The Neurological Pupil index for outcome prognostication in people with acute brain injury (ORANGE): a prospective, observational, multicentre cohort study

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Summary

Background Improving the prognostication of acute brain injury is a key element of critical care. Standard assessment includes pupillary light reactivity testing with a hand-held light source, but findings are interpreted subjectively; automated pupillometry might be more precise and reproducible. We aimed to assess the association of the Neurological Pupil index (NPi)—a quantitative measure of pupillary reactivity computed by automated pupillometry—with outcomes of patients with severe non-anoxic acute brain injury.

Methods ORANGE is a multicentre, prospective, observational cohort study at 13 hospitals in eight countries in Europe and North America. Patients admitted to the intensive care unit after traumatic brain injury, aneurysmal subarachnoid haemorrhage, or intracerebral haemorrhage were eligible for the study. Patients underwent automated infrared pupillometry assessment every 4 h during the first 7 days after admission to compute NPi, with values ranging from 0 to 5 (with abnormal NPi being <3). The co-primary outcomes of the study were neurological outcome (assessed with the extended Glasgow Outcome Scale [GOSE]) and mortality at 6 months. We used logistic regression to model the association between NPi and poor neurological outcome (GOSE ≤4) at 6 months and Cox regression to model the relation of NPi with 6-month mortality. This study is registered with ClinicalTrials.gov, NCT04490005.

Findings Between Nov 1, 2020, and May 3, 2022, 514 patients (224 with traumatic brain injury, 139 with aneurysmal subarachnoid haemorrhage, and 151 with intracerebral haemorrhage) were enrolled. The median age of patients was 61 years (IQR 46–71), and the median Glasgow Coma Scale score on admission was 8 (5–11). 40 071 NPi measurements were taken (median 40 per patient [20–50]). The 6-month outcome was assessed in 497 (97%) patients, of whom 160 (32%) patients died, and 241 (47%) patients had at least one recording of abnormal NPi, which was associated with poor neurological outcome (for each 10% increase in the frequency of abnormal NPi, adjusted odds ratio 1·42 [95% CI 1·27–1·64]; p<0·0001) and in-hospital mortality (adjusted hazard ratio 5·58 [95% CI 3·92–7·95]; p<0·0001).

Interpretation NPi has clinically and statistically significant prognostic value for neurological outcome and mortality after acute brain injury. Simple, automatic, repeat automated pupillometry assessment could improve the continuous monitoring of disease progression and the dynamics of outcome prediction at the bedside.

Funding NeurOptics.

Introduction

Assessment of pupillary light reactivity is part of the daily clinical practice in managing patients with acute brain injury[1] and has strong diagnostic and prognostic value.[2] Pupillary light reactivity could be modified in the critical care setting by pain, use of opioids, and increased intracranial pressure.[3] Typically, pupillary light reactivity is performed using a hand-held light source, such as a penlight or flashlight, which provides a non-standardised qualitative measurement. These subjective assessments of pupillary light reactivity could lead to imprecision, mainly because of significant inter-observer variability and heterogeneity in the technique used.[4] Automated infrared pupillometry[3,5] provides a standardised, quantitative, highly reproducible measurement of the pupillary light reactivity and other pupillary variables, including amplitude, latency, constriction, and dilation velocity. These measures can be integrated automatically by the pupillometer device to compute a risk score. The Neurological Pupil index (NPi)[6,7] is a composite numerical index with scores ranging from 0 to 5, which can be used to measure pupillary reactivity. The NPi is minimally affected by sedation in comparison with other directly measured pupillometric variables, and it has been often reported in critical care studies. The main factor that might alter the NPi is increased intracranial pressure.[8,9]

So far, outcome studies in the critical care setting have been performed primarily in patients with

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See Online for appendix

Research in context

Evidence before this study

We searched PubMed from database inception to May 1, 2023, for studies published in English, excluding experimental studies, case reports, and reviews, using the terms ("Traumatic brain injury [Title]" OR "subarachnoid haemorrhage [Title]" OR "intracerebral haemorrhage [Title]") AND ("neurological pupil index [Title]" OR "automated pupillometry"). Automated pupillometry has been shown to enhance the precision and reproducibility of the standard pupillary light reactivity test, which has traditionally relied on a hand-held light source and subjective interpretation. Automated pupillometry quantitatively measures pupillary reactivity and global midbrain function, which can be integrated into the Neurological Pupil index (NPi), with scores ranging from 0 to 5 (score <3 is judged abnormal). Single-centre retrospective cohort studies have suggested a potential prognostic value of NPi in patients with severe acute brain injuries, but large prospective cohorts are needed to confirm these findings.

Added value of this study

The ORANGE study was a large, prospective, observational cohort study at 13 hospitals in eight countries in Europe and North America. Logistic regression was done to model the association between NPi and poor neurological outcome (extended Glasgow Outcome Score ≤4) at 6 months. Cox regression was done to model the relation of NPi with 6-month mortality. Models were adjusted for age, the primary cause of injury, and the initial severity of cerebral damage.

Implications of all the available evidence

Abnormal NPi was strongly associated with increased mortality and poor neurological outcome after an acute brain injury, whereas improvements in NPi heralded better neurological outcomes. NPi could, therefore, be a valuable predictor of disease trajectories in patients with acute brain injury. Findings support the use of NPi as a standardised quantitative measurement of pupillary reactivity and global midbrain function. Implementing simple automatic evaluation of pupil reactivity (such as NPi) could improve the continuous evaluation of disease progression and the dynamics of outcome prediction at the bedside.

hypoxic-ischaemic brain injury, including in a large multicentre European study using NPi for early prognosis after cardiac arrest.[7,8,10–13] Emerging data from single-centre retrospective cohort studies in patients with severe non-anoxic acute brain injury suggest the potential prognostic value of NPi.[12–15] However, confirmatory studies from large prospective cohorts are lacking.

Therefore, we conducted an international, multicentre, prospective, observational study in patients with acute brain injury, including traumatic brain injury, aneurysmal subarachnoid haemorrhage, and intracerebral haemorrhage, with the primary aim to assess the association of NPi with 6-month neurological outcome and mortality.

Methods

Study design and setting

The Outcome Prediction of Acute Brain Injury Using the Neurological Pupil Index (ORANGE) study was a prospective, observational, multicentre, international cohort study with a published protocol.[16] The study was performed in 13 European and US academic or teaching hospitals localised in eight countries (Belgium [one centre], France [one], Germany [one], Italy [three], Norway [one], Spain [two], Switzerland [one], and the USA [three]) where NPi was already part of standard monitoring. Ethics committee approvals were obtained at the coordinating and participating centres, and written informed consent was obtained according to local regulations. The study protocol was approved at the sponsor site by the Ethics Committee Brianza at the Azienda Socio Sanitaria Territoriale (ASST; Monza, Italy) on July 16, 2020. Patients were screened for inclusion in the study and followed up for 6 months after the injury for outcome assessment.

Patients were included if they were aged 18 years or older, had a diagnosis of acute traumatic brain injury, intracerebral haemorrhage, or aneurysmal subarachnoid haemorrhage requiring intensive care unit admission, intubation, and mechanical ventilation due to the neurological condition (these conditions are recognised as crucial and prevalent causes of intensive care unit admissions following acute brain injury, as they all have intracranial hypertension and brainstem compression as a potential cause of deterioration), and had automatic infrared pupillometry used as part of the standard evaluation practice.

Patients were excluded if they had facial trauma that could alter the use of automated infrared pupillometry. Clinical teams were not blinded to the NPi measurements, as it was part of the standard clinical practice. Conversely, the outcome assessors were blinded. Furthermore, there was no explicit indication in the study protocol regarding providing any specific therapy in the event of low NPi values. The study is reported according to the STROBE and TRIPOD statements.

The ORANGE Study has been performed according to the Helsinki Declaration and the International Conference on Harmonization for Good Clinical Practice. Since patients in a coma could not provide informed consent at the time of study recruitment, each centre referred to the local or national law on the lack of capacity. If the patients

regained capacity at the follow-up visit, they had to provide written informed consent for the use of sensitive data and follow-up, or decline research participation. The local investigators at the international study sites obtained approvals according to the local regulations. Dates of approval in the other recruiting centres and of data transfer agreements are available in the appendix (p 20). The copy of all the local ethics approvals and data transfer agreements are stored at Milano-Bicocca University and are available upon request.

Procedures

Data collected included age, gender, comorbidities, the reason for intensive care unit admission, acute clinical presentation, severity of acute brain injury, clinical information on intensive care unit interventions, intracranial pressure (whenever available), therapeutic intensity level,[17] and neuroimaging.

In the intensive care unit, most recent evidence focused on the NPi, an ordinal index ranging from 0 to 5, derived from several directly measured pupillary variables (size, latency, constriction, and dilation velocity), which are integrated by a proprietary algorithm to reflect pupillary reactivity. The behaviour is scalar, with an NPi of 1 being more pathological than an NPi of 3.[18] In concordance with previous literature, NPi scores below 3 were deemed pathological in the protocol,[16] indicating deviations from the established norms of pupillary reactivity. NPi is only minimally affected by sedative and analgesic agents in comparison with directly measured pupillometric variables,[19] and for this reason it was used as the single variable of interest for outcome associations in our study.

As part of clinical practice, pupillometry was tested at least every 4 h in both eyes by the investigators, from intensive care unit admission until day 7, because most acute problems (ie, brainstem compression due to herniation) occur in the first week after admission.

