Perspectives of a catheter-based nitric oxide sensor for the evaluation of endothelial function

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Key words:
Endothelial function, Nitric oxide, Flow-mediated dilation, Nitric oxide sensor

Introduction

Vascular endothelial cells, which are considered to be the largest endocrine organ, secrete various vasoactive agents including nitric oxide (NO), to maintain homeostasis of the blood vessel wall. In particular, NO not only diffuses into underlying smooth muscles, causing them to relax and regulate vessel diameter, but also regulates inflammatory cell adhesion, vascular permeability, and coagulation via the fibrinogenolysis system. Thus, NO is regarded as an anti-atherosclerotic agent, and such homeostatic roles represent the fundamental "endothelial function". Endothelial dysfunction is the initial step for vascular failure leading to cardiovascular events. Namely, the quantity of NO produced by vascular endothelial cells via the endothelial nitric oxide synthase (eNOS) directly reflects endothelial function. However, the quantitative measurement of NO is very difficult, because the life span of NO is very short. Alternatively, flow-mediated dilation (FMD) has been highlighted as a method to assess NO-dependent endothelial function. The FMD is widely used as an increasing of the diameter of the target artery, which is measured by high-resolution external vascular ultrasound in response to the increase in blood flow that occurs during ischemic reactive hyperemia. Reperfusion during FMD measurement increases shear stress on endothelium. Therefore, NO released from endothelial cells in response to increased shear stress diffuses into smooth muscles to promote relaxation of vasomotor tone, and consequently, the artery becomes dilated. However, NO bioavailability is not directly represented by the process of ischemia to reactive hyperemia in FMD measurement, because the FMD depends on, not only the release of endothelial NO-release, but also other conditions of the target artery, including smooth muscle function.

Circulating NO is assessed by the measurement of plasma levels of NOx, i.e., a composite of nitrite (NO₂⁻) and nitrate (NO₃⁻), which are stable metabolites of NO. However, the NO, reflects not only eNOS-derived NO but also NO synthesized by the neuronal or inducible NOS isozymes, so does not directly represent endothelial function alone. On the other hand, plasma levels of asymmetric dimethylarginine (ADMA), known as an eNOS inhibitor, are also often measured. In general, ADMA competitively inhibits the activity of eNOS and reduces the production of NO in endothelial cells; thus, increased ADMA levels are thought to represent endothelial dysfunction. However, measurement of these biomarkers cannot provide information about the kinetics of circulating endothelial cell-derived NO in real-time.

Catheter-based NO measurement system

Recently an in-vivo direct measurement system for circulating blood NO concentrations using a catheter-based NO sensor has been developed. With this system, several investigators have attempted to evaluate in-vivo NO bioavailability in real-time in the blood stream. This system is comprised of an amperometric NO-selective sensor (amino-700 XL, Innovative Instruments, Tampa, FL, USA), bonded at the tip of a 4-Fr catheter with a monorail lumen (1200 mm long; Hirakawa Hewtech, Ibaraki, Japan). Polyurethane was attached to the detection tip to prevent physical damage of the vessel wall, and two metal wires were attached along the detection tip to provide mechanical support for the elec-
trodes. The oxidative current produced by NO is monitored using a NO monitoring system (model in NO-T, Innovative Instruments) (Figure 1). Both the working and reference electrodes are integrated in a single element. The sensing electrode is covered with a NO gas permeable membrane. Thus, the NO gas diffuses through the electrode surface causing an electrical current that is monitored and recorded after A/D conversion in the interface. The sensor does not measure the absolute levels of circulating NO, because it measures the electrical potential differences between the reference site and the monitoring site, and therefore, converts milliamperes to nanomole (nM) according to a previously-installed calibration scale.

**In-vivo circulating NO kinetics during ischemia/reactive hyperemia**

At present, the in-vivo NO kinetics during the FMD procedure have not been proven. We hypothesized that a novel maneuver for the direct-estimation of NO kinetics using the catheter-based NO measurement system in the endothelial response to ischemia/reactive hyperemia could be used to evaluate the conformity of FMD. Therefore, we measured circulating NO levels in the forearm artery directly using this system.

To measure circulating NO levels, we deployed the NO sensor catheter into the forearm arteries of two patients with coronary artery disease, during diagnostic coronary angiography procedures, which were performed on the day after the FMD measurement, in 2010. These procedures were performed after approval of the hospital ethical review committee, and written informed consent was obtained from each of the two patients. Following insertion of a sheath by the radial approach, the NO sensor catheter mounted on a 0.014-inch guidewire with the occlusion balloon (PercuSurge GuardWire®; Medtronic Inc.) was positioned in the brachial artery. In this study, we generated an ischemia/reactive hyperemia model, similar to the FMD maneuver, by inflation/deflation of the occlusion balloon at the proximal site of the catheter-tip NO sensor. At the distal site, a pressure/flow wire (Combo-wire®; VOLCANO U.S.) was positioned for continuous monitoring of blood pressure and blood flow. The time required to obtain a high-quality baseline scan was over 5 min. The balloon was inflated for 5 min until the blood pressure and blood flow disappeared in downstream monitoring, and following a deflation period of 2 min, in succession. This ischemia/reactive hyperemia model was based on the procedure for FMD measurement. Throughout this process, circulating NO levels were monitored every 5 sec continuously using the NO sensor catheter (Figure 2). The FMD measurement was performed, according to a previously reported method.³ The normal FMD value was regarded as > 6.0%.³

The first case was a 69 year-old male with a normal FMD value of 6.9%. In this case, the baseline NO level was 52.2 nM. After occlusion of the vessel by inflation of the balloon, the NO level increased immediately and remarkably to the peak value of 76.4 nM (46% increase) and then gradually recovered to the baseline level. Following balloon deflation, the NO level increased gradually and slightly to 55.2 nM (5.7% increase) and then returned to the baseline level (Figure 3-A). We speculate that the immediate and transient NO elevation after balloon inflation might be caused by hypoxic stress or change of shear stress accompanied with occlusion of the blood flow. We also speculate that the gradual secondary elevation of NO levels, might be a part of the response to reactive hyperemia. We consider that these circulating NO kinetics might be representative in cases in which vascular endothelial function is maintained. The second case was a 61 year-old male with a decreased FMD value of
2.6%, in whom the baseline circulating NO level was high, 80.6 nM. Despite the occlusion of the vessel by balloon inflation, the NO level increased only slightly to 81.8 nM (a 1.4% increase). Moreover, there was no significant change in the NO level following deflation of the balloon (Figure 3-B). We can see that the baseline NO level was already elevated, although its mechanism is unclear, and speculate that there was no capacity for it to increase further. The NO kinetics, as described in the first case, might be absent in cases in which the vascular endothelial function is extremely impaired; although the precise mechanisms responsible for the differences between cases with and without vascular endothelial dysfunction remain to be elucidated.

Clinical implications and limitation of current system

In our experiments, we attempted to reproduce ischemia/reactive hyperemia using an original model in which blood flow was blocked by balloon inflation and restored following balloon deflation, as an alternative to the inflation/deflation of forearm cuff in the FMD procedure. Although there is a procedural difference in the ischemia/reactive hyperemia models between our experiment and FMD procedure, i.e., vessel compression or luminal occlusion, we used this model to observe real-time dynamics of blood NO levels during the ischemia/reactive hyperemia maneuver. As a result, in our first case with normal vascular endothelial function based on the FMD, we observed an immediate transient increase in NO levels after occlusion of the blood flow and a gradual secondary increase in NO levels after restoration of the blood flow. We consider that the secondary response of the NO increase might correspond to the FMD response. Therefore, we suggest that the balloon inflation/deflation model could reproduce ischemia/reactive hyperemia, similar to FMD. However, the current catheter-based system for the measurement of circulating NO appears to have many potential limitations for the real-time assessment of blood NO dynamics. First, even if the secondary response of the NO level represents endothelial function, this response appears to be attenuated, compared with the FMD response, as shown in our first case (5.7% vs 6.9%). As such, our approach to evaluate vascular endothelial function through the assessment of circulating NO kinetics with the NO sensor catheter would be less sensitive, compared with the FMD. A fundamental question arises as to how much of the endothelium-derived NO is released into blood stream; although it is certain that it diffuses into the vessel wall and relaxes the vascular smooth muscles. Furthermore, it is uncertain whether the change in blood NO represents a role in the endothelium-dependent regulation of vascular tone. Secondly, even if the NO is released into the blood vessel, with respect to fluid dynamics of the blood vessel, it is unclear whether the NO concentration would be identical between the central stream and the marginal stream. In addition, it remains to be determined whether the NO sensor is able to detect NO in plasma vs. that in red blood cells, or both.

It is well known that red-blood cell hemoglobin transfers NO to peripheral tissue as a transporter. The endothelial NO, that is released to vascular cavity, momentarily combines with an iron (Fe^{2+}) of hemoglobin and is scavenged immediately, so it still remains unclear how much the circulating NO represents endothelium-released NO. We hypothesized that endothelium-released NO into the blood stream may be detected more precisely in the marginal blood stream close to the endothelium. However, with the current NO sensor
catheter system, it is not possible to determine where the catheter-tip NO sensor is positioned, in the central stream or in the marginal stream. Thus, we are now working to improve the system to detect NO levels in the marginal stream as close as possible to the endothelium. In addition to above mentioned limitations, there are many other problems to be resolved in the catheter-based NO sensor system. However, it would be possible to elucidate more precisely the mechanism of NO-dependent vasodilation and its response to various vasoactive drugs, if we can assess the circulating NO kinetics. We believe that when the system is successfully improved, the clinical application of the NO sensor catheter will provide us with novel information regarding vascular failure.

Figure 3. Circulating NO kinetics during ischemia/reactive hyperemia in the forearm artery. A. A 69 year-old male with a normal FMD value (6.9%). The baseline NO level was 52.2 nM. After occlusion of the vessel by balloon inflation, the NO level immediately increased to the peak value of 76.4 nM and gradually recovered to the baseline level thereafter. Following balloon deflation, the NO level increased gradually and slightly again to 55.2 nM and then recovered to the baseline level. B. A 61 year-old male with a decreased FMD value (2.6%). The baseline circulating NO level was 80.6 nM. Despite vessel occlusion by balloon inflation, the NO level increased only slightly to 81.8 nM. Moreover, there was no noteworthy change in the NO level following deflation of the balloon.

