total, 108 porcine eyes, 108 PMMA ablations, and 36 PET ablations were performed in a span of 3 days, encompassing a total of 9 climate settings. Due to technical limitations of the climate chamber, at very low temperatures settings (18°C), extreme relative humidity could not be achieved despite continued attempts. Therefore, not all the climate settings in the design of the experiment could be reached. A comparison of the aimed and reached climate settings is graphically presented in Figure 2.

Table 3 shows all the achieved output values, for each test setting realized in the climate chamber. All these output values were normalized based on the average value of the metric to develop a multiple linear regression ablation model.

Ablation Model

The ablation model for each output metric is presented in Table 4.

Temperature was not a significant predictor for any output parameter. Relative humidity was a significant predictor for energy (pre-ablation and post-ablation) and PET ablation performance at both energy settings. Time stamp (time elapsed since the beginning of the tests) was a significant predictor for all output parameters, except for refractive change achieved on porcine cornea. The mean coefficient of determination of the ablation model was 0.8, suggesting a good predictability of each output parameters based on the predictive factors.

The impact of climate settings on various metrics is elaborated below, and depicted with the help of surface plots encompassing a broad range of temperature (18°C to 30°C) and relative humidity conditions (20% to 80% RH). The direction of the maximum rate of change was included in the plots to indicate the environmental gradients leading to the maximum response. These surface plots were created based on the output values resulting from the ablation model, by inputting a 4×4 matrix of values for the predictive factors (temperature 18°C, 22°C, 26°C, 30°C; relative humidity 20%, 40%, 60%, 80%). The time stamp (time elapsed since the beginning of the tests) was taken out from the ablation model as a covariate, and its influence was eliminated from the analysis. Hence, a fixed value of average time stamp (average time elapsed since the beginning of the tests) was used for the analysis.

Influence of Climate Settings on Single Laser Pulse Energy

The impact of the climate settings on single laser pulse energy is presented in Figure 3 (Top), for the pre-ablation and post-ablation condition. Between the pre-ablation and post-ablation condition, there was a time gap of ~2 hours, in which a total of 12 porcine eyes, 12 PMMA, and 4 PET ablations were performed. The change in temperature did not affect the single pulse energy, for a constant relative humidity. However, changing the relative humidity from 20% to 80% resulted in changing the single pulse energy from 105% to 95% pre-ablation, and 106% to 92% post-ablation. Dry climate conditions resulted in higher single pulse energy, compared with moist climate conditions, resulting in lower single pulse energy.

Influence of Climate Settings on Single Laser Pulse Fluence

The impact of the climate settings on single laser pulse fluence for the two energy settings (high and low fluence) is

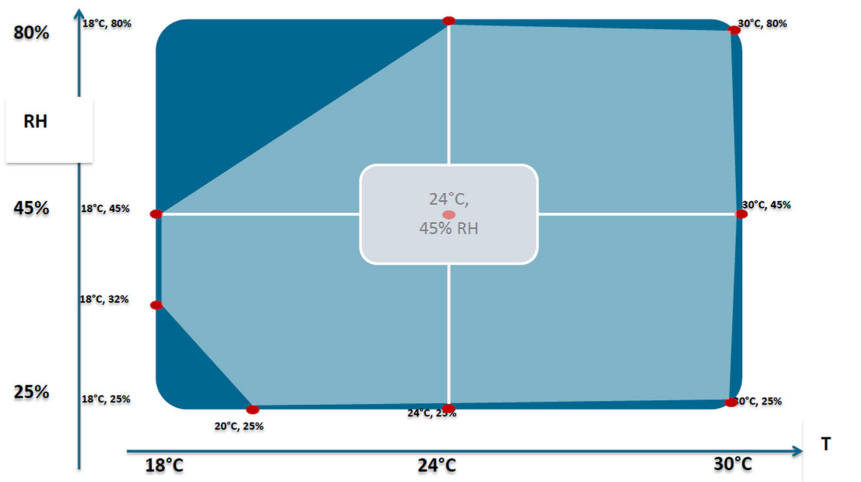

Fig. 2. A graphic representation of the attempted climate settings in the design of experiment (dark blue) versus the achieved climate settings (light blue).