NPi-200 and (after July, 2021) NPi-300 pupillometers (NeurOptics, Irvine, CA, USA) were used in the participating centres. The two devices have a high level of agreement,[20] and can be used interchangeably.

For all included patients, we collected data in an electronic case report form developed in REDCap and hosted at the University of Milano-Bicocca. NPi data were imported automatically into the electronic case report form using smart-card technology (SmartGuard Reader; NeurOptics) to avoid errors related to manual data transfer. Data were securely held at the University of Milano-Bicocca, and all the procedures complied with the European Union Regulation 2016/679 on protecting participants regarding personal data processing and movement.

Outcomes

The co-primary endpoints of this study were functional neurological outcome and mortality, assessed at 6 months post-injury. Scores on the extended Glasgow Outcome Scale (GOSE)[21] were collected by trained personnel who were blinded to the pupillometry results, using a validated questionnaire via telephone-structured interviews with patients or family members. A poor neurological outcome was defined as a GOSE score of four or less (ie, low and upper disability, vegetative state, and death). The date and the cause of death were also collected.

Statistical analysis

Between-group comparisons were performed through the Wilcoxon or χ^2 test, as appropriate. We collected NPi values for both the right and left eye. For modelling clinical outcomes, we specifically focused on the lowest measure obtained from each assessment, which was deemed most pathological. To assess whether they were overlapping over time, we performed a longitudinal linear mixed model on delta NPis.

The association between NPi and a poor neurological outcome at 6 months was evaluated through logistic regression, adjusting for age, acute brain injury diagnosis, and motor Glasgow Coma Scale (mGCS) score on admission. We also considered alternative confounders to adjust for illness severity (ie, worst mGCS score, picking the lowest daily mGCS over the week, and pathological radiographical examinations at baseline defined as a Marshall classification[22] of three or more for traumatic brain injury, a modified Fisher[23] grade of three or more for aneurysmal subarachnoid haemorrhage, and a volume of 30 mL or greater for intracerebral haemorrhage) as supportive analysis. The longitudinal NPi values during the first week were summarised with the following quantities: the relative frequency of NPi less than 3, defined as abnormal by the protocol,[16] and the relative frequency of NPi equalling 0. The results were presented as odds ratios (ORs) and 95% CIs.

To evaluate the relationship of NPi with 6-month mortality, the extended version of the Cox regression model was used, entering NPi as a time-dependent covariate and age, acute brain injury diagnosis, and mGCS on intensive care unit admission as fixed covariates. To account for individual dynamic variations of NPi over the first week of assessment, NPi was considered in the following three ways: first, categorised in two (NPi <3 *vs* NPi ≥3, as defined by the protocol,[16] and NPi=0 *vs* NPi >0) or three levels (NPi <3 *vs* NPi 3–4 *vs* NPi ≥4); second, as a continuous variable; and third, considering the actual (NPi[t_i]) and the preceding (NPi[t_{i-1}]) NPi measurement, defining four categories according to the presence of NPi equal to 0 or not on both occasions. The assumption of proportional hazards was assessed by visually inspecting the plots of Schoenfeld residuals and using appropriate statistical tests for all covariates; the linearity of the effect for continuous variables was evaluated using splines. The results are presented as hazard ratios (HRs) and 95% CIs.

All tests were performed with a two-sided significance level of 5%, but we adjusted the NPi p values according to

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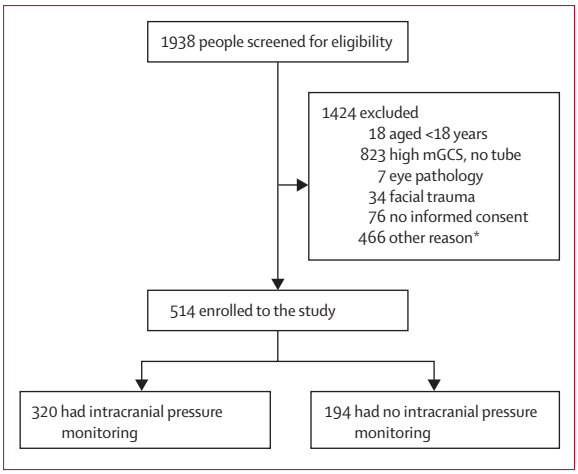

Figure 1: **Study profile**
mGCS=motor Glasgow Coma Scale. *Includes participants with a diagnosis of brain death at intensive care unit admission or withdrawal of care (n=111), participants enrolled by staff not trained to perform the study (n=73), NPi not available (n=68), primary diagnosis different from acute brain injury (n=53), management issues in NPi evaluation (n=48), no download of the SmartGuard due to infections (n=44), no possibility of long term follow-up (n=11), intubation for causes different from neurological deterioration (n=10), language barrier (n=8), participation in other clinical trials (n=4), pregnancy (n=1), and unknown (n=35).

	All (n=514)	Traumatic brain injury (n=224)	Aneurysmal subarachnoid haemorrhage (n=139)	Intracerebral haemorrhage (n=151)
Age, years	61 (46–71)	54 (34–72)	59 (51–71)	64 (54–71)
Gender				
Male	309 (60%)	170 (76%)	46 (33%)	93 (62%)
Female	205 (40%)	54 (24%)	93 (67%)	58 (38%)
Glasgow Coma Scale score				
3–5	141 (29%)	69 (33%)	38 (29%)	34 (24%)
6–8	131 (27%)	70 (33%)	21 (16%)	40 (28%)
9–15	211 (44%)	72 (34%)	71 (55%)	68 (48%)
NA	31	13	9	9
Motor Glasgow Coma Scale				
None	120 (23%)	63 (28%)	31 (22%)	26 (17%)
Extension	40 (8%)	14 (6%)	13 (9%)	13 (9%)
Abnormal flexion	27 (5%)	12 (5%)	5 (4%)	10 (7%)
Normal flexion	63 (12%)	35 (16%)	11 (8%)	17 (11%)
Localises/obeys	263 (51%)	99 (44%)	79 (57%)	85 (56%)
NA	1	1	0	0
Pupil reactivity				
Reactive	414 (82%)	186 (85%)	117 (85%)	111 (75%)
One unreactive	32 (6%)	11 (5%)	9 (7%)	12 (8%)
Both unreactive	60 (12%)	22 (10%)	12 (9%)	26 (17%)
NA	8	5	1	2
Pathological severity by radiographical examinations				
Pathological	334 (65%)	117 (52%)	121 (87%)	96 (64%)
Non-pathological	180 (35%)	107 (48%)	18 (13%)	55 (36%)

(Table 1 continues on next page)

the Benjamini-Hochberg approach to account for multiple testing. For internal validation, we fitted 100 models randomly, excluding 10% of the sample, and pooled the relative coefficients. The analyses were conducted using R statistical software (version 4.2.2).

This trial is registered with ClinicalTrials.gov, NCT04490005.

Role of the funding source

NeurOptics funded this research with an unrestricted grant to Milano-Bicocca University. The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript, or in the decision to submit for publication.

Results

Between Nov 1, 2020 and May 3, 2022, we screened 1938 patients with acute brain injury, of whom 514 (27%) were enrolled (figure 1). 224 (44%) had traumatic brain injury, 139 (27%) had aneurysmal subarachnoid haemorrhage, and 151 (29%) had intracerebral haemorrhage. The median age was 61 years (IQR 46–71), and 309 (60%) patients were male (table 1). The median Glasgow Coma Scale score on admission was 8 (IQR 5–11). The number of patients enrolled at each centre is presented in the appendix (p 7). 6-month outcomes were available for 497 (97%) patients: 206 (41%) were alive with a good neurological outcome (GOSE >4), and 291 (59%) had a poor outcome (GOSE ≤4), of whom 160 (32%) died (appendix p 7).

40 071 pupillometry examinations from both eyes were collected, with a median of 40 measurements per patient (IQR 20–50) during the study period. At baseline and over time, right and left NPi values were not significantly different. The overall distribution of NPi values is presented in figure 2A, showing a distribution with two peaks at 0 and 4·7 and a median NPi of 4·3 (IQR 3·7–4·7). The distribution of the 20 194 lowest NPi values at each timepoint is shown in figure 2B with a similar distribution, with two peaks at 0 and 4·7 and a median NPi of 4·2 (IQR 3·5–4·6). Among the NPi values, utilising the lowest measure at each assessment between the two eyes, abnormal NPi was observed at least once in 241 (47%) of 514 patients (table 2). At least one NPi equal to 0 was recorded in 132 (26%) of 514 patients (two of whom did not have a 6-month outcome), most frequently in the intracerebral haemorrhage group.

Monitoring of intracranial pressure was performed in 320 (62%) of 514 patients, accounting for a median of 46 (IQR 35–91) paired intracranial pressure and NPi measurements. When intracranial pressure exceeded 20 mm Hg, an abnormal NPi value was more frequently observed (170 [25%] of 691 measurements higher than 20 mm Hg, *vs* 1069 [13%] of 8001 measurements lower than 20 mm Hg) than for lower intracranial pressure values. NPi values equal to 0 were also more frequently observed when intracranial pressure was higher than

20 mm Hg than for lower intracranial pressure values (135 [20%] of 691, *vs* 586 [7%] of 8001).

Figure 2C illustrates the distribution of NPi values according to different GOSE outcome scores. Median NPi was lower in patients with a poor neurological outcome (4·0 [IQR 3·3–4·5]) compared with those with a good neurological outcome (4·3 [3·9–4·6]; p<0·0001). 113 (39%) of 291 patients with a poor neurological outcome had at least one NPi equal to 0, and 17 (8%) of 206 patients with a good neurological outcome had at least one NPi equal to 0 (p<0·0001). An abnormal NPi was measured at least once in 179 (62%) of 291 patients with a poor neurological outcome and in 59 (29%) of 206 patients with a good neurological outcome (p<0·0001).

