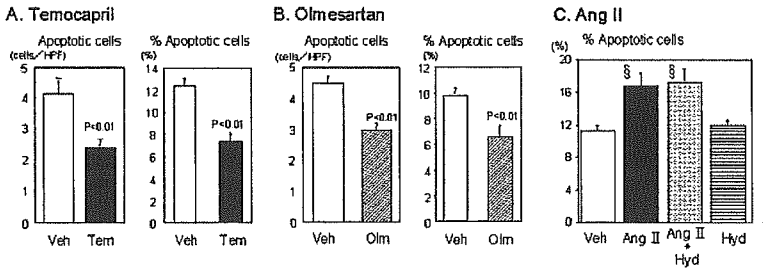


**Figure 2.** Effects of temocapril (A), olmesartan (B), and Ang II (C) on EC apoptosis after $H_2O_2$ treatment in rat carotid artery. The number of apoptotic ECs was counted per high power field (HPF; ×200), and the ratio of the apoptotic cell number to the intact cell number was calculated using *en face* specimens of the carotid artery stained with Hoechst 33342. A and B, Temocapril (Tem; 10 mg/kg per day; n=12), olmesartan (Olm; 1 mg/kg per day; n=8), or their vehicle (Veh; n=10 and n=6, respectively) was administered orally for 3 days before $H_2O_2$ treatment. C, Ang II (0.7 mg/kg per day) or its vehicle was administered subcutaneously for 3 days using an osmotic minipump alone (n=8 for Ang II and n=10 for vehicle) or in combination with oral administration of hydralazine (Hyd; 25 mg/kg per day; n=6 for Ang II and n=6 for vehicle; single administration for 5 days and coadministration with Ang II for 3 days) before $H_2O_2$ treatment. §$P<0.01$ vs vehicle. Values are expressed as mean±SEM.

## Effect of RAS Inhibitors and Ang II on EC Apoptosis After $H_2O_2$ Treatment in Rats

The effects of an ACE inhibitor, temocapril, and an AT1 receptor blocker, olmesartan, on EC apoptosis were examined at 24 hours after $H_2O_2$ treatment because the peak of apoptosis was observed at 6 to 24 hours.[24] Administration of 10 mg/kg per day temocapril or 1 mg/kg per day olmesartan for 3 days before $H_2O_2$ treatment did not significantly change body weight, heart rate, or blood pressure, but this dose of temocapril effectively inhibited plasma ACE activity (data not shown). The number and percentage of apoptotic cells, as determined using *en face* specimens with Hoechst 33342 staining, were significantly decreased by temocapril compared with vehicle (Figure 2A; supplemental Figure I, available online at http://www.hypertensionaha.org). Olmesartan showed a comparable inhibitory effect on EC apoptosis (Figure 2B).

Ang II was administered for 3 days in combination with hydralazine to eliminate the effect of Ang II on blood pressure. Consequently, systolic blood pressure was higher in rats administered Ang II alone (161±5 mm Hg; $P<0.01$) than in the other groups of rats: 123±3 mm Hg in the vehicle group, 129±7 mm Hg in the Ang II plus hydralazine group, and 114±4 mm Hg in the hydralazine group. In contrast to RAS inhibitors, Ang II administration augmented EC apoptosis independent of the pressor effect because coadministration of hydralazine did not influence EC apoptosis (Figure 2C).

## Inhibitory Effect of Temocapril on Neointimal Formation

We examined whether inhibition of EC apoptosis by temocapril would result in a reduction of neointimal formation. To do so, histological analysis of the carotid artery was performed 2 weeks after $H_2O_2$ treatment. Temocapril significantly decreased the neointimal area and the intima/media area ratio: intima/media area ratio was 0.18±0.02 in the vehicle group versus 0.12±0.02 in the temocapril group (n=9; $P<0.05$; supplemental Figure II). Because temocapril was administered for only 3 days before $H_2O_2$ treatment, it is suggested that inhibition of EC apoptosis may play a mechanistic role in attenuation of neointimal formation, although ACE inhibitors have various effects such as anti-inflammation and antimigration as well.

## Effect of RAS Inhibitors on $H_2O_2$-Induced EC Apoptosis in Culture

To reproduce oxidative stress–induced EC apoptosis in culture, we applied 0.2 mmol/L $H_2O_2$ to cultured ECs derived from a bovine carotid artery for 1.5 hours based on dose- and time-response experiments. EC apoptosis, as determined by DNA fragmentation and caspase-3 activity, was induced at 24 hours after $H_2O_2$ treatment. Comparable to in vivo experiments, temocapril inhibited EC apoptosis in a dose-dependent manner (Figure 3A and 3B). The inhibitory effect on EC apoptosis was mimicked by 10 μmol/L olmesartan (Figure 3C), but an AT2 receptor blocker, PD123319, did not influence EC apoptosis (supplemental Figure IIIA). The involvement of NO in the effect of temocapril was examined using an NO synthase inhibitor, L-NAME, because ACE inhibitors stimulate NO production via the inhibition of bradykinin degradation.[12] However, L-NAME did not influence the effect of temocapril (supplemental Figure IIIB).

To make the interaction between $H_2O_2$ and Ang II clear, dose response and combined effects of both agents on EC apoptosis and 8-isoprostane formation, a marker of oxidative stress, were examined. As shown in Figures 3D and 4A, combination of Ang II and $H_2O_2$ dose-dependently stimulated EC apoptosis and 8-isoprostane formation. Conversely, temocapril and olmesartan restrained 8-isoprostane formation (Figure 4B) and intracellular DCF formation (Figure 4C; supplemental Figure IV) induced by $H_2O_2$, suggesting that endogenous Ang II also interacts with $H_2O_2$ to elevate oxidative stress levels.

ACE activity and the expression of AT1 receptor mRNA in cultured ECs were determined. ACE activity calibrated by the protein concentration was not changed after $H_2O_2$ treatment: 106±9% at 3 hours and 103±8% at 24 hours after $H_2O_2$ treatment compared with the values at baseline and 3 hours after vehicle treatment (100±3% and 96±13%, respectively; n=3). The relative amount of the AT1 receptor to the housekeeping gene G3PDH, as measured by real-time PCR analysis, was not significantly changed after $H_2O_2$ treatment: 91±2% at 1.5 hours during the treatment, 99±5% at 3 hours, and 102±4% at 6 hours after $H_2O_2$ treatment compared with vehicle treatment (100±6%; n=3). Considering negative regulation in vascular smooth muscle cells[27,28] together, upregulation of the AT1 receptor is not likely to occur in response to $H_2O_2$ treatment.

