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A multiparameter panel method for outcome prediction following aneurysmal subarachnoid hemorrhage

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Abstract *Purpose:* Accurate early anticipation of long-term irreversible brain damage during the acute phase of patients with aneurysmal subarachnoid hemorrhage (aSAH) remains difficult. Using a combination of clinical scores together with brain injury-related biomarkers (H-FABP, NDKA, UFD1 and S100β), this study aimed at developing a multiparameter prognostic panel to facilitate early outcome prediction following aSAH. *Methods:* Blood samples of 141 aSAH patients from two separated cohorts (sets of 28 and 113 patients) were prospectively enrolled and analyzed with 14 months of delay. Patients were admitted within 48 h following aSAH onset. A venous blood sample was withdrawn within 12 h after admission. H-FABP, NDKA, UFD1, S100β and troponin I levels were determined using classical immunoassays. The World Federation of Neurological Surgeons (WFNS) at admission and the Glasgow Outcome Score (GOS) at 6 months were evaluated. *Results:* In the two cohorts, blood concentration of H-FABP, S100β and troponin I at admission significantly predicted unfavorable outcome (GOS 1–2–3). A multivariate analysis identified a six-parameter panel, including WFNS, H-FABP, S100β, troponin I, NDKA and UFD-1; when at least three of these parameters were simultaneously above cutoff values, prediction of unfavorable outcome reached around 70% sensitivity in both cohorts for 100% specificity. *Conclusion:* The use of this panel, including four brain injury-related proteins, one cardiac marker and a clinical score, could be a valuable tool to identify aSAH patients at risk of poor outcome.

Keywords Aneurysmal subarachnoid hemorrhage · H-FABP · NDKA · S100β · Prognosis

Abbreviations

H-FABP	Heart-fatty acid binding protein
NDKA	Nucleoside diphosphate kinase A
UFD-1	Ubiquitin fusion degradation protein-1
aSAH	Aneurysmal subarachnoid hemorrhage
SE	Sensitivity
SP	Specificity

Introduction

Besides the high early mortality associated with aSAH, long-term neurological morbidity is also a significant problem in a substantial proportion of these patients [1, 2]. Identification of prognostic factors, aimed to predict patient outcome, would help in the management and decision making within this population. Clinical scores, such as WFNS classification, demonstrated an association between prognosis following aSAH and the patient's clinical neurological status at hospital admission [3]. Biochemical markers may provide additional information about specific pathological disruptions and recovery processes that occur in the central nervous system following aSAH. In conjunction with clinical status, these biomarkers may also inform prognosis and guide therapeutic decisions to optimize treatments [4, 5].

Over the past few years, a large number of biomarkers, present in the blood and CSF, have raised interest in the detection of aSAH patients with poor clinical outcome. Nevertheless, the majority of these markers displayed either low sensitivity or specificity to anticipate the detection of patients with poor outcome [6, 7].

We recently explored post-mortem CSF as a model of massive brain insult [8, 9]. In these studies, heart-fatty acid binding protein (H-FABP), nucleotide diphosphate kinase A (NDKA) and ubiquitin fusion degradation protein 1 (UFD-1) were over-expressed in post-mortem compared to ante-mortem CSF and were validated as potential brain damaged biomarkers [10–12]. In the present study, we hypothesized that such a reliable plasmatic marker may provide quantitative information reflecting the prediction of aSAH patient outcome. The objective of this study was to determine, immediately at the hospital admission, S100β, H-FABP, troponin I, NDKA and UFD-1 protein blood concentrations of patients with spontaneous aSAH obtained in two separated cohorts from the same institution. In addition to specific clinical parameters, their potential predictive power to detect poor 6-month outcome following aSAH was evaluated [13–15].

Patients and methods

Population

The inclusion period was from July 2004 to December 2006 in the Pitié-Salpêtrière Hospital (Paris, France). Inclusion criteria were clinical history of aSAH within the last 2 days before admission with evidence of bleeding in CT and presence of an aneurysm at cerebral angiography, age above 18 years old and treatment by surgery or coiling within 48 h after admission. Each eligible patient was admitted in the intensive care unit (ICU) within the 2 days after aSAH symptom onset (mean 7 ± 18 h, min 3 h and max 48 h), and a unique venous blood sample was withdrawn within 12 h after ICU admission (mean 24 ± 13.9 h). Fifty-nine patients were excluded due to either a delay of more than 48 h after the onset of symptoms ($n = 55$) or missing clinical information ($n = 3$). A total of 199 consecutive patients were evaluated, and 141 were finally enrolled in this study.

Samples were sent from Paris to Geneva in two distinct sets of samples with a 14-month period delay. As samples were analyzed immediately in Geneva, results between the two sets displayed a 14-month period delay explaining why the two sets were considered separately. The selection set had 28 patients (8 men and 20 women; age range 26–84 years) and the verification set 113 patients (42 men and 71 women; age range 18–81 years). Fifty patients (35.4% of the study sample) had an unfavorable outcome at 6 months (GOS score 1–3), and 91 (65%) patients had a favorable outcome (GOS score 4–5). The two sets are described in Table 1.

The local ethical committee (Comité de Protection des Personnes, Pitié-Salpêtrière, Paris, France) approved the study. In accordance with the Helsinki Declaration, written informed consent was obtained from the patient or patient's relatives.