The median NPi value was lower in non-survivors (3·9 [IQR 3·0–4·5]) than in survivors (4·3 [3·7–4·6]; p<0·0001); 80 (50%) of 160 non-survivors had at least one NPi equal to 0, and 50 (15%) of 337 survivors had at least one NPi value equal to 0 (p<0·0001). None of the 35 patients who had all NPi measurements equal to 0 survived (median number of measures 7 [IQR 5–10]; median time to death 2·1 days [IQR 1·3–2·5]). An abnormal NPi was measured at least once in 113 (71%) of 160 non-survivors and in 125 (37%) of 337 survivors (p<0·0001).

The analysis of the relationship of NPi with the neurological outcome is reported in table 3, and the complete results of these multivariable-adjusted models are in the appendix (pp 8–9). A 10% increase in the frequency of abnormal NPi was associated with poor neurological outcome (OR 1·42 [95% CI 1·27–1·64]; p<0·0001). Similarly, a 10% increase in the frequency of NPi equal to 0 was associated with poor neurological outcome (OR 1·70 [1·37–2·38]; p<0·0001; appendix p 9). These results were consistent when traumatic brain injury, aneurysmal subarachnoid haemorrhage, and intracerebral haemorrhage were considered separately (appendix pp 15–18). The sensitivity analyses adjusting for the worst mGCS score and pathological severity by radiographical examinations further confirmed the results (appendix p 19).

The analysis of NPi and mortality is presented in table 3, and the complete results of other multivariable-adjusted models are in the appendix (pp 10–14). After adjusting for covariates, abnormal NPi was associated with an increased risk of mortality (HR 5·58 [95% CI 3·92–7·95]; p<0·0001). An association was also found for NPi equal to 0 (12·05 [7·86–18·48]; p<0·0001; appendix p 11). A one-unit decrease in the NPi value was independently associated with a higher risk of mortality (1·80 [1·62–1·99]; p<0·0001; appendix p 12). In the analysis including individual dynamic NPi variations over time and using two consecutive values of NPi greater than 0 as the reference category, the occurrence of two successive NPi values equal to 0 was associated with an increased risk of mortality (13·92 [8·94–21·67]; p<0·0001; appendix p 13). Also, deterioration of an NPi value to 0 was associated with an increased risk of mortality (8·37 [2·52–27·87]; p=0·0007), and the risk of mortality was not increased when NPi improved from 0 to a higher value (1·32 [0·32–5·41]; p=0·70). Finally, NPi less than 3 (7·10 [4·77–10·57]; p<0·0001), and NPi values between 3 and 4 (1·70 [1·13–2·56]; p=0·0186) were associated with a higher risk of mortality than were higher NPi values (≥4; appendix p 14). Internal cross-validation showed robustness in the results of the multivariable models that were performed on both outcomes.

	All (n=514)	Traumatic brain injury (n=224)	Aneurysmal subarachnoid haemorrhage (n=139)	Intracerebral haemorrhage (n=151)
(Continued from previous page)				
Any cardiovascular disease				
Yes	248 (48%)	83 (37%)	71 (51%)	94 (62%)
No	266 (52%)	141 (63%)	68 (49%)	57 (38%)
Any endocrine disturbances				
Yes	106 (21%)	34 (15%)	30 (22%)	42 (28%)
No	408 (79%)	190 (85%)	109 (78%)	109 (72%)
Any liver diseases				
Yes	20 (4%)	6 (3%)	5 (4%)	9 (6%)
No	494 (96%)	218 (97%)	134 (96%)	142 (94%)
Any neurological diseases				
Yes	72 (14%)	23 (10%)	21 (15%)	28 (29%)
No	442 (86%)	201 (90%)	118 (85%)	123 (81%)
Any oncological diseases				
Yes	44 (9%)	14 (6%)	12 (9%)	18 (12%)
No	470 (91%)	210 (94%)	127 (91%)	133 (88%)
Any respiratory diseases				
Yes	31 (6%)	9 (4%)	5 (4%)	17 (11%)
No	483 (94%)	215 (96%)	134 (96%)	134 (89%)
Any psychiatric disturbances				
Yes	47 (9%)	26 (12%)	13 (9%)	18 (11%)
No	467 (91%)	198 (88%)	126 (91%)	133 (89%)
Any renal diseases				
Yes	15 (3%)	2 (1%)	3 (2%)	10 (7%)
No	499 (97%)	222 (99%)	136 (98%)	141 (93%)
Any eye diseases				
Yes	16 (3%)	7 (3%)	4 (3%)	5 (3%)
No	498 (97%)	217 (97%)	135 (97%)	146 (97%)

Data are n (% of available data) or median (IQR). NA=not available.

Table 1: **Baseline characteristics**

Discussion

To the best of our knowledge, the ORANGE study is the most extensive prospective study to investigate the prognostic value of NPi as a standardised quantitative measurement of pupillary reactivity and global midbrain function in patients with non-anoxic brain injury. Overall, our results strongly suggest that repeatedly abnormal NPi values, including the most extreme values of 0, in the first week after acute brain injury predict poor

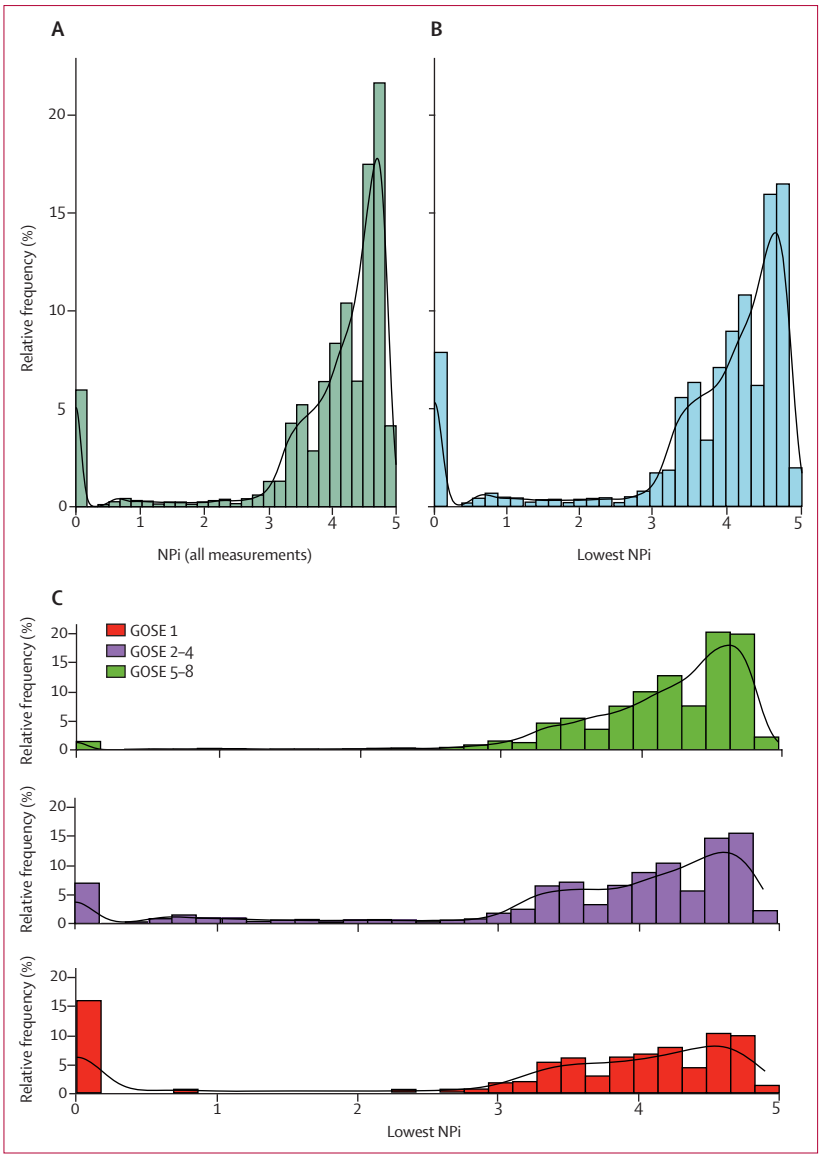

Figure 2: **Distribution of NPi measurements**
(A) Distribution of all (n=40 071) NPi measurements in 514 patients. (B) Distribution of lowest NPi measurements (n=20 194). (C) Distribution of the 19 427 lowest NPi measurements by 6-month neurological outcome (GOSE 1 [dead], n=160 patients, n=5725 NPi measurements; GOSE 2–4 [poor outcome], n=131 patients, n=5997 NPi measurements; GOSE 5–8 [good outcome], n=206 patients, n=7705 NPi measurements). GOSE=extended Glasgow Outcome Scale. NPi=Neurological Pupil index.

outcome. Abnormal NPi (<3) and NPi values of 0 were more frequently observed in patients with poor (GOSE ≤4) versus good (GOSE >4) outcomes. An increase in the number of abnormal NPi measurements over time was associated with a higher probability of poor neurological outcome. Two consecutive NPi measurements equal to 0, or deterioration of NPi to a value of 0, were associated with an increased mortality risk. By contrast, the mortality risk was not increased when an NPi value of 0 recovered to a higher value. These findings indicate the importance of the trajectories of NPi. Finally, NPi values between 3 and 4 were significantly associated with a greater risk of mortality than were NPi values greater than 4.

