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ORIGINAL ARTICLE

Neurodegenerative cerebrospinal fluid biomarkers tau and amyloid beta predict functional, quality of life, and neuropsychological outcomes after aneurysmal subarachnoid hemorrhage

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Abstract Cerebrospinal fluid (CSF) biomarkers might be useful in predicting outcome after aneurysmal subarachnoid hemorrhage (aSAH). It was the aim to determine whether tau and amyloid beta CSF concentrations predict functional, health-related quality of life (hrQoL), and neuropsychological outcomes after aSAH. Ventricular CSF was obtained from n = 24 aSAH patients at admission (D0), day 2 (D2), and day 6 (D6). CSF total (t)Tau, phosphorylated (p)Tau$_{(181P)}$, and amyloid beta$_{(1\text{-}40 \text{ and } 1\text{-}42)}$ (Aβ40/Aβ42) levels were compared between patients with favorable and unfavorable functional (modified Rankin Scale (mRS)), hrQoL (Euro-Qol (EQ-5D)), and neuropsychological outcomes at 3 (3 m) and 12 months (12 m). Patients with unfavorable functional (mRS 4–6) and hrQoL outcome (EQ-5D z-score ≤ − 1.0) at 3 and 12 m had higher CSF tTau/pTau and lower Aβ40/Aβ42 at D0, D2, and D6 with varying degrees of statistical significance. In terms of predicting neuropsychological outcome, CSF pTau showed a statistically significant correlation with the z-scores of executive function (r = − 0.7486, p = 0.008), verbal memory (r = − 0.8101, p = 0.002), attention (r = − 0.6498, p = 0.030), and visuospatial functioning (r = − 0.6944, p = 0.017) at 3 m. At 12 m, CSF pTau had statistically significant correlations with the z-scores of verbal memory (r = − 0.7473, p = 0.008) and visuospatial functioning (r = − 0.6678, p = 0.024). In conclusion, higher tTau/pTau and lower Aβ40/Aβ42 CSF levels predict unfavorable long-term functional and hrQoL outcomes. Neuropsychological deficits correlate with increased CSF tTau and pTau concentrations.

Keywords Amyloid beta protein · Biomarker · Cerebrospinal fluid · Neuropsychological outcome · Subarachnoid hemorrhage · Tau protein

Introduction

Cognitive and functional sequelae of aneurysmal subarachnoid hemorrhage (aSAH) or secondary events such as delayed cerebral ischemia and hydrocephalus [12] are profound in many who survive, even years following the ictus [1]. The establishment of reliable predictive outcome parameters remains challenging, especially during the acute phase. Cerebrospinal fluid (CSF) biomarkers gain increasing interest among researchers and clinicians. Their predictive capacity for clinical outcome has been demonstrated for traumatic brain injury (TBI) [9], but less so for aSAH [3, 8, 16]. Tau and amyloid beta proteins are among the more recently studied CSF biomarkers. Tau proteins exist in six isoforms that are involved in the formation of microtubule

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bundles building the structural basis of the axonal cytoskeleton. This enables axoplasmatic flow of proteins between the axon terminal and cell body. Brain injury results in proteolytic cleavage of these six isoforms [9]. Furthermore, tau contains several phosphorylation sites. Pathological hyperphosphorylation of tau has a central role in the pathogenesis of neurodegenerative disorders [9]. Amyloid beta $_{(1\text{-}40 \text{ and } 1\text{-}42)}$ (Aβ40/Aβ42) is a cell adhesion protein in synaptic membranes, which bears two caspase-cleaved breakdown products from amyloid precursor protein with 40- and 42-amino acid lengths, respectively [9]. The regular function of Aβ40/Aβ42 is not well understood [4], but it is suggested that overall protective effects prevail [10].

It was the aim of this study to determine whether the CSF biomarkers tau and amyloid beta are useful predictive surrogate biomarkers for the functional, health-related quality of life (hrQoL), and neuropsychological outcome up to 1 year after aSAH.

Materials and methods

Patient recruitment and inclusion and exclusion criteria

In this prospective observational study, consecutive aSAH patients between May 2013 and February 2015 were screened for study eligibility. Exclusion criteria were lack of computed tomographic angiography and/or digital subtraction angiography confirmation of a ruptured aneurysm, such as in non-aneurysmal perimesencephalic SAH. Also, moribund patients with absent brain stem reflexes and bilateral fixed mydriasis unlikely to survive until completion of further assessments were excluded. As this study investigated CSF biomarkers, only patients requiring an external ventricular drain (EVD) for acute hydrocephalus were analyzed.

The study was approved by the Ethics Committee St. Gallen, Switzerland (EKSG 13/011/1B). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was registered at ClinicalTrials.gov (Identifier: NCT02129010; URL https://clinicaltrials.gov/ct2/show/NCT02129010).

