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ORIGINAL ARTICLE

# Plasma SPARC Elevation in Delayed Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage

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**Summary**
Matricellular proteins have been implicated in pathologies after subarachnoid hemorrhage (SAH). To find a new therapeutic molecular target, the present study aimed to clarify the relationships between serially measured plasma levels of a matricellular protein, secreted protein acidic and rich in cysteine (SPARC), and delayed cerebral ischemia (DCI) in 117 consecutive aneurysmal SAH patients with admission World Federation of Neurological Surgeons (WFNS) grades I–III. DCI developed in 25 patients with higher incidences of past history of hypertension and dyslipidemia, preoperative WFNS grade III, modified Fisher grade 4, spinal drainage, and angiographic vasospasm. Plasma SPARC levels were increased after SAH, and significantly higher in patients with than without DCI at days 7–9, and in patients with VASOGRADE-Yellow compared with VASOGRADE-Green at days 1–3 and 7–9. However, there were no relationships between plasma SPARC levels and angiographic vasospasm. Receiver-operating characteristic curves differentiating DCI from no DCI determined the cut-off value of plasma SPARC $\geq$ 82.1 ng/ml at days 7 − 9 (sensitivity, 0.800; specificity, 0.533; and area under the curve, 0.708), which was found to be an independent determinant of DCI development in multivariate analyses. This is the first study to show that SPARC is upregulated in peripheral blood after SAH, and that SPARC may be involved in the development of DCI without angiographic vasospasm in a clinical setting.

**Keywords** Delayed cerebral ischemia · Early brain injury · Matricellular protein · SPARC · Subarachnoid hemorrhage · VASOGRADE

## Introduction

Aneurysmal subarachnoid hemorrhage (SAH) remains a life-threatening disease with a poor prognosis, and delayed cerebral ischemia (DCI) is an important factor for possible therapeutic intervention [1–3]. However, effective treatments for DCI are limited, and the early diagnosis and therapeutic targets are under intense research. Recently, matricellular proteins (MCPs) were reported to be associated with the development of DCI in experimental and clinical studies [4–8]. MCPs are nonstructural extracellular matrix proteins that have a fast turnover and are involved in cell–cell interactions [9].

Secreted protein acidic and rich in cysteine (SPARC) is one of MCPs, and was reported to be upregulated in the wall of intracranial aneurysms in autopsy specimens [10]. In SAH, an experimental study reported an increased expression of SPARC in association with early brain injury (EBI) in a rat SAH model [8]. However, no studies have investigated the relationship between SPARC and DCI after SAH. SPARC has been reported to induce inflammatory reactions, which may cause microcirculatory disturbances following EBI in SAH [11, 12]. As microcirculatory disturbance is an important cause of DCI [3, 12], the authors hypothesized that SPARC may be induced and involved in the development of DCI after aneurysmal SAH. Thus, this study was aimed to investigate if plasma SPARC levels are increased in association with the development of DCI after aneurysmal SAH. In this study, the authors analyzed only non-severe SAH patients with admission World Federation of

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Neurological Surgeons (WFNS) grades I–III [13], because DCI or a delayed change in neurological status is easily detectable in such patients.

## Methods

The Institutional Ethics Committee approved this study, and written informed consent was obtained from the relatives.

### Study Population

The present study retrospectively analyzed consecutive patients registered in the prospective registry for searching mediators of neurovascular events after aneurysmal SAH (pSEED) that was conducted in 9 primary stroke centers in Mie prefecture in Japan from September 2013 to December 2016 [4–7, 14]. The inclusion criteria of this study were as follows: ≥ 20 years of age at onset, modified Rankin scale (mRS) 0–2 before onset, SAH on computed tomography (CT) or lumber puncture, saccular aneurysm as the cause of SAH confirmed on CT angiography or digital subtraction angiography (DSA), aneurysmal obliteration by clipping or coil embolization within 48 h of onset, and admission WFNS grades I–III. The following cases were excluded: unknown etiology of SAH, SAH by causes such as ruptured fusiform, dissecting, traumatic, mycotic, or arteriovenous malformation-related aneurysms, aneurysmal treatment with parent artery occlusion, angiographic or treatment-related complications such as cerebral infarction or hemorrhage, serious pre-morbidities such as heart, respiratory, or renal failure that precluded protocolized anti-DCI treatment, inflammatory diseases that are known to upregulate SPARC [11, 15], and missing data of at least one outcome measure (angiographic vasospasm, DCI, delayed cerebral infarction, and/or 90-day mRS). Timing, treatment modality (clipping or coil embolization) of aneurysmal obliteration, and other medical management or treatment strategies depended on the on-site investigators. In the registry, as plasma samples were collected with minimal stasis from peripheral veins early in the morning at post-onset days 1 – 3, 4 – 6, 7 – 9, and 10 – 12 after aneurysmal obliteration and stored at – 78 °C in addition to clinical data, the present study used the stocked plasma samples to measure plasma SPARC levels, and investigated the relationships between plasma SPARC levels and clinical variables, especially DCI.