TABLE 3. Achieved Output Values, for Each Test Setting Realized in the Climate Chamber

T achieved (°C)	RH achieved (%)	Energy Pre (mJ)	HF Pre (mJ/cm^2)	LF Pre (mJ/cm^2)	PMMA mean (%)	PMMA SD (%)	Defocus mean (D)	Defocus SD (D)	HF Post (mJ/cm^2)	LF Post (mJ/cm^2)	Energy Post (mJ)
24	25	0.98	663	576	98	1.8	−15.46	0.37	639	532	0.91
30	25	0.92	644	541	93	4	−15.73	0.62	639	535	0.89
30	80	0.83	596	500	98	3.2	−14.87	0.54	576	481	0.76
18	32	0.85	603	513	98	1.8	−14.71	0.31	613	520	0.85
18	45	0.85	607	514	94	3	−14.57	0.32	588	495	0.79
24	80	0.78	592	491	94	2.2	−13.98	0.35	556	481	0.73
20	25	0.82	613	499	95	2.1	−14.93	0.47	600	489	0.80
24	45	0.80	588	489	91	3.2	−13.24	0.32	555	477	0.75
30	45	0.76	584	471	92	3.1	−15.20	0.52	562	470	0.72

Pre and Post represent pre-ablation and post-ablation measurements, respectively. The high and low fluence are presented as HF and LF, respectively. All these output values were normalized based on the average value of the metric to develop a multiple linear regression ablation model.

presented in Figure 3 (middle and bottom), for the pre-ablation and post-ablation condition. Between the pre-ablation and post-ablation condition, there was a time gap of ~1.75 hours, in which a total of 12 porcine eyes and 12 PMMA ablations were performed. The change in temperature did not affect the single laser pulse fluence, for a constant relative humidity. The High and low fluence setting behaved similarly. Furthermore, the fluence behaved similarly pre-ablation and post-ablation. Dry climate conditions (20% RH) resulted in higher single laser pulse fluence (~103%), compared with moist climate conditions (80% RH), resulting in lower single laser pulse fluence (~95-97%).

Influence of Climate Settings on Laser Ablation Efficiency on PMMA

The impact of the climate settings on Laser ablation efficiency on PMMA is presented in Figure 4 (top), for the mean values and standard deviation in ablation efficiency. The mean ablation efficiency remained stable and close to 100% for a wide window of climate settings. For extremely low humidity and high temperatures (30°C with 20% RH, dry and hot conditions), reduced performance (98%) was recorded, compared with increased efficiency (103%) at low temperatures and high humidity (18°C with 80% RH, cold and dry conditions). The trend was similar for the standard deviation in ablation efficiency, however, percentage changes were large in comparison, since they depict a change in very small numbers compared with the mean values.

Influence of Climate Settings on Laser Ablation Efficiency on Porcine Cornea Tissue

The impact of the climate settings on porcine cornea ablation is presented in Figure 4 (bottom), for the mean values and standard deviation in achieved refractive change. For extremely low humidity and high temperatures (30°C with 20% RH, dry and hot conditions), a higher refractive change (106%) was recorded, compared with reduced efficiency (93%) at low temperatures and high humidity (18°C with 80% RH, cold and dry conditions). The trend was similar for the standard deviation in refractive change, however, percentage changes were large in comparison, since they depict a change in very small numbers compared with the mean values.

