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Pupilometer efficacy in monitoring anxiety in undergraduate medical students during high-fidelity clinical simulation

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The aim of the present work was to determine the correlation between the State-Trait Anxiety Inventory (STAI) score and pupillary diameter and whether this correlation exists to develop a predictive model of anxiety with the pupillary diameter of students exposed to high-fidelity clinical simulation. This was a randomized, blinded, simulation-based clinical trial. The study was conducted at the Advanced Clinical Simulation Center, Faculty of Medicine, Valladolid University (Spain), from February 1 to April 15, 2023, and involved volunteer sixth-year undergraduate medical students. The STAI score, vital signs (oxygen saturation, perfusion index, blood pressure, heart rate, and temperature), and pupillary response were assessed. The primary outcomes were the delta (pre/postsimulation) of the state STAI and the delta of the pupillary diameter. Sixty-one sixth-year students fulfilled the inclusion criteria. There was no difference regarding the clinical scenario. There was a statistically significant correlation between the state STAI score and pupillary diameter. The predictive model had an AUC of 0.876, with the delta diameter of the pupillary being the only statistically significant variable for anxiety prediction. Our results showed that both the pupillary response and the STAI score allowed the identification of students with disabling anxiety. These results could pave the way for appropriate protocol development that allows for personalized tutoring of students with elevated anxiety levels.

Keywords Anxiety, High-fidelity clinical simulation, STAI, Pupillary diameter, Learning

Undergraduate medical students can experience different forms and levels of anxiety related to performing regular hospital internships or high-fidelity clinical simulations[1,2]. The COVID-19 pandemic, among other things, has focused attention on mental health[3]. Severe anxiety affects an estimated 15% of medical health care workers, and 4.8% of them experience symptoms of moderate to severe levels of psychological distress[4]. These data presented by professionals may be just the tip of the iceberg, and an underrated problem may also exist for students.

In high-fidelity clinical simulation laboratories, undergraduate medical students are challenged by fast-paced, highly dynamic situations centered on emergency and critical care scenarios. In addition, students encounter an unfamiliar environment with a variety of foreign materials and equipment[5]. To improve learning, interventions are video recorded and observed in real time by peers or tutors to finish with a debriefing of the session. All

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these inputs may significantly disrupt the behavior and performance of students and generate a heightened level of anxiety[6,7].

The autonomic nervous system is triggered (sympathetic unloading) in dangerous or perceived dangerous situations, and the secretion of epinephrine and norepinephrine activates the physiological response. Sympathetic activation triggered an entire automatic battery of responses to increase bioavailable energy intake, redirecting oxygen flow to the heart, brain, and musculoskeletal system. As a result, among other changes, by putting the body in self-defense mode, the heart rate increases, the airways dilate, peripheral vasoconstriction occurs, insulin secretion is inhibited, glycogenolysis is increased, sweating escalates[8], and/or pupils dilate to improve long-range vision[9,10]. Pupillary responsiveness can be interpreted as an indicator of cognitive and emotional performance. In other words, pupillary size may be influenced by various input signals, such as the psychosensory reflex, which causes pupillary dilation associated with any relevant trigger, e.g., a stressful situation such as high-fidelity clinical simulations[11]. Despite being a constantly changing research topic, current studies have confirmed that pupillary contraction in response to unpleasant stimuli is not a common response; in contrast, all psychological and sensory stimuli, with the exception of light, dilate the pupil, and no one contracts the pupil[12].

When the automatic signal-averaged response is prolonged over time or becomes unsustainable, the mechanism of assistance in dangerously sensed events may become self-defeating and significantly impair the cognitive capacity of students[13–15]. An increasing demand has been expressed by the educational community to determine the anxiety response experienced by students in simulation scenarios. This burden is measured by different predesigned instruments, e.g., the State-Trait Anxiety Inventory (STAI)[16] or vital sign monitoring[17].

Recently, an innovative system with the capacity to perform noninvasive neurological monitoring, the pupillometer, has emerged[18]. As previously reported, sympathetic nervous system triggering, among other effects, may lead to pupillary dilatation. Therefore, the purpose of the present study was to analyze the correlation between the STAI score and pupillary diameter and to determine whether a positive correlation is able to predict whether pupillary size is useful for estimating anxiety increases in undergraduate medical students during a clinical simulation. The secondary objective was to analyze the associations between clinical scenarios and before and after simulation changes in the variables measured.