### Clinical Variables

Baseline demographic and clinical data included age, sex, pre-onset mRS, past medical history (hypertension, diabetes mellitus, dyslipidemia, and cerebral infarction), regular use of statin, smoking, alcohol, family history of SAH, admission and preoperative WFNS grades [13], modified Fisher grade [16], acute hydrocephalus, and ruptured aneurysm location. Acute hydrocephalus was documented when there was evidence of ventriculomegaly obtained by CT on admission, which was considered to cause neurological impairments such as disturbance of consciousness [6]. Treatment-related variables included treatment modality (clipping or coiling) used for aneurysmal obliteration, cerebrospinal fluid (CSF) drainage including ventricular, cisternal, and/or spinal drainage to manage hydrocephalus and/or to promote hematoma clearance, prophylactic medications for DCI (intravenous fasudil hydrochloride, oral cilostazol, eicosapentaenoic acid, and statin), treatments for angiographic vasospasm or DCI (intra-arterial fasudil hydrochloride, percutaneous transluminal angioplasty, and augmentation of triple-H [hypertension, hypervolemia, and hemodilution] therapy).

Outcome measures included angiographic vasospasm, delayed cerebral infarction, shunt-dependent hydrocephalus, and 90-day mRS, in addition to DCI. DCI was defined as otherwise unexplained clinical deterioration (i.e., focal neurological impairments, a decrease of at least 2 points on the Glasgow Coma Scale, or both), which continued for at least 1 h [17], and other potential causes of clinical deterioration, such as hydrocephalus, rebleeding, or seizures, were rigorously excluded. Angiographic vasospasm was defined as more than 50% constriction in the baseline vessel diameter of major cerebral arteries on CT angiography or DSA regardless of clinical symptoms. Delayed cerebral infarction was defined as a newly developed cerebral infarct on CT scans that was not recognized on CT scans on the day after the operation or intervention. Shunt-dependent hydrocephalus was diagnosed when there were no detectable causes of persistent conscious disturbance or neurological deterioration other than hydrocephalus that occurred after day 14 post-SAH and when ventricular size progressively increased and the Evans index became greater than 0.30, being treated by CSF shunting [4]. Determination of these events was made at each center, and the organizing committee qualified them.

### Measurement of Plasma SPARC

Collected blood samples were immediately centrifuged at 3000G for 5 min, and the supernatant fluid (plasma) was stored at – 78 °C until measurement. Experienced technicians unaware of the clinical information determined plasma levels of full-length SPARC at post-SAH days 1 – 3, 4 – 6, 7 – 9, and 10 – 12 using a commercially available enzyme-linked immunosorbent assay kit for human full-length SPARC (Code No. DSP00; R&D Systems, Minneapolis, USA). As a control, plasma samples were obtained from 10

patients (5 males and 5 females; mean age, 66.7 ± 8.9 years) with unruptured cerebral aneurysms who provided written informed consent prior to any invasive procedure, and plasma SPARC levels were measured as well.

VASOGRADE is a grading scale for prediction of DCI after SAH, and is classified into a low risk (*green*; admission WFNS grades I and II, and modified Fisher grades 1 and 2), an intermediate risk (*yellow*; admission WFNS grades I – III, and modified Fisher grades 3 and 4), and a high risk (*red*; admission WFNS grades IV and V, and any modified Fisher grade) [18]. The relationship between the VASOGRADE and plasma SPARC levels was also examined.

## Statistical Analysis

Variables were recorded as categorical or continuous variables. Categorical variables were reported as a percentage, and were compared between patients with and without DCI using chi-square or Fisher's exact tests, as appropriate. Continuous variables were reported as a mean ± standard deviation (SD) or standard error of the mean (SEM; for graphs), and compared between the two groups using unpaired *t* test, and among more than three groups using one-way analysis of variance (ANOVA) followed by Tukey–Kramer post hoc tests. Correlation among plasma SPARC levels and various factors was evaluated by Pearson's correlation coefficient for continuous variables or point biserial correlation coefficient for categorical variables, and $r > 0.4$ was considered significant. Cut-off values of plasma SPARC levels were obtained by receiver-operating characteristic (ROC) curve analyses using the Youden index and the point on the curve closest to the (0, 1) point for DCI development [19]. Multivariate logistic regression analyses with DCI as the dependent variable were performed by the stepwise forward selection method using any variable with $p$ value of $< 0.05$ on univariate analyses except for outcome measures, although only a variable with the smallest $p$ value was used as a candidate variable among similar clinical variables that were intercorrelated. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were calculated, and independence of variables was verified using the likelihood ratio test on reduced models. All statistical analyses were performed with SPSS 8 (IBM; Armonk, NY, USA). $p$ value $< 0.05$ was considered significant.