DISCUSSION

Valuable contributions related to the topics in calibration systems and their influence and advancements in the field or laser vision correction have been published in the past [19–22]. Several groups have tested the impact of hydration and room temperature in human eyes undergoing refractive surgery, reporting different influences on post-ablation outcomes [23–26]. Using univariate and multivariate analysis, Walter and Stevenson [27] found that LASIK enhancement rates strongly correlated with procedure room humidity, 2-week preparative mean outdoor humidity, outdoor temperature, and age; suggesting that these factors should be taken into account while planning the LASIK procedure. In another study, a modified LASIK procedure was performed

TABLE 4. Ablation Model for Output Metrics Single Laser Pulse Energy, Single Laser Pulse Fluence, Ablation Efficiency on PMMA, and Ablation Efficiency on Porcine Cornea Tissue, Based on the Predictive Factors Temperature (T), Relative Humidity (RH), and Time Stamp (Since the Beginning of the Test)

Metric	Energy pre	HF Pre	LF Pre	PMMA	Porcine	HF Post	LF Post	Energy Post
m1 – T	−0.032%	0.073%	−0.091%	−0.185%	0.462%	0.023%	−0.055%	−0.106%
	($P=0.87$)	($P=0.63$)	($P=0.61$)	($P=0.33$)	($P=0.17$)	($P=0.83$)	($P=0.72$)	($P=0.46$)
m2 – RH	**−0.164%**	**−0.106%**	−0.098%	0.035%	−0.128%	**−0.163%**	**−0.115%**	**−0.233%**
	($P=0.01$)	**($P=0.03$)**	($P=0.07$)	($P=0.46$)	($P=0.14$)	**($P=0.00$)**	**($P=0.02$)**	**($P=0.00$)**
m3 – Time	**−0.834%**	**−0.390%**	**−0.657%**	**−0.288%**	−0.257%	**−0.470%**	**−0.459%**	**−0.762%**
	($P=0.00$)	**($P=0.01$)**	**($P=0.00$)**	**($P=0.05$)**	($P=0.24$)	**($P=0.00$)**	**($P=0.00$)**	**($P=0.00$)**
C	**126%**	111%	**120%**	**109%**	**100%**	**117%**	**116%**	**129%**
R^2	0.94	0.88	0.92	0.58	0.63	0.96	0.90	0.97

Pre and Post represent pre-ablation and post-ablation measurements, respectively. The high and low fluence are presented as HF and LF, respectively. The statistically significant predictive factors for each output metric are shown in green color; all other predictive factors did not show statistical significance. The P-values for each output parameter and predictive factors are presented in brackets. The bold values indicate the statistically significant values, with $P < 0.05$.

on the corneal surface that was kept relatively dry by blotting of the stromal surface between sets of laser pulses [28]. It was reported that for less hydrated corneas, ablation effects were greater than for corneas not blotted during the procedure, but these patients appeared to undergo greater myopic regression. In contrast to these results, studies with other laser platforms could not demonstrate any significant difference in patients grouped according to season at time of treatment [29].

Model simulations have shown that laser energy absorption (up to 7% of the available energy) occurs along the path of laser beam, into the existent space between the laser beam source and the patient's eye, due to the environmental temperature and relative humidity. In comparison, our results suggest laser energy absorption of 10–14% in the range of tested climate conditions (Fig. 3 (top), Range of variability 10% for Energy Pre and 14% for Energy Post). A theoretical model of the water vapor absorption at 193 nm wavelength has been proposed to quantitatively assess the influence of environmental parameters on the laser energy that actually reaches the corneal surface. [30,31]

A critical interplay exists between several factors affecting the corneal temperature and hydration during the refractive surgery, namely geographical location and patient lifestyle [32–34], fluid distribution, photoablation time, temperature [35] etc. Intraoperatively, the presence of excess fluid in the central cornea appears as a shiny area, which may reduce the rate of central ablation by reflecting and absorbing a significant amount of the incident excimer laser light. [36] Photoablation has been shown to increase the refractive index of the stroma, and the increase is influenced by the treatment time [37,38]. Furthermore, cooling and rehydration of the cornea with chilled balanced salt solution between passes during PRK significantly reduces haze in patients with baseline myopia between −6.00 and −9.75 D. [39]

