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Role of Endothelin-1 in Human Aneurysmal Subarachnoid Hemorrhage: Associations with Vasospasm and Delayed Cerebral Ischemia

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Abstract

Background—Endothelin-1 (ET-1) is a potent vasoconstrictor implicated in the pathogenesis of vasospasm and delayed cerebral ischemia (DCI) in aneurysmal subarachnoid hemorrhage (aSAH) patients. The aim of this study was to investigate the relationship between cerebrospinal fluid (CSF) ET-1 levels and angiographic vasospasm and DCI.

Methods—Patients with aSAH were consented ($n = 106$). Cerebral vasospasm was determined by angiography. DCI was determined by transcranial Doppler (TCD) results and/or angiogram results with corresponding clinical deterioration. CSF ET-1 levels over 14 days after the initial insult was quantified by ELISA. ET-1 analysis included a group-based trajectory analysis and ET-1 exposure rate during 24, 48, and 72 h prior to, as well as 72 h post angiography, or clinical deterioration.

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Results—Trajectory analysis revealed two distinct groups of subjects with 56% of patients in the low ET-1 trajectory group (mean at day 1 = 0.31 pg/ml; SE = 0.04; mean at day 14 = 0.41 pg/ml; SE = 0.15) and 44% of patients in the high ET-1 trajectory group (mean at day 1 = 0.65 pg/ml; SE = 0.08; mean at day 14 = 0.61 pg/ml; SE = 0.06). Furthermore, we observed that ET-1 exposure rate 72 h before angiography and clinical spasm was a significant predictor of both angiographic vasospasm and DCI, whereas, ET-1 exposure after angiography and clinical spasm was not associated with either angiographic vasospasm or DCI.

Conclusion—Based on these results we conclude that ET-1 concentrations are elevated in a subgroup of patients and that the acute (72 h prior to angiography and clinical neurological deterioration), but not chronic, elevations in CSF ET-1 concentrations are indicative of the pathogenic alterations of vasospasm and DCI in aSAH patients.

Keywords

aSAH; CSF; Endothelin-1; Vasospasm; DCI

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating neurological insult affecting approximately 30,000 people per year with a 25% mortality rate. Angiographic vasospasm or arterial vasospasm indicating vascular constriction is present in approximately 70% of patients. Roughly, 20–30% of aSAH patients develop DCI. It is a common sequela of aSAH and is a significant contributor to overall morbidity and mortality. Survivors of the initial insult suffer long-term functional and neuropsychological impairment due to DCI that significantly affects their ability to resume previously held roles [1–3]. Although advances have been made in understanding the pathology of aSAH, there has been limited success in identifying strategies for prevention of DCI or improving neurological outcomes. This is due, in large part, to the paucity of studies evaluating molecular mechanisms in human pathogenesis and the lack of predictive factors involved in DCI. Absent in knowledge of causative mechanisms, it remains difficult to develop interventions to optimally manage this patient population.

Preclinical and clinical studies have implicated metabolites that regulate cerebrovasculature as important mediators of reduced blood flow and subsequent development of vasospasm after aSAH. ET-1 is one such vasoactive substance that has been implicated in the development of vasospasm; elevated levels of ET-1 in cerebrospinal fluid (CSF) have been suggested as causal factors in the pathogenesis of cerebral vasospasm in several clinical studies [4–7]. However, evidence for the association between ET-1 and vasospasm is inconsistent [8–11], in part, due to study design differences in evaluating the temporal profile of ET-1 relative to vasospasm diagnosis. Based on these conflicting reports, the potential relationship between the temporal profile of ET-1 concentrations and pathologic alterations in vascular response remains unclear.

A full understanding of the utility of ET-1 in patients with aSAH remains elusive due to clinical difficulty in measuring ET-1 at the peak interval for clinical vasospasm or over a longer time interval, inadequate power to detect changes of interest due to small sample size, and inconsistencies in the operationalization of DCI by clinical and angiographic evidence. Based on these issues, we evaluated the temporal relationship between CSF ET-1 concentrations and evidence of angiographic vasospasm and DCI in a large cohort of aSAH patients. Specifically, the purpose of this study was to: (1) examine changes in CSF ET-1 levels during the first 14 days following aSAH, (2) determine whether CSF ET-1 levels over the first 14 days following aSAH as well as ET-1 levels during the 24, 48, and 72 h prior to

angiography or clinical neurological deterioration were associated with vasospasm and DCI. In this study, we observed that ET-1 CSF exposure 72 h prior to angiography and clinical neurological deterioration is predictive of both angiographic vasospasm and DCI whereas, total ET-1 CSF exposure was not associated with either vasospasm or DCI. These results suggest that the temporal profile of ET-1 CSF concentrations is essential in determining the relationship between ET-1 and vascular alterations after aSAH.