Clinical monitoring and treatment

At admission, clinical severity was assessed using the WFNS score [16]. The initial CT was reviewed by an independent radiologist blinded to clinical history and classified according to the original Fisher score [17] modified as follows: grade 1, no subarachnoid blood; grade 2, broad diffusion of subarachnoid blood; grade 3, with clots or thick layers of blood; grade 4, intraventricular hemorrhage or intracerebral hematoma, no clot; grade 5, intraventricular hemorrhage or intracerebral hematoma with clot [18–20] and qualified presence or absence of acute hydrocephalus. The neurological outcome was assessed by phone interviews using the Glasgow Outcome Scale (GOS) [21] at 6 months. The type of treatment (surgery or coiling) was decided according to both location and size of the aneurysm by the neurosurgeon and the neuro-radiologist. Seizures were routinely prevented by gabapentin (600 mg t.i.d., per os). A central venous line and an arterial catheter were inserted in most of the patients before and/or after surgery or coiling. An external ventricular drain (Sophysa, Orsay, France) was inserted in patients with CT evidence of hydrocephalus, high WFNS grade or a trans-cranial Doppler (TCD) pulsatility index greater than 1.2, suggesting intracranial pressure (ICP) elevation. The line was

Table 1 Main characteristics of the population

	28-Patient set			113-Patient set		
	GOS 1–2–3 ($N = 9$)	GOS 4–5 ($N = 19$)	P^a	GOS 1–2–3 ($N = 41$)	GOS 4–5 ($N = 72$)	P^a
Gender			1			0.07
♂ *n%*	3 (33.3)	5 (26.3)		20 (48.8)	22(30.6)	
♀ *n%*	6 (66.4)	14 (73.7)		21(51.2)	50(69.4)	
Age (years)			0.86			0.043
Median (range)	56 (49–75)	57 (26–84)		55.0 (31–81)	49.5 (18–76)	
Mean (±SD)	56.9 (±7.4)	53.5 (±14.1)		54.9 (±13.3)	48.9 (±13.8)	
Time of blood drawing (h)			0.74			0.73
Median (range)	24 (6–24)	22.5 (11–48)		24 (10–48)	24 (5–48)	
Mean (±SD)	20.4 (±6.3)	22.9 (±11.8)		21.8 (±10.5)	20.9 (±9.9)	
WFNS score			0.026			<0.0001
1–2 *n%*	4 (44.4)	18 (94.8)		14 (34.1)	57 (79.2)	
3–4–5 *n%*	5 (55.6)	1 (5.2)		27 (67.5)	15 (20.8)	
Modified Fisher score			0.14			<0.0001
1–2 *n%*	0 (0.0)	5 (26.3)		0 (0.0)	19 (26.4)	
3–4–5 *n%*	9 (100.0)	14 (73.8)		41 (100.0)	53 (73.6)	
Vasospasm			0.08			0.48
No *n%*	6 (66.7)	18 (94.8)		27 (65.9)	54 (75.0)	
Yes *n%*	3 (33.3)	1 (5.2)		14 (34.1)	19 (25.0)	
Location			0.23			0.32
MCA *n%*	3 (33.3)	1 (5.2)		12 (29.3)	11 (15.3)	
CA *n%*	3 (33.3)	10 (52.7)		19 (46.3)	36 (50.0)	
ICA/PCA *n%*	3 (33.3)	7 (36.9)		10 (24.4)	23 (31.9)	
VBS *n%*	0	1 (5.2)		0 (0.0)	2 (2.8)	
Treatment			0.12			0.29
No *n%*	1 (11.1)	0		2 (4.9)	2 (2.8)	
Coiling *n%*	6 (66.7)	18 (94.8)		29 (70.7)	60 (83.3)	
Surgery *n%*	2 (22.2)	1 (5.2)		10 (24.4)	10 (13.9)	
Seizures			1			0.89
No *n%*	6 (66.7)	13 (68.4)		33 (80.5)	58 (80.0)	
Yes *n%*	3 (33.3)	6 (31.6)		8 (19.5)	14 (19.4)	

Age non-parametric Mann–Whitney *U* test

MCA middle cerebral artery, *CA* cerebral anterior artery, *ICA* internal carotid artery, *PCA* posterior communicating artery, *VBS* vertebro basilar system

[a] Fisher exact test

connected to an external pressure strain gauge to monitor ICP. Early ICP elevation was defined as ICP above 20 mmHg under sedation but without drainage. Monitoring and treatment of vasospasm are described in Online Data Supplement 1.

H-FABP, S100β, NDKA, UFD1 and troponin I measurements

Cardiac troponin I serum concentration was systematically measured using the Stratus Analyzer (Dade, Massy, France). S100β concentration was measured with an immunoluminometric sandwich assay on a LIA-mat 300 analyzer (Byk-Sangtec France Laboratories, Le Mée sur Seine, France) using the manufacturer's reagents [22]. H-FABP concentration was determined with a commercially available enzyme-linked immunosorbent assay (ELISA) (Hycult Biotechnology, Uden, The Netherlands) according to the manufacturer's instructions. The concentrations of NDKA and UFD1 were determined by home-made ELISA as previously described by Allard et al. [11, 12]. For more details, see the Online Data Supplement 2.