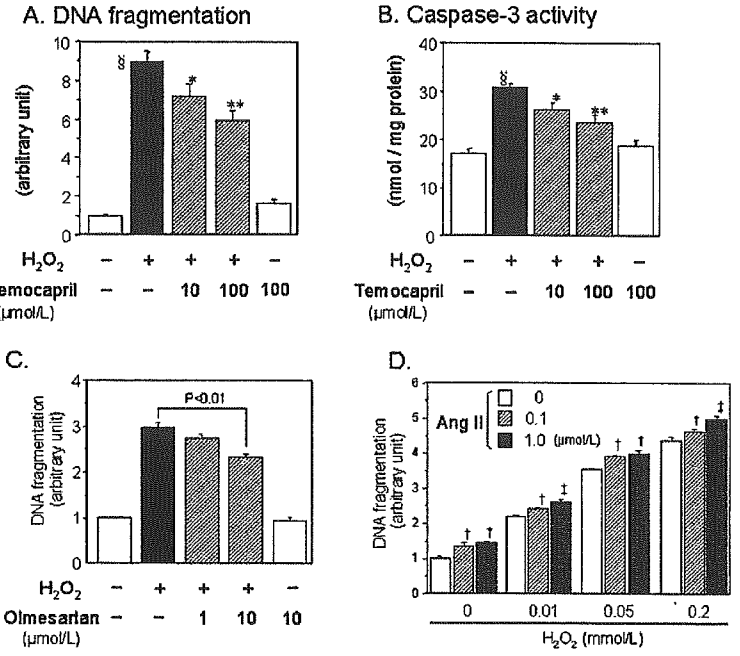


**Figure 3.** Effects of temocapril (A and B), olmesartan (C), and Ang II (D) on $H_2O_2$-induced EC apoptosis in culture. A through D, Temocapril, olmesartan, Ang II, or their vehicle was added to the culture medium 24 hours before $H_2O_2$ treatment until assay. EC apoptosis was evaluated 24 hours after $H_2O_2$ treatment (0.2 mmol/L in A through C; 0.01 to 0.2 mmol/L in D) by means of DNA fragmentation (A, C, and D; n=3) and caspase-3 activity (B; n=4). §$P<0.01$ vs $H_2O_2$ (−). *$P<0.05$; **$P<0.01$ vs $H_2O_2$ (+) + temocapril (−). †$P<0.05$ vs Ang II (−). ‡$P<0.05$ vs Ang II 0.1 μmol/L. Values are expressed as mean±SEM. Similar results were obtained in 3 independent experiments.

## Discussion

This study was conducted to elucidate the role of the RAS in oxidative stress–induced EC apoptosis using a rat model and cultured ECs. Treatment with $H_2O_2$ did not increase ACE activity or Ang II in the rat carotid artery during the acute phase. However, administration of an ACE inhibitor, temocapril, and an AT1 receptor blocker, olmesartan, inhibited EC apoptosis in vivo. Furthermore, we demonstrated using cultured ECs that combination of Ang II and $H_2O_2$ dose-dependently increased EC apoptosis and 8-isoprostane formation. In addition, temocapril and olmesartan reduced but not canceled EC apoptosis and 8-isoprostane formation induced by $H_2O_2$, suggesting that endogenous Ang II interacts with $H_2O_2$ to elevate oxidative stress levels and EC apoptosis.

In vascular lesions such as atherosclerosis and intimal hyperplasia, the production of reactive oxygen species[4,5] as well as the components of the RAS[9–12] are upregulated, suggesting a possible interaction between them. A number of investigations have clarified that Ang II induces oxidative stress in vascular cells. Ang II stimulates the production of reactive oxygen species in ECs by upregulating the subunits of NAD(P)H oxidase: gp91 phox[17] and p47 phox.[18] It has been reported that the RAS enhances EC apoptosis in vitro[20,21] and contributes to endothelial dysfunction in patients with renovascular hypertension through the oxidant-dependent mechanism.[19] Conversely, it remains unknown whether oxidative stress could regulate the RAS; only 1 report has shown the modulation of ACE by oxidative stress.[29] Usui et al[29] reported that the inhibition of NO synthesis by chronic administration of L-NAME in rats augmented superoxide production and ACE activity in aortic ECs, and these effects were eliminated by treatment with

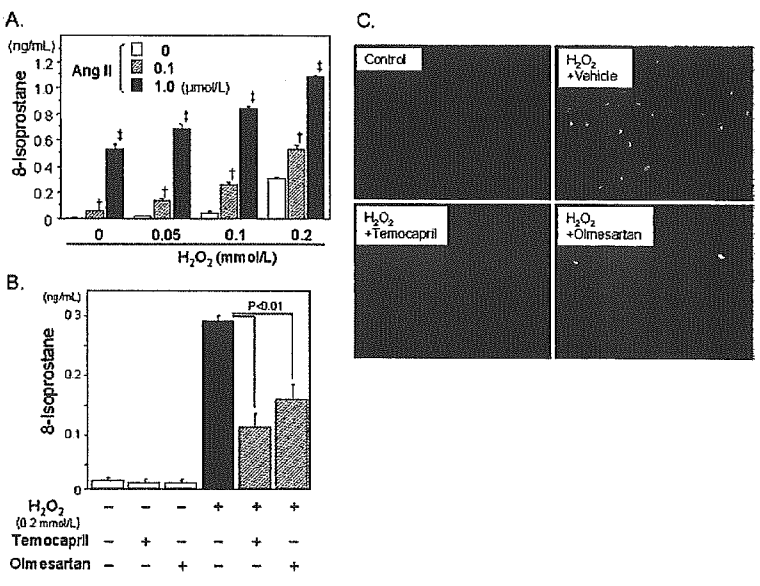


**Figure 4.** Effects of Ang II (A), temocapril, and olmesartan (B and C) on 8-isoprostane and DCF formation in cultured ECs. Ang II, temocapril (100 μmol/L), olmesartan (10 μmol/L), or their vehicle was added to the culture medium 24 hours before $H_2O_2$ treatment until assay. Then 8-isoprostane concentration in the culture supernatant and intracellular DCF intensity were measured 3 hours after $H_2O_2$ treatment. †$P<0.05$ vs Ang II (−). ‡$P<0.05$ vs Ang II 0.1 μmol/L. Values are expressed as mean±SEM (n=3). Similar results were obtained in 3 independent experiments.

antioxidants. In the present study, ACE activity in the carotid artery was not increased until 24 hours after $H_2O_2$ treatment. We also found that ACE activity was not changed after $H_2O_2$ treatment in cell culture experiments. Furthermore, the expression of AT1 receptor mRNA in cultured ECs, as measured using real-time PCR, was not increased after $H_2O_2$ treatment. Together, it is not likely that Ang II production or its receptor expression was upregulated in response to $H_2O_2$.

However, an ACE inhibitor, temocapril, and an AT1 receptor blocker, olmesartan, inhibited $H_2O_2$-induced EC apoptosis in rats as well as in cell culture experiments. No influence of L-NAME on the antiapoptotic effect of temocapril in cell culture studies indicates that the effect of temocapril was attributable to the inhibition of Ang II synthesis. An AT2 receptor blocker, PD123319, did not influence $H_2O_2$-induced EC apoptosis either. This result appears to be inconsistent with the previous finding[30] but suggests a minimal contribution of the AT2 receptor in $H_2O_2$-induced EC apoptosis or minimal expression of the AT2 receptor in the cultured ECs used in the present study. Reduction in 8-isoprostane formation by temocapril and olmesartan suggests that endogenous Ang II adds to the oxidative stress levels on top of exogenous $H_2O_2$; otherwise temocapril and olmesartan would have antioxidant effects independent of Ang II through currently unknown mechanisms, although the in vivo role of bradykinin/NO in the effect of ACE inhibitors and that of the AT2 receptor remain to be addressed.

