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Title Page

Title: Hydroperoxide in internal jugular venous blood reflects occurrence of subarachnoid hemorrhage-induced delayed cerebral vasospasm

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Abstract

Background: To investigate the association between subarachnoid hemorrhage-induced delayed cerebral vasospasm (DCVS) and oxidative stress, an oxidation product, hydroperoxide, was measured in 3 specimens: peripheral arterial blood, cerebrospinal fluid (CSF), and internal jugular venous blood (IJVB).

Methods: Hydroperoxide was measured using the Diacron-Reactive Oxygen Metabolites test (d-ROMs test). The hydroperoxide levels were evaluated based on the rate of change in the d-ROMs test value on Day 6 relative to that on Day 3 (d-ROMs change rate).

Results: The subjects were 20 patients. The d-ROMs change rate in IJVB was significantly higher in patients with DCVS on Day 6 than in those without it ($p<0.01$). When the patients were classified into the following 3

groups: Group A (no DCVS occurred throughout the clinical course), Group B (DCVS occurred, but no cerebral infarction [CI] was induced), and Group C (DCVS occurred and caused CI), the d-ROMs change rate in IJVB was the highest in Group C, followed by Group B then A ($p<0.01$). The d-ROMs change rates in peripheral arterial blood and CSF were not related to the development of DCVS.

Conclusion: It was concluded that more severe DCVS occurs and is more likely to progress to CI as the IJVB hydroperoxide level rises early after the development of SAH.

Keywords: hydroperoxide; delayed cerebral vasospasm; oxidative stress; internal jugular venous blood; subarachnoid hemorrhage

Introduction

The cause of subarachnoid hemorrhage (SAH) is rupture of cerebral aneurysm in most cases[1]. Rerupture of cerebral aneurysm in the acute phase markedly influences the prognosis, for which surgical treatments with aneurysmal neck clipping and coil embolization have achieved favorable outcomes[2]. Another serious complication influencing the prognosis of SAH patients is cerebral vasospasm. This is classified into early cerebral vasospasm, which occurs early (within 24 hours) after the development, and delayed cerebral vasospasm (DCVS), which occurs 4-14 days after the development[3]. DCVS occurs in about 70% of SAH patients, and 20-30% are symptomatic. Severe neurological deficits remain and may be life-threatening[4]. Many points have not been clarified with regard to the pathology, and no therapeutic method completely inhibiting it has been established.

DCVS is considered to be induced by hemoglobin in hematomas present around the cerebral arteries in the subarachnoid space[5,6], and it has been clarified that red blood cell-derived reactive oxygen species cause peroxidation and induce DCVS[7,8]. Recent studies clarified that free radicals

released from oxyhemoglobin contained in red blood cells induce lipid peroxidation of the cell membranes of vascular smooth muscles, resulting in activation of calcium channel, protein kinase C, and Rho-kinase, which may be a mechanism of DCVS[9-15]. However, in vivo evaluation of oxidative stress is difficult because of instability of free radicals, and only a few studies have quantified it[16-19]. Thus, we quantitatively evaluated oxidative stress by measuring an oxidation product, hydroperoxide, in 3 specimens of SAH patients: peripheral arterial blood, cerebrospinal fluid (CSF), and internal jugular venous blood (IJVB), using a free radical analytical system (FRAS) (Wismerll Co., Ltd., Tokyo, Japan) capable of measuring it simply. In addition, the association between the hydroperoxide levels and development of DCVS and progression to cerebral infarction (CI) was investigated.

Materials and Methods

The subjects were consecutive SAH patients (WFNS grade III-V) who underwent surgical treatment for the acute phase at Toho University Omori Medical Center and Misato Central General Hospital between April 2011 and March 2013. At our institution, a radial arterial catheter is inserted to

monitor direct blood pressure (BP), a lumbar CSF drain is applied to irrigate subarachnoid blood, and a jugular bulb venous oxygen saturation monitoring (SjO_2) catheter is inserted into the internal jugular vein to measure cerebral hemodynamics in patients with WFNS grade III or more severe SAH as a routine practice. Through these catheters, peripheral arterial blood, CSF, and internal jugular venous blood (IJVB) were collected, respectively, and an oxidation product, hydroperoxide, was quantitatively measured to evaluate the in vivo oxidative stress level. Head computed tomography (CT) and cerebral angiography were performed in all patients as preoperative neuroradiological examinations, and surgical aneurysmal neck clipping or coil embolization was performed within 48 hours after the onset. The catheter for direct BP monitoring, lumbar CSF drain, and SjO_2 catheter were inserted during or immediately after surgery, and peripheral arterial blood, CSF, and IJVB were collected through the catheters, respectively. Regarding the choice of which of the bilateral jugular bulbs to use to insert the SjO2 catheter, the side with greater venous blood flow to the jugular bulb on preoperative cerebral angiography was selected. When their blood flow was similar, the right side was selected[20)21)]. The samples were collected between

8:00 and 9:00 a.m. in consideration of diurnal fluctuations of the oxidative stress level. Since intravenous anesthetics, such as propofol, have been reported to increase the antioxidant power[22], patients not treated with an intravenous anesthetic after surgery were selected. To evaluate oxidative stress, the samples were subjected to the Diacron-Reactive Oxygen Metabolites test (d-ROMs test) using FRAS, and the hydroperoxide levels were spectrophotometrically measured. The hydroperoxides are intermediate oxidative products of lipids, peptides, and amino acids, and their levels constitute an index of oxidative injury of cellular components. The hydroperoxide levels were measured as follows[23,24]: 20 μL of blood and CSF was dissolved in an acetate-buffered solution (pH 4.8). The hydroperoxide groups react with the transition metal ions liberated from the proteins in the acidic medium and are converted to alkoxy and peroxy radicals, according to the Fenton reaction. These newly formed radicals, the quantities of which are directly proportional to those of the peroxides present in serum and CSF, are trapped chemically with chromogen (N,N-dimethyl para-phenylenediamine), leading to formation of the corresponding radical cation. The concentration of this persistent species can be determined at 505

nm using a spectrophotometer. Results are expressed in Carratelli (Carr) units, where 1 Carr unit corresponds to 0.8 mg/L of hydrogen peroxide. The measured values are presented as the d-ROMs test values below.

