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Persistence of NPi differential predicts outcome at intensive care unit discharge: a single-center retrospective analysis

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Abstract

This retrospective study explores the prognostic significance of the frequency and persistence of Neurological Pupil index (NPi) differentials (NPi-d) in patients with acute brain injury admitted to the intensive care unit. A total of 57 patients with traumatic brain injury, subarachnoid hemorrhage, or intracerebral hemorrhage were included, all of whom underwent serial quantitative pupillometry. An NPi-d was defined as a difference of ≥ 0.7 between the NPi of the two pupils, while the persistence of the differential (pNPi-d) captured sustained asymmetry over time, regardless of whether the threshold was reached. The analysis revealed that both the number of NPi-d events and the pNPi-d were significantly associated with unfavorable neurological outcomes and mortality at intensive care unit (ICU) discharge. Multinomial logistic regression showed that an increased number of NPi-d was associated with a more than threefold increased risk of poor outcome and death. Similarly, higher pNPi-d values correlated with greater odds of poor outcome, death, elevated need for therapeutic interventions to manage intracranial hypertension, decompressive craniectomy, and longer ICU stay. These findings suggest that, beyond static NPi thresholds, dynamic measures such as the frequency and persistence of NPi asymmetries provide valuable prognostic information. Incorporating these pupillometry-based metrics into routine neuromonitoring could enhance early risk stratification and guide treatment strategies in critically ill neurological patients.

Keywords Neurological Pupil index, Pupillar light reflex, Acute brain injuries, Prognosis, NPi differential

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Introduction

Several studies have demonstrated an association between the Neurological Pupil Index (NPi) and neurological outcomes in patients with acute brain injury (ABI) [1–5]. An NPi value below the normative value of 3 in at least one eye during the first days following ABI is associated with poor neurological outcomes at discharge and during long-term follow-up [6–8]. Furthermore, a decrease in NPi values during an ICU stay can predict neurological deterioration [9].

Although the prognostic value of the NPi is well-established, only a few studies have explored whether asymmetry in NPi between the two eyes, referred to as the NPi differential, could serve as an early prognostic tool in critically ill patients with ABI [10, 11]. Privitera et al. [10] demonstrated that patients with ABI who experienced at least one episode of NPi differential (NPi-d), with a difference between the NPi values of the two eyes ≥ 0.7 , even when the individual NPi values were within the normal range above 3, had a higher modified Rankin Scale (mRS) score at discharge.

In this study, we aim to further investigate these findings by examining additional characteristics of the NPi differential that have not yet been explored. The first is the frequency or number of differential episodes, to determine if this has prognostic significance. Additionally, we aim to analyze the trend of the differential over time, rather than focusing solely on the absolute difference at a specific moment. Specifically, we want to evaluate whether the persistence of asymmetry—where one pupil's NPi remains consistently smaller than the other over a prolonged period—even when the difference does not reach the 0.7 threshold, may also indicate a poor outcome.

Finally, we explore the possibility to quantify the amount of this difference over time, with a novel quantitative index that we term persistency of NPi differential (pNPi-d).

Methods

Study design and setting

This is a retrospective, observational, cohort study conducted at the General Intensive Care Unit (ICU) and Neurointensive Care Unit (NICU) of the Sant'Anna Hospital in Como (ASST Lariana), with a data collection period spanning from 2020 to 2024 (approximately 46 months).

The study documentation was submitted to the competent Ethics Committee (CET 5), which did not issue

a negative opinion within the timeframe established by current regulations. Therefore, in accordance with the applicable legal provisions for retrospective observational studies, the absence of a formal objection was interpreted as tacit approval (silence-assent principle).

The study design, the execution and the report meet the STROBE criteria (STrengthening the Reporting of OBservational Studies in Epidemiology, also known as STROBE).