Patient management and data collection

Neurointensive care unit patient management followed the current guidelines for aSAH [2] and has been described previously [5]. Patient baseline parameters with common risk factors for aSAH, as well as pre-ictal functional status (modified Rankin Scale (mRS)) and hrQoL using the Euro-Qol questionnaire (EQ-5D) were ascertained. Admission World Federation of Neurological Surgeons (WFNS) score and disease-specific parameters were recorded. Clinical deterioration and cerebral infarcts attributable to delayed cerebral ischemia were defined according to Vergouwen et al. [13, 14]. Functional outcome assessment was performed at discharge, 3 (3 m) and 12 months (12 m) using the mRS. hrQoL was estimated with the EQ-5D at 3 and 12 m. In accordance with Swiss national standard, a neuropsychological assessment was obtained, using the Montreal Cognitive Assessment (MoCA) at days 14–21 and 3 and 12 m. This was followed by a more detailed evaluation of patients with a MoCA of at least 15 points at 3 and 12 m [18]. To calculate *z*-scores of the neuropsychological domains executive function, verbal memory, attention, and visuospatial functioning, a standardized test battery was used that included the following tests, adjusted for age and education whenever possible: computerized Test of Attentional Performance (TAP 2.3) using the subtests alertness, divided attention, Go/NoGo (1 out of 2), neglect (92 trials with switching letters); verbal and visual span—forward; Color-Word Interference Test (Victoria version); Verbal fluencies (semantic and phonemic); Design fluency; cognitive flexibility (Trail Making Test B); Auditiv-verbaler Lern und Gedächtnistest (as adaptation of the Rey Auditory Verbal Learning Test); Rey-Osterrieth Complex Figure Test [18].

CSF sample handling

Following EVD insertion, CSF samples were obtained on admission (day 0; D0) as well as on day 2 (D2) and 6 (D6) by discarding the first 20 drops and then collecting 2 ml CSF in a polypropylene collection tube (Tube Sarstedt PP, 10 ml ref. 62.610.201, Sarstedt AG & Co., Nümbrecht, Germany). CSF was directly processed by centrifugation at 2000*g* for 10 min at controlled room temperature. CSF aliquots were stored in 500-μL tubes (Tube Sarstedt PP, 500 μL ref. 72.730.006, Sarstedt AG & Co., Nümbrecht, Germany) at − 80 °C until enzyme-linked immunosorbent assay (ELISA) analysis for total (t)Tau including the six isoforms (352 to 441 amino acids), phosphorylated (p)Tau$_{(181P)}$, and Aβ40/Aβ42 (Fujirebio Europe N.V., Ghent, Belgium). No lumbar drain CSF was used as there is no comparative data regarding the comparability of CSF levels obtained from EVD and lumbar drains, respectively.

Statistical methods

CSF biomarker levels were compared between patients with favorable and unfavorable functional and hrQoL, as well as neuropsychological outcome, at 3 and 12 m using student's *t* tests. In order to adjust for case severity (WFNS score), an additional analysis of covariance (ANCOVA) model was run.

Functional outcome was dichotomized into favorable (mRS 0–3) and unfavorable (mRS 4–6), as done previously by Helbok et al. [3]. For the hrQoL outcomes, the EQ-5D index was transformed into age- and sex-adjusted z-scores, based on the normal population data. From here, patients with a z-score ≤ -1.0 (one standard deviation below population norm) were considered unfavorable, and those with a z-score > -1.0 favorable. Again, deceased patients at follow-up were considered to have an unfavorable hrQoL outcome for statistical analysis. The cutoff for a favorable and unfavorable neuropsychological outcome for the screening test was set at the generally accepted 26 points on the MoCA [15]. Death at follow-up was considered as neuropsychologically impaired for statistical analysis. For the detailed neuropsychological outcome, Pearson correlations were run to analyze the relationship between adjusted z-scores of executive function, verbal memory, visuospatial functioning, and attention in all available patients alive at follow-up and the neurodegenerative CSF biomarker levels (average value of D0, D2, and D6 combined). Probability values ≤ 0.05 were considered statistically significant. Stata v.14 (College Station, TX, USA) was used for analysis.

Estimation of sample size

Based on the results of Helbok et al. [3] and Zanier et al. [16], a group size of $n = 10$ and $n = 9$ patients was required to detect different CSF tau levels to predict outcome with a power of 80% and alpha set at 0.05. As our study included more low-grade aSAH patients than the previous studies and detailed outcome parameters, we aimed to include at least 25 patients in total.

Results

During the study period, 84 patients admitted for spontaneous SAH were screened for study participation, and 45 patients (53.6%) were found to have SAH due to a ruptured aneurysm and to be eligible. Seven patients/next of kin refused consent to participation. Thus, a remainder of $n = 38$ patients was included, of which $n = 30$ required ventriculostomy using an EVD. CSF biomarkers could not be obtained when polypropylene tubes were not at hand in the emergency setting (D0: $n = 6$), or at D2/D6 in patients with slit ventricles and EVD in place ($n = 4$), and when the EVD was removed or replaced by a lumbar drain ($n = 2$). Thus, data for neurodegenerative CSF biomarkers was available for $n = 24$ (80%) patients on D0, $n = 20$ (66.7%) on D2, and $n = 18$ (60%) on D6. While there was no missing data for the mRS and EQ-5D index, neuropsychological outcome at 3 and 12 m was obtained in 19 of 22 surviving patients (86.4%) as the remaining three patients did not attend the follow-up assessment. A flow chart of the study patients is given in Fig. 1.

