ROZPRAWA DOKTORSKA

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Zmiany stężenia biomarkerów uszkodzenia mózgu u pacjentów z krwotokiem podpajęczynówkowym z pękniętego tętniaka.

Changes in the concentration of brain injury biomarkers in patients with aneurysmal subarachnoid hemorrhage.

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Spis treści **strona**

Wykaz skrótów użytych w pracy:

aSAH	aneurysmal subarachnoid hemorrhage
APACHE II	Acute Physiology and Chronic Health Evaluation II
AUC	area under the ROC curve
CUN	centralny układ nerwowy
DCI	delayed cerebral ischemia
EVD	external ventricular dreinage
GCS	Glasgow Coma Scale
GFAP	glial fibrillary acidic protein
GOS	Glasgow Outcome Scale
MAPT	microtubule associated protein tau
NSE	neuron- specific enolase
OIT	Oddział Intensywnej Terapii
PMR	płyn mózgowo- rdzeniowy
ROC	receiver operating characteristic
WFNS	World Federation of Neurological Surgeons grade

1. WSTĘP

Krwotok podpajęczynówkowy z pękniętego tętniaka naczyń mózgowych (aneurysmal subarachnoid hemorrhage, aSAH), to choroba o nagłym i ciężkim przebiegu.

Krwotok podpajęczynówkowy stanowi ok. 5% wszystkich udarów mózgu [1]. Najczęstszą przyczyną samoistnych krwotoków podpajęczynówkowych jest pęknięty tętniak tętnicy mózgowej. Choroba dotyczy najczęściej osób w wieku produktywnym, obarczona jest znaczną śmiertelnością oraz powstaniem trwałych deficytów neurologicznych, czy zaburzeń kognitywnych u tych, którzy przeżyją krwotok pierwotny [2, 3]. W aSAH dochodzi do wynaczynienia się krwi do przestrzeni między oponą pajęczą i oponą miękką w wyniku pęknięcia tętniaka tętnicy mózgowej. Rozległość krwotoku może być różna- od niewielkiego do masywnego krwawienia z powstaniem krwiaka śródmózgowego lub przebiciem się krwi do układu komorowego. Wiążą się z tym różnego stopnia wczesne uszkodzenia tkanki mózgowej rozwijające się w pierwszych 72 godzinach. Są one spowodowane zarówno wynaczynieniem się krwi pod wysokim ciśnieniem, jak i przez ratunkowe mechanizmy powodujące niedokrwienie, konieczne do ograniczenia lub zahamowania krwotoku, takie jak odruchowy skurcz w obszarze uszkodzonego naczynia i gwałtowny wzrost ciśnienia wewnątrzczaszkowego. Krwiakowi śródmózgowemu towarzyszy obrzęk powodujący ucisk przyległych tkanek i przemieszczający struktury mózgu. Natomiast krwotok do układu komorowego może prowadzić do powstania ostrego wodogłowia obturacyjnego i gwałtownego wzrostu ciśnienia wewnątrzczaszkowego [4, 5].

Poza urazem powstałymi we wczesnej fazie choroby, u części pacjentów dochodzi do wystąpienia późnego niedokrwienia i zawałów mózgu (delayed cerebral ischemia, DCI) w obszarze naczyniowym tętniaka, a nawet w oddalonych od niego naczyniach. Obecnie uważa się, że przyczyną DCI jest nie tylko skurcz naczyń mózgowych rozwijający się zwykle między trzecią a dwunastą dobą od aSAH, ale szereg czynników generowanych we wczesnej fazie krwotoku, takich jak uszkodzenie bariery krew- mózg, stres oksydacyjny, reakcja zapalna i rozprzestrzeniająca się depolaryzacja (spreading depolarization) [6, 7].

Pomimo dużego postępu w diagnostyce obrazowej, leczeniu neurochirurgicznym i intensywnej terapii, aSAH jest nadal poważnym wyzwaniem dla medycyny. Około 50% pacjentów umiera we wczesnej fazie choroby, a około połowa tych, którzy przeżyli pierwotny krwotok doznaje trwałych deficytów neurologicznych [2].

Ocenę ciężkości krwotoku podpajęczynówkowego przeprowadza się na początku leczenia przy użyciu powszechnie stosowanych skal. Określają one stan kliniczny pacjenta

biorąc pod uwagę jakościowe i ilościowe deficyty neurologiczne (skala Glasgow Coma Scale, GCS; skala Hunta- Hessa, skala World Federation of Neurological Surgeons, WFNS) oraz rozległość krwotoku w badaniu tomografii komputerowej (skala Fishera). Ocena jakościowych i ilościowych zaburzeń neurologicznych we wczesnej fazie leczenia pacjentów z aSAH na Oddziale Intensywnej Terapii (OIT) ma jednak ograniczoną wartość. Wynika to z konieczności stosowania głębokiej analgosedacji, wykorzystywanej jako jeden z elementów terapii ograniczającej uraz wtórny po krwotoku podpajęczynówkowym lub po operacji neurochirurgicznej oraz w celu zniesienia dolegliwości bólowych, nieprzyjemnych doznań i odruchów przy stosowaniu inwazyjnych technik leczenia np. wentylacji mechanicznej.

W ostatnich latach pojawiły się nowe możliwości oceny uszkodzenia komórek mózgowych wykorzystujące oznaczanie we krwi i płynie mózgowo- rdzeniowym (PMR) stężenia białek specyficznych dla tych komórek. Białka te, zawarte w dużej ilości w komórkach mózgowych, nie występują fizjologicznie we krwi lub ich stężenie jest bardzo niskie. W sytuacji, kiedy dochodzi do uszkodzenia tkanki mózgowej uwalniają się one z uszkodzonych komórek do PMR, a przy przerwaniu bariery krew- mózg przedostają się do krwi, gdzie można je zidentyfikować oraz zmierzyć ich stężenie [8]. Najczęściej wykorzystywanymi w badaniach białkami specyficznymi dla komórek układu nerwowego są:

enolaza neuronalna (NSE, neuron- specific enolase)- białko enzymateczne zawarte w cytoplazmie komórek neuronalnych centralnego układu nerwowego (CUN), katalizujące jeden z etapów glikolizy beztlenowej,

białko tau (MAPT, microtubule associated protein tau)- proteina biorąca udział w budowie cytoszkieletu, odpowiadająca głównie za utrzymywanie stabilności mikrotubul w aksonach komórek neuronalnych CUN,

białko S100beta (S100B)- białko regulatorowe wiążące wapń, charakterystyczne dla komórek glejowych CUN, głównie dla astrocytów,

kwaśne włókienkowe białko gleju (GFAP, glial fibrillary acidic protein)- białko cytoplazmatyczne, składnik cytoszkieletu komórek glejowych, charakterystyczne dla astrocytów.

Od wielu lat białka te są przedmiotem badań w różnych schorzeniach CUN, jak na przykład udar niedokrwienny, urazowe uszkodzenie mózgu, krwotok podpajęczynówkowy, a także choroby neurodegeneracyjne [9-12]. W wielu pracach wykazano wzrost stężenia tych białek zarówno w PMR jak i we krwi, jednakże nie ustalono punktów odcięcia dla poszczególnych biomarkerów uszkodzenia mózgu, które mogłyby być przydatne w praktyce klinicznej do prognozowania wyników leczenia.

Zdefiniowanie panelu specyficznych biomarkerów możliwych do oznaczenia we krwi, które odzwierciedlałyby wielkość uszkodzenia mózgu analogicznie do stężenia troponiny w zawale mięśnia sercowego, mogłoby być użytecznym narzędziem w praktyce klinicznej. Byłoby pomocne w diagnostyce i planowaniu intensywności nadzoru nad chorym. Umożliwiałoby monitorowanie skuteczności leczenia w ostrej fazie choroby oraz przewidywanie wczesnych i późnych wyników leczenia.

2. CELE PRACY

Celem przedstawionego cyklu prac była:

1. Analiza zmian stężeń wybranych biomarkerów uszkodzenia mózgu w grupie pacjentów z krwotokiem podpajęczynówkowym z pękniętego tętniaka hospitalizowanych na oddziale intensywnej terapii.
2. Zbadanie zależności między oznaczonymi stężeniami biomarkerów, a ciężkością krwotoku ocenianego za pomocą rutynowo stosowanych skal klinicznych.
3. Ocena przydatności oznaczonych biomarkerów uszkodzenia mózgu w prognozowaniu wczesnych wyników leczenia u pacjentów z krwotokiem podpajęczynówkowym z pękniętego tętniaka.

3. PUBLIKACJE STANOWIĄCE PRACĘ DOKTORSKĄ

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NEUROCRITICAL CARE SOCIETY

ORIGINAL WORK

Biomarkers of Neurological Outcome After Aneurysmal Subarachnoid Hemorrhage as Early Predictors at Discharge from an Intensive Care Unit

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Abstract

Background: Subarachnoid bleeding is associated with brain injuries and ranges from almost negligible to acute and life threatening. The main objectives were to study changes in brain-specific biomarker levels in patients after an aneurysmal subarachnoid hemorrhage (aSAH) in relation to early clinical findings, severity scores, and intensive care unit (ICU) outcome. Analysis was done to identify specific biomarkers as predictors of a bad outcome in the acute treatment phase.

Methods: Analysis was performed for the proteins of neurofilament, neuron-specific enolase (NSE), microtubule-associated protein tau (MAPT), and for the proteins of glial cells, S100B, and glial fibrillary acidic protein (GFAP). Outcomes were assessed at discharge from the ICU and analyzed based on the grade in the Glasgow Outcome Scale (GOS). Patients were classified into two groups: with a good outcome (Group 1: GOS IV–V, $n=24$) and with a bad outcome (Group 2: GOS I–III, $n=31$). Blood samples were taken upon admission to the ICU and afterward daily for up to 6 days.

Results: In Group 1, the level of S100B (1.0, 0.9, 0.7, 2.0, 1.0, 0.3 ng/mL) and NSE (1.5, 2.0, 1.6, 1.2, 16.6, 2.2 ng/mL) was significantly lower than in Group 2 (S100B: 4.7, 4.8, 4.4, 4.5, 6.6, 6.8 ng/mL; NSE: 4.0, 4.1, 4.3, 3.8, 4.4, 2.5 1.1 ng/mL) on day 1–6, respectively. MAPT was significantly lower only on the first and second day (83.2 ± 25.1, 132.7 ± 88.1 pg/mL in Group 1 vs. 625.0 ± 250.7, 616.4 ± 391.6 pg/mL in Group 2). GFAP was elevated in both groups from day 1 to 6. In the ROC analysis, S100B showed the highest ability to predict bad ICU outcome of the four biomarkers measured on admission [area under the curve (AUC) 0.81; 95% CI 0.67–0.94, $p<0.001$]. NSE and MAPT also had significant predictive value (AUC 0.71; 95% CI 0.54–0.87, $p=0.01$; AUC 0.74; 95% CI 0.55–0.92, $p=0.01$, respectively). A strong negative correlation between the GOS and S100B and the GOS and NSE was recorded on days 1–5, and between the GOS and MAPT on day 1.

Conclusion: Our findings provide evidence that brain biomarkers such as S100B, NSE, GFAP, and MAPT increase significantly in patients following aSAH. There is a direct relationship between the neurological outcome in the acute treatment phase and the levels of S100B, NSE, and MAPT. The detection of brain-specific biomarkers in conjunction with clinical data may constitute a valuable diagnostic and prognostic tool in the early phase of aSAH treatment.

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Keywords: Brain-specific biomarkers, Glial fibrillary acidic protein, Microtubule-associated protein tau, Neuron-specific enolase, S100B protein, Subarachnoid hemorrhage

Introduction

Subarachnoid bleeding is associated with brain injuries and ranges from almost negligible to acute and life threatening. An acute brain injury after an aneurysmal subarachnoid hemorrhage (aSAH) leads to the destruction of brain cells and the release of brain-specific proteins to the cerebrospinal fluid and systemic blood circulation. Brain proteins can pass directly through a damaged blood–brain barrier into the systemic circulation, where they can be detected [1, 2]. Therefore, the proteins abundant in the neurons and glia of the central nervous system (CNS) can be sensitive markers of brain damage caused by aSAH in the acute and long-term treatment phases. The severity of the initial brain damage is one of the most important factors associated with outcome after aSAH. Brain ischemia associated with an increase in intracranial pressure (ICP) and focal brain ischemia, caused by tissue compression, are the main mechanisms of initial brain damage after aSAH [3, 4]. Therefore, the identification of biomarkers that are known to be released during brain ischemia after aSAH could be useful in the clinical setting [5]. The concept of brain-specific biomarkers refers to substances found in high concentration in the central nervous system (CNS) and absent or present in low concentration in blood. Proteins abundant in the neurons and glia of the CNS are neuron-specific enolase (NSE), S100B protein, microtubule-associated protein tau (MAPT), and glial fibrillary acidic protein (GFAP), and these proteins may be considered to be brain-specific biomarkers that can be used to assess brain damage caused by a ruptured aneurysm in the acute and long-term phases of treatment.

Outcome prediction using clinical scores recognized in neurological critical care, such as the Glasgow Coma Scale score, WFNS, and Hunt and Hess grade, has been useful in patients with SAH. However, the clinical evaluation is of limited value to ICU physicians, when there is prolonged sedation of a patient that is often required to treat elevated intracranial pressure or in patients on mechanical ventilation. The scientific evidence to date suggests that SAH-associated brain injury is a complex, multifactorial process, where early brain damage affects secondary damage and the end result of treatment. Thus, independent mechanisms from vasoconstriction, such as early brain damage, spreading depolarization, oxidative stress, inflammation, and blood–brain barrier disruption, can have a much greater effect on delayed cerebral ischemia and outcome than the mere presence of cerebral vasospasm [6, 7]. Defining a panel of brain-specific biomarkers that would reflect the degree of brain damage could be useful in planning and determining therapeutic directions at the ICU. In the early phase after aSAH, reliable laboratory tools for outcome prediction could be particularly helpful and could facilitate the management of the patient. In very few studies, the patient outcome was evaluated in the acute phase of aSAH treatment in relation to the patient's clinical condition at discharge from the ICU; most studies evaluated the predictive value of brain biomarkers in relation to the long-term outcome after SAH. The primary goal of this paper was to predict outcome within the very early phase after SAH. Analysis was done to identify specific biomarkers that are predictors of a bad outcome at ICU discharge. Early changes in brain-specific biomarker levels were assessed in patients after aSAH, in the acute phase of treatment, in relation to clinical findings, severity scores, and ICU outcome.

Materials and Methods

Study Population

This observational, prospective study included patients with aSAH admitted to the Intensive Care Unit (ICU) at the University Hospital in Wroclaw between July 2014 and January 2017.

1. Inclusion criteria: age $\geq$ 18 years, ICU admission within 48 h after clinical diagnosis of aSAH. An aneurysm in cerebral arteries as the cause of bleeding was confirmed using computer tomography angiography (CTA), magnetic resonance imaging (MRI) or conventional angiography,
2. Exclusion criteria: previously diagnosed neurological disease.

Patients with previously diagnosed aneurysms, with a history of stroke, epilepsy, neurodegenerative diseases such as Parkinson's disease, dementia, cerebrovascular diseases, motor neuron diseases, traumatic brain injury, schizophrenia, or depression were excluded.

Exclusion criteria were based on previously published data, indicating that levels of some brain-specific biomarkers may be elevated in these diseases [8–12].

Outcome Assessment, Study Groups

The neurological condition of the patients was graded according to the 5-grade Glasgow Outcome Scale (GOS). The GOS is the most commonly used outcome measurement after an acute brain injury. To calculate the GOS, the best source of available information should be used. Therefore, for this study, the parameters of patient conditions at discharge from the ICU were used to calculate the GOS, and the GOS results were dichotomized into two categories: good or bad outcome.

Study Group 1 consisted of patients with a good outcome (GOS IV–V).

Study Group 2 consisted of patients with a bad outcome (GOS I–III) (Table 1).

Clinical Evaluation and Management

Patients were given treatment according to a standardized management protocol [13], and study procedures were previously described in detail [14]. Briefly, all patients diagnosed with a subarachnoid hemorrhage caused by a ruptured **c**erebral aneurysm were admitted to the ICU. Neurosurgical intervention was undertaken within the first 24 h after aSAH. The decision on the type of procedure (surgical clipping or endovascular coiling,) as well as the time of the procedure was made by a team of neurosurgeons and interventional neuroradiologists based on medical indications, such as the results of the complete blood count, size and location of the aneurysm, and the neurological status of the patient. In patients assessed as a IV or V on the WFNS scale, for early management of hydrocephalus and elevated intracranial pressure, external continuous ventricular drainage (EVD) with of the cerebrospinal fluid was first implanted. Then, after stabilization of the general and neurological condition, endovascular coiling was performed. In patients with uncontrolled elevated intracranial pressure (ICP), due to an intracerebral hematoma or edema, open surgical clipping was done. The decision to place the sensor for measuring intracranial pressure, EVD or decompressive craniectomy was made individually for each patient. After securing the aneurysm, the EVD was removed as quickly as clinically feasible. Most often, intermittent drainage of cerebrospinal fluid was used, with an early attempt to clamp. At the ICU, all treatment algorithms included euvolemia, analgesia, sedation, and inotropic support, when indicated. Nimodipine was administered to all patients with aSAH to improve neurological outcome. Depending on the indications, patients were either mechanically ventilated or they remained on passive oxygen therapy. A control CT scan was performed within 24 h after excision or collapse of the aneurysm. Neurological examinations were performed by an ICU physician daily to detect neurological impairment (movement disorders, mental disorders, aphasia). Demographic data, medical history, and baseline clinical parameters were obtained shortly after admission. At the ICU, the patient's clinical status was assessed with the APACHE II score (Acute Physiology and Chronic Health Evaluation II). Neurological status was assessed with the GCS (Glasgow Coma Scale). To classify the severity of aSAH based on the patient's clinical condition, the Hunt–Hess scale was used, and the extent of the hemorrhage on the CT was graded with the Fisher scale [15]. Additionally, the patient's follow-up was done with a Glasgow Outcome Scale (GOS) at hospital discharge and with a Glasgow Outcome Scale Extended (GOSE) after 6 months.