The inclusion criteria were patients aged ≥ 18 years; presence of traumatic brain injury (TBI), or spontaneous subarachnoid hemorrhage (SAH), or intraparenchymal hemorrhage (ICH); spontaneous coma with Glasgow Coma Scale (GCS) ≤ 8 or rapid neurological deterioration requiring orotracheal intubation for airway protection. More specifically, rapid neurological deterioration describes a patient who, within minutes, loses airway or ventilatory competence, or develops signs consistent with increased intracranial pressure or brainstem compromise (such as Cushing's reflex). In these situations, there is no time for a structured neurological reassessment, and the GCS is typically in the low 3 s. Note that among all subjects for whom a GCS was determined at enrollment, values ranged from 3 to 12, with a mean of 7 and an interquartile range (IQR) of 5.3–8.8. All eligible patients were required to have bilateral NPi recordings during the first ten days of their ICU/NICU stay, as part of the routine clinical practice.

Exclusion criteria were non-reactive bilateral mydriasis at presentation and/or GCS value of 3 performed after stabilization of vital parameters and without sedation, thus reflecting the patient's spontaneous neurological status; clinical signs of brain death at ICU admission; primary decompressive craniectomy; penetrating skull injuries; chronic or active injuries of the eye or ophthalmologic pathologies, including trauma to the orbit; hemorrhagic infarction in ischemic stroke; primary or metastatic brain tumors; central nervous system infections and cardiac arrest. To minimize confounding factors, patients with pupillary areflexia (NPi=0) in one or both eyes were excluded from the analysis.

Additionally, to ensure an adequate volume of pupillometry data for all patients and to minimize variability within the population, patients with fewer than five total pupillometry measurements were excluded from the statistical analysis. Finally, to avoid further confounding, patients who developed a central nervous system infection during their ICU stay (e.g., post-external ventricular drainage infection, as confirmed by cerebrospinal fluid culture) were also excluded from the analysis.

Data collection

Clinical records included age, sex, Body Mass Index (BMI), comorbidities, diagnosis at ICU admission, use of antiplatelet and anticoagulant medications, neurological status at ICU admission and discharge assessed by the GCS, ICU length of stay and discharge department.

Additionally, mean arterial pressure (MAP), heart rate (HR), oxygen saturation (SpO₂), body temperature and partial pressure of oxygen PaO₂, intracranial pressure (ICP) and cerebral perfusion pressure (CPP) were recorded every 2 h. The therapeutic intensity level (TIL) was documented in accordance with guidelines [12–14]. The presence of seizures, confirmed by electroencephalogram (EEG) or continuous EEG monitoring (cEEG) and all neuroimaging findings were also recorded. These data were used to compare the three-outcome population (see below).

All data were anonymously extracted from MargheritaTre, the electronic medical record system powered by GiViTi, used in our ICU/NICU.

Quantitative pupillometry was performed bilaterally using the NeuroOptics® NPi®–200 pupillometer. An NPi differential (NPi-d ≥ 0.7) was defined, in accordance with [10], as a difference of 0.7 or greater between the left and the right NPi.

We define the persistency of the NPi differential (pNPi-d) as the time integration of the differentials of all paired measurements in which the sign of the difference (indicating the eye with the lower NPi) remains the same, reflecting a persistently lower NPi in one eye. In other words, if the differential is defined as *left NPi* – *right NPi*, the sign of this difference must remain constant over consecutive measurements—negative when the right NPi is larger and positive when the left NPi is larger—for those measurements to be included in the persistency calculation. The pNPi-d does not account for the absolute value of the difference but

rather its persistency (of the sign) over time, even when the difference is below the threshold of 0.7 previously proposed as pathological [10]—and it must be present in at least two consecutive measurements.

For example, if two consecutive differentials are NPi-d₁ = 0.3 and NPi-d₂ = 0.4, and in both cases the left pupil NPi is greater than the right pupil NPi, and these two measurements are taken 2 h apart, then the amount of persistency for this pair of measurements is calculated as the mean of the two differentials (0.35) multiplied by the time interval (2 h), resulting in 0.7. If the next measurement also has a differential (NPi-d₃) with the same sign (left NPi still larger), the same average area is calculated between NPi-d₂ and NPi-d₃. The final pNPi-d is then obtained by summing all these averaged areas. The unit of persistency is, in theory, the product of time (hours) and the NPi differential. However, since NPi itself is a statistical score without a physical unit, we prefer not to assign a unit to persistency and instead refer to it simply as a unitless measure. Lastly, differentials smaller than 0.2 are not included in the persistency calculation.