In this work, we could establish a well-fitting multi-linear model to estimate the impact of changes in temperature and relative humidity on laser energy, fluence, and ablation efficiency on PMMA and tissue. According to this model, time stamp (time elapsed since the beginning of the tests) was a significant predictor for all output parameters, except for refractive change achieved on porcine cornea, suggesting that the system performance changed significantly with time. One possible explanation for this could be that the rapidly changing room environment conditions affected the performance of the laser system dramatically. To analyze the impact of temperature and humidity, independently, and eliminate the influence of time, the time was taken out as a covariate in our analysis. This can be regarded as a limitation of the ablation model. There are some further limitations to our work; the tests and resulting ablation model was established based on only one laser system used over a series of days of testing, and a range of climate conditions. The inter-system variability was not included in our analysis, however, PMMA ablation efficiency being the calibration method for SCHWIND AMARIS laser systems, a comparison of the ablation efficiency in PMMA and porcine corneal tissue was a more critical analysis goal. Our ablation model, followed

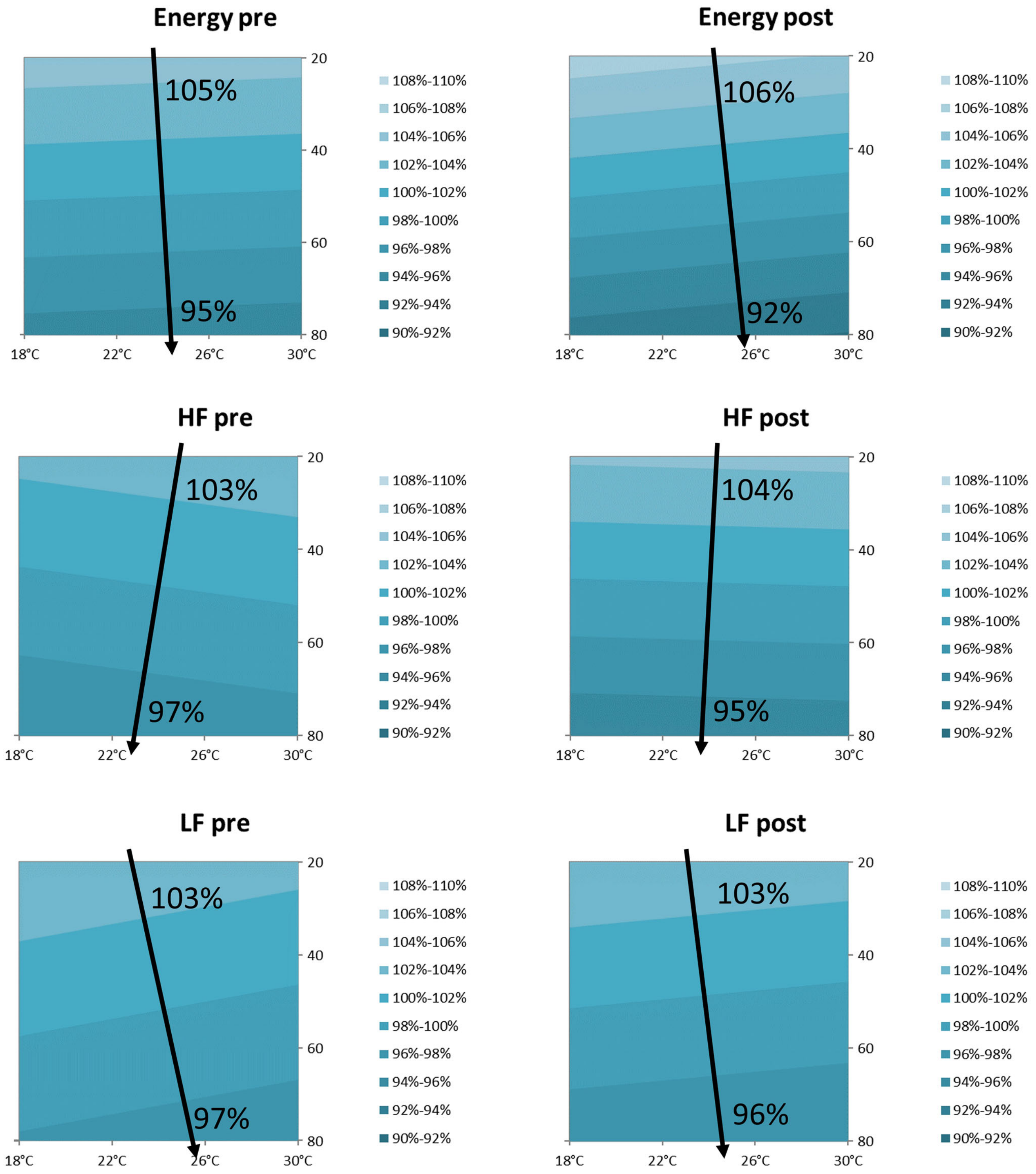

Fig. 3. Surface plots presenting the impact of change in relative humidity (in *Y*-axis) and temperature (in *X*-axis) on single laser pulse energy (**top**) and single laser pulse fluence (**middle**: high fluence; **bottom**: low fluence), pre-ablation (**left**) and post-ablation (**right**).

multi-linear approximation of the deviations in the normalized data. The deviations in other metrics (like median, averages, etc.) were not analyzed. In our methods, we describe the dependencies via a linear approximation. The analysis was repeated for an exponential model, which showed very comparable results to the linear approximation. However, due to obtained good correlation, further modeling approaches (exponential, sigmoid, etc.) were not tested. In