Data analysis and statistics

SPSS software (version 15, SPSS Inc., Chicago, IL), R (URL http://www.R-project.org) and PERL (ActivePerl version 5.8.8.820, ActiveState Software Inc.) were used for data analysis.

Because protein concentrations did not show normal distributions (Kolmogorov–Smirnov test), between-group differences were tested with the non-parametric Mann–Whitney *U* test. The Fisher exact test was used for categorical variables. Statistical significance was set at 0.05 (two-tailed tests).

The dichotomized 6-month GOS score was considered as the main outcome variable, with ranges 1–2–3 and 4–5 reflecting unfavorable and favorable outcome, respectively.

The different markers (H-FABP, S100β, troponin I, NDKA, UFD-1) as well as clinical data were considered as possible predictors.

For each individual predictor, a receiver-operating characteristic (ROC) curve was determined in each cohort, and a cutoff value was selected as the threshold predicting poor outcome with specificity >90%. Partial ROC AUCs (pAUC) [23, 24] and 95% confidence intervals (CI) were calculated using an adaptation of previously described algorithms [25]. pAUCs were restricted between 90 and 100% specificity considering that an efficient predictor in clinical practice should be able to identify clearly at least nine out of ten patients as having a favorable prognosis when the test was negative. *P* values for the difference between two pAUCs were computed based on [26] where standard deviation was determined by bootstrap as described above.

Univariate and multivariate logistic regressions with stepwise backward selection were performed using SPSS software and are described in Online Data Supplements 3 and 4, respectively.

Panel development

Panel selection was performed essentially as described by Reynolds et al. [27, 28]. Briefly, the optimized cutoff values were obtained by iterative permutation-response calculations using all available parameters. Each cutoff value was changed iteratively by quantiles of 2% increment, and sensitivity was determined after each iteration until a maximum of sensitivity was achieved for 100% specificity. Binary clinical parameters (hydrocephaly, vasospasm, sex and statin treatment) were recorded as 0/1 (absent/present), and a unique cutoff of 0.5 was used.

Results

Patients with favorable and unfavorable outcomes did not significantly differ with respect to gender. Age of patients with poor outcome at 6 months was slightly higher in the 113-patient set, suggesting that age might be considered as a prognostic factor. WNFS score was significantly higher in patients with a poor outcome than favorable outcome (Fisher's exact test, $P = 0.026$ and <0.0001 in the 28- and 113-patient sets, respectively). A modified Fisher score, estimating severity of aSAH, did not significantly differ according to outcome in the 28-patient set, whereas in the 113-patient set, severe aSAH (high modified Fisher score) was significantly associated with unfavorable outcome (Fisher's exact test, $P < 0.0001$). No associations were found between long-term neurological outcome and time course of blood samples drawings, post-hemorrhagic seizures, location of the aneurysm, occurrence of vasospasm and treatment modality (coiling vs. surgery). Demographic characteristics are shown in Table 1.

As shown in Table 2, baseline H-FABP, S100β and troponin I levels were significantly elevated in the blood of patients with an unfavorable outcome compared to patients with a favorable outcome. Initial NDKA and UFD-1 levels were unable to discriminate between favorable and unfavorable outcome in the 28-patient set, but in the 113-patient set, the NDKA level was marginally higher in patients with a poor 6-month outcome

Table 2 H-FABP, S100β, troponin I, NDKA and UFD-1 concentrations (µg/l) at admission according to the patient outcome at 6 months in the 28- and 113-patient sets

	28-Patient set			113-Patient set		
	GOS 1–2–3 ($N = 9$)	GOS 4–5 ($N = 19$)	*P*	GOS 1–2–3 ($N = 41$)	GOS 4–5 ($N = 72$)	*P*
H-FABP (µg/l)						
Median (range)	4.65 (1.73–62.2)	1.79 (0.86–9.03)	0.01	3.59 (0.63–67.36)	1.35 (0–44.43)	<0.0001
Mean (±SD)	12.06 (±19.16)	2.82 (±2.13)		10.33 (±16.30)	3.50 (±7.33)	
S100β (µg/l)						
Median (range)	0.33 (0.17–0.46)	0.15 (0.06–0.32)	0.04	0.30 (0.03–2.07)	0.11 (0.04–0.5)	<0.0001
Mean (±SD)	0.32 (±0.14)	0.163 (±0.84)		0.39 (±0.37)	0.16 (±0.13)	
Troponin I (µg/l)						
Median (range)	0.50 (0.04–6.4)	0.05 (0.04–2.62)	0.04	0.36 (0.03–155)	0.05 (0.03–4.4)	<0.0001
Mean (±SD)	1.92 (±2.54)	1.15 (±3.18)		5.51 (±24.2)	0.32 (±0.77)	
NDKA (µg/l)						
Median (range)	13.74 (0–46.39)	13.98 (2.31–32.81)	0.92	13.56 (3.9–419.2)	10.95 (3.0–80.3)	0.05
Mean (±SD)	15.6 (±13.76)	15.9 (±10.45)		28.08 (±64.2)	14.86 (±12.8)	
UFD-1 (µg/l)						
Median (range)	71.0 (1.83–24.55)	12.6 (0.39–33.8)	0.33	83.73 (3.61–1792)	84.48 (10.4–553.2)	0.99
Mean (±SD)	11.23 (±8.14)	15.06 (±10.21)		169.3 (±291.6)	108.3 (±87.9)	

P = Non-parametric Mann–Whitney U test. $P < 0.05$ is considered significant

($P = 0.073$, Mann–Whitney U test). No significant difference was observed in the molecule concentrations as a function of time of blood drawing (data not shown).