Methods

Patient Sample

Data were obtained from an ongoing NIH trial. Participants were recruited from a neurovascular intensive care unit. Eligibility criteria were: (1) 18–75 years of age, (2) diagnosed with an aSAH via cerebral angiogram and head computed tomography; (3) Fisher grade ≥ 2 and/or Hunt and Hess grade ≥ 3 ; (4) no history of neurological disorders; and (5) ventriculostomy in place. Exclusion criteria were traumatic SAH, mycotic aneurysm, or arteriovenous malformation. The protocol was approved by the Institutional Review Board; informed consent was obtained from the patient/proxy prior to data collection.

Data Collection

A total of 1 cc of CSF was withdrawn from the ventriculostomy port closest to the patient each morning (8 am $\pm$ 1 h) and evening (8 pm $\pm$ 1 h) until (1) the ventriculostomy was removed, (2) the patient expired/was discharged, or (3) 14 days elapsed from the initial hemorrhage. CSF samples were also collected from patients who received lumbar drains. Specimens were stored at -80° for batch analysis. Socio-demographic and clinical data were collected via chart review.

The majority of patients were Caucasian females with a Hunt and Hess score of 3–5 and a Fisher score of 3–4 (Table 1). Patients (28%) who had Hunt and Hess scores 1 and 2 had ventriculostomy in place because of the presence of hydrocephalus, subsequent neurological deterioration, or mental status change. Mean duration of ventriculostomy placement was 7 ± 3.4 days; 11.7 ± 6.5 CSF samples were obtained per patient. All 106 participants had CSF available for ET-1 analysis over time, 79 patients had CSF drains via an external ventricular catheter (EVD), 13 had lumbar drains (LD), and 14 had initial EVD followed by LD. Angiographic data were available on 71 participants (67%) and DCI data were available on 85 patients (80%).

Measures

Endothelin-1 Levels—ET-1 levels were quantified using QuantiGlo® Human Endothelin-1 Immunoassay kit (R & D Systems, Minneapolis, MN) with a modified standard curve range yielding interday and intraday reproducibility ($<15\%$ CV) with a limit of quantitation of 0.195 pg/ml. ET-1 chemiluminescence detection was performed using a Wallace Victor2 1420 MultiLabel Counter (PerkinElmer Wallace, Gaithersburg, MD). Each sample was analyzed separately. A repeated measures model test showed ET-1 levels did not differ significantly between two samples per day, so the mean value was used. The ET-1 exposure rate was determined at 24, 48, and 72 h prior to angiography or clinical neurological deterioration as follows:

$$\text{Exposure rate (pg/h)} = \frac{\text{Concentration of sample (pg/ml)} \times \text{CSF drainage (ml)}}{\text{Time period (h)}}$$

Delayed Cerebral Ischemia

Angiography was performed as standard care on admission, as post-operative follow-up care, and/or in the event of neurological deterioration. All patients received standard medical care including nimodipine and triple H therapy, and early surgical or endovascular intervention. Angiographic vasospasm was determined from cerebral angiograms read and coded by neurosurgeons blinded to participant identity and patients were grouped as either “negative” (0–24% narrowing of cerebral blood vessels) or “positive” ($\geq 25\%$ narrowing of cerebral blood vessels). To determine DCI, the following variables were examined: angiographic results when present, daily TCD ultrasound results (mean systolic velocities >200 and/or Lindegaard ratios >3) accompanied by clinical deterioration as recorded by the nurse (decrease of 2 or more from the previous assessment in the National Institutes of Health Stroke Scale (NIHSS)) [12, 13]. Patients were grouped as: (1) DCI positive—elevated vessel velocities on TCD ultrasound and/or angiographic vasospasm with corresponding clinical deterioration, or (2) DCI negative—the absence of neurologic deterioration or neurologic deterioration with no changes in cerebral vessel narrowing.