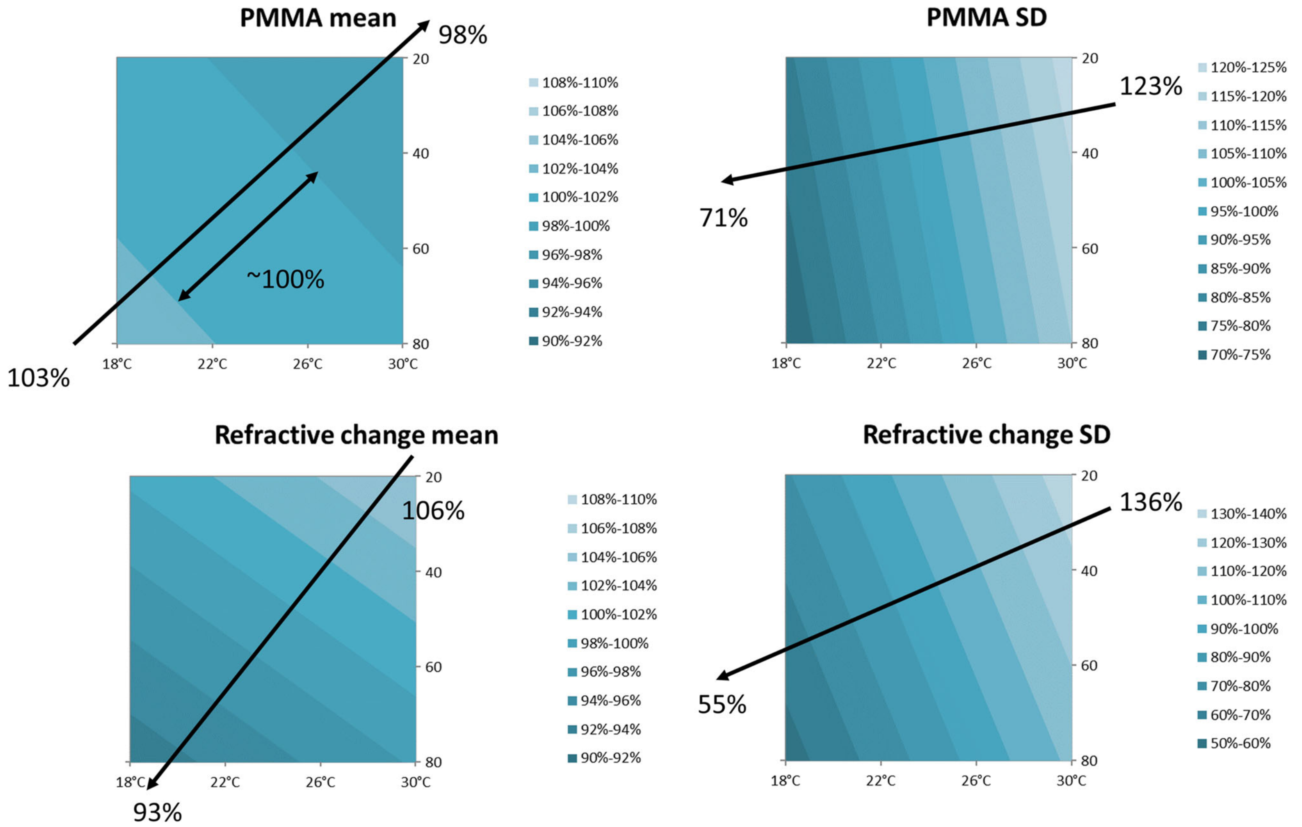

Fig. 4. Surface plots presenting the impact of change in relative humidity (in *Y*-axis) and temperature (in *X*-axis) on mean (**left**) and standard deviation (**right**) of ablation efficiency in polymethylmethacrylate (PMMA) (**top**) and refractive change achieved in porcine cornea (**bottom**).

addition, the goodness of the ablation model was tested for a narrow window of parameters, however, surpassing the extremes of the potential working conditions for refractive surgery laser systems. No further conditions were tested to qualify the model and recognize the outliers. An ablation model based on human cornea would be ideal, however, our tests were conducted on enucleated porcine eyes; hence, the results shall be extrapolated from porcine to human cornea. The pressure in the eye was subjectively checked by touching the corneal surface with a fingertip instead of an objective measurement through a pressure sensor. During the evaluation of the test set-up prior to the beginning of the tests, it was discovered that using a pressure column to maintain the pressure with an infusion was logistically problematic, for orienting the porcine eyes properly under the laser. A lack of measurement of eye pressure could have caused variability in the achieved refractive change in the porcine cornea, however, given the number of eyes in the test protocol, it is unlikely that this may induce a systematic bias. Actually, the increased variability may mask a difference as non-significant although there is one, so if at all, this may lead to a type II statistical error. The porcine eyes were refrigerated, and then were serially placed in the climate chamber, ablated, and discarded. It would have been optimal to have the porcine eyes at normal room temperature and measure the corneal temperature at the time of ablation, to precisely determine the effect of temperature and humidity on ablation rate. However, due to the logistical issues and a long test protocol, porcine eyes were refrigerated until being ablated. This protocol was