Automated pupillometry to uncover signs of consciousness in acute brain injury: statistical analysis plan

Authors: Marwan H. Othman, Markus Harboe Olsen, Kirsten Møller, Jesper Kjærgaard, Daniel Kondziella

ABSTRACT

Background: Automated pupillometry is well-established in the clinical setting for quantifying pupil motility with great precision at the bedside. Mental tasks such as mental arithmetic lead to pupillary dilation in conscious people, and pupillary dilation in this context is considered a surrogate marker for cognitive and emotional processes. Using a paradigm of standardized stimuli to evoke pupillary dilation, we investigated if automated pupillometry could detect covert consciousness in clinically unresponsive patients with acute brain injury. ***Methods:*** We established an automated pupillometry paradigm presenting patients (n=91) and age- and sex-matched healthy volunteers (n=25) to the following stimuli: 1) their own facial reflection in a mirror, 2) a series of three different sounds, and 3) a mental task based on mental arithmetic to produce cognitive load. Pupillary responses before and after stimuli were recorded by automated pupillometry. Here, we present a pre-specified statistical analysis plan (SAP) for our automated pupillometry study. The SAP aims to increase transparency and validity by stating the author's hypothesis, objective, and analysis approach before accessing the data. Also, the SAP limits p-hacking and researcher bias and discern data-driven interpretations from the pre-planned analysis. ***Conclusions:*** We hypothesize that automated pupillometry may contribute to the clinical evaluation of unresponsive patients with acute brain injury by detecting covert consciousness in a subset of patients with cognitive-motor dissociation.

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INTRODUCTION

Approximately 15% of patients diagnosed with unresponsive wakefulness syndrome (UWS) show signs of consciousness using advanced functional MRI (fMRI) and electroencephalography (EEG) [1]. In other words, patients with higher-level cognition with complete motor paralysis, i.e., cognitive-motor dissociation (CMD), cannot be identified by standard neurological evaluation [2-4]. Misclassifying the clinical state of these patients could have significant consequences associated with outcome prediction and may put these patients at risk of premature withdrawal of life-sustaining therapy or limited access to neurorehabilitation [5, 6].

Manual pupillometry is part of the current bedside neurological exam, but in recent years automated pupillometry has shown promising results in quantifying pupil constriction and dilation [7, 8]. It is well-known that cognitive and emotional processes evoke pupillary dilation [9-14]. In a recent proof-of-concept study, we displayed the feasibility of combining automated pupillometry with mental arithmetic exercises to generate such activation [15]. This leads us to believe that automated pupillometry may have a future role in assessing consciousness if proven sensitive enough to detect responses to standardized stimuli in acute brain injury.

HYPOTHESIS

Primary hypothesis:

- Automated pupillometry combined with active paradigms can detect CMD in approximately 15-20%, of clinically unresponsive patients with brain injury, which would be comparable with previous data derived from active EEG and fMRI paradigms

Secondary hypotheses:

A) Automated pupillometry combined with passive paradigms detects a greater proportion of responsive patients than active paradigms (e.g., 25-30% vs. 15-20%)

B) Response rates for active automated pupillometry paradigms in healthy conscious volunteers (control group 1) are about 70-90% in line with success rates for active fMRI/EEG paradigms.

C) Response rates for active paradigms in conscious hospitalized patients (control group 2) are lower than those in healthy volunteers, e.g., 50-80%.

Exploratory hypotheses:

A) CMD patients (those who respond to active paradigms as assessed by pupillometry) and responsive patients (those who respond to passive, but not to active paradigms) have a better clinical outcome at discharge compared to VS/UWS patients without signs of responsiveness.

B) Overall pupillary response rates across all patient groups (i.e., coma, VS/UWS, and MCS) are associated with levels of consciousness – the more responses, the higher the level of consciousness.

C) Overall pupillary response rates across all patient groups (i.e., coma, VS/UWS, and MCS) are associated with the functional clinical level (as measured by FOUR score) at discharge – the more responses, the better the clinical state.

OBJECTIVES

Aim: To search for preserved consciousness in unresponsive patients suffering from acute brain injury in the ICU using automated pupillometry combined with passive and active paradigms.