Conclusion

We describe a novel approach to evaluate the vascular endothelial response to ischemia/reactive hyperemia based upon blood NO kinetics using a catheter-based NO sensor with occlusion/release of blood flow with a balloon. Although this system currently has many problems that remain to be resolved, we believe that when it is successfully improved, the clinical application of this system will provide with novel information regarding vascular endothelial function.

Conflicts of Interest

There is no conflict of interest to disclose.

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Obstructive sleep apnea as a risk factor for the onset and progression of aortic dissection

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Abstract:

Background: Aortic dissection is thought to develop and progress due to hypertension and atherosclerosis, but the detailed mechanisms of the onset and progression are still unknown. In this study, we investigated the relationship between type of aortic dissection and the atherosclerotic risk factors including obstructive sleep apnea (OSA) and discussed potential mechanisms. Methods: There were 52 consecutive patients with aortic dissection who were admitted to our hospital, and a sleep study was performed to look for OSA in 42 of them (27 men and 15 women, age: 67 ± 12 years, BMI: 24 ± 4, DeBakey type I: n=6, type IIIa: n=7, type IIIb: n=29). Results: In the 42 patients who had a sleep study, OSA was seen in 36 patients (86%). OSA was more frequent in type IIIb (n=27) than in type IIIa (n=4) aortic dissection (93% vs 57%, p=0.01). Univariate logistic regression analysis indicated that the presence of OSA could distinguish type IIIb from IIIa (odds ratio: 10.125, 95% confidence interval: 1.272-80.623, P=0.029). Conclusion: OSA was frequently associated with aortic dissection and its prevalence was higher in type IIIb than type IIIa, suggesting that OSA may be associated with the development and progression of aortic dissection.

Key words: Aortic dissection, Atherosclerosis, Obstructive sleep apnea, Risk factor

Introduction

Aortic dissection is a life-threatening disease caused by a tear in the intimal layer of the aorta or bleeding within the aortic wall, resulting in the dissection of the layers of the aortic wall. Aortic dissection is most common in patients 65-75 years old, with an incidence of 0.035% per year in this population. Except for genetic connective tissue disorders, such as Marfan syndrome, Ehlers-Danlos syndrome or Takayasu disease, aortic dissection is thought to develop and progress due to hypertension and atherosclerosis, but the detailed mechanisms of its onset and progression are still unknown.

Obstructive sleep apnea (OSA) is characterized by repetitive partial or complete obstruction of the pharynx during sleep. Despite increasing breathing efforts, upper airway collapse results in episodes of obstructive hypopnea or apnea affecting sleep architecture and the entire body via both immediate and long-term mechanisms. OSA has been confirmed to be a causal factor in the pathogenesis of hypertension and atherosclerosis. In addition, OSA is thought to be related to aortic disease including aortic dissection. However, the pathophysiological role of OSA in the onset and progression of aortic dissection is not well understood.

In the present study, we investigated the prevalence of OSA in patients with aortic dissection and tried to elucidate the clinical significance of OSA as a complication of aortic dissection.
Methods

Study protocol

We recruited 52 consecutive patients with acute aortic dissection who were admitted to our hospital between April 2013 and March 2016. The diagnosis and DeBakey type classification of aortic dissection was based on contrast-enhanced computed tomography. Patients who had been diagnosed with clinical OSA before admission were excluded. Of these 52 patients, we performed a sleep study in 42 patients (27 men and 15 women, age: 67 ± 12 years, BMI: 24 ± 4, DeBakey type I: n=6, type IIIa: n=7, type IIIb: n=29) for screening of subclinical OSA as a complication. In the 6 patients with DeBakey type I dissection, 4 underwent emergent surgical treatment. In these patients, the sleep study was performed only after there was a stable post-operative status. The remaining 2 patients with type I dissection and 36 with type III dissection underwent conservative treatment with antihypertensive therapy, and a sleep study was performed after the acute phase had passed. We analyzed clinical features in these patients. The local institutional review board approved the study protocol, and written informed consent was obtained from each patient.

Sleep study

Overnight pulse oximetry was performed while the patients were breathing room air under stable conditions as a screening test for sleep-related breathing disorders. An oxygen saturation monitor (Pulsox-M24®, Konica Minolta Sensing Inc., Osaka, Japan) was attached to the left fourth finger to determine oxygen saturation (SpO2) and pulse rate from 10 pm to 6 am. The frequency of reductions in SpO2 by ≥ 3% per hour (3% oxygen desaturation index: 3% ODI) and the lowest SpO2 were used as parameters of sleep-related breathing disorders. For the patients who had ≥ 5 ODI events, portable polysomnography with electroencephalography (Sleep Watcher®, Compumedics Ltd, Abbotsford, Australia) was performed to assess OSA. The parameters were analyzed by 2 experienced technicians who were unaware of the study design. A respiratory amplitude reduction ≥ 50% was defined as hypopnea and ≥ 80% as apnea, and the number of apnea or hypopnea events/hour was determined as the apnea-hypopnea index (AHI). OSA was defined as an AHI ≥ 5, based on the recommendation of the American Academy of Sleep Medicine Task Force.

Assessment of clinical features

Prevalence of atherosclerotic risk factors such as smoking habits, hypertension, diabetes mellitus and dyslipidemia, and underlying medications at the onset of aortic dissection were assessed as categorical variables via the patients’ history and medical examinations. Body mass index was calculated as weight (kg)/[height (m)]². Blood pressure, fasting blood glucose and lipid profiles including low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride at admission were assessed. Hypertension was defined as pointing out at previous health check or by medical institutions somewhere, receiving antihypertensive medications, or systolic blood pressure (SBP) > 140 mmHg and/or diastolic blood pressure (DBP) > 90 mmHg measured in the supine position at admission. Diabetes was defined as a fasting blood glucose level ≥ 126 mg/dl and/or a hemoglobin A1c level ≥ 6.5% and/or a random blood sugar level ≥ 200 mg/dl and/or receiving anti-diabetic agents. The LDL-cholesterol level ≥ 140 mg/dl, HDL-cholesterol level ≤ 40 mg/dl, or triglyceride level ≥ 150 mg/dl and/or receiving lipid lowering drugs were included in dyslipidemia. History of coronary artery disease and stroke was also assessed.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD) for continuous variables or as the number (percent) of patients for categorical variables. Normality for the distribution of continuous variables was assessed using the Shapiro-Wilk test. Comparisons between two groups were performed using an unpaired t test for continuous variables and a Chi-square test for categorical variables. The comparisons among 3 groups were performed using one-way analysis of variance followed by a post-hoc Bonferroni test for continuous variables and Chi-square test for categorical variables. Univariate logistic regression analysis was performed to determine the variables that could distinguish type IIIb from type IIIa. All statistical analyses were performed using the statistical package for Social Science (Dr. SPSS II for Windows, SPSS Inc., Tokyo, Japan). P<0.05 was considered significant.

Results

In a total of 42 patients with aortic dissection who had a sleep study, OSA was seen in 36 patients (86%), in whom AHI was 34 ± 24. In these patients, mild OSA (AHI< 15) was seen in 8 patients, moderate OSA (15 ≤ AHI< 30) in 10, and severe OSA (30 ≤ AHI) in 18. There were no differences in age, gender, prevalence of current smoking, diabetes and dyslipidemia between the 36 patients with OSA and the 6 without OSA. Blood pressure and lipid profiles at admission were also comparable between the 2 groups with and without OSA. BMI and the prevalence of hypertension were higher in patients with than without OSA. Although a history of coronary artery disease was comparable between the two groups, a history of stroke was more frequent in patients without OSA. Regarding DeBakey type, 75% of patients with OSA showed type IIIb, whereas 50% of patients without OSA showed type IIIa (Table 1).

Among the patient groups with type I, IIIa and IIIb, there were no differences in age, gender, prevalence of current smoking, hypertension and dyslipidemia. Blood pressure and lipid profiles at admission were also comparable among the 3 groups. The BMI was higher in the group with type I than the type IIIa and type IIIb groups. When the BMI was com-
pared between type IIIa and type IIIb groups, it was higher in the type IIIb group. The prevalence of diabetes was higher in the type I than the type IIIb group. Regarding underlying medications, the use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers and that of statins were comparable among the 3 groups. However, the use of calcium channel blockers was more frequent in the type I and type IIIa groups, compared with type IIIb group. The use of anti-diabetic drugs was more frequent in the type I group, compared with type IIIb group. Although a history of coronary artery disease was comparable among the 3 groups, a history of stroke was more frequent in the type IIIa than the type IIIb group. Regarding underling medications, the use of angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CAD, coronary artery disease

Discussion

In this small cohort study, we demonstrated that OSA was highly prevalent (86%) in patients with acute aortic dissection. The prevalence of OSA was higher in DeBakey type IIIb than type IIIa. In addition, the presence of OSA could distinguish between type IIIa and IIIb in univariate analysis. These results suggest the presence of OSA might be related to the development and progression of aortic dissection.