# Results

## Baseline and Treatment-Related Variables

Three hundred and five consecutive SAH patients were registered, and 117 patients were included in the present study (Fig. 1). Plasma SPARC levels were measured at all-time points in all patients, and were significantly higher in SAH patients (106.2 ± 88.9 ng/ml) compared with control patients (46.7 ± 20.7 ng/ml, $p < 0.001$). As data of regular use of statin (12 patients), smoking (11 patients), alcohol (14 patients), and family history of SAH (11 patients) were missing, the analyses were performed except for the missing data.

The mean age of 117 patients was 61.2 ± 13.4 years, and this study included 82 female patients (70.0%), 5 patients (4.3%) with pre-onset mRS 1 – 2, 9 patients (7.7%) with admission WFNS grade III, 30 patients (25.6%) with modified Fisher grade 4, and 27 patients (23.1%) with acute hydrocephalus. DCI

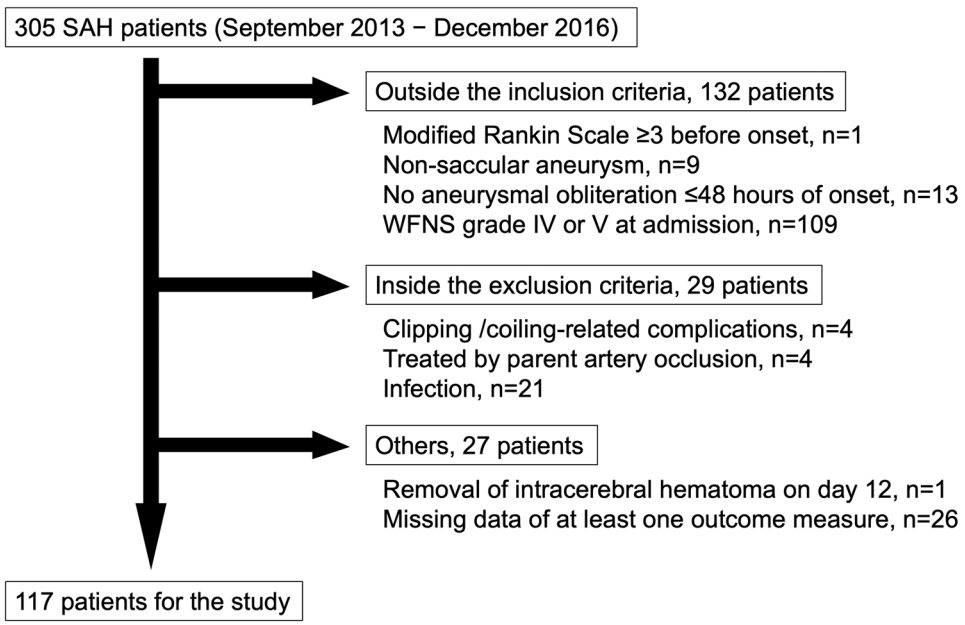


**Fig. 1** A flow chart indicating the included and excluded patients in this study. SAH = subarachnoid hemorrhage, WFNS = World Federation of Neurological Surgeons

occurred in 25 patients (21.4%), and 96 patients (82.0%) had 90-day mRS 0 – 2 with the mortality rate of 2.6% (3 patients).

As to baseline demographic and clinical variables, patients with DCI were associated with significantly higher incidences of past medical history of hypertension, dyslipidemia and regular use of statin, as well as preoperative WFNS grade III and modified Fisher grade 4, although other factors such as age, smoking, ruptured aneurysmal location, and acute hydrocephalus were not different between patients with and without DCI (Table 1). In treatment-related variables, treatment modality (clipping or coiling) and prophylactic medications for DCI were not different between patients with and without DCI (Table 2). However, patients with DCI were more frequently treated with spinal drainage, intra-arterial injections of fasudil hydrochloride, and triple-H therapy, and more associated with angiographic