Primary objective

- What is the prevalence of CMD detected by active automated pupillometry paradigms in patients with UWS (approximately 15% as detected by EEG or fMRI)?

Secondary objectives

A) What is the prevalence of responsiveness detected by examination with *passive* automated pupillometry paradigms in patients with UWS (approximately 25-30% reported in the existing EEG/fMRI literature)?

B) How often does automated pupillometry show pupillary dilation in response to passive and active stimuli in healthy volunteers? (Around 70-90% success rates for passive and active fMRI/EEG paradigms in conscious healthy volunteers

C) Compared to non-hospitalized healthy volunteers, how often does automated pupillometry in hospitalized conscious patients show pupillary dilation in response to passive and active stimuli? In other words, is hospitalization associated with affected pupillary responses?

EXPLORATORY QUESTIONS

A) Are CMD patients (active paradigms) and responsive patients (passive paradigms) identified by automated pupillometry associated with a more favorable outcome at discharge than VS/UWS patients without signs of responsiveness?

B) Are pupillary response rates across all patient groups (i.e., coma, VS/UWS, and MCS) associated with consciousness levels?

C) Are pupillary response rates across all patient groups (i.e., coma, VS/UWS, and MCS) associated with a higher level of consciousness at discharge?

METHODS

We recorded automated pupillometry on acute brain injury with 46 neurological patients from the Neuro-ICU and 45 out-of-hospital cardiac arrest patients from the Cardiac-ICU. All patients were recruited from ICUs located at Rigshospitalet, Copenhagen University Hospital. Twenty-five healthy and fully conscious volunteers served as controls; hence 116 participants total.

Exclusion criteria

Patient population

- Age < 18 years
- Pre-existing disorder of consciousness before the onset of acute brain injury
- Clinical recovery of the ability to follow commands before enrolment
- Deafness, blindness, or bilateral eye surgery before the onset of acute brain injury
- Participants or families who actively refuse to participate in the study

Control population

- Age < 18 years
- Known significant neurological disorder
- Deafness, blindness, or previous bilateral eye surgery

Clinical examination

The clinical examination consisted of an automated pupillometry paradigm to four consecutive stimuli while recording pupillary size and motion using a PLR®-3000 pupillometer (NeurOptics, Laguna Hills, CA, USA) over the course of approximately 10 minutes.

The first (passive) stimulus was performed using a mirror held at a distance of 50 centimeters from the eyes of the patient. The patients were exposed to their facial reflection for 10 seconds to stimulate arousal and a sympathetic response seen as a pupillary dilation. Before and after this exposure, they were shown the backside of the mirror, which had a blank paper with a red dot in the middle to maintain focus on the object at the same distance and thereby adjust for potential accommodation.

The second (passive) stimulus contained a series of three different sounds, each played for 10 seconds with 20 seconds of white noise played before, in between, and after to minimize confounding from background noise. The first sound played was Aaron Copland's Rodeo – Four Dance

Episodes, inspired by a study documenting how its frequent tempo shifts can arouse consciousness [16]. The second sound was from a crying baby, and the third was a burglary alarm, both included for their properties to evoke arousal and alertness in adults.

The third and fourth (active) stimuli included two mental arithmetic tasks of increasing complexity. These aimed to induce a cognitive load that was sufficient to induce pupil dilation detectable by automated pupillometry [15]. The first mental arithmetic stimulus consisted of five tasks at a moderate level (4 x 36; 8 x 32; 3 x 67; x 6 x 37; 7 x 43) and the second consisted of five hard level tasks (21 x 22; 33 x 32; 55 x 54; 43 x 44; 81 x 82). Task duration was set at 20 seconds with 20 seconds rest periods before, between, and after.

Pupillary dilation

A pupillary dilation was defined as a significantly larger pupil compared to the resting period before and after. All pupil measurements were included for each period of interest and compared using the Student's t-test with an alpha of 0.05. Even though the period comparison includes autocorrelated measurements, we used the unpaired t-test, which is generally more conservative in the estimates and is not limited by requiring an equal number of measurements in each group. All recordings will be investigated for artifacts with a visual inspection of the data; however, presence or absence of pupillary dilations was blinded to the primary investigator and revealed only after publication of this statistical analysis plan.