Blood Sample Collection and Biomarker Detection

For each patient, blood samples were collected at the time of admission (day 1), and on day 2, 3, 4, 5, and 6 after aSAH. A blood sample was drawn from an intravenous catheter to a tube (2.7 mL). Each blood sample was centrifuged after 30 min (10,000 rpm for 15 min), and the supernatant was aliquoted and stored at − 70 °C until assayed. A solid phase enzyme linked-immuno-sorbent assay (ELISA) was used to measure serum levels of S100B (Cloud-Clone Corp., Katy, TX, USA), GFAP (Elabscience, Houston, Texas, USA), MAPT (Cusabio TECHNOLOGY LLC, Houston, TX,

Table 1 Classifying study groups based on the Glasgow Outcome Scale (GOS)

Study groups	GOS rating	Definition
Group 1	5: Good recovery	Resumption of normal life despite minor deficits
	4: Moderate disability	Disabled but independent. Can work in sheltered setting
Group 2	3: Severe disability	Conscious but disabled. Dependent for daily support
	2: Persistent vegetative	Minimal responsiveness
	1: Death	Nonsurvival

USA), and NSE (R&D Systems, Minneapolis, MN, USA). Concentrations of mediators were measured in duplicate with appropriate controls, according to the manufacturer's instructions using an ELx800 absorbance microplate reader (BioTek, Winooski, VT, USA).

Statistical Analysis

All analysis was performed with Statistica 13 software (StatSoft, Inc. Tulsa, USA). The distribution was not normal based on the Shapiro–Wilk test. Therefore, statistical analysis was performed using nonparametric tests. Comparisons of biomarker levels within a single group among different time points (day 1, 2, 3, 4, 5, and 6) were performed using the Friedman analysis of variance (ANOVA) and Kendall's coefficient of concordance test. Categorical variables were analyzed using the Chi-square test. The Mann–Whitney U test was used for comparison of continuous variables between study groups at each time point. The Spearman rank test was used for correlations. A comparison of the predictive accuracy of the biomarkers measured on admission to the ICU was made using receiver operating characteristics curve (ROC) analysis, by calculating the area under the curve (AUC), including 95% confidence intervals (CI), to determine sensitivity and specificity. Multivariate logistic regression analysis was performed to evaluate the association between baseline S100B, NSE, MAPT, and GFAP and covariates age, gender, GCS, APACHEII, WFNS scale, Hunt and Hess scale, Fisher scale) and ICU outcome; the results were reported as odds ratio (OD) and 95% confidence intervals (CI). The first prepared model included all biomarkers and all selected covariates (age, gender, GCS, APACHEII, WFNS scale, Hunt and Hess scale, Fisher scale).The collinearity of the variables was tested. Four of the covariates (GCS, WFNS scale, Hunt and Hess scale, Fisher scale) were collinear, so those features were excluded from the analysis. The choice of the best model was proposed based on the Akaike information criterion and the backward selection of the model. From the four biomarkers (S100B, NSE, MAPT, and GFAP) and the three covariates (age, gender, APACHEII), the procedure of minimizing Akaike criterion chose the model with two biomarkers (S100B, NSE) and two covariates (APACHEII, gender). The statistical analysis was conducted using R 3.6.01: R Core Team (2013). Continuous variables were reported as mean values ± standard error and minimum–maximum. All the tests were conducted with a 5% significance level.

Results

Out of the 60 patients with aSAH who met the inclusion criteria, 5 were excluded due to an incomplete acquisition of samples. The analysis was performed on 55 patients (Group 1, $N=24$ and Group 2, $N=31$). The mean admission GCS was 11.6 (range 4–15), a WFNS grade of I–III was recorded in 36 patients (65%) and a WFNS grade of IV–V in 19 patients (35%). The results of the general clinical assessment scores WFNS, GCS, Hunt–Hess, and the Fisher scale were significantly better in Group 1 than in Group 2. The APACHE II score, used for the classification of disease severity in ICU patients, was significantly lower in Group 1, indicating a better clinical status on admission to the ICU (Group 1: 9.6 ± 0.8 pts, Group 2: 17.1 ± 1.2 pts., $p<0.001$). A summary of patient baseline characteristics is given in Table 2.

Table 2 Characteristics of the study population on admission to the ICU

Parameter	All ($N=55$)	Group 1 ($N=24$)	Group 2 ($N=31$)	p
Age (years)	58.8 ± 1.9 (25–83)	52.9 ± 2.0 (25–82)	63.3 ± 2.4 (30–83)	0.005
Gender (F/M)	36/19	17/7	19/12	0.460
APACHE II	13.8 ± 0.9 (2–29)	9.6 ± 0.8 (2–21)	17.1 ± 1.2 (4–29)	< 0.001
WFNS scale [*n* (%)]				0.020
I–III	36 (65)	20 (83)	16 (52)	
IV–V	19 (35)	4 (17)	15 (48)	
Initial CT Fisher scale (subarachnoid blood) [*n* (%)]				0.002
Grade I (none)	0	0	0	
Grade II (diffuse only)	12 (22)	10 (42)	2 (6)	
Grade III (clot or thick layer)	15 (27)	7 (29)	8 (26)	
Grade IV (diffuse or none, with cerebral or ventricular blood)	28 (51)	7 (29)	21 (68)	
GCS	11.6 ± 0.54 (4–15)	13.6 ± 0.4 (5–15)	10.0 ± 0.8 (4–15)	< 0.001
Hunt and Hess scale, (the severity of subarachnoid hemorrhage) [*n* (%)]				0.011
Grade I	10 (18)	8 (33)	2 (6)	
Grade II	8 (15)	4 (17)	4 (13)	
Grade III	15 (27)	8 (33)	7 (23)	
Grade IV	10 (18)	3 (15)	7 (23)	
Grade V	12 (22)	1 (4)	11 (35)	
Treatment [*n* (%)]				0.227
Neurosurgical clipping	23 (42)	9 (38)	14 (45)	
Endovascular embolization	27 (49)	14 (58)	13 (42)	
Conservative	5 (9)	1 (4)	4 (13)	

Data are presented as mean ± standard error (min–max), unless other stated; CT, computed tomography; GCS, Glasgow Coma Score; LOS, length of stay; WFNS, the World Federation of Neurological Surgeons; *p* value represents a comparison between groups

Aneurysm Treatment

All patients had an aneurysm in a cerebral artery which was confirmed as the cause of bleeding using computer tomography angiography (CTA), magnetic resonance imaging (MRI) or conventional angiography. Twenty-three patients (42%) underwent neurosurgical clipping of the aneurysm, 27 (49%) underwent endovascular embolization, and the other patients were treated conservatively ($N=5$, 9%) (Table 2). Patients treated conservatively (H–H score 5, Fisher score 4) had only external ventricular drain (EVD) in the acute phase of treatment as decided by the attending neurosurgeons. Lack of improvement in the neurological condition was the reason for disqualification of these patients from further neurosurgical interventions. There was no statistically significant difference in the distribution of the treatment methods between Group 1 and Group 2 ($p=0.227$). Patients, who underwent either embolization or clipping, did not differ significantly in age, gender, and the results of the clinical scores on admission to the ICU (APACHE II $p=0.952$, WFNS p-0.912, GCS $p=0.629$, Hunt and Hess $p=0.503$, and Fisher scale $p=0.401$). S-100B, NSE, GFAP, and MAPT levels recorded on days 1, 2, 3, 4, 5, and 6 were not significantly different in patients whose aneurysms were coiled and clipped. The Glasgow outcome score at ICU discharge was not different for patients who underwent clipping or embolization (respectively, 2.95 ± 0.239 and 3.18 ± 0.238, $p=0.451$). Vasospasm occurred in 15 patients (62%) in Group 1 and in 18 patients (58%) in Group 2 ($p=0.739$). S-100B, NSE, GFAP, and MAPT levels were not significantly different in patients with and without vasospasm.

Biomarker Kinetics in the Acute Treatment Phase

S100B, MAPT, and NSE levels were correlated with the neurological outcome of the patients evaluated with the GOS at discharge from the ICU. At baseline, there was a significant increase in the level of the biomarkers in the group of patients with a bad outcome (Group 2) but not in Group 1 (S100B: $1,0\pm0.3$ vs. $4.7\pm0.1.1$ ng/mL; MAPT: 83.2 ± 25.1 vs. $625,0\pm250.7$ pg/mL; NSE: 1.5 ± 0.3 vs. 4.0 ± 1.2 ng/mL in Group 1 and 2, respectively). In Group 2, S100B and NSE levels remained significantly elevated during the whole study period (S100B: 4.8 ± 1.4, 4.4 ± 0.8, 4.5 ± 1.3, 6.6 ± 3.3, 6.8 ± 3.2 ng/mL; NSE: 4.1 ± 0.9, 4.3 ± 1.1, 3.8 ± 1.0, 4.4 ± 1.1, 2.5 ± 1.1 ng/mL on day 2, 3, 4, 5, and 6, respectively) and stayed low in Group 1 (S100B: 0.9 ± 0.3, 0.7 ± 0.1, 2.0 ± 1.3, 1.0 ± 0.4, 0.3 ± 0.2 ng/mL, NSE: 2.0 ± 0.5, 1.6 ± 0.4, 1.2 ± 0.3, 16.6 ± 0.4, 2.2 ± 0.4 ng/mL on day 2, 3, 4, 5, and 6, respectively). The MAPT level remained elevated only until the second day of observation in Group 2 (617.4 ± 391.6, 195.0 ± 115.6, 379.9 ± 277, 315.5 ± 167.0, 86.9 ± 34.8 pg/mL on day 2, 3, 4, 5, and 6, respectively) and stayed low in Group 1 (132.7 ± 88.1, 438.7 ± 316.9, 102.9 ± 62.2, 79.7 ± 14.7, 8.1 ± 8.0 pg/mL on day 2, 3, 4, 5, and 6, respectively). The difference between groups in S100B and NSE was significant during the whole study period, and the difference in the MAPT level between groups was significant on day 1 and 2. An aSAH increased the blood concentration of GFAP significantly from day 1 and remained high till the end of the observation period (Group 1: 5.7 ± 1.5, 6.9 ± 1.6, 6.9 ± 1.5, 7.3 ± 1.5, 8.5 ± 1.7, 6.5 ± 3.3 ng/mL; Group 2: 6.6 ± 1.1, 7.0 ± 1.2, 7.8 ± 1.3, 8.1 ± 1.2, 7.5 ± 2.4, 5.3 ± 1.9 ng/mL on day 1, 2, 3, 4, 5, and 6, respectively); however, no significant differences were observed between the groups studied. The temporal course in biomarkers in Groups 1 and 2 is presented in Fig. 1. No significant correlation was found between a patient's age or gender and the levels of GFAP, MAPT, S100B or NSE.

Prediction of Clinical Outcome After aSAH

Based on the value of the GOS recorded upon discharge from the ICU, 44% of the study population had a good outcome (Group 1, GOS IV–V) and 56% had a bad outcome (Group 2, GOS I–III). The majority of patients within Group 1 were graded with a GOS of IV (79%), and in Group 2, the majority were graded with a GOS of III (61%). Table 3 displays a comparison between patients with a good versus bad clinical status based on the GOS recorded at ICU discharge. There were no patients with a grade 2 on the GOS.

Based on the value of the GOS recorded at hospital discharge, the majority of patients within Group 1 (good ICU outcome) were graded with a GOS of V (75%) indicating a good hospital outcome; in Group 2 (bad ICU outcome), 42% of patients died (GOS of I), and among the survivors, the majority were graded with a GOS of III and IV (22% and 29%, respectively) (Table 3). At 6-month follow-up, based on the value of the Glasgow Outcome Scale Extended (GOSE), the majority of patients within Group 1 were graded with a GOSE of VII (50%) and VIII (33%) indicating improvement in the clinical condition and only one patient died (GOSE of I). In Group 2, there were two additional deaths (15 deaths in total, 48%), and the majority of survivors were graded with a GOSE of IV (6%) or V (23%) indicating severe or moderate disability, respectively (Table 3).

The receiver operating characteristic curves for outcome prognosis of the baseline S100B, NSE, MAPT, and GFAP in the serum of patients after an aneurysmal subarachnoid hemorrhage are shown in Fig. 2. In the ROC curve analysis, S100B (AUC 0.813; 95% CI 0.677–0.948, $p<0.001$) showed the highest ability to predict bad ICU outcome among the single biomarkers

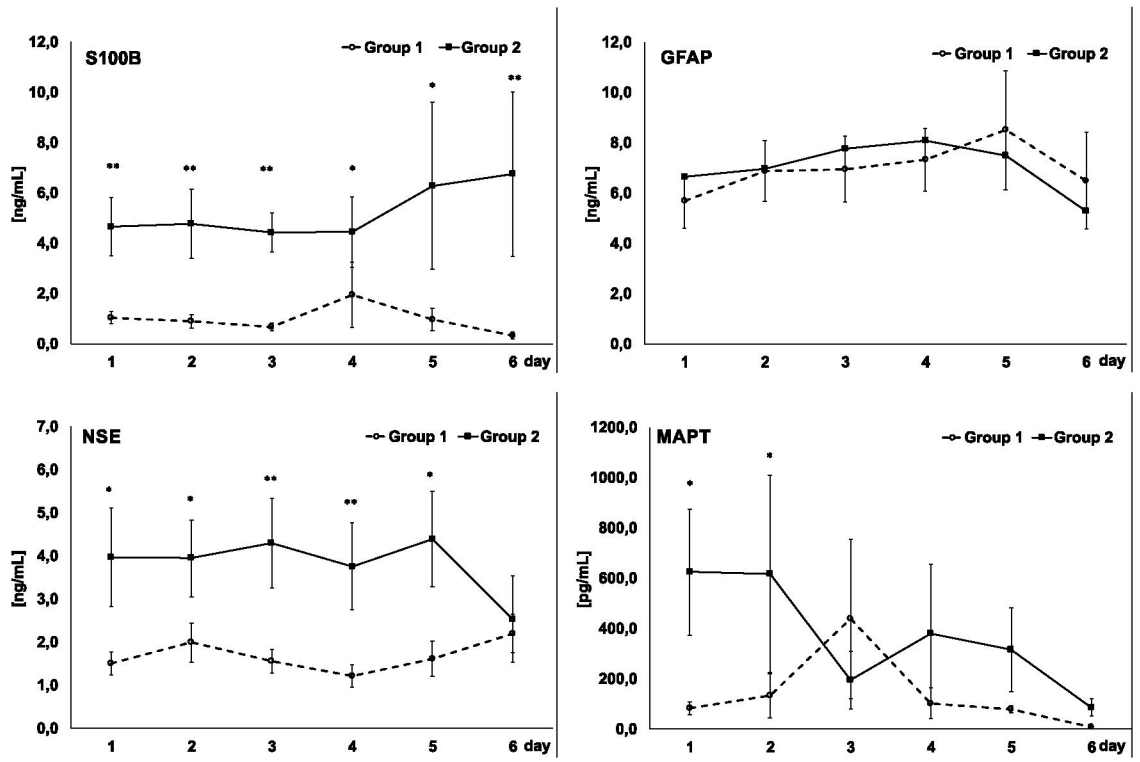

Fig. 1 Temporal course of S100B, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), and microtubule-associated protein tau (MAPT) in patients with a good outcome (Group 1) and in patients with a bad outcome (Group 2), based on the grade in the Glasgow Outcome Scale at discharge from the ICU. (*$p < 0.05$; **$p < 0.001$)

measured on admission. The optimal threshold value for the baseline S100B was 0.625 ng/mL, with sensitivity of 0.913 (95% CI 0.833–1.067) and specificity of 0.625 (95% CI 0.651–1.249). The baseline NSE also had significant predictive value (AUC 0.706; 95% CI 0.541–0.871, $p = 0.015$); however, for the optimal threshold value of 1.613 ng/mL, the sensitivity (0.667; 95% CI 0.719–1.180) of the marker was no longer as good as for S100B, and specificity was 0.733 (95% CI 0.689–1.211). The baseline MAPT had significant predictive value (AUC 0.735; 95% CI 0.550–0.892, $p = 0.012$); for the optimal threshold value of 240.7 pg/mL, the sensitivity was low (58.8%; 95% CI 0.647–1.253), but the specificity of the marker was very good (0.909; 95% CI 0.830–1.070). The baseline GFAP prediction value was not significant ($p = 0.46$).

In addition, a multivariate logistic regression analysis was performed to create a model predicting bad outcome. The choice of the variables from the set of biomarkers (S100B, NSE, MAPT, and GFAP) and covariates (age, gender, GCS, APACHEII, WFNS scale, Hunt and Hess scale, Fisher scale) was determined by the minimizing of the Akaike information criterion and the backward selection of the model; collinear covariates were excluded from the analysis. Therefore, the only variables that were included in the final model were the initial S100B, NSE, gender, and APACHEII. The initial S 100B, APACHEII, and gender were significant predictors of bad outcome. The initial NSE had no statistical significance in the model ($p = 0.088$). Results of analysis are presented in Table 4.

Correlations of Brain Biomarkers with GOS

At ICU discharge, a strong negative correlation between the GOS and the level of S100B was recorded on days 1–6, between the GOS and the level of NSE on days 1–5 (Table 5), and between the GOS and the level of MAPT on day 1; there was no correlation between GOS and GFAP. There was no correlation between any of the biomarkers measured on day 1, and the severity scores recorded on admission to the ICU (APACHE II, GCS, H–H, Fisher, and WFNS).