**Table 1** Baseline clinical characteristics in patients with and without delayed cerebral ischemia (DCI)

| Variable | All ($n=117$) | DCI ($n=25$) | No DCI ($n=92$) | $p$ value |
|---|---|---|---|---|
| Age, mean ± SD (years) | 61.2 ± 13.4 | 65.4 ± 13.6 | 60.0 ± 13.2 | 0.076 |
| Sex, female | 82 (70.0%) | 20 (80.0%) | 62 (67.4%) | 0.222 |
| Pre-onset mRS 1 – 2 | 5 (4.3%) | 5 (20.0%) | 0 (0.0%) | 0.294 |
| Past history | | | | |
| SAH | 7 (6.0%) | 1 (4.0%) | 6 (6.5%) | 1.000 |
| Cerebral infarction | 3 (2.6%) | 0 (0.0%) | 3 (3.3%) | 1.000 |
| Hypertension | 57 (48.7%) | 17 (68.0%) | 40 (43.5%) | 0.030 |
| Dyslipidemia | 13 (11.1%) | 7 (28.0%) | 6 (6.5%) | 0.006 |
| Regular use of statin | 9 (7.7%) | 5 (20.0%) | 4 (4.3%) | 0.012 |
| Diabetes mellitus | 7 (6.0%) | 2 (8.0%) | 5 (5.4%) | 0.641 |
| Smoking | 30 (25.6%) | 7 (28.0%) | 23 (25.0%) | 0.797 |
| Alcohol | 22 (18.8%) | 2 (8.0%) | 20 (21.7%) | 0.148 |
| Family history of SAH | 17 (14.5%) | 2 (8.0%) | 15 (16.3%) | 0.515 |
| Admission WFNS grade | | | | |
| I | 56 (47.9%) | 10 (40.0%) | 46 (50.0%) | 0.375 |
| II | 52 (44.4%) | 12 (48.0%) | 40 (43.5%) | 0.678 |
| III | 9 (7.7%) | 3 (12.0%) | 6 (6.5%) | 0.400 |
| Preoperative WFNS grade | | | | |
| I | 47 (40.2%) | 7 (28.0%) | 40 (43.5%) | 0.162 |
| II | 51 (43.6%) | 11 (44.0%) | 40 (43.5%) | 0.963 |
| III | 13 (11.1%) | 6 (24.0%) | 7 (7.6%) | 0.022 |
| IV | 6 (5.1%) | 1 (4.0%) | 5 (5.4%) | 1.000 |
| Modified Fisher grade | | | | |
| 1 | 20 (17.1%) | 0 (0.0%) | 20 (21.7%) | 0.005 |
| 2 | 3 (2.6%) | 0 (0.0%) | 3 (3.3%) | 0.483 |
| 3 | 64 (54.7%) | 14 (56.0%) | 50 (54.3%) | 0.883 |
| 4 | 30 (25.6%) | 11 (44.0%) | 19 (20.7%) | 0.018 |
| Acute hydrocephalus | 27 (23.1%) | 9 (36.0%) | 18 (19.6%) | 0.084 |
| Ruptured aneurysm location | | | | |
| AcomA | 32 (27.3%) | 4 (16.0%) | 28 (30.4%) | 0.151 |
| ACA | 6 (5.1%) | 1 (4.0%) | 5 (5.4%) | 1.000 |
| ICA | 53 (45.3%) | 13 (52.0%) | 40 (43.5%) | 0.448 |
| MCA | 18 (15.3%) | 7 (28.0%) | 11 (12.0%) | 0.063 |
| Posterior circulation | 6 (5.1%) | 0 (0.0%) | 6 (6.5%) | 0.339 |
| Others | 2 (1.7%) | 0 (0.0%) | 2 (2.2%) | 1.000 |

Data are number of patients (% of total patients per group) unless otherwise specified. $p$ value, comparison between DCI and no DCI by chi-square or Fisher's exact test or unpaired $t$ test

*ACA* anterior cerebral artery, *AcomA* anterior communicating artery, *ICA* internal carotid artery, *MCA* middle cerebral artery, *mRS* modified Rankin Scale, *SAH* subarachnoid hemorrhage, *SD* standard deviation, *WFNS* World Federation of Neurological Surgeons

**Table 2** Treatment-related variables and outcome measures in patients with and without delayed cerebral ischemia (DCI)