Since d-ROMs test values markedly vary among individuals, the absolute values cannot be directly compared. Thus, the rate of change in this value over time in each patient was investigated[25,26]. Moreover, surgery increases oxidative stress[27,28], and this influence has to be eliminated. Since brain injury-induced oxidative stress is reduced within 48 hours[29], regarding the d-ROMs test value on Day 3 as the control, oxidative stress was evaluated based on the rate of change in the d-ROMs test value on Day 6 (d-ROMs change rate).

The following 2 items were investigated based on the values measured in peripheral arterial blood, CSF, and IJVB: Firstly, the presence or absence of DCVS on Day 6 was investigated, and the d-ROMs change rates (Day 6/Day 3 d-ROMs test value) at this time point were compared. Secondly, the patients were divided into 3 groups: patients in whom no DVC occurred throughout the course (Group A), DCVS occurred but did not induce CI (Group B), and DCVS occurred and caused CI (Group C), and these 3

groups and the d-ROMs change rates on Day 6 were compared. Oxidative stress increases in CI[30-33], which may produce an error in analysis of the relationship between the development of DCVS and oxidative stress level. To avoid this, the patients who developed CI were excluded from the later investigation of the d-ROMs test value. Since the d-ROMs change rate on Day 3 was regarded as the baseline, patients who had already developed cerebral vasospasm on Day 3 were also excluded.

This clinical study was performed after approval by the Ethics Committees of Toho University School of Medicine and Misato Central General Hospital. The catheters and drain used to collect samples were applied for postoperative management, not specially performed for this study.

DCVS and CI evaluation methods

To confirm the presence or absence of DCVS, cerebral angiography was performed in all patients on Day 6. In addition, three-dimensional CT angiography was conducted on Day 10, and cerebral angiography was performed when DCVS was suspected. A condition in which 25% or more narrowing was noted in one or more major arteries on preoperative cerebral

angiography was defined as DCVS. A condition in which the territory of an artery with DCVS was visualized as a low-density area on head CT or a high-intensity area on diffusion-weighted magnetic resonance imaging (MRI) was defined as CI. The images were judged by 2 neurosurgeons (H.U. and C.M.).

Statistical analysis

All measured values are presented as the mean ± standard deviation (SD). For analysis of the significance of differences, the t-test was employed for comparison between 2 groups, and one-way analysis of variance (ANOVA) was employed for comparison among 3 groups. The significance level was set at 5%, and when the p-value was less than 5%, the difference in the comparison was regarded as significant.

Results

The subjects were 20 patients (5 males and 15 females) aged 41-86 years. The WFNS grade was III in 4, IV in 9, and V in 7. Peripheral arterial blood, CSF, and IJVB were collected on Days 3 and 6 from all patients and subjected to the d-ROMs test. Since the d-ROMs change rate on Day 3 was

regarded as the baseline, 3 patients who had already developed DCVS on Day 3 were excluded, and the remaining 17 patients were evaluated (Table 1).

DCVS had developed by Day 6 in 12 patients, and it had not occurred in 5. No DCVS occurred throughout the course in 4 (Group A). DCVS occurred by Day 8 in all 13 patients who developed it. CI did not occur in 6 of them (Group B), but it occurred in 7 (Group C). No significant difference was noted in the age, sex, WFNS classification, Fisher grade, location of aneurysm, surgical treatment method, or Glasgow Outcome Scale among the 3 groups (Tables 1, 2).

I. Peripheral arterial blood

No significant difference was noted in the peripheral arterial blood d-ROMs change rate between the groups with and without DCVS on Day 6 [DCVS (-): 100.2±10.9%, DCVS (+): 109.9±17.8%, p=0.283, t-test, Fig. 1], nor was there a significant difference among Groups A, B, and C [Group A: 102.9±10.5%, Group B: 112.2±17.0%, Group C: 104.9±19.4%, p=0.644, one-way ANOVA, Fig. 2].

II. CSF

No significant difference was noted in the CSF d-ROMs change rate between the groups with and without DCVS on Day 6 CSF [DCVS (-): 103.0±16.6%, DCVS (+): 115.2±24.6%, p=0.33, t-test, Fig. 3], nor was there a significant difference among Groups A, B, and C [Group A: 96.2±7.7%, Group B: 104.6±20.9%, Group C: 126.4±22.9%, p=0.0572, one-way ANOVA, Fig. 4].

III. Internal jugular venous blood (IJVB)

The IJVB d-ROMs change rate was significantly higher in the group with DCVS on Day 6 than in the group without DCVS [DCVS (-): 93.0±12.0%, DCVS (+): 116.0±11.2%, p=0.00182, t-test, Fig. 5]. A significant difference was noted among Groups A, B, and C, and the rate was the highest in Group C, followed by Group B and then A [Group A: 95.5 ± 12.3%, Group B: 103.8 ± 13.5%, Group C: 121.8 ± 8.4%, p=0.0049, one-way ANOVA, Fig. 6].

The SjO2 catheter was inserted into the right side in 14 and left side in 3 of the 17 patients. No significant difference due to the side of insertion was noted in the incidence of DCVS on Day 6 [p=0.6765, Fisher's exact test], nor was there a significant difference in the number of cases among Groups A, B, and C [p=0.6376, Mann–Whitney U-test].

Discussion

Various studies on DCVS have been performed, but the elucidation of how oxidative stress is involved in DCVS is still incomplete. It has been clarified that the fundamental cause of DCVS is the presence of hematoma in the subarachnoid space, and consensus has been reached that oxidative stress is partially involved[7,8]. Actually, prevention of cerebral vasospasm by administration of free radical scavengers, such as nicaraven, ebselen, and edaravone, has been reported[10,34-36]. Studies on quantitation of free radicals have also been performed[37], but its clinical application is difficult because measurement devices are expensive and require specific conditions for installation. Thus, oxidation products of body components have been generally measured to evaluate oxidative stress[16,19,38-43]. Oxidation products include 8-hydroxy-2'deoxyguanosine (8-OHdG) as a DNA oxidative stress marker, 3-nitrotyrosine (3-NT) and carbonyl protein as protein or amino acid markers, 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde as lipid markers, and 8-iso-prostaglandin F2α (8-iPGF2α) as an arachidonic acid marker[38-42]. Of these, the 8-OHdG and 8-iPGF2α levels in urine and CSF have been reported to be correlated with the occurrence of DCVS[16,19,43].