The relationship between aortic dissection and OSA has not been assessed widely in large scale epidemiological studies. In several small cohort studies, however, a higher prevalence of OSA was observed in patients with aortic dissection than in control subjects. Naito et al. demonstrated that the 3% ODI, a major component of OSA, was closely associated with aortic dissection. Hata et al. observed a higher prevalence of sleep disorders including OSA in younger active patients with aortic dissection. Zhang et al. demonstrated that OSA was highly prevalent and independently associated with Stanford type B (i.e., DeBakey type III aortic dissection). In addition, Wan et al. demonstrated that the severity of OSA was significantly associated with an increased risk of partial false lumen thrombosis in type III aortic dissection. The results of our study support the results of these previous studies. However, our finding of a higher prevalence of OSA in type IIIb than type IIIa is a novel finding. Recently, Zhou et al. performed a meta-analysis of observational studies using a systematic search of PubMed, Embase and Cochrane Library. In their meta-analysis, information on 424 cases of aortic dissection among 56,291 patients was obtained from one cohort, four case-control and

### Table 1. Baseline characteristics in patients with and without obstructive sleep apnea

|                                | Patients with OSA (n=36) | Patients without OSA (n=6) | P value |
|--------------------------------|--------------------------|---------------------------|---------|
| Age: yrs                       | 67±12                    | 72±13                     | 0.319   |
| Male gender: n (%)             | 24 (67)                  | 3 (50)                    | 0.440   |
| BMI: kg/m²                      | 24±4                     | 20±3                      | 0.040   |
| Current smoking: n (%)         | 23 (64)                  | 4 (67)                    | 0.965   |
| Hypertension: n (%)            | 27 (75)                  | 2 (33)                    | 0.029   |
| Diabetes: n (%)                | 3 (5)                    | 0 (0)                     | 0.469   |
| Dyslipidemia: n (%)            | 9 (25)                   | 3 (50)                    | 0.237   |
| SBP at admission: mmHg         | 170±32                   | 156±12                    | 0.295   |
| DBP at admission: mmHg         | 85±19                    | 85±19                     | 0.406   |
| LDL-cholesterol: mg/dl         | 111±33                   | 117±50                    | 0.693   |
| HDL-cholesterol: mg/dl         | 49±14                    | 53±12                     | 0.536   |
| Triglyceride: mg/dl            | 135±115                  | 127±97                    | 0.877   |
| Underling medications: n (%)   |                          |                           |         |
| ACE inhibitors/ARBs            | 13 (36)                  | 1 (17)                    | 0.350   |
| Calcium channel blockers       | 12 (33)                  | 4 (67)                    | 0.120   |
| Statins                        | 12 (33)                  | 2 (33)                    | 1.000   |
| Anti-diabetic drugs            | 3 (8)                    | 1 (17)                    | 0.520   |
| History of CAD: n (%)          | 1 (3)                    | 1 (17)                    | 0.154   |
| History of stroke: n (%)       | 7 (17)                   | 4 (67)                    | 0.016   |
| DeBakey type: n (%)            |                          |                           | 0.050   |
| I                              | 5 (14)                   | 1 (17)                    |         |
| IIIa                           | 4 (11)                   | 3 (50)                    |         |
| IIIb                           | 27 (75)                  | 2 (33)                    |         |

OSA, obstructive sleep apnea; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CAD, coronary artery disease
Obstructive sleep apnea as a risk factor for the onset and progression of aortic dissection

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Two cross-sectional studies. As a result, OSA was associated with an overall significant 60% increase in the risk of aortic dissection, compared with the absence of OSA (odds ratios 1.60; 95% confidence interval: 1.01-2.53), and there was a significantly higher AHI (mean difference: 10.71; 95% confidence interval: 7.46-13.96) in those with OSA. Therefore, the presence of OSA appears to be a major risk factor for the development and progression of aortic dissection and may also be related to its pathogenesis.

The underlying mechanisms by which OSA may promote the development and progression of aortic dissection are not fully understood. Studies investigating the impact of OSA on aortic disease have discussed several pathogenetic mechanisms, including intrathoracic pressure changes leading to shear stress on the aortic wall, intermittent hypoxia leading to oxidative stress and sympathetic stimulation, and arousal-induced sympathetic activation with subsequent repetitive blood pressure surges. OSA results in an inspiratory effort against an occluded airway, producing negative intrathoracic pressure swings. These forces support an elevated blood pressure that stretches the aortic wall where blood pressure surges are highest and lead to pathological shear stress. It is known that systolic blood pressure promotes the fragmentation of fibrin and collagen deposition, leading to stiffening of the aortic wall. Additional physical dilation or shear stress itself might cause development of aortic dissection. These mechanisms could explain the close association of OSA with DeBakey type III, because the descending aorta might be susceptible to the influence of intrathoracic pressure. A higher prevalence of OSA, especially in type IIIb aortic dissection in our results, suggests that OSA might accelerate the progression of aortic dissection.

In a mouse model, intermittent hypoxia increased chemoreflex and depressed baroreflex sensitivity, resulting in sympathoadrenal hyperactivity. In humans, intermittent hypoxia due to OSA has been proposed to induce hypertension via increased release of vasoactive substances and peripheral chemoreceptor activation. In OSA patients, the sever-
ity of OSA is independently associated with oxidative stress caused by apneic events[17]. In several studies, the decrease in arterial oxygen saturation measured by pulse oximetry was correlated with the degree of aortic stiffness[18,19], which might be associated with development of aortic dissection. In OSA, each apneic event is accompanied by an arousal, which leads to transient increases in blood pressure despite a fall in cardiac output[20]. These events are caused by sympathetic vasoconstriction and their consequences include catecholamine release[21], endothelial dysfunction[22] and probably aortic dissection[23]. However, these mechanisms are somewhat speculative, so further investigation is needed to establish the causal relation between OSA and aortic dissection.

Continuous positive airway pressure (CPAP), a standard treatment for OSA, can reduce acute hemodynamic changes during sleep and inhibit atherosclerosis progression[24]. To fully understand the effect of CPAP treatment, it is important to know whether aortic dissection is caused by the acute effects of apneic episodes or the chronic structural and autonomic changes in OSA patients[25]. CPAP treatment can abolish the acute blood pressure surges and/or intrathoracic pressure swings and lower diurnal blood pressure in OSA patients. However, there is no evidence whether CPAP treatment prevents the onset of acute aortic dissection. Even if CPAP treatment is effective for disease prevention, patient adherence to this therapy is only about 30-60%, and this is a significant limitation[26]. Further investigation is needed to determine the value of CPAP treatment in the primary and secondary prevention of aortic dissection in OSA patients.

### Study limitations

The present study has several potential limitations. The biggest limitation is that sample size was too small to draw a conclusion regarding the association between aortic dissection and OSA. Also, in the 42 study patients, 29 patients (69%) had DeBakey type IIIb, but there were only a few type I and IIIa patients (6 patients: 14% and 7 patients: 17%, respectively). Therefore, it is possible that the difference in the prevalence of OSA between type IIIa and IIIb patients might be due to a type I error because of the small sample size. Although in the present study we performed univariate logistic regression analysis, multivariate analysis data are absent. It is important to note that this study was merely an observational study, in which we showed a high prevalence of OSA in patients with aortic dissection. A prospective event-driven study, such as registration research of OSA patients, would be useful to assess whether OSA is an independent risk factor for aortic dissection. In addition, more precise information regarding aortic dissection (onset time, blood pressure and/or hemodynamics during sleep) should be analyzed, although we could not collect such data in the present study.

### Conclusion

OSA was frequently associated with aortic dissection, and its prevalence was higher in type IIIb than type IIIa, suggesting that OSA may be associated with the development and progression of aortic dissection.

### Conflicts of Interest

Teruo Inoue has received honorariums from Mochida and Bayer; research grants from Astellas, Abbott Vascular, Boehringer Ingelheim, Bayer, Boston Scientific, Sanwa Kagaku Kenkyusho, Teijin Pharma, Takeda, Mitsubishi Tanabe and Medtronic. For the remaining authors none were declared.

### Table 3. Univariate logistic regression analysis to distinguish DeBakey type IIIb from type IIIa aortic dissection

|                | Wald $\chi^2$ | Odds ratio | 95% confidential interval | P value |
|----------------|--------------|------------|---------------------------|---------|
| Age            | 1.840        | 0.938      | 0.855-1.029               | 0.175   |
| Male gender    | 0.088        | 0.760      | 0.124-4.644               | 0.766   |
| BMI            | 4.719        | 1.426      | 1.035-1.963               | 0.025   |
| Current smoking| 0.088        | 0.760      | 0.124-4.644               | 0.766   |
| Hypertension   | 0.743        | 0.370      | 0.039-3.545               | 0.389   |
| Dyslipidemia   | 0.953        | 0.424      | 0.076-2.374               | 0.329   |
| SBP at admission| 0.029      | 1.003      | 0.973-1.033               | 0.864   |
| DBP at admission| 0.481      | 0.978      | 0.917-1.042               | 0.488   |
| LDL-cholesterol| 1.251        | 0.987      | 0.966-1.010               | 0.263   |
| HDL-cholesterol| 0.168        | 0.988      | 0.933-1.046               | 0.682   |
| Triglyceride   | 0.063        | 1.001      | 0.993-1.009               | 0.802   |
| ACE inhibitors/ARBs | 0.003   | 0.952      | 0.153-5.943               | 0.958   |
| Calcium channel blockers | 7.141 | 0.043      | 0.004-0.434               | 0.0075  |
| Statins        | 2.660        | 0.239      | 0.043-1.335               | 0.103   |
| History of stroke| 7.633      | 0.064      | 0.009-0.450               | 0.0057  |
| OSA            | 4.783        | 10.125     | 1.272-80.623              | 0.029   |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; OSA, obstructive sleep apnea
Obstructive sleep apnea as a risk factor for the onset and progression of aortic dissection

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Influence of Maintenance of Face-Down Positioning on Physiological and Psychological Factors

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Abstract:

Background: The purpose of this study was to investigate the influence of maintenance of Face-down positioning (FDP) on physiological and psychological responses of patients who require FDP after vitrectomy. Methods: The study included 22 healthy volunteers (21.9±2.6 years old) and two conditions: maintenance of FDP or maintenance of the sitting chair positioning (SCP). Study participants were evaluated for Profile of Mood States (POMS), subjective pain, blood pressure, heart rate, heart rate variability, shoulder muscle rigidity, skin temperature, and skin blood flow. Results: The change in POMS “fatigue” score before and after positioning was significantly greater in FDP than in SCP. Subjective pain increased over time in both FDP and SCP, but the increase was more pronounced in FDP and an interaction was observed between the neck and shoulders. Shoulder skin temperature decreased significantly over time in both FDP and SCP. In contrast, back skin temperature increased significantly in SCP, but decreased significantly in FDP. Conclusions: The psychological and physiological burdens are significant in FDP. Our results suggest that proactive support for relieving pain and promoting blood flow from the neck to the back region may be helpful in patients maintaining FDP.