Methods

Design

This was a randomized, blinded, simulation-based clinical trial involving volunteer sixth-year undergraduate medical students conducted at the Advanced Clinical Simulation Center, Faculty of Medicine, Valladolid University (Spain), from February 1 to April 15, 2023. The trial was approved by the Ethics Committee for Research with Medicines of the Valladolid West Health Area (PI-033/18). All the subjects signed written informed consent, in line with the Declaration of Helsinki. No one received compensation or was offered any incentive to participate in this study. The trial was performed in accordance with the Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines.

Using a high-fidelity manikin, we explored the correlation between vital signs and anxiety levels in relation to the pupillary response in two simulated acute life-threatening clinical scenarios: major trauma and anaphylactic shock. The participants were randomly allocated to one of the two simulated clinical scenarios.

Participants

Eligible participants were recruited from among sixth-year undergraduate medical students (Valladolid University, Spain) interested in participating in the study on a voluntary basis.

Participants who performed the STAI during the previous year or had a medical history of functional disability or visual or hearing impairments impeding completion of the simulation procedure, major surgery in the previous 30 days, or who were taking anticonvulsants or anxiolytics were excluded.

Randomization

Nonreplacement randomization with a 1:1 ratio was stratified by clinical scenario by using R, version 4.1.2 (package Dplyr). The participants were unaware of the scenario until they prebriefed and entered the simulation lab (participants had previously completed the STAI, and baseline measurements of pupillary reactions and vital signs were performed). Specifically, the initial STAI questionnaire was administered after randomization and before any intervention was given to the students (including taking vital signs).

Intervention

Before randomization, a survey with epidemiologic demographic data, the STAI (unrestricted time), baseline vital signs (oxygen saturation, perfusion index, blood pressure, heart rate, and temperature), and pupillary response assessment were completed by each participant on the day of the test. All the participants attended a 20-minute, standardized, fully video-recorded presentation of the simulation manikin characteristics and the correct way to complete the anxiety questionnaire. A simulated scenario was performed by the participant accompanied by another student (only the team leader was evaluated). The subjects were asked to handle, following current guidelines, a clinical scenario of major trauma or anaphylactic shock, depending on randomization. The simulated clinical scenario lasted for a maximum of 10 min. Immediately afterward, a postsimulation evaluation was performed, which included pupillary response, a vital sign set, and the STAI (unrestricted time).

The validated Spanish version of the STAI[19] is a self-reported scale composed of two subscales (20 items per subscale), state (how the person feels at that specific moment) and trait (how the person usually feels), and was used for measuring anxiety levels. Oxygen saturation, perfusion indices, blood pressure, heart rate, and temperature were measured and logged via a DEFIGARD Touch 7 multiparametric monitor (Schiller AG, Baar, Switzerland) by applying a blood pressure cuff, a pulse oximeter (which also measures the perfusion index),

and three adhesive electrodes to monitor heart rate. The pupillary response was measured with an NPi-300 Pupillometer (NeurOptics, Inc., California, USA). In addition, prior to any intervention, body mass index was also recorded (using a scale and a height meter), as were lifestyle habits and laboratory environmental conditions.

Outcomes

The primary outcome was the delta of anxiety level (pre- and postsimulation) on the STAI state subscale, which exhibited strong internal consistency (Cronbach's = 0.9–0.93)[20]. A high score suggests elevated anxiety. Similarly, the physiological delta response was also compared pre- and postsimulation. The primary predictive variable was the delta pupillary diameter (pre/postsimulation) in both the right and left eyes.

The group to which each subject was assigned was not masked, but to avoid possible biases, the researchers involved in the analysis were unaware of the initial allocation, and the results were not unblinded until the final phase of the analysis. The training needed for the questionnaire coding and correction, standardized acquisition of vital signs, use of the digital pupillometer, and gathering and transfer of data were ensured.