Findings of a few single-centre retrospective cohort studies have indicated that NPi values less than 3 on admission, defined as abnormal in our protocol, are associated with a higher likelihood of in-hospital mortality or unfavourable outcome at discharge disposition.[3,19,24–27] Patients with abnormal NPi could have either a direct brainstem injury or brainstem compression due to intracranial hypertension.[6,18] In patients suffering hypoxic-ischaemic brain injury after cardiac arrest, the most likely cause of abnormal NPi is direct and irreversible brainstem anoxia and a low NPi, which is associated with poor neurological outcome.[11,14,28] In these patients, an NPi measurement of less than 3 at least 24 h after the initial injury is highly predictive of poor outcome. Conversely, after non-anoxic acute brain injury, the brainstem injury is more likely to be secondary to brainstem compression or herniation and, therefore, could potentially be reversed by therapeutic interventions, such as osmotherapy, with subsequent improvement in NPi values.[6,29] Indeed, in literature and in our study, about two-thirds of patients with an NPi of 0 during their stay also had values greater than 3, reinforcing the importance of repeated NPi measurements when assessing coma prognostication after acute brain injury.

In patients with traumatic brain injury and aneurysmal subarachnoid haemorrhage, NPi values have been shown to correlate with the severity of the injury.[12] However, the added predictive value of NPi to baseline characteristics remains poorly defined. Our study was designed to assess the prognostic value of multiple NPi measurements over time after adequately adjusting for other known baseline predictors. We used a dataset with high data granularity, a minimum of six NPi assessments per day, an automated digital system for data downloading, and a rigorous, effective, and blindly evaluated long-term outcome follow-up, all of which added to the robustness of the current study analysis. In patients with non-anoxic brain-injuries, repeated NPi measurements are crucial for predicting outcome. Indeed, serial NPi assessments offer a more comprehensive and accurate understanding of the evolution of brain damage over time in this context.

Our approach had several advantages. Detecting NPi changes allows clinicians to monitor patient conditions and identify improvements, deteriorations, or persistent abnormal NPi values. This information can help to identify patients at high risk of poor outcome (ie, those with a high percentage of abnormal NPi measurements over time). Additionally, including reliable non-invasive neurological monitoring in clinical practice is highly beneficial, as it represents a safe alternative to invasive procedures, reducing the associated risks and complications for the patient. Also, pupillometry could be more accessible and easier to perform than invasive methods such as intracranial pressure monitoring, allowing for

broad utilisation across various health-care settings. Finally, NPi assessment can provide near real-time data, enabling health-care providers to promptly identify any neurological changes or abnormalities.

Our analysis also showed that NPi variations between two consecutive measurements have significant prognostic value, particularly in patients with NPi deterioration without recovery or with persistent NPi measurements at a value of 0. Due to the dynamic changes of NPi over time, a single abnormal measure should prompt health-care providers to retest NPi to minimise measurement errors. Furthermore, as NPi can improve over time, repeated measurements would enable clinicians to assess the efficacy of therapeutic interventions over time. Our results also offer further valuable insights into the interpretation of NPi values. Although the absence of pupillary light reactivity (ie, an NPi value of 0) is a well established indicator of poor outcome, an abnormal NPi has been previously defined as below 3. Repeated NPi measurements enhanced the sensitivity analysis and identified an NPi range between 3 and 4 already associated with an increased mortality risk. In this setting, NPi monitoring might identify at-risk patient populations that would benefit from careful intensive observation to manage secondary brain deterioration and target specific interventions before irreversible damage can occur. In this context, instead of focusing on a single measurement or cut-off, clinicians could view NPi as a tool for quantifying in a timely fashion the extent of midbrain dysfunction, ranging from very severe (NPi=0), to severe (NPi <3) and moderate (NPi 3–4). Integrating NPi with other available tools for assessing the severity of brain injury could ultimately lead to targeted and effective diagnostic and treatment strategies for patients with varying degrees of acute brain injury.

Lower NPi values were observed in cases of elevated intracranial pressure, consistent with previous reports.[30] This finding highlights the significance of NPi in evaluating patients with reduced brain tolerance to increased intracranial pressure; in particular, patients exhibiting elevated intracranial pressure alongside altered NPi values might have an increased risk of brainstem injury, necessitating immediate intervention. Conversely, patients with elevated intracranial pressure but relatively normal NPi measurements could potentially have better brain tolerance to the increased pressure levels. This hypothesis warrants further investigation in future studies, as it could represent a crucial advancement in personalised intracranial pressure management for brain-injured patients. By distinguishing between patients with different degrees of brain tolerance to increased intracranial pressure, clinicians can tailor therapeutic interventions more effectively, ensuring optimal management of each patient.

Our study has several limitations that should be acknowledged. First, the observational design and the lack of standardised treatment protocols across centres might compromise the robustness of certain results. The study strives to depict real-life situations accurately.

	All (n=514)	Traumatic brain injury (n=224)	Aneurysmal subarachnoid haemorrhage (n=139)	Intracerebral haemorrhage (n=151)
NPi first measure right eye				
Median (IQR)	4·0 (3·4–4·5)	4·0 (3·4–4·5)	4·0 (3·4–4·4)	3·9 (2·5–4·4)
n	514	224	139	151
NPi first measure left eye				
Median (IQR)	4·0 (3·4–4·5)	4·1 (3·4–4·5)	4·1 (3·4–4·5)	3·7 (3·2–4·5)
n	514	224	139	151
NPi right eye				
Median (IQR)	4·4 (3·8–4·7)	4·4 (3·9–4·7)	4·4 (3·8–4·7)	4·3 (3·6–4·6)
n	19 976	8466	6112	5398
NPi left eye				
Median (IQR)	4·3 (3·7–4·7)	4·4 (3·8–4·6)	4·4 (3·8–4·7)	4·3 (3·6–4·6)
n	20 095	8640	6124	5511
Lowest NPi value				
Median (IQR)	4·2 (3·5–4·6)	4·2 (3·6–4·6)	4·2 (3·6–4·6)	4·1 (3·4–4·5)
n	20 194	8499	6163	5532
Patients with at least one NPi=0	132 (26%)	45 (20%)	32 (23%)	55 (36%)
0	382 (74%)	179 (80%)	107 (77%)	96 (64%)
1	22 (4%)	7 (3%)	5 (4%)	10 (7%)
2	12 (2%)	7 (3%)	3 (2%)	2 (1%)
3	8 (2%)	4 (2%)	0	4 (3%)
>3	90 (18%)	27 (12%)	24 (17%)	39 (26%)
Patients with at least one NPi <3	241 (47%)	89 (40%)	70 (50%)	82 (54%)
0	273 (53%)	135 (60%)	69 (50%)	69 (46%)
1	57 (11%)	23 (10%)	17 (12%)	17 (11%)
2	22 (4%)	8 (4%)	6 (4%)	8 (5%)
3	24 (5%)	9 (4%)	6 (4%)	9 (6%)
>3	138 (27%)	49 (22%)	41 (30%)	48 (32%)

Data are n (%), unless otherwise stated. NPi=Neurological Pupil index.

Table 2: **NPi values in the first 7 days of admission to the intensive care unit**

	Effect size (95% CI)	p value
Model 1 (NPi and GOSE ≤4)		
10% increase in the frequency of NPi <3	OR 1·42 (1·27–1·64)	<0·0001
Model 2 (NPi and mortality)		
NPi <3	HR 5·58 (3·92–7·95)	<0·0001
NPi ≥3	1 (ref)	..

Table shows the association between NPi value and 6-month poor neurological outcome (GOSE ≤4; model 1) and mortality (model 2), adjusted for age, acute brain injury diagnosis, and motor Glasgow Coma Scale on admission to the intensive care unit. Model 1 included data for 497 patients, of whom 291 had a GOSE score of 4 or less. Model 2 included data for 19 427 NPi measurements, and 160 patients died. p values are Benjamini-Hochberg adjusted. The complete results for the multivariable-adjusted models (1 and 2) are shown in the appendix (pp 8–9). GOSE=extended Glasgow Outcome Scale. HR=hazard ratio. NPi=Neurological Pupil index. OR=odds ratio.

Table 3: **Results of protocol-defined outcome models**

The staff conducting the study were not blinded to the NPi evaluation because NPi evaluation is an integral part of the clinical evaluation practice. As a result, the observed NPi changes could have influenced some actions. Moreover, the blinded evaluation of NPi changes and the effect on outcome enhances the reliability of the findings. This approach reinforces the integrity of the assessment by minimising potential biases. Second, our focus was solely on NPi. We did not assess the potential value of other variables obtained from automated infrared pupillometry assessment, such as pupillary constriction or dilation velocities. However, this limitation could be seen as an advantage since NPi, contrary to pupillary constriction or dilation velocities, is only minimally influenced by sedatives and analgesics. Third, it is yet to be determined whether the measurement duration over the 7 days following admission to the intensive care unit was the most optimal approach. It remains uncertain whether the findings from our study can be extrapolated to other types of brain injuries. Finally, although our data provide robust associations between NPi and patient prognosis using a large dataset and internal cross-validation, additional confirmation of these findings in diverse settings is needed, including in centres with varying expertise in pupillometry utilisation or different protocols regarding the limitation of life-sustaining therapies. Based on this evidence, we expect that future trial designs will explore the potential of automated pupillometry as both a diagnostic tool for decision-making and an interventional tool in conjunction with standardised therapy.