Key words: Face-down positioning, Chair sitting positioning, After vitrectomy, Young healthy adults

Background

Face-down positioning (FDP) is a common posture assumed when using a laptop computer or smartphone. However, FDP is burdensome; flexing the neck 15-60 degrees for long periods of time appears to cause chronic neck and shoulder pain in computer and smartphone users.¹² On the other hand, patients with retinal diseases such as retinal detachment and macular holes are often forced to maintain FDP after vitrectomy. Vitrectomy often involves intracocular tamponade using expansible gases such as sulfur hexafluoride (SF6) or propane octafluoride (C3F8), and FDP is required to buoy the gas to mechanically push the retina into the pigment epithelium after surgery.³⁵ Recently, the duration of FDP after vitrectomy was shortened after it was reported that FDP is an intense burden for patients, but maintenance of FDP for as long as 10 days is still recommended.⁶⁰ Several studies have reported that patients who must maintain FDP complain of physical pain including headaches, neck pain, shoulder pain, and backache, as well as mental pain including anxiety, stress, and sleeplessness.³⁰,¹¹ Some strategies to reduce the pain caused by FDP include assistance devices such as pillows and desks,¹²,¹³ and aromatherapy massage can reduce short-term FDP-related neck and shoulder pain. However, no concrete investigations have examined how FDP influences the physiological and psychological responses of patients after vitrectomy. We thus sought to evaluate the effectiveness of mitigation methods based on both subjective and objective measures.

The purpose of this study was to investigate the physiological and psychological responses of patients who must maintain FDP after vitrectomy.
Methods

This study was conducted between August and December 2014 in the Department of Medicine, Saga University (room temperature 26.4±0.6 degrees Celsius, humidity 49.6±6.3%, mean±SD). The study was conducted according to the declaration of Helsinki and was approved by the ethical Committee of the Department of Medicine, Saga University. Written informed consent was obtained from all participants.

Participants

The peak incidence of retinal detachment, which is an indication for vitrectomy, occurs in the 3rd and 7th decades of life(14-16), so this study targeted adults in their 20s. Healthy adult volunteers were recruited via poster and flyer. Inclusion criteria included the following: 1) clear understanding of the study instructions, 2) physically independent, 3) able to maintain a static position, 4) no use of cardiovascular medications, and 5) no wounds, disorders, or pain in the neck, shoulder, waist, or hip joints. Participants were instructed to sleep well the night before the test, and avoid high-intensity physical activity, alcohol, smoking, and excessive eating and drinking the day before and the day of the test. Participants were instructed to stop oral intake two hours before the test. Participants self-declared compliance with the instructions prior to testing. Power calculations, estimated from our previous study using change in subjective pain as the primary outcome, indicated that 24 volunteers were required to observe a significant between-group difference. However, two people were excluded after the interview, and 22 subjects participated in the study (11 males: 22.6±2.6 years old, height 167.3±6.0, body weight 61.5±6.3 kg, 11 females: 21.1±2.4 years old, height 158.7±4.9 cm, body weight 54.1±7.3 kg).

Positioning

In a crossover design, all participants were required to complete two days of testing. Maintenance of FDP or sitting chair positioning (SCP) occurred on different days, and the order of the conditions was randomly assigned. As shown in Figure 1, participants wore the same garments (100% cotton T-shirt and clothing) and measuring instruments. Each participant rested 30 minutes and then held their assigned posture for 90 minutes. Heart rate (HR), heart rate variability (HRV), skin temperature, and skin blood flow rate were measured continuously, while blood pressure (BP), shoulder muscle rigidity, and visual analog scale (VAS) were measured every 15 minutes from baseline (0 minutes) to 90 minutes. The Profile of Mood States (POMS) was completed at baseline and 90 minutes later. In FDP, participants sat at the edge of a bed with their head on a cushion placed on the over-bed table. In SCP, participants sat in a chair and watched a DVD (Ocean world, Jean-Jacques Mantell, 2012) with playback equipment placed on the over-bed table.

Psychological Assessment

In this study, the brief Japanese version of POMS (Kaneko Shobo, Japan) and subjective pain with VAS were used as psychological evaluations before and after the protocol. POMS is a popular tool used widely among psychologists and scientists from many fields that consists of six mood-state factors constituting affect, represented by six subscales: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor, Fatigue, and Confusion. Participants were asked to answer 30 questions on current mood using a 5-point scale ranging from 0 (not at all) to 4 (extremely). They were also asked to indicate pain in the neck, shoulder, and back using a 10-point scale ranging from 0 (no pain at all) to 10 (unacceptable pain) in a continuous 10-cm VAS.

Heart rate and heart rate variability

HR was calculated using the HRV analysis program (MEMcalc/TARAWA; GMS, Japan) by capturing the RR interval on an electrocardiogram obtained from the HR monitor (BSM-2401; NIHONKODEN, Japan) at a sampling interval of 250 Hz. The average HR was calculated every minute. HRV was calculated by frequency analysis based on the maximum entropy method using the same HRV analysis program. The target frequency was 0.04 to 0.40 Hz, and the high-frequency component (HF) (0.15 to 0.40 Hz), low-
frequency component (LF) (0.04 to 0.15 Hz), and component ratios between LF and HF (LF/HF) were obtained every minute. The HF component was used as an index of parasympathetic activity and LF / HF was used as an index of sympathetic nerve activity.

Blood pressure
BP was measured on the upper arm by sphygmomanometer (HEM-7210; Omron, Japan).

Shoulder muscle rigidity
Muscle rigidity of the right and left shoulder regions was measured by a muscle rigidity meter (NEUTONE TDM-Z1, TRY-ALL, Japan) midway between the right and left neck bottom and the seventh cervical vertebra, and a bilateral mean was calculated every 15 minutes.

Skin blood flow
Skin blood flow in the back (midpoint of the line connecting the left iliac crest and the spine) and in the left shoulder (scapula) was measured by laser Doppler flowmetry (ALF-21, ADVANCE, Japan) every 15 minutes during the study.

Skin temperature
Skin temperature was measured by thermistor probes at four body sites: right and left shoulder (scapula), and right and left back (midpoint of the line connecting the iliac crest and spine). Skin temperature was monitored every minute by a data logger (LT-8A, Gram Corporation, Japan), and the left and right temperatures were averaged every 15 minutes.

Statistical analysis
Data were analyzed using IBM SPSS Statistics version 22. All data are expressed as the mean±standard deviation, and P<0.05 was considered significant. A paired t-test was used to compare the POMS scores before and after the study, and Student’s t-test was used to compare the change in POMS scores before and after the study. For time course analyses, average values were assessed at baseline, and at 15, 30, 45, 60, 75, and 90 minutes. Physiological indices were calculated as the ratio of each time point relative to baseline, and VAS scores were calculated as the difference between each time point and baseline. The time course was analyzed with a repeated-measures one-way analysis of variance (ANOVA), and differences between baseline and each point were evaluated by Dunnett’s one-to-many post-hoc test. Comparison of the time course between the two conditions was analyzed by a two-way repeated measure ANOVA, and comparisons at each point were made using Student’s t-test with the Bonferroni correction.

Results

POMS
The “Tension-Anxiety” score was significantly lower after the positioning test (P=0.023) in SCP (Figure 2). The “Vigor” score was significantly lower after the positioning test in both FDP and SCP (P<0.001 in both). The “Fatigue” score was significantly higher after the positioning test in both FDP and SCP (P<0.001 and P=0.003, respectively). Finally, the change in “Fatigue” score was significantly higher in FDP than in SCP (P=0.027).

Subjective pain (VAS)
The subjective pain time course is shown in Figure 2. In the neck, shoulder, and back region, VAS scores significantly increased over time in both FDP and SCP (P<0.001 in all cases). In the neck and shoulder, significant interactions (P<0.001 and P=0.013, respectively) were observed between position and time course. Significant differences were found between positions at each point from 15 to 90 minutes in the neck, and from 15 and 60 minutes in the shoulder. In the back region, no significant interaction was observed (P=0.434).

HR and HRV
Baseline HR was 69.7±7.7 beats per minute (bpm) in FDP and 67.8±7.7 bpm in SCP. HR in FDP slightly increased from baseline to 90 minutes (ratio 1.04, HR at 90 minutes 72.8±15.4 bpm), but did not significantly change over time. Likewise, HR in SCP slightly increased from baseline to 90 minutes (ratio 1.08, HR at 90 minutes 73.1±16.5 bpm), but did not significantly change over time. There was no significant interaction between position and time course (P=0.404) (see Figure 3).

Baseline HF was 736.6±627.7 msec⁻¹ in FDP and 917.0±1080.3 msec⁻¹ in SCP, and LF/HF was 2.8±2.4 in FDP and 2.5±1.7 in SCP. The HRV time course is shown in Figure 3. HF in SCP significantly decreased (P=0.001) over time. An interaction was observed between position and time course (P=0.008). There were significant differences between FDP and SCP at 30 minutes (P=0.012). LF / HF significantly increased in SCP (P=0.001) over time, and an interaction was observed between position and time course (P=0.016). Likewise, there were significant differences between FDP and SCP at 90 minutes (P<0.001).

Blood pressure (BP)
Baseline systolic BP was 104.3±10.5 mmHg in FDP and 108.9±12.3 mmHg in SCP, and baseline diastolic BP was 61.3±6.1 mmHg in FDP and 61.9±7.5 mmHg in SCP. As shown in Figure 3, systolic BP initially decreased and then increased in both FDP and SCP (P=0.003 and P=0.001, respectively), but there were no significant interactions between position and time course (P=0.280). Likewise, diastolic BP significantly increased after an initial decline in
A: Profile of Mood States

![Graphs showing time courses of psychology responses](image)

Figure 2. Time courses of psychology responses
Values are mean±SD, FDP: face down positioning, SCP: sitting chair positioning
a: Profile of Mood States, Δ: difference between before and after, * P<0.05, ** P<0.01, *** P<0.001 before vs. after score, § P<0.05 FDP vs. SCP
b: Subjective pain, * P<0.05, ** P<0.01, *** P<0.001, vs. baseline, § P<0.05, §§ P<0.01, §§§ P<0.001, FDP vs. SCP

Shoulder muscle rigidity

Shoulder muscle rigidity at baseline was 28.9±8.1 points in FDP and 28.7±7.8 points in SCP. Shoulder muscle rigidity in FDP slightly increased from baseline to 90 minutes (ratio 1.035), and a significant difference (one-way ANOVA, P=0.020) was observed, but there was no significant difference in the post-hoc analysis. In SCP, there was a slight increase (ratio 1.091) from baseline to 90 minutes, but no significant difference was observed. There was no interaction between position and time course (P=0.279).