An example of persistence is shown in the left panel (Fig. 1); the red line represents the right NPi, and the blue line represents the left NPi. The gray vertical bar on the left indicates an NPi-d differential where the right NPi is equal to or greater than 0.7 lower than the left pupil. The shaded red areas indicate intervals where the right pupil is consistently weaker than the left pupil, even if the difference does not reach the critical threshold of 0.7. The final value of the pNPi-d is simply the total summed area of all these red-shaded areas.

Outcomes at the ICU/NICU discharge were categorized as Good outcome, Glasgow (GCS 15–14); Poor outcome, (GCS between 3 and 13); and Death, in accordance with prior studies [15, 16].

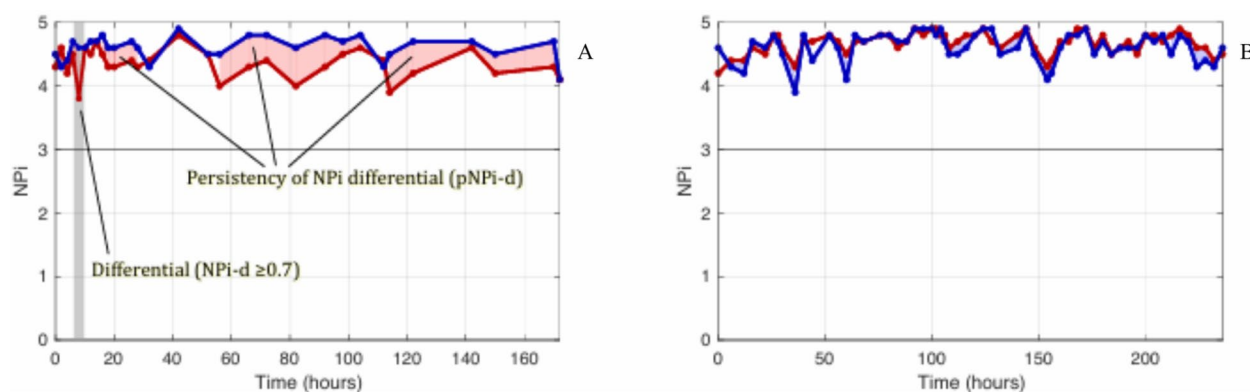


Fig. 1 The right and left NPi values are shown on the same time axis, with red representing the right pupil and blue representing the left pupil (A). The initial gray vertical bar on the left indicates a differential where the right NPi is equal to or greater than 0.7 lower than the left pupil. The shaded red areas represent intervals where the right pupil is consistently lower than the left pupil, even if the difference does not reach the critical threshold of 0.7. The final value of the persistence is calculated as the sum of all these red-shaded areas. For comparison, a patient with no differentials and no significant persistence is shown (B)

Endpoints and statistical analysis

The primary endpoint of the study was to assess the association between the number of NPi differentials (NPi-d), persistence of differential (pNPi-d) and outcomes at ICU discharge. Secondary endpoints included the association between the persistence of differential (pNPi-d) and (i) development of intracranial hypertension (HICP) and therapeutic intensity level (TIL) required to manage HICP; (ii) secondary decompressive craniectomy; (iii) duration of ICU stay.

A descriptive analysis of patient characteristics was performed. Categorical variables were shown as either absolute or relative frequencies. Continuous variables were summarized using the mean $\pm$ standard deviation (SD) or the median and interquartile range (IQR), depending on the distribution, as assessed by the Shapiro–Wilk test. Comparisons between groups with different outcomes (primary endpoint) were conducted using one-way analysis of variance (ANOVA) or the Kruskal–Wallis rank test for continuous variables, based on the distribution. For categorical variables, the chi-squared test or Fisher’s exact test was applied, as appropriate.