We measured the level of hydroperoxide, an oxidation product of lipids,

peptides, and amino acids, in peripheral arterial blood, CSF, and IJVB (d-ROMs test) to evaluate oxidative stress in DCVS. The d-ROMs change rate in IJVB was significantly higher in patients who developed DCVS, clarifying that intracranial oxidative stress is strongly associated with the development of DCVS, showing the possibility of using the d-ROMs test value in IJVB as a biomarker of DCVS. The correlation of the d-ROMs test value early after SAH development with the later development of DCVS-induced CI was then investigated. Of the patients with DCVS on Day 6, CI occurred in those with a high d-ROMs change rate in IJVB before the development of CI, showing that the oxidative stress level was high from the early phase of SAH. These findings suggest that intracranial oxidative stress is involved in the development of DCVS, and more severe DCVS occurs and progresses to CI as the stress level rises. In addition, the possibility of using the d-ROMs test value in IJVB early after the development of SAH as a marker to predict the development of DCVS-induced CI was revealed.

In contrast, no significant relationship of the d-ROMs change rate in peripheral arterial blood with the development of DCVS or CI was noted, suggesting that the association between systemic oxidative stress and DCVS

is weak. No significant relationship was also noted between the d-ROMs change rate in CSF and the development of DCVS or CI. Many previous reports pointed out the presence of an association between biological markers in CSF and cerebral vasospasm[44-50]. It has been reported that the membrane-bound tissue factor (mTF) level in CSF was abnormally high immediately before DCVS-induced ischemic stroke at an 80% or higher probability[44,47], and the 14-3-3 ß, calpain-derived α-spectrin N (CCSntf), NSE, and S-100B levels in CSF were useful to predict moderate or more severe cerebral vasospasm[48-50]. The absence of a significant difference in the d-ROMs change rate in CSF may have been due to the small number of patients, but it is also possible that oxidative stress was not accurately measured because CSF was collected from a lumbar CSF drain, which is distant from the spastic blood vessels.

Hydroperoxide was measured using the d-ROMs test. High reliability of the d-ROMs test as an oxidative stress evaluation method has been demonstrated by Alberti et al. through comparison with direct free radical measurement using electron spin resonance[23]. Its accuracy has also been confirmed by comparison with measurement of other oxidation products,

such as lipid hydroperoxides[24,51]. This measurement method can be performed rapidly and simply, which is an advantage of it. Since the sample volume is small and measurement takes only a few minutes, it can be used for continuous bedside oxidative stress monitoring. This study suggested that continuous measurement of the d-ROMs test value in IJVB facilitates early discovery of DCVS and may predict aggravation causing CI.

The subjects were limited to patients with WFNS grade III or more severe SAH because insertions of the direct BP monitoring catheter, lumbar CSF drain, and SjO_2 catheter to collect samples are clinically not always necessary for grade I and II patients. Reportedly, cerebral vasospasm and CI are less likely to occur in low- than high-WFNS-grade cases[52,53]. Thus, the intracranial oxidative stress level may be low in these cases. It may be necessary to investigate how to measure the intracranial oxidative stress level in WFNS grade I and II patients.

Conclusions

To investigate the correlation between SAH-induced DCVS and oxidative stress, hydroperoxide was measured in peripheral arterial blood, CSF, and

IJVB using the d-ROMs test. It was clarified that patients with a high oxidative stress level in IJVB early after the development of SAH are likely to develop DCVS and subsequent CI. Using the d-ROMs test, the oxidative stress level can be measured simply and rapidly, suggesting its clinical usefulness as a marker.

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Figure legends

Figure 1.

Comparison of the d-ROMs change rate in peripheral arterial blood on Day 6 between the DCVS (-) and DCVS (+) groups

No significant difference was noted between the 2 groups (p=0.283, t-test).

Abbreviations: DCVS, delayed cerebral vasospasm; d-ROMs,

Diacron-Reactive Oxygen Metabolites; ns, no significant.

Figure 2.

Comparison of the d-ROMs change rate in peripheral arterial blood among Groups A, B, and C

Group A: Patients who did not develop DCVS throughout the course

Group B: Patients in whom DCVS developed but did not induce cerebral infarction (CI) during the course

Group C: Patients who developed CI during the course

No significant difference was noted among the groups (p=0.644, one-way ANOVA). Abbreviations: d-ROMs, Diacron-Reactive Oxygen Metabolites; ns, no significant; DCVS,delayed cerebral vasospasm; CI, cerebral infarction.

Figure 3.

Comparison of the d-ROMs change rate in CSF on Day 6 between the DCVS (-) and DCVS (+) groups

No significant difference was noted between the 2 groups (p=0.330, t-test). Abbreviations: CSF, cerebrospinal fluid; DCVS, delayed cerebral vasospasm;

d-ROMs, Diacron-Reactive Oxygen Metabolites; ns, no significant.

Figure 4.

Comparison of the d-ROMs change rate in CSF among Groups A, B, and C

Group A: Patients who did not develop DCVS throughout the course

Group B: Patients in whom DCVS developed but did not induce cerebral infarction (CI) during the course

Group C: Patients who developed CI during the course

No significant difference was noted among the groups ($p=0.0572$, one-way ANOVA). Abbreviations: CSF, cerebrospinal fluid; d-ROMs, Diacron-Reactive Oxygen Metabolites; ns, no significant; DCVS, delayed cerebral vasospasm; CI, cerebral infarction.

Figure 5.

Comparison of the d-ROMs change rate in internal jugular venous blood (IJVB) on Day 6 between the DCVS (-) and DCVS (+) groups

A significant difference was noted between the DCVS (-) and DCVS (+) groups ($p<0.01$, t-test). Abbreviations: IJVB, internal jugular venous blood;

DCVS, delayed cerebral vasospasm; d-ROMs, Diacron-Reactive Oxygen Metabolites.

Figure 6.