In conclusion, in this prospective international multicentre study, abnormal NPi was strongly associated with long-term mortality and a poor neurological outcome after an acute brain injury, irrespective of age, primary diagnosis, and severity of cerebral damage. Repeated NPi measurements provided relevant prognostic information. Our study also identified novel NPi pathological thresholds (<4) following acute brain injury that could assist clinicians in detecting brain damage and monitoring the response to therapeutic interventions in this setting.

Contributors

MO contributed to the conceptualisation, protocol definition, funding acquisition, patient enrolment, writing of the original draft, and writing-review and editing of the revised manuscript. FST contributed to the conceptualisation, protocol definition, funding acquisition, patient enrolment, and writing-review and editing of the revised manuscript. MP contributed to the data verification, formal analysis, writing of the original draft, and writing-review and editing of the revised manuscript. RB, ABO, PB, AC, RMC, ACF, NBH, JCH, JK, FR, JIS, contributed to patient enrolment and writing-review and editing of the revised manuscript. FE contributed to patient enrolment, data verification, and writing-review and editing of the revised manuscript. AV contributed to patient enrolment, project administration, data access and verification, and writing-review and editing of the revised manuscript. PR contributed to the formal analysis, writing of the original draft, and writing-review and editing of the revised manuscript. SG contributed to the formal analysis, data access and verification, writing of the original draft, and writing-review and editing of the revised manuscript. GC contributed to the conceptualisation, protocol definition, funding acquisition, patient enrolment, data access and verification, writing of the original draft, and writing-review and editing of the revised manuscript, and participated in the manuscript preparation during his personal involvement in the Italian Minister of University MUR Dipartimenti di Eccellenza 2023–2027 (l. 232/2016, art. 1, commi 314 - 337). All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

GC reports institutional research grants from Integra and NeurOptics; and received personal fees as a speakers' bureau member and advisory board member from Integra, NeurOptics, Biogen, Idorsia, and Invex Therapeutics, all outside the submitted work; and is the Editor-in-Chief of *Intensive Care Medicine*. FST received consulting and lecture fees from NeurOptics; and received personal fees as an Advisory Board Member from NeurOptics, all outside the submitted work. MO received fee payments for consultancy roles for NeurOptics and honoraria for lectures by NeurOptics; received personal fees as an advisory board member from NeurOptics, all unrelated to the submitted work; and received institutional grants from the Swiss National Science Foundation. JIS reported personal fees as a member of the Clinical Endpoint Committee for the REACT Study funded by Idorsia; is member of the data safety and monitoring board for the INTREPID study; is a member of the board of directors of the Neurocritical Care Foundation and the Neurocritical Care Society; and is member of the editorial board of *Stroke*. NB-H received honoraria for lectures from ORION Pharma. ABO received honoraria for educational events from BD, support for attending meetings from Pfizer, and received equipment from NeurOptics. PB received honoraria for lectures from LFB Company and equipment from NeurOptics. RMC received institutional grants from the National Institutes of Health (National Institute of Child Health and Human Development; Fogarty International Centre). SG participated as a data safety and monitoring board member at Division of Cell Matrix Biology and Regenerative Medicine, University of Manchester, without payment. All other authors declare no competing interests.

Data sharing

Data supporting the study's findings are available on reasonable request after approval of a proposal from the corresponding author (GC). Data collected for the analysis, including deidentified individual participant data and a data dictionary defining each field in the set, will be made available to others on request. Related documents such as the study protocol, statistical analysis plan, and informed consent form will also be available on request. The University of Milano-Bicocca is the study sponsor and has the property of the data collected.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Oddo M, Taccone FS, Petrosino M, et al. The Neurological Pupil index for outcome prognostication in people with acute brain injury (ORANGE): a prospective, observational, multicentre cohort study. *Lancet Neurol* 2023; published online Aug 28. https://doi.org/10.1016/S1474-4422(23)00271-5.

Supplemental Material

The Neurological Pupil index for outcome prognostication in people with acute brain injury (ORANGE): a prospective, observational, multicentre cohort study

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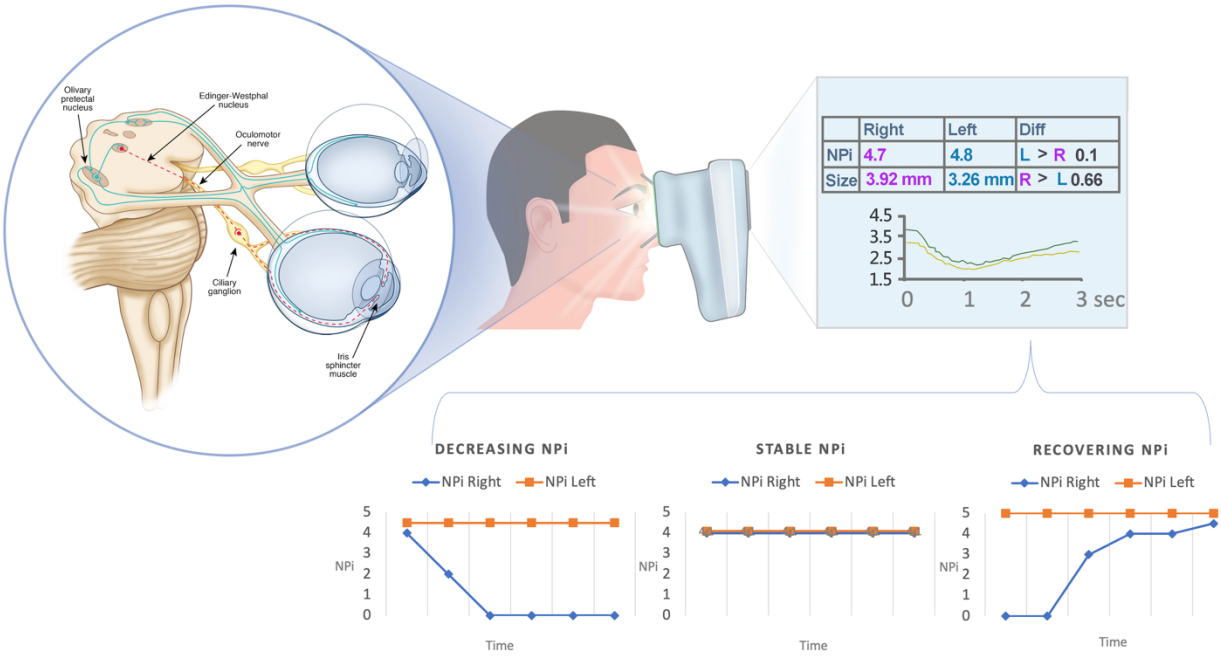
DRAWING OF THE DEVICE

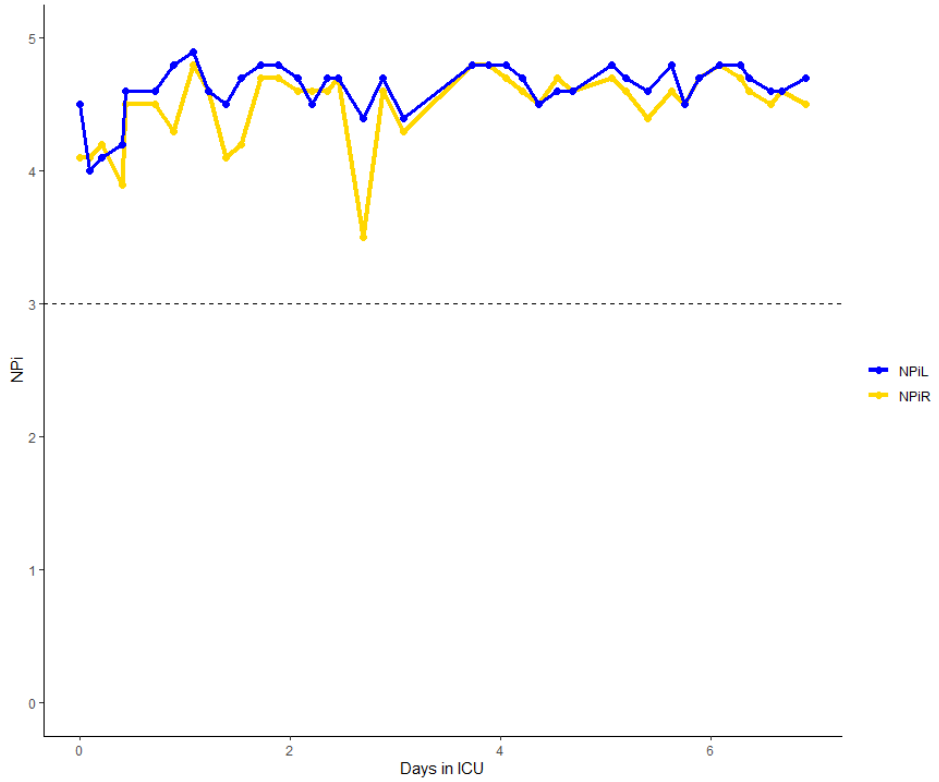
Anatomy of the pupillary light reflex and visual representation of the pupillary light response on a pupillometer screen are depicted. Once light stimulates the retina, a neural signal travels through the optic nerve to the pretectal area of the midbrain and reaches the pretectal olivary nucleus (OPN). From there, fibers extend to the Edinger-Westphal nucleus. This impulse activates parasympathetic preganglionic cells, which travel along the oculomotor nerve and stimulate the ciliary ganglion. The postganglionic fibers of the ciliary ganglion innervate the iris sphincter muscle, leading to pupillary constriction. The fibers from the OPN on each side also activate the Edinger-Westphal nuclei on both sides, ensuring a bilateral (consensual) pupillary reflex.