Skin blood flow

Skin blood flow in the shoulder was analyzed in only 17 participants due to malfunction of the measuring equipment. Back skin blood flow was analyzed in all 22 participants. Baseline skin blood flow in the shoulder was 3.3±1.1 ml/
min/100 g in FDP and 3.0±1.0 ml/min/100 g in SCP, and baseline skin blood flow in the back was 2.6±0.7 ml/min/100 g in FDP and 2.9±1.5 ml/min/100 g in SCP.

Blood flow in the shoulder decreased significantly over time in FDP \((P<0.001)\), but increased at 45 minutes then decreased at 90 minutes in SCP \((P<0.001)\) (Figure 3). In addition, a significant interaction was observed between position and time course \((P=0.005)\). However, there was no significant difference between FDP and SCP at any time point. Blood flow in the back did not significantly change over time in either FDP or SCP, and no significant difference between positions was observed. Furthermore, there was no significant interaction between position and time course \((P=0.279)\).

**Skin temperature**

Baseline skin temperature of the shoulder was 35.5±0.6°C in FDP and 35.5±0.5°C in SCP, and baseline skin temperature of the back was 35.2±0.7°C in FDP and 35.1±0.7°C in SCP.

Shoulder skin temperature in both FDP and SCP significantly decreased over time \((P<0.001)\), and there was no significant interaction between position and time course \((P=0.195)\) (Figure 3). Back skin temperature significantly decreased over time in FDP \((P<0.001)\). In contrast, back skin temperature in SCP initially decreased and then increased, and the change was significant \((P<0.001)\). Furthermore, there was a significant interaction between position and time course \((P<0.001)\). Significant differences were found between positions at each point from 30 to 90 minutes \((P=0.012, P<0.001, P<0.001, P<0.001, P<0.001)\), respectively.

**Discussion**

In this study, we investigated the influence of maintaining FDP on physiological and psychological responses by comparing FDP with SCP. Psychologically, participants felt more fatigued in FDP than in SCP, and neck and shoulder pain increased markedly over time in FDP. In this study, FDP was based on the posture required after vitrectomy, so participants were instructed to direct their eyes downward and bend forward about 60 degrees, which is the maximum curvature at which the neck was maintained. Therefore, the
significant interaction was observed. Okubo et al. reported the force on the adult neck is 10-12 pounds (4-6 kg) at 0 degrees, 27 pounds (12 kg) at 15 degrees forward bend, and 60 pounds (27 kg) at 60 degrees forward bend. In this study, in FDP the forehead was supported by a cushion, so it is unlikely that the force on the neck was 27 kg. However, compared to SCP, FDP was a heavy burden and led to a feeling of psychological fatigue.

We further investigated the physiological responses in FDP based on HR, HRV, shoulder muscle rigidity, skin temperature, and skin blood flow. Both FDP and SCP led to a slight increase in HR over time, but there was no significant change and no interaction was observed. We previously reported that maintenance of FDP for 120 minutes in healthy adults caused a significant rise in HR by 7% at 120 minutes compared to baseline. In this study, however, maintaining the posture for 90 minutes did not appear to significantly change HR. In a clinical setting, patients maintain FDP after vitrectomy for about 90 to 120 minutes, and our result suggested that the posture should not exceed 90 minutes.

We found no significant change in HRV over time in FDP. However, compared with SCP, HF, a marker of parasympathetic nervous activity, was high, and LF/HF, a marker of sympathetic nerve activity, was low, and a statistically significant interaction was observed. Okubo et al. reported that sympathetic nervous activity increases and parasympathetic nervous activity decreases in a sitting position in which the neck is not supported, compared with the supine position and with a sitting position in which the back and neck are supported. Therefore, it is likely that that SCP caused a decrease in HF and an increase in LF/HF that did not occur in FDP.

Both FDP and SCP initially decreased BP, but then increased BP by 90 minutes. No interaction was observed. However, in FDP, diastolic BP decreased markedly and was significantly lower than baseline until 15 to 75 minutes. Since peripheral vasoconstriction is dominated by sympathetic nervous activity, it is possible that low LF/HF in FDP caused peripheral vascular dilation and diastolic BP reduction. However, details of this finding need to be investigated further.

Shoulder skin temperature significantly decreased relative to baseline in both FDP and SCP until 15 to 90 minutes. Similarly, shoulder skin blood flow significantly decreased from 30 to 90 minutes. Incidentally, in SCP, the value at 45 minutes was elevated, but the individual variation in values was large and this elevation was not significant. Neither FDP nor SCP allowed shoulder movement for 90 minutes, so skin blood flow decreased and skin temperature, likewise, decreased. In support of these findings, particularly in FDP, there was a slight but significant rise in shoulder muscle rigidity. On the other hand, in the back region, there were conflicting results and significant interaction. In FDP, skin temperature significantly decreased until 90 minutes, but in SCP, skin temperature initially decreased then significantly increased until 90 minutes. Therefore, when patients maintain FDP, it is important to support their shoulder and back regions, which are particularly susceptible to pain. The method of support should be an intervention that raises skin temperature and skin blood flow, e.g. massage, hot compress, stretch etc.

This study has several limitations. Generally, sympathetic nervous activity is activated by pain stimulus. Although there was a tendency for pain and BP and autonomic functions, there were no significant correlations in this study (HF; $P=0.153$, LF/HF; $P=0.151$, SBP; $P=0.299$, DBP; $P=0.217$). It is thought that there were few samples and did not lead to such a drastic change. In addition, the pain on FDP in this study was small increase, thus it is possible that it did not become a significant change to sympathetic nerve activity. SCP, which is often done in daily life, was used as a control in order to examine the psychological and physiological influence of SCP. However, since SCP was accompanied by DVD viewing, it induced a cervix tilting posture of about 15 degrees, and the influence of this posture on autonomic nervous activity was observed. Furthermore, since DVD viewing occurred only in SCP, the possibility of an influence of DVD viewing cannot be excluded. Moreover, it is highly probable that changes in fatigue level, muscle rigidity, and psychogenic factors would be observed if the positions were maintained for longer periods of time that more realistically reflected the posture requirements after vitrectomy.

**Conclusions**

In this study, the influence of FDP on psychological and physiological factors was examined by comparing FDP with SCP. We found that FDP causes significant psychological burdens including an increase of fatigue, and neck and shoulder pain, as well as physiological burdens such as decreased skin temperature and blood flow in the shoulder and back and increased shoulder muscle rigidity. Our results suggest that proactive support for relieving pain and promoting blood flow from the neck to the back region is necessary when maintaining FDP.

**Abbreviations**

ANOVA: Analysis of variance; BP: Blood Pressure; FDP: Face-Down Positioning; HF: High Frequency component; HR: Heart Rate; HRV: Heart Rate Variability; LF: Low Frequency component; LF/HF: component ratio between LF and HF; POMS: Profile of Mood States; SCP: Sitting Chair Positioning; VAS: Visual Analog Scale

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Availability of data and materials

The datasets analyzed in this study are not publicly available due to a privacy policy but are available from the corresponding author on reasonable request.

Authors’ contributions

CF conceived and designed the study, performed the experiments and the statistical analysis, and drafted the manuscript. TN helped to carry out the experiments. JO helped to perform the data analysis. JO, MA, NK, and KN revised the manuscript critically. All authors have given their final approval.

Ethics approval and consent to participate

Participants provided written informed consent to participate in this study after receiving a detailed explanation regarding the purpose, method, freedom of participation and interruption, maintenance of privacy, and risks involved with participation. The experimental procedures of the study were conducted according to the declaration of Helsinki and were approved by the ethical Committee of Department of Medicine, Saga University (approval number 26-34), Saga, Japan.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare that they have no competing interests.

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The relationship between estimated salt intake and central systolic blood pressure in Japanese outpatients with hypertension

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Abstract:
Background: Recent reports suggest that central blood pressure (BP) may be instrumental in the diagnosis and management of hypertension. Several reports have shown an association between salt intake and central hemodynamics, especially central systolic BP; however, this relationship remains unclear in Japanese outpatients with hypertension. Therefore, this study investigated the relationship between central systolic BP and salt intake in Japanese outpatients with hypertension.

Methods: We recruited 141 Japanese outpatients with hypertension. Their daily salt intake was estimated using spot urine samples. Their central systolic BP was measured using an Omron HEM-9000AI device.

Results: The median estimated salt intake was 9.81 (range, 8.34-11.47) g/day. The mean brachial systolic/diastolic BP and central systolic BP were 131.2 ± 16.5 /78.1 ± 10.9 mmHg and 135.6 ± 17.3 mmHg, respectively. The estimated salt intake was divided into four quartiles, with central systolic BP significantly higher in Q3 and Q4 than that in Q1 (P < 0.01). A significant positive correlation was observed between central systolic BP and estimated salt intake (r=0.275, P=0.001). Multiple regression analysis of central systolic BP showed that the estimated salt intake and BMI were significant factors (P = 0.014 and P = 0.027, respectively).

Conclusions: We found that our Japanese outpatients with hypertension consumed higher amounts of salt than the target value recommended by Japanese guidelines. In addition, there was a moderate relationship between central systolic BP and the estimated salt intake. Therefore, a decrease in salt intake is important, even in outpatients with hypertension receiving antihypertensive medication.

Key words: Salt intake, Hypertension, Central blood pressure

Introduction

The number of patients with hypertension has increased to ~43 million in Japan, representing one-third of the Japanese adult population in 20101. Hypertension is a crucial risk factor in the progression of cardiovascular and cerebrovascular diseases and mortality2-4 and appropriate blood pressure (BP) control improves the prognosis of hypertensive patients5-6.

BP measured using the brachial artery has typically been used for patient evaluation and management. However, in recent years, attention has shifted to the central BP, defined as the sum of the forward pressure wave created by ventricular contraction and the pressure wave reflected from the peripheral arterial system. The central BP reflects the true load on the heart, kidneys, and brain and the central blood flow influences the local flow into these vital organs. Recent technological advances have enabled the noninvasive evaluation of pulsatile hemodynamics in the central aorta7-8 and it has become clear that the central BP is more closely related to cardiovascular events and target organ damage compared to brachial BP. The results of several studies have suggested

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that central systolic BP is an important parameter and a prognostic risk factor\(^{2,14}\). Additionally, in patients with similar brachial BP but different central systolic BP, lower central systolic BP levels resulted in more favorable clinical outcomes. These results suggest that central systolic BP has better predictive power for future cardiovascular events than that of brachial BP.