Data Analysis

Gender, age, race, Hunt and Hess and Fisher grade were obtained from medical records and education information from the participant/proxy. Due to the clinical nature of the disease and standards of care following aSAH (e.g., placement of a ventriculostomy and use of cerebral angiography), different subgroups of the same sample were used for each analysis, allowing the most parsimonious way to present the data. To examine homogeneous latent trajectory classes in CSF ET-1 levels during the first 14 days following aSAH ($n = 106$), group-based trajectory analysis was performed with the PROC TRAJ macro in SAS version 9.1 [14, 15]. This procedure identifies distinct subgroups within the population, conducts an approximation of the general distribution of the data, and groups similarly behaving patterns together. Trajectory groups are a convenient statistical device for summarizing trajectories. Log transformation which remained consistent with the distribution of the raw data was applied to reduce sample variation and skewness for better model fitting. The Bayesian Information Criterion (BIC) and the substantive utility of the classes (e.g., distinctiveness of the trajectories, proportion assigned to a given class) were used to determine the optimal solution for the number of trajectory groups. Chi-square analyses were completed to determine the overall association between ET-1 levels over time identified by the trajectory analysis and angiographic vasospasm or DCI. Binary logistic regression models were created to examine the association between angiographic vasospasm and ET-1 exposure rates 24, 48, and 72 h prior to detection of angiographic vasospasm and DCI after controlling for sex, age, race, Hunt and Hess grade, and Fisher grade. Independent t-tests were completed after log transformation of the data to determine the association between angiographic vasospasm and ET-1 exposure rate 72 h prior to spasm and after spasm in spasm negative and positive groups. Similar analysis was performed for ET-1 exposure rates in DCI positive and negative patients 72 h prior and after DCI.

Results

CSF ET-1 Levels During the First 14 Days Following aSAH

CSF ET-1 concentrations over the first 14 days after initial insult (Fig. 1a, $n = 106$) differentiated the population into high and low groups as determined by a trajectory analysis (Fig. 1b). Participants in Group 1 ($n = 59$, 56%) had relatively low ET-1 values (mean at day 1 = 0.31 pg/ml; SE = 0.04; mean at day 14 = 0.41 pg/ml; SE = 0.15); Group 2 ($n = 47$, 44%) had higher ET-1 values (mean at day 1 = 0.65 pg/ml; SE = 0.08; mean at day 14 = 0.61 pg/ml; SE = 0.06). Within the high and low ET-1 groups, there was no significant change from day 1 to day 14. A two-group trajectory model was defined (Fig. 1b), suggesting two groups

of participants with significantly ($P < 0.01$) different ET-1 values. To explore potential reasons for this finding, chi-square analyses and t tests were used to evaluate potential between-group differences. No significant between-group differences were found related to socio-demographic or clinical characteristics including method of treatment of original aneurysm and CSF drainage methods (Table 2).

Cerebral Vasospasm

To determine whether CSF ET-1 levels were associated with vasospasm, two indicators were utilized: angiographic vasospasm and DCI. First, chi-square analyses were completed to determine the overall association between ET-1 levels over time identified by the trajectory analysis (high versus low ET-1 levels) and angiographic spasm and DCI. No significant differences were found (Table 2). Next, binary logistic regression models were used in a subset of patients to determine the association between positive angiographic vasospasm and ET-1 exposure rates at 24, 48, and 72 h prior to angiographic detection and positive DCI and ET-1 exposure rates at 72 h after positive angiographic spasm and DCI detection. The subset was chosen based on the availability of CSF collection time data and other covariates. The exposure rate is the actual concentration of ET-1 after controlling for CSF drainage. The rate accounts for the variability in the amount of CSF drainage between patients and provides the concentration of the sample per hour so that a relationship can be drawn with vasospasm. The average CSF output did not significantly differ between spasm positive and negative, DCI positive and negative, and ET-1 high and low patient groups (Table 3). This conclusion is based on independent t test results. After controlling for age and Fisher grade, there were significant relationships between angiographic vasospasm and ET-1 exposure rates during the 48 and 72 h prior to angiography ($P = 0.05$ and 0.03), and between DCI and exposure rates 72 h prior to DCI ($P = 0.05$) with odds ratios ranging from 1.23 to 2.95 (Table 4). The average exposure rate for the 72 h before angiography was significantly higher in vasospasm positive patients ($n = 16$) compared to vasospasm negative ($n = 24$) patients ($P = 0.03$). The average ET-1 exposure rate for the 72 h before angiography was 4.35 ± 3.02 pg/h for the vasospasm negative group and 7.94 ± 6.47 pg/h for the vasospasm positive group (Fig. 2a). However, the average exposure rate for the 72 h after angiography did not significantly differ between vasospasm positive ($n = 16$) and negative groups ($n = 19$). Similarly, the average exposure rate for the 72 h before DCI was significantly greater in DCI positive patients ($n = 26$) compared to DCI negative ($n = 11$) groups ($P = 0.02$). The average ET-1 exposure rate for the 72 h before DCI was 4.40 ± 4.07 pg/h for the DCI negative group and 7.12 ± 5.43 pg/h for the DCI positive group (Fig. 2b). There was no significant association between DCI and ET-1 exposure rate 72 h after DCI.