Discussion

In this report, we describe a panel of proteins abundant in the cells of the central nervous system that increase in the blood following an aSAH, and they may be early predictors of the neurological outcome in the acute

Table 3 A comparison of patients with a good (Group 1) versus poor (Group 2) status based on the value of the GOS recorded at discharge from the ICU

Parameter	Group 1 ($N=24$)	Group 2 ($N=31$)	p
GOS at ICU discharge [n (%)]			NA
Grade 1	–	12 (39)	
Grade 3	–	19 (61)	
Grade 4	19 (79)	–	
Grade 5	5 (21)	–	
ICU LOS (day)	10.4 ± 1.7 (2–44)	17.9 ± 2.5 (3–62)	0.029
Hospital LOS (day)	25.3 ± 6.5 (9–145)	52.3 ± 7.1 (9–120)	< 0.001
ICU survival [n (%)]	24 (100)	19 (61)	< 0.001
Hospital survival [n (%)]	24(100)	18 (58)	0.001
Patient's status, discharge from hospital [n (%)]			< 0.05
Dead	0	13 (42)	
Home	20 (83)	13 (42)	
Rehabilitation ward	1 (4)	2 (6)	
Another hospital	3 (13)	3 (10)	
Follow-up:			
GOS at hospital discharge [n (%)]			< 0.001
Grade 1	–	13 (42)	
Grade 2	–	1 (3)	
Grade 3	–	7 (22)	
Grade 4	6 (25)	9 (29)	
Grade 5	18 (75)	1(3)	
GOSE at 6-month follow-up [n (%)]			< 0.001
Grade 1	1 (4)	15 (48)	
Grade 4	–	2 (6)	
Grade 5	1 (4)	7 (23)	
Grade 6	2 (8)	2 (6)	
Grade 7	12 (50)	4(13)	
Grade 8	8 (33)	1 (3)	

Data are presented as mean ± standard error (min–max), unless other stated; GOS, Glasgow Outcome Scale; GOSE, Glasgow Outcome Scale Extended; LOS, length of stay; p value represents a comparison between groups

treatment phase. In this study, we limited the question of outcome prognostication to the acute phase of aSAH treatment, i.e., at discharge from the ICU, and the biomarker concentrations were evaluated for six consecutive days after aSAH. Evaluating aSAH patients using clinical scores becomes more difficult with ICU patients receiving sedatives and analgesics. Biomarkers assessments might be an independent, additional tool to support the clinical evaluation at the ICU. Analysis was performed for the proteins of neurofilament, neuron-specific enolase (NSE) and microtubule-associated protein tau (MAPT), and for the proteins of glial cells, S100B and glial fibrillary acidic protein (GFAP). Outcomes were assessed at discharge from the ICU and analyzed based on the grade in the Glasgow Outcome Scale. Patients were classified into two groups: patients with a good outcome (GOS IV–V) and patients with a bad outcome (GOS I–III). The main findings are: (1) brain biomarkers S100B, NSE, GFAP, and MAPT increase markedly following an aSAH, (2) the baseline S100B shows the highest ability to predict bad ICU outcome after aSAH, and (3) in acute phase of aSAH treatment, S100B and NSE correlates with neurological outcome.

For practical reasons, all biomarker measurements were performed in serum samples. A high level of neuronal biomarkers in the blood of patients with an aSAH is a consequence of the damage to the neurons and to the blood–brain barrier, in which biomarkers are allowed to pass from the cerebrospinal fluid into the bloodstream. The ability to measure the level of brain damage markers in serum instead of in the cerebrospinal fluid seems more useful and universal, because patient blood samples can be collected regardless of elevated intracranial pressure. Moreover, it has been found that the duration of external ventricular drainage (EVD) and CSF sampling frequency

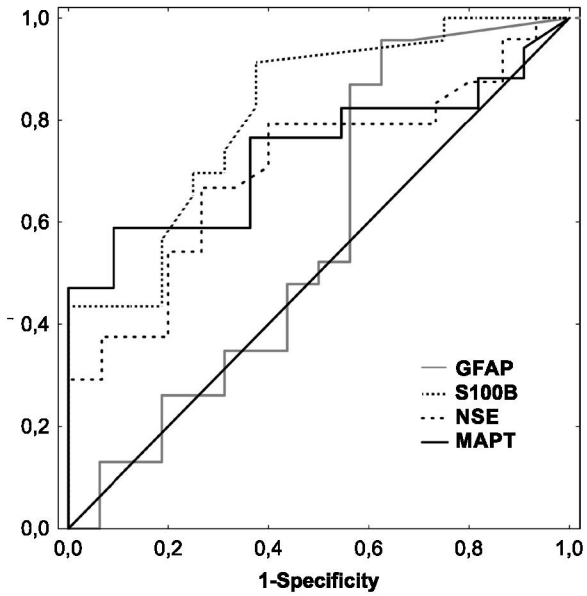

Fig. 2 Receiver operating characteristic curves for outcome prognosis of the baseline S100B (AUC 0.813), neuron-specific enolase (NSE; AUC 0.706), microtubule-associated protein tau (MAPT; AUC 0.735), and glial fibrillary acidic protein (GFAP; AUC 0.575) levels in the serum of patients after an aneurysmal subarachnoid hemorrhage

Table 4 Results of a multivariate logistic regression analysis model predicting bad ICU outcome in patients after aSAH

	Odds ratio	95% confidence intervals	*p* value
S100B	2.229	1.275–5.168	0.023
NSE	1.354	1.062–2.020	0.088
APACHE II	1.303	1.119–1.601	0.002
Gender	0.089	0.008–0.575	0.022

Table 5 *The Spearman's* rank *correlation coefficient* between neuronal biomarkers and the Glasgow Outcome Scale

Day	S100B and GOS	NSE and GOS	MAPT and GOS	GFAP and GOS
1	− 0.6**	− 0.4*	− 0.4*	n.s.
2	− 0.5**	− 0.4*	n.s.	n.s.
3	− 0.7**	− 0.6**	n.s.	n.s.
4	− 0.4*	− 0.5**	n.s.	n.s.
5	− 0.6*	− 0.5*	n.s.	n.s.
6	− 0.8*	n.s.	n.s.	n.s.

*$p < 0.05$; **$p < 0.001$, n.s. statistically non-significant

were significant risk factors for EVD-related infections, i.e., meningitis or ventriculitis (Hoefnagel et al. [16].

S100B, NSE, GFAP, and MAPT have previously been used as markers of neuronal damage, such as in stroke [17, 18], traumatic brain injury [19, 20], and aneurysmal SAH [21, 22]. Though a substantial number of studies have been performed to examine serum and CSF levels in patients with a range of neurological disorders, findings have been inconsistent regarding the changes in concentration of brain-specific biomarkers after an aneurysmal type SAH. Several clinical investigations have shown that the concentrations of S100B, NSE, GFAP, and MAPT are elevated in the serum and CSF samples of patients with an aSAH; however, large variations in the concentrations of the markers were reported. In an early study by Nylén et al. [23], increased s-GFAP levels were seen in a majority of aSAH patients, but the correlation with a WFNS was weak on admission (correlation coefficient < 0.4). Vos et al. [24] demonstrated increased glial (S100b and GFAP) but not neuronal (NSE) protein levels in peripheral blood at hospital admission. Additionally, high S100b and GFAP serum concentrations found after SAH were associated with the clinical severity of the initial injury, as measured by the WFNS scale. In another study, an early temporal profile of the S100B concentration after an aSAH was characterized by peak initial values followed by a decrease during the ensuing days post-injury [25]. Moreover, a threshold of initial S100B levels of > 0.7 µg/dl in serum was associated with 100% mortality. Our results also show large variation in concentrations of all the tested markers, from negligible to very high. However, certain kinetic patterns can be identified. Changes in S100B and NSE can be characterized with a similar pattern: S100B and NSE increased significantly at baseline and remained elevated throughout the study period in the group of patients with a bad outcome, while it stayed low in patients with a good outcome. The MAPT level was significantly elevated during the first 2 days of observation in the group of patients with a bad outcome, while it remained low in patients with a good outcome; the fifth and sixth day of observation had a similar pattern: the mean MAPT level decreased in both study groups. The mean GFAP level was elevated throughout the study period, and this pattern was seen in both groups, without significant differences in the kinetics. It should be noted that the initial hemorrhage in aSAH patients can be accompanied by a number of other factors, including intracerebral hematomas, re-bleedings, secondary ischemic events, and complications after surgery. All these factors may contribute to changes in the brain-specific biomarkers measured in the serum at different points in time. According to the multivariate logistic regression analysis, the best model

predicting bad ICU outcome after SAH included initial S100B, NSE, APACHEII score and gender. An elevated S100B, and APACHE II score, and male gender indicated a significantly higher risk of bad ICU outcome, resulting in severe disability, persistent vegetative state or death (GOS rating 1–3).

Findings of other authors regarding the prognostic potential of S100B, NSE, GFAP, and MAPT after an aSAH have been inconsistent. Our results indicate that S100B and NSE were strongly associated with neurological outcome measured with the GOS at discharge from the ICU. In the group of patients with a bad outcome, S100B and NSE levels increased significantly at baseline and remained elevated throughout the study period. The strong relationship of serum S100B, NSE, and MAPT levels with patient status at ICU discharge (AUC = 0.813, AUC = 0.706, AUC = 0.735, respectively) indicates that these two proteins may be potential predictors of a bad outcome in the clinical setting. We found that baseline S100B with a cutoff value of 0.625 ng/mL provided very good sensitivity of 91.3% and the NSE with a cutoff value of 1.613 ng/mL provided a sensitivity of 66.7% in predicting bad ICU outcome. It was notable that the baseline MAPT provided a specificity of 90.9% in predicting a good ICU outcome.

Our findings are in line with some previously published results. However, in very few studies was patient outcome evaluated in the acute phase of aSAH treatment, i.e., at discharge from the ICU; most studies evaluated the predictive value of brain biomarkers in relation to the long-term outcome after SAH. Based on earlier data on the utility of measuring biomarkers with aSAH, there is some controversy regarding the threshold value for the prediction of a bad outcome. Moritz et al. [26] found that mean and peak values of S100B (cutoff 0.17 and 0.23 μg/L, respectively) provided the ability to distinguish between patients with good and bad outcome at ICU discharge, while NSE did not provide predictive value.

In a study of fifty-one SAH patients, an S100B that was higher than 1 μg/L within the first 3 days after SAH was predictive of an unfavorable outcome at 6-month follow-up, and the NSE concentration was not related to the outcome [27]. Sanchez-Peña et al. [28] observed that an elevated level of S100B over the first 15 days after a subarachnoid aneurysmal hemorrhage was associated with a bad outcome after SAH at 12-month follow-up and the best cutoff for the mean 15-day S100B value was 0.23 μg/L (specificity 90%, sensitivity 91%), which was much lower than in the study by Oertel and in our study. Abboud et al. [29] found that S100B and NSE levels measured daily for the first 3 days after a hemorrhage accurately predicted the neurological outcome in poor-grade aSAH patients at 6-month follow-up. The best cutoff for the mean 3-day S100B value was 1.172 μg/L with a specificity of 75%, and for the mean 3-day NSE value 14.6 μg/L, with a specificity of 71.4%.

Quite opposite results have recently been published. Kiiski et al. [30] found that S100B and NSE measured during the first 24 h were not associated with neurological outcome evaluated at 6 months after an aSAH. Similar results were published by Olivecrona et al. [31]; based on the biomarker concentrations determined twice daily for five consecutive days, there was no significant clinical value of S100B and NSE as predictors of clinical outcome at 3 and 12-month follow-up.

As shown above, the time at which the determination of the biomarker concentrations would have the highest prognostic value and be of the greatest importance in identifying patients at risk of poor results remains a matter of dispute. Moreover, different values were used for the analyses: the value from the first day only, the mean of all measurements, the peak value. Different follow-up points were taken into consideration: short-term, such as ICU discharge, hospital discharge, 3-month follow-up, and long-term, such as 6- or 12-month follow-up.

The significant differences in the published results and the importance of biomarkers in predicting long-term clinical outcome after an aSAH may be also due to differences in the management of patients after the completion of ICU treatment. Rehabilitation content is a challenge, requires interdisciplinary cooperation, and may vary depending on the patient's clinical status. In addition, rehabilitation programs specializing in neurologic disorders after an aSAH may differ from country to country and may not be equally available to patients.

The limitation of this study is the relatively small size of the group, and a larger population of patients should be included to increase the statistical power of the findings. Like most previous studies, our study is based on the experiences from one clinical center; therefore, it may not be possible to generalize to a larger population.

Conclusions

Detecting the initial S100B, NSE, and MAPT in blood samples may prove to be a valuable diagnostic and prognostic tool in the very early phase of aSAH treatment. Tools for early outcome prediction in individual patients with SAH are needed, especially in the population of ICU patients receiving sedatives and analgesics, to more accurately assess clinical status, to direct care, and provide families with the most accurate information. Our findings provide further evidence that brain biomarkers such as S100B, NSE, GFAP, and MAPT increase markedly in patients following an aSAH. There is a direct relationship between the neurological outcome in the acute treatment

phase and the levels of S100B, NSE, and MAPT. Measuring biomarkers should be considered as a potential additional tool, supporting but not replacing the results of clinical scales such as the APACHEII, WFNS, GCS, Hunt–Hess scale, the Fisher scale, and GOS.

Abbreviations

aSAH: Aneurysmal subarachnoid hemorrhage; GFAP: Glial fibrillary acidic protein; MAPT: Microtubule-associated protein tau; NSE: Neuron-specific enolase.

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Author Contributions

JK contributed to conceptualization, methodology, investigation, writing—review and editing; MB contributed to conceptualization, methodology; WG contributed to writing—review and editing; AK contributed to conceptualization, funding acquisition, writing—review and editing; KK contributed to methodology, writing—review and editing; BA contributed to conceptualization, methodology, investigation, writing—review and editing, supervision.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical Approval

The protocol was approved by the Bioethics Committee of the Medical University in Wroclaw (KB–688/2014) and complies with the Declaration of Helsinki of the World Medical Association. In all cases, written informed consent was obtained from the patient or a legally authorized representative.

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References

1. Zanier ER, Zoerle T, Fiorini M, Longhi L, Cracco L, Bersano A, Branca V, Benedetti MD, De Simoni MG, Monaco S, Stocchetti N. Heart-fatty acid-binding and tau proteins relate to brain injury severity and long-term outcome in subarachnoid haemorrhage patients. Br J Anaesth. 2013;111(3):424–32. https://doi.org/10.1093/bja/aet149.
2. Lenski M, Huge V, Schmutzer M, Ueberschaer M, Briegel J, Tonn JC, Schichor C, Thon N. Inflammatory markers in serum and cerebrospinal fluid for early detection of external ventricular drain-associated ventriculitis in patients with subarachnoid hemorrhage. J Neurosurg Anesthesiol. 2019;31(2):227–33. https://doi.org/10.1097/ANA.0000000000000496.
3. Cossu G, Messerer M, Stocchetti N, Levivier M, Daniel RT, Oddo M. Intracranial pressure and outcome in critically ill patients with aneurysmal subarachnoid hemorrhage: a systematic review. Minerva Anestesiol. 2016;82(6):684–96.
4. Daou BJ, Koduri S, Thompson BG, Chaudhary N, Pandey AS. Clinical and experimental aspects of aneurysmal subarachnoid hemorrhage. CNS Neurosci Ther. 2019;25(10):1096–112. https://doi.org/10.1111/cns.13222.
5. Montaner J, Mendioroz M, Delgado P, García-Berrocoso T, Giralt D, Merino C, Ribó M, Rosell A, Penalba A, Fernández-Cadenas I, Romero F, Molina C, Alvarez-Sabín J, Hernández-Guillamon MJ. Differentiating ischemic from hemorrhagic stroke using plasma biomarkers: the S100B/RAGE pathway. Proteomics. 2012;75(15):4758–65. https://doi.org/10.1016/j.jprot.2012.01.033.
6. Zemke D, Farooq MU, Mohammed Yahia A, Majid A. Delayed ischemia after subarachnoid hemorrhage: result of vasospasm alone or a broader vasculopathy? Vasc Med. 2007;12(3):243–9. https://doi.org/10.1177/1358863X07081316.
7. Geraghty JR, Testai FD. Delayed cerebral ischemia after subarachnoid hemorrhage: beyond vasospasm and towards a multifactorial pathophysiology. Curr Atheroscler Rep. 2017;19(12):50. https://doi.org/10.1007/s11883-017-0690-x.
8. Mori T, Tan J, Arendash GW, Koyama N, Nojima Y, Town T. Overexpression of human S100B exacerbates brain damage and periinfarct gliosis after permanent focal ischemia. Stroke. 2008;39(7):2114–21. https://doi.org/10.1161/STROKEAHA.107.503821.
9. Kleine TO, Benes L, Zöfel P. Studies of the brain specificity of S100B and neuron-specific enolase (NSE) in blood serum of acute care patients. Brain Res Bull. 2003;61(3):265–79. https://doi.org/10.1016/s0361-9230(03)00090-x.
10. Rothermundt M, Peters M, Prehn JH, Arolt V. S100B in brain damage and neurodegeneration. Microsc Res Tech. 2003;60(6):614–32. https://doi.org/10.1002/jemt.10303.
11. Steinacker P, Weidehaas K, Cepek L, Feneberg E, Kretzschmar HA, Otto M. Influence of the blood-CSF-barrier function on S100B in neurodegenerative diseases. Acta Neurol Scand. 2013;128(4):249–56. https://doi.org/10.1111/ane.12113.
12. Tanaka Y, Marumo T, Omura T, Yoshida S. Relationship between cerebrospinal and peripheral S100B levels after focal cerebral ischemia in rats. Neurosci Lett. 2008;436(1):40–3. https://doi.org/10.1016/j.neulet.2008.02.056.
13. Connolly ES Jr, Rabinstein AA, Carhuapoma JR, Derdeyn CP, Dion J, Higashida RT, Hoh BL, Kirkness CJ, Naidech AM, Ogilvy CS, Patel AB, Thompson BG, Vespa P. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2012;43(6):1711–37. https://doi.org/10.1161/STR.0b013e3182587839.
14. Burzynska M, Uryga A, Kasprowicz M, Kedziora J, Szewczyk E, Wozniak J, Jarmundowicz W, Kübler A. Changes in the level of cardiac troponine and disorders in pulmonary gas exchange as predictors of short- and long-term outcomes of patients with aneurysm subarachnoid haemorrhage. Br J Neurosurg. 2017;31(6):653–60. https://doi.org/10.1080/02688697.2017.1339301.
15. Claassen J, Bernardini GL, Kreiter K, Bates J, Du YE, Copeland D, Connolly ES, Mayer SA. Effect of cisternal and ventricular blood on risk of delayed cerebral ischemia after subarachnoid hemorrhage: the fisher scale revisited. Stroke. 2001;32(9):2012–20.
16. Hoefnagel D, Dammers R, Ter Laak-Poort MP, Avezaat CJ. Risk factors for infections related to external ventricular drainage. Acta Neurochir (Wien). 2008;150(3):209–14. https://doi.org/10.1007/s00701-007-1458-9.
17. Deboevere N, Marjanovic N, Sierecki M, Marchetti M, Dubocage M, Magimel E, Mimoz O, Guenezan J. Value of copeptin and the S-100B protein assay in ruling out the diagnosis of stroke-induced dizziness pattern in emergency departments. Scand J Trauma Resusc Emerg Med. 2019;27(1):72. https://doi.org/10.1186/s13049-019-0651-1.