The prediction performances of individual molecules, neurological scales and age for predicting a poor outcome were evaluated with ROC curves and pAUC (Fig. 1). Thresholds of individual predictors were chosen to provide specificity above 90% except for WFNS and modified Fisher where the cutoff value was fixed to separate patients according to their clinical pattern. With a threshold strictly above 2, WFNS allowed to discriminate patients with poor and favorable outcome with 55.6% sensitivity (SE) and 97.4% specificity (SP) in the 28-patient set and 67.5% SE for 79.2% SP in the 113-patient set. The modified Fisher scale (threshold >2) provided perfect 100% sensitivity in the two sample sets but low specificity (26.4 vs. 29.0%).

In the 28-patient set, H-FABP, S100β and troponin I displayed 44.4, 33.0 and 22.2% SE for 94.7, 94.0 and 94.7% SP, respectively. Similar performances were obtained in the 113-patient set. NDKA, UFD1 and age led to relatively poor prediction of outcome at 6 months in the two sets (Tables 3, 4). Univariate and multivariate logistic regressions with stepwise backward selection were used to

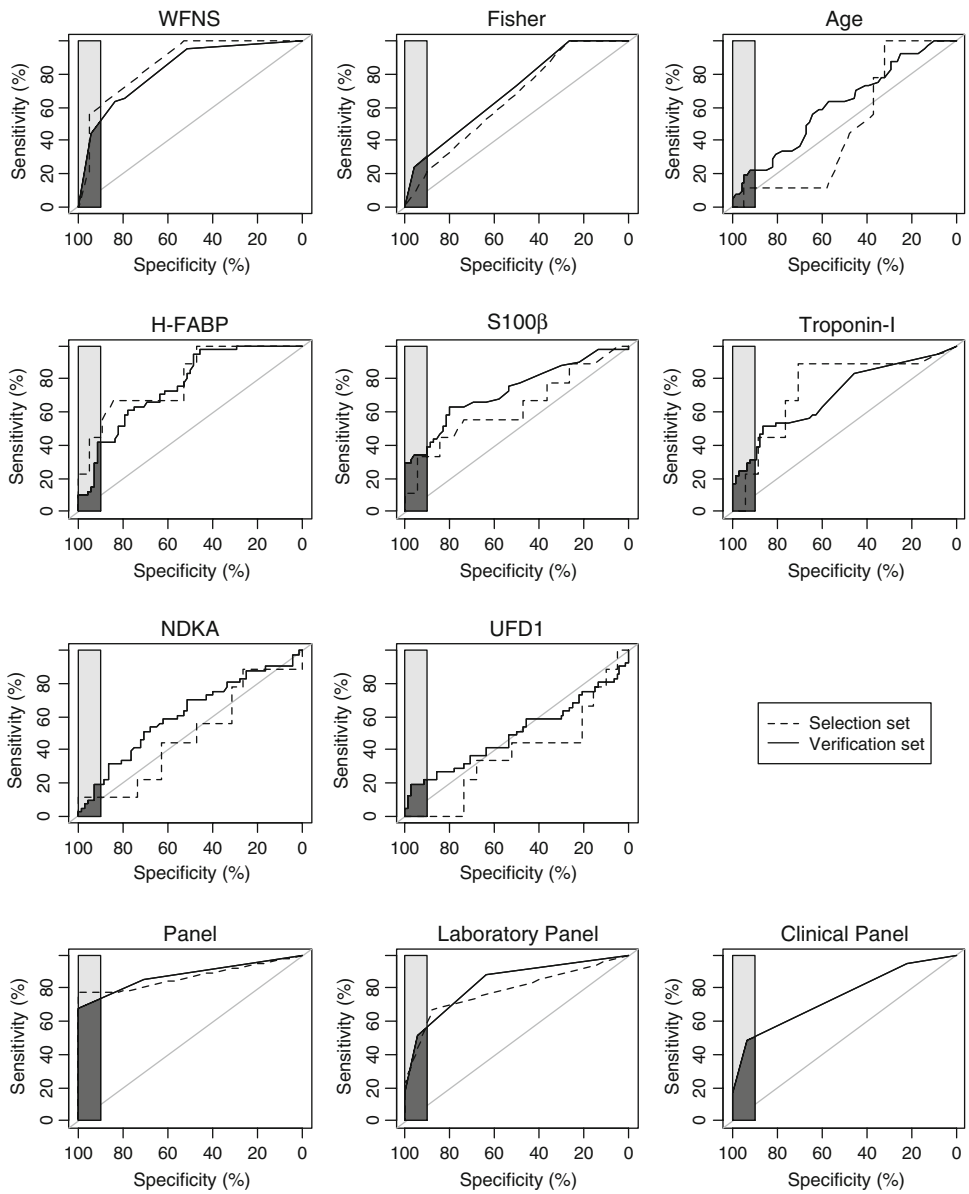

Fig. 1 pAUC of the different parameters on the selection ($N = 28$) and verification ($N = 113$) sets. *Grey boxes* correspond to the maximal area (10%) between 90 and 100% SP if a perfect ROC curve was obtained. *Dark boxes* correspond to the partial area under the curve