Discussion

aSAH is a complex, multifaceted disease whose pathogenesis progresses over days to weeks. Angiographically evidenced vasospasm, a definable event in the acute course, develops in 70% of the patients between post-bleed days 3 and 14 with a maximum peak at 4–7 days [16]. This window provides an excellent opportunity for protracted CSF sampling for biomarker analysis.

The current study demonstrated that high CSF ET-1 concentrations are associated with vasospasm and DCI. We have observed the following two significant findings. First, our population was clearly dichotomized into high and low groups with respect to CSF ET-1 concentrations over time. This difference was not influenced by gender, race, aneurysm side or locale, method of treatment for original aneurysm, Hunt and Hess or Fisher grade, age, or education level, which suggests that there may be genetic or other predictors of CSF ET-1 concentrations after aSAH. Second, samples obtained 72 h prior to angiographic vasospasm or change in clinical neurological status had higher ET-1 concentrations which suggest that

ET-1 levels are temporally related to vascular complications after aSAH. Collectively, these results suggest that ET-1 concentrations are elevated in the time period immediately preceding vascular complications.

Previous research has focused on the role of ET-1 as a pathological mediator of cerebral vasospasm. Clinical and animal studies have shown ET-1 to be a highly potent vasoconstrictive mediator involved in vascular reactivity after aSAH and a likely contributor to the development of vasospasm [17, 18]. Several clinical studies have demonstrated that CSF ET-1 levels are markedly elevated in patients with clinical manifestations of vasospasm compared to patients without vasospasm [6, 8, 18–20]. Specifically, the temporal profiles of ET-1 levels in CSF of aSAH patients have been shown to significantly increase 4–7 days after hemorrhage. In posttraumatic patients ($n = 10$) with aSAH, CSF ET-1 concentrations showed a significant elevation on day 2 and remained elevated until day 14 [21]. Our findings extend prior investigations by demonstrating the significant relationship between elevated ET-1 exposure rate and cerebral vasospasm in a larger number of patients than previously reported. Based on these findings, ET-1 is an indicator of vascular complications after aSAH.

In our study, we utilized trajectory analysis to determine changes in ET-1 concentrations over time by identifying subpopulations that follow specified patterns of change over that time frame. The analysis approaches a set of individual trajectories by grouping those which closely resembles one another thus simplifying longitudinal data. The identified subpopulations can subsequently be evaluated for their relationship with patient outcomes. This approach has been shown to be a more sensitive method of evaluating biomarker outcome relationships over time as compared to traditional point estimate methods. Recently, a similar approach has been successfully used in identifying trajectories or developmental course in clinical and social research [14, 15, 22–26].

The role of endothelins in spasm associated with SAH and impaired cerebral hemodynamics after traumatic brain injury is well supported by basic science and clinical observations. Selective antagonists of the two ET-1 receptors (ET_A and ET_B) have been used for the treatment of SAH-induced vasospasm in many animal models, and these studies have proven that vasospasm can be limited by blocking the detrimental effects of endogenous ET-1. Several human clinical studies and experimental animal model studies of SAH have shown that endothelin receptor antagonists reduce vasospasm [27–30]. Preclinical studies suggest that ET_A -selective agents might be more efficacious in treating angiographic vasospasm. Specifically, clazosentan, a nonpeptide ET_A receptor antagonist, significantly reduced moderate to severe angiographic vasospasm in a randomized phase-2 clinical trial in aSAH patients [31, 32]. A recent meta-analysis of randomized controlled trials assessing the use of ET_A antagonists in 867 SAH patients reported marked reduction in radiographic vasospasm without any improvement in neurological outcome [33]. Furthermore, ET_A receptor antagonists have been shown to reverse several functional impairments including artery constriction following traumatic brain injury [34–36].