18. Ng GJL, Quek AML, Cheung C, Arumugam TV, Seet RCS. Stroke biomarkers in clinical practice: a critical appraisal. Neurochem Int. 2017;107:11–22. https://doi.org/10.1016/j.neuint.2017.01.005.
19. Egea-Guerrero JJ, Revuelto-Rey J, Murillo-Cabezas F, et al. Accuracy of the S100β protein as a marker of brain damage in traumatic brain injury. Brain Inj. 2012;26(1):76–82. https://doi.org/10.3109/02699052.2011.635360.
20. Wang KK, Yang Z, Zhu T, Shi Y, Rubenstein R, Tyndall JA, Manley GT. An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Rev Mol Diagn. 2018;18(2):165–80. https://doi.org/10.1080/14737159.2018.1428089.
21. Zheng YK, Dong XQ, Du Q, Wang H, Yang DB, Zhu Q, Che ZH, Shen YF, Jiang L, Hu W, Wang KY, Yu WH. Comparison of plasma copeptin and multiple biomarkers for assessing prognosis of patients with aneurysmal subarachnoid hemorrhage. Clin Chim Acta. 2017;475:64–9. https://doi.org/10.1016/j.cca.2017.10.009.
22. Watson E, Ding D, Khattar NK, Everhart DE, James RF. Neurocognitive outcomes after aneurysmal subarachnoid hemorrhage: identifying inflammatory biomarkers. J Neurol Sci. 2018;15(394):84–93. https://doi.org/10.1016/j.jns.2018.06.021.
23. Nylén K, Csajbok LZ, Ost M, Rashid A, Blennow K, Nellgård B, Rosengren L. Serum glial fibrillary acidic protein is related to focal brain injury and outcome after aneurysmal subarachnoid hemorrhage. Stroke. 2007;38(5):1489–94.
24. Vos PE, van Gils M, Beems T, Zimmerman C, Verbeek MM. Increased GFAP and S100beta but not NSE serum levels after subarachnoid haemorrhage are associated with clinical severity. Eur J Neurol. 2006;13(6):632–8.
25. Kellermann I, Kleindienst A, Hore N, Buchfelder M, Brandner S. Early CSF and serum S100B concentrations for outcome prediction in traumatic brain injury and subarachnoid hemorrhage. Clin Neurol Neurosurg. 2016;145:79–83. https://doi.org/10.1016/j.clineuro.2016.04.005.
26. Moritz S, Warnat J, Bele S, Graf BM, Woertgen C. The prognostic value of NSE and S100B from serum and cerebrospinal fluid in patients with spontaneous subarachnoid hemorrhage. J Neurosurg Anesthesiol. 2010;22(1):21–31. https://doi.org/10.1097/ANA.0b013e3181bdf50d.
27. Oertel M, Schumacher U, McArthur DL, Kästner S, Böker DK. S-100B and NSE: markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. J Clin Neurosci. 2006;13(8):834–40. https://doi.org/10.1016/j.jocn.2005.11.030.
28. Sanchez-Peña P, Pereira AR, Sourour NA, Biondi A, Lejean L, Colonne C, Boch AL, Al Hawari M, Abdennour L, Puybasset L. S100B as an additional prognostic marker in subarachnoid aneurysmal hemorrhage. Crit Care Med. 2008;36(8):2267–73. https://doi.org/10.1097/CCM.0b013e3181809750.
29. Abboud T, Mende KC, Jung R, Czorlich P, Vettorazzi E, Priefler M, Kluge S, Westphal M, Regelsberger J. Prognostic value of early S100 calcium binding protein B and neuron-specific enolase in patients with poor-grade aneurysmal subarachnoid hemorrhage: a pilot study. World Neurosurg. 2017;108:669–75. https://doi.org/10.1016/j.wneu.2017.09.074.
30. Kiiski H, Långsjö J, Tenhunen J, Ala-Peijari M, Huhtala H, Hämäläinen M, Moilanen E, Peltola J. S100B, NSE and MMP-9 fail to predict neurologic outcome while elevated S100B associates with milder initial clinical presentation after aneurysmal subarachnoid hemorrhage. J Neurol Sci. 2018;390:129–34. https://doi.org/10.1016/j.jns.2018.04.030.
31. Olivecrona M, Rodling-Wahlström M, Naredi S, Koskinen LO. S-100B and neuron specific enolase are poor outcome predictors in severe traumatic brain injury treated by an intracranial pressure targeted therapy. J Neurol Neurosurg Psychiatry. 2009;80(11):1241–7. https://doi.org/10.1136/jnnp.2008.158196.

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Brain-Specific Biomarkers as Mortality Predictors after Aneurysmal Subarachnoid Haemorrhage

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Abstract: Aneurysmal subarachnoid haemorrhage (aSAH) is a serious condition with a high mortality and high permanent disability rate for those who survive the initial haemorrhage. The purpose of this study was to investigate markers specific to the central nervous system as potential in-hospital mortality predictors after aSAH. In patients with an external ventricular drain, enolase, S100B, and GFAP levels were measured in the blood and cerebrospinal fluid (CSF) on days 1, 2, and 3 after aSAH. Compared to survivors, non-survivors showed a significantly higher peak of S100B and enolase levels in the blood (S100B: 5.7 vs. 1.5 ng/mL, $p = 0.031$; enolase: 6.1 vs. 1.4 ng/mL, $p = 0.011$) and the CSF (S100B: 18.3 vs. 0.9 ng/mL, $p = 0.042$; enolase: 109.2 vs. 6.1 ng/mL, $p = 0.015$). Enolase showed the highest level of predictability at 1.8 ng/mL in the blood (AUC of 0.873) and 80.0 ng/mL in the CSF (AUC of 0.889). The predictive ability of S100B was also very good with a threshold of 5.7 ng/mL in the blood (AUC 0.825) and 4.5 ng/mL in the CSF (AUC 0.810). In conclusion, enolase and S100B, but not GFAP, might be suitable as biomarkers for the early prediction of in-hospital mortality after aSAH.

Keywords: subarachnoid haemorrhage; outcome; S100B protein; enolase; GFAP; brain damage markers; cerebrospinal fluid

1. Introduction

In patients who have suffered brain injury, the concentrations of certain markers in the cerebrospinal fluid (CSF) and blood are correlated with the severity of brain damage and the outcome [1–3]. Markers specific to the central nervous system (CNS) have been the focus of research as potential post-injury outcome biomarkers, in particular those derived from neurons and astrocytes [4]. Neuron-specific enolase is a cytoplasmic enzyme of neurons and has no extracranial sources. Therefore, NSE has the potential to be useful as a marker of destructive processes in the central nervous system. Another marker, S100B, is a protein found predominantly in astrocytes, glial and Schwann cells in the CNS. S100B is released after brain injury and high levels of S100B can be detected in a variety of pathological injuries to the CNS, such as subarachnoid haemorrhage (SAH), acute brain injury, traumatic brain injury (TBI), and acute ischemic stroke [5,6]. Glial fibrillary acid protein (GFAP), a cytoskeleton protein, is another promising marker of brain injury. It serves as an intermediate filament in numerous cell types of the CNS, including mature astrocytes. The concentration of GFAP is higher in brain pathologies such as stroke, intracerebral haemorrhage, dementia, and SAH [7].

Aneurysmal subarachnoid haemorrhage (aSAH), which is subarachnoid bleeding resulting from an intracranial aneurysm, is a serious condition with a high mortality rate and high permanent disability rate for those who survive the initial haemorrhage. Previously published studies indicated that aSAH occurs at a relatively young age and has a mortality rate of 8% to 67% [8,9]. Outcome indicators of aSAH evaluate the patient's condition on admission and most often include age, admission neurologic grade (assessed with the World Federation of Neurological Surgeons grade and Hunt and Hess score) and the appearance of SAH on admission with a computed tomography scan (assessed with the Fisher grade or a similar classification). Failure of cerebral autoregulation and delayed cerebral ischemia also affects the overall prognosis [10]. In addition to clinical parameters and clinical grades, the use of biomarkers after brain injury may be of interest not only for diagnosis and identification of intracranial lesions, but also for assessing severity, treatment effectiveness, and prognosis. An increase in some biomarkers can be detected before the presentation of neurological deterioration [11]. Consequently, patients can be identified earlier in the acute period after aSAH, as a high risk for a bad outcome and death [12]. Patients with a bad prognosis on admission would require long-term intensive care with frequent neurological examinations and continuous monitoring.

Markers specific to the central nervous system, enolase, S100B, and GFAP were the focus of this research as potential outcome predictors after aSAH [13,14]. In the majority of previously published studies, brain biomarkers were assessed in blood samples of patients diagnosed with aSAH, and only a few studies investigated S100B, enolase, and GFAP in the cerebrospinal fluid (CSF). The procedure of collecting CSF in aSAH patients is performed for clinical reasons, such as acute hydrocephalus, severe intraventricular haemorrhage or to measure and treat elevated intracranial pressure [15,16]. It should also be emphasized that a Hunt and Hess grade ≥ 3 and Glasgow Coma Score ≤ 12 are the thresholds for strong consideration of placing an external ventricular drain [15]. Some studies suggested that CSF drainage reduced the vasospasm-related delayed ischemic neurological deficit and improved outcomes. On the other hand, external ventricular drain management influences the rate of complications, such as infection, a ventriculo-peritoneal shunt, CSF leak, or intracranial subdural hygroma or haematoma; moreover, prolongation of the ICU and hospital stay and the deterioration of cognitive outcome were observed in SAH survivors [17,18]. However, in cases when CSF was collected for a clinical reason, measuring the concentration of brain-specific biomarkers in a CSF sample might provide additional valuable information about the degree of brain cell damage, while measuring brain-specific markers in the blood relies on the assumption that the blood-brain barrier has been damaged, allowing proteins to pass from the cerebrospinal fluid into the bloodstream.

It is important to have sufficiently sensitive markers for the brain that can be determined in the CSF and blood. An accurate interpretation of brain biomarkers in relation to predicting mortality in the acute phase of aSAH treatment requires a deeper understanding of the patterns in which they are released. Therefore, the aim of this study was to investigate whether enolase, S100B, and GFAP would be suitable as sensitive biomarkers for the early prediction of in-hospital mortality after aSAH. We analysed biomarker concentrations in the cerebrospinal fluid and blood in aSAH patients who were initially treated with an external ventricular drain and correlated this to patient outcome at hospital discharge.

2. Experimental Section

2.1. Patient Population

Seventy patients with a diagnosis of spontaneous aSAH were admitted to the Neuro-Intensive Care Unit (ICU) from 2014 to 2017. Patients who were treated with an external ventricular drain and met the inclusion criteria were included in the study.

Inclusion criteria: age ≥ 18 years, an aneurysm in the cerebral arteries as the cause of bleeding confirmed using computer tomography angiography (CTA), magnetic resonance imaging (MRI) or

conventional angiography, ICU admission within 24 h after the clinical diagnosis of aSAH, and CSF that was accessible for collection for three consecutive days after the SAH.

Exclusion criteria: previously diagnosed neurological disease, such as previously diagnosed aneurysms, stroke, epilepsy; neurodegenerative diseases such as Parkinson's disease, dementia, cerebrovascular diseases, motor neuron diseases, and traumatic brain injury.

Cerebrospinal fluid was collected only if the procedure was medically justified and safe for a patient.

Overall, sixteen patients met the inclusion criteria and were included in the final analysis. The study flowchart is shown in Figure 1.

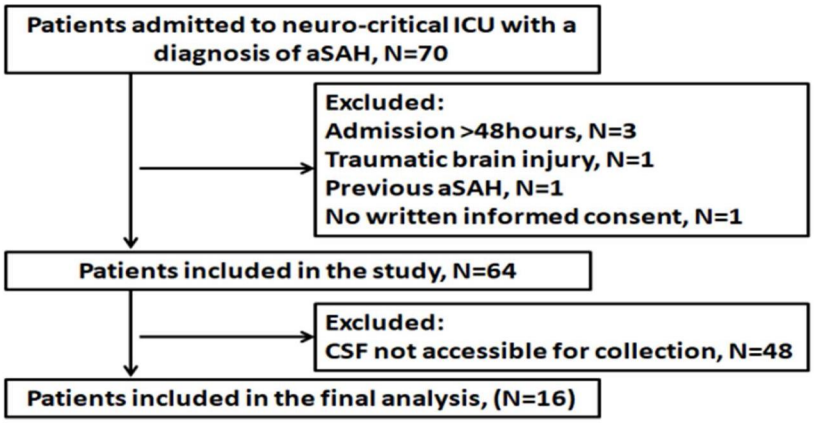

Figure 1. Flow diagram of the study.

2.2. Ethics

The protocol was approved by the Bioethics Committee of the Medical University in Wroclaw (KB–688/2014) and complies with the Declaration of Helsinki of the World Medical Association. In all cases, written informed consent was obtained from the patient or a legally authorized representative.

2.3. Clinical Assessment

All patients had an aneurysm in a cerebral artery which had been confirmed as the cause of bleeding by using computer tomography (CT), magnetic resonance imaging (MRI) or conventional angiography. Patients underwent either early clipping or coiling of the detected ruptured aneurysm, within 72 h after the initial bleeding.

The following data were collected: patient age, sex, medical history, aneurysm location, Hunt and Hess, Fisher, WFNS, and GCS grades on admission to the ICU, the timing of surgery or coiling, postoperative complications, the length of stay in the ICU and hospital, and outcomes. A standard clinical treatment protocol was used to manage patients, including early CT angiography and/or three-dimensional digital subtraction angiography, and early surgery when possible, based on aneurysm characteristics, neurological and the general status of the patient. Poor-grade patients with acute hydrocephalus or severe intraventricular haemorrhage were treated with supplementary external ventricular drainage (EVD). Postoperative management included computed tomography of the brain to detect postoperative complications (performed within 24 h after surgical or endovascular treatment), analgesia, sedation, mechanical ventilation, and euvolemia and catecholamine support, where indicated. Induced hypertension was used to improve cerebral circulation and perfusion pressure in patients with cerebral vasospasm (CV) or *delayed* cerebral ischemia (DCI). All patients received nimodipine. Screening for cerebral vasospasm (CVS) was performed daily using neurological examination and transcranial Doppler ultrasound (TCD) measurements. Cerebral vasospasm was defined as a mean cerebral blood flow velocity (CBFV) in the middle cerebral artery that exceeded 120 cm/s or a daily increase of CBFV of approximately 20%.

Focal neurological impairment and weakness or paralysis in the arms and legs were investigated daily by physical examination. DCI was defined as a new focal or global neurological impairment lasting for at least one hour, together with a cerebral infarction that was not caused by other factors (e.g., systemic or surgical complications). On admission, patients were assessed using the Glasgow Coma Scale (GCS). Subarachnoid haemorrhage severity was evaluated with the Hunt and Hess (H-H) scale and defined as severe (4–5) or moderate (1–3). The extent of haemorrhaging was assessed with a computerized tomography (CT) scan of the head and classified using the Fisher scale, where 1 = no subarachnoid (SAH) or intraventricular haemorrhage (IVH) detected, 2 = diffuse thin (<1 mm) SAH, no clots, 3 = localized clots and/or layers of blood >1 mm in thickness, no IVH, and 4 = diffuse or no SAH an intracerebral or intraventricular clot. Outcomes were assessed at discharge from the hospital using the Glasgow Outcome Scale (GOS). Long-term outcomes were assessed with GOS at six months after discharge.

2.4. Blood and CSF Sampling and Measurement of Biomarkers

CSF samples (4.5 mL) were collected via an external ventricular drain at the ICU on days 1, 2, and 3. CSF samples were treated with an anticoagulant, centrifuged and the supernatant was aliquoted and kept at −80 degrees Celsius. Blood samples were collected using an intravenous catheter at the ICU on days 1, 2, and 3. Samples were centrifuged, and the supernatant was aliquoted and kept at −80 degrees Celsius. An ELISA assay was used to measure the concentration of S100B (Cloud-Clone Corp., Katy, TX, USA), GFAP (Elabscience, Houston, TX, USA), and enolase (R&D Systems, Minneapolis, MN, USA). The concentrations of the mediators were measured in duplicate with appropriate controls, according to the manufacturer's instructions using an ELx800 absorbance microplate reader (BioTek, Winooski, VT, USA). As we did not measure CSF biomarkers in another group of neurosurgical patients, we used reference ranges previously reported in the literature. Hajduková et al. established reference ranges for S100B and enolase in CSF based on the results of a study involving a group of 601 patients with no pathological findings (inflammatory, vascular, degenerative, or traumatic impairment of CNS) and without clinical or CT/MRI signs of CNS tissue damage; biochemical, cytological, and immunological values in CSF were normal [19]. The following reference intervals were calculated as 2.5th and 97.5th percentiles: for S100B, 0.3–1.6 ng/mL, and for enolase, 3.5–22.9 ng/mL.

2.5. Groups

Patients were categorized based on their status at hospital discharge into two groups: survivors and non-survivors. After 6 months, the clinical status of the patients was assessed with the Glasgow Outcome Scale. To explore the relationship between the CSF and blood biomarkers and outcome, the maximum (peak) values were determined in each patient for the analysed biomarkers, based on values recorded on days 1, 2, and 3.

2.6. Statistical Analysis

Continuous variables are presented as the median (interquartile range between the 25th and 75th percentiles); categorical data are presented as numbers and percentages. All analysis was performed with Statistica, version 13 (StatSoft Inc., Tulsa, OK, USA). The distribution was not normal based on the Shapiro–Wilk test. Therefore, statistical analysis was performed using nonparametric tests. The Mann–Whitney U test was used for the comparison of continuous variables between the study groups. For categorical variables, Fisher's exact test was used to check the level of significance in small sample sizes; contingency tables were used to summarize the relationship between several categorical variables. The Friedman ANOVA test was used to detect differences in biomarker concentrations across multiple test attempts (day 1, 2, and 3). The peak values were determined in each patient for S100B, enolase, and GFAP in the blood and CSF, based on concentrations recorded on days 1, 2, and 3. Spearman's rank correlation was used to determine the relationship between the CSF and blood biomarkers and the clinical scores. Receiver operating characteristic (ROC) curve analysis was used to

measure the ability of the biomarkers measured in the CSF and blood to discriminate between hospital death and survivors by calculating the area under the curve (AUC), including 95% confidence intervals (CI), to determine sensitivity and specificity. A "p" value of < 0.05 was accepted as significant.