Linear correlations were assessed using the r^2 coefficient (R Squared), with Spearman or Pearson tests, depending on the distribution. A multinomial logistic regression model was used for the categorical dependent variable outcome as above mentioned. Logistic regression models were used for the dependent variables in secondary endpoints (e.g., increase in ICP therapy), and Cox proportional hazards models were applied to examine the relationships between the time to outcome event and a set of explanatory variables. Statistical significance was considered for p -values < 0.05 . All analyses were performed using STATA 13 software.

Results

Between August 1, 2020, and May 31, 2024, a total of 344 patients with ABI were screened and monitored with quantitative pupillometry, with approximately 12,000 pupillometric examinations performed. Among these, 91 patients met the inclusion criteria. Data were collected according to the study protocol, resulting in the registration of 5293 pupillometric measurements.

We then excluded patients with areflexia in accordance with the criteria used to define the differential, which does not contemplate cases where either pupil is already extremely insulted with no signs of response (NPi = 0). In fact, among these excluded patients with NPi = 0 ($n = 24$), 22 (92%) had a negative neurological outcome, with 19 (79%) dying during hospitalization and 3 (13%) presenting a GCS between 4 and 6 at discharge. Ten additional patients were excluded because they did not meet the

minimum required number of pupillometry measurements ($n = 8$) or affected by Cerebral nervous system infection during their ICU stay ($n = 2$).

As a result, 57 patients were finally enrolled in the analysis, of whom 26 (46%) were admitted for traumatic brain injury (TBI), 20 (35%) for intracerebral hemorrhage (ICH), and 11 (19%) for subarachnoid hemorrhage (SAH) (Fig. 2).

The studied population had a mean age of 62.9 ± 16.8 years, 33 (58%) were male. The most prevalent comorbidities in the study population were cardiovascular diseases, observed in 27 patients (47%). Most of the patients were not on chronic therapy with antiplatelet agents (used by 9 patients, 16%) or anticoagulants (used by 2 patients, 3.5%).

The mean GCS score at ICU admission was 7, although it was not available for 7 patients (12%) due to missing data in the medical records. The three patient groups are homogeneous, differing only for the ICU length of stay, ICP monitoring, NPi-d frequency and NPi-d persistence (see all baseline characteristics in Tables 1 and 2).

During hospitalization, 36 patients (63%) underwent invasive ICP monitoring. More than half of those discharged with a poor neurological outcome (67%) and nearly all patients who died during their ICU stay (90%) had ICP monitoring. Eight patients (14%) had documented epileptic seizures during their ICU stay. The median length of stay for the study population was 12 days (IQR 5–21), with longer stays observed in patients who died (18 days, IQR 11–40) or were discharged with poor outcomes (11 days, IQR 7–17). Forty-seven patients (82%) were alive at the ICU discharge, with a median GCS score of 13 (IQR 11–15). Twenty-three patients (40%) were discharged with a GCS of 14–15,

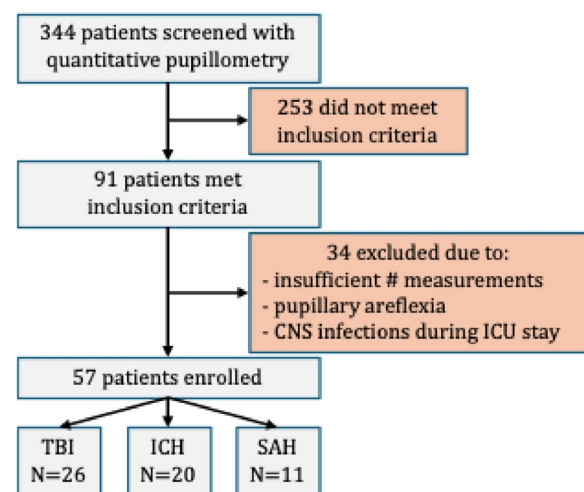


Fig. 2 Flow diagram of enrollment

Table 1 Baseline characteristics. Numbers in square brackets indicate 95% confidence intervals. age is presented with standard deviation. Asterisk indicates statistical significance according to one-way analysis of variance (ANOVA) or the Kruskal–Wallis rank test