Table 3 Partial area under the curve (pAUC), sensitivities (SE) and specificities (SP) for individual parameters and the panel on the 28-patient set

28-Patient set				
	Partial AUC (95% CI)	Threshold	SE (%) (95% CI)	SP (%) (95% CI)
WFNS	3.0% (0.0–8.2)	>2[a]	55.6 (20.0–88.9)	94.1 (81.3–100)
Modified Fisher	1.7% (0.0–3.8)	>2[a]	100 (100–100)	29.4 (8.3–52.9)
Age	1.1% (0.0–3.6)	72.5 years old	11.1 (0.0–33.3)	100 (100–100)
H-FABP	5.1% (1.7–8.8)	6.3 μg/l	44.4 (12.5–80.0)	100 (100–100)
S100β	2.0% (0.0–6.7)	0.37 μg/l	33.3 (0.0–66.7)	94.1 (80.0–100)
Troponin I	0.9% (0.0–6.7)	5.3 μg/l	22.2 (0.0–50.0)	94.1 (80.0–100)
NDKA	1.1% (0.0–3.8)	31.9 μg/l	11.1 (0–37.5)	100 (100–100)
UFD-1	0.0% (0.0–1.4)	24.87 μg/l	0 (0–0)	76.5 (53.8–94.4)

[a] A threshlod strictly above two for the neurological scores means that patients have been dichotomized into two groups: patients with WFNS 1–2 and patients with WFNS 3–5 or patients with modified Fisher 1–2 and patients with modified Fisher 3–5

Table 4 Partial area under the curve (pAUC), sensitivities (SE) and specificities (SP) for individual parameters and the panel on the two sets

113-Patient set				
	Partial AUC (95% CI)	Threshold	SE (%) (95% CI)	SP (%) (95% CI)
WFNS	3.3% (1.7–5.4)	>2[a]	65.9 (51.1–79.2)	79.1 (69.6–88.1)
Modified Fisher	2.1% (0.9–3.6)	>2[a]	100 (100–100)	26.4 (16.7–36.8)
Age	1.5% (0.6–2.6)	67.5 years old	20.4 (9.5–32.6)	92.0 (86.0–97.4)
H-FABP	1.9% (0.5–4.0)	5.9 μg/l	41.4 (26.5–56.8)	91.7 (84.7–97.3)
S100β	3.3% (1.9–4.9)	0.48 μg/l	31.7 (17.8–46.5)	97.2 (92.9–100)
Troponin I	2.5% (1.2–4.3)	1.56 μg/l	29.3 (15.6–43.9)	93.1 (86.8–98.6)
NDKA	1.0% (0.2–2.5)	30.4 μg/l	19.5 (8.3–32.4)	93.1 (86.5–98.6)
UFD-1	1.7% (0.6–3.0)	271.5 μg/l	19.5 (7.9–32.5)	97.2 (93.0– 100)

[a] A threshlod strictly above two for the neurological scores means that patients have been dichotomized in two groups: patients with WFNS 1–2 and patients with WFNS 3–5 or patients with modified Fisher 1–2 and patients with modified Fisher 3–5

validate predictors of poor outcome. Results are presented in Online Data Supplements 3 and 4, respectively.

Provided the low sensitivity obtained with individual predictors, we tested combinations of all parameters on the 28-patient set to select a panel that could improve outcome prediction. The iterative permutation-response approach led to a six-parameter panel including WFNS, H-FABP, S100β, troponin I, NDKA and UFD-1. The panel result was defined as positive if at least three out of the six selected parameters were simultaneously above threshold with 77% (95% CI: 50.0–100.0%) SE for 100% (95% CI: 100.0–100.0) SP. This panel tested on the 113-patient set presented extremely similar performances with 68.3% (95% CI: 53.5–82.2) SE for 100% (95% CI: 100.0–100.0) SP. The panel was confirmed by a ten-fold cross-validation (data not shown). The six-parameter panel allowed to increase sensitivity by about 25% when compared to the best single predictor (H-FABP, SE: 45%). In addition, pAUC of the panel was significantly higher than pAUC of WFNS ($P < 0.0002$). The relative performance of each marker was also evaluated by removing them one by one from the panel and recalculating sensitivity and specificity. The results obtained are shown in Online Data Supplement 5.

Importantly including both clinical and laboratory variables into the same panel was found to be superior to approaches combining either purely clinical or merely laboratory variables. Indeed, a clinical panel including WFNS, modified Fisher scale and age displayed only 22% SE for 100% SP to predict poor outcome in the 113-patient set. The presence of hydrocephaly and occurrence of vasospasm in the clinical panel did not improve the discriminating performance of the clinical panel. In line with this, a panel containing only the five laboratory variables reached only 50% SE for 100% SP (Fig. 1). pAUC of our panel was significantly higher than pAUC of both purely clinical and laboratory panels ($P = 0.0004$ and 0.05, respectively.)

Discussion

In this prospective study, including 141 patients from two separated cohorts of patients presenting aSAH, we have

demonstrated for the first time that elevated serum concentration of H-FABP at hospital admission was able to predict unfavorable clinical prognosis at 6 months. More importantly, the development of a multiparameter panel strategy, using a combination of blood-borne biomarkers together with a clinical score (WFNS), considerably improved unfavorable outcome prediction compared to solely clinical parameters, alone or in combination, by allowing identification of poor neurological outcome in patients with a sensitivity around 70% and a specificity of 100% following aSAH.