Comparison of the d-ROMs change rate in IJVB among Groups A, B, and C

Group A: Patients who did not develop DCVS throughout the course

Group B: Patients in whom DCVS developed but did not induce cerebral infarction (CI) during the course

Group C: Patients who developed CI during the course

A significant difference was noted among the 3 groups, and the order of the rate was Group A<Group B<Group C ($p<0.01$, one-way ANOVA).

Abbreviations: IJVB, internal jugular venous blood; d-ROMs, Diacron-Reactive Oxygen Metabolites; DCVS, delayed cerebral vasospasm; CI, cerebral infarction.

Table1. Time of development of delayed cerebral vasospasm and cerebral infarction and the numbers of patients

Abbreviations: WFNS, World Federation of Neurosurgical Societies; DCVS,

delayed cerebral vasospasm; CI, cerebral infarction.

Table2.

Summary of the 3 groups

Group A: Patients who did not develop DCVS throughout the course

Group B: Patients in whom DCVS developed but did not induce cerebral infarction (CI) during the course

Group C: Patients who developed CI during the course

Abbreviations: DCVS, delayed cerebral vasospasm; CI, cerebral infarction; WFNS, World Federation of Neurosurgical Societies; ICA, internal carotid artery; ACA, anterior cerebral artery; MCA, middle cerebral artery; BA basilar artery; VA vertebral artery; GOS, Glasgow Outcome Scale; GR good recovery; MD, moderate disability; SD, severe disability; VS, vegetative state; D, dead.

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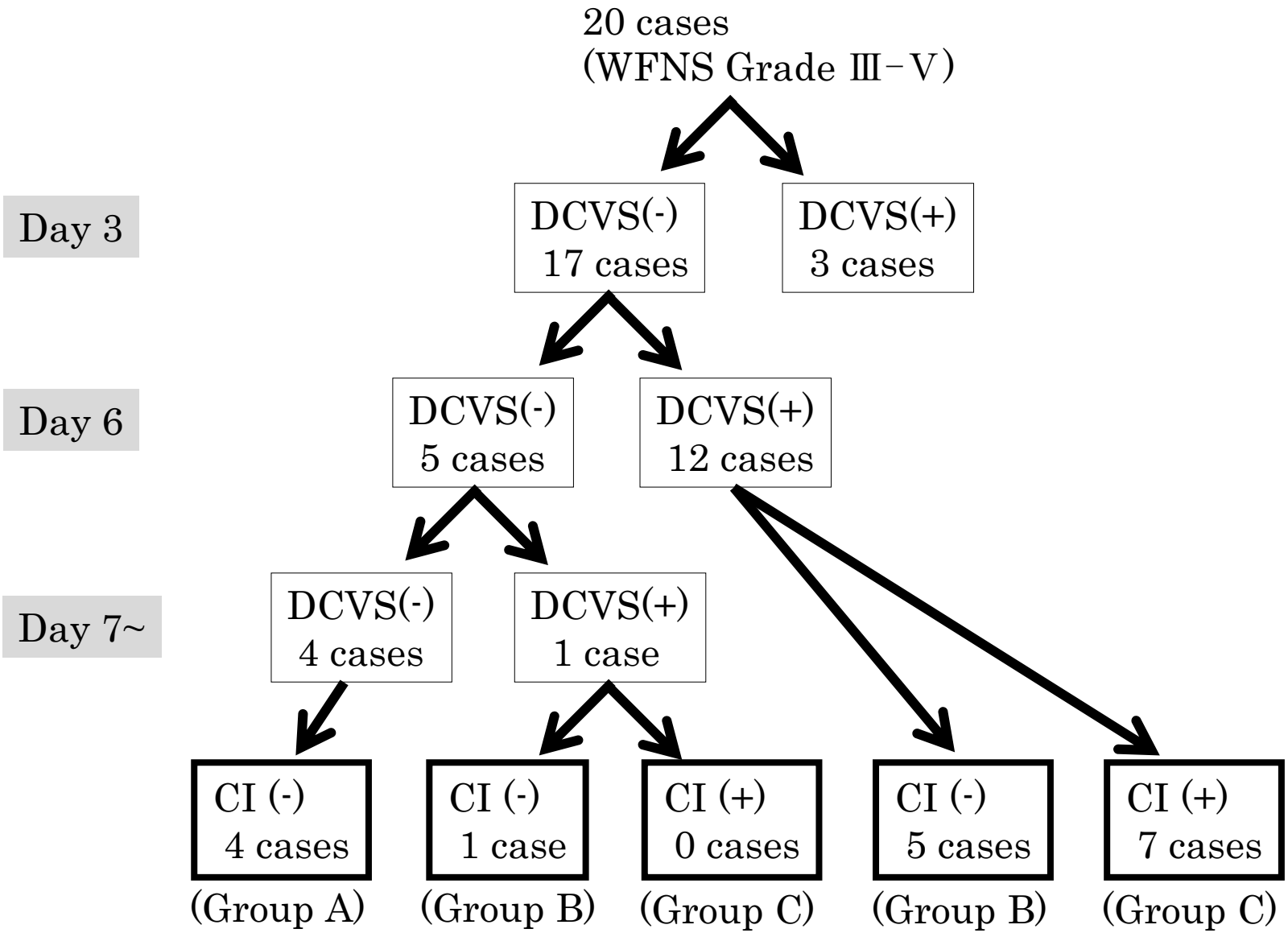
Table 1


20 cases
(WFNS Grade Ⅲ－Ⅴ)
Day 3
DCVS(-)
17 cases
DCVS(+)
3 cases
Day 6
DCVS(-)
5 cases
DCVS(+)
12 cases
Day 7~
DCVS(-)
4 cases
DCVS(+)
1 case
CI (-)
4 cases
(Group A)
CI (-)
1 case
(Group B)
CI (+)
0 cases
(Group C)
CI (-)
5 cases
(Group B)
CI (+)
7 cases
(Group C)