	All	Good outcome GCS 15–14 N = 23 (40%)	Poor outcome GCS 13–3 N = 24 (42%)	Death N = 10 (18%)	p-value
Age	62.9 ± 16.8	58.2 ± 19.2	65.7 ± 16.6	67.1 ± 7.43	0.21
Gender					0.07
Male	33 (58%)	11 (48%)	13 (54%)	9 (90%)	
Female	24 (42%)	12 (52%)	11 (46%)	1 (10%)	
BMI	26 [23.5–28.4]	24.2 [22.4–26.7]	26.6 [23.9–29.5]	29.6 [26.4–32.3]	0.12
Diagnosis					0.38
TBI	26 (46%)	12 (52%)	9 (37.5%)	5 (50%)	
ICH	20 (35%)	6 (26%)	9 (37.5%)	5 (50%)	
SAH	11 (19%)	5 (22%)	6 (25%)	0	
Therapy					
Antiplatelet	9 (16%)	2 (9%)	3 (12.5%)	4 (40%)	0.07
Anticoagulant	2 (3.5%)	2 (9%)	0	0	0.22
Medical history					
Cardiovascular	27 (47.4%)	9 (39.1%)	13 (54.1%)	5 (50%)	0.58
Respiratory	5 (9%)	1 (4.3%)	2 (8.3%)	2 (20%)	0.34
Diabetes mellitus	7 (12.3%)	3 (13%)	3 (12.5%)	1 (10%)	0.97
Other comorbidities	25 (43.8%)	12 (52.2%)	9 (37.5%)	4 (44.4%)	0.60
Days in ICU	12 [5–21]	9 [3–22]	11 [7–17]	18 [11–40]	0.01*
GCS at admission	7 [5.3–8.8]	7 [6–9]	7 [5–8.3]	7 [6–8]	0.66
GCS at discharge	13 [11–15]	15 [15–15]	11 [10–11]	-	NA
ICP monitor	36 (63.1%)	10 (43.5%)	16 (66.6%)	9 (90%)	0.01*
Seizures	8 (14%)	2 (8.7%)	4 (16.6%)	2 (20%)	0.61

Table 2 Endpoints. Numbers in square brackets indicate 95% confidence intervals. Median, maximum, and minimum values are reported for the number of NPi-d events. Asterisk indicates statistical significance according to one-way analysis of variance (ANOVA) or the Kruskal–Wallis rank test

	All	Good outcome GCS 15–14 N = 23 (40%)	Poor outcome GCS 13–3 N = 24 (42%)	Death N = 10 (18%)	p-value
Patients with NPi-d	43 (75.4%)	12 (52%)	22 (92%)	9 (90%)	0.004*
No. of NPi-d	1 [1–4]	1 [0–1]	4 [1.75–8]	3 [1–7.5]	< 0.001*
Persistence of NPi-d	9.8 [3.5–24.1]	3 [1.6–5.8]	16.4 [9.8–16.0]	22.9 [9.6–43.1]	< 0.001*

twenty-four patients (42%) with a GCS between 3 and 13, and ten patients (18%) died during their ICU stay, including 2 who were declared brain dead. All patients who died due to brain death were in the TBI group.

As summarized in Table 2, 43 patients (75%) had at least one episode of a differential (NPi-d). A differential occurred in 92% of patients discharged with poor neurological outcomes, 90% of those who died during ICU stay, and 52% of patients discharged with good neurological outcomes.

The multimodal model showed that the number of differentials is statistically associated with a 3.5-fold

increased risk of poor outcome at discharge and a 3.4-fold increased risk of death during ICU stay (Table 3). Similarly, the persistence of NPi differentials is associated with unfavorable outcomes or death during ICU stay. Specifically, after adjusting for other covariates (not shown in the table but including gender, diagnosis, age, and medical history), a one-unit increase in persistence significantly increases the risk of poor outcome by 61% (1.61-fold increase) and the risk of death by 60% (1.60-fold increase, Table 3).