Identification of predictors is an important aspect of the management and study of patients with aSAH. Several clinical factors have been identified as independent predictors of patient outcome following aSAH [3, 29, 30]. Among them, clinical scores describing the patient's neurological condition at hospital admission were reported to correlate with long-term outcome [31, 32]. In line with these observations, we also showed that, when tested individually, the WFNS score at hospital admission appeared to be the strongest predictor of neurological outcome in our patient sample. In agreement with previous studies [17, 33], we found a significant correlation between the amount of blood observed in the initial CT scan and long-term neurological outcome. Although the majority of earlier studies designed age as a major independent prognostic factor [34, 35], a recent, prospectively conducted trial including 177 poor WFNS grade patients with aSAH did not find a significant association between age and outcome [36]. In our cohort, the age of patients presenting a poor GOS at 6 months was slightly but significantly higher than those with a favorable course, suggesting a potential influence of age as a prognostic factor. In contrast, we found no significant association between occurrence of vasospasm and seizure activity during hospital stay and long-term GOS outcome. Also, neither the aneurysm location site nor the treatment modality (i.e., clipping versus coiling) showed significant association with patient prognosis.

To our knowledge, this is the first study investigating the role of the recently identified, brain-related biomarkers H-FABP, NDKA and UFD-1 in the context of aSAH. These molecules have recently been shown to be reliable early blood biomarkers in ischemic stroke. H-FABP is a well-known marker for myocardial injury [37, 38] and also appears to be a potential biomarker of stroke [10, 39]. Results of the present study revealed that H-FABP was one of the best outcome predictors at 6 months (42.5% SE and 92% SP), and its performance was as high as WFNS. In addition, H-FABP was an important parameter of the panel, since its absence induces a decrease of the sensitivity from 70 to 47%.

NDKA (also called NM23-H1) is an ubiquitous enzyme that catalyzes the transfer of the terminal phosphate of ATP to (deoxy)nucleotide triphosphates via the formation of a high-energy phosphorylated intermediate. Specific expression pattern and enzymatic activity of this protein have been demonstrated in the brain [40]. In stroke, NDKA was described as an early biomarker since its level was already elevated in blood of patients within 3 h after the stroke onset [11]. In the present aSAH study, NDKA alone appeared to be an unsatisfactory predictor of outcome at 6 months. However, in combination with other parameters, its presence drastically increased the sensitivity of the panel, suggesting that its strength resides in the detection of patients not included by other predictors.

Several studies highlighted an increasing interest in S100β, a calcium-binding protein, in various brain damage disorders, and especially in aSAH [6, 41]. In these studies, elevated levels of S100β correlated with neurological deficit and outcome at 6 months or 1 year [18–20]. Our present results are in line with these observations, showing a 35% SE and 96% SP of this protein in the prediction of neurological outcome at 6 months.

The commonly used cardiac biomarker troponin I, also known as cardiac isoform of troponin I (cTnI), has previously been reported to be correlated with neurological outcome following aSAH [42]. In fact, cardiopulmonary dysfunctions could occur after aSAH, but their impacts in the mortality rate or outcome remain controversial [43, 44]. In our study, troponin I permitted to discriminate patients according to their outcome with 30% SE and 93% SP, and, in combination with other markers, it increased the sensitivity of the panel from 42 to 70%.

Outcome prediction based on a single measured biomarker or clinical score has led so far to unsatisfactory levels of sensitivity and, more importantly, specificity. Therefore, there is an urgent need to combine multiple parameters to achieve higher sensitivity without sacrificing specificity. Many studies have evaluated a multitude of classification approaches to improve the prediction performance [45]. In the present study, we used a multiparametric combination of blood-borne protein values and clinical scores. The iterative permutation-response highlighted that a six-parameter panel comprising WFNS, H-FABP, S100β, troponin I, NDKA and UFD-1 could be used for the prediction of aSAH outcome at 6 months. The six-parameter panel provided increasing prognosis sensitivity (70%) for 100% SP compared with any other parameter individually or purely clinical and laboratory panels (22% and 50% SE, respectively), when at least three out of the six predictors are above their cutoff values.

The future challenge for these biomarkers and panel is their translation in clinical practice. Several drawbacks must be solved to consider their real prospective impact in the management of SAH patients. Among them, the development of multiplex point-of-care systems should considerably reduce the time of analyses (between 15 and 30 min), making possible their use in routine clinical practice. Alternatively, new emerging ELISA technologies, such as bead suspension arrays, can also quantitate simultaneously several biomarkers in a unique patient

sample, restricting the volume need for analyses, and this in a fast and reproducible manner. Finally, the panel interpretation (binary response: positive or negative) is simple enough to be used in clinical practice.