3. Results

Seventy consecutive patients with a diagnosis of aSAH were screened for inclusion/exclusion criteria. Of this number, 64 patients met the inclusion criteria. The 16 patients in whom cerebrospinal fluid was available for collection for three consecutive days after SAH were included in the final analysis. The flow diagram of the study is presented in Figure 1.

The median age was 67 (range 53–76) and 8 patients (50%) were female. On admission to the ICU, 7 patients (44%) were in poor clinical condition (WFNS 4–5). CT scans on admission were graded as follows: 2 patients were graded II (diffuse blood only) on the Fisher scale, 2 were graded III (localized clots and/or vertical layers of blood 1 mm or greater in thickness), and 12 were graded IV (diffuse or no subarachnoid blood with cerebral or ventricular blood). Aneurysms were treated with endovascular coiling in 3 patients and with surgical clipping in 13 patients (81%). During treatment at the ICU, 2 patients showed ventriculitis, which was closely related to site leakage and the duration of catheterization. Both patients received early aggressive treatment, including re-insertion of the drain and treatment with antibiotics. A good outcome was obtained in 1 of these patients; the *ventriculoperitoneal* shunt (*VPS*) was inserted in this patient. The second patient was in poor neurological condition, with an intracerebral hematoma and a blood clot in the ventricles. In both patients, we kept the EVD open with a gradual EVD weaning approach. Attempts were made to close the drain, but due to clinical symptoms such as nausea/vomiting, headache, and altered mental status, the drain was reopened and finally the VPS was inserted. The decision to repeat a clamp trial or place a VPS after a failed clamp trial was made by the attending neurosurgeon.

Nine patients survived and 7 died (44%); all deaths occurred at the ICU. After a 6-month follow-up, the mortality rate had increased to 56% with 2 additional deaths.

GCS on admission to the ICU was significantly lower in non-survivors than in survivors (4 vs. 14 points, $p = 0.022$); the results of other clinical scales (WFNS, Hunt–Hess, and Fisher) did not differ significantly between survivors and non-survivors. The length of stay in the hospital for non-survivors (109 days) was 5× longer than for survivors (21 days). The demographic and clinical data are shown in Table 1.

Table 1. Characteristics of patients with aneurysmal subarachnoid haemorrhage (aSAH).

	Total	**Non-Survivors**	**Survivors**	***p***
	(*N* = 16)	**(*N* = 7)**	**(*N* = 9)**	
Age, [years]	67 (53–76)	75 (38–82)	67 (56–67)	0.606
Gender male/female, [*n*]	8/8	3/4	4/5	0.500
Glasgow Coma Scale	11 (4–14)	4 (4–12)	14 (10–14)	0.022
WFNS, severity of symptoms, [*n* (%)]				0.141
I-III	7 (44)	1	6	
IV-V	9 (56)	6	3	
Hunt–Hess grade, severity of symptoms, [*n* (%)]				0.216
Mild headache	2 (12)	0	2	
Severe headache	2 (12)	1	1	
Lethargy or confusion	2 (12)	0	2	
Stupor	3 (19)	2	1	
Coma	7 (44)	4	3	
Fisher grade, SAH on CT scan, [*n* (%)]				0.411
none	0			
diffuse only	2 (12)	1	1	
clot or thick layer	2 (12)	0	2	
diffuse or none, with cerebral/ventricular blood	12 (75)	6	6	

Table 1. *Cont.*

	Total	Non-Survivors	Survivors	*p*
	(*N* = 16)	(*N* = 7)	(*N* = 9)	
Delayed cerebral vasospasm, [*n* (%)]	11(69)	5	6	0.634
Intensive Care Unit LOS [day]	15 (8–21)	13 (8–18)	19 (13–28)	0.210
Hospital LOS [day]	43 (20–109)	109 (23–114)	21 (18–57)	0.055

Data are presented as the median (25th–75th percentiles), unless otherwise stated; SAH, subarachnoid haemorrhage; LOS, length of stay.

3.1. Blood and CSF Biomarkers Concentration

The peak value is the highest value of the biomarker recorded on days 1 to 3. Non-survivors showed significantly higher peaks of S100B and enolase levels compared to survivors (S100B: 5.7 vs. 1.5 ng/mL, $p = 0.031$; enolase: 6.1 ng/mL vs. 1.4 ng/mL, $p = 0.011$) in the blood. Similar relationships were observed in the CSF: in non-survivors the peak S100B and peak enolase levels were significantly higher compared to survivors (S100B: 18.3 vs. 0.9 ng/mL, $p = 0.042$; enolase: 109.2 vs. 6.1 ng/mL, $p = 0.015$). It is worth noting that, in the CSF, the peak value of S100B and enolase was within the reference range in the group of survivors and exceeded the reference range several times in the group of non-survivors (S100B ref. range: 0.3–1.6 ng/mL; enolase ref. range: 3.5–22.9 ng/mL).

There were no significant differences in the GFAP peak values between the study groups, neither in the blood samples nor the CSF. Detailed data on the peak values of individual biomarkers, including the interquartile range, are presented in Table 2.

Table 2. The peak values determined in each patient for S100B, enolase, and GFAP in the blood and CSF based on values measured on days 1, 2, and 3.

	Blood			CSF		
	Non-Survivors	Survivors	*p*	Non-Survivors	Survivors	*p*
S100B [ng/mL]	5.7 (1.6–11.2)	1.5 (0.7–2.3)	0.031	18.3 (8.9–78.1)	0.9 (0.6–13.4)	0.042
Enolase [ng/mL]	6.1 (1.9–10.8)	1.4 (1.4–1.6)	0.011	109.2 (31.7–269.0)	6.1 (4.8–16.5)	0.015
GFAP [ng/mL]	4.4 (3.0–15.6)	4.5 (2.3–9.1)	0.469	1.37 (0.03–4.88)	0.05 (0.03–0.09)	0.438

Data are presented as the median (25th–75th percentiles); *p*-value, the difference between non-survivors and survivors.

The biomarker concentrations on consecutive days after the SAH showed significantly higher levels of S100B in non-survivors than in survivors, both in the blood samples (day 1: 1.7 vs. 0.7 ng/mL, $p = 0.041$; day 2: 5.6 vs. 0.7 ng/mL, $p = 0.007$; day 3: 3.6 vs. 0.3 ng/mL $p < 0.001$, in non-survivors vs. survivors, respectively) and the CSF (day 1: 21.9 vs. 3.9 ng/mL, $p = 0.031$; day 2: 25.6 vs. 0.6 ng/mL, $p = 0.002$; day 3: 23.9 vs. 0.6 ng/mL, $p = 0.002$, in non-survivors vs. survivors, respectively). Similar patterns were observed in the changes in enolase concentrations in the blood (day 1: 6.0 vs. 1.3 ng/mL, $p = 0.046$; day 2: 4.2 vs. 0.9 ng/mL, $p = 0.005$; day 3: 3.4 vs. 1.1 ng/mL, $p = 0.049$, in non-survivors vs. survivors, respectively) and the CSF (day 1: 52.6 vs. 5.4 ng/mL, $p = 0.029$; day 2: 16.9 vs. 4.1 ng/mL, $p = 0.035$; day 3: 80.0 vs. 3.4 ng/mL, $p = 0.031$, in non-survivors vs. survivors, respectively). It is worth noting, that on all days, the median of both biomarkers was higher in the cerebrospinal fluid than in the blood. In contrast, the concentrations of GFAP were not statistically different for non-survivors and survivors (Figure 2). A log scale was used to plot the data in order to compare the concentrations using the same range of scales for individual biomarkers in the blood and cerebrospinal fluid. The Friedman ANOVA test did not detect significant differences in the biomarker concentrations between days 1, 2, and 3.

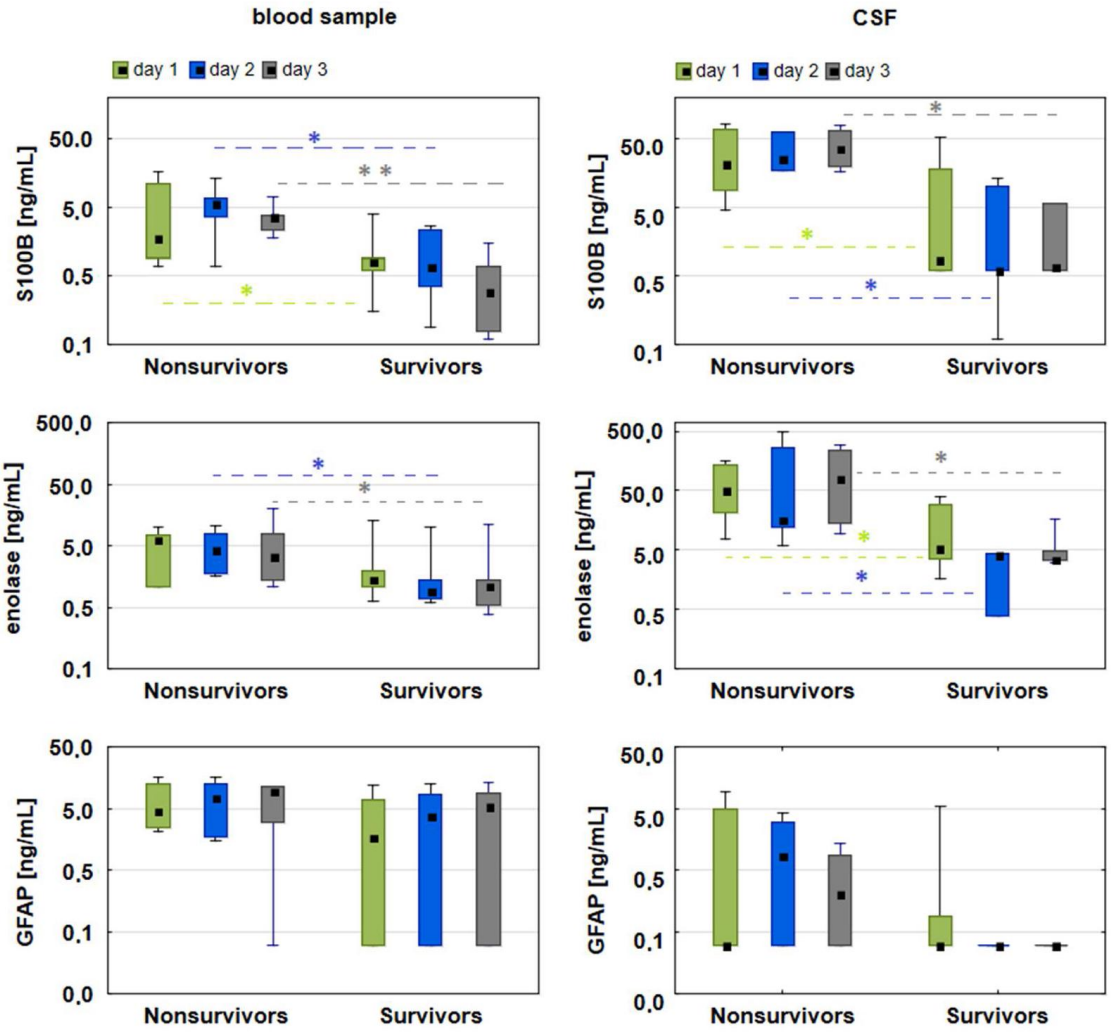

Figure 2. Graphs comparing the levels of S100B, enolase, and GFAP in the blood (**left panel**) and CSF (**right panel**) of non-survivors and survivors. The statistically significant differences in the levels of biomarkers between non-survivors and survivors on corresponding days were marked with * ($p < 0.05$) or ** ($p < 0.001$). A logarithmic scale was used to plot the data. The box plots represent the median values (midpoint) with upper and lower quartiles (box); the whiskers represent the minimum and maximum values.

3.2. Association between Biomarkers and Severity Scores

The peak concentrations of enolase recorded in the CSF were strongly correlated with GCS (R = 0.7, $p = 0.005$), the Hunt–Hess score (R = 0.6, $p = 0.027$), the Fisher score (R = 0.6, $p = 0.009$), and the WFNF score (R = 0.7, $p = 0.003$) calculated on admission to the ICU. The peak concentrations of enolase in the blood and the peak concentrations of S100B in the blood or CSF did not show a significant correlation to the clinical scores.

3.3. Biomarkers as Outcome Predictors

In the ROC curve analysis, the peak concentrations of enolase recorded in the blood (AUC 0.873; 95% CI 0.682–1.000, $p = 0.001$) and the CSF (AUC 0.889; 95% CI 0.716–1.000, $p < 0.001$) showed the highest ability to predict hospital mortality (Figure 3). The optimal threshold value for the peak concentration of enolase in the blood was 1.8 ng/mL, with sensitivity of 100% and specificity of 78% and in the CSF it was 80.0 ng/mL, with sensitivity of 67% and specificity of 100%. The peak concentrations

of S100B in the blood (AUC 0.825; 95% CI 0.592–1.000, $p = 0.006$; CSF) and the CSF (AUC 0.810; 95% CI 0.595–1.000, $p < 0.004$) also had significant predictive value. The optimal threshold value for the peak concentration of S100B in the blood was 5.7 ng/mL, with sensitivity of 71% and specificity of 100%, and in the CSF it was 4.5 ng/mL, with sensitivity of 100% and specificity of 56%. The GFAP prediction value was not significant either in the blood ($p = 0.488$) or CSF ($p = 0.443$). For comparison, among clinical scores calculated on admission to the ICU, only GCS showed the ability to predict hospital mortality (AUC 0.833; 95% CI 0.632–1.000, $p = 0.001$), while the Hunt–Hess grade, the Fisher grade, and the WFNS score results of the ROC curve analysis were not significant. The optimal threshold value for the GCS was 13 points, with sensitivity of 100% and specificity of 55%.

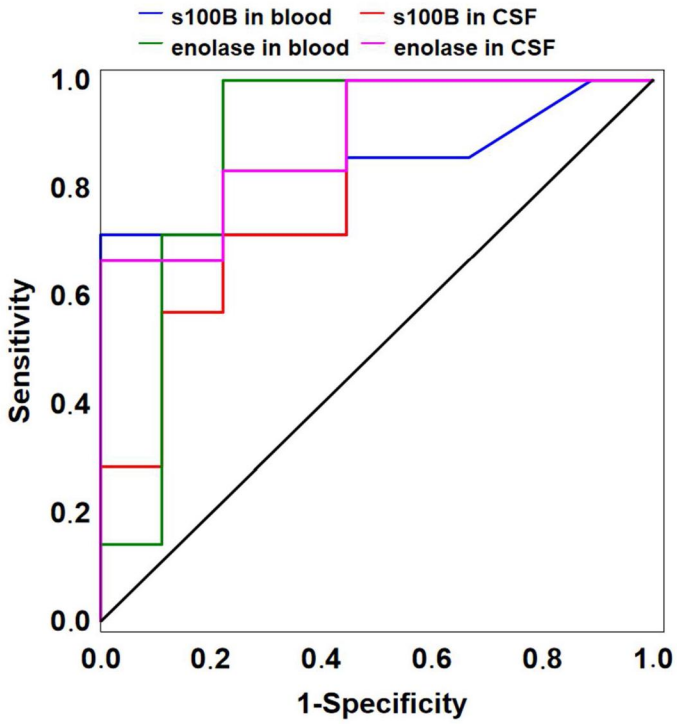

Figure 3. Receiver operating characteristic curve analysis of the peak concentration of S100B in blood (AUC = 0.825) and CSF (AUC = 0.810) and enolase in blood (AUC = 0.873) and CSF (AUC = 0.889) as mortality predictors after aSAH.

4. Discussion

The results of this study are consistent with the hypothesis that enolase and S100B measured in the cerebrospinal fluid and blood are sensitive biomarkers for predicting in-hospital mortality in patients with aSAH. This study focused on the first 3 days after SAH. We found that S100B and enolase were significantly higher in patients who died than in those who survived, and the peak concentrations of these biomarkers recorded in the cerebrospinal fluid and blood showed a clear ability to predict in-hospital mortality. The authors found very few published reports evaluating the usefulness of cerebrospinal fluid and blood biomarker measurements in predicting mortality in the acute period after aSAH, i.e., at hospital discharge.

Brain cells are damaged to varying degrees by SAH. As a result, various proteins are released into the subarachnoid space from damaged neurons, oligodendrocytes and glial cells [20]. Depending on their molecular weight and the integrity of the blood–brain barrier, these proteins pass to the subarachnoid space and/or to the bloodstream. Proteins that are not physiologically present in the cerebrospinal fluid or blood, or are present at a very low level, can be indicators of brain damage. Here, we showed that determining the concentration of S100B and enolase in the CSF and blood

samples could support the early prediction of mortality after aSAH; the concentrations of S100B and enolase measured in the CSF in the early phase of treatment were several times higher in non-survivors than in survivors. Moreover, a similar pattern was observed in the blood samples, indicating damage to the blood–brain barrier, showing that SAH disrupted the integrity of the blood-brain barrier and resulted in the leakage of endogenous proteins. In a previous study by Kellerman et al., when assessing the prognostic value of early blood and CSF concentrations of S100B after subarachnoid haemorrhage, significant differences were found between patients with a bad and good outcome [13]. S100B concentrations in the CSF and blood were significantly higher in patients with an unfavourable outcome (GOS 1–3) in comparison to patients with a good outcome (GOS 4–5), and similar to our results, the concentration of S100B in the CSF was markedly higher than in the blood samples on corresponding days. In a study by Petzold et al., S100B concentrations in the blood measured on admission to the ICU and then at day 1 were significantly higher in patients with a fatal outcome compared with survivors [11]. Importantly, early S100B measurements predicted patient mortality 3–4 days before high intracranial pressure (ICP) readings predicted the same. Quite the opposite results were documented recently by Kiiski et al., who found that elevated S100B at day 1 was strongly associated with a less severe initial clinical representation of aSAH, and the study concluded that plasmatic biomarkers should not be used to guide clinical decision-making in patients with aSAH, in prognostication based on S100B [3].

The time course of blood–brain barrier disruption after SAH has not been fully investigated. The results of both experimental and clinical studies indicate that it is most useful to monitor the biomarkers of brain damage in the first 2–3 days after SAH [21,22]. In our study, the longitudinal profile of biomarkers was rather constant in both non-survivors and survivors over the 3 days of observation. Previously, the time course of barrier breakdown was assessed in an animal model of SAH using Evan's blue dye extravasation [21]. The breakdown to the barrier began at 36 h, peaked at 48 h, and resolved 3 days after the SAH. In a clinical study by Petzold et al., non-survivors maintained high levels of S100B for over 24 h, slowly decreased on day 2 and reached a plateau on days 3–6 [11]. Similarly, in a recent study by Balanca, a significant decline in S100B in the blood was noted on day 3 after an aSAH diagnosis [22]. The decrease in blood levels of S100B found in several studies suggests that there may be renal clearance into the urine.