	Group A	Group B	Group C	total	p-value
Age	59 ± 14	66 ± 22	58 ± 17	61 ± 25	0.6944
Sex					0.8778
M	1	1	2	4	
F	3	5	5	13	
WFNS classification					0.3466
3	1	2	1	4	
4	1	4	4	9	
5	2	0	2	4	
Fisher Grade					0.6297
1	0	0	0	0	
2	2	2	3	7	
3	2	2	4	8	
4	0	2	0	2	
Location of aneurysm					0.4121
ICA	1	2	5	8	
ACA	1	2	2	5	
MCA	1	1	0	2	
BA	0	1	0	1	
VA	1	0	0	1	
Therapy					0.4174
clipping	3	5	7	15	
embolization	1	1	0	2	
GOS					0.4071
GR	4	2	3	9	
MD	0	1	0	1	
SD	0	1	2	3	
VS	0	1	0	1	
D	0	1	2	3	

table 2

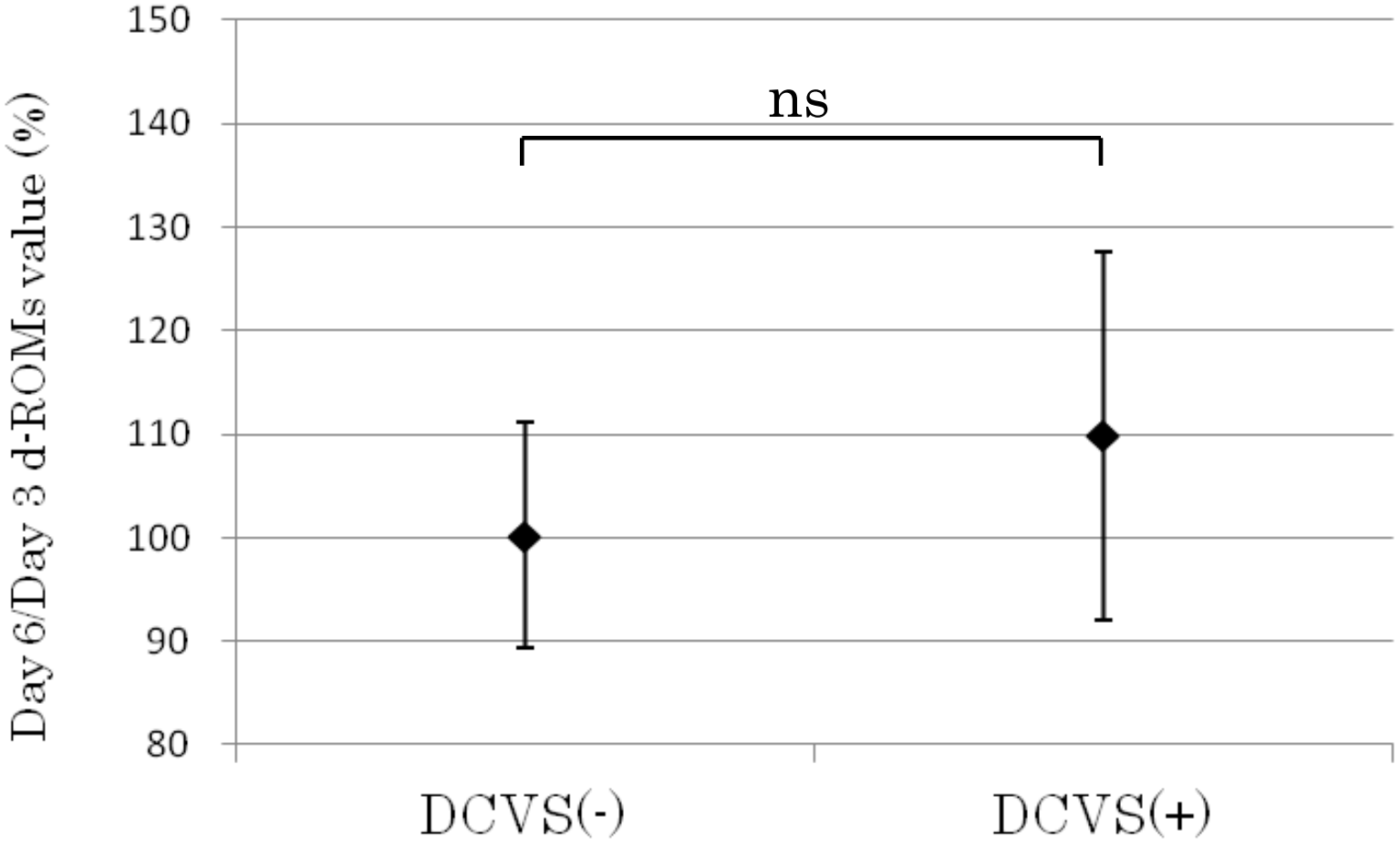

Day 6/Day 3 d-ROMs value (%)
150
140
130
120
110
100
90
80
ns
DCVS(-)
DCVS(+)