Scoring system

The different tasks had different thresholds for success (**Table 1**). In the first passive stimulus, the mirror, one pupillary dilation was sufficient for the patient to pass. The full 10 seconds of the stimulus period was compared to the 5 seconds immediately before and after.

In the second passive stimulus, the music, at least two of the three possible pupillary dilations were required to pass. The full 10 seconds of the stimuli were compared to the 5 seconds immediately before and after.

For the third and fourth active stimuli, moderate and hard arithmetic, at least three of the five possible pupillary dilations were required to pass. The mental arithmetic recordings will compare the 20 seconds of stimuli and the 20 seconds of the pre-and post-stimulus. As a sensitivity analysis, we will compare the results gained with a threshold of three or four pupillary dilations *(see Questions 2 and 4).*

Lastly, we will exploratory investigate the prognostic and diagnostic value when assessing the sensitivity of each stimulus by comparing 1) each dilation of the fourteen stimuli, and 2) the overall number of each passed test (*see Exploratory Questions A-C)*.

Table 1: Illustration of the scoring system

Pupillary dilations	Mirror	Auditory	MA moderate	MA hard
0 significant	Failed	Failed	Failed	Failed
1 significant	Passed	Failed	Failed	Failed
2 significant	-	Passed	Failed	Failed
3 significant	-	Passed	Passed	Passed
4 significant	-	-	Passed	Passed
5 significant	-	-	Passed	Passed

Abb. MA=mental arithmetic

Study setup

The study setup included one clinical examination per healthy volunteer, up to four clinical examinations per patient, and measures of pupil reaction to light for each participant using the NPi®-200 pupillometer (NeurOptics, Laguna Hills, CA, USA). Recordings of the neurological pupil index (NPi) were documented. The patients were examined at admission while sedated (T_0), repeated within 48h after discontinuation of sedatives (T_1), and finally at least 24 hours after the second session (T_2). If possible, a fourth session was added days after completing a series of three (T_3). The patients were furthermore examined using the Full Outline of UnResponsiveness (FOUR) scale before each session to assess their clinical consciousness state and, finally, at discharge to note any significant changes in clinical consciousness.

Sample size and power calculations

The sample size was calculated based on preliminary data from our feasibility study [15]. When using automated pupillometry, compliance with mental arithmetic tasks was detected in 14 of 20 (70%) healthy volunteers and 17 of 43 (39.5%) neurological patients. To detect a clinically relevant difference in pupillary dilations between resting and stimulation periods, we used a power of 80% and a probability of type 1 error (α) of 0.05. We calculated a required case and control sample size of 41, respectively. As each participant serves as their own control and to compensate for potential dropouts, a total of at least 50 participants was deemed necessary for this study.

STATISTICAL ANALYSIS AND OUTCOMES

Statistical analysis will be performed using the latest version of R (R foundation for statistical computing, Vienna, Austria). All patients and healthy volunteers will be included in the analysis of pupil responsiveness to passive and active paradigms and clinical consciousness scores. In contrast, follow-up data analysis will solely rely on patient data. A p-value of ≤0.05 is set as the significant threshold. The summary of data recordings will be presented in table, displaying the number of recordings performed for each level of consciousness (LoC).

Primary Objective
Do *active* automated pupillometry paradigms in patients with UWS identify approx. 15% of CMD patients (which would be consistent with the CMD rates detected by EEG or fMRI?)

Outcome: The proportion of clinically classified UWS patients with at least 3 of 5 (60%) significantly larger pupillary size during mental arithmetic trials as compared to pupillary size during the rest periods, indicating intact ability to follow commands.