systematically followed for all the eyes. The porcine eyes were stored in glucose and saline solution until being ablated. No pachymetry data was recorded to evaluate the influence of hydration on the thickness of the corneal tissue. This factor could have even affected the influence of the humidity conditions in the room on to the effective hydration of the cornea. As fresh porcine eyes were procured and tested on each day, the variability between the days would not be as critical, compared with the changes throughout the day as the eyes are being stored in the solution for nearly 8 hours.

Based on the ablation model, the temperature changes did not affect laser pulse energy, fluence, and ablation efficiency (on PMMA or porcine corneal tissue) significantly. This was confirmed by the surface plots shown in Figure 3. A diagonal trend was observed in surface plots shown in Figure 4, showing an influence of the temperature change, although not achieving statistical significance ($P = 0.33$ for PMMA and $P = 0.17$ for Porcine cornea, although the lowest of all the parameters predicted by the temperature change yet $P > 0.05$). Changes in relative humidity were more critical and significantly affected the laser pulse energy, high fluence and low fluence, however, these changes did not significantly show up in the ablation efficiency on PMMA and porcine cornea. As per the design of SCHWIND AMARIS, the output of PET ablations calibrates the PMMA ablations in the short term (interval of 2 hours). Therefore, the internal compensation of SCHWIND AMARIS (preset energy, pulses) could be a factor for reducing the influence of temperature and relative humidity on PMMMA and porcine to a non-significant impact. In any modern commercially available refractive laser system, calibration cycles are repeated at different frequencies depending on the frequency of the system feedback [21]. In one form or another, as all laser systems follow the same principle of short term calibration, the results of our tests on PMMA and porcine cornea, and their inter-relationship would still remain valid.