Although ET-1 is implicated in the development of vasospasm and ET-1 antagonist is shown to be effective in the prevention of acute vasospasm, its precise role in DCI is still controversial. The efficacy against angiographic vasospasm did not translate into improved outcome in the clazosentan clinical trial. There was no significant effect on outcome based on the modified Rankin scale at 3 months. The possible explanations are that (1) the study is under-powered to detect relatively small improvements in outcome especially when assessed using relatively insensitive measures such as modified Rankin and Glasgow outcome scales (2) the adverse effects of treatment affected the beneficial effects of the drug. In addition, several studies indicate that the development of DCI cannot be fully attributed to the

occurrence of angiographic vasospasm and not all patients with DCI have angiographic vasospasm, and DCI is associated with poor outcome whereas angiographic vasospasm alone is not. Recent advances in elucidating the pathogenesis of DCI have indicated several alternative causes of neurological deterioration and poor outcome after aSAH including microcirculatory dysfunction, cortical spreading depression (CSD), and thromboembolism [37–39]. Apart from being a vasoconstrictor, ET-1 is a neuronal and astroglial modulator. It has been shown that ET-1 is a potent inducer of CSD and ischemia triggered by spreading neuronal activation is induced by ET-1 in rats. It is suggested that the mechanism that triggers spreading neuronal/astroglial depolarization waves is important in the pathogenesis of cortical spreading ischemia [40]. It is hypothesized that this mechanism may be through reduction in Na^+/K^+ -ATPase activity which accounts for half of cerebral energy consumption. Indeed, ET-1 has been shown to inhibit Na^+/K^+ -ATPase from cerebral cortex preparation in vitro [41]. In addition to its action through ET_A receptor, ET-1 may elicit deleterious effects through ET_B receptor which is the predominant subtype in neuronal/astroglial network. ET-1 has been reported to be released from astrocytes after hypoxia [11]. Therefore, it may be speculated that ET-1 released from neuronal and glial cells after early ischemic damage may later contribute to DCI. ET-1 may also cause microvessel spasm contributing to ischemia after aSAH. However, the question whether ET-1 is a causative factor in the pathogenesis of DCI or an indicator of ischemic cell damage remains an area for future investigation.

These results provide preliminary evidence that acute elevations in CSF ET-1 concentrations are associated with vasospasm and DCI. However, further studies are needed to fully elucidate the role of ET-1 in vasospasm and DCI. Although this is the largest study to evaluate ET-1 and vasospasm and DCI in aSAH patients reported to date, there are a few limitations that should be noted. Patients were enrolled from one center and outcomes may have reflected patient management specific to this setting. The study limited enrollment to patients with a ventriculostomy; more stable patients are less likely to have a ventriculostomy and may have responded differently.

Conclusion

Our results suggest that ET-1 is a pathogenic mediator of aSAH. In combination with results of prior studies demonstrating an association between acute elevations in CSF ET-1 and development of vasospasm and DCI, our findings suggest that therapy to inhibit ET-1 may be beneficial in preventing the acute development of vasospasm and DCI. Future studies are necessary to fully elucidate the effects of ET-1 in aSAH patients.

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Fig. 1.

Raw population values of CSF ET-1 concentrations (pg/ml) over the first 14 days from the time of insult from 106 aSAH patients (a). Raw data are denoted as samples from high (filled circle) and low (open circle) groups as identified by trajectory analysis (a). Log ET-1 concentrations versus time from insult (days) in high (filled circle) and low (filled diamond) groups as identified by trajectory analysis (b). Data are presented as mean log ET-1 values measured per day. The model shows two groups of patients with significantly different ($P < 0.001$) ET-1 values. Patients in low group ($n = 59$, 56%) have relatively low ET-1 values (mean at day 1 = 0.31 pg/ml; SE = 0.04; mean at day 14 = 0.41 pg/ml; SE = 0.15) and those in high group ($n = 47$, 44%) have higher ET-1 values (mean at day 1 = 0.65 pg/ml; SE = 0.08; mean at day 14 = 0.61 pg/ml; SE = 0.06)

Fig. 2.