Analysis: A clinically classified UWS patient who engages in mental arithmetic (moderate or hard difficulty) as detected by at least 3 of 5 pupillary dilations is considered to have preserved consciousness and, therefore, a patient with CMD. The analysis will consist of a 1-sample proportion test counting UWS patients with a passed mental arithmetic paradigm test, which we expect to be approximately 15-20%, in line with other reports of CMD patients assessed with EEG/fMRI combined with active paradigms (null hypothesis) [1]. The same analysis will be performed with a cut-off set at

4 of 5 pupillary dilations. As a sensitivity analysis, we will also investigate the two abovementioned thresholds using only one recording per patient to analyze the potential influence of multiple measurements. Primary analysis will include first instance of UWS for each patient. Furthermore, two sensitivity analyses will be carried out using the best and worst performance over time.

Secondary Objective (A)
Does examination with *passive* automated pupillometry paradigms in patients with UWS identify pupillary responses in >15% of the population (e.g., 25-30% consistent with previous EEG/fMRI literature)?

Outcome: The proportion of clinically classified UWS patients with passed passive test (i.e., mirror and/or auditory), indicating responsiveness to passive paradigm.

Analysis: The analysis will consist of a 1-sample proportion test counting UWS patients with passed mirror and/or auditory paradigm test, which we expect to be approximately 25-30% for each test in line with other reports of CMD patients assessed with EEG/fMRI combined with passive paradigms (null hypothesis). Two sensitivity analyses will be carried out using the best and worst performance over time.

Secondary Objective (B)
How often does automated pupillometry show pupillary dilation in response to passive and active stimuli in healthy volunteers? (Successful response rates in passive and active EEG/fMRI paradigms in conscious healthy volunteers is around 70-90%).

Outcome: The proportion of successfully passed passive and active test among healthy volunteers.

Analysis: The proportion of successfully passed passive and active tests will be calculated using the 1-sample proportions test with continuity correction, which results in an estimate and a 95% confidence interval. We expect the proportion to be around 70-90% consistent with the response rates in passive and active EEG/fMRI paradigms. Two sensitivity analyses will be carried out using the best and worst performance over time.

Secondary Objective (C)
Compared to non-hospitalized healthy volunteers, does automated pupillometry in hospitalized conscious patients show pupillary dilation in response to passive and active stimuli less or equally often? In other words, does hospitalization affect pupillary response?

Primary outcome: The proportion of passed passive and active tests among healthy volunteers compared to the proportion of passed tests in both fully conscious (n=12) and ≥MCS (n=51) patients suffering from acute brain injury.

Analysis: We will use Fisher's exact test to compare the total count of passed tests between healthy volunteers and fully conscious patients. As a subgroup analysis, we will compare the performance of each test (i.e., mirror vs. mirror; auditory vs. auditory, etc.) based on the rates of passed and failed between the two groups to determine if specific tests are more successful in generating pupil dilations.

Secondary outcome: The proportion of passed passive and active tests among healthy volunteers compared to the proportion of passed tests in ≥MCS patients suffering from acute brain injury.

Analysis: We will use Fisher's exact test to compare the total count of passed tests between healthy volunteers and ≥MCS patients. As a subgroup analysis, we will compare the performance of each test (i.e., mirror vs. mirror; auditory vs. auditory, etc.) based on the rates of passed and failed between the two groups to determine if specific tests are more successful in generating pupil dilations.

Exploratory Question (A)
D) Are CMD patients (active paradigms) and responsive patients (passive paradigms) identified by automated pupillometry associated with a more favorable outcome at discharge than VS/UWS patients without signs of responsiveness?

Primary outcome: Functional outcome at discharge among CMD patients, using a dichotomized mRS (i.e., mRS ≤ 2 (moderate disability or better), and mRS ≥ 3 (moderately severe disability or worse) [17], compared to VS/UWS patients without signs of consciousness.

Analysis: To assess whether CMD patients have a better functional outcome at discharge, we will use the Fisher's exact test to compare their functional outcome based on dichotomized mRS with unresponsive UWS patients.

Secondary outcome: Functional outcome at discharge among responsive patients, using a dichotomized mRS (i.e., mRS ≤ 2 (moderate disability or better), and mRS ≥ 3 (moderately severe disability or worse), compared to VS/UWS patients without signs of consciousness.

Analysis: To assess whether responsive patients have a better functional outcome at discharge, we will use the Fisher's exact test to compare their functional outcome based on dichotomized mRS with unresponsive UWS patients.