| Variable | All ($n=117$) | DCI ($n=25$) | No DCI ($n=92$) | $p$ value |
|---|---|---|---|---|
| Treatment modality | | | | |
| Clipping | 91 (77.8%) | 22 (88.0%) | 69 (75.0%) | 0.166 |
| Coil embolization | 26 (22.2%) | 3 (12.0%) | 23 (25.0%) | |
| Cerebrospinal fluid drainage | 34 (29.1%) | 10 (40.0%) | 24 (26.1%) | 0.174 |
| Ventricular | 21 (18.0%) | 6 (24.0%) | 15 (16.3%) | 0.386 |
| Cisternal | 12 (10.3%) | 2 (8.0%) | 10 (10.9%) | 1.000 |
| Spinal | 13 (11.1%) | 6 (24.0%) | 7 (7.6%) | 0.032 |
| Prophylactic medication of DCI | | | | |
| i.v. fasudil hydrochloride | 115 (98.3%) | 24 (96.0%) | 91 (99.0%) | 0.383 |
| Cilostazol | 95 (81.2%) | 19 (76.0%) | 76 (82.6%) | 0.564 |
| Eicosapentaenoic acid | 62 (53.0%) | 10 (40.0%) | 52 (56.5%) | 0.142 |
| Statin | 28 (23.9%) | 7 (28.0%) | 21 (22.8%) | 0.591 |
| Treatment of vasospasm or DCI | 25 (21.4%) | 18 (72.0%) | 7 (7.6%) | <0.001 |
| i.a. fasudil hydrochloride | 9 (7.7%) | 8 (32.0%) | 1 (1.0%) | <0.001 |
| PTA | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | |
| Triple-H therapy | 16 (13.7%) | 10 (40.0%) | 6 (6.5%) | <0.001 |
| Angiographic vasospasm | 38 (32.5%) | 21 (84.0%) | 17 (18.5%) | <0.001 |
| Delayed cerebral infarction | 24 (20.5%) | 18 (72.0%) | 6 (6.5%) | <0.001 |
| Shunt-dependent hydrocephalus | 26 (22.2%) | 12 (48.0%) | 14 (15.2%) | <0.001 |
| 90-day modified Rankin Scale | | | | |
| 0 | 64 (54.7%) | 5 (20.0%) | 59 (64.1%) | |
| 1 | 18 (15.4%) | 1 (4.0%) | 17 (18.5%) | |
| 2 | 12 (10.3%) | 5 (20.0%) | 7 (7.6%) | |
| 3 | 8 (6.8%) | 3 (12.0%) | 5 (5.4%) | |
| 4 | 6 (5.1%) | 3 (12.0%) | 3 (3.3%) | |
| 5 | 6 (5.1%) | 5 (20.0%) | 1 (1.1%) | |
| 6 | 3 (2.6%) | 3 (12.0%) | 0 (0.0%) | |
| 0 – 2 | 96 (82.0%) | 12 (48.0%) | 84 (91.3%) | <0.001 |

Data are number of patients (% of total patients per group). $p$ value, comparison between DCI and no DCI by chi-square or Fisher's exact test

*i.a.* intra-arterial, *i.v.* intravenous, *PTA* percutaneous transluminal angioplasty, *triple-H* hypertension, hypervolemia, and hemodilution

vasospasm, delayed cerebral infarction, shunt-dependent hydrocephalus, and 90-day poor outcomes.

## Plasma SPARC Levels in Patients with DCI

Post-SAH plasma SPARC levels at days 1 – 3, 4 – 6, 7 – 9, and 10 – 12 were neither related to age ($r=-0.001$, 0.060, 0.080, and – 0.022, respectively; Pearson's correlation coefficient) nor sex ($p=0.579$, 0.912, 0.561, and 0.883, respectively; unpaired $t$ test). After SAH, plasma SPARC levels were elevated and significantly higher at days 4 – 6, 7 – 9, and 10 – 12 compared with those in control patients ($p<0.001$, respectively; Fig. 2a).

When compared between patients with and without DCI, plasma SPARC levels were significantly higher in patients with DCI at days 7 – 9 (Fig. 2b). ROC curve analyses of plasma SPARC levels to differentiate DCI from no DCI showed that the area under the curve (AUC) was the largest at days 7 – 9 (Fig. S1), and that the cut-off value of plasma SPARC was 82.1 ng/ml at days 7 – 9 (sensitivity, 0.800; specificity, 0.533; and AUC, 0.708) when the Youden index was used (Fig. 3). When the point on the ROC curve closest to the (0, 1) point was used, the cut-off value of plasma SPARC at days 7 – 9 was 115.7 ng/ml (sensitivity, 0.640; and specificity, 0.685; Fig. 3).

In contrast, plasma SPARC levels were not significantly related to the presence or absence of angiographic vasospasm and delayed cerebral infarction at any sampling point (Fig. S2).

## Plasma SPARC Levels and VASOGRADE

As the present study consisted of patients with admission WFNS grades I to III, they were classified into VASOGRADE-Green