Furthermore, still adjusting for covariates, a unit increase of persistence (pNPi-d) is associated with a

Table 3 Multivariate multinomial logistic regression: association between the number of NPi-d events and persistence of NPi-d (pNPi-d) with neurological outcome at discharge. Good outcome is not included, as it serves as the reference outcome for the analysis. Values in square brackets indicate 95% confidence intervals. The analysis is adjusted for age, gender, diagnosis (TBI ICH SAH), therapy (antiplatelet, anticoagulant), medical condition (cardiovascular respiratory, diabetes, other)

	Poor outcome	Death
No. of NPi-d	3.58 [1.49–8.60] $p=0.004^*$	3.44 [1.42–8.33] $p=0.006^*$
Persistence of NPi-d	1.61 [1.12–2.32] $p=0.011^*$	1.60 [1.11–2.31] $p=0.011^*$

4% increased risk of requiring therapy for ICP control (Table 4, first row, logistic regression); a 5% increased risk of undergoing decompressive craniectomy (Table 4, second row, Cox regression); a 23% increase in the length of ICU stay (Table 4, third row, linear regression).

Discussion

Our findings suggest that the number of differentials (NPi-d) is strongly correlated with clinical outcomes at ICU discharge. A higher number of differentials is associated with increased mortality during the ICU stay or worse neurological outcomes (GCS 3–13) at ICU discharge, while patients without differentials tend to have more favorable short-term neurological outcomes (GCS 14–15) at ICU discharge. We also demonstrated that the persistence of the NPi differential (pNPi-d) is associated with neurological outcomes; each unit increase in persistence is associated with a higher likelihood of requiring intensified therapy for intracranial hypertension and secondary decompressive craniectomy. Elevated values of pNPi-d are also correlated with prolonged ICU stays.

Quantitative pupillometry allows for more accurate assessments of the pupil light reflex, reducing the risk of interrater variability errors. Studies have shown for example that only 33.3% of pupils identified as non-reactive by clinicians are truly non-reactive when measured with pupillometry [17–19]. Furthermore, quantitative

pupillometry detects pupillary abnormalities earlier than traditional evaluations, enabling the early identification of neurological deterioration. Integrating pupillometry into multimodal neuromonitoring facilitates early therapeutic interventions to prevent or minimize secondary brain injury in patients with acute brain injury [20].

In clinical practice, the NPi has become the standard parameter for evaluating the pupillary light reflex in critically ill patients [6, 9, 21–24]. However, nearly all existing literature focuses on an absolute assessment—specifically, whether one or both pupils have an NPi below 3 at any given time. A recent study [10] suggests that the presence of at least one differential, NPi-d—defined as a difference between the NPi values of the two pupils equal to or greater than 0.7—is indicative of poor neurological outcomes, even if both NPi values remain above the normative threshold of 3.

In this study, we aimed to build upon this early finding by investigating whether the frequency or number of differentials also contributes to prognosis. Additionally, we analyzed the persistence of the differential, disregarding the pathological threshold of 0.7 proposed in [10], and instead focusing on the persistence of the sign of the difference over time. The persistence of the NPi differential is defined as the time-integrated difference between the NPi values of the two pupils (as evidenced by the shaded area in Fig. 1A). Under normal conditions, the NPi values of the two pupil light reflexes are expected to be symmetric. In pathological conditions, such as in the TBI patients studied in this article, the NPi of one eye may be persistently lower than that of the other. We refer to this as the persistence of the NPi differential, pNPi-d.

Persistence is not defined when one eye has an NPi = 0. The reason we omitted areflexia (NPi = 0) in the logic of the analysis is that it already represents a severe pupillary event, well recognized in clinical practice. For a neurological patient, a non-reactive pupil is, by itself, a well-established poor prognostic sign. In fact, among the excluded patients with NPi = 0, the vast majority (92%) had poor neurological outcomes. Our intention here is to focus instead on cases in which the NPi is non-zero—it may even fall within the normal range above 3—but where