Administration of Ang II provided evidence that Ang II can interact with $H_2O_2$ to elevate oxidative stress levels and induce EC apoptosis. In rat experiments, a high and pressor dose of Ang II was used in combination with hydralazine[31] because 3-day administration of lower doses of Ang II (0.1 to 0.2 mg/kg per day) did not show significant effects on EC apoptosis (data not shown). The cell culture experiments to examine the effect of submaximal doses of Ang II and $H_2O_2$ on apoptosis and 8-isoprostane formation gave us clear information that AT1 receptor signaling augments EC apoptosis by an interaction with oxidative stress. Although the doses of $H_2O_2$ and the time duration of exposure were optimized on the basis of the time- and dose-response experiments, the conditions in cell culture studies were different from those in animal studies. However, it has been reported that cigarette smoke, oxidized lipoproteins, and polymorphonuclear leukocytes, which play important roles in atherogenesis, can generate $H_2O_2$ concentrations of 0.05 to 0.2 mmol/L in vitro.[32] These reports suggest that the dosages of $H_2O_2$ used in the present study do not far exceed the physiological range, although direct comparison of physiological or pathophysiological conditions with those in our experiments may be inappropriate.

Considering the stimulatory effect of Ang II on free radical production,[17–19] our finding that endogenous Ang II exacerbates EC apoptosis induced by exogenous $H_2O_2$ is not surprising. In fact, a number of reports have shown experimentally that RAS inhibitors can reduce the production of reactive oxygen species in pathological conditions such as peripheral arteries in rats with chronic heart failure,[33] rat diabetic nephropathy,[34] and kidney mitochondria in aged rats.[35] In the clinical setting, it is reported that administration of an AT1 receptor blocker (losartan) to patients with chronic renal disease reduced urinary excretion of oxidized albumin and malondialdehyde.[36] Also, 4-week treatment with losartan or an ACE inhibitor (ramipril) in patients with coronary artery disease diminished the response of endothelium-dependent vasodilation to intracoronary administration of antioxidant vitamin C in parallel with improvement of basal endothelium-dependent vasodilation,[37] indicating that RAS inhibitors can improve endothelial function in association with a reduction of oxidative stress. In the present study, we investigated EC apoptosis, an important process that leads to endothelial dysfunction and atherosclerosis[22,23] using an in vivo model. Moreover, our finding that RAS inhibitors attenuated EC apoptosis suggests broad end-organ protective effects of RAS inhibitors, which have been used for the treatment of hypertension and heart failure.

## Perspectives

We found using an in vivo model and cultured ECs that Ang II elevated oxidative stress levels and increased EC apoptosis, whereas RAS inhibitors restrained them. These findings will add new information for cardiovascular research and the clinical application of RAS inhibitors.

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and specificity of EV were higher for those aged 60 and older than for those younger than 60; the sensitivity and specificity were 75.0% and 68.5% in the former and 47.9% and 56.0% in the latter. From ROC curve analysis, 14 mm was chosen as the cutoff value of EV for those aged 60 and older, and 16 mm was chosen as the cutoff value of EV for those younger than 60. The sensitivity and specificity of EV in diagnosing Graves' disease were not good in the total 113 untreated patients with Graves' disease studied, but the sensitivity and specificity of EV for the diagnosis of Graves' disease was good for those aged 60 and older.

No association was noted between EV and TRAb.

## DISCUSSION

We demonstrated the clinical usefulness of exophthalmos measurements for the diagnosis of Graves' disease in older Japanese people. Although elderly patients with Graves' disease have been said not to have exophthalmos, they do when their EV is compared with that of similarly aged people without Graves' disease. Findings were that (1) exophthalmos has a diagnostic value for Graves' disease in those aged 60 and older, (2) no association was noted between EV and TRAb, and (3) EV changed with age in control subjects (the values were highest in those aged 20–29 and then gradually decreased with age) but did not in patients with Graves' disease.

EV changed with age in normal control subjects but not in patients with Graves' disease. The differences in EV between patients with Graves' disease and control subjects were significant in those aged 60 and older. ROC curve analysis showed that the sensitivity and specificity of EV were higher in those aged 60 and older than in those younger than 60. Exophthalmos has a diagnostic value for Graves' disease in older patients.

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**Author Contributions:** Takashi Nakamatsu measured exophthalmos and assayed TRAb, analyzed and interpreted the data, and prepared the manuscript. Nobuyuki Takasu assayed TRAb, analyzed and interpreted the data, and prepared the manuscript. Ken Nakachi measured TRAb, analyzed and interpreted the data, and prepared the manuscript.

**Sponsor's Role:** None.

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## TAKO-TSUBO LEFT VENTRICULAR DYSFUNCTION CAUSED BY A FALL

*To the Editor*: We read with interest the systematic review of the psychological outcomes of falling by Jorstad et al.[1] Most of the physical complications resulting from falls are head injuries and fractures.[2,3] In addition, psychological problems sometimes induce physical complications. Falls can induce chest pain and heart failure. There have been some reports of patients with transient left ventricular dysfunction after emotional or physical stress, mostly in older women.[4–6] Here, we present a case of transient heart failure that occurred in an elderly woman after a fall.

An 85-year-old Japanese woman was referred to the University of Tokyo Hospital in July 2004 with gait disturbance, bradykinesia, anorexia, weakness, and dyspnea. Her daughter claimed that her gait disturbance had appeared a few months before and gradually worsened. She had fallen in her bedroom 2 days before and was not able to get up until the care worker visited her house the next morning. Fortunately, she had no fractures.

She exhibited stiffness and slowness of movement, stooped posture, and a mask-like facial expression and was disabled because of rigidity and slight tremor that were more marked in the right upper extremity. She had no dementia. Brain computed tomography revealed several small infarctions in the thalamus and basal nuclei, suggesting that her parkinsonism was due to multiple cerebral infarctions. Chest radiograph showed right pleural effusion but no cardiomegaly, and electrocardiogram (ECG) showed deep negative T-waves in leads I, AVL, AVF, and $V_{2-6}$ (Figure 1A). Echocardiography revealed akinesis of the apical wall of the left ventricle, although the rest of the left ventricle was normokinetic (Figure 1C). Myocardial infarction was