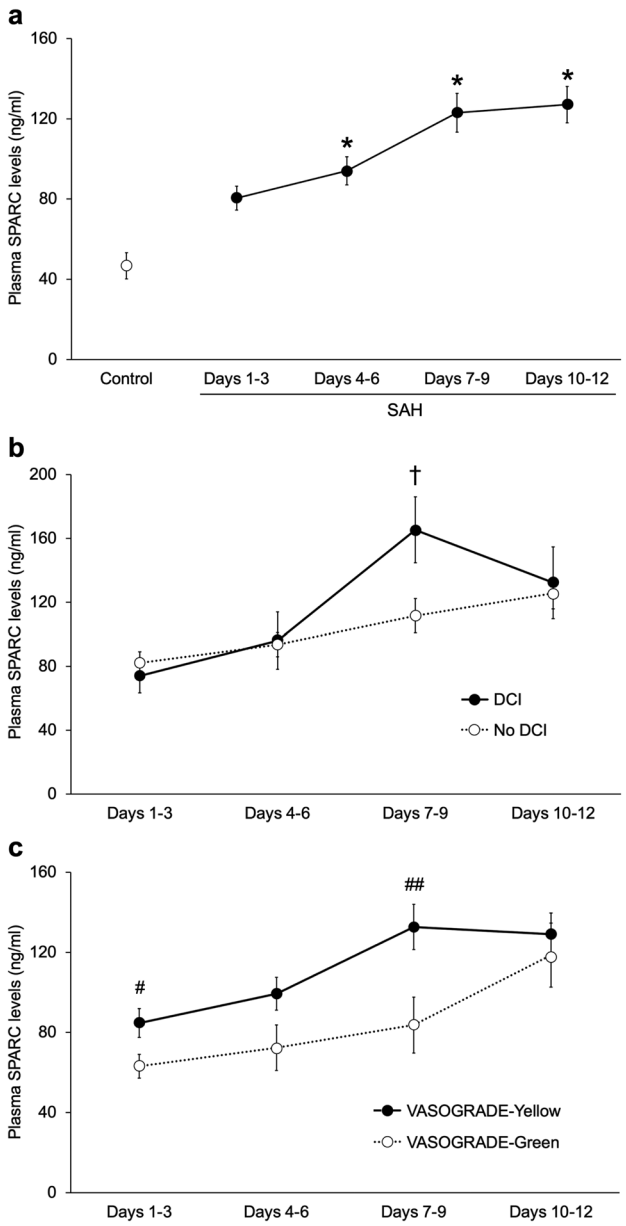


**Fig. 2** The relationships of plasma levels of secreted protein acidic and rich in cysteine (SPARC) between control patients ($n=10$) and patients with subarachnoid hemorrhage (SAH, n=117; **a**), between patients with ($n=25$) and without delayed cerebral ischemia (DCI) after SAH ($n=92$; **b**), and between patients with SAH of VASOGRADE-Yellow ($n=94$) and VASOGRADE-Green ($n=23$; **c**). Data are expressed as means ± standard error of the mean and compared using unpaired *t* test: $^{*}p<0.001$ versus control patient; $^{\dagger}p<0.05$ versus no DCI; and $^{\#}p<0.05$, and $^{\#\#}p<0.01$ versus VASOGRADE-Green

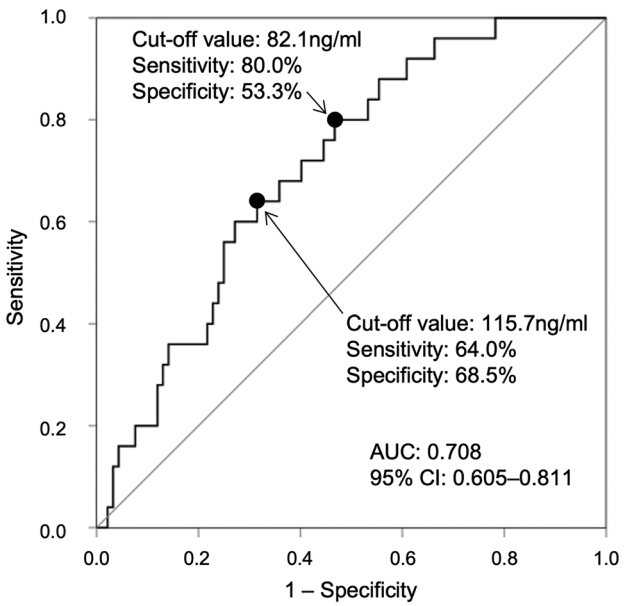


**Fig. 3** Receiver operating characteristic curve analyses of plasma secreted protein acidic and rich in cysteine (SPARC) levels at days 7 – 9 after subarachnoid hemorrhage to differentiate delayed cerebral ischemia (DCI) from no DCI. The cut-off value of plasma SPARC is 82.1 ng/ml when determined by the Youden index, and 115.7 ng/ml when determined by the point on the curve closest to the (0, 1) point. AUC, area under the curve; CI, confidence interval

(23 patients) or VASOGRADE-Yellow (94 patients). As expected, VASOGRADE-Yellow patients had a significantly higher frequency of DCI development ($p=0.002$) and 90-day mRS 3–6 ($p=0.005$). Plasma SPARC levels were significantly higher in patients with VASOGRADE-Yellow than in patients with VASOGRADE-Green at days 1 – 3 ($p=0.024$) and 7 – 9 ($p=0.008$) (Fig. 2c). However, plasma SPARC levels were not significantly different among admission WFNS grades and modified Fisher grades at any sampling point (Fig. S3).