Finally, we will then estimate the AUC for each outcome, and the results will be visualized with a figure presenting the category of patients on the x-axis (CMD, $UWS_{Responsive}$, and $UWS_{Unresponsive}$) and AUC confidence intervals on the y-axis. Furthermore, ROC curves will highlight the results by including the dichotomous mRS as the outcome as a function of 1) the number of pupil dilations in passive and active tests (0-14 dilations), and 2) the number of passed tests (0-4).

Exploratory Question (B)
Are pupillary response rates across all patient groups (i.e., coma, VS/UWS, and MCS) associated with consciousness levels?

Primary outcome: Frequency of passed passive and active tests between LoC's (i.e., coma, VS/UWS, and MCS).

Analysis: A Kruskal-Wallis test will be used to compare the number of passed tests across groups (e.g., Coma vs. UWS vs. MCS minus vs. MCS plus. vs. ≥MCS). Bonferroni corrections will be applied to account for multiple comparisons of pupillary dilations between groups. Finally, we will use Spearman correlation with a figure to test for correlation (r) between LoC (categorical – ordinal, x-axis) and pupillary dilations measured by the count of passed tests in each category of consciousness (numeric, y-axis).

Secondary outcome: Frequency of pupil dilations among the four consecutive stimuli in each LoC (i.e., coma, VS/UWS, and MCS).

Analysis: A Kruskal-Wallis test will be used to compare the number of pupil dilations in all stimuli across groups (e.g., Coma vs. UWS vs. MCS minus vs. MCS plus. vs. ≥MCS). Bonferroni corrections will be applied to account for multiple comparisons of pupillary dilations between groups. Finally, we will use Spearman correlation with a figure to test for correlation (r) between LoC (categorical – ordinal, x-axis) and the number of pupillary dilations in each category of consciousness (numeric, y-axis).

Exploratory Question (C)
Are pupillary response rates across all patient groups (i.e., coma, VS/UWS, and MCS) associated with a higher level of consciousness at discharge?

Primary outcome: Frequency of passed passive and active tests in LoC's compared to the clinical state of consciousness at the time of discharge.

Analysis: We will use a logistic regression including the state of consciousness at the time of discharge based on a dichotomized scale of consciousness (≤UWS or ≥MCS) as the outcome. The number of passed tests for each LoC (i.e., coma, VS/UWS, and MCS) will be the independent variables. The model will present odds ratios and estimates of 95% confidence intervals to display whether an increase in the number of pupillary dilations is correlated with a higher level of consciousness.

Secondary outcome: Frequency of pupillary dilations in the automated pupillometry paradigm for each LoC's compared to the clinical state of consciousness at the time of discharge.

Analysis: We will use a logistic regression including the state of consciousness at the time of discharge based on a dichotomized scale of consciousness (≤UWS or ≥MCS) as the outcome. The number of pupillary dilations in each LoC (i.e., coma, VS/UWS, and MCS) will be the independent variables. The model will present odds ratios and estimates of 95% confidence intervals to display whether an increase in the number of pupillary dilations is correlated with a higher level of consciousness.

Visualization of data

Trends or tendencies between the number of pupillary dilations or passed/failed tests and the state of consciousness will also be interpreted through plots (i.e., spaghetti, lasagna, clusters, alluvial, etc.)

The illustrations of our results will contain clinical examinations and automated pupillometry from several time points, thereby displaying our results in series with relation to pupil dilations and consciousness state.