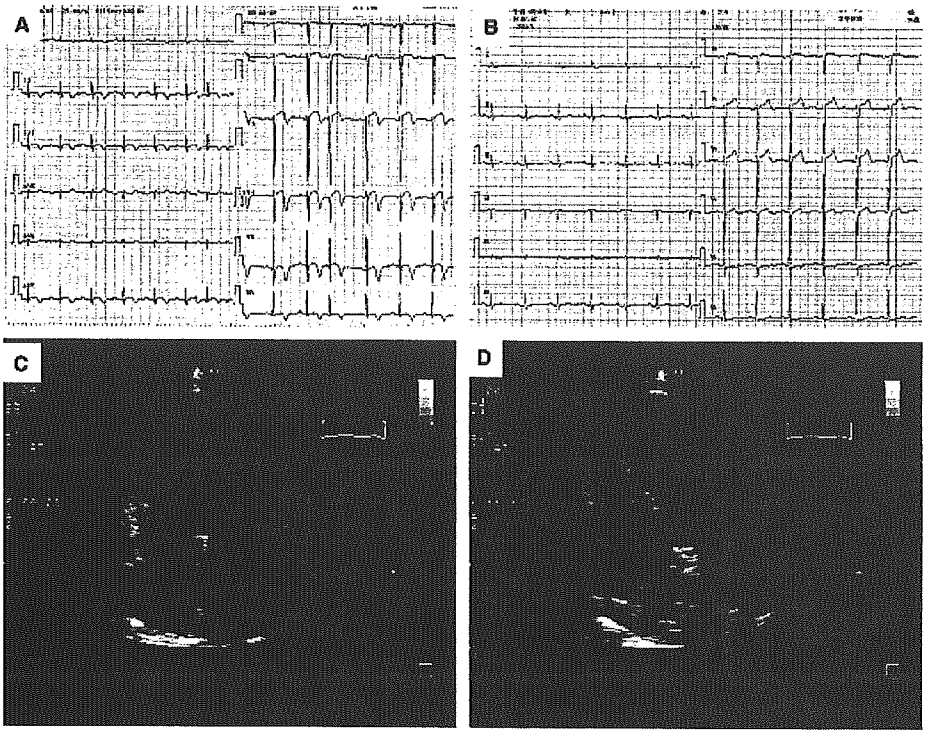


Figure 1. A. Initial electrocardiogram (ECG) showing deep negative T-waves in I, AVL, AVF, and $V_{2-6}$. B. ECG showing improvement of T-waves 6 months later. C and D. Echocardiography showing akinesis of the apical wall and normokinesis of the basal region of the left ventricle (C. systolic; D. diastolic).

suspected, but there was no increase in creatine kinase, troponin T, or myosin light chain. Thallium scintigraphy was performed, but no evidence of myocardial ischemia was detected. Her dyspnea improved, and the pleural effusion had disappeared by the third day without any medication.

Two weeks later, the echocardiography showed normal ventricular wall motion, although negative T-waves on the ECG remained unchanged. Finally, the ECG recovered to normal (Figure 1B) 6 months later, although she did not have any further cardiovascular medications.

After excluding drug-related cardiomyopathy, myocarditis, and myocardial infarction, based on her medical history, laboratory data, and scintigram, "tako-tsubo" (which means an octopus trap in Japanese) left ventricular dysfunction[5] was diagnosed. Transient left ventricular apical ballooning, or tako-tsubo left ventricular dysfunction, is a transient reversible cardiomyopathy with the unique feature of being induced by physical or emotional stress. The patient felt desperate when she fell and could not get up by herself, so her emotional stress may have induced this transient heart failure. Elderly women are reported to be susceptible to this disorder.[4–6] Its symptoms are similar to those of myocardial infarction, although sometimes there are none. In most cases, cardiac dysfunction is transient and recovers rapidly, although cardiogenic shock due to ventricular septal perforation[7] has been reported. Its mechanism is still unknown, although some reports suggest that reversible coronary microvascular impairment[8] or sudden surges in circulating catecholamine levels[4] are involved in this disease.

The incidence of this disease is unknown, but it is likely to be more common than generally thought. In this case, a fall resulting from parkinsonism was the cause of psychological distress, which was considered the only possible cause of tako-tsubo left ventricular dysfunction. Falls could cause psychological difficulties in older people and could be the trigger for this disorder. Fortunately the patient's heart failure was mild and did not need medical attention, but falls might cause chest pain or heart failure, as well as head injuries and fractures.

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## ALZHEIMER'S DISEASE AND MEDICAL DISEASE CONDITIONS: A PROSPECTIVE COHORT STUDY

*To the Editor:* Patients with early Alzheimer's disease (AD) represent a heterogeneous cohort, with some patients progressing faster to end-stage dementia and some others progressing much more slowly (Table 1). It has been postulated that various factors such as Mini-Mental State Examination (MMSE) score on initial presentation, educational level, age of onset of AD, female sex, poor performance on activities of daily living, family history, and presence of psychiatric symptoms explain this difference.[1,2] A prospective cohort study was designed to study the coexisting medical diseases at various stages of AD and to determine whether they have any effect on the progression of AD.

## METHODS

A cohort of community-residing elderly persons aged 60 to 85 who fulfilled the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition,* (DSM-IV) criteria for primary degenerative dementia of the Alzheimer's type[3] and National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association criteria[4] for probable AD at baseline were followed longitudinally at intervals of 3 to 4 years. Medical, neurological, psychiatric, psychometric, and neuroradiological evaluations were conducted at baseline to exclude patients with other dementing illnesses. The Global Deterioration Scale (GDS)[5] and the MMSE were used to assess the cognitive and functional capacity at all evaluations. Further evaluations were not performed once the person reached the final, most severe stage of AD (GDS 7). The course of dementia was considered rapid if GDS 7 was reached within 4 years of the time of baseline evaluation.[6]

Criteria for exclusion at baseline included history of head trauma; seizures or other neurological disorders; mental retardation; a diagnosis of multiinfarct dementia; significant alcohol abuse; schizophrenia, depression, or major affective disorders; and cardiac, pulmonary, vascular, metabolic, or hematological conditions or other impairments of sufficient severity to adversely affect cognition or functioning.

The medical disease conditions (MDCs) were categorized as cardiovascular, endocrine, respiratory, nervous system (except dementia), hematological, neoplastic, gastrointestinal, dermatological and connective tissue disorders, allergic, history of surgeries, injuries and fractures, eye and ear, genitourinary and gynecological, and musculoskeletal disorders. The MDCs were reviewed at each visit, and their effect on the course of AD was investigated.

**Table 1. Comparison Between Groups Depending on the Rapidity of Course of Dementia**

| | Overall (n = 40) | | Faster Course (n = 28) | | Slower Course (n = 12) | |
|---|---|---|---|---|---|---|
| Variable | Baseline | Final | Baseline | Final | Baseline | Final |
| Number of medical diseases, mean ± SD | 5.1 ± 3.2 | 7.9 ± 3.9 | 6.0 ± 2.5* | 8.1 ± 3.6 | 3.9 ± 3.2 | 7.8 ± 4.1 |
| Geriatric Depression Scale score, mean ± SD | 4.8 ± 0.9 | 7.0 ± 0.0 | 4.9 ± 0.9 | 7.0 ± 0.0 | 4.8 ± 0.8 | 7.0 ± 0.0 |
| Mini-Mental State Examination score, mean ± SD, mean ± SD | 13.2 ± 7.3 | 0.2 ± 0.8 | 13.3 ± 7.6 | 0.0 ± 0.0 | 13.0 ± 7.1 | 0.2 ± 0.9 |
| Age, mean ± SD | 69.9 ± 3.2 | 75.1 ± 5.8 | 70.6 ± 3.8 | 73.8 ± 3.9 | 68.9 ± 3.0 | 75.8 ± 4.2 |
| Nursing home residence, n (%) | 0 (0) | 29 (73) | 0 (0) | 20 (71) | 0 (0) | 9 (75) |
| Peripheral vascular disease, n (%) | 10 (25) | 11 (28) | 10 (36)* | 10 (36)* | 0 (0) | 1 (8) |
| Atherosclerotic heart disease, n (%) | 12 (30) | 17 (43) | 11 (39)* | 13 (46) | 1 (8) | 4 (33) |
| Pressure mellitus, n (%) | 10 (25) | 11 (28) | 8 (29) | 8 (29) | 2 (17) | 3 (25) |
| Hypertension, n (%) | 23 (58) | 27 (68) | 18 (64) | 20 (71) | 5 (42) | 7 (58) |
| Pressure ulcer, n (%) | 0 (0) | 15 (38) | 0 (0) | 11 (39) | 0 (0) | 4 (33) |
| Contracture, n (%) | 0 (0) | 8 (20) | 0 (0) | 6 (21) | 0 (0) | 2 (17) |
| Hip fracture, n (%) | 0 (0) | 10 (25) | 0 (0) | 8 (29) | 0 (0) | 2 (17) |

*$P < .05$ for differences between faster and slower progression of Alzheimer's disease.
SD = standard deviation.