## Independent Determinants for DCI Development

Although plasma SPARC levels at days 1 – 3 and 7 – 9 were potentially related to DCI development (Fig. 2b, c), plasma SPARC levels ≥ 82.1 ng/ml at days 7 – 9 were selected as a candidate factor for DCI development according to the ROC curve analyses using the Youden index (Figs. 3 and S1). Among significant variables on univariate analyses (Table 1), because $r=0.596$ of correlation was observed between dyslipidemia and regular use of statin, dyslipidemia with a smaller *p* value on univariate analyses was used for subsequent multivariate analyses. Treatments for vasospasm and DCI, as well as outcome measures (delayed cerebral infarction, shunt-dependent hydrocephalus, and 90-day mRS) were not used for multivariate analyses, because these variables appeared as a result of or after DCI development. Multivariate analyses revealed that dyslipidemia (aOR, 43.808; 95% CI, 3.706 – 517.843; $p=0.003$), preoperative WFNS grade III (aOR, 18.198; 95% CI, 1.590 – 208.223; $p=0.020$), angiographic vasospasm (aOR, 90.225; 95% CI, 9.986 – 815.208; $p<0.001$), and plasma

**Table 3** Multivariate logistic regression analyses with delayed cerebral ischemia (DCI) as the dependent variable using plasma secreted protein acidic and rich in cysteine (SPARC) levels at days 7 − 9 ≥ 82.1 ng/ml and significant variables related to DCI development on univariate analyses

| Variable | aOR | 95% CI | *p* value |
|---|---|---|---|
| Dyslipidemia | 43.808 | 3.706–517.843 | 0.003 |
| Preoperative WFNS grade III | 18.198 | 1.590–208.223 | 0.020 |
| Angiographic vasospasm | 90.225 | 9.986–815.208 | < 0.001 |
| Plasma SPARC levels at days 7 − 9 ≥ 82.1 ng/ml | 5.488 | 1.209–24.921 | 0.027 |
| Hypertension | | | 0.242 |
| Modified Fisher grade 4 | | | 0.616 |
| Spinal drainage | | | 0.465 |

*aOR* adjusted odds ratio, *CI* confidence interval, *WFNS* World Federation of Neurological Surgeons

SPARC levels at days 7 − 9 ≥ 82.1 ng/ml (aOR, 5.488; 95% CI, 1.209 − 24.921; $p = 0.027$) were independent factors for DCI development (Table 3).

## Discussion

The novel findings in the present study were as follows: (1) plasma SPARC levels were increased after SAH; (2) patients with DCI had significantly higher plasma SPARC levels at days 7 − 9 after SAH; (3) plasma SPARC levels at days 1 − 3 and 7 − 9 were significantly higher in patients with VASOGRADE-Yellow compared with VASOGRADE-Green; 4) plasma SPARC levels ≥ 82.1 ng/ml at days 7 − 9 were an independent factor for DCI development; and 5) plasma SPARC levels had no relationships with angiographic vasospasm. These findings suggest that SPARC is induced after SAH and may contribute to the development of DCI without angiographic vasospasm.

SPARC, also known as osteonectin or basement-membrane protein 40, is a 43 kDa calcium and collagen-binding glycoprotein and is a member of SPARC family [20–22]. Elevated plasma levels of SPARC have been reported in many types of malignancies, diabetes mellitus, and systemic sclerosis, possibly by reflecting changes in a microenvironment or micro-vasculopathy, including endothelial cell dysfunction, inflammatory processes and recruitment of leukocytes, and platelet activation and aggregation [23–26]. In the adult central nervus system, SPARC is expressed in astrocytes and microglia, and maintains extracellular matrix integrity, synaptic stability, and cortical lamination [20, 27, 28]. Li et al. reported that SPARC was upregulated in the wall (medial smooth muscle cells) of unruptured and ruptured intracranial aneurysms, but not in the cerebral arterial wall; and that the degree of SPARC expression was not related to the severity of SAH using human autopsy specimens, suggesting that SPARC upregulation may be involved in the formation of intracranial aneurysms [10]. Another autopsy study showed SPARC as one of highly expressed aneurysm-specific markers: SPARC was considered to express in vascular endothelium, smooth muscle, and fibroblasts, and to inhibit endothelial cell adhesion and proliferation and/or to regulate remodeling of extracellular matrix [29]. Jiang et al. also reported that SPARC may be useful in distinguishing normal patients from patients with unruptured intracranial aneurysms [30]. As to the relationships between SPARC and SAH pathologies, in contrast, little information is available. In an experimental SAH study in rats, SPARC was reported to cause blood–brain barrier (BBB) disruption in association with post-SAH EBI [8]. According to Li et al., SPARC expression was limited in cerebral arteries even after SAH [10], suggesting that SPARC may have no relationships with the development of angiographic vasospasm. Although no studies have investigated the relationships between SPARC and DCI or vasospasm in a clinical setting of aneurysmal SAH, the above findings are consistent with the findings in the present study that SPARC upregulation was not associated with angiographic vasospasm, but with DCI possibly by microcirculatory disturbances following EBI.