Salt, a ubiquitous food ingredient, is important for both food preservation and taste enhancement, leading to its consumption in large amounts. Epidemiological studies have found that excessive salt intake is associated with high brachial BP and an increased prevalence of hypertension\(^{13,10}\). In a recent Cochrane meta-analysis of data from 35 trials, a 100-mmol reduction in the 24-h urinary sodium level led to a reduction not only in the systolic/diastolic BP of 5.4/2.8 mmHg in hypertensive individuals but also of 2.4/1.0 mmHg in normotensive individuals\(^{17}\). In a study by Cook et al., high sodium intake was associated with an increased risk of mortality and was directly related to total mortality\(^{10}\). Therefore, implementing a salt-restrictive diet should become a target to reduce health problems associated with salt overuse.

The World Health Organization currently recommends limiting salt intake to less than 5 g/day\(^{20}\) and the Japanese guidelines for the management of hypertension 2014 (JSH 2014) recommend limiting salt intake to 6 g/day in patients with hypertension\(^{20}\). However, the actual salt intake among Japanese outpatients with hypertension has not been clearly established. Additionally, several reports have shown an association between salt intake and central hemodynamics\(^{21,22}\), however, the relationship remains unclear in Japanese outpatients with hypertension.

Therefore, this study aimed to investigate the actual salt intake and its relationship to central systolic BP in Japanese outpatients with hypertension.

**Methods**

Patients with essential hypertension who visited the cardiovascular division of Shimane University Hospital between December 2012 and May 2016 were included in this study. The patients’ conditions were stable and they had been taking a stable dosage of antihypertensive medication for at least one month. Patients who had recently changed any medications at the discretion of their attending physicians, those with apparent decompensated heart failure, those with acute or severe chronic renal failure (estimated glomerular filtration rate <15 mL/min/1.73 m\(^2\)), those on hemodialysis, those with infectious disease, and those with atrial fibrillation were excluded. Eventually, 141 outpatients with hypertension (65 men and 76 women; mean age, 67.6 ± 9.4 years) were enrolled in the study.

Body height and weight were measured and body mass index (BMI) (weight [kg]/height [m]\(^2\)) was calculated for all patients.

Patients with systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg and those taking antihypertensive medications were considered to have hypertension. Patients were considered to have diabetes mellitus if their fasting plasma glucose level was ≥126 mg/dL, HbA1c was ≥6.5% in a recent blood sampling test, or if they used anti-diabetic medication. Patients with a low-density lipoprotein cholesterol level ≥140 mg/dL, high-density lipoprotein cholesterol level <40 mg/dL, and triglycerides level ≥150 mg/dL in a recent blood sampling test or those taking anti-dyslipidemic drugs were considered to have dyslipidemia. Those with a uric acid level ≥7.0 mg/dL in a recent blood sampling test or on anti-hyperuricemic medication were considered to have hyperuricemia.

The ethics committee of Shimane University approved this study protocol and written informed consent was obtained from all patients.

**Estimation of salt intake**

The daily dietary salt intake was estimated based on sodium (Na) and creatinine (Cr) concentrations measured in spot urine samples. This approach was selected for convenience because the estimation based on Na and Cr content in 24-h pooled urine, which is the most reliable method of evaluation of salt intake, is difficult to perform in outpatients in routine medical practice. The daily salt intake was measured using Tanaka’s equation\(^{27}\). The estimated 24-h urinary salt excretion (g/day) was calculated as follows: 21.98 × [Na (mEq l\(^{-1}\))/ Cr (mg l\(^{-1}\))] in spot urine × expected 24-h Cr excretion\(^{20}\), where the expected 24-h Cr excretion (mg/day) = −2.04 × age (years) + 14.89 × weight (kg) + 16.14 × height (cm) − 2244.45. The Japanese Society of Hypertension (JSH 2014) recommends the use of Tanaka’s equation for the estimation of salt intake\(^{20}\). Spot urine samples were collected at the time of patient visits, between 08:30 and 11:00 am.

**Measurement of brachial and central systolic BP**

All patients were examined in a quiet temperature-controlled room while in a seated position with their backs supported. After 5 minutes of seated rest, brachial systolic, diastolic, and central systolic BP were measured by a laboratory technician.

An automated device (HEM-9000AI; Omron Healthcare, Kyoto, Japan) was used to simultaneously record radial artery pressure waveforms and brachial BP, while brachial BP was measured using Omron’s built-in oscillometric sphygmomanometer\(^{15}\). This device automatically estimates central systolic BP, augmentation index (AI), and augmentation index normalized to a heart rate of 75 bpm (AIP75) using applanation tonometry of the radial artery. In clinical situations, the procedure is simple and does not require an operator, which facilitates the assessment of central BP. The details of the measurements and reproducibility of this automatic method have been described previously\(^{22,26}\).
Salt and central systolic blood pressure

Table 1. Characteristics of the study participants

|                          | All          |
|--------------------------|-------------|
| Number of patients       | 141         |
| Age (years)              | 67.6±9.4    |
| Gender (female, %)       | 76 (54%)    |
| Body height (cm)         | 158.4±7.9   |
| Body weight (kg)         | 59.1±10.2   |
| Body mass index          | 23.4±3.4    |
| Brachial systolic BP (mmHg) | 131.2±16.5 |
| Brachial diastolic BP (mmHg) | 78.1±10.9  |
| Central systolic BP (mmHg) | 135.6±17.3 |
| Heart rate (beats/min)   | 73.4±11.7   |
| Estimated salt intake (g/day) | 9.8 (8.31-11.47) |

Comorbidities

- Dyslipidemia: 82 (58%)
- Diabetes mellitus: 35 (25%)
- Hyperuricemia: 19 (14%)
- Chronic kidney disease: 16 (11%)
- Coronary heart disease: 24 (17%)

Antihypertensives

- ACEIs and/or ARBs: 82 (58%)
- Ca antagonist: 74 (52%)
- β-blocker: 17 (12%)
- α-blocker: 3 (2%)
- Diuretics: 6 (4%)

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker

Statistical analysis

Continuous data are expressed as means ± SD or as medians (interquartile range), depending on data distribution. Categorical data are expressed as numbers (%). To analyze salt intake as a categorical variable, four quartiles representing estimated salt intake were established. To compare the baseline characteristics of patients assigned to the quartiles, analysis of covariance was used for continuous variables and χ² tests for dichotomous and categorical variables.

SPSS 22.0 for Windows (SPSS Japan, Tokyo, Japan) was used for statistical analysis. All P-values were two-tailed. P-values < 0.05 were considered to indicate statistical significance.

Results

The clinical characteristics of the 141 outpatients are shown in Table 1. The mean age, body height, body weight, and BMI were 67.6 ± 9.4 years, 158.4 ± 7.9 cm, 59.1 ± 10.2 kg, and 23.5 ± 3.4 kg/m², respectively. Eighty-two patients had dyslipidemia (58%), 35 had diabetes mellitus (25%), 19 had hyperuricemia (14%), 16 had chronic kidney disease (11%), and 24 had coronary heart disease (17%). Renin-angiotensin system blockade were the most commonly prescribed antihypertensive drugs (82 patients, 58%), followed by Ca-channel blockers (74 patients, 52%), β-blockers (17 patients, 12%), diuretics (6 patients, 4%), and α-blockers (3 patients, 2%).

Discussion

In this study, our Japanese outpatients with hypertension consumed more salt than the target value recommended by the Japanese guidelines. To our knowledge, this is the first study to show the relationship between central systolic BP and estimated salt intake in Japanese outpatients with hypertension.

Although the average salt intake in Japan has decreased in recent years, it remains high, at up to 9.9 g/day in 2016 according to a recent national survey. The JSH 2014 guidelines recommend that salt intake be reduced to 6 g/day in...
patients with hypertension; however, the actual salt intake in Japanese outpatients with hypertension remains to be precisely determined.

In the present study, the median salt intake in our Japanese outpatients with hypertension was 9.81 (range, 8.34-11.47) g/day, which greatly exceeds the 6.0 g/day limit recommended by the JSH 2014 guidelines. Only seven patients (5%) in our study had a daily salt intake of <6.0 g. Previous studies reported the salt intake in Japanese outpatients with hypertension to range from 9.6 to 9.7 g/day. Recently, we also demonstrated a mean salt intake in 236 Japanese outpatients with hypertension of 9.72 ± 2.43 g/day and only 4% of the patients had a daily salt intake of <6.0 g. Therefore, the results of this study appear to reflect the unpleasant truth regarding excessive salt intake in Japanese outpatients with hypertension, which has not yet decreased sufficiently.

For many years, BP has generally been measured at the brachial artery and the brachial BP has been widely used for the diagnosis and treatment of hypertension. However, central BP has recently attracted attention. Central BP is speculated to reflect the true burden to the heart, kidneys, and brain and also influences the local flow to these vital organs. Additionally, the elevation of central BP may have a direct adverse effect on the target organs and, consequently, on the cardiovascular prognosis of patients with hypertension.