Patient cohort and treatment results

Baseline patient characteristics and disease-specific and treatment parameters are described in Table 1. The cohort had a mean age of 57.1 years, 53.3% were female, and the vast majority had a good functional, general health, and hrQoL status before the ictus. Almost all patients had a Fisher-III aSAH and intraventricular hemorrhage and hydrocephalus were common radiological findings at admission. The most frequent risk factors were hypertension and smoking. The occurrences of Terson's syndrome [5] and sentinel headache [6] were previously reported on. Aneurysm localization was typical with almost a third situated at the anterior communicating artery complex, followed by the middle cerebral artery, carotid artery, and posterior circulation. Aneurysm occlusion was undertaken in 28 patients (93.3%), with coiling preferred over clipping in situations of equipoise. Chronic hydrocephalus was treated by shunt placement in 36.7% of patients.

Complications and outcomes are disclosed in Table 2. During hospitalization (mean 24.5 days), vasospasm as detected by transcranial doppler occurred in 56.7% of patients, neurological deterioration and infarcts attributable to delayed cerebral ischemia in 30% and 16.7%, respectively. Mortality was 26.7%, with $n = 5$ dying from brain injury due to the hemorrhage itself, and $n = 3$ dying from

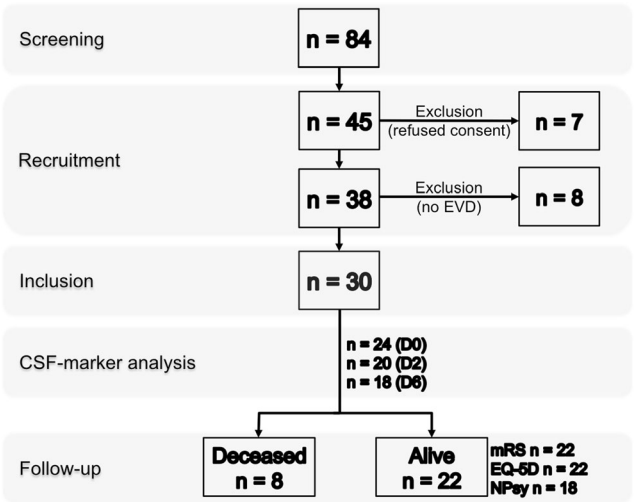


Fig. 1 Flow chart of $n = 45$ patients with aneurysmal subarachnoid hemorrhage eligible for study participation. After exclusion of $n = 7$ patients who or whose next of kin refused consent and $n = 8$ patients who did not require an external ventricular drain (EVD), $n = 30$ patients remained for analysis. Cerebrospinal fluid (CSF) biomarkers were obtained in $n = 24$ patients on day (D0), $n = 20$ on D2, and $n = 18$ on D6. Modified Rankin Scale (mRS) and EQ-5D were available in $n = 22$ survivors at 3 and 12 months. Three patients refused follow-up neuropsychological assessment (NPsy)

Table 1 Basic demographics and disease-specific and treatment parameters at hospital admission of $n = 30$ patients with aneurysmal subarachnoid hemorrhage

Age in years	57.1	11.5
Sex		
Male	14	46.7%
Female	16	53.3%
Delay ictus to admission		
0 day	24	80.0%
1 day	5	16.7%
> 1 day	1	3.3%
WFNS degree		
1	2	6.7%
2	14	46.7%
3	2	6.7%
4	2	6.7%
5	10	33.2%
Fisher degree		
3	29	96.7%
4	1	3.3%
Epileptic seizure		
No	26	86.7%
Yes	4	13.3%
Intubated		
No	19	63.3%
Yes	11	36.7%
Imaging features		
Intraventricular hemorrhage	20	66.7%
Intracerebral hemorrhage	6	20.0%
Hydrocephalus	28	93.3%
Pre-morbid mRS		
0–1	29	96.7%
≥ 2	1	3.3%
Pre-morbid EQ-5D[a]		
≤ − 1.0	–	0.0%
− 0.99–0.99	27	96.7%
≥ 1.0	1	3.3%
Aneurysm site		
A1/A2	3	10.0%
Acom	9	30.0%
ICA/Pcom	7	23.3%
M1/M2	7	23.3%
VA/BA/PICA	4	13.4%
Aneurysm size in mm		
Dome	7.7	2.9
Neck	3.5	1.6
Aneurysm multiplicity		
No	21	70.0%
Yes	9	30.0%
Risk factors		
Arterial hypertension	14	46.7%
Active/former cigarette smoking	11/5	36.7/16.7%
High alcohol consumption[b]	5	16.7%
Illegal drug abuse	2	6.7%
Previous aSAH	1	3.3%
Sentinel headache	6	20.0%
Treatment details		
Endovascular coiling	17	56.7%
Surgical clipping	11	36.7%
Decompressive craniectomy	4	13.3%
Lumbar drainage	4	13.3%
VP shunt	11	36.7%
	$n = 30$ (100%)	