The 28-day mortality of patients with SAH was reported to range from 26% to 40% and half of those who survived sustained irreversible brain damage [23,24]. Patients with SAH often have loss of consciousness from temporary global ischaemia, are often sedated and require respiratory support, so clinical symptoms and the calculation of clinical scores may have a limited role in predicting in-hospital mortality. The complex pathophysiology of SAH indicates the need for additional prognostic tools that can help identify patients at high risk of death. In addition to assessing the clinical status of patients through scoring, measuring biomarkers of brain damage in the acute phase aSAH could help predict a high risk of death, especially in unconscious patients. Using biomarkers may improve our ability to distinguish between those who are at risk of death and those who are not, which may affect the treatment strategy. In a study by Rodrigues et al., the results of calculated clinical scores on admission with the WFNS (I–II and II–IV) and Fisher scales (I–II and II–IV) were similarly distributed between survivors and non-survivors and were not associated with in-hospital mortality [25]. In our study, among several clinical scores calculated on admission to the ICU, only GCS was significantly lower in patients who ultimately died compared to those who survived (4 vs. 14 pts.). Other grades (WFNS, H-H, and Fisher) did not differ significantly between survivors and non-survivors. In addition, the ROC probability curve indicated that only GCS could distinguish between survivors and non-survivors, while the results of the WFNS, H-H, and Fisher grades were not significant. Therefore, measuring brain markers in the cerebrospinal fluid and blood could be an additional tool to help identify patients at high risk of in-hospital death after aSAH. In our study, the receiver operating characteristic curve analysis was used to evaluate the suitability of the studied biomarkers for classifying patient outcome, and the Youden index was used as a direct measure of the maximum diagnostic accuracy that each biomarker could

achieve. The peak concentrations of enolase showed the highest performance at 1.8 ng/mL threshold in the blood (AUC of 0.873) and at 80.0 ng/mL in the CSF (AUC of 0.889). The predictive ability of the S100B peaks was also very good, with a threshold of 5.7 ng/mL in the blood (AUC 0.825) and 4.5 ng/mL in the CSF (AUC 0.810). For comparison, the clinical scores calculated on admission did not distinguish between a bad and good outcome at hospital discharge, except for GCS, which predicted hospital mortality with high probability (AUC 0.833) and an optimal threshold of 13 pts. There is controversy in the current literature about S-100B and enolase threshold values for predicting an unfavourable outcome or death after aSAH. In a study by Oertel et al., average S-100B values in the blood were significantly higher in patients who died than those who survived and all patients with S-100B > 1 ng/mL experienced an unfavourable outcome (GOS 2–3) or death [26]. In the same study, the outcome was not related to the enolase concentration, contrary to our results. In another study by Kellerman et al., 100% mortality was reported with blood S100B values > 0.7 ng/mL after SAH, but the same relationship was not reported for values in the CSF [13].

In patients with aSAH, both lumbar (LD) and external ventricular (EVD) drains are used for CSF drainage. The main indications for EVD are acute hydrocephalus and intracranial hypertension. EVD drainage might result in drainage-related complications and the total incidence of drainage complications was reported to be approximately 5.3% [27]. These complications include ventriculitis or meningitis, headache due to the ICP drop, intracerebral haemorrhage, deterioration of neurological functions, the need for *ventriculoperitoneal* shunting, and a prolonged stay in the ICU. Lumbar drainage appears to be safer and more effective in removing blood clots from the subarachnoid space, but it is more commonly used in patients with better neurological status [28]. Another problem is EVD management: whether the EVD should be kept open to drainage or only open when needed. It is believed that continuous drainage with low ICP is associated with more complications, including the need for ventricular VPS insertion. Continuous drainage and low ICP may preclude reaching the pressure gradient necessary to restore the natural drainage pathways of the CSF [29]. Rapid EVD wean and intermittent CSF drainage is safe, reduces complications, and shortens the ICU and hospital length of stay as compared to gradual weaning and continuous drainage. In the Qian et al. study, it was found that CSF drainage reduced the incidence of both angiographic and symptomatic cerebral vasoconstriction and significantly reduced the incidence of DIND and cerebral infarction [27]. In addition, the group with CSF drainage had better long-term outcomes and a lower mortality rate. Further work is needed to answer the question of whether there are differences in the results obtained with ventricular and lumbar drainage. The results of previous studies indicate statistically significant differences in white blood cell counts, glucose, and total protein concentrations in the cranial and spinal fluid samples, which may suggest that there may also be differences in biomarker levels [30]. Moreover, the presence of blood clots in the intracranial space may disturb the dynamics of CSF circulation and affect the levels of biomarkers [28].

There are certain limitations to our study. Routine CSF examination only to predict prognosis is impractical due to the invasiveness of the CSF collection procedure. The limitation of this study is the lack of availability of CSF in all patients treated for aSAH, and thus the small size of the study sample. However, it should be emphasized that drainage of the CSF is one of the methods of treating patients with aSAH. About 7–65% of patients with aSAH require placement of EVD to facilitate CSF drainage [31]. Fluid drainage is used to relieve acute hydrocephalus, measure and treat elevated intracranial pressure, and to remove blood from the subarachnoid space [15]. Blood clots in the subarachnoid space are among the pathogenic mechanisms of vasoconstriction and the development of delayed ischemic neurological deficit (DIND), which is the main cause of poor treatment outcomes after aSAH [32]. In some studies, CSF drainage reduced the DIND associated with vasoconstriction and improved treatment outcomes. In our ICU, the procedure of collecting CSF in aSAH patients is performed for clinical reasons only. In patients with CSF drainage, measuring the concentration of S100B and enolase in a fluid sample may provide valuable additional information about the degree of brain damage in the period of early brain injury following aSAH and its relationship to secondary

changes such as cerebral vasospasm or the formation of secondary ischemic changes. In addition, due to the small sample size, the impact of the type of intervention (surgical clipping or endovascular coiling) on the release of biomarkers and the prediction of in-hospital mortality after aSAH could not be analysed. Our study was retrospective and conducted in a single centre, which presents a challenge to generalizing our findings. These are preliminary data with interesting results that should encourage further patient recruitment.

5. Conclusions

As our study and several previous studies have shown that brain biomarker levels correlate with the clinical outcome of patients after aSAH, these results could be helpful in guiding families and physicians in choosing the most appropriate treatment and comfort measures. However, the clinical utility of biomarker testing will require more consistent and reliable information that indicates what the threshold for a bad outcome would really be. Exact thresholds for increases in enolase and S100B that could predict death after aSAH are still in development.

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References

1. Siman, R.; Giovannone, N.; Toraskar, N. Evidence that a Panel of Neurodegeneration Biomarkers Predicts Vasospasm, Infarction, and Outcome in Aneurysmal Subarachnoid Hemorrhage. *PLoS ONE* **2011**, *6*, e28938. [CrossRef] [PubMed]
2. Vos, P.E.; Van Gils, M.; Beems, T.; Zimmerman, C.; Verbeek, M.M. Increased GFAP and S100β but not NSE serum levels after subarachnoid haemorrhage are associated with clinical severity. *Eur. J. Neurol.* **2006**, *13*, 632–638. [CrossRef] [PubMed]
3. Kiiski, H.; Långsjö, J.; Tenhunen, J. S100B, NSE and MMP-9 fail to predict neurologic outcome while elevated S100B associates with milder initial clinical presentation after aneurysmal subarachnoid hemorrhage. *J. Neurol. Sci.* **2018**, *390*, 129–134. [CrossRef] [PubMed]
4. Hong, C.M.; Tosun, C.; Kurland, D.B.; Gerzanich, V.; Schreibman, D.; Simard, J.M. Biomarkers as outcome predictors in subarachnoid hemorrhage—A systematic review. *Biomarkers* **2014**, *19*, 95–108. [CrossRef] [PubMed]
5. Sorci, G.; Bianchi, R.; Riuzzi, F. S100B Protein, a Damage-Associated Molecular Pattern Protein in the Brain and Heart, and Beyond. *Cardiovasc. Psychiatry Neurol.* **2010**, *2010*, 13. [CrossRef] [PubMed]
6. Thelin, E.P.; Nelson, D.W.; Bellander, B.M. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. *Acta Neurochir.* **2017**, *159*, 209–225. [CrossRef] [PubMed]
7. Brenner, M. Role of GFAP in CNS injuries. *Neurosci. Lett.* **2014**, *565*, 7–13. [CrossRef]
8. Lantigua, H.; Ortega-Gutierrez, S.; Schmidt, J.M. Subarachnoid hemorrhage: Who dies, and why? *Crit. Care* **2015**, *19*. [CrossRef]

9. Nieuwkamp, D.J.; Setz, L.E.; Algra, A.; Linn, F.H.; de Rooij, N.K.; Rinkel, G.J. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: A meta-analysis. *Lancet Neurol.* **2009**, *8*, 635–642. [CrossRef]
10. Budohoski, K.P.; Czosnyka, M.; Smielewski, P. Monitoring cerebral autoregulation after subarachnoid hemorrhage. *Acta Neurochir. Suppl.* **2016**, *122*, 199–203. [CrossRef]
11. Petzold, A.; Green, A.J.E.; Keir, G. Role of serum S100B as an early predictor of high intracranial pressure and mortality in brain injury: A pilot study. *Crit. Care Med.* **2002**, *30*, 2705–2710. [CrossRef] [PubMed]
12. Kedziora, J.; Burzynska, M.; Gozdzik, W.; Kübler, A.; Kobylinska, K.; Adamik, B. Biomarkers of Neurological Outcome After Aneurysmal Subarachnoid Hemorrhage as Early Predictors at Discharge from an Intensive Care Unit. *Neurocrit. Care* **2020**. [CrossRef] [PubMed]
13. Kellermann, I.; Kleindienst, A.; Hore, N.; Buchfelder, M.; Brandner, S. Early CSF and Serum S100B Concentrations for Outcome Prediction in Traumatic Brain Injury and Subarachnoid Hemorrhage. *Clin. Neurol. Neurosurg.* **2016**, *145*, 79–83. [CrossRef]
14. Quintard, H.; Leduc, S.; Ferrari, P.; Petit, I.; Ichai, C. Early and persistent high level of PS 100β is associated with increased poor neurological outcome in patients with SAH: Is there a PS 100β threshold for SAH prognosis? *Crit. Care* **2016**, *20*, 1–9. [CrossRef] [PubMed]
15. Gigante, P.; Hwang, B.Y.; Appelboom, G.; Kellner, C.P.; Kellner, M.A.; Connolly, E.S. External ventricular drainage following aneurysmal subarachnoid haemorrhage External ventricular drainage following aneurysmal subarachnoid haemorrhage. *Br. J. Neurosurg.* **2010**. [CrossRef] [PubMed]
16. Chung, D.Y.; Leslie-Mazwi, T.M.; Patel, A.B.; Rordorf, G.A. Management of External Ventricular Drains after Subarachnoid Hemorrhage: A Multi-Institutional Survey. *Neurocrit. Care* **2017**, *26*, 356–361. [CrossRef]
17. Dasic, D.; Hanna, S.J.; Bojanic, S.; Kerr, R.S.C. External ventricular drain infection: The effect of a strict protocol on infection rates and a review of the literature. *Br. J. Neurosurg.* **2006**. [CrossRef]
18. Rao, S.S.; Chung, D.Y.; Wolcott, Z. Intermittent CSF drainage and rapid EVD weaning approach after subarachnoid hemorrhage: Association with fewer VP shunts and shorter length of stay HHS Public Access. *J. Neurosurg.* **2019**, 1–6. [CrossRef]
19. Hajduková, L.; Sobek, O.; Prchalová, D. Biomarkers of Brain Damage: S100B and NSE Concentrations in Cerebrospinal Fluid—A Normative Study. *BioMed Res. Int.* **2015**, *2015*. [CrossRef]
20. Geraghty, J.R.; Davis, J.L.; Testai, F.D. Neuroinflammation and Microvascular Dysfunction After Experimental Subarachnoid Hemorrhage: Emerging Components of Early Brain Injury Related to Outcome. *Neurocrit. Care* **2019**, *31*, 373–389. [CrossRef]
21. Germanò, A.; D'Avella, D.; Imperatore, C.; Caruso, G.; Tomasello, F. Time-course of blood-brain barrier permeability changes after experimental subarachnoid haemorrhage. *Acta Neurochir.* **2000**, *142*, 575–581. [CrossRef] [PubMed]
22. Balança, B.; Ritzenthaler, T.; Gobert, F. Significance and Diagnostic Accuracy of Early S100B Serum Concentration after Aneurysmal Subarachnoid Hemorrhage. *J. Clin. Med.* **2020**, *9*, 1746. [CrossRef] [PubMed]
23. Ingall, T.; Asplund, K.; Mähönen, M.; Bonita, R. A multinational comparison of subarachnoid hemorrhage epidemiology in the WHO MONICA stroke study. *Stroke* **2000**, *31*, 1054–1061. [CrossRef] [PubMed]
24. Connolly, E.S.; Rabinstein, A.A.; Carhuapoma, J.R. Guidelines for the management of aneurysmal subarachnoid hemorrhage: A guideline for healthcare professionals from the american heart association/ american stroke association. *Stroke* **2012**, *43*, 1711–1737. [CrossRef] [PubMed]
25. Rivero Rodríguez, D.; Scherle Matamoros, C.; Cúe, L.F.; Miranda Hernández, J.L.; Pernas Sánchez, Y.; Pérez Nellar, J. Predictor's of mortality in patients with aneurysmal subarachnoid haemorrhage and reebleding. *Neurol. Res. Int.* **2015**, *2015*. [CrossRef]
26. Oertel, M.; Schumacher, U.; McArthur, D.L.; Kästner, S.; Böker, D.K. S-100B and NSE: Markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. *J. Clin. Neurosci.* **2006**, *13*, 834–840. [CrossRef]
27. Qian, C.; Yu, X.; Chen, J. Effect of the drainage of cerebrospinal fluid in patients with aneurismal subarachnoid hemorrhage: A meta-analysis. *Medicine* **2016**, *95*. [CrossRef]
28. Maeda, Y.; Shirao, S.; Yoneda, H. Comparison of lumbar drainage and external ventricular drainage for clearance of subarachnoid clots after Guglielmi detachable coil embolization for aneurysmal subarachnoid hemorrhage. *Clin. Neurol. Neurosurg.* **2013**, *115*, 965–970. [CrossRef]

29. Chung, D.Y.; Olson, D.W.M.; John, S. Evidence-Based Management of External Ventricular Drains. *Curr. Neurol. Neurosci. Rep.* **2019**, *19*, 94. [CrossRef]
30. Podkovik, S.; Kashyap, S.; Wiginton, J. Comparison of Ventricular and Lumbar Cerebrospinal Fluid Composition. *Cureus* **2020**, *12*. [CrossRef]
31. Wang, Y.M.; Lin, Y.J.; Chuang, M.J. Predictors and outcomes of shunt-dependent hydrocephalus in patients with aneurysmal sub-arachnoid hemorrhage. *BMC Surg.* **2012**, *12*. [CrossRef] [PubMed]
32. Reilly, C.; Amidei, C.; Tolentino, J.; Jahromi, B.S.; Macdonald, R.L. Clot volume and clearance rate as independent predictors of vasospasm after aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **2004**, *101*, 255–261. [CrossRef] [PubMed]

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4. OMÓWIENIE PUBLIKACJI STANOWIĄCYCH PRACĘ DOKTORSKĄ

W pierwszej z prezentowanych publikacji "*Biomarkers of Neurological Outcome After Aneurysmal Subarachnoid Hemorrhage as Early Predictors at Discharge from an Intensive Care Unit. Kedziora J, Burzynska M, Gozdzik W, Kübler A, Kobylinska K, Adamik B. Neurocrit Care. 2020 Sep 25. doi: 10.1007/s12028-020-01110-2. PMID: 32978732*" przedstawiono wyniki badania nad oceną zmian stężeń we krwi biomarkerów specyficznych dla uszkodzenia komórek nerwowych u pacjentów z krwotokiem podpajęczynówkowym z pękniętego tętniaka oraz przedstawiono wyniki oceny zależności pomiędzy stężeniem biomarkerów, a ciężkością krwotoku ocenianego przy użyciu skal klinicznych i przydatności dokonanych pomiarów w przewidywaniu wczesnych wyników leczenia.

Ostre uszkodzenie mózgu w krwotoku podpajęczynówkowym z pękniętego tętniaka prowadzi do zniszczenia komórek mózgowych i uwolnienia specyficznych białek do płynu mózgowo- rdzeniowego. Białka te mogą przenikać przez uszkodzoną barierę krew- mózg do układu krążenia, gdzie można je wykryć i zmierzyć ich stężenie. Zmiany stężeń tych białek mogą być zatem wskaźnikiem ciężkości uszkodzenia mózgu spowodowanego przez aSAH we wczesnej fazie leczenia. Ocena stanu klinicznego oraz prognozowanie wyników leczenia na podstawie powszechnie stosowanych w neurointensywnej terapii skal klinicznych uwzględniających jakościowe i ilościowe zaburzenia neurologiczne, takich jak GCS, WFNS i skala Hunta- Hessa, ma ograniczoną wartość w przypadku pacjentów leczonych w OIT, poddanych analgosedacji i wymagających wentylacji mechanicznej. Dlatego określenie panelu specyficznych dla mózgu biomarkerów, które odzwierciedlałyby stopień uszkodzenia mózgu, mogłoby być użytecznym narzędziem w praktyce klinicznej. Ułatwiłoby planowanie i monitorowanie leczenia we wczesnej fazie po krwotoku, a także przewidywanie wyników leczenia. W większości opublikowanych badań oceniano wartość predykcyjną biomarkerów w odniesieniu do odległych wyników leczenia aSAH (po 6 i 12 miesiącach) [13-15]. Tylko w niewielu pracach zbadano wartość rokowniczą stężenia biomarkerów mózgowych w przewidywaniu wyników leczenia w bardzo wczesnej fazie choroby, ograniczonej do leczenia i wypisu z OIT [16, 17].