Table 4 Multivariate regression analysis between persistence of NPi-d and increased Therapeutic Intensity Level (TIL) to manage intracranial hypertension (HICP), first row, logistic regression; secondary decompressive craniectomy within the first 10 day, second row, Cox regression; duration of ICU stay, last row, linear regression. Values in square brackets indicate 95% confidence intervals. The analysis is adjusted for age, gender, diagnosis (TBI ICH SAH), therapy (antiplatelet, anticoagulant), medical condition (cardiovascular respiratory, diabetes, other)

Regression table	Persistence of NPi-d
Therapeutic Intensity Level (TIL) to manage intracranial hypertension (HICP)	1.04 [1.00–1.08] $p=0.036^*$
Secondary decompressive craniectomy within the first 10 day	1.05 [1.01–1.08] $p=0.002^*$
Duration of ICU stay	0.23 [0.068–0.39] $p=0.006^*$

persistency is present. This phenomenon, not previously explored, may represent an early clinical alarm that should not be overlooked. How areflexia is connected with the onset of an NPi-d or pNPi-d is of great interest and, although beyond the scope of the present study, warrants further investigation to better understand the temporal dynamics and sequencing of these abnormalities and how these patterns relate to patient outcomes.

The presence of an NPi differential between the two eyes may be attributed to several factors, including increased lesion volume with associated mass effect, brainstem compression, or vascular impairment due to altered cerebral autoregulation. These conditions can lead to asymmetric intracranial dynamics, resulting in uneven pressure or dysfunction along the two oculomotor pathways that mediate the pupillary light reflex in the midbrain. Such asymmetry may indicate localized injury, differential hemispheric compliance, or early signs of herniation syndromes. As a result, the NPi differential could serve as a physiological marker, providing early insight into evolving intracranial pathology—even in the absence of overt clinical signs or changes in absolute NPi values. Recognizing and monitoring this asymmetry over time may support more timely and targeted therapeutic interventions.

Although the pathophysiological mechanisms have not yet been fully elucidated, low NPi values have been associated with decreased CPP in septic patients [25]. Given that the physiology of the pupillary light reflex reflects the functional integrity of the autonomic nervous system, it has been suggested that impaired cerebral autoregulation in sepsis may be mediated by autonomic dysfunction. Consequently, automated pupillometry has been proposed as a potential tool for the assessment of cerebral hemodynamics in this clinical context [25]. As a result, another possible pathophysiological explanation for the NPi differential may relate to the perfusion status of the brainstem. In severe acute brain injuries, impaired autoregulation can cause rapid changes in perfusion, potentially leading to a sudden weakening of the pupillary reflex that is not equally distributed across the two efferent pupil pathways. This weakening may be followed by a rapid recovery when perfusion improves. In this context, the frequency of NPi-d and the increase in pNPi-d could reflect the fragility of the patient's brain perfusion, resulting in transient impairments of the pupillary reflex when perfusion becomes critically compromised [25].

While this study offers valuable new insights into the many ways NPi can be interpreted and integrated into clinical practice, there are several important considerations to note. The main limitations of the study are its

small sample size and retrospective design. Additionally, the persistence of NPi differentials may have been influenced by the specific protocol used, such as the frequency of pupillometry measurements. Pupil data in this study were collected with relatively good consistency across and within patients, with an average interval of 3.4 h between assessments. In fact, 85% of all measurements were taken between 2 and 4 h apart, and only 6% were occasionally delayed by more than ten hours. Timing is, of course, a crucial factor when evaluating persistency. Long gaps between measurements, for example, may impair not only the detection of persistency but also its accurate quantification. While the modest variability in measurement intervals in this study is unlikely to have significantly affected the findings, the potential bias it introduces should be carefully considered in the design of future prospective studies.

GCS is a clinical evaluation scale rather than an outcome assessment tool. Nevertheless, we chose to use the GCS for two main reasons. First, it was not always possible to obtain a Glasgow Outcome Score extended (GOSE) or a Modified Rankin Score (mRS) evaluation for all enrolled patients. Second, our interest was focused on capturing an early clinical outcome relevant for in-hospital prognostic evaluation. The GCS was consistently reported and, when missing, could be easily reconstructed from the clinical documentation.