The pupillary response curve, known as the pupillogram, represents the changes in pupillary diameter before, during, and after light stimulation.

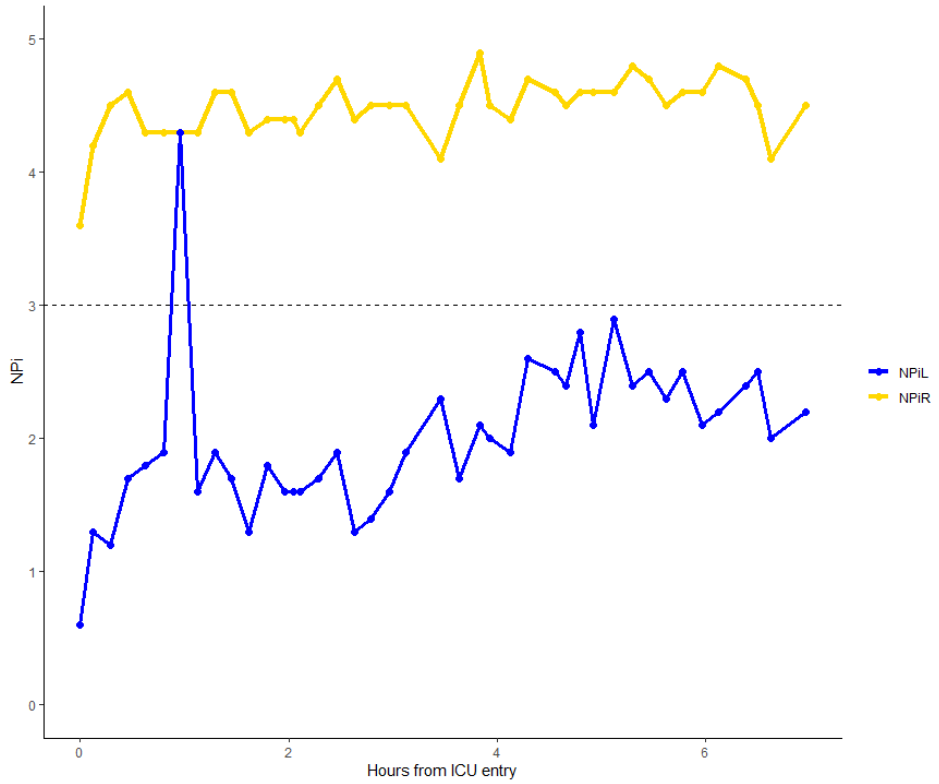
The pupillometer condenses these variables into a proprietary parameter called the neurological pupil index (NPi), with values ranging from 0 to 5. Modern pupillometers not only display a graphical representation of the change in pupillary size during the test, referred to as the pupillogram but they also offer a video playback of the elicited pupillary reflex. Furthermore, they store patient data and provide a trend of the NPi values over time, allowing clinicians to track the patient's clinical trajectory. As a portable and non-invasive monitoring tool, the pupillometer proves useful at the bedside.



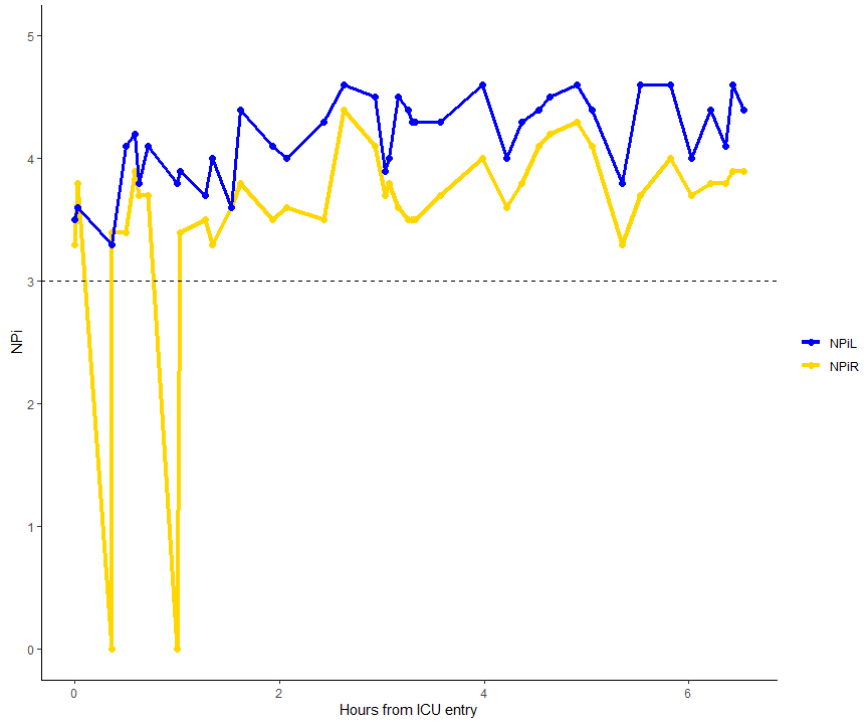
EXEMPLARY RECORDINGS OF NPi TRACING IN REAL PATIENTS IN THE FIRST WEEK



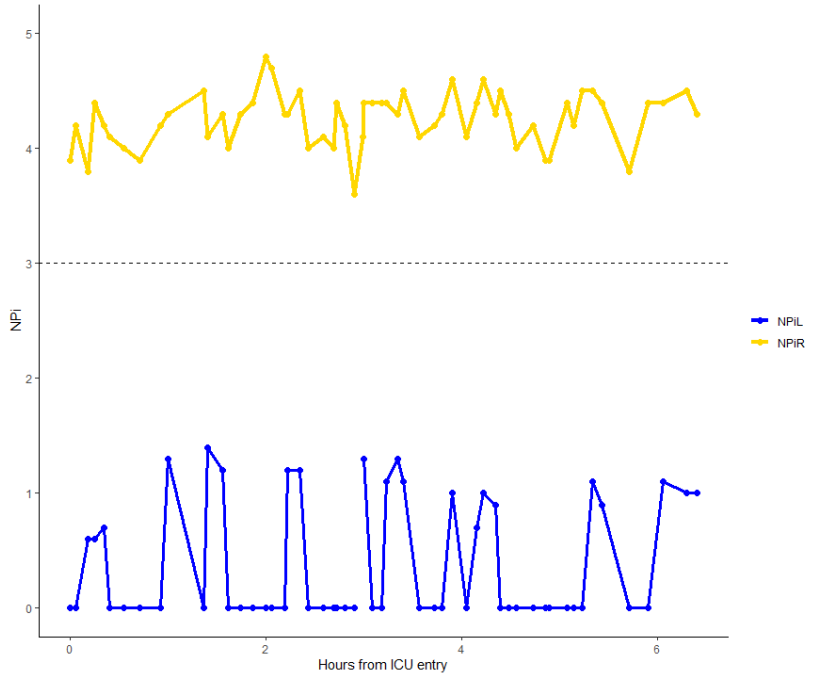
Trace 1 Tracing of the pupillometry of the two eyes (different colors). All the data are in the range 3-5.



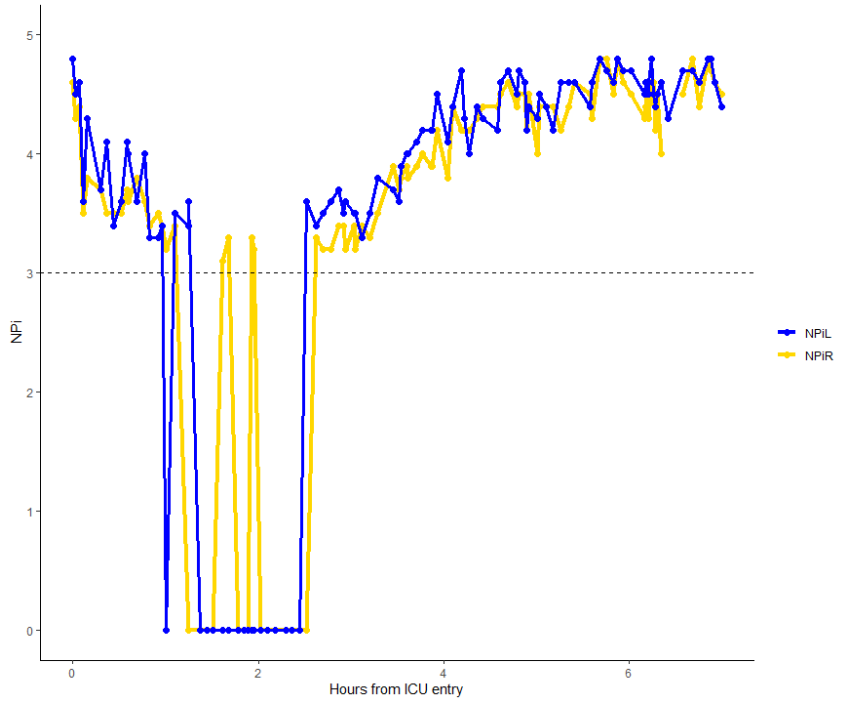
Trace 2 Tracing of the pupillometry of the two eyes (different colors). Left eye had a lower NPi in all the examinations.



Trace 3 Tracing of the pupillometry of the two eyes (different colors). Right eye had 2 episodes of 0 and returned in a range > 3.