Statistical analysis

Descriptive results and the associations between clinical scenarios and between baseline and postsimulation evaluations were assessed via paired t tests, the Mann–Whitney U test or the chi-square test, when appropriate. Absolute values and percentages were used for categorical variables, and median interquartile ranges (IQRs) were used for continuous variables because they did not follow a normal distribution. Cronbach's alpha was also calculated to assess the reliability of the STAI. Note that for the following calculations, the mean value between both eye parameters was used. The correlation between the STAI score and pupillary diameter was assessed as follows: Due to the difference in the statistical characteristics of the two variables, the delta (post–pre) value was binarized according to increase (>0), decrease or no change (≤ 0), and a chi-square test was applied to assess the chi-square statistic value and the p value to contrast the null hypothesis and assess the relationship between these two parameters. Furthermore, the ability of the delta pupillary diameter to predict an increase in anxiety was assessed by logistic regression with forward and backward stepwise variable selection (for this purpose, the *step* function of R was used, which uses the Akaike information criterion for the selection of the model; the input and output p value is equivalent to 0.15). The outcome of the logistic regression was a binarization of the STAI according to whether the participants presented an increase in anxiety (>0) or not (≤ 0). The results from the logistic regression were evaluated via the area under the curve (AUC) or the receiver operating characteristic curve (AUC); moreover, the results were internally validated by bootstrapping (1000 iterations).

The data were collected and registered in a database generated with IBM SPSS Statistics for Apple version 20.0 software. (IBM Corp, Armonk, NY, USA). The caseload entry system was tested to delete unclear or ambiguous items and to verify the adequacy of the data collection system. The data did not present missing values. The sample size needed for the present study was $n = 8$ on the basis of the following considerations: statistical power (1 - β) of 80%, significance level (α) of $p = 0.05$, and standard deviation of difference (σ_d) = 0.9.

All calculations and analyses were performed by using our own codes, R packages and base functions in R, version 4.2.2 (http://www.R-project.org; the R Foundation for Statistical Computing, Vienna, Austria).

Results

Sixty-one sixth-year undergraduate medical students with a median age of 24 years (IQR: 23–24; range 23–41), 45 females (73.8%), and 41 participants (67.2%) with previous simulation experience were randomly assigned to the two clinical scenarios. In total, 29 subjects were randomized to the major trauma scenario, and 32 were randomized to the anaphylactic shock simulated clinical scenario, with no missing data and one dropout (Fig. 1). The demographic characteristics and environmental conditions are described in supplementary Table S1.

The intercomparison by clinical scenario yielded no significant differences regarding any of the variables analyzed (supplementary Table S2).

Primary outcome

The STAI state subscale displayed considerable differences pre- and postsimulation (globally, not by scenario). The median pretest score was 56 points (IQR: 49–63), whereas it was 66 points (IQR: 58–71) postsimulation (17% increase). When the same subscale was analyzed by clinical scenario, no differences were detected, with a median of 56 points in both simulated scenarios ($p = 0.387$). However, no differences between the scenarios were noted at the physiological threshold. Globally, the delta was more pronounced in terms of the perfusion index (38% decrease), with improvements in blood pressure and decreases in heart rate and temperature maintained (Table 1). The STAI presented good reliability (alpha = 0.88, 0.89, 0.84, and 0.88, respectively, for state STAI presimulation, state STAI postsimulation, trait STAI presimulation, and trait STAI postsimulation).

Primary predictive variable

Pupillary diameter presented clear differences before and after simulation (Table 1) but not between the simulated scenarios (supplementary Table S2). All the parameters related to the pupillary response, except for the dilatation velocity of the left eye, were significantly different between the pre- and postsimulation comparisons (Table 1). The right eye had a median presimulation pupil size of 4.71 mm (IQR: 4.09–5.19), and postsimulation measurements revealed a pupillary diameter of 5.69 mm (IQR: 4.94–6.25), with a delta of 0.955. The left eye had a median presimulation pupil size of 4.65 mm (IQR: 4.05–5.08) and 5.71 mm (IQR: 4.93–6.37) for the post stimulation, with a delta of 1.020. The minimum-maximum pupillary diameter difference was for the right eye presimulation 31% (IQR: 26–36) and 36% (IQR: 31 – 29) for postsimulation, with a delta of 3.442. For the left eye, the presimulation minimum-maximum pupillary diameter difference was 33% (IQR: 29–37) vs. 37% (IQR: 32–40) for postsimulation, with a delta of 3.672.