Fig.1

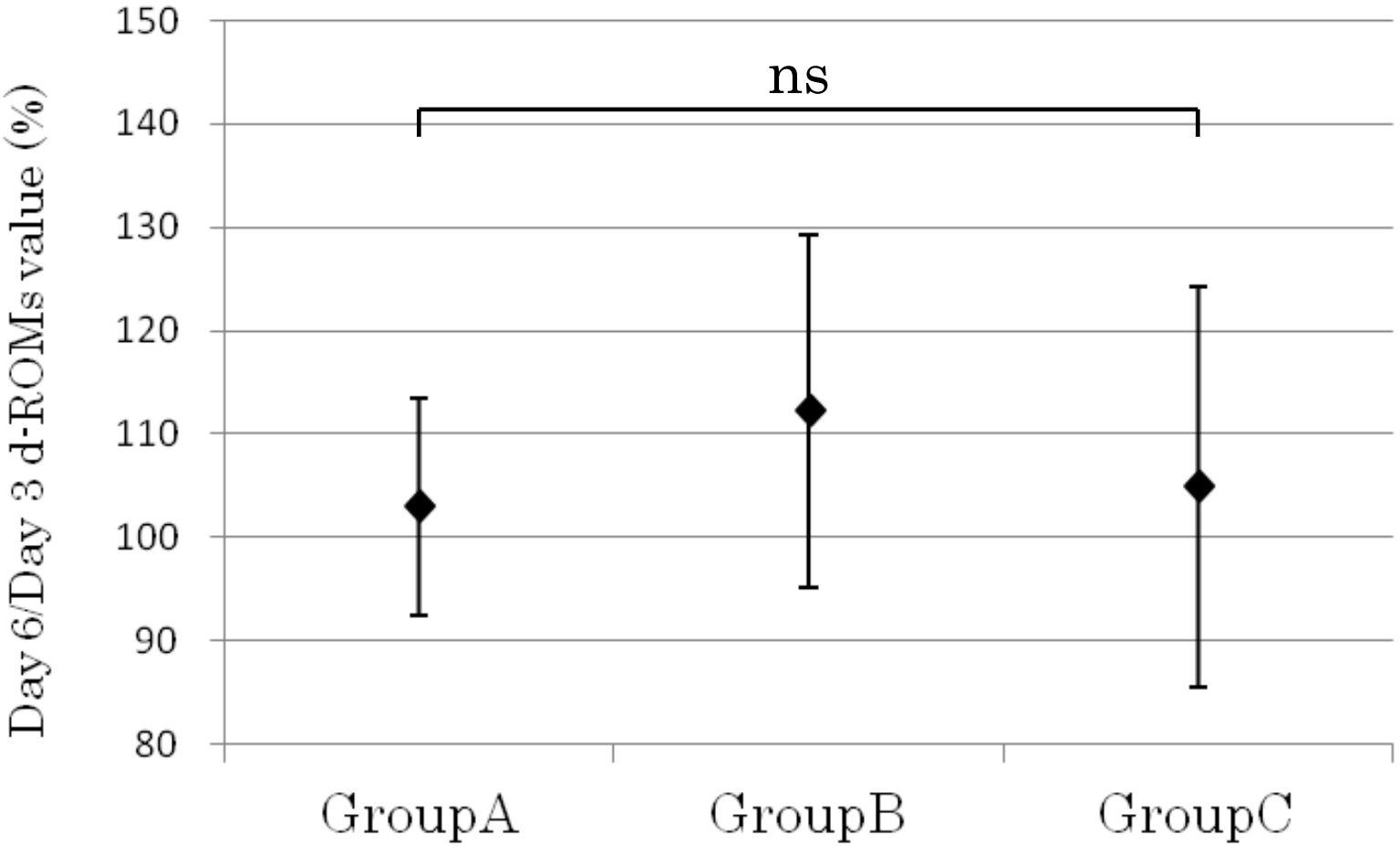

Day 6/Day 3 d-ROMs value (%)
150
140
130
120
110
100
90
80
ns
GroupA
GroupB
GroupC

Fig.2

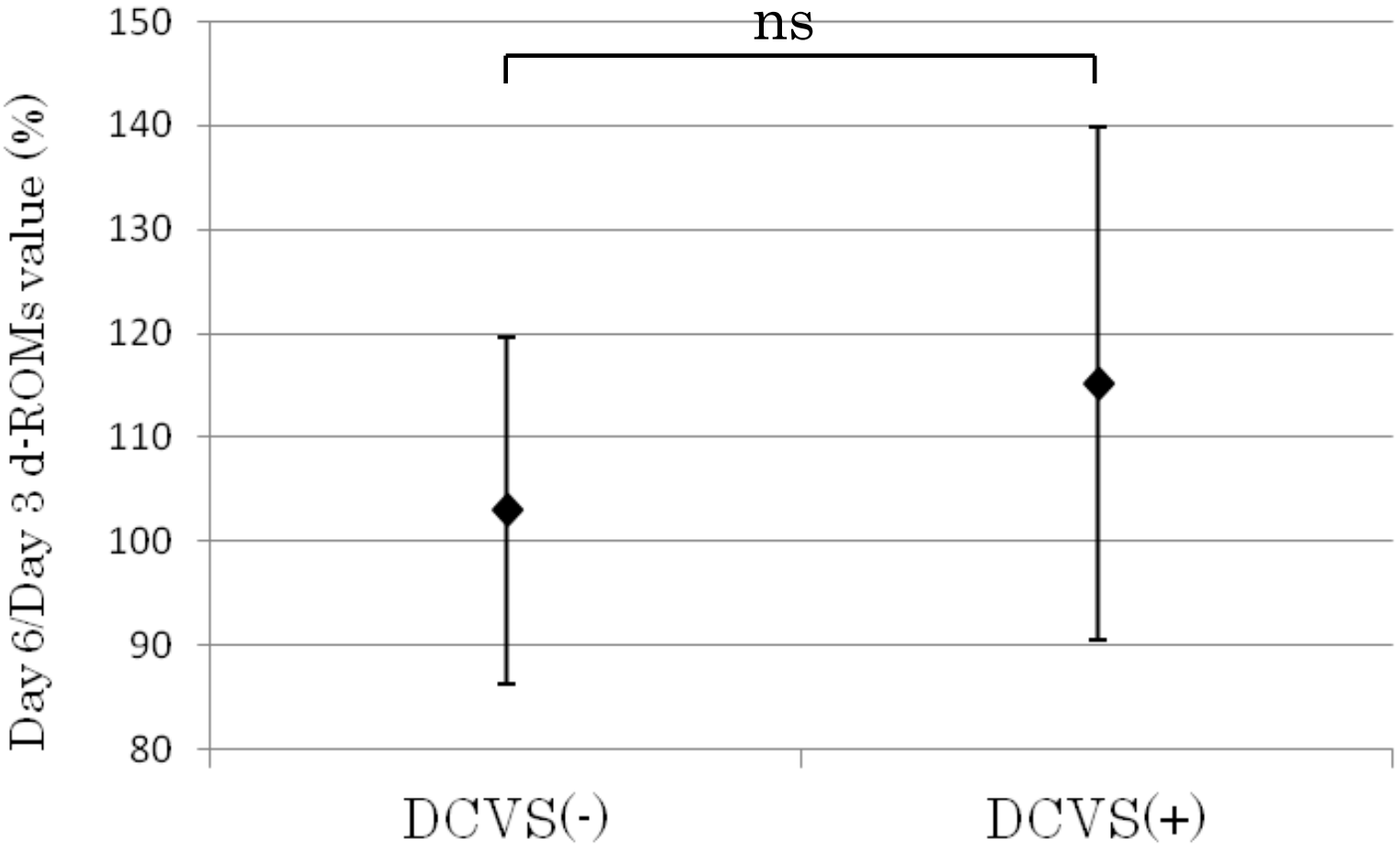

ns
150
140
130
120
110
100
90
80
Day 6/Day 3 d-ROMs value (%)
DCVS(-)
DCVS(+)