Data is presented in mean (standard deviation) or count (percent)

A1/A2 pre- and postcommunicating segment of anterior cerebral artery, *Acom* communicating segment of anterior cerebral artery, *aSAH* aneurysmal subarachnoid hemorrhage, *EQ-5D* Euro-Qol 5 D, *ICA/Pcom* internal carotid artery/posterior communicating artery, *M1/M2* Middle cerebral artery segments, *mm* millimeter, *mRS* modified Rankin Scale, *VA/BA/PICA* vertebral artery/basilar artery/posterior inferior cerebellar artery, *VP* ventriculoperitoneal, *WFNS* World Federation of Neurological Surgeons

[a] Result expressed as age- and sex-adjusted *z*-score

[b] > 1 drink per day for women and >2 drinks per day for men

medical complications ($n = 2$ pulmonary embolism, $n = 1$ cardiac arrest). Mortality remained stable until 12 m, with the fraction of patients with favorable functional and hrQoL outcome increasing over time. The impairment rate on neuropsychological screening with the MoCA decreased from 79% at 3 m to 52.6% at 12 m. On detailed neuropsychological testing, the most severely affected domains were verbal memory, followed by executive function, visuospatial function, and attention.

CSF biomarkers and functional and hrQoL outcome at 3 and 12 m

Patients with unfavorable functional outcome (mRS 4–6) at the 3 and 12 m of follow-up had higher CSF tTau at D6 (3 m: $p = 0.023$), higher pTau at D0 (12 m: $p = 0.015$) and D2 (3 m: $p = 0.019$), lower Aβ40 at D2 (3 m: $p = 0.044$), and lower Aβ42 at D2 (3 m: $p = 0.011$) and D6 (12 m: $p = 0.021$) as shown in Fig. 2. After adjustment for case severity, patients with unfavorable functional outcome at the 3 and 12 m of follow-up had higher CSF pTau at D0 (12 m: $p = 0.015$) and D2 (3 m: $p = 0.048$).

Patients with unfavorable hrQoL outcome (EQ-5D *z*-score ≤ − 1.0) at the 3 and 12 m of follow-up had higher CSF tTau at D0 (3 m: $p = 0.014$; 12 m: $p = 0.017$) and D6 (12 m: $p = 0.018$), higher pTau at D2 (12 m: $p = 0.049$), lower Aβ40 at D2 (12 m: $p = 0.038$), and lower Aβ42 at D2 (12 m: $p = 0.035$) as shown in Fig. 2. After adjustment for case severity, patients with unfavorable hrQoL outcome at the 3 and 12 m of follow-up had higher CSF tTau at D0 (3 m: $p = 0.015$; 12 m: $p = 0.023$) and D6 (12 m: $p = 0.042$).

Table 2 Complications and outcomes of $n = 30$ patients with aneurysmal subarachnoid hemorrhage

Complications		
Transcranial doppler mean blood flow velocity > 120 cm/s	17	56.7%
Delayed ischemic neurologic deficit	9	30.0%
Delayed cerebral ischemia	5	16.7%
Seizure	2	6.7%
Aneurysm re-hemorrhage	2	6.7%
Hyper/hyponatremia	10	33.3%
Cardiac complication	2	6.7%
Pneumonia	4	13.3%
Urinary infection	4	13.3%
Sepsis	2	6.7%
Follow-up		
Intensive Care Unit length of stay	16.7	9.0
Hospital length of stay	24.5	12.5
Hospital mortality	8	26.7%
Rehabilitation discharge	19	63.3%
Home discharge	3	10.0%
Functional and hrQoL outcome		
mRS at 3 months		
0–1	2	6.7%
2–3	16	53.3%
4–5	4	13.3%
6	8	26.7%
EQ-5D index at 3 months[a]		
− 1.0	14	46.7%
− 0.99–0.99	16	53.3%
≥ 1.0	–	0.0%
mRS at 1 year		
0–1	10	33.3%
2–3	10	33.3%
4–5	2	6.7%
6	8	26.7%
EQ-5D index at 1 year[a]		
− 1.0	12	40.0%
− 0.99–0.99	18	60.0%
≥ 1.0	–	0.0%
Neuropsychological outcome		
At 14–21 days[a]		
MoCA raw score	12.7	9.5
MoCA < 26 points	22	95.7%
At 3 months[b]		
MoCA raw score	20.9	5.9
MoCA < 26 points	15	79.0%
z-score executive function	− 1.67	1.49
z-score verbal memory	− 2.00	1.64
z-score attention	− 0.91	1.28
z-score visuospatial function	− 1.15	1.44
At 1 year[c]		
MoCA raw score	24.4	5.7
MoCA < 26 points	10	52.6%
z-score executive function	− 0.52	1.06
z-score verbal memory	− 1.05	1.21
z-score attention	− 0.25	0.81
z-score visuospatial function	− 0.37	1.14
	$n = 30$ (100%)	