However, until quite recently, central hemodynamics were rarely estimated because their assessment required invasive catheterization. The development of noninvasive methods has removed this requirement, making it easy to measure central hemodynamics. One of the recently introduced auto-

### Table 2. Clinical characteristics of the patients according to the quartiles of salt intake

|                      | All (n=141) | Q1 (n=35) SI, <8.34 | Q2 (n=36) SI, 8.34-9.81 | Q3 (n=35) SI, 9.81-11.47 | Q4 (n=35) SI, 11.47< | P-values |
|----------------------|------------|---------------------|------------------------|-------------------------|---------------------|----------|
| Age (years)          | 67.6±9.4   | 68.7±11.3           | 68.4±9.0               | 66.9±8.1                | 66.6±9.2           | 0.740    |
| Sex (men)            | 65 (46%)   | 17 (49%)            | 17 (47%)               | 14 (40%)                | 17 (49%)           | 0.870    |
| Dyslipidemia         | 82 (58%)   | 19 (54%)            | 21 (58%)               | 20 (57%)                | 22 (63%)           | 0.908    |
| Diabetes mellitus    | 35 (25%)   | 8 (23%)             | 6 (17%)                | 11 (31%)                | 10 (29%)           | 0.487    |
| Hyperuricemia        | 19 (14%)   | 6 (17%)             | 2 (6%)                 | 5 (14%)                 | 6 (17%)            | 0.429    |
| Chronic Kidney Diseases | 16 (11%) | 1 (3%)              | 5 (14%)                | 2 (6%)                  | 8 (23%)            | 0.038    |
| Coronary Heart Diseases | 24 (17%) | 8 (23%)             | 8 (22%)                | 2 (6%)                  | 6 (17%)            | 0.195    |
| ACEIs and/or ARBs    | 82 (58%)   | 21 (60%)            | 20 (56%)               | 17 (49%)                | 24 (69%)           | 0.387    |
| Ca antagonist        | 74 (52%)   | 22 (63%)            | 18 (50%)               | 15 (43%)                | 19 (54%)           | 0.400    |
| β-blocker            | 17 (12%)   | 4 (11%)             | 4 (11%)                | 5 (14%)                 | 4 (11%)            | 0.974    |
| α-blocker            | 3 (2%)     | 0 (0%)              | 1 (3%)                 | 1 (3%)                  | 1 (3%)             | 0.798    |
| Diuretics            | 6 (4%)     | 1 (3%)              | 1 (3%)                 | 1 (3%)                  | 3 (9%)             | 0.546    |
| Body height (cm)     | 158.4±7.9  | 155.7±8.2           | 158.7±8.2              | 159.6±5.8               | 159.7±8.6          | 0.114    |
| Body weight (kg)     | 59.1±10.2  | 55.7±10.4           | 57.4±8.5               | 60.9±10.3               | 62.5±10.5*         | 0.019    |
| Body mass index      | 23.5±3.4   | 22.9±3.9            | 22.7±2.9               | 23.8±3.1                | 24.5±3.5           | 0.090    |
| Brachial systolic BP (mmHg) | 131.2±16.5 | 125.7±16.2           | 129.9±15.0              | 133.1±16.3              | 136.3±17.4*        | 0.048    |
| Brachial diastolic BP (mmHg) | 78.1±10.9 | 72.8±11.1            | 77.4±8.8               | 81.2±9.3**               | 81.2±12.2**        | 0.002    |
| Central systolic BP (mmHg) | 135.6±17.3 | 127.9±17.3            | 134.4±16.3              | 139.6±16.1*              | 140.5±17.4*        | 0.008    |
| Heart Rates (beats/min) | 73.4±11.7 | 74.1±11.7            | 72.1±13.8               | 73.5±11.7               | 74.0±9.4           | 0.879    |

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, *vs. Q1, p<0.05, **vs. Q1, p<0.01

### Figure 2. Relationships between the quartiles of estimated salt intake, brachial systolic/diastolic BP, and central systolic BP

- **Brachial systolic BP**: P=0.037
- **Brachial diastolic BP**: P=0.005
- **Central systolic BP**: P=0.012
Estimated devices for the estimation of central hemodynamics is the HEM-9000AI. This device automatically records the radial pulse waveform, while the BP equivalent to the second systolic peak is measured by adjustment against the brachial BP. A regression equation is then used to estimate the central systolic BP. Owing to the simplicity of these measurements, this device is now widely used in Japan. These non-invasive estimates are closely correlated with the results of invasive measurements of central BP in the ascending aorta. Studies have demonstrated that central BP measured non-invasively predicts cardiovascular events and target organ damage independently and is considered a better indicator than conventional brachial BP for the treatment of hypertension. Studies have also suggested that central systolic BP is an important parameter and a prognostic risk factor. Additionally, Williams et al. demonstrated that lower central systolic BP levels resulted in more favorable clinical outcomes in patients with similar brachial systolic BP but different central systolic BP. These results suggest that central systolic BP has a better predictive power for future cardiovascular events than that of brachial BP.

Epidemiological studies have shown a correlation between excessive salt intake, BP measured at the brachial artery, and the prevalence of hypertension. A recent Japanese study also demonstrated a significant positive correlation between estimated salt intake and brachial BP in both treated and untreated individuals with hypertension. However, the relationship between central hemodynamics and salt intake in Japanese outpatients with hypertension was unclear. The results of the present study showed the moderate relationship between salt intake and not only brachial systolic/diastolic BP but also central systolic BP. This finding emphasizes the importance of a reduction in salt intake in Japanese outpatients with hypertension, even in those taking antihypertensive medication. Additionally, because elevated central systolic BP is associated with future cardiovascular events, the
A reduction of salt intake may be an important strategy to suppress the elevation of central systolic BP.

Central aortic pressure comprises a forward traveling wave from the heart and a reflected wave from the periphery. AI is widely used as an index of pulse wave reflection; that is, arterial stiffness. Several reports demonstrated that AI predicts cardiovascular events and mortality independent of peripheral BP\(^{(3,33)}\). Additionally, recent reports demonstrated that high salt intake may have a BP-independent effect on vascular wall function\(^{(2,34)}\). The present study demonstrated the significant relationship between estimated salt intake and AIP75 (Figure 4). The results suggest that salt intake influences arterial stiffness, similar to the results of previous studies, and may be one of the mechanisms by which salt intake increases central systolic BP.

### Limitations

This study has several limitations. First, its cross-sectional nature resulted in an inability to speculate on the causality between the estimated salt intake and central systolic BP. Second, the number of patients was very small and only patients from the cardiovascular division of Shimane University Hospital were recruited. These patients might slightly differ in terms of clinical characteristics from those of patients visiting a general medical practitioner. Third, the measurement of daily urinary salt excretion in a spot urine sample might be less accurate than that made using 24-h urine collection. Although 24-h urine collection is the most accurate method for evaluating salt intake, it is difficult to obtain a complete and accurate sample in outpatients.

Fourth, both central systolic BP and estimated salt intake were measured only once. Finally, although the study evaluated the relationship between central systolic BP and estimated salt intake, it did not determine whether a decrease in salt intake reduces central systolic BP.

Therefore, further studies are required to validate this method of estimating daily salt intake and to evaluate the relationship between central systolic BP and estimated salt intake in detail.

### Conclusion

We found that our Japanese outpatients with hypertension consumed higher amounts of salt than the target value recommended by Japanese guidelines. In addition, there was a moderate relationship between the central systolic BP and the estimated salt intake. These results suggest that a reduction in salt intake is important, even in outpatients with hypertension receiving antihypertensive medication.

### Conflicts of Interest

The authors declare no conflicts of interest.

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### Table 3

| Comorbidities                      | Univariate analysis | Multivariate analysis |
|------------------------------------|--------------------|----------------------|
|                                    | β                  | P-value   | β                  | P-value   |
| Dyslipidemia                       | 0.078              | 0.356     |                    |          |
| Diabetes mellitus                  | 0.069              | 0.413     |                    |          |
| Hyperuricemia                      | 0.068              | 0.422     |                    |          |
| Chronic kidney disease             | 0.184              | 0.029     | 0.116              | 0.162    |
| Coronary heart disease             | -0.160             | 0.059     |                    |          |
| Antihypertensives                  |                    |          |                    |          |
| ACEIs and/or ARBs                  | -0.084             | 0.323     |                    |          |
| Ca antagonist                      | -0.111             | 0.190     |                    |          |
| β-blocker                          | -0.008             | 0.928     |                    |          |
| α-blocker                          | 0.060              | 0.477     |                    |          |
| Diuretics                          | 0.060              | 0.481     |                    |          |

ACEI: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, β: standard partial regression coefficient
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Significance of D-dimer and soluble fibrin testing in screening of incident venous thromboembolism

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Abstract:
Background: D-dimer (DD) is useful for excluding diagnosis of venous thromboembolism (VTE) because of high sensitivity and high negative predictive value. Recently, soluble fibrin (SF) has been introduced for clinical use to examine coagulation status. However, the significance of SF in screening or diagnosis for VTE is uncertain. Thus, we examined DD and SF levels in incident VTE patients with ultrasonographic examination in their lower extremities. Subjects and Methods: We have conducted simultaneous measurement of DD and SF in 141 inpatients in our hospital between December 2013 and November 2014. Among them, we further selected 46 patients who were examined by lower extremity ultrasonography 1 month before or after the measurement of DD and SF. Incident VTE was diagnosed based on acute or subacute symptoms, the presence of thrombus in compression ultrasonography, and/or results from contrast-enhanced CT. Results: Incident VTE was found in 18 patients. SF levels were similar in VTE (+) and VTE (-) groups, while DD levels were significantly higher in VTE (+) group than those of VTE (-) group (17.7±30.4 μg/mL vs 5.1±5.2 μg/mL, p<0.05). When patients were classified based on surgical intervention, no significant difference in SF or DD levels was observed in peri-operative patients. However, DD levels in non-operative patients tended to be higher in VTE (+) group, compared to VTE (-) group. No such tendency was observed in SF levels. Conclusion: Measurement of DD but not SF may be beneficial for screening of incident VTE, especially in non-operative inpatients. Further study is necessary to determine the significance of DD and/or SF testing in VTE screening, diagnosis, and treatment in peri-operative patients.

Key words: Venous thromboembolism, D-dimer, Soluble fibrin, Ultrasonography

1. Introduction

Venous thromboembolism (VTE) includes deep vein thrombosis (DVT) and pulmonary thromboembolism (PTE), which is a life-threatening disorder. It has been demonstrated that VTE occurs after long-term bed rest or sitting, and even watching television for more than 5 hours¹⁶. Although accurate incidence rate is difficult to survey, it is considered to be lower in Japanese than that of Western population. However, it is reported that currently the incidence rate in Japan is remarkably increasing⁷.

Diagnosis of VTE is suggested by suspicion from nonspecific signs or symptoms and laboratory data such as elevated D-dimer (DD) level. Further, VTE is diagnosed mostly by contrast-enhanced CT, angiography, and compression ultrasonography⁸. Since DD level is not specifically elevated by VTE, DD testing is useful for exclusion of VTE¹⁰. However, elevation of DD level is often seen in subjects with cancer, pregnant women, aged people, and hospitalized patients. Therefore, it has been reported that DD testing is not suitable for the screening of incident VTE, especially for hospitalized patients because of highly false positive results¹⁰.

Soluble fibrin (SF) has been introduced for clinical use to
examine coagulation status. Recently, SF complex or SF monomer is examined in patients and reported by several researchers, and SF testing can be used for a disease marker of VTE\(^{13-17}\). However, little is known about significance of SF testing in the screening of VTE in hospitalized patients. Thus, we conducted a retrospective cross-sectional study to compare SF and DD levels in patients with and without VTE.