*Circ J* 2005; **69:** 221–226

# Amelioration of Vascular Endothelial Dysfunction in Obstructive Sleep Apnea Syndrome by Nasal Continuous Positive Airway Pressure

## — Possible Involvement of Nitric Oxide and Asymmetric NG,NG-Dimethylarginine —

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**Background** Asymmetric NG,NG-dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide (NO) synthase and its plasma concentration is elevated in patients with cardiovascular risk factors, including hyperlipidemia, hypertension, diabetes, and hyperhomocysteinemia. Obstructive sleep apnea syndrome (OSAS) has been attracting attention as a risk factor for cardiovascular disorders because it often accompanies hypertension, obesity, glucose impairment, and dyslipidemia, all of which are factors in metabolic syndrome and risk factors for cardiovascular disease.

**Methods and Results** In the present study, flow-mediated vasodilatation (FMD) of the brachial artery and plasma concentrations of ADMA were measured before and after nasal continuous positive airway pressure (nCPAP) therapy, which abrogates apnea, in 10 male patients aged 36–69 years old, who were given a diagnosis of OSAS by polysomnography. The percent FMD (%FMD) improved significantly from 3.3±0.3% to 5.8±0.4% ($p<0.01$) and 6.6±0.3% ($p<0.01$), before, 1 week, and 4 weeks after nCPAP, respectively. At the same time, the plasma NOx concentrations, metabolites of NO, tended to increase, but the plasma ADMA concentration decreased inversely to %FMD and NOx. A negative correlation between %FMD and plasma ADMA concentration, and a positive correlation between %FMD and plasma NOx concentrations were observed.

**Conclusion** Nasal CPAP improves endothelial function, in part by the decreasing ADMA concentration, thereby potentiating NO production. (*Circ J* 2005; **69:** 221–226)

**Key Words:** Asymmetric NG,NG-dimethylarginine (ADMA); Flow-mediated dilatation; Nasal continuous positive airway pressure; Obstructive sleep apnea syndrome

Endothelial dysfunction is recognized as an early phase of arteriosclerosis[1] and an important cause of that dysfunction is impaired nitric oxide (NO) release from the endothelium. Endothelial NO is a key regulator of vascular homeostasis; it induces vasorelaxation by generating cyclic GMP in the underlying smooth muscle cells, and prevents monocyte adhesion to the endothelium, platelet activation, and smooth muscle cell proliferation. Hence, impaired NO release from injured endothelial cells is regarded as an initiator and promoter of arteriosclerosis.

Endothelial NO is produced when L-arginine is converted to L-citrulline by the enzyme endothelial nitric oxide synthase (eNOS). Endothelial NOS is inhibited by endogenous inhibitors, NG-monomethyl-L-arginine (L-NMMA) and asymmetric dimethylarginine (ADMA), which are structural analogues of L-arginine[2,3] Plasma ADMA is eliminated by renal excretion and by degradation to citrulline and dimethylamine by the enzyme dimethylarginine dimethylaminohydrolase (DDAH).[4] Increased plasma concentration of ADMA is associated with hypertension,[5] pulmonary hypertension,[6] hypercholesterolemia,[7,8] carotid intima–media thickening,[9] severe peripheral artery occlusive disease,[10] and the clustering of coronary risk factors.[9] These findings suggest that ADMA is responsible for endothelial dysfunction.

Obstructive sleep apnea syndrome (OSAS) has been recently attracting attention as a significant disorder. Frequent apnea/hypopnea attacks followed by arousal results in insufficient sleep at night, causing daytime sleepiness, leading to work inefficiency, and even traffic accidents. In addition, OSAS often accompanies hypertension, obesity, glucose intolerance, and dyslipidemia, all of which are factors in metabolic syndrome. Hence, OSAS is recognized as a risk factor for cardiovascular disease.[11–14] It has been

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**Table 1 Clinical Characteristics of the Study Subjects With Obstructive Sleep Apnea Syndrome**

| | *Before nCPAP* | *1 week after nCPAP* | *4 weeks after nCPAP* |
|---|---|---|---|
| *Body mass index (kg/m²)* | *29.1±4.8* | *28.8±4.6* | *28.0±4.7* |
| *Systolic BP (mmHg)* | *131±15.2* | *128±10.3* | *133±8.8* |
| *Diastolic BP (mmHg)* | *79±9.3* | *75±6.2* | *81±6.5* |
| *Heart rate (beats/min)* | *74±14* | *72±10* | |
| *Creatinine (mg/dl)* | *0.85±0.21* | | *0.83±0.22* |
| *Uric acid (μmol/L)* | *387±59* | *387±65* | *363±48* |
| *Total cholesterol (mmol/L)* | *5.23±0.72* | *4.79±0.61* | *4.71±0.62* |
| *HDL-cholesterol (mmol/L)* | *1.06±0.20* | *1.04±0.19* | *1.19±0.26* |
| *Triglyceride (mmol/L)* | *1.66±0.55* | *1.60±0.75* | *1.28±0.39* |
| *LDL-cholesterol (mmol/L)* | *3.39±0.72* | *3.03±0.69* | *2.92±0.72* |
| *Fasting plasama glucose (mmol/L)* | *5.83±1.28* | *5.72±1.40* | *5.77±1.00* |
| *HemoglobinA1c (%)* | *5.9±1.2* | *5.9±1.4* | *5.5±1.0* |
| *Apnea/hypopnea index* | *33±14.7* | *3.9±3.7* | |
| | *p<0.01* | | |
| *Desaturation index* | *11.1±8.2* | *0.26±0.43* | |
| | *p<0.01* | | |

*All values are presented as mean±SEM. Other than the apnea/hypopnea index and desaturaion index, none were statistically significant before or after nCPAP (1 week and 4 weeks). BP, blood pressure; desaturation index, duration of $SpO_2$ <90%/total sleep time (%); HDL, high-density lipoprotein; LDL, low-density lipoprotein; nCPAP, nasal continuous positive airway pressure.*

also reported that endothelium-dependent vasodilatation is impaired in OSAS patients![15,16] Currently, the most effective therapy for OSAS is nasal continuous positive airway pressure (nCPAP), which eliminates the upper airway obstruction.

In the present study, we examined the impact of nCPAP on endothelial function by measuring the flow-mediated vasodilatation (FMD) of the brachial artery before and after nCPAP. We also examined the temporal change in both the NO metabolites (NOx) and ADMA to understand the mechanism of improvement in FMD by nCPAP.