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References

1. Mocco J, Ransom ER, Komotar RJ, Schmidt JM, Sciacca RR, Mayer SA, Connolly ES Jr (2006) Preoperative prediction of long-term outcome in poor-grade aneurysmal subarachnoid hemorrhage. Neurosurgery 59:529–538
2. Cesarini KG, Hardemark HG, Persson L (1999) Improved survival after aneurysmal subarachnoid hemorrhage: review of case management during a 12-year period. J Neurosurg 90:664–672
3. Saveland H, Brandt L (1994) Which are the major determinants for outcome in aneurysmal subarachnoid hemorrhage? A prospective total management study from a strictly unselected series. Acta Neurol Scand 90:245–250
4. Nylen K, Csajbok LZ, Ost M, Rashid A, Karlsson JE, Blennow K, Nellgard B, Rosengren L (2006) CSF-neurofilament correlates with outcome after aneurysmal subarachnoid hemorrhage. Neurosci Lett 404:132–136
5. Nylen K, Csajbok LZ, Ost M, Rashid A, Blennow K, Nellgard B, Rosengren L (2007) Serum glial fibrillary acidic protein is related to focal brain injury and outcome after aneurysmal subarachnoid hemorrhage. Stroke 38:1489–1494
6. Stranjalis G, Korfias S, Psachoulia C, Kouyialis A, Sakas DE, Mendelow AD (2007) The prognostic value of serum S-100B protein in spontaneous subarachnoid haemorrhage. Acta Neurochir (Wien) 149:231–237, discussion 237–238
7. Oertel M, Schumacher U, McArthur DL, Kastner S, Boker DK (2006) S-100B and NSE: markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. J Clin Neurosci 13:834–840
8. Lescuyer P, Allard L, Zimmermann-Ivol CG, Burgess JA, Hughes-Frutiger S, Burkhard PR, Sanchez JC, Hochstrasser DF (2004) Identification of post-mortem cerebrospinal fluid proteins as potential biomarkers of ischemia and neurodegeneration. Proteomics 4:2234–2241
9. Burgess JA, Lescuyer P, Hainard A, Burkhard PR, Turck N, Michel P, Rossier JS, Reymond F, Hochstrasser DF, Sanchez JC (2006) Identification of brain cell death associated proteins in human post-mortem cerebrospinal fluid. J Proteome Res 5:1674–1681
10. Zimmermann-Ivol CG, Burkhard PR, Le Floch-Rohr J, Allard L, Hochstrasser DF, Sanchez JC (2004) Fatty acid binding protein as a serum marker for the early diagnosis of stroke: a pilot study. Mol Cell Proteomics 3:66–72
11. Allard L, Burkhard PR, Lescuyer P, Burgess JA, Walter N, Hochstrasser DF, Sanchez JC (2005) PARK7 and nucleoside diphosphate kinase A as plasma markers for the early diagnosis of stroke. Clin Chem 51:2043–2051
12. Allard L, Turck N, Burkhard PR, Walter N, Rosell A, Gex-Fabry M, Hochstrasser DF, Montaner J, Sanchez JC (2007) UFD1 as a blood marker for the early diagnosis of ischemic stroke. Biomarker Insights 2:155–164
13. Turck N, Vutskits L, Sanchez-Pena P, Robin X, Hainard A, Gex-Fabry M, Fouda C, Mueller M, Puybasset L, Sanchez JC (2009) A multiparameter panel method for outcome prediction following aneurysmal subarachoid hemorrhage. In: 5th International conference on biochemical markers for brain damage, 11–14 May, Lund, Sweden
14. Turck N, Vutskits L, Sanchez-Pena P, Robin X, Hainard A, Gex-Fabry M, Fouda C, Mueller M, Puybasset L, Sanchez JC (2008) From the proteomics to the clinical study: identification of a multiparameter panel for outcome prediction following aneurysmal subarachnoid hemorrhage. In: 8th Siena meeting from genome to proteome: integration and plateform completion: August 31st–September 4th, Siena, Italy
15. Turck N, Vutskits L, Sanchez-Pena P, Robin X, Hainard A, Gex-Fabry M, Fouda C, Mueller M, Puybasset L, Sanchez JC (2008) A multiparameter panel method for outcome prediction following aneurysmal subarachoid hemorrhage. In: XVII European stroke conference, 13–16 May, Nice, France
16. Drake C (1988) Report of World Federation of Neurological Surgeons Committee on a universal subarachnoid hemorrhage grading scale. J Neurosurg 68:985–986
17. Fisher CM, Kistler JP, Davis JM (1980) Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 6:1–9
18. Weiss N, Sanchez-Pena P, Roche S, Beaudeux JL, Colonne C, Coriat P, Puybasset L (2006) Prognosis value of plasma S100B protein levels after subarachnoid aneurysmal hemorrhage. Anesthesiology 104:658–666
19. Pereira AR, Sanchez-Pena P, Biondi A, Sourour N, Boch AL, Colonne C, Lejean L, Abdennour L, Puybasset L (2007) Predictors of 1-year outcome after coiling for poor-grade subarachnoid aneurysmal hemorrhage. Neurocrit Care 7:18–26
20. Sanchez-Pena P, Pereira AR, Sourour NA, Biondi A, Lejean L, Colonne C, Boch AL, Al Hawari M, Abdennour L, Puybasset L (2008) S100B as an additional prognostic marker in subarachnoid aneurysmal hemorrhage. Crit Care Med 36:2267–2273
21. Jennett B, Bond M (1975) Assessment of outcome after severe brain damage. Lancet 1:480–484
22. Beaudeux JL, Leger P, Dequen L, Gandjbakhch I, Coriat P, Foglietti MJ (2000) Influence of hemolysis on the measurement of S-100beta protein and neuron-specific enolase plasma concentrations during coronary artery bypass grafting. Clin Chem 46:989–990
23. McClish DK (1989) Analyzing a portion of the ROC curve. Med Decis Making 9:190–195
24. Fawcett T (2006) An introduction to ROC analysis. Pattern Recogn Lett 27:861–874
25. Carpenter J, Bithell J (2000) Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. Stat Med 19:1141–1164