Data is presented in mean (standard deviation) or count (percent)

DCI delayed cerebral ischemia, *DIND* delayed ischemic neurological deficit, *EQ-5D* Euro-Qol 5 D, *hrQoL* health-related quality of life, *ICU* intensive care unit, *LOS* length of stay, *mBFV* mean blood flow velocity, *MoCA* Montreal Cognitive Assessment, *mRS* modified Rankin Scale

[a] Result expressed as age- and sex-adjusted z-score. Assessment available in $n = 23$ patients

[b] Assessment available in $n = 19$ patients

[c] Assessment available in $n = 19$ patients

CSF biomarkers and neuropsychological screening at 3 and 12 m

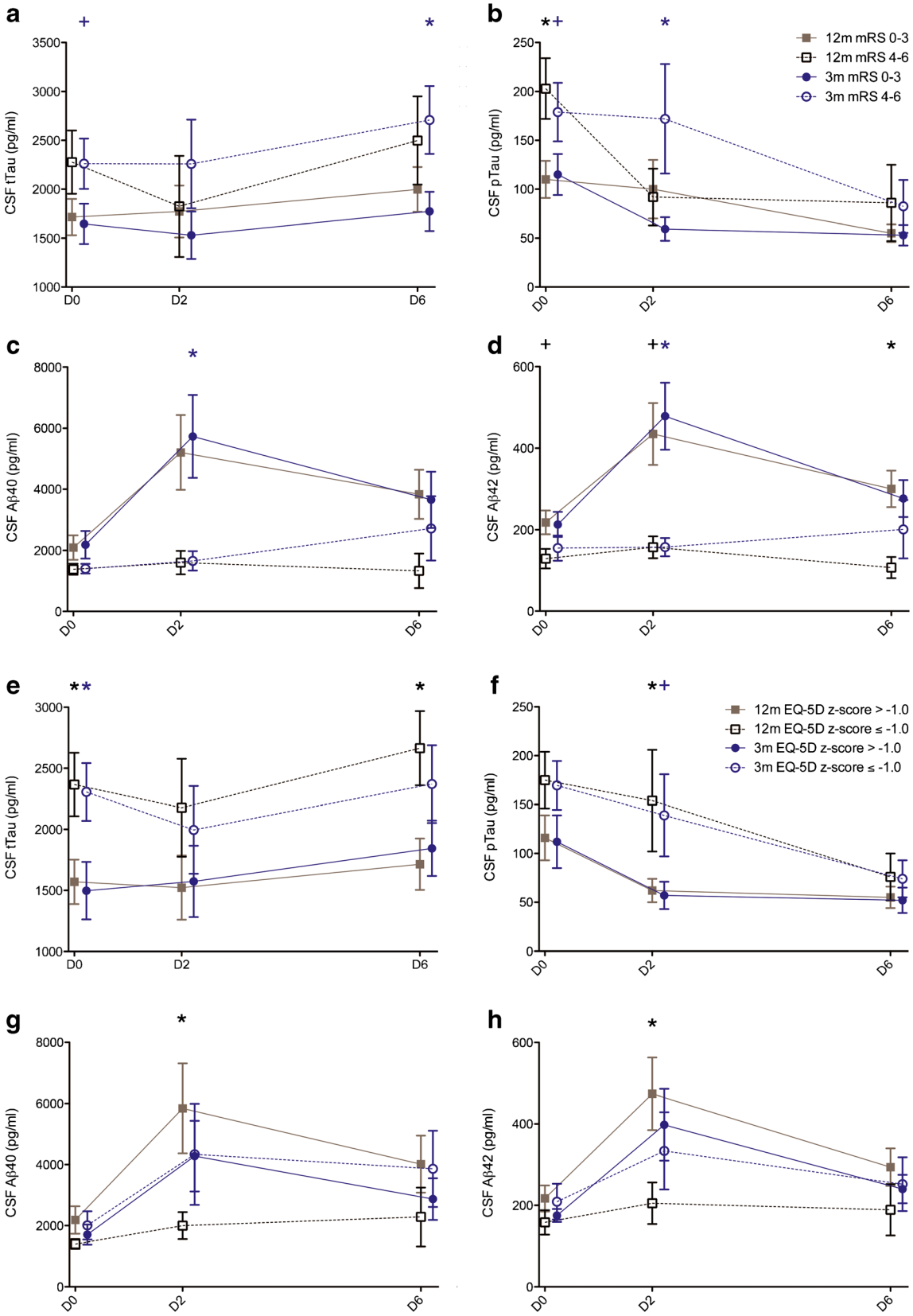
Patients with a MoCA < 26 points at the 3 and 12 m of follow-up tended to have (non-significant) higher CSF tTau and pTau and lower Aβ40 and Aβ42 at D0, D2, and D6 displayed in Supplementary Fig. 1. Also, after adjustment for case severity, the relationships remained non-significant. It becomes evident that discriminative power for tau protein was generally better than for the amyloid protein to predict neuropsychological impairment at 3 and 12 m.