## Methods

*Patients*

This study was performed in 10 men, aged 36–69 years (53.3±10.5, mean±SD), who were admitted to the Department of Geriatric Medicine, The University of Tokyo Hospital, given a diagnosis of OSAS by polysomnography and then treated with nCPAP (Table 1). Seven patients had hypertension, 5 of whom were on medication; 4 patients were diabetic, one of whom was on oral medication. The pressure for the nCPAP was adjusted by titration with auto-CPAP followed by manual titration. Measurements of FMD, plasma ADMA, and NOx were performed before, 1, and 4 weeks after nCPAP. The diagnostic criterion for OSAS was either an apnea/hypopnea index (AHI) >10 or $SpO_2$ <90% for more than 5 min or more than 1% of total sleep time![17] The indication for nCPAP was (1) AHI >20 or (2) $SpO_2$ <90% more than 20 min or more than 5% of total sleep time. The study protocol was approved by the ethics committee of The University of Tokyo Hospital, and written informed consent was obtained from each patient.

*Measurement of Flow-Mediated Vasodilatation*

Percent flow-mediated, endothelium-dependent, vasodilatation (%FMD) and percent nitroglycerine-mediated, endothelium-independent, vasodilatation (%NTG) were determined by ultrasound![18,19] The subjects rested quietly for 15–20 min on a bed in a temperature-controlled room. The blood flow and vessel diameter of the upper right brachial artery were measured using a 7.5-MHz ultrasound linear array transducer. Forearm ischemia was induced by a blood pressure Manschette tourniquet set at 250 mmHg for 5 min and subsequent rapid deflation of the tourniquet resulted in FMD. The change in diameter caused by FMD was expressed as the percent change relative to that of the initial resting scan. After the measurement of FMD, the subjects rested quietly for 15 min and complete recovery of the vessel diameter was confirmed. A sublingual spray of NTG (Myocor spray; 0.3 mg/spray) was administered, and the flow rate and vessel diameter of the same vessel were determined 3–5 min later. The diameter of the upper brachial artery was determined by taking the mean values determined from 4 images. Changes in diameter of 0.1–0.2 mm can be detected accurately with this method![20,21] The coefficient of variation for the measurements of FMD was 5.84±0.25% and that for NTG-induced dilation was 3.97±0.24%, as reported previously![20,21] The coefficient of variation for reproducibility of this ultrasound determination of FMD was 9.77±0.82% and that of NTG-induced dilation was 7.24±0.49%.

*Measurement of ADMA*

The plasma concentration of ADMA was measured by high-performance liquid chromatography (HPLC) with a fluorescent detection method![22] using blood samples that had been collected in EDTA tubes. The plasma was separated and 10 μl was added to 40 μl of mobile phase solution for HPLC. The arginine analogues were adsorbed in the positive ion-exchange column, after which the column was switched and the sample was injected into a separation column. NMMA, ADMA, and symmetric dimethylarginine (SDMA) were separated by ion-pair chromatography and then detected by adding fluorescent derivatization reagent, O-phthalaldehyde and thiol. The NMMA, ADMA, and SDMA calibration curves straightened over the range of 0.05–5.0 μmol/L and their respective detection limits were 0.005 μmol/L, 0.008 μmol/L, and 0.01 μmol/L (S/N=2). The intra-day variations for NMMA, ADMA, and SDMA were 4.6, 4.3, and 6.4%, respectively, and 6.1, 5.8, and 7.0%, for the inter-day variations. The HPLC solid and mobile phases were as follows: positive ion-exchange column: Capcell Pak MF-SCX (10×40 mm, inner diameter, 5 μm,

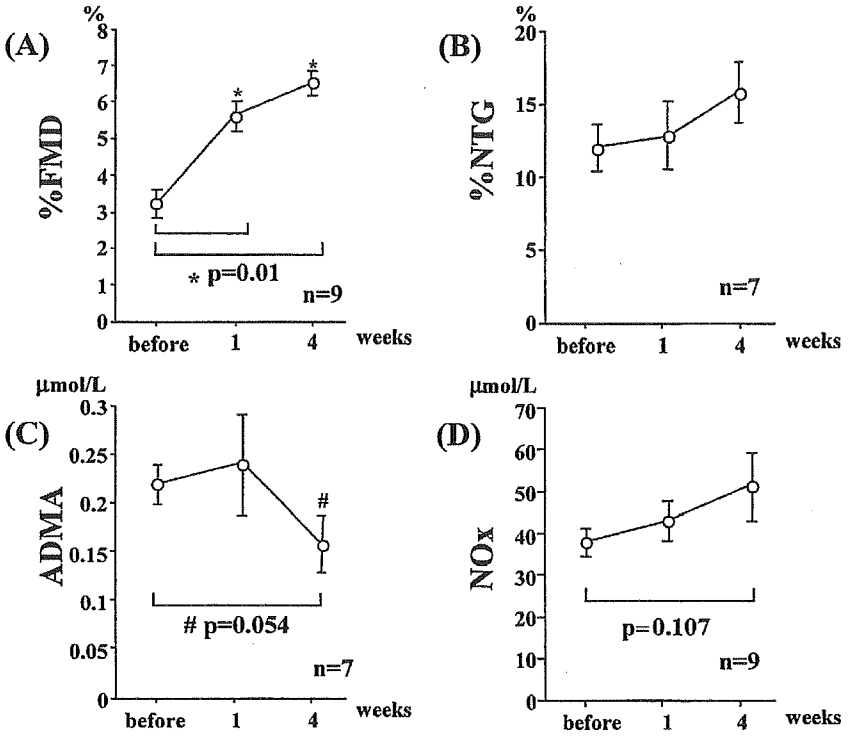


Fig 1. Changes in %FMD (A), %NTG (B), plasma ADMA concentrations (C), and plasma NOx concentrations (D) before and after nCPAP in OSAS patients. All measurements were performed before, 1 and 4 weeks after nCPAP. All values are presented as mean±SEM.

Shiseido, Tokyo); separation column: Capcell Pak MG-C18 (250×4.6 mm, inner diameter, 5 μm, Shiseido); mobile phase: 70 mmol/L sodium phosphate buffer (pH 6.7) containing 15 mmol/L cyclohexanecarboxylate and 1.5 mmol/L octanoate; flow rate: 1.0 ml/min; derivatization reagent solution: 3.7 mmol/L 2-mercaptopropionic acid in 0.2 mol/L borate buffer (pH 9.8) 3.0 mmol/L O-phthaldehyde in 0.2 mol/L borate buffer (pH 9.8); flow rate: 0.3 ml/min. The elute was monitored at 450 nm with excitation set at 337 nm.

*Measurement of Nitrate and Nitrite (NOx)*

NOx was measured at SRL Co, Ltd, Tokyo, by the Griess method. The samples were deproteinated and separated into nitrates and nitrites. After all the nitrates were reduced to nitrites, the samples were reacted with naphthylethylamine, and the product was determined by absorbance at 540 nm.

*Statistics*

All values are presented as the mean±SEM. The data were analyzed by one-factor ANOVA and the Student Newman-Keuls test was performed to test the significance of the differences. Statistical significance was made when $p<0.05$.