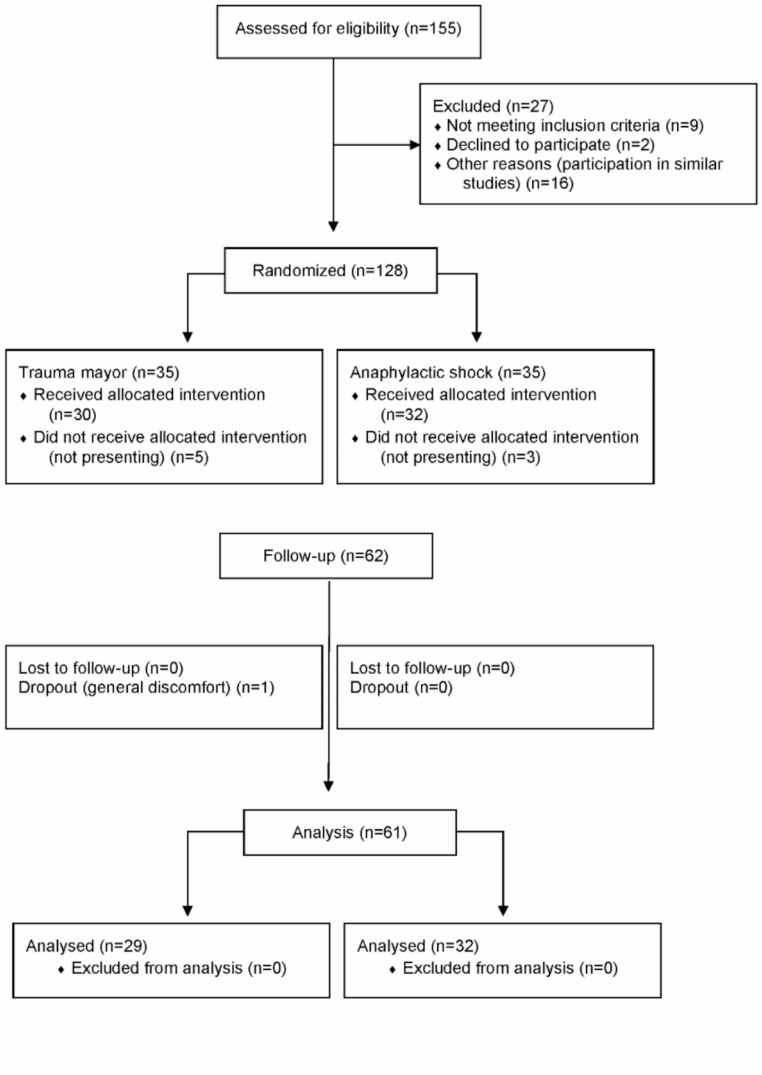

Fig. 1. CONSORT diagram of study participation.

The correlation between the delta of the State STAI and the delta of the pupillary maximum diameter presented a chi-square test statistic of 28.9 and a p value of $p < 0.001$, rejecting the null hypothesis and allowing us to conclude that there is a relationship between these two parameters.

The logistic regression used to evaluate the predictive validity of the parameters analyzed to determine patient anxiety initially included age, sex, simulation scenario, delta of oxygen saturation, delta of perfusion index, delta of systolic and diastolic blood pressure, delta of heart rate, delta of temperature, delta of pupillary diameter, and the binarized state STAI as the outcome. Only the difference in the diameter of the pupillary delta diameter was significant ($p = 0.049$) (Table 2). The full logistic regression results before the stepwise procedure with further statistical details are shown in Table 3. The predictive validity of the model, derived from multivariate logistic regression, presented an AUC of 0.876 (95% confidence interval: 0.706-1) (Fig. 2). Further details on the internal validation can be found in supplementary Table S3.