2. Subjects and Methods

2.1. Subjects

This retrospective cross-sectional study was performed by the collaboration of Department of Laboratory Medicine, Shimane University, and Central Clinical Laboratory and Vascular Lab, Shimane University Hospital. We included 141 patients in this study who had been simultaneously measured DD and SF from December 2013 to November 2014. Among them, we further selected 46 patients who examined ultrasonography in lower extremities 1 month before or after the measurement of DD and SF.

2.2. Ethics

The study protocol was approved by the local ethics committee of Shimane University and in accordance to the Declaration of Helsinki.

2.3. Data collection

Patient information such as age, sex, levels of DD and SF, was obtained from medical records. Incident VTE was diagnosed based on acute or subacute symptom, the presence of thrombus in compression ultrasonography, and/or results from contrast-enhanced CT and angiography.

Plasma concentration of DD and SF was measured using LIA’s Auto D-dimer Neo\(^{\text{®}}\) kit (Sysmex Co, Kobe, Japan) and Nano-pia\(^{\text{®}}\) SF kit (Sekisui Medical Co, Tokyo, Japan), both of which were based on latex agglutination immunoassay. According to the manufacturer’s information, the reference range of DD and SF were <1.0 μg/mL and <7.0 μg/mL, respectively.

2.4. Statistics

Data were expressed as mean ± standard deviation (SD). ANOVA was employed in univariate analyses of age in 4 groups classified by VTE and gender. The Student’s t-test was employed in comparison of DD and SF levels between patients with and without VTE. All statistical analyses were performed using the SAS system statistical software (PASW Statistics 18). Statistical significance was defined as \(p<0.05\).

3. Results

3.1. Background data

Clinical background of the patients is shown in Table 1. Incident VTE was found in 14 women and in 4 men. Mean age of all patients was 69.6±16.6 years, and no difference was found among 4 groups categorized according to the gender and presence of VTE. Surgical operation was done on 17 patients, whereas 29 patients did not receive any surgical intervention. The detail distribution of VTE (+) and VTE (-) patients with or without operation is shown in Table 1.

 Presence or absence of VTE, comorbidity or surgery, timing of blood sampling, and DD and SF levels in patients with surgical operation were shown in Table 2. Among the 29 patients without operation, comorbid diseases were as follows: 10 patients with cancer (ovarian cancer n=3, uterus cancer n=3, prostate cancer n=1, colon cancer n=1, and other cancers n=2), 4 patients with cerebral infarction, 2 patients with peripheral arterial disease (PAD), 2 patients with diabetes mellitus, and 11 patients with other diseases. These findings indicate that the causative disorders of VTE frequently include 1) cancer in pelvic organ such as urology, gynecology, and rectum or sigmoid colon, 2) vascular diseases such as aortic aneurysm, dissection, heart valve disease, and PAD, 3) local or systemic inflammation, and 4) immobilization for long time due to cerebral infarction, severe illness or some other reasons.

3.2. SF and DD levels

Results of SF and DD levels in patients with or without VTE are shown (Figure 1). Regarding SF, no difference was observed between VTE (+) and VTE (-) groups (15.9±23.6 μg/mL vs 13.4±23.7 μg/mL, \(p=0.72\)), while DD level was significantly higher in VTE (+) group than that of VTE (-) group (17.7±30.4 μg/mL vs 5.1±5.2 μg/mL, \(p<0.05\)). When cut-off value for SF was set at 7.0 μg/mL, the sensitivity and specificity for VTE (+) were 38.9% (7/18) and 64.3% (18/28), respectively. In the case of DD, the sensitivity and specificity for VTE (+) were 94.4% (17/18) and 21.4% (6/28), respectively, when the cut-off value was set at 1.0 μg/mL. Also, the negative and positive predictive values

### Table 1. Clinical background in patients with or without VTE

|                  | VTE (-) |     | VTE (+) |     |
|------------------|---------|-----|---------|-----|
|                  | Women   | Men | Women   | Men |
| All cases (N)    | 15      | 13  | 14      | 4   |
| Age              | 67.2±19.7 | 71.2±15.2 | 70.7±16.0 | 69.8±15.8 |
| Peri-operative cases (N) | 4      | 5   | 6       | 2   |
| Non-operative cases (N) | 11     | 8   | 8       | 2   |

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Table 2. Presence of VTE, comorbid disease or surgery, timing of blood sampling, and DD and SF levels in 17 peri-operative patients

| VTE | Disease/Surgery                  | Timing of blood sampling (Days before or after surgery) | DD (μg/mL) | SF (μg/mL) |
|-----|----------------------------------|--------------------------------------------------------|------------|------------|
| Pre-operative group | Rectal herniation                  | -36                                                   | 3.0        | 28.7       |
| (+) | Lung cancer                       | -29                                                   | 3.4        | 7.3        |
| | Rectal herniation                  | -15                                                   | 2.1        | 0.9        |
| | Lung cancer                        | -9                                                    | 1.6        | 4.9        |
| | Sigmoidal colon cancer             | -5                                                    | 8.2        | 4.0        |
| Abdominal aneurism in aorta        | -35                                                   | 5.0        | 3.5        |
| Rectal cancer                      | -29                                                   | 4.6        | 9.6        |
| Ovarian cancer                     | -23                                                   | 1.6        | 0.6        |
| Rectal cancer                      | -22                                                   | 4.1        | 11.1       |
| Sigmoidal colon cancer             | -22                                                   | 2.2        | 7.8        |
| Uterus cancer                      | -21                                                   | 2.0        | 51.4       |
| Sigmoidal colon cancer             | -18                                                   | 2.4        | 1.8        |
| Post-operative group | Hematoma in soft tissue            | 1                                                    | 26.9       | 17.8       |
| (+) | Kidney transplantation            | 13                                                   | 80.4       | 85.2       |
| | Kidney transplantation            | 27                                                   | 4.6        | 4.8        |
| (-) | Spinal subdural hematoma          | 1                                                    | 20.8       | 99.3       |
| Aortic dissection                  | 4                                                    | 4.1        | 5.7        |

Figure 1. Comparison of DD and SF levels in all the patients with or without VTE Parenthesis indicates the number of patients.

Next, we analyzed SF and DD levels in patients classified by the timing of operation and blood testing. Among 17 patients with surgical operation, 12 patients were examined by blood testing before surgery. In the pre-operative patients, no significant difference in SF or DD levels was shown between VTE (+) and VTE (-) groups (Figure 2). At least in 5 patients examined blood testing after surgery, there was no difference in SF or DD levels between VTE (+) and VTE (-) groups (Figure 2).

We further performed analysis of 29 patients without surgical operation. Although SF levels in VTE (+) and VTE (-) groups were similar (13.3±20.4 μg/mL vs 10.2±17.5 μg/mL, p=0.66), DD level in VTE (+) group was marginally higher than that of VTE (-) group (18.9±34.2 μg/mL vs 4.7±4.9 μg/mL, p=0.08) (Figure 3).

Figure 2. Comparison of DD and SF levels in peri-operative patients with or without VTE Parenthesis indicates the number of patients.

4. Discussion

In this cross-sectional study, measurement of DD but not SF seems to be beneficial for screening of incident VTE in hospitalized patients. However, these findings do not deny the significance of SF testing in patients with VTE. Since SF level is rapidly and tentatively elevated after VTE onset, the interval between VTE onset and blood testing remarkably affects our results. Although we tried to address this issue, it was difficult to get conclusive results regarding SF
testing due to retrospective study design.

We showed that DD may be beneficial for screening of incident VTE among inpatients except surgical operation. Since DD is a marker of endogenous fibrinolysis, it is suitable to detect VTE patients with a high negative predictive value\textsuperscript{10}. Meanwhile, Righini et al. pointed out in their narrative review that DD must be integrated in comprehensive sequential diagnostic strategies including clinical probability assessment and imaging techniques, due to its poor specificity\textsuperscript{10}. Shigemi et al. reported that DD might be useful as a pre-operative screening of VTE in gynecologic patients\textsuperscript{18}. They classified patients into 3 groups by plasma DD level to find pre-operative VTE occurrence in 0%, 2.7%, and 23.7% in patients with ≤0.5 μg/mL, 0.6-0.9 μg/mL, and ≥1.0 μg/mL of DD, respectively. Thus, further study is necessary to determine the significance of DD measurement in pre-operative screening for VTE.

In the present study, we could not find any significant difference in SF levels of patients with or without VTE. In a previous study, however, both DD and SF increased the sensitivity in SF levels of patients with or without VTE. In a operative screening for VTE. To determine the significance of DD measurement in pre-operative screening of VTE in gynecologic patients\textsuperscript{18}. Shigemi et al. reported that DD might be useful as a pre-operative screening of VTE in gynecologic patients\textsuperscript{18}. They classified patients into 3 groups by plasma DD level to find pre-operative VTE occurrence in 0%, 2.7%, and 23.7% in patients with ≤0.5 μg/mL, 0.6-0.9 μg/mL, and ≥1.0 μg/mL of DD, respectively. Thus, further study is necessary to determine the significance of DD measurement in pre-operative screening for VTE.

In the present study, we could not find any significant difference in SF levels of patients with or without VTE. In a previous study, however, both DD and SF increased the sensitivity and specificity for the diagnosis of VTE\textsuperscript{10}. Another study reported that measurement of SF might be effective to early detection of incident VTE in post-operative patients with total knee arthroplasty, when combined with DD measurement\textsuperscript{10}. This discrepancy seems to be mediated by the characteristics of SF. Because SF reflects the very early phase of a thrombotic event, the sensitivity becomes low when measured more than 3 days after the onset\textsuperscript{10}. Indeed, in our study, an 88-years old woman, who showed 28.7 μg/mL in SF and 3.0 μg/mL in DD before surgical operation, was diagnosed acute DVT by fresh thrombus in her soleal veins in both sides after ultrasonography. Thus, simultaneous measurement of DD and SF is recommended in patients with high risk of VTE. In addition, SF is responsible for the coagulation status earlier than DD, indicating that SF is more suitable for treatment efficacy of VTE, compared with DD\textsuperscript{10}. The authors would like to thank all the members of the Department of Clinical Laboratory Medicine of Shimane University Hospital for their skillful assistance.

Conflicts of Interest
All authors have no conflicts of interest.

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