Głównym celem prezentowanej pracy była analiza zmian stężeń biomarkerów uszkodzenia komórek nerwowych i określenie przydatności pomiaru biomarkerów do prognozowania stanu neurologicznego pacjenta w bardzo wczesnej fazie aSAH. Wczesne

zmiany stężeń biomarkerów oceniano w ostrej fazie leczenia, w odniesieniu do wyników skal klinicznych i stanu pacjenta przy wypisie z OIT.

Do badania zakwalifikowano chorych z aSAH leczonych pomiędzy lipcem 2014 r. a styczniem 2017 r. w Klinice Anestezjologii i Intensywnej Terapii Uniwersyteckiego Szpitala Klinicznego we Wrocławiu. Kryteria włączenia do badania obejmowały: wiek ≥ 18 lat; aSAH jako przyczynę krwawienia potwierdzony za pomocą tomografii komputerowej z kontrastem, rezonansu magnetycznego lub konwencjonalnej angiografii; przyjęcie do OIT w ciągu 48 godzin od rozpoznania aSAH. Kryteria wykluczenia obejmowały wcześniej zdiagnozowaną chorobę neurologiczną. Od każdego pacjenta pobierano próbki krwi w dniu przyjęcia (1 doba) oraz przez 5 kolejnych dni (2, 3, 4, 5 i 6 doba). W pobranych próbkach krwi oznaczono stężenie następujących biomarkerów: S100B, NSE, MAPT i GFAP.

Uzyskane wyniki oceniono w odniesieniu do ciężkości stanu ogólnego pacjenta przy przyjęciu do OIT oraz ciężkości krwotoku zwalidowanych za pomocą skal klinicznych: APACHE II, GCS, WFNS, Hunta- Hessa i Fishera. Określono także przydatność pomiarów stężenia biomarkerów do prognozowania wyników leczenia w bardzo wczesnej fazie, którego efekt oceniono w pięciostopniowej skali Glasgow Outcome Scale (GOS) przy wypisie z OIT. Dodatkowo przeprowadzono ocenę wczesnych wyników leczenia z użyciem tej samej skali przy wypisie ze szpitala (GOS I- zgon; GOS II- stan wegetatywny; GOS III- ciężka niepełnosprawność, pacjent zależny od osób trzecich w codziennych czynnościach; GOS IV- umiarkowana niepełnosprawność, pacjent niezależny od osób trzecich w życiu codziennym; GOS V- pacjent bez deficytu neurologicznego, powrót do normalnego funkcjonowania pomimo niewielkiego deficytu neurologicznego). W zależności od wyniku leczenia ocenianego w skali GOS przy wypisie z OIT, każdego pacjenta przyporządkowano do jednej z dwóch grup:

Grupa badana 1: pacjenci z dobrym wynikiem leczenia (GOS IV – V),

Grupa badana 2: pacjenci ze złym wynikiem leczenia (GOS I – III).

Spośród 60 pacjentów z aSAH, którzy spełniali kryteria włączenia, 5 zostało wykluczonych z powodu braku kompletu pobranego materiału badawczego. Analizę przeprowadzono w grupie 55 pacjentów.

Na podstawie wartości GOS ocenianej przy wypisie z OIT grupę z dobrym wynikiem leczenia (Grupa 1, GOS IV-V) stanowiło 24 pacjentów (44%), a grupę ze złym wynikiem (Grupa 2, GOS I-III) 31 pacjentów (56%). Przy wypisie ze szpitala wszyscy pacjenci z Grupy 1 osiągnęli dobry wynik leczenia (GOS V 75%, GOS IV 25%), natomiast większość chorych

z Grupy 2 (77%) stanowili pacjenci ze złym wynikiem (GOS I- III): 42% zmarło (GOS I), 3% GOS II, 22% GOS III.

Stężenia biomarkerów S100B, MAPT i NSE były istotnie wyższe w grupie pacjentów ze złym wynikiem leczenia (Grupa 2) w porównaniu z pacjentami z Grupy 1. W pierwszym dniu badania (stężenie wyjściowe) obserwowano istotną różnicę w stężeniach biomarkerów pomiędzy Grupą 1 i 2 (odpowiednio Grupa 1 i 2: S100B: 1.0 vs. 4.7 ng/ml; MAPT: 83.2 vs. 625.0 pg/ml; NSE: 1.5 vs. 4,0 ng/ml). W Grupie 2 wartości S100B i NSE były istotnie podwyższone przez cały okres badania (S100B: 4.8, 4.4, 4.5, 6.6, 6.8 ng/ml; NSE: 4.1, 4.3, 3.8, 4.4, 2.5 ng/ml, w dniach 2-6) a pozostawały niskie w Grupie 1 (S100B: 0.9, 0.7, 2.0, 1.0, 0.3 ng/ml, NSE: 2.0, 1.6, 1.2, 16.6, 2.2 ng/ml, w dniach 2-6). Poziom MAPT był podwyższony tylko do 2 doby obserwacji w Grupie 2 (617.4, 195.0, 379.9, 315.5, 86.9 pg/ml, w dniach 2-6), a pozostał niski w Grupie 1 (132.7, 438.7, 102.9, 79.7, 8.1 pg/ml, w dniach 2-6). Różnica stężeń S100B i NSE między grupami była statystycznie istotna przez cały okres badania, a różnica w stężeniu MAPT między grupami była istotna w dniu 1 i 2. Stężenie GFAP w obydwu badanych grupach było wysokie od pierwszego dnia do końca okresu obserwacji (Grupa 1: 5.7, 6.9, 6.9, 7.3, 8.5, 6.5 ng/ml; Grupa 2: 6.6, 7.0, 7.8, 8.1, 7.5, 5.3 ng/ml, w dniach 1-6); nie stwierdzono istotnych różnic w stężeniu GFAP między badanymi grupami.

Nie stwierdzono istotnej korelacji między stężeniem GFAP, MAPT, S100B, NSE a wiekiem lub płcią pacjentów. Poziomy białek S100B, NSE, GFAP i MAPT zarejestrowane w dniach 1-6 nie różniły się istotnie w zależności od zastosowanej techniki operacyjnej (embolizacja lub klipsowanie).

Przy przyjęciu do OIT wyniki oceny klinicznej w skalach GCS, APACHE II, WFNS, Hunta- Hessa i Fishera były istotnie lepsze w Grupie 1 niż w Grupie 2 (GCS: 13.6 vs. 10.0 pkt., $p<0.0011$; APACHE II: 9.6 vs. 17.1 pkt., $p<0.001$). WFNS I-III odnotowano u 83% chorych w Grupie 1 i u 52% w Grupie 2, a stopień WFNS IV-V u 17% w Grupie 1 i 48% w Grupie 2 ($p=0.020$). Ciężkość krwotoku podpajęczynówkowego oceniana w skali Hunta-Hessa była statystycznie istotnie większa ($p=0.001$) w Grupie 2 (stopień IV-V u 58% pacjentów) niż w Grupie 1 (stopień IV-V u 19% pacjentów). Ciężkość aSAH oceniona przy przyjęciu w tomografii komputerowej wg skali Fishera była istotnie większa w Grupie 2, w porównaniu do zmian obserwowanych w Grupie 1 ($p=0.002$).

Do oceny wartości predykcyjnej poszczególnych biomarkerów zastosowano analizę ROC (*receiver operating characteristic),* wyniki porównywano w oparciu o wartości pola pod krzywą AUC (*area under the ROC curve*). Wykazano, że wyjściowe stężenie S100B najlepiej przewiduje zły wynik (GOS I-III) przy wypisie z OIT (AUC 0.813; 95% CI 0,677–0.948, p

<0.001); optymalna wartość progowa dla S100B wynosiła 0.625 ng/ml, z czułością 0.913 (95% CI 0.833–1.067) i swoistością 0.625 (95% CI 0.651–1.249). Wyjściowe stężenie NSE również miało istotną wartość predykcyjną (AUC 0.706; 95% CI 0.541–0.871, p=0.015), jednakże dla optymalnej wartości progowej wynoszącej 1.613 ng/ml, czułość markera (0.667; 95% CI 0.719–1,180) nie była już tak dobra jak dla S100B, a swoistość wynosiła 0.733 (95% CI 0.689–1.211). Pomiar MAPT w pierwszej dobie miał istotną wartość predykcyjną (AUC 0.735; 95% CI 0.550–0.892, p= 0.012), dla optymalnej wartości progowej 240.7 pg/ml czułość była niska (0.588; 95% CI 0.647–1.253), ale swoistość markera była bardzo dobra (0.909; 95% CI 0.830–1,.070). GFAP nie miało istotnej wartości prognostycznej (p=0.460).

W celu stworzenia modelu prognostycznego do przewidywania złego wyniku leczenia (GOS I-III) wykonano wieloczynnikową analizę regresji logistycznej. Początkowo do analizy włączono zmienne ze zbioru biomarkerów (S100B, NSE, MAPT i GFAP) i zmiennych towarzyszących (wiek, płeć, skale APACHE II, GCS, WFNS, Hunta-Hessa, Fishera), następnie wykluczono z analizy zmienne skorelowane. Ostatecznie jedynymi zmiennymi, które zostały uwzględnione w modelu było wyjściowe stężenie S100B i NSE oraz płeć i APACHE II. Istotnymi predyktorami złego wyniku były: wyjściowe stężenie S100B (OR= 2.229, p=0.023), APACHE II (OR=1.303, p=0.002) oraz płeć (OR=0.089, p=0.022). Wyjściowe stężenie NSE nie miało istotności statystycznej w modelu (p=0.088).

Wyniki przeprowadzonego badania dostarczają dalszych dowodów na to, że u pacjentów z krwotokiem podpajęczynówkowym z pękniętego tętniaka obserwuje się we krwi wzrost stężenia biomarkerów uszkodzenia komórek nerwowych S100B, NSE, GFAP i MAPT. Wykazano związek pomiędzy wysokimi stężeniami biomarkerów, a ciężkim stanem klinicznym pacjenta oraz ciężkością krwotoku ocenianymi przy pomocy skal używanych w praktyce klinicznej. Wykazano bezpośredni związek między wynikiem leczenia we wczesnej fazie po aSAH, tzn. przy wypisie z OIT, a stężeniami S100B, NSE i MAPT.

Z przeprowadzonej analizy wynika, że pomiar stężenia S100B, NSE i MAPT we krwi przy przyjęciu do OIT mógłby być cennym narzędziem diagnostycznym i prognostycznym w bardzo wczesnej fazie leczenia pacjentów z aSAH. Dodatkowe parametry biochemiczne uzupełniające ocenę kliniczną, zwłaszcza w populacji pacjentów OIT, którzy są nieprzytomni lub sedowani, ułatwiałyby: ocenę przebiegu leczenia we wczesnym okresie, wdrożenie adekwatnego leczenia i monitorowania oraz udzielanie rodzinom pacjentów dokładniejszych informacji na temat prowadzonej terapii i rokowania. Pomiar stężenia biomarkerów należy traktować jako potencjalne dodatkowe narzędzie wspierające, ale nie zastępujące skal klinicznych APACHE II, WFNS, GCS, skala Hunta- Hessa, skala Fishera i GOS.

W drugiej z prezentowanych publikacji *„Brain-Specific Biomarkers as Mortality Predictors after Aneurysmal Subarachnoid Haemorrhage. Kedziora J, Burzynska M, Gozdzik W, Kübler A, Uryga A, Kasprowicz M, Adamik B. J Clin Med. 2020 Dec 20;9(12):4117. doi: 10.3390/jcm9124117. PMID: 33419282*" przeanalizowano zmiany stężeń biomarkerów uszkodzenia mózgu w PMR i krwi u pacjentów z aSAH, u których jedną z zastosowanych metod leczenia neurochirurgicznego był zewnętrzny drenaż komorowy (EVD, external ventricular drain). Poprzez porównanie uzyskanych wyników z efektem leczenia przy wypisie ze szpitala, oceniono przydatność biomarkerów w przewidywaniu śmiertelności wewnątrzszpitalnej u pacjentów z aSAH.

Drenaż PMR u pacjentów z aSAH jest procedurą leczniczą wykonywaną wyłącznie ze wskazań klinicznych, takich jak ostre wodogłowie, ciężki krwotok dokomorowy lub w celu pomiaru i leczenia podwyższonego ciśnienia wewnątrzczaszkowego [18, 19]. EVD obarczony jest też ryzykiem powikłań, takich jak infekcja, wyciek PMR, wodniak lub krwiak wewnątrzczaszkowy. Ponadto u chorych leczonych EVD obserwowano wydłużenie hospitalizacji oraz pogorszenie wyników poznawczych [20-22]. Jednakże u pacjentów leczonych EVD, pomiar stężenia specyficznych biomarkerów mózgowych w PMR może dostarczyć dodatkowych cennych informacji o stopniu uszkodzenia komórek mózgowych.

Badanie przeprowadzono w grupie chorych, którzy byli leczeni w Klinice Anestezjologii i Intensywnej Terapii Uniwersyteckiego Szpitala Klinicznego we Wrocławiu między lipcem 2014 r. a styczniem 2017 r.

Kryteria włączenia do badania obejmowały: wiek ≥18 lat; aSAH jako przyczynę krwawienia potwierdzony za pomocą tomografii komputerowej z kontrastem, rezonansu magnetycznego lub konwencjonalnej angiografii; przyjęcie do OIT w ciągu 48 godzin od rozpoznania aSAH, obecność zewnętrznego drenażu komorowego umożliwiającego pobieranie PMR przez trzy kolejne doby od przyjęcia do OIT. Kryteria wykluczenia obejmowały wcześniej zdiagnozowaną chorobę neurologiczną.

W trakcie leczenia na Oddziale Intensywnej Terapii (OIT) stan kliniczny pacjentów oraz ciężkość krwotoku oceniano stosując rutynowe skale kliniczne (GCS, WFNS, Hunta- Hessa, Fishera). Próbki PMR pobierano przez zewnętrzny drenaż komorowy w 1, 2 i 3 dniu leczenia w OIT. W tych samych punktach czasowych pobierano próbki krwi. W pobranym materiale oznaczono stężenie następujących białek: S100B, NSE i GFAP. W prezentowanym badaniu pacjentów podzielono oceniając wynik leczenia przy wypisie ze szpitala na dwie grupy:

Grupa badana 1: pacjenci, którzy przeżyli,

Grupa badana 2: pacjenci, którzy zmarli.

Z grupy 70 pacjentów z aSAH, do analizy końcowej włączono 16 pacjentów, u których możliwe było pobieranie płynu mózgowo- rdzeniowy przez trzy kolejne doby od dnia przyjęcia do OIT. Grupa 1 (pacjenci, którzy przeżyli) składała się z 9 pacjentów, Grupa 2 składała się z 7 pacjentów. Wszystkie zgony miały miejsce w OIT.

Mediana wieku pacjentów wynosiła 67 lat (53–76), 50% badanych stanowiły kobiety. W chwili przyjęcia do OIT 44% chorych było w złym stanie klinicznym (WFNS 4–5). Ciężkość krwotoku podpajęczynówkowego w skali Fishera przedstawiała się w następujący sposób: 2 pacjentów otrzymało stopień 2, 2 pacjentów stopień 3, a 12 oceniono w stopniu 4. GCS przy przyjęciu do OIT był istotnie niższy u pacjentów, którzy nie przeżyli niż u osób, które przeżyły (4 vs. 14 pkt., p = 0.022). Wyniki innych skal klinicznych (WFNS, Hunta-Hessa i Fishera) nie różniły się istotnie między grupami. Długość pobytu w szpitalu w grupie pacjentów, którzy zmarli była 5- krotnie dłuższa niż u tych, którzy przeżyli (109 vs. 21 dni, p=0.055).

W przebadanej populacji pacjentów stwierdzono wysoki poziom stężenia biomarkerów uszkodzenia mózgu we krwi i PMR w kolejnych dniach po aSAH, jednak stężenia tych białek w PMR były znacznie wyższe, niż we krwi. Stężenie S100B w kolejnych dniach po aSAH było istotnie wyższe u pacjentów, którzy zmarli (Grupa 2) zarówno w próbkach krwi (dzień 1: 1.7 vs. 0.7 ng/ml, p=0.041; dzień 2: 5.6 vs. 0.7 ng/ml, p=0.007; dzień 3: 3.6 vs. 0.3 ng/ml, p<0.001, odpowiednio zmarli vs. przeżyli), jak i w PMR (dzień 1: 21.9 vs. 3.9 ng/ml, p=0,031; dzień 2: 25.6 vs. 0.6 ng/ml, p=0.002; dzień 3: 23.9 vs. 0.6 ng/ml, p=0.002, odpowiednio zmarli vs. przeżyli). Podobne zmiany obserwowano w stężeniach enolazy we krwi (dzień 1: 6.0 vs. 1.3 ng/ml, p=0.046; dzień 2: 4.2 vs. 0.9 ng/ml, p=0.005; dzień 3: 3.4 vs. 1.1 ng/ml, p=0.049, odpowiednio zmarli vs. przeżyli) i w PMR (dzień 1: 52.6 vs. 5.4 ng/ml, p=0.029; dzień 2: 16.9 vs. 4.1 ng/ml, p=0.035; dzień 3: 80.0 vs. 3.4 ng/ml, p=0.031, odpowiednio zmarli vs. przeżyli). Nie stwierdzono istotnych statystycznie różnic w wartościach GFAP między badanymi grupami ani w próbkach krwi, ani PMR. Dodatkowo analizowano wartości maksymalne biomarkerów. Maksymalna wartość, to najwyższa wartość zarejestrowana w dniach od 1 do 3. Pacjenci, którzy nie przeżyli, mieli we krwi znacznie wyższe wartości maksymalne S100B i enolazy w porównaniu z osobami, które przeżyły (S100B: 5.7 vs. 1.5 ng/ml, p=0.031; NSE: 6.1 vs. 1.4 ng/ml, p=0.011). Podobne zależności obserwowano w PMR. Wartości maksymalne S100B i enolazy w Grupie 2 były istotnie wyższe, niż w Grupie 1 (S100B: 18.3 vs. 0.9 ng/ml, p=0.042; NSE: 109.2 vs. 6.1 ng/ml, p=0.015). Nie stwierdzono istotnych różnic w wartościach maksymalnych GFAP między badanymi grupami ani w próbkach krwi, ani PMR.