Trace 4 Tracing of the pupillometry of the two eyes (different colors). Left eye remained between 0 and 2 during all the examinations.



Trace 5 Tracing of the pupillometry of the two eyes (different colors). Both eyes presented a falling NPi that returned in an area > 3-4

Supplemental Table 1. Number of patients enrolled in each centre.

Centre	Number of enrolled patients
Monza, Italy	118
Lausanne, Switzerland	80
Roma, Italy	62
Oslo, Norway	51
Erlangen, Germany	46
Grenoble, France	41
Bruxelles, Belgium	27
Madrid, Spain	25
Brescia, Italy	17
Washington, US	16
UCSF, US	13
John Hopkins, US	10
Valencia, Spain	8

Supplemental Table 2. Six-month outcomes.

Mortality, overall and divided by pathology, and 6 months neurological outcome evaluated with the GOSE.

		ALL (n=514)	TBI (n=224)	SAH (n=139)	ICH (n=151)
Mortality, n (%)	Survivors	337 (68)	155 (72)	97 (71)	85 (59)
	Non-survivors	160 (32)	61 (28)	39 (29)	60 (41)
	NA	17	8	3	6
Neurological outcome, n (%)	Poor (GOS-E 1-4)	291 (59)	106 (49)	79 (58)	106 (73)
	Good (GOS-E 5-8)	206 (41)	110 (51)	57 (42)	39 (27)
	NA	17	8	3	6

**17 patients were lost to follow-up

Supplemental Table 3. Model 1.

Logistic regression model's odds ratios with 95% CI of GOSE at 6-month follow-up for 10% increment for frequency of NPi<3, age, ABI diagnosis (Subarachnoid haemorrhage, Intracranial haemorrhage, Traumatic Brain Injury), GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.04 (0.02-0.10)	<0.0001
Age	1.04 (1.03-1.05)	<0.0001
NPi		
10% increment for frequency of NPi<3	1.42 (1.27-1.64)	*<0.0001
Diagnosis		
Subarachnoid haemorrhage	1.32 (0.80-2.18)	0.2735
Intracranial haemorrhage	2.29 (1.36-3.89)	0.0021
Traumatic Brain Injury	1	..
GCS Motor Score		
None	1.87 (1.07-3.32)	0.0306
Extension	2.84 (1.15-7.56)	0.0284
Abnormal flexion	1.02 (0.39-2.66)	0.9680
Normal flexion	1.88 (0.98-3.71)	0.061
Localizes/obeys	1	..
Number of patients = 497, GOSE<=4 = 291		

* Benjamini-Hochberg adjusted

Supplemental Table 4. Model 2.

Logistic regression model's odds ratios with 95% CI of GOSE at 6-month follow-up for 10% increment for frequency of NPi=0, age, ABI diagnosis (Subarachnoid hemorrhage, Intracranial hemorrhage, Traumatic Brain Injury), GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.06 (0.02-0.13)	<0.0001
Age	1.04 (1.03-1.05)	<0.0001
NPi		
10% increment for frequency of NPi=0	1.70 (1.37-2.38)	*<0.0001
Diagnosis		
Subarachnoid haemorrhage	1.33 (0.81-2.19)	0.2548
Intracranial haemorrhage	2.27 (1.36-3.85)	0.0020
Traumatic Brain Injury	1	..
GCS Motor Score		
None	2.02 (1.16-3.57)	0.0143
Extension	2.75 (1.12-7.22)	0.0320
Abnormal flexion	1.14 (0.44-2.92)	0.7830
Normal flexion	1.83 (0.95-3.57)	0.0728
Localizes/obeys	1	..
Number of patients = 497, GOSE<=4 = 291		

* Benjamini-Hochberg adjusted

Supplemental Table 5. Model 3.

Extended Cox regression model's hazard ratios with 95% CI of mortality at 6-month follow-up for NPi over the first week categorized in two (using a cut-off of 3), age, ABI diagnosis (Subarachnoid haemorrhage, Intracranial haemorrhage, Traumatic Brain Injury), GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Hazard ratio (95% CI)	**p-values**
Age	1.05 (1.04-1.07)	<0.0001
NPi		
$NPi(t_i)<3$	5.58 (3.92-7.95)	*<0.0001
$NPi(t_i)>=3$	1	..
Diagnosis		
Subarachnoid haemorrhage	0.78 (0.52-1.18)	0.2353
Intracranial haemorrhage	1.20 (0.83-1.75)	0.3287
Traumatic Brain Injury	1	..
GCS Motor Score		
None	1.92 (1.28-2.85)	0.0014
Extension	2.87 (1.70-4.83)	<0.0001
Abnormal flexion	1.26 (0.61-2.61)	0.5248
Normal flexion	1.72 (1.04-2.85)	0.0341
Localizes/obeys	1	..
n= 19427, number of deaths= 160		

* Benjamini-Hochberg adjusted

Supplemental Table 6. Model 4.

Extended Cox regression model's hazard ratios with 95% CI of mortality at 6-month follow up for NPi over the first week categorized in two (using a cut-off of 0), age, ABI diagnosis (Subarachnoid haemorrhage, Intracranial haemorrhage, Traumatic Brain Injury), GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Hazard ratio (95% CI)	**p-values**
Age	1.05 (1.04-1.07)	<0.0001
NPi		
NPi(t_i)=0	12.05 (7.86-18.48)	*<0.0001
NPi(t_i)>0	1	-
Diagnosis		
Subarachnoid haemorrhage	0.88 (0.59-1.32)	0.5439
Intracranial haemorrhage	1.14 (0.78-1.65)	0.5015
Traumatic Brain Injury	1	..
GCS Motor Score		
None	1.46 (0.95-3.96)	0.0828
Extension	2.28 (1.34-3.90)	0.0025
Abnormal flexion	1.95 (0.96-3.96)	0.0655
Normal flexion	1.52 (0.91-2.51)	0.1061
Localizes/obeys	1	..
n= 19427, number of deaths= 160		

* Benjamini-Hochberg adjusted

Supplemental Table 7. Model 5.

Extended Cox regression model's hazard ratios with 95% CI of mortality at 6-month follow-up for NPi over the first week as a continuous variable, age, ABI diagnosis (Subarachnoid hemorrhage, Intracranial hemorrhage, Traumatic Brain Injury), GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Hazard ratio (95% CI)	**p-values**
Age	1.05 (1.04-1.07)	<0.0001
NPi		
NPi(t_i)	1.80 (1.62-1.99)	*<0.0001
Diagnosis		
Subarachnoid haemorrhage	0.79 (0.53-1.19)	0.2640
Intracranial haemorrhage	1.05 (0.72-1.54)	0.7813
Traumatic Brain Injury	1	..
GCS Motor Score		
None	1.30 (0.84-1.99)	0.2345
Extension	2.24 (1.32-3.83)	0.0030
Abnormal flexion	1.50 (0.73-3.05)	0.2680
Normal flexion	1.49 (0.90-2.47)	0.1234
Localizes/obeys	1	..
n= 19427, number of deaths= 160		

* Benjamini-Hochberg adjusted

Supplemental Table 8. Model 6.

Extended Cox regression model's hazard ratios with 95% CI of mortality at 6-month follow up for NPi over the first week considering the actual [NPi(ti)] and the preceding [NPi(t_{i-1})] NPi, defining four categories according to a cut-off of 0 on both, age, ABI diagnosis (Subarachnoid haemorrhage, Intracranial haemorrhage, Traumatic Brain Injury), GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Hazard ratio (95% CI)	**p-values**
Age	1.05 (1.04-1.07)	<0.0001
NPi		
NPi(t_{i-1})=0 & NPi(t_i)=0	13.92 (8.94-21.67)	*<0.0001
NPi(t_{i-1})=0 & NPi(t_i)>0	1.32 (0.32-5.41)	*0.6995
NPi(t_{i-1})>0 & NPi(t_i)=0	8.37 (2.52-27.87)	*0.0007
NPi(t_{i-1})>0 & NPi(t_i)>0	1	..
Diagnosis		
Subarachnoid haemorrhage	0.89 (0.59-1.34)	0.5787
Intracranial haemorrhage	1.07 (0.73-1.56)	0.7205
Traumatic Brain Injury	1	..
GCS Motor Score		
None	1.30 (0.83-2.02)	0.2480
Extension	2.16 (1.25-3.73)	0.0059
Abnormal flexion	2.01 (0.99-4.09)	0.0533
Normal flexion	1.57 (0.95-2.58)	0.0762
Localizes/obeys	1	..
n =18947, number of deaths =156		

* Benjamini-Hochberg adjusted

Supplemental Table 9. Model 7.

Extended Cox regression model's hazard ratios with 95% CI of mortality at 6-month follow up for NPi over the first week categorized in three levels (<3, 3-4 and 4-5), age, ABI diagnosis (Subarachnoid haemorrhage, Intracranial haemorrhage, Traumatic Brain Injury), GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Hazard ratio (95% CI)	**p-values**
Age	1.06 (1.04-1.07)	<0.0001
NPi		
$0 <= NPi(t_i) < 3$	7.10 (4.77-10.57)	*<0.0001
$3 <= NPi(t_i) < 4$	1.70 (1.13-2.56)	*0.0186
$NPi(t_i) >= 4$	1	..
Diagnosis		
Subarachnoid haemorrhage	0.74 (0.49-1.12)	0.1519
Intracranial haemorrhage	1.11 (0.76-1.62)	0.5855
Traumatic Brain Injury	1	..
GCS Motor Score		
None	1.70 (1.13-2.56)	0.0102
Extension	2.68 (1.59-4.52)	0.0002
Abnormal flexion	1.20 (0.58-2.48)	0.6256
Normal flexion	1.72 (1.05-2.83)	0.0315
Localizes/obeys	1	..
n= 19427, number of deaths= 160		

* Benjamini-Hochberg adjusted

Supplemental Table 10. Model 8.