Discussion

This randomized, blinded, simulation-based clinical trial was performed to determine the association between pupillary size recorded by a digital pupillometer and anxiety level measured by the gold standard, the State-Trait Anxiety Inventory (STAI). The pupillary diameter presented clear differences pre- and postsimulation but not between simulated scenarios (trauma mayor or anaphylactic shock), revealing a correlation between the binarized delta STAI and binarized delta of the pupillary maximum diameter.

To the best of our knowledge, this is the first study conducted with sixth-year undergraduate medical students and, similarly, the first trial to assess pupillometer efficacy in measuring anxiety levels. Pupillometer application for several clinical purposes has emerged as an ongoing practice. In previous reports, Yamaguchi J et al.[21] explored the relationship between the neuromodulated pain response and pupillary feedback. Okamoto

Variable[a]	Baseline evaluation	Postsimulation evaluation	Delta	*p* value[b]
State-Trait Anxiety Inventory				
State, points	56 (49–63)	66 (58–71)	9.409	< 0.001
Trait, points	41 (38–48)	47 (39–51)	3.786	< 0.001
Vital signs				
SpO2, %	98 (97–98)	98 (97–99)	0.147	0.586
Perfusion index, %	2.11 (0.99–4.41)	1.30 (0.80–2.60)	−1.319	< 0.001
SBP, mmHg	125 (114–135)	132 (122–137)	5.737	0.001
DBP, mmHg	77 (71–86)	84 (77–91)	5.114	< 0.001
HR, beats/min	91 (75–104)	88 (75–100)	−1.819	0.274
Temperature, °C	37 (36.6–37.3)	36.8 (36.5–37.2)	−0.096	0.125
Pupilar response				
Right eye				
Max diameter, mm	4.71 (4.09–5.19)	5.69 (4.94–6.25)	0.995	< 0.001
Max/min difference, %	31 (26–36)	36 (31 – 29)	3.442	< 0.001
CV, mm/sg	2.93 (2.39–3.50)	3.22 (2.85–3.56)	0.310	< 0.001
DV, mm/sg	1.27 (1.01–1.42)	1.33 (1.11–1.47)	0.086	0.016
Left eye				
Max diameter, mm	4.65 (4.05–5.08)	5.71 (4.93–6.37)	1.020	< 0.001
Max/min difference, %	33 (29–37)	37 (32–40)	3.672	< 0.001
CV, mm/sg	2.93 (2.36–3.60)	3.64 (3.17–3.95)	0.517	< 0.001
DV, mm/sg	1.31 (1.15–1.53)	1.36 (1.12–1.57)	0.040	0.347

Table 1. Vital signs, pupillary response and anxiety level, baseline, and postsimulation by clinical scenario. SPO2: oxygen saturation; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; Max: maximum; Min: minimum; CV: constriction velocity; DV: dilation velocity. [a]Values are expressed as the total number (percentage) and median (25th-75th percentile), as appropriate. [b]The Mann–Whitney U test or chi-squared test was used as appropriate.

	Odds ratios	5%CI	95%CI	*p* value
Age	5,936	1,338	41,783	0,081
Delta of systolic pressure	1,062	0,996	1,153	0,162
Delta of heart rate	1,098	1,008	1,229	0,111
Delta of pupillary diameter	7,049	1,750	49,161	0,049

Table 2. Odds ratios derived from the multivariate logistic regression after Stepwise selection. CI: Confidence interval.

	Estimate	Standard Error	z value	*P* value
Age	3.39795	2.23259	1.522	0.128
Sex	−2.54626	2.47514	−1.029	0.304
Aleatorization	−2.99905	2.31542	−1.295	0.195
Delta of SpO2	−0.54127	0.46045	−1.176	0.240
Delta of perfusion index	−0.17572	0.39057	0.450	0.653
Delta of systolic blood pressure	0.08220	0.06528	1.259	0.208
Delta of diastolic blood pressure	−0.16356	0.11604	−1.409	0.159
Delta of heart rate	0.11756	0.07780	1.511	0.131
Delta of temperature	0.96805	1.46188	0.662	0.508
Delta of pupillary diameter	3.72660	1.92623	1.935	0.053

Table 3. The logistic regression results before the Stepwise procedure showing all the variables.