CSF biomarkers and detailed neuropsychological examination at 3 and 12 m

Patient-specific CSF values for tTau, pTau, Aβ40, and Aβ42 averaged over D0–D6 were correlated to standardized *z*-scores of the following cognitive domains: (1) executive function, (2) verbal memory, (3) attention, and (4) visuospatial functioning.

At the 3 m of assessment, CSF tTau had a statistically non-significant correlation with the *z*-scores of executive function ($r = -0.3121$, $p = 0.35$), verbal memory ($r = -0.5287$, $p = 0.09$), attention ($r = -0.2893$, $p = 0.38$), and visuospatial functioning ($r = -0.3984$, $p = 0.22$). CSF pTau was best at predicting neuropsychological outcome at 3 m as it showed a moderate to high statistically significant correlation with the *z*-scores of executive function ($r = -0.7486$, $p = 0.008$), verbal memory ($r = -0.8101$, $p = 0.002$), attention ($r = -0.6498$, $p = 0.030$), and visuospatial functioning ($r = -0.6944$, $p = 0.017$; Fig. 3a–d). The correlations between CSF Aβ40 and Aβ42 with the *z*-scores of neuropsychological domains at 3 m were weak and non-significant.

At the 12 m of assessment, CSF tTau had a statistically non-significant correlation with the *z*-scores of executive function ($r = -0.5379$, $p = 0.08$), verbal memory ($r = -0.3474$, $p = 0.29$), attention ($r = -0.4359$, $p = 0.18$),


a
b
c
d
e
f
g
h
CSF tTau (pg/ml)
CSF pTau (pg/ml)
CSF Aβ40 (pg/ml)
CSF Aβ42 (pg/ml)
D0
D2
D6
12m mRS 0-3
12m mRS 4-6
3m mRS 0-3
3m mRS 4-6
12m EQ-5D z-score > -1.0
12m EQ-5D z-score ≤ -1.0
3m EQ-5D z-score > -1.0
3m EQ-5D z-score ≤ -1.0

◀ **Fig. 2** Cerebrospinal fluid (CSF) measurements of total Tau (tTau), phosphorylated Tau (pTau), and amyloid beta 40 (Aβ40) and 42 (Aβ42) at admission day (D0), day 2 (D2), and day 6 (D6) after aneurysmal subarachnoid hemorrhage in patients with favorable (modified Rankin Scale (mRS) 0–3) and unfavorable functional outcome (mRS 4–6; **a**–**d**) as well as with favorable (Euro-Qol 5D index (EQ-5D) *z*-score > − 1.0) and unfavorable health-related quality of life outcome (EQ-5D *z*-score ≤ − 1.0; **e**–**h**) at three (3 m; blue circles) and 12 months (12 m; gray squares). Results are presented in mean picogram per milliliter and standard error of mean. * = $p < 0.05$; + = $p < 0.10$

and visuospatial functioning ($r = -0.5274$, $p = 0.09$). CSF pTau had moderate to high statistically significant correlations with verbal memory ($r = -0.7473$, $p = 0.008$) and visuospatial functioning ($r = -0.6678$, $p = 0.024$), but statistically non-significant correlations with the *z*-scores of executive function ($r = -0.5356$, $p = 0.08$) and attention ($r = -0.4941$, $p = 0.12$; Fig. 3e–h). The correlations between CSF Aβ40 and Aβ42 with the *z*-scores of neuropsychological domains at 12 m were weak and non-significant.

Correlation coefficients of CSF biomarkers at each time point (D0–D6) with the domain-specific *z*-scores at 3 and 12 m are provided in Supplementary Table 1. The data indicate that, in particular, the pTau measurements obtained at D2 and D6 relate well to the neuropsychological outcome, whereas those obtained at D0 do not.

Discussion

This prospective study demonstrated an association of higher tTau and pTau, as well as lower Aβ40 and Aβ42 CSF levels in the acute phase of aSAH with unfavorable mid- and long-term functional, hrQoL, and neuropsychological outcome. The present results thus confirm the findings of previous reports that demonstrated functional and neuropsychological outcomes as a function of the levels of neurodegenerative CSF biomarkers, both in TBI and aSAH research [3, 7, 8, 16, 17]. In addition, for the first time, a similar relationship could be established with hrQoL.