During a single episode of persistency, the sign of the differential cannot change, as this would break the definition of the phenomenon. However, patients may experience multiple episodes of persistency, and these episodes could, in theory, have opposite signs (the left NPi more reactive than the right in one episode, and the right more reactive than the left in another. Note that this is not the case in Fig. 1, where all episodes had the same sign). When all episodes of persistency are summed to derive the final measure for a patient, episodes with different signs could partially or completely cancel each other out. We may want to ponder this possibility more carefully in future analyses—perhaps by considering only the strongest episode of persistency or by taking the absolute value of each episode before summing them.

Both the differential NPi-d and the persistency pNPi-d represent differences between the two eyes' NPi values, independent of the absolute values of the individual NPis. In other words, a persistency or differential occurring when both NPis are above the pathological threshold of 3 is treated the same as when both are below it. We performed an additional exploratory analysis examining the effect of absolute NPi values (above vs. below the

threshold of 3) on differential and persistency and found no difference in outcome GCS. This finding is consistent with the rationale proposed by Privitera et al. [10], which suggests that the occurrence of an asymmetry in NPi—whether expressed as a differential or as persistency—represents a neurologically relevant event that supersedes the absolute NPi value itself. However, our cohort size is relatively small compared to that of Privitera et al. A more definitive analysis to fully capture the interplay among multiple variables—differential, persistency, pathological NPi, and non-responsive NPi—and their combined impact on outcomes will require larger studies. For this reason, we chose not to explore this aspect in the present work, although it remains an important question to be addressed in future studies.

There is, in the notion of persistency, an interesting conceptual parallel with the concept of ICP burden or Pressure-Time Dose (PTD), which refers to the cumulative effect of both the magnitude of ICP elevation above a threshold and the duration of that elevation—a combination that is associated with poor prognosis even when ICP does not reach refractory or extremely high levels (see for example [26]). Similarly, in persistency, the two NPis may remain above the normative cutoff of 3, yet the duration of the asymmetry over time carries clinical significance. Of course, with the cause–effect relationship inverted, PTD refers to an intracranial event that can cause a series of clinical observations, including NPi dysfunction, while persistency represents the externally observable effect of an underlying process. This parallel underscores the importance of time and duration in intracranial neurophysiological phenomena, which may be more alarming than short, even if more severe, isolated events.

Hemodynamic and cerebral perfusion data, as well as systemic and tissue oxygenation values, were not assessed—limiting the ability to evaluate potential confounding factors such as hemodynamic instability, inadequate cerebral perfusion, or hypoxia when interpreting differentials. Multicenter studies with larger sample sizes are needed to confirm these findings and to further investigate their pathophysiological significance, including the correlation between NPi-d events and other clinical determinants of neuroworsening such as HICP, TIL or imaging.

Conclusion

Our study highlights the importance of adopting a more holistic approach to interpreting the NPi. The literature provides extensive evidence on the significance of the value of NPi for a single pupil at any given time. In this study, we demonstrate that not only the presence of a differential but, more importantly, the frequency and total

persistence of the differential are strong early prognostic indicators of short-term outcomes. Integrating these factors into clinical assessments could improve the management of acute brain injury patients. Future studies aimed at identifying the specific threshold for the differential and its persistence in relation to diagnostic and therapeutic interventions are warranted.

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None.

Authors' contributions

S.M.Z. and C.M.P. conceptualised the study. S.M.Z. and S.M.M. designed the study protocol. S.M.M. and F.L. and S.R. collected clinical data. F.F. and A.D. designed statistical strategies and data analysis. All authors contribute to results interpretation.

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Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located and secured at ASST Lariana hospital in Como (Italy) owner of data collection.

Declarations

Ethics approval and consent to participate

The study documentation was submitted to the competent Ethics Committee (CET 5), which did not issue a negative opinion within the timeframe established by current regulations. Therefore, in accordance with the applicable legal provisions for retrospective observational studies, the absence of a formal objection was interpreted as tacit approval (silence-assent principle).

Consent for publication

Not applicable.

Competing interests

CMP is the Chief Scientist of NeurOptics. All other authors declare no additional financial nor non-financial competing interests.

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