The role of SPARC in DCI development remains unclear, but it is well known that DCI occurs following EBI, and that inflammatory reactions may be important mediators between EBI and DCI [12, 31]. Among inflammatory mediators, experimental and clinical studies have repeatedly indicated that proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α are major contributors of post-SAH pathologies and prognostic factors of aneurysmal SAH [32, 33]. The proinflammatory cytokines may induce microvascular spasm, microthrombosis formation, and BBB disruption, resulting in microcirculatory disturbances to cause DCI [12]. SPARC is one of MCPs, which can act as inflammatory regulators, although its action in SAH is largely unknown [34]. However, in recent years, there have been increasing reports that some MCPs are involved in EBI or DCI after SAH: in experimental or clinical SAH, most of reported MCPs (tenascin-C, periostin, and galectin-3) have been considered to be harmful, sometimes by interacting with other MCPs and proinflammatory cytokines [4, 6, 7, 32, 35], while another MCP osteopontin has been neuroprotective [5, 14, 36]. At least, SPARC was reported to be neurotoxic in experimental ischemic stroke in mice [27] and EBI after SAH in rats [8]. Previous reports have shown that SPARC is involved in inflammation through a variety of mechanisms, and induces IL-1β, IL-6, and TNF-α [37, 38], which in turn may upregulate SPARC [39]. Thus, the authors consider that SPARC may be a causative mediator of DCI by microcirculatory disturbances, for which SPARC may interact with proinflammatory cytokines such as IL-1β, IL-6, and TNF-α as well as other MCPs, and induce microvascular pathologies such as microvascular spasm, microthrombosis and BBB disruption [8, 12, 26, 37–39].

Another interesting finding in the present study was the relationship between plasma SPARC levels and VASOGRADE. Poor WFNS grades at admission and high modified Fisher grades were both predictors of DCI development after SAH [40]. Moreover, WFNS grades I to III at admission and modified Fisher grades 1 to 2 were independent factors of the absence of DCI [41]. Thus, VASOGRADE was proposed to evaluate these combinations, and was validated as a prognostic scale [18]. On the other hand, the initial clinical severity (poor WFNS grades) and increased amount of SAH (high modified Fisher grades) have been reported as surrogate markers of EBI [35, 42]. Therefore, it can be said that VASOGRADE not only predicts the development of DCI but also reflects the severity of EBI. The present study confirmed that VASOGRADE is a good predictor of DCI development. In addition, plasma SPARC levels were significantly higher in patients with VASOGRADE-Yellow than those with VASOGRADE-Green at days 1 – 3 and 7 – 9, suggesting that the higher plasma SPARC levels may be related to the development of EBI and DCI, respectively.

In this study, the numbers of DCI and non-DCI patients were very unbalanced, although this was inevitable given that the frequency of DCI is 15 – 20% in SAH patients with admission WFNS grades I–III [18]. However, the unbalance may have led to the low specificity of the cutoff value determined by the Youden index (82.1 ng/ml at days 7 – 9; sensitivity, 0.800; and specificity, 0.533). When the cutoff value was determined by the point on the ROC curve closest to the (0, 1) point, the specificity was improved with decreased sensitivity (115.7 ng/ml at days 7 – 9; sensitivity, 0.640; and specificity, 0.685). As early detection and early treatment are important for countermeasures against DCI, the former cutoff value is considered effective for screening of DCI, and the latter one may be effective for the diagnosis of DCI and the assessment of therapeutic effects against DCI. However, the timing of DCI onset varies somewhat among individual patients, so the timing of measurement of SPARC values will be an issue for clinical application. On the other hand, since this study showed that SPARC may be one of causative factors for DCI, it may lead to the development of a new therapeutic method using SPARC as a molecular target through basic research.

The limitations in the present study include a relatively small number of patients, exclusion of patients with factors that potentially affect plasma SPARC levels, and no inclusion of poor-grade patients. In addition, although this study examined plasma SPARC measurements in the early morning and there is no known circadian variation in plasma SPARC levels, it is necessary to investigate whether blood sampling time affects plasma SPARC levels. It is also unknown where SPARC is produced and how SPARC influences DCI. Measurement of SPARC concentrations in cerebrospinal fluid (CSF) may be informative, but it is invasive to test in all SAH patients, and it has the disadvantage of being biased to measure them only in SAH patients who require CSF drainage for acute hydrocephalus. However, this study first showed that plasma SPARC level was an independent determinant of the development of DCI without angiographic vasospasm, and that SPARC may be a novel therapeutic target to prevent DCI. Although racial differences in plasma SPARC levels are beyond the scope of this study, only Japanese patients with SAH were examined in this study. As baseline plasma SPARC levels and the incidence of post-SAH DCI are similar between the Japanese and non-Japanese Asians or Westerners [24, 26], it seems likely that the findings in this study are not restricted to only Japanese patients. Further experimental and clinical studies are awaited to clarify the functions of SPARC after SAH.

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**Data Availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of Interest** Dr. H. Suzuki reported personal fees from Eisai and Kowa, and a research fund from Japan Blood Products Organization outside the submitted work. The other authors declare that they have no conflict of interest.

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