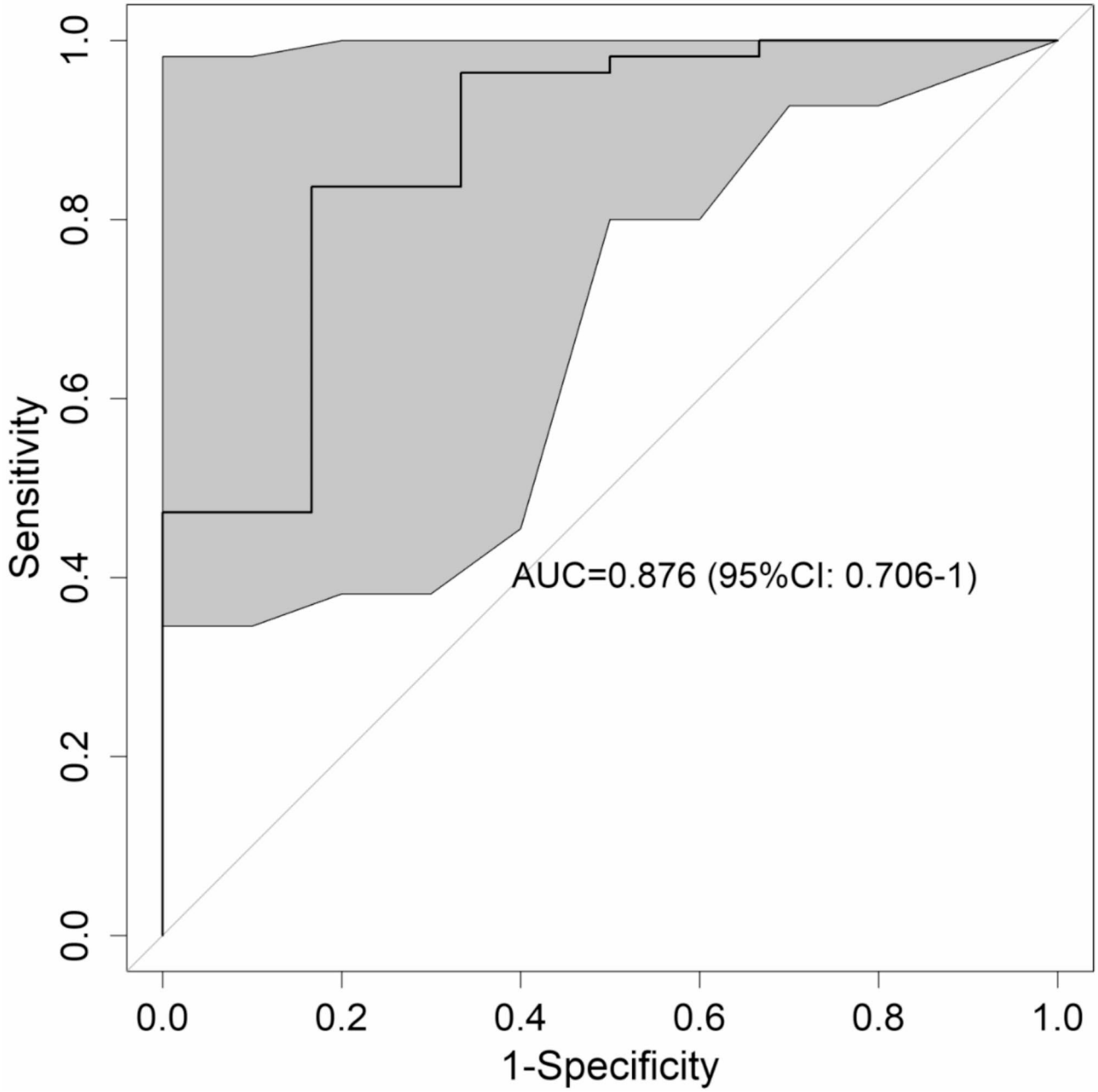

Fig. 2. Receiver operating characteristic (ROC) curve of the predictive model. The gray area represents the 95% confidence interval.

S et al.[22] analyzed the usefulness of the pupillometer for predicting ICU delirium, or Peluso L et al.[23] used the neurological pupil index to predict outcomes after cardiac arrest, among other studies.