Fig.3

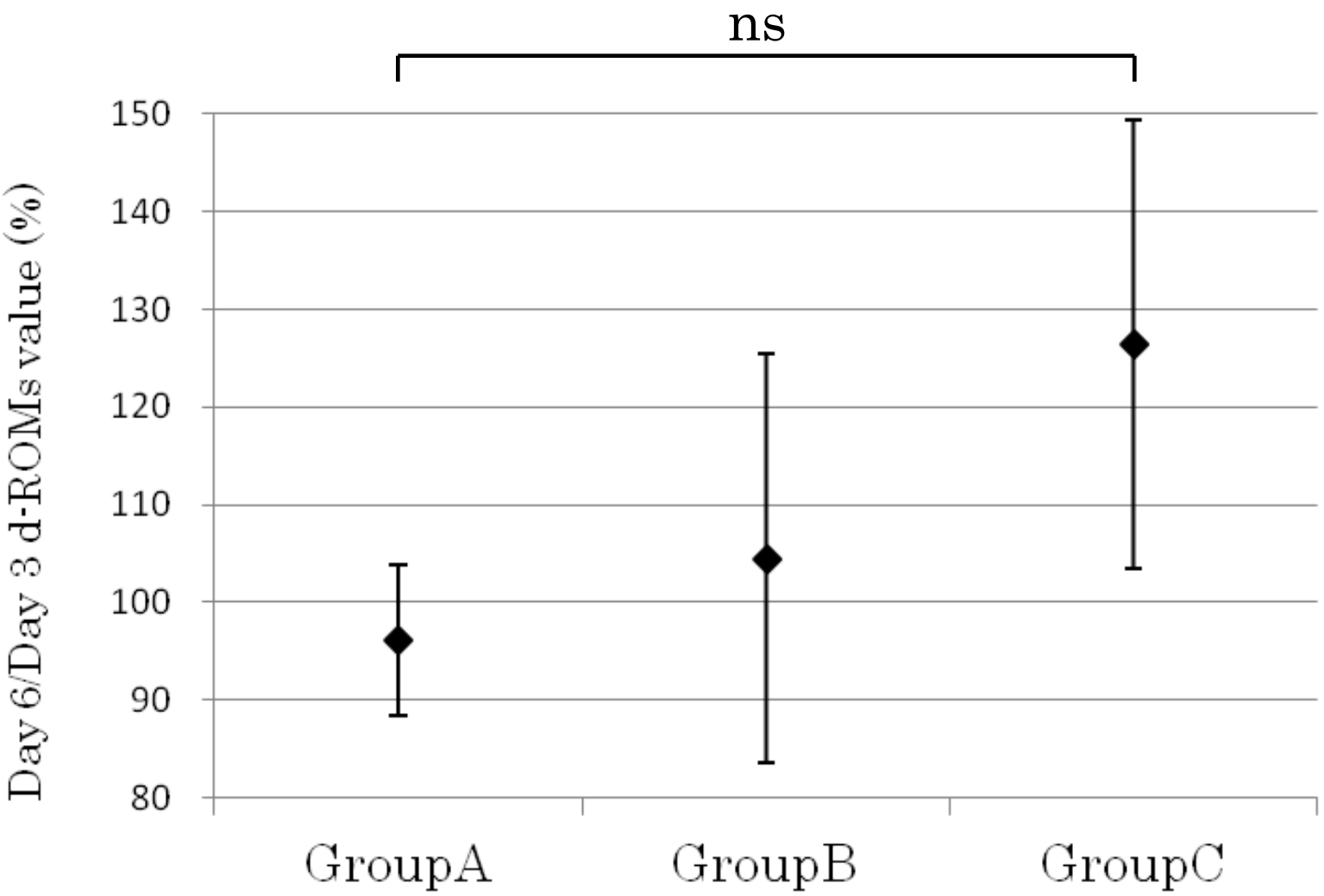

ns
150
140
130
120
110
100
90
80
Day 6/Day 3 d-ROMs value (%)
GroupA
GroupB
GroupC