26. Hanley JA, McNeil BJ (1983) A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 148:839–843
27. Reynolds MA, Kirchick HJ, Dahlen JR, Anderberg JM, McPherson PH, Nakamura KK, Laskowitz DT, Valkirs GE, Buechler KF (2003) Early biomarkers of stroke. Clin Chem 49:1733–1739
28. Hainard A, Tiberti N, Robin X, Lejon V, Ngoyi DM, Matovu E, Enyaru JC, Fouda C, Ndung'u JM, Lisacek F, Muller M, Turck N, Sanchez JC (2009) A Combined CXCL10, CXCL8 and H-FABP panel for the staging of human African Trypanosomiasis patients. PLoS Negl Trop Dis 3:e459
29. Lagares A, Gomez PA, Lobato RD, Alen JF, Alday R, Campollo J (2001) Prognostic factors on hospital admission after spontaneous subarachnoid haemorrhage. Acta Neurochir (Wien) 143:665–672
30. Yoshikai S, Nagata S, Ohara S, Yuhi F, Sakata S, Matsuno H (1996) A retrospective analysis of the outcomes of patients with aneurysmal subarachnoid hemorrhages: a focus on the prognostic factors. No Shinkei Geka 24:733–738
31. Gerber CJ, Lang DA, Neil-Dwyer G, Smith PW (1993) A simple scoring system for accurate prediction of outcome within four days of a subarachnoid haemorrhage. Acta Neurochir (Wien) 122:11–22
32. Hirai S, Ono J, Yamaura A (1996) Clinical grading and outcome after early surgery in aneurysmal subarachnoid hemorrhage. Neurosurgery 39:441–446 discussion 446–447
33. Brouwers PJ, Dippel DW, Vermeulen M, Lindsay KW, Hasan D, van Gijn J (1993) Amount of blood on computed tomography as an independent predictor after aneurysm rupture. Stroke 24:809–814
34. Deruty R, Pelissou-Guyotat I, Mottolese C, Amat D, Bognar L (1995) Level of consciousness and age as prognostic factors in aneurysmal SAH. Acta Neurochir (Wien) 132:1–8
35. Lagares A, Gomez PA, Alen JF, Lobato RD, Rivas JJ, Alday R, Campollo J, de la Camara AG (2005) A comparison of different grading scales for predicting outcome after subarachnoid haemorrhage. Acta Neurochir (Wien) 147:5–16 discussion 16
36. Laidlaw JD, Siu KH (2003) Poor-grade aneurysmal subarachnoid hemorrhage: outcome after treatment with urgent surgery. Neurosurgery 53:1275–1280 discussion 1280–1282
37. Suzuki M, Hori S, Noma S, Kobayashi K (2005) Prognostic value of a qualitative test for heart-type fatty acid-binding protein in patients with acute coronary syndrome. Int Heart J 46:601–606
38. Tanaka T, Sohmiya K, Kitaura Y, Takeshita H, Morita H, Ohkaru Y, Asayama K, Kimura H (2006) Clinical evaluation of point-of-care-testing of heart-type fatty acid-binding protein (H-FABP) for the diagnosis of acute myocardial infarction. J Immunoassay Immunochem 27:225–238
39. Wunderlich MT, Hanhoff T, Goertler M, Spener F, Glatz JF, Wallesch CW, Pelsers MM (2005) Release of brain-type and heart-type fatty acid-binding proteins in serum after acute ischaemic stroke. J Neurol 252:718–724
40. Dabernat S, Larou M, Masse K, Hokfelt T, Mayer G, Daniel JY, Landry M (1999) Cloning of a second nm23–M1 cDNA: expression in the central nervous system of adult mouse and comparison with nm23–M2 mRNA distribution. Brain Res Mol Brain Res 63:351–365
41. Vos PE, van Gils M, Beems T, Zimmerman C, Verbeek MM (2006) Increased GFAP and S100beta but not NSE serum levels after subarachnoid haemorrhage are associated with clinical severity. Eur J Neurol 13:632–638
42. Yarlagadda S, Rajendran P, Miss JC, Banki NM, Kopelnik A, Wu AH, Ko N, Gelb AW, Lawton MT, Smith WS, Young WL, Zaroff JG (2006) Cardiovascular predictors of in-patient mortality after subarachnoid hemorrhage. Neurocrit Care 5:102–107
43. Schuiling WJ, Dennesen PJ, Tans JT, Kingma LM, Algra A, Rinkel GJ (2005) Troponin I in predicting cardiac or pulmonary complications and outcome in subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry 76:1565–1569
44. Ramappa P, Thatai D, Coplin W, Gellman S, Carhuapoma JR, Quah R, Atkinson B, Marsh JD (2008) Cardiac troponin-I: a predictor of prognosis in subarachnoid hemorrhage. Neurocrit Care 8:398–403
45. Germanson TP, Lanzino G, Kongable GL, Torner JC, Kassell NF (1998) Risk classification after aneurysmal subarachnoid hemorrhage. Surg Neurol 49:155–163