## Results

All patients were given a diagnosis of OSAS and were indicated for nCPAP treatment, which remarkably improved both the apnea/hypopnea index and desaturation index (Table 1). Neither renal function nor the factors related to metabolic syndrome changed significantly after nCPAP (Table 1), indicating that short-term treatment does not improve patients' metabolic status. However, the %FMD changed significantly from 3.3±0.3% before nCPAP to

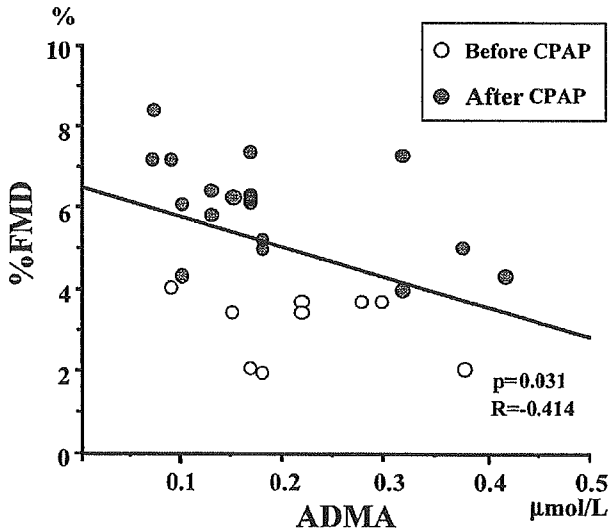


Fig 2. Correlation between %FMD and plasma ADMA concentrations before and after nCPAP. Black circles indicate values before nCPAP and white circles indicate values after nCPAP.

5.8±0.4% ($p<0.01$) at 1 week, and 6.6±0.3% ($p<0.01$) 4 weeks after nCPAP (Fig 1A). We confirmed that the basal diameter of the brachial artery was the same before and after nCPAP. No significant change was observed in %NTG, suggesting that nCPAP does not affect endothelium-independent vasodilatation (Fig 1B). The plasma concentrations of ADMA, the endogenous inhibitor of eNOS, decreased inversely to the improvement of %FMD at 4 weeks after nCPAP: 0.22±0.27 μmol/L before nCPAP, 0.21±0.44 μmol/L at 1 week, and 0.16±0.27 μmol/L at 4 weeks after nCPAP ($p=0.054$ by a paired 2 group test) (Fig 1C).

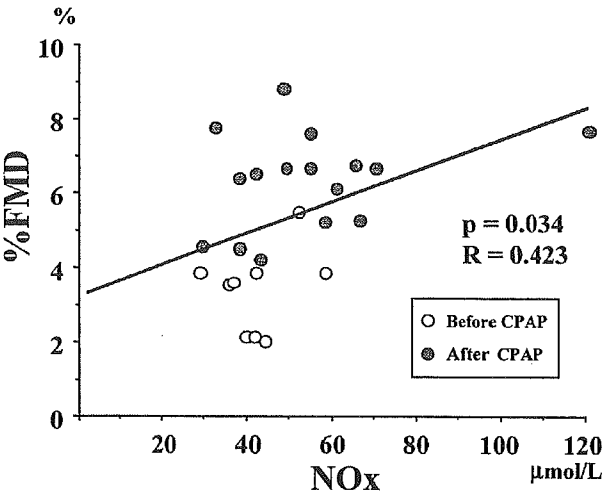


Fig 3. Correlation between %FMD and plasma NOx concentrations before and after nCPAP. Black circles indicate values before nCPAP and white circles indicate values after nCPAP.

Plasma NOx concentrations increased slightly, though not significantly, in parallel with the improvement of %FMD: 37.9±2.8 μmol/L before nCPAP, 43.0±4.6 μmol/L at 1 week after nCPAP, and 51.4±8.3 μmol/L at 4 weeks after nCPAP (Fig 1D).

Although neither the decrease in plasma ADMA concentration nor the increase in plasma NOx concentration was statistically significant, the results imply that the decreased plasma ADMA concentration lead to the improvement in %FMD by increasing NO production in the vessel wall. In fact, a negative correlation was found between %FMD and plasma ADMA concentration when all values (before, 1 week, and 4 weeks after nCPAP) were analyzed (Fig 2). Although the plasma NOx concentrations did not change significantly after nCPAP, a positive correlation was found between them and %FMD when all values (before, 1 week, and 4 weeks after nCPAP) were analyzed (Fig 3).

## Discussion

Patients with cardiovascular risk factors show diminished endothelium-dependent vasodilatation[23] and elevated plasma ADMA concentrations[9] the latter being also increased in patients with vasospastic angina;[24] and in those with hypercholesterolemia, hypertriglyceridemia and diabetes mellitus, concomitant with impaired endothelium-dependent vasodilatation.[7,25,26] Endothelium-dependent vasodilatation has also been found to be impaired in patients with OSAS;[15,27–29] but their plasma ADMA concentrations were not determined in those studies. In the present study, endothelium-dependent vasodilatation was ameliorated with the improvement of apnea by nCPAP. Concomitantly, the improvement in endothelium-dependent vasodilatation paralleled negatively the plasma ADMA concentration and positively paralleled the plasma NOx concentration. The decrease in ADMA did not appear to be mediated by improved renal function or sympathetic nerve activity, known regulators of plasma ADMA, according to our data for serum creatinine and heart rate (Table 1). Our findings suggest that nCPAP reduces the concentration of ADMA, thereby enhancing NO production and leading to an improvement of endothelium-dependent vasodilatation.

One of the most deleterious features of OSAS is nocturnal hypoxia. It has been reported that serum NOx concentrations decline and there is a negative correlation between serum NOx concentration and the severity of OSAS.[30] We did not find such a negative correlation in the present study (data not shown). However, with respect to ADMA, we found a negative correlation between the difference in the plasma ADMA concentrations before and after nCPAP (ΔADMA) and the desaturation index (DI) before nCPAP, and ΔADMA and the difference of DI before and after nCPAP (ΔDI) (data not shown). The ΔADMA did not correlate with ΔAHI, which indicates that the plasma ADMA concentration parallels the severity of OSAS with regard to desaturation, but to apnea.

With respect to hypoxia and ADMA, it is reported that the expression of the dimethylarginine dimethylaminohydrolases (DDAH), the enzymes that catalyze the degradation of ADMA, is decreased in hypoxia-induced pulmonary hypertension[9,31] but it is not known whether DDAHs are directly downregulated by hypoxia or upregulated by oxygenation, leading to the reduction of ADMA. Another plausible mechanism for impaired endothelium-dependent vasodilatation in OSAS is the reduction in eNOS protein, based on the finding that eNOS protein was decreased in the rat aorta under low oxygen tension;[32] and that hypoxia decreases the expression of eNOS mRNA and protein in cultured endothelial cells.[33] In addition, the present result that %FMD increased but plasma ADMA and NOx concentrations were unchanged at 1 week after nCPAP (Fig 1A,C, and D) suggests that FMD starts to improve before ADMA and NOx start to change. This indicates that FMD is regulated in part by the ADMA–NO axis.