Fig.4

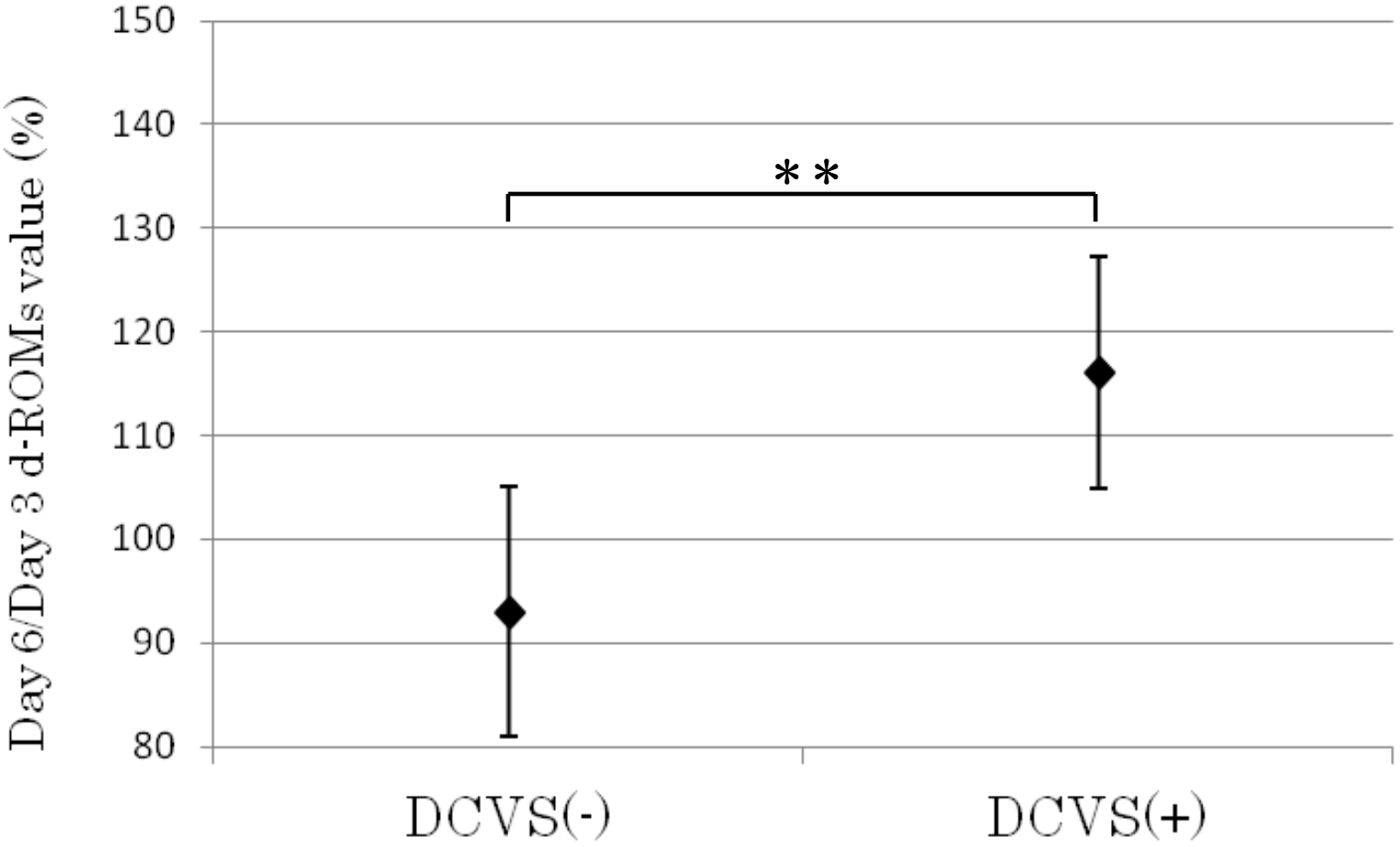


Fig.5

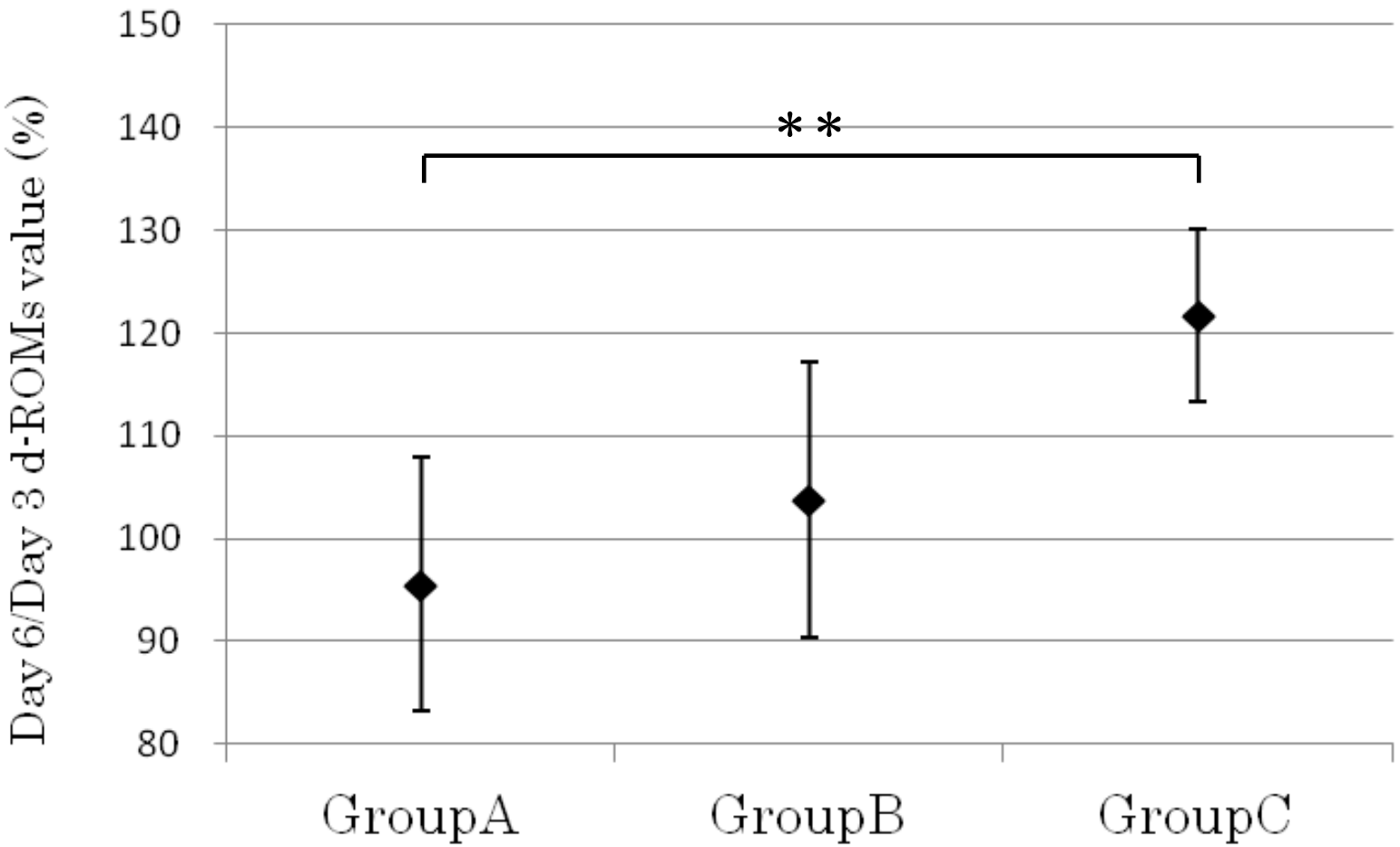

Day 6/Day 3 d-ROMs value (%)
150
140
130
120
110
100
90
80
**
GroupA
GroupB
GroupC

Fig.6