Determining the anxiety level among simulation session participants is a common topic of interest[24]. Low-medium levels of anxiety are known to facilitate concentration and improve short-term results; similarly, high levels of anxiety are known to dampen alertness (for overstimulation), hinder problem resolution, and disrupt the decision-making process dramatically[1].

A variety of instruments have been applied to measure anxiety, e.g., questionnaires, biomarkers, and scales[25]. The STAI questionnaire is one of the most widely used questionnaires and, consequently, is considered the gold standard[16,19]. Contrasting competence can be used to measure both anxiety in isolation and pre- and postsimulation delta anxiety, and it is increasingly used in simulation sessions[26]. Nonetheless, the STAI contains certain handicaps. Unrestricted time to complete survey items is needed; this process should not be conducted repeatedly since participants might memorize the questions and adjust the answer; somehow, it is a subjective questionnaire that the participant voluntarily accepts to respond sincerely. Instead, pupillometry allows for consecutive application as many times as deemed necessary, as it is a noninvasive technique with an extremely easy training curve for providers and is not dependent on intersubjective or interpersonal factors to measure

stress-related sympathetic responses in the pupil. However, alternative biomarker-based methods for measuring anxiety are available, with particular emphasis on the use of salivary cortisol variations[27]. In response to a sensed warning, the hypothalamus–adrenal axis triggers a surge of hormones such as cortisol, improving glucose bioavailability. Stressors may, however, be permanently prevalent, allowing alarm feedback to run rampant and leading to cortisol overshoot[28].

Undoubtedly, the usefulness of the pupillometer has been demonstrated in neurological response assessment, and high-fidelity clinical simulation has been proven to stimulate sympathetic output[27,29,30]. Therefore, after observing the correlation between the delta maximum diameter and delta STAI, we hypothesize that a possible novel use of the pupillometer could be to estimate anxiety levels. Indeed, as shown by our results, the only statistically significant variable in the predictive model was the delta of pupil diameter, which was not affected by confounding variables and presented an excellent predictive capacity to identify changes in anxiety (AUC = 0.876).

High-fidelity clinical simulation has become an irreplaceable resource that can decisively help in training in the decision-making process at critical moments. This unique form of face-to-face education could induce significant levels of anxiety[31]. Students are exposed to uncertain scenarios, unfamiliar teamwork, and direct observation by both faculty and colleagues[14]. Indeed, this was the stressful response induced by clinical simulation in which the trait subscale scores, presimulation vs. postsimulation, even increased significantly. Anxiety as a trait theoretically should not increase or experience discrete changes; nevertheless, our data and similar evidence reported in cases of anxiety analysis via objective structured clinical examination reported increases in anxiety as a trait[32]. This increase could be caused by a variety of factors, such as self-consciousness, fear of making mistakes, criticism from professors or classmates, and high self-expectations. However, all of these factors are psychological in nature, which explains the increase in anxiety as a trait and, in turn, explains the slight variation in vital signs postsimulation, as it is a self-limiting stressor.

The ongoing pandemic caused by coronavirus disease 2019 (COVID-19) has resulted in a shock to health systems worldwide. This disruption has been carried out in all sectors of society, including unsurprisingly, education, especially advanced education in the healthcare sciences[33]. Despite performing high-fidelity clinical simulations under rigorous biosafety protocols (mandatory face masks, regular hand washing, frequent surface cleaning, social distancing, forced air ventilation, air quality monitoring, antigen testing, temperature control, small groups of eight students, and two teachers in a laboratory of 68 m^2)[34], the challenge of safeguarding the health of everyone became a permanent anxiety point.