Similar conclusions regarding the influence of temperature and humidity on refractive outcomes were presented in a study with 237 patients who underwent LASIK. [40] The results at 15 and 60 days after LASIK were compared according to different levels of temperature and humidity in the operating room during the procedure. The linear regression coefficient showed that lower temperature levels were associated with lower spherical equivalent refractions at 60 days after LASIK. The evaluation of humidity indicated an influence at 15 days after LASIK as well as at 60 days. Although humidity showed a higher influence compared with temperature, both factors showed no statistical significance on spherical equivalent refraction. Few differences can be pointed out between this clinical study and our experimental set up. In this clinical setting, the natural variability in refraction was used, while in our setting always the same ablation was performed. Further to that, the methodology was retrospective, leading to one single instance (treatment) per setting; comparatively, in our approach, the chamber was tempered and stabilized before repeating the test 12 times. In the clinical study, the temperature and humidity readings were recorded from normal monitors, while in this study a NIST traceable calibrated chamber was used. Further to the clinical study we also evaluated the impact on typical calibration materials such as PET or PMMA, as well as on the laser energy.

In a study by Hood et al. [41], involving 458 consecutive patients, influence of temperature and humidity was retrospectively evaluated on postoperative visual acuity. It was reported that no significant association existed between temperature and uncorrected distance visual acuity (UDVA) or corrected distance visual acuity (CDVA) or between humidity and UDVA ($P > 0.05$ for all). However, increased humidity was associated with a small but statistically significant improvement in CDVA after LASIK at 1 day, 1 month, 3 months, and 1 year postoperatively.

In our results, moist climate conditions resulted in lower single pulse energy, this could be due to higher UV absorption in moist air present in the environment. Similarly, reduced ablation efficiency was recorded on porcine corneal tissue, at low temperatures and high humidity.

Comparison of PMMA Ablation to Porcine Cornea Tissue

A comparison of PMMA and porcine corneal tissue in terms of the influence of temperature and relative humidity, for all the points in the 4×4 matrix of input values of predictive factors is presented in Figure 5. An opposite trend was observed between the performance on PMMA and porcine cornea. This finding was counterintuitive, as principally, the behavior on calibration material is expected to follow the same trend as tissue ablation. Further explorations are needed to explain this findings, but a potential explanation may be that PMMA has a much lower water content than the cornea, therefore, PMMA regardless of the tested setting tends to moisten (faster for larger humidity differences), while cornea regardless of the tested setting tends to dry out (faster for larger humidity differences). Nevertheless, these opposite trends may amplify each other, since calibrating in dry and hot conditions would make the laser underperform on PMMA, which after potentially being adjusted to 100%, would result in over-performance on porcine cornea in dry and hot conditions. In the most extreme case, such a calibration may lead to an over-correction of 8% (2% adjustment due to PMMA calibration at 30°C and 80% RH, increasing the correction on porcine cornea from 6% to 8%, Fig. 5) on porcine cornea, or conversely an under-performance on PMMA by the same amount. It must be also pointed here, that principal diagonal in PMMA and porcine tissue surface plots (Fig. 4, dry-hot to humid-cold), represents a "90°

PMMA	18°C	22°C	26°C	30°C
20	101%	100%	99%	98%
40	101%	101%	100%	99%
60	102%	101%	101%	100%
80	103%	102%	101%	101%

Defocus	18°C	22°C	26°C	30°C
20	100%	102%	104%	106%
40	98%	100%	102%	103%
60	95%	97%	99%	101%
80	93%	95%	96%	98%

Fig. 5. A comparison of polymethylmethacrylate and porcine corneal tissue in terms of the influence of temperature and relative humidity, for all the points in the 4×4 matrix of input values of predictive factors.