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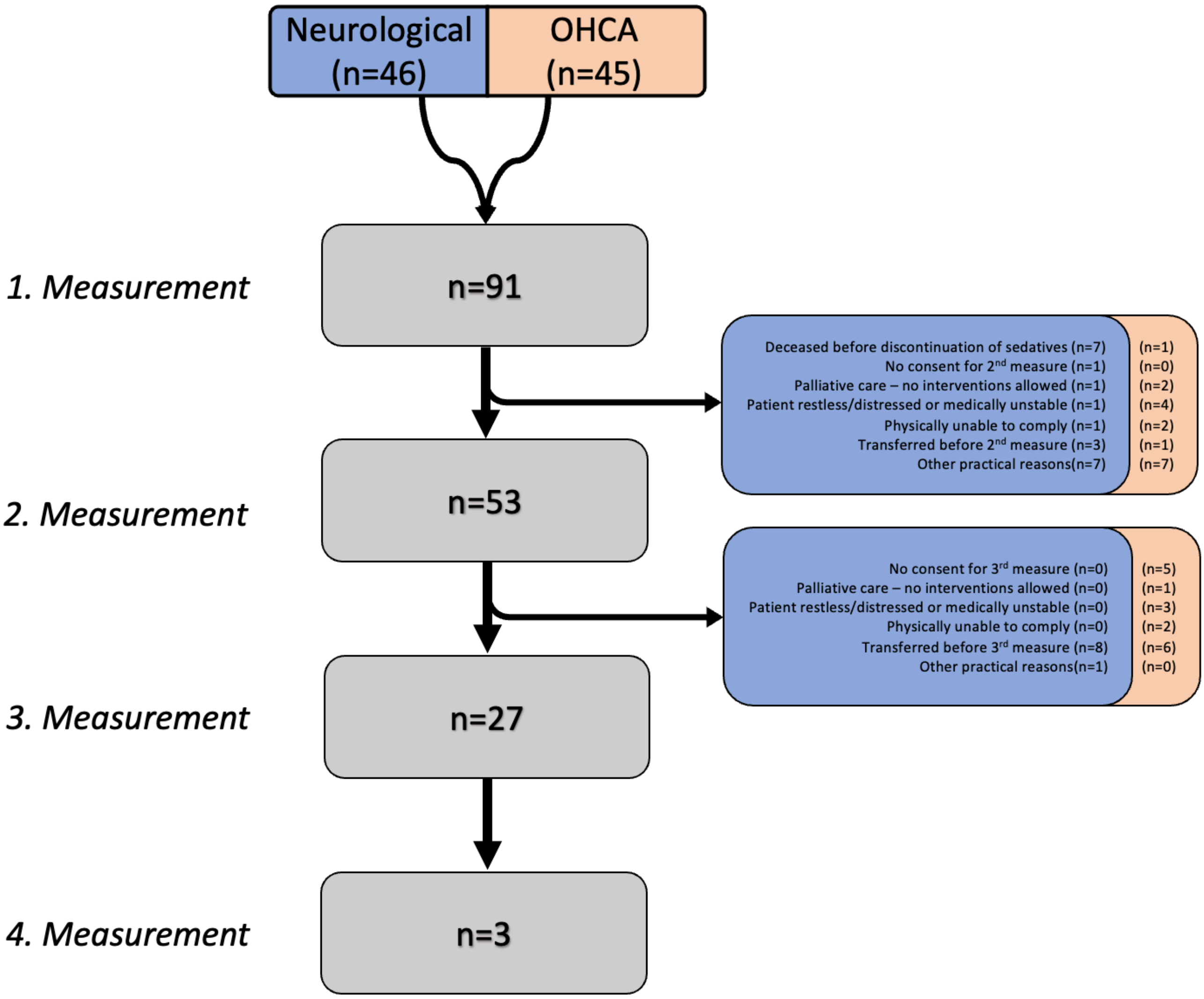
APPENDIX



Neurological (n=46)
OHCA (n=45)
1. Measurement
n=91
Deceased before discontinuation of sedatives (n=7) (n=1)
No consent for 2nd measure (n=1) (n=0)
Palliative care – no interventions allowed (n=1) (n=2)
Patient restless/distressed or medically unstable (n=1) (n=4)
Physically unable to comply (n=1) (n=2)
Transferred before 2nd measure (n=3) (n=1)
Other practical reasons(n=7) (n=7)
2. Measurement
n=53
No consent for 3rd measure (n=0) (n=5)
Palliative care – no interventions allowed (n=0) (n=1)
Patient restless/distressed or medically unstable (n=0) (n=3)
Physically unable to comply (n=0) (n=2)
Transferred before 3rd measure (n=8) (n=6)
Other practical reasons(n=1) (n=0)
3. Measurement
n=27
4. Measurement
n=3