The present trial presented an important strength, that is, its generalizability to each educational system, since the anxiety measurement is immediate and can be repetitively performed. This study has the following limitations. First, many questionnaires, tests, surveys, etc., are available to determine anxiety levels (e.g., the Beck Anxiety Inventory, Coping Responses Inventory-Adult Form, and Job Stress Survey). The STAI was selected because the prevalence of implementation has been generalized, and a validated version of the STAI is available in Spanish[19]. Second, the range of clinical scenarios available to rate the response and modification of anxiety levels is unlimited. Two clinical scenarios were randomized: major trauma and anaphylactic shock; critical situations that require accurate and appropriate management quickly; and a 10-minute evolution that allows the patient to be appraised[35,36]. Despite the discretionary nature of the choice of scenarios, there were no significant differences between them, so we believe that the type of scenario simulated does not have a decisive influence on the increase in anxiety. Third, recruitment was based on opportunity criteria involving volunteer sixth-year undergraduate medical students, so there may have been sampling bias. The cohort had a median age of 24 years and was two-thirds female. In future studies, we propose expanding the sample to include students from other courses and healthcare providers to assess the efficacy of the pupillometer accurately. Fourth, aspects such as drug use, psychiatric disorders, borderline personality, previous trauma or even economic conditions were not considered. For future studies, express authorization will be requested from the ethics committee to collect these data and their subsequent analysis. Fifth, the sample size was small and was based on a homogeneous cohort (for instance, similar ages and education levels); these circumstances limit the generalizability of the study. Finally, pupillometer measurements can be influenced by the light reflex and the near reflex. Lighting variations in the room may provoke significant changes in pupil size; increases in brightness trigger rapid pupil contraction. On the other hand, as an object approaches our eye, the pupil dynamically adjusts to improve visual focus on the object (in this case, the pupillometer)[37,38]. To minimize possible biases, the pre- and postsimulation measurements were performed in the same laboratory under identical environmental conditions; likewise, there were no significant differences between the simulated clinical scenarios. While the amount of ambient light can modulate the pupillary response, the trial was performed under controlled environmental conditions, with the same conditions for all participants. This study was embedded in the mandatory medicine studies teaching plan; therefore, including a placebo group (with no exposure to simulation cases) was not possible.

Conclusion

In summary, recognizing students with disabling anxiety levels when performing the simulated clinical scenario to the fullest extent could be extremely valuable to facilitators. When these learners are more predisposed to developing raised anxiety thresholds, different interventions could be performed to improve their response, e.g., a previous visit to the simulation laboratory, visualization of a presession, and relaxation exercises by breathing control. In this sense, the well-known STAI questionnaire and the newest digital pupilometer device are effective aids for assisting in screening students for elevated anxiety levels.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to the inclusion of confidential data but are available from the corresponding author upon reasonable request.

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Author contributions

F.M-R. conceptualized the project, managed and coordinated the project, assisted with the design of the methodology, analysed the data, and prepared the initial and final drafts of the manuscript. A.S-G. takes responsibility for the data and their analysis. C.P.V., R.L-I., E.S.A., S.G.V., L.A.D.L., S.A.O., R.C.I., R.M-S. and M.Á.C.V. assisted with the management and coordination of the project, assisted with the design of the methodology, and helped review the manuscript. All the authors performed a critical review and approved the final manuscript for interpretation of the data and important intellectual input.

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Declarations

Competing interests

All the signing authors meet the requirements of authorship and declare the nonexistence of potential conflicts of interest. Carlos del Pozo Vegas, Raul Lopez-Izquierdo, Eduardo Silva Alvarado, Santos Gracia Villar, Luis Alonso Dzul López, Silvia Aparicio Obregón, Rubén Calderon Iglesias, Francisco Martín-Rodríguez, Ancor Sanz-García, Rafael Martín Sánchez and Miguel Ángel Castro Villamor report no conflicts of interest. On behalf of the other authors, the corresponding author guarantees the accuracy, transparency, and honesty of the data and information contained in the study; that no relevant information has been omitted; and that all discrepancies between authors have been adequately resolved and described.

Ethics approval and consent to participate

The study was approved by the Ethics Committee for Research with Medicines of the Valladolid West Health Area (PI-033/18). The review protocol was registered as ISRCTN32132176 (https://doi.org/10.1186/ISRCTN32132176) (03/01/2020). Details of the study design, statistical analysis plan and raw data are available online. All subjects signed written informed consent, in line with the Declaration of Helsinki. No one received compensation or was offered any incentive to participate in this study.

Consent for publication

This article is original work, has not been published before and is not being considered for publication elsewhere in its final form in either printed or electronic media. It is not based on any previous communication to a society or meeting.

Additional information

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