Do zbadania zależności pomiędzy stężeniami biomarkerów a ciężkością stanu neurologicznego i krwotoku ocenionymi z użyciem skal klinicznych zastosowano analizę

korelacji Spearman'a. Maksymalne stężenia enolazy zarejestrowane w PMR były silnie skorelowane z wynikami w skalach GCS (R=0.7, p=0.005), Hunta- Hessa (R=0.6, p=0.027), WFNS (R=0.7, p=0.003) i Fishera (R=0.6, p=0.009) obliczonymi przy przyjęciu do OIT. Maksymalne stężenie enolazy we krwi oraz maksymalne stężenia S100B we krwi i PMR nie wykazały istotnej korelacji z wynikami skal klinicznych.

Do oceny wartości predykcyjnej poszczególnych biomarkerów zastosowano analizę ROC. Wyniki porównywano w oparciu o wartości pola pod krzywą AUC. Wykazano, że stężenia enolazy oznaczone we krwi (AUC 0.873; 95% CI 0.682–1.00, p=0.001) i w PMR (AUC 0.889; 95% CI 0.716–1.000, p <0.001) najlepiej przewidują śmiertelność szpitalną. Optymalna wartość progowa dla enolazy we krwi wynosiła 1.8 ng/ml, przy czułości 100% i swoistości 78%, a w PMR 80.0 ng/ml, przy czułości 67% i swoistości 100%. Stężenie S100B we krwi (AUC 0.825; 95% CI 0.592–1.000, p = 0.006) i PMR (AUC 0.810; 95% CI 0.595–1.000, p<0.004) również miało istotną wartość predykcyjną. Optymalna wartość progowa dla S100B we krwi wynosiła 5.7 ng/ml, przy czułości 71% i swoistości 100%, a w PMR 4.5 ng/ml, przy czułości 100% i swoistości 56%. Wartość predykcyjna GFAP nie była istotna ani we krwi (p=0,488), ani w PMR (p=0.443). Dla porównania, spośród skal klinicznych stosowanych przy przyjęciu chorych do OIT, tylko skala GCS wykazała zdolność do przewidywania śmiertelności szpitalnej (AUC 0.833; 95% CI 0.632–1.000, p=0.001). Oceny w skalach Hunta- Hessa, WFNS i Fishera były nieistotne statystycznie. Optymalna wartość progowa dla GCS wyniosła 13 punktów przy czułości 100% i swoistości 55%.

Uzyskane w przeprowadzonym badaniu wyniki są zgodne z hipotezą, że białka NSE i S100B mierzone w PMR i krwi we wczesnym okresie choroby są czułymi biomarkerami do przewidywania śmiertelności wewnątrzszpitalnej u pacjentów z aSAH. Stężenia S100B i NSE mierzone w PMR we wczesnej fazie leczenia aSAH były kilkakrotnie wyższe u pacjentów, którzy zmarli niż u tych, którzy przeżyli. Podobną zależność zaobserwowano w próbkach krwi, co wskazuje na uszkodzenie bariery krew-mózg i przedostawanie się białek charakterystycznych dla komórek mózgowych do krążenia. Pomiar markerów mózgowych w PMR i krwi mógłby być dodatkowym narzędziem pomocnym w identyfikacji pacjentów z wysokim ryzykiem zgonu szpitalnego w aSAH. Jednak przed wdrożeniem pomiarów biomarkerów do praktyki klinicznej, potrzebne są dalsze badania, które pozwolą na ustalenie takich wartości progowych enolazy i S100B, które z wysokim prawdopodobieństwem wskazywałyby ryzyko zgonu.

5. PODSUMOWANIE

Obie przedstawione publikacje koncentrują się na problematyce przydatności pomiarów stężeń biomarkerów uszkodzenia mózgu u pacjentów z aSAH w odniesieniu do wczesnych parametrów klinicznych, punktacji skal klinicznych oraz wyniku leczenia przy wypisie z OIT i ze szpitala. Dodatkowe parametry biochemiczne uzupełniające ocenę kliniczną, zwłaszcza w populacji pacjentów OIT, którzy są nieprzytomni lub sedowani, ułatwiałyby ocenę przebiegu leczenia we wczesnym okresie, wdrożenie adekwatnego leczenia i monitorowania oraz udzielanie rodzinom pacjentów dokładniejszych informacji na temat prowadzonej terapii i rokowania. Najważniejsze wnioski z prezentowanego cyklu prac są następujące:

1. U pacjentów z aSAH obserwuje się we wczesnej fazie choroby wzrost stężenia biomarkerów uszkodzenia komórek CUN zarówno w PMR jak i we krwi, co wskazuje na uszkodzenie bariery krew-mózg i przedostawanie się białek charakterystycznych dla komórek mózgowych do krążenia ogólnoustrojowego.

2. Oznaczanie biomarkerów we krwi jest możliwe u wszystkich pacjentów z aSAH, natomiast pomiar stężenia tych białek w PMR ogranicza się do pacjentów z implantowanym ze wskazań medycznych zewnętrznym drenażem PMR.

3. Istnieje zależność między wysokim stężeniem biomarkerów uszkodzenia komórek CUN we wczesnej fazie aSAH, a złym stanem pacjentów ocenianym przy przyjęciu do OIT za pomocą standardowych skal klinicznych, biorących pod uwagę jakościowe i ilościowe zaburzenia neurologiczne. Oznaczanie stężeń biomarkerów może być więc przydatnym narzędziem uzupełniającym badanie kliniczne w populacji chorych OIT, którzy z uwagi na ciężki stan ogólny i inwazyjne techniki leczenia wymagają stosowania leków sedujących.

4. Istnieje zależność między stężeniem biomarkerów uszkodzenia mózgu w ostrej fazie aSAH, a wczesnymi wynikami leczenia ocenianymi przy wypisie z OIT oraz ze szpitala. Stężenie biomarkerów w ostrej fazie krwotoku podpajęczynówkowego może być zatem predyktorem wczesnych wyników leczenia.

5. Stosując wieloczynnikową analizę regresji logistycznej utworzono model dla przewidywanie stanu neurologicznego w ostrej fazie leczenia aSAH. Parametry takie jak płeć, stężenie biomarkera S100B oraz stan kliniczny oceniany w skali APACHE II przy przyjęciu okazały się istotnymi elementami modelu predykcyjnego.

6. W przeprowadzonym badaniu określono optymalne wartości progowe poszczególnych biomarkerów dla przewidywania złego wyniku leczenia przy wypisie z OIT. Należy jednak podkreślić, iż w celu potwierdzenia przydatności wartości progowych dla przewidywania złego wyniku leczenia konieczne jest dalsze kontynuowanie badań, szczególnie projektów wieloośrodkowych z udziałem dużych grup pacjentów.

7. Pomiar stężenia biomarkerów należy traktować jako potencjalne dodatkowe narzędzie wspierające, ale nie zastępujące skal klinicznych APACHE II, WFNS, GCS, skala Hunta-Hessa, skala Fishera i GOS.

6. STRESZCZENIE

Wstęp: Krwotok podpajęczynówkowy z pękniętego tętniaka naczyń mózgowych (aSAH) jest poważnym schorzeniem o wysokiej śmiertelności i wysokim wskaźniku trwałego inwalidztwa u osób, które przeżyły pierwotny krwotok. Ostre uszkodzenie mózgu po aSAH prowadzi do uszkodzenia komórek mózgowych i uwolnienia białek specyficznych dla mózgu do PMR i krążenia ogólnoustrojowego. Białka mózgowe mogą przechodzić bezpośrednio przez uszkodzoną barierę krew-mózg do krążenia ogólnoustrojowego, gdzie można je wykryć i zmierzyć. Dlatego białka obecne w komórkach neuronalnych i glejowych OUN mogą być czułymi markerami uszkodzenia mózgu spowodowanego przez aSAH w ostrej fazie leczenia. Głównym celem projektu było zbadanie zmian w stężeniach biomarkerów specyficznych dla mózgu u pacjentów po aSAH w odniesieniu do wczesnych parametrów klinicznych, punktacji skal klinicznych oraz wyniku leczenia przy wypisie z OIT i ze szpitala. Przeprowadzono analizę w celu zidentyfikowania określonych biomarkerów jako predyktorów złego wyniku leczenia w ostrej fazie po aSAH.

Materiał i metoda: Próbki krwi i PMR pobierano w celu oznaczenia biomarkerów przy przyjęciu na OIT oraz w kolejnych dniach. Analizę przeprowadzono dla białek specyficznych dla komórek neuronalnych NSE (neuron-specific enolase) i MAPT (microtubule associated protein tau) oraz dla komórek glejowych S100B i GFAP (glial fibrillary acidic protein). Stan kliniczny pacjenta oceniono stosując skale APACHEII oraz GCS. Do klasyfikacji ciężkości aSAH posłużono się skalami Hunta-Hessa i WFNS, a nasilenie krwotoku w obrazie tomografii komputerowej oceniano w skali Fishera. Przydatność biomarkerów do prognozowania złego wyniku leczenia skutkującego ciężką niepełnosprawnością, stanem wegetatywnym lub zgonem (GOS 1-3) oceniano przy wypisie z OIT i ze szpitala.

Wyniki: Stężenia biomarkerów uszkodzenia mózgu były podwyższone po aSAH, a pacjenci ze złym wynikiem leczenia wykazywali znacząco wyższe poziomy S100B, NSE i MAPT we krwi i PMR w porównaniu z pacjentami z dobrym wynikiem. Wyniki punktacji w skalach klinicznych (skala GCS, APACHE II, WFNS, Hunta-Hessa i Fishera) obliczone przy przyjęciu do OIT były istotnie gorsze u pacjentów z wysokim poziomem biomarkerów. Analiza wieloczynnikowj regresji logistycznej wykazała, że podwyższona wartość S100B i APACHE II oraz płeć męska wskazywały na istotnie wyższe ryzyko złego wyniku leczenia przy wypisie z OIT (GOS 1-3).

Wnioski: Prezentowane badania dostarczają dowodów, że biomarkery mózgowe, takie jak S100B, NSE, GFAP i MAPT, są podwyższone u pacjentów po aSAH. Istnieje bezpośredni związek między stanem neurologicznym pacjenta w ostrej fazie leczenia aSAH i poziomem tych biomarkerów. Ocena pacjentów z aSAH za pomocą skal klinicznych staje się trudniejsza, gdy pacjenci wymagają sedacji. Pomiar biomarkerów może być niezależnym, dodatkowym narzędziem diagnostycznym i prognostycznym wspomagającym ocenę kliniczną w OIT.

7. SUMMARY

Introduction: Aneurysmal subarachnoid haemorrhage (aSAH) is a serious condition with a high mortality and high permanent disability rate for those who survive the initial haemorrhage. An acute brain injury after an aSAH leads to the destruction of brain cells and the release of brain-specific proteins to the cerebrospinal fluid (CSF) and systemic blood circulation. Brain proteins can pass directly through a damaged blood–brain barrier into the systemic circulation, where they can be detected and measured. Therefore, the proteins abundant in the neurons and glia of the central nervous system (CNS) can be sensitive markers of brain damage caused by aSAH in the acute treatment phase. The main objectives were to study changes in brain-specific biomarker levels in patients after an aSAH in relation to early clinical findings, severity scores, and ICU and hospital outcome. Analysis was done to identify specific biomarkers as predictors of a bad outcome in the acute treatment phase.

Material and Methods: Blood and CSF samples were collected for biomarker determination on admission to the ICU and on the following days. Analysis was performed for the proteins of neurofilament, neuron-specific enolase (NSE), microtubule-associated protein tau (MAPT), and for the proteins of glial cells, S100B, and glial fibrillary acidic protein (GFAP). Outcomes were assessed at discharge from the ICU and from the hospital. The patient's clinical status was evaluated with the APACHE II score and the GCS. To classify the severity of aSAH, the Hunt–Hess and WFNS scales were used, and the intensity of the hemorrhage on the CT scans was graded with the Fisher scale.

Results: Brain biomarkers increase markedly following aSAH and patients with a bad outcome showed significantly higher levels of S100B, NSE and MAPT in blood and CSF compared to patients with a good outcome. The results of clinical scores (GCS, APACHE II, WFNS, Hunt-Hess and Fisher scale) calculated on admission to the ICU were significantly worse in patients with a high levels of biomarkers. According to the multivariate logistic regression analysis, an elevated S100B and APACHE II score, and male gender indicated a significantly higher risk of a bad ICU outcome, resulting in severe disability, persistent vegetative state or death (GOS rating 1–3).

Conclusion: Our findings provide evidence that brain biomarkers such as S100B, NSE, GFAP, and MAPT increase significantly in patients following aSAH. There is a direct relationship between the neurological outcome in the acute treatment phase and the levels of these biomarkers. Evaluating aSAH patients using clinical scores becomes more difficult with ICU patients receiving sedatives and analgesics. Biomarkers assessments might be an independent, additional diagnostic and prognostic tool to support the clinical evaluation at the ICU.

8. PIŚMIENNICTWO

1. Van Gijn J , Rinkel G J. Subarachnoid haemorrhage: diagnosis, causes and management. Brain **2001,**124:249-78.
2. Lantigua, H.; Ortega-Gutierrez, S.; Schmidt, J.M. Subarachnoid hemorrhage: Who dies, and why? Crit. Care **2015**, 19.
3. Nieuwkamp, D.J.; Setz, L.E.; Algra, A.; Linn, F.H.; de Rooij, N.K.; Rinkel, G.J. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: A meta-analysis. Lancet Neurol. **2009**, 8, 635–642.
4. Cossu G, Messerer M, Stocchetti N, Levivier M, Daniel RT, Oddo M. Intracranial pressure and outcome in critically ill patients with aneurysmal subarachnoid hemorrhage: a systematic review. Minerva Anestesiol. 2016;82(6):684–96.
5. Daou BJ, Koduri S, Thompson BG, Chaudhary N, Pandey AS. Clinical and experimental aspects of aneurysmal subarachnoid hemorrhage. CNS Neurosci Ther. 2019;25(10):1096–112.
6. Zemke D, Farooq MU, Mohammed Yahia A, Majid A. Delayed ischemia after subarachnoid hemorrhage: result of vasospasm alone or a broader vasculopathy? Vasc Med. 2007;12(3):243–9.
7. Geraghty JR, Testai FD. Delayed cerebral ischemia after subarachnoid hemorrhage: beyond vasospasm and towards a multifactorial pathophysiology. Curr Atheroscler Rep. 2017;19(12):50.
8. Montaner J, Mendioroz M, Delgado P, García-Berrocoso T, Giralt D, Merino C, Ribó M, Rosell A, Penalba A, Fernández-Cadenas I, Romero F, Molina C, Alvarez-Sabín J, Hernández-Guillamon MJ. Differentiating ischemic from hemorrhagic stroke using plasma biomarkers: the S100B/RAGE pathway. Proteomics. 2012;75(15):4758–65
9. Hong, C.M.; Tosun, C.; Kurland, D.B.; Gerzanich, V.; Schreibman, D.; Simard, J.M. Biomarkers as outcome predictors in subarachnoid hemorrhage—A systematic review. Biomarkers **2014**, 19, 95–108.
10. Sorci, G.; Bianchi, R.; Riuzzi, F. S100B Protein, a Damage-Associated Molecular Pattern Protein in the Brain and Heart, and Beyond. Cardiovasc. Psychiatry Neurol. **2010**, 2010, 13.
11. Thelin, E.P.; Nelson, D.W.; Bellander, B.M. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochir. **2017**, 159, 209–225
12. Brenner, M. Role of GFAP in CNS injuries. Neurosci. Lett. **2014**, 565, 7–13
13. Zanier ER, Zoerle T, Fiorini M, Longhi L, Cracco L, Bersano A, Branca V, Benedetti MD, De Simoni MG, Monaco S, Stocchetti N. Heart-fatty acid-binding and tau proteins relate to brain injury severity and long-term outcome in subarachnoid haemorrhage patients. Br J Anaesth. 2013;111(3):424–32
14. Moritz S, Warnat J, Bele S, Graf BM, Woertgen C. The prognostic value of NSE and S100B from serum and cerebrospinal fluid in patients with spontaneous subarachnoid hemorrhage. J Neurosurg Anesthesiol. 2010;22(1):21–31
15. Sanchez-Peña P, Pereira AR, Sourour NA, Biondi A, Lejean L, Colonne C, Boch AL, Al Hawari M, Abdennour L, Puybasset L. S100B as an additional prognostic marker in subarachnoid aneurysmal hemorrhage. Crit Care Med. 2008;36(8):2267–73.
16. Kiiski H, Långsjö J, Tenhunen J, Ala-Peijari M, Huhtala H, Hämäläinen M, Moilanen E, Peltola J. S100B, NSE and MMP-9 fail to predict neurologic outcome while elevated S100B associates with milder initial clinical presentation after aneurysmal subarachnoid hemorrhage. J Neurol Sci. 2018;390:129–34.
17. Olivecrona M, Rodling-Wahlström M, Naredi S, Koskinen LO. S-100B and neuron specific enolase are poor outcome predictors in severe traumatic brain injury treated by

an intracranial pressure targeted therapy. J Neurol Neurosurg Psychiatry. 2009;80(11):1241–7.
18. Gigante, P.; Hwang, B.Y.; Appelboom, G.; Kellner, C.P.; Kellner, M.A.; Connolly, E.S. External ventricular drainage following aneurysmal subarachnoid haemorrhage External ventricular drainage following aneurysmal subarachnoid haemorrhage. Br. J. Neurosurg. **2010**
19. Chung, D.Y.; Leslie-Mazwi, T.M.; Patel, A.B.; Rordorf, G.A. Management of External Ventricular Drains after Subarachnoid Hemorrhage: A Multi-Institutional Survey. Neurocrit. Care **2017**, 26, 356–361.
20. Dasic, D.; Hanna, S.J.; Bojanic, S.; Kerr, R.S.C. External ventricular drain infection: The effect of a strict protocol on infection rates and a review of the literature. Br. J. Neurosurg. **2006**.
21. Rao, S.S.; Chung, D.Y.; Wolcott, Z. Intermittent CSF drainage and rapid EVD weaning approach after subarachnoid hemorrhage: Association with fewer VP shunts and shorter length of stay HHS Public Access. J. Neurosurg. **2019**, 1–6.
22. Hoefnagel D, Dammers R, Ter Laak-Poort MP, Avezaat CJ. Risk factors for infections related to external ventricular drainage. Acta Neurochir (Wien). 2008;150(3):209–14.

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