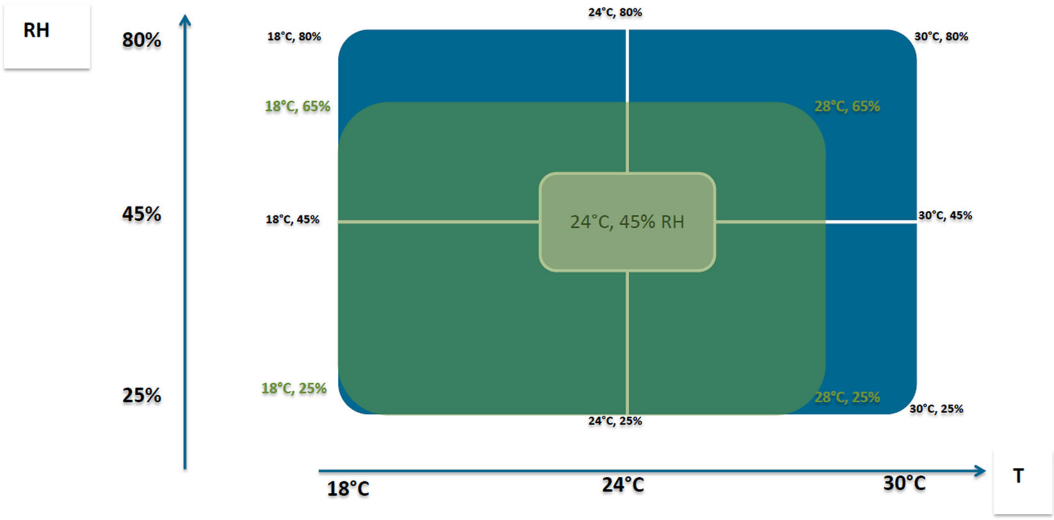

Fig. 6. Definition of a working window of climate settings for an optimum operation of the laser system, with a maximum deviation of ±2.5% in ablation efficiency in polymethylmethacrylate and porcine corneal tissue.

shift" from the summer-winter diagonal (typically going from hot-humid to cold-dry and vice-versa). Therefore, in reality, the laser system shall be unlikely subjected to such an extreme change in the climate.

The effect of both temperature and relative humidity accounted for a deviation of ±2.5% in PMMA. Assuming the same maximum deviation of ±2.5% in refractive change in porcine cornea, a working window of climate settings can be defined for an optimum operation of the laser system. This is presented in Figure 6.

CONCLUSION

For a refractive correction of −12D (maximum correction available in most commercial refractive laser systems), −10.5D is targeted at the cornea (assuming a vertex distance of 12 mm). Therefore, a maximum deviation of ±2.5% will result in a deviation of 0.25D at the cornea (±2.5% of 10.5D). Given the current clinical success criteria for a deviation in refractive surgery, a system achieving this refraction in all climate conditions can be regarded as well calibrated. According to our linear model, a working window for optimum operation has been found with a temperature range of 10°C (±5°C from a reference value) relative humidity range of 40% (±20% from a reference value) for a maximum deviation of ±2.5% in ablation efficiency in PMMA and porcine corneal tissue. The reference values should be ideally the values corresponding to the manufacturer's last technical calibration performed in situ. For the sake of simplicity and assuming from experience typical comfort values in the surgical rooms of ~23°C and 45% relative humidity, this window can be fixed to temperature 18% to 28°C and relative humidity 25% to 65% for maximum deviation of ±2.5% in ablation efficiency in PMMA and porcine corneal tissue.

The relationship between calibration materials like PMMA and corneal tissue should be analyzed cautiously before designing the calibration routine, to obtain optimum outcomes with minimum deviations.

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AUTHORS' CONTRIBUTIONS

SV was involved in the conception and design of the study, data collection, analysis and interpretation of data, writing the manuscript, critical revision of the manuscript, providing statistical expertise, obtaining funding, and providing administrative support. TK was involved in data collection, analysis and interpretation of data, critical revision of the manuscript, and providing statistical expertise. JH was involved in supervision, analysis and interpretation of data, critical revision of the manuscript, obtaining funding, and providing administrative support. SAM was involved in supervision, conception and design of the study, analysis and interpretation of data, critical revision of the manuscript, providing statistical expertise, obtaining funding, and providing administrative support.

AVAILABILITY OF DATA AND MATERIAL

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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