The average ET-1 exposure rate 72 h prior and after angiography in angiographic cerebral vasospasm (CV) positive and negative patients (a) and the average ET-1 exposure rate 72 h prior and after DCI in DCI positive and negative patients (b). Independent t tests revealed significant differences ($P < 0.05$) in ET-1 exposure rates between CV positive and negative patients 72 h prior vasospasm and between DCI positive and negative patients 72 h prior DCI development after normalization of data by log transformation

Table 1Socio-demographic and clinical characteristics of total sample, $N = 106$

Characteristic	N	%
Gender		
Female	75	71
Male	31	29
Ethnicity		
Caucasian	92	87
Non-Caucasian	14	13
Hunt and Hess grade upon admission		
I–II	30	28
III–IV	76	72
Fisher grade upon admission		
II	26	25
III	51	48
IV	29	27
Vasospasm		
None or mild	31	29
Moderate or severe	25	24
No post-intervention angiogram performed	50	47

	Range	Mean (SD)
Age	24–75	53.9 (10.6)
Years of education	3–20	12.5 (2.6)
Number of days ventriculostomy intact/patient	1–14	7 (3.4)
Number of CSF samples available/patient	1–25	11.7 (6.5)

Table 2
Socio-demographic and clinical characteristics of patients with high and low CSF ET-1 levels

Characteristic	Group		P value
	Low N = 59 N (%)	High N = 47 N (%)	
Gender			0.59
	Male	16 (27%)	15 (32%)
	Female	43 (73%)	32 (68%)
Race			0.49
	Non-white	9 (15%)	5 (11%)
	White	50 (85%)	42 (89%)
Aneurysm side			0.40
	Left	10 (29%)	10 (40%)
	Right	24 (71%)	15 (60%)
Aneurysm locale			0.99
	Anterior	44 (75%)	35 (74%)
	Posterior	15 (25%)	12 (26%)
Intervention			0.11
	Clipping	31 (53%)	32 (68%)
	Coiling	28 (47%)	15 (32%)
Drainage device			0.95
	External ventricular (EVD)	43 (42%)	36 (34%)
	Lumbar (LD)	8 (7%)	5 (5%)
	EVD then LD	8 (7%)	6 (6%)
Hunt and Hess grade			0.32
	I-II	19 (32%)	11 (23%)
	III-V	40 (68%)	36 (77%)
Fisher grade			0.66
	2	16 (27%)	10 (21%)
	3	26 (44%)	25 (53%)
	4	17 (29%)	12 (26%)
Vasospasm			0.10
	Negative	19 (32%)	21 (45%)
	Positive	16 (27%)	15 (32%)
	Unavailable	24 (41%)	11 (23%)
DCI			0.21
	Negative	28 (48%)	15 (32%)
	Positive	22 (37%)	20 (43%)
	Unavailable	9 (15%)	12 (25%)

	Low			High			P value
	N	Mean (SD)	Range	N	Mean (SD)	Range	
Age	59	53 (11)	29–75	47	55 (10)	24–74	0.50
Years of education	34	12 (3)	3–20	23	13 (3)	3–16	0.88

Table 3

Average CSF output in ET-1, spasm, and DCI groups

Average exposure rate determination time	Group	N	Average CSF volume (ml)		P value
			Mean	SD	
48 h before angiographic spasm	ET-1	Low	154.46	74.89	0.12
		High	119.16	67.01	
	Spasm	No	124.97	72.79	0.25
		Yes	152.37	70.30	
72 h before angiographic spasm	ET-1	Low	146.53	76.21	0.33
		High	124.29	65.59	
	Spasm	No	123.85	69.53	0.23
		Yes	151.36	71.68	
72 h before clinical spasm	ET-1	Low	115.45	51.36	0.39
		High	128.82	49.67	
	DCI	No	107.40	49.68	0.30
		Yes	127.00	52.06	

Table 4

Results from binary logistic regression for spasm positive versus spasm negative groups

Average exposure rate determination time	N	Odds ratio (with 95% CI)	SE	P value
48 h before angiographic spasm	40	1.23 (1.00–1.51)	0.11	0.05
72 h before angiographic spasm	40	1.24 (1.03–1.51)	0.10	0.03
72 h after angiographic spasm	35	1.18 (0.91–1.53)	0.21	0.21
48 h before clinical spasm	38	1.12 (0.62–2.00)	0.30	0.72
72 h before clinical spasm	37	2.95 (1.00–8.80)	0.56	0.05
72 h after clinical spasm	36	1.08 (0.33–3.55)	0.61	0.90