Logistic regression model's odds ratios with 95% CI for **TBI patients** of GOSE at 6-month follow-up for 10% increment for frequency of NPi<3, age, GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.03 (0.01-0.11)	<0.0001
Age	1.04 (1.03-1.07)	<0.0001
NPi		
10% increment for frequency of NPi<3	1.51 (1.23-1.84)	<0.0001
GCS Motor Score		
None	2.02 (0.90-4.54)	0.0884
Extension	4.21 (1.03-17.10)	0.0445
Abnormal flexion	0.46 (0.08-2.55)	0.3743
Normal flexion	1.48 (0.59-3.73)	0.4054
Localizes/obeys	1	..
Number of patients = 216, GOSE<=4 = 106		

Supplemental Table 11. Model 9.

Logistic regression model's odds ratios with 95% CI for **TBI patients** of GOSE at 6-month follow-up for 10% increment for frequency of NPi=0, age, GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.05 (0.02-0.16)	<0.0001
Age	1.04 (1.03-1.06)	<0.0001
NPi		
10% increment for frequency of NPi=0	1.42 (1.27-1.64)	0.0056
GCS Motor Score		
None	2.32 (1.06-5.09)	0.0358
Extension	3.53 (0.89-14.00)	0.0728
Abnormal flexion	0.45 (0.08-2.48)	0.3581
Normal flexion	1.40 (0.57-3.45)	0.4654
Localizes/obeys	1	..
Number of patients = 216, GOSE<=4 = 106		

Supplemental Table 12. Model 10.

Logistic regression model's odds ratios with 95% CI for **SAH patients** of GOSE at 6-month follow-up for 10% increment for frequency of NPi=0, age, GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.06 (0.01-0.39)	0.0030
Age	1.04 (1.01-1.07)	0.0064
NPi		
10% increment for frequency of NPi<3	1.37 (1.06-1.78)	0.0169
GCS Motor Score		
None	2.17 (0.76-6.17)	0.1467
Extension	3.18 (0.58-17.35)	0.1817
Abnormal flexion	1.93 (0.26-14.12)	0.5172
Normal flexion	4.82 (0.94-24.78)	0.0593
Localizes/obeys	1	..
Number of patients = 136, GOSE<=4 = 79		

Supplemental Table 13. Model 11.

Logistic regression model's odds ratios with 95% CI for **SAH patients** of GOSE at 6-month follow-up for 10% increment for frequency of NPi=0, age, GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.08 (0.01-0.48)	0.0058
Age	1.04 (1.01-1.07)	0.0097
NPi		
10% increment for frequency of NPi=0	1.38 (0.99-1.92)	0.0610
GCS Motor Score		
None	2.43 (0.87-6.80)	0.0911
Extension	3.90 (0.75-20.32)	0.1060
Abnormal flexion	2.12 (0.30-15.10)	0.4526
Normal flexion	4.74 (0.93-24.17)	0.0611
Localizes/obeys	1	..
Number of patients = 136, GOSE<=4 = 79		

Supplemental Table 14. Model 12.

Logistic regression model's odds ratios with 95% CI **for ICH patients** of GOSE at 6-month follow-up for 10% increment for frequency of NPi<3, age, GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.18 (0.03-1.32)	0.0925
Age	1.04 (1.00-1.07)	0.0342
NPi		
10% increment for frequency of NPi<3	1.33 (1.08-1.64)	0.0066
GCS Motor Score		
None	1.12 (0.33-3.48)	0.8582
Extension	1.42 (0.26-7.70)	0.6833
Abnormal flexion	1.57 (0.28-8.76)	0.6070
Normal flexion	1.69 (0.42-6.82)	0.4625
Localizes/obeys	1	..
Number of patients = 145, GOSE<=4 = 106		

Supplemental Table 15. Model 13.

Logistic regression model's odds ratios with 95% CI for **ICH patients** of GOSE at 6-month follow-up for 10% increment for frequency of NPi=0, age, GCS Motor (no motor response, extension to pain, abnormal flexion, normal, localizes/obey).

	Odds ratio (95% CI)	**p-values**
Intercept	0.14 (0.02-1.10)	0.0612
Age	1.04 (1.01-1.08)	0.0223
NPi		
10% increment for frequency of NPi=0	3.05 (1.05-8.85)	0.0403
GCS Motor Score		
None	0.99 (0.28-3.53)	0.9848
Extension	0.99 (0.16-5.91)	0.9874
Abnormal flexion	2.01 (0.35-11.39)	0.4314
Normal flexion	1.65 (0.40-6.79)	0.4896
Localizes/obeys	1	..
Number of patients = 145, GOSE<=4 = 106		

Supplemental Table 16. Model 14.

Logistic regression model's odds ratios with 95% CI of GOSE at 6-month follow-up for 10% increment for frequency of NPi<3, age, pathological severity by radiographic examinations, worst GCS motor score (no motor response, extension to pain/abnormal flexion, normal, localizes/obey).

	Odds Ratio (95 % CI)	**p-values**
Age	1.04 (1.03-1.06)	<0.0001
NPi		
- 10% increase in the frequency of NPi<3	1.43 (1.26-1.62)	<0.0001
Pathological severity by radiographic examinations		
- Pathologic	1.44 (0.93-2.22)	0.1005
- Nnon-Pathologic	1	..
Worst GCS Motor Score		
- None	2.26 (1.42-3.61)	0.0006
- Extension/Abnormal	2.90 (0.93-9.01)	0.0658
- Normal	2.90 (1.05-7.98)	0.0391
- Localizes/Obeys	1	..
Number of patients = 497, GOSE<=4 = 291		

Supplemental Table 17. Model 15.

Logistic regression model's odds ratios with 95% CI of GOSE at 6-month follow-up for 10% increment for frequency of NPi=0, age, pathological severity by radiographic examinations, worst GCS motor score (no motor response, extension to pain/abnormal flexion, normal, localizes/obey).

	Odds Ratio (95 % CI)	**p-values**
Age	1.04 (1.03-1.06)	<0.0001
NPi		
- 10% increase in the frequency of NPi=0	1.75 (1.32-2.32)	<0.0001
Pathological severity by radiographic examinations		
- Pathologic	1.35 (0.88-2.07)	0.1637
- Non Pathologic	1	..
Worst GCS Motor Score		
- None	2.13 (1.35-3.38)	0.0012
- Extension/Abnormal	3.07 (1.00-9.41)	0.0496
- Normal	3.48 (1.27-9.56)	0.0155
- Localizes/Obeys	1	..
Number of patients = 497, GOSE<=4 = 291		

Local ethical approvals and Data Transfer Agreements with the University Milano-Bicocca

PRINCIPAL INVESTIGATOR		HOSPITAL	TYPE OF INSTITUTION	IRB APPROVAL DATE	DTA FINALIZATION DATE
GIUSEPPE CITERIO	ITALY	Fondazione IRCCS San Gerardo dei Tintori, Monza	Academic/Teaching hospital	16/07/20	//
RAFAEL BADENES	SPAIN	Hospital Clinic Universitari de Valencia, University of Valencia, Valencia	Academic/Teaching hospital	17/12/20	04/06/21
J. CLAUDE HEMPHILL	USA	University of California San Francisco, San Francisco, CA	Academic/Teaching hospital	29/01/21	27/01/21
KJETIL SUNDE	NORWAY	Oslo University Hospital Ullevål, Oslo	Academic/Teaching hospital	10/02/21	12/05/21
PIERRE BOUZAT	FRANCE	Grenoble-Alpes University Hospital (CHU), Grenoble	Academic/Teaching hospital	19/03/21	10/05/21
ANSELMO CARICATO	ITALY	Fondazione Policlinico Universtario "A. Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Roma	Academic/Teaching hospital	15/04/21	08/07/21
RANDAL M. CHESNUT	USA	Harborview Medical Center, University of Washington, Seattle, Washington	Academic/Teaching hospital	20/04/21	23/08/21
STEFAN SCHWAB	GERMANY	University of Erlangen-Nuremberg, Erlangen	Academic/Teaching hospital	06/05/21	04/10/21
MAURO ODDO	SWITZERLAND	CHUV-Lausanne University Hospital and University of Lausanne, Lausanne	Academic/Teaching hospital	03/06/21	20/05/21
AARON BLANDINO	SPAIN	Ramón Y Cajal University Hospital, Madrid	Academic/Teaching hospital	09/07/21	13/10/21
JOSE' IGNACIO SUAREZ	USA	The Johns Hopkins University School of Medicine, Baltimore, Maryland	Academic/Teaching hospital	20/07/21	22/11/21
FRANK RASULO	ITALY	Spedali Civili University Affiliated Hospital of Brescia	Academic/Teaching hospital	10/11/21	17/02/22
FABIO SILVIO TACCONE	BELGIUM	Hôpital Universitaire de Bruxelles (HUB), Université Libre de Bruxelles, Bruxelles	Academic/Teaching hospital	07/02/22	23/03/22

The copy of all the Local ethical approvals and Data Transfer Agreements are safely stored at Milano-Bicocca University and are available upon request.