CSF biomarkers and functional outcome

Following pioneer work on the association between elevated intracranial pressure, clinical outcome, and tau protein in TBI [17], Kay et al. [7, 8] further investigated the temporal alterations of CSF biomarkers in $n = 19$ patients with aSAH. Ventricular CSF tau concentration was significantly higher on day 2 (3400 ± 4400 pg/ml) as compared to a control group of patients with suspected shunt dysfunction or compensated chronic hydrocephalus (190 ± 200 pg/ml). Comatose patients had significantly higher CSF levels than those with Glascow Coma Score > 8. Intriguingly, Aβ40 and Aβ42 CSF levels were found to be lower during the acute phase after aSAH. Based on previous findings [7], the authors speculated that increased receptor-mediated uptake of apoE interacting with amyloid beta may explain this observation [8]. The maximum tau and minimum Aβ levels significantly correlated with an unfavorable 3-month Glasgow Outcome Score [8]. Zanier et al. [16] detected significantly lower mean tau levels in the ventricular CSF of $n = 27$ aSAH patients with mild WFNS (1–3) aSAH (1572 pg/ml) than in those with severe WFNS (4–5) aSAH (7194 pg/ml). The only outcome parameter was dead or alive at discharge from hospital for which significantly different tau peak concentrations were found (9066 vs. 2260 pg/ml). Helbok et al. [3] discriminated between unfavorable and favorable outcome in poor-grade patients using cerebral microdialysis with a cutoff tau level of ≥ 1259 pg/ml on day 2. In an attempt to make our data comparable to their study, we adopted the same functional outcome criteria (mRS 0–3 vs. 4–6) and long-term follow-up (12 m). Although significantly different on D0 and D6, our patients' mean CSF tTau levels were well above the previously reported cutoff of ≥ 1259 pg/ml at any given point in time, irrespective of the outcome. Interestingly, tTau and amyloid beta seem to be further increased and decreased on D6, respectively (Fig. 2). These presumed differences could be explained by additional brain damage secondary to vasospasm, delayed cerebral ischemia, or surgical interventions such as EVD changes and aneurysm clipping, but were not statically significant and have not been reported before. In general, comparability of our results to those of Helbok et al. [3] is limited as CSF samples were taken from different intracranial spaces (see below).

CSF biomarkers and hrQoL

Previous studies have not addressed CSF biomarkers in the context of hrQoL outcome after aSAH. Our study reports this important facet of a comprehensive outcome assessment and establishes a relationship of higher tTau/pTau and lower Aβ40/Aβ42 CSF levels with poor hrQoL outcome.

CSF biomarkers and neuropsychological outcome

Helbok et al. [3] established a relationship between tau protein levels and neuropsychological outcome for the first time. A positive association for tTau with the Mini-Mental State Examination < 24 and impaired performance in Trail Making Test-A and Clock drawing was ascertained, while no association was found for pTau at 12 m after aSAH. In the current study, the MoCA was used as a screening tool, which previously had been shown to be more sensitive for the detection of aSAH-associated cognitive impairment [11]. Our data show a good correlation of averaged tTau and pTau values obtained in the most vulnerable period after aSAH (D0–D6) with executive function, verbal memory, attention,

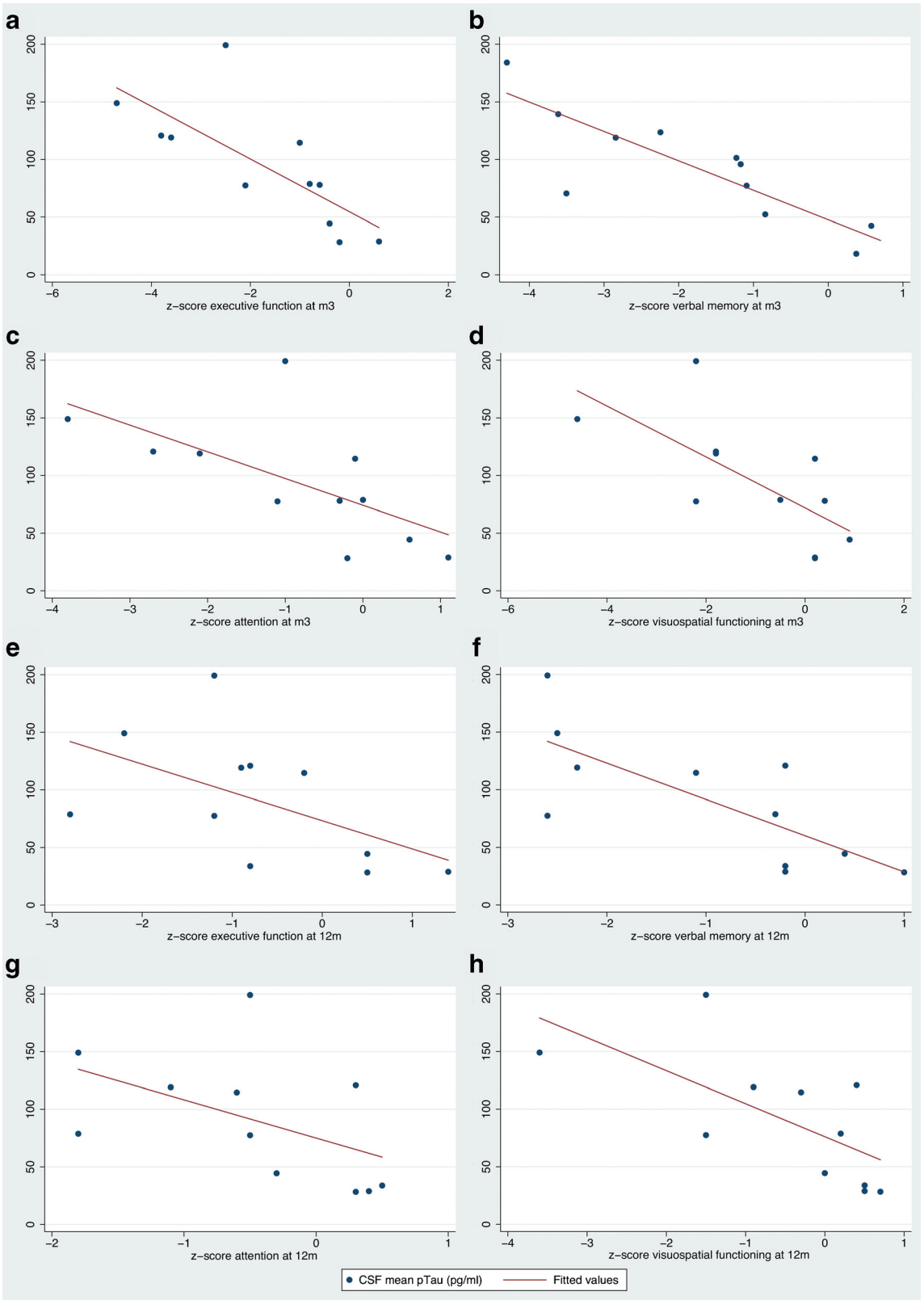


Fig. 3 Correlation of phosphorylated Tau (pTau in pg/ml; *y*-axis) with *z*-scores of executive function ($r = -0.7486$, $p = 0.008$; **a**), verbal memory ($r = -0.8101$, $p = 0.002$; **b**), attention ($r = -0.6498$, $p = 0.030$; **c**), and visuospatial functioning ($r = -0.6944$, $p = 0.017$; **d**), assessed 3 months (3 m), and *z*-scores of executive function ($r = -0.5356$, $p = 0.08$; **e**), verbal memory ($r = -0.7473$, $p = 0.0082$; **f**), attention ($r = -0.4941$, $p = 0.12$; **g**), and visuospatial functioning ($r = -0.6678$, $p = 0.0247$; **h**), assessed 12 months (12 m) after aneurysmal subarachnoid hemorrhage

and visuospatial function, while none of the CSF biomarkers turned out to predict favorable or unfavorable neuropsychological outcome as defined by the MoCA cutoff. Furthermore, our data suggest that CSF pTau levels obtained during the first days after aSAH relate better to the detailed neuropsychological outcome than at admission (Supplementary Table 1).

Strengths and limitations

In this prospective study, standardized outcome measures were applied in a limited, but sufficiently large sample. Despite the established positive associations, the results of the present analysis need to be interpreted with caution. Data spread was larger than expected and these large ranges of measurements make it difficult to reliably predict outcomes in the individual patient. For now, despite the academically interesting findings that provide new insight into the pathophysiology of aSAH, it would currently not be feasible to predict functional, neuropsychological, and hrQoL outcomes on the grounds of CSF biomarkers in daily clinical practice. Several points need to be taken into consideration for the interpretation of our results: similar to previous studies [3, 16], most of the patients in the study cohort had poor admission scores. Naturally, multimodal monitoring and EVDs are not required in those who are amenable to clinical assessment, and therefore, CSF biomarker levels for these patients are usually lacking. As a consequence, our data underlies selection bias and cannot be generalized to the whole population of aSAH patients. CSF space sampling and processing might differ and limits the comparability between studies that used the subarachnoid [3] or intraventricular space [8, 16]. Helbok et al. [3] reported on higher tTau levels from catheters placed next to a lesion as compared to into normal appearing brain tissue. In order to alleviate this local variability, ventricular CSF was sampled in the current study. The presence of intraventricular hemorrhage could potentially influence obtained CSF biomarker concentrations. Additional analyses at each time point did not reveal significant differences in patients with or without intraventricular hemorrhage in the present sample (data not shown). However, it is possible that CSF biomarker concentrations are subject to various other (yet unknown) factors during treatment of patients with aSAH and sampling at more time points would have been desirable. Zanier et al. [16] showed that a "wash-out" effect was not the case for surgical clipping, but other factors such as age, gender, comorbidities, and complications need to be further looked at.

Conclusion

Higher tTau/pTau and lower Aβ40/Aβ42 CSF levels in the acute phase of aSAH predict unfavorable long-term functional and hrQoL outcomes. Neuropsychological deficits at 3 and 12 m of follow-up correlate well with increased CSF tTau and pTau concentrations. In combination with a panel of other CSF biomarkers and more data from validation studies, tau and amyloid beta might be of value to estimate prognosis in aSAH in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Ethics Committee St. Gallen, Switzerland (EKSG 13/011/1B). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was registered at ClinicalTrials.gov (Identifier: NCT02129010; URL https://clinicaltrials.gov/ct2/show/NCT02129010).

Informed consent Informed consent was obtained from all individual participants or his/her next of kin or substitute decision maker.

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