There are several studies showing that OSAS is a risk factor for cardiovascular disease, but only a few have reported the prognosis of OSAS. He et al showed that in 385 untreated patients, the 9-year survival rate was significantly lower in those with apnea index (AI) >20 compared with those with an AI <20.[11] Noda et al showed that the survival rate is low in OSAS patients depending on their age; the prognosis in the middle-aged OSAS patients depends on the complication of hypertension and severity of the oxygen desaturation, but not on AHI.[34] OSAS patients suffer from a high incidence of cardiovascular and cerebrovascular diseases;[12–14] and the mortality rate increases with the severity of OSAS;[11,14] with the main causes of death being ischemic heart disease and cerebrovascular disease. Compared with the general population, OSAS patients have a 2-fold greater incidence of hypertension, 2–3-fold incidence of ischemic heart disease, and 3–5-fold incidence of cerebrovascular disease;[35] and the mortality rate from total vascular events is 2.7-fold greater than that of non-OSAS patients. In addition, it was recently reported that sleep apnea is common in patients with idiopathic cardiomyopathy.[36]

Hypertension is a frequent complication of OSAS. It has been reported that the mean blood pressure increases with the severity of the sleep apnea.[37] Hypertension is significantly associated with a lower survival rate in the middle-aged population.[34] Hypertension can be induced in rats by putting them in hypoxic conditions for 8 h daily;[38] and in dogs by repetitive airway occlusion while sleeping.[39] Although the precise mechanism of this induction is not understood, augmented sympathetic nerve tension following repetitive hypoxia is thought to be one of the causes for the rise in blood pressure. Indeed, it is reported that urinary

catecholamine excretion during the night is increased in OSAS patients. Augmented sympathetic nerve activity during the night may also elevate vessel tone, leading to elevated blood pressure not only at night, but also during the day. Furthermore, the serum NOx concentration negatively parallels blood pressure in OSAS patients,[30] indicating that NO production from endothelial cells plays a significant role in the elevation of blood pressure. With respect to the effect of nCPAP on blood pressure, it has been shown that blood pressure falls after adequate treatment with nCPAP,[40,41] and the assumed mechanism is reduced peripheral vascular resistance.[42] As well, nCPAP changes the diurnal pattern of blood pressure from non-dipper to dipper, which presumably reduces the risk of coronary events.[43] In the present study, we did not find any decline in systolic or diastolic pressure over the 4 weeks and it may take longer before the effect on blood pressure becomes apparent.

The importance of vascular endothelial cells in OSAS has been shown in relation to vascular events.[30,44] OSAS patients are exposed to hypoxia for prolonged periods, which would damage endothelial cells, evidenced by the enhanced release of thrombomodulin and von Willebrand factor (vWF). Plasma vWF concentrations, which are high in OSAS patients compared with control subjects, were reduced significantly at 1 month after nCPAP, which is in agreement with our study result that endothelial dysfunction is improved by nCPAP.

*Study Limitations*

We were unable to find unequivocal causal relationships of ADMA, NOx, and FMD; a negative correlation between ADMA and FMD, and a positive correlation between NOx and FMD were discovered when all values (before, 1 week, and 4 weeks after nCPAP) were analyzed, although no correlation was found when the values were analyzed separately. This result is related to the limited number of subjects and in this sense, the occurrence of type I and type II errors is the default of the current study. Another limitation of the present study is the lack of an age-matched, and BMI-matched control group because of the very high BMI of the study subjects in relation to the rest of the Japanese population. We previously showed that %FMD declines significantly in subjects with one or more coronary risk factors from 6.7±0.3% (control group; 56.8±1.0 years old) to 4.8±0.5%.[23] The ages of those subjects were close to those of the patients in the current study. From these data, the %FMD of 3.3±0.3% before nCPAP in OSAS patients is considered very low compared with the %FMD of healthy subjects in our previous study. From this viewpoint, the endothelial function appears to be impaired in the present OSAS patients and we need to set up a more precise control group to clarify this.

## Conclusion

We found that nCPAP improves endothelial function (FMD), in part by reducing ADMA, thereby increasing L-arginine availability and NO production. In this regard, nCPAP is effective for treating vascular dysfunction as well as sleep disorders. OSAS is now recognized as a vicious cluster of sleep, metabolic, and vascular disorders and we propose that measuring FMD, ADMA, and NOx is useful for evaluating the vascular conditions.

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〈症例報告〉

# 原因不明の発熱で発症し，赤芽球癆が先行した高齢者悪性リンパ腫の1例

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〈要　約〉 症例は76歳女性．抗生剤（CEZ，PIPC）が無効な不明熱に対する精査目的で2002年6月第1回入院．39℃に至る発熱，CRP上昇，汎血球減少を認めた．貧血については，骨髄穿刺で赤芽球癆と診断された．原因の特定に至らないまま，1カ月後に胆嚢腫脹，胆道系酵素の上昇を認め，胆嚢穿刺を行ったところ発熱は軽快，その後赤芽球癆も軽快し退院となった．しかし2003年4月，再び発熱，CRP上昇，汎血球減少を認めたため第2回入院．検索の結果，脾腫・異型リンパ球の出現とともに骨髄穿刺の所見からびまん性大細胞型B細胞リンパ腫（骨髄浸潤）の診断に至った．高齢者は典型的な症状を示しにくく，発熱のみを主症状とする節外性の悪性リンパ腫の場合には，他疾患との鑑別がきわめて困難である．高齢者の不明熱においては血液悪性腫瘍，とくに悪性リンパ腫が潜在している可能性を念頭におき，精査を行うことが必要と考えられた．

**Key words**：不明熱，赤芽球癆，高齢者，悪性リンパ腫

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## 緒　言

不明熱の鑑別診断は若年者と同様に感染症，悪性腫瘍，膠原病などが主因を占める．しかし高齢者は自覚症状に乏しく，若年者のような典型的な症状を示しにくい．また高齢者は発熱により消耗すると全身状態が極めて悪化してしまうことがあり，結果として十分な検査が行えず，不明熱と表現されてしまうこともある．我々は赤芽球癆と原因不明の発熱を示し，1年の経過観察で悪性リンパ腫と診断しえた高齢者の一症例を経験したので報告する．

## 症　例

症例：76歳，女性．

主訴：発熱，食欲不振．

既往歴：高脂血症・高血圧．

家族歴：食道癌（父），胃癌（母）．

生活歴：出身地は広島，被爆歴なし．飲酒歴，喫煙歴なし．

第1回入院の経過：2002年5月頃から食欲低下，自立歩行不能となり近医に緊急入院．38℃台の発熱・炎症反応上昇（CRP 6mg/dl程度）に対し，2週間にわたり抗生剤を使用されたがスパイク状の発熱が連日続き，また著明な貧血（Hb 6.7g/dl）も認められたため，精査目的に6月3日東大病院老年病科に第1回入院となった．理学所見上，皮疹や表在リンパ節の腫脹は認めなかった．入院時検査所見はTable 1の通り．汎血球減少と異型リンパ球の出現，総蛋白，アルブミンの低値，LDH，フェリチン，CRPの高値を認めた．また可溶性IL-2レセプターは3,227U/mlと著明な高値であった．末梢血の細胞表面マーカーは明らかにCD8優位で，HLA-DR陽性のCD8陽性リンパ球が60%を占めていた．入院当初の各種培養はすべて陰性で，各種自己抗体は陰性．各種ウイルス抗体価はいずれも陰性，または既感染パターンであった．画像所見としては，頭部，胸部，腹部のCTで軽度の脾腫，慢性胆嚢炎を疑わせる壁肥厚の所見を認めたが，深部のリンパ節腫脹を認めず，ガリウムシンチでも異常な集積増加は認められなかった．上部・下部消化管内視鏡検査，気管支鏡検査でも特記すべき所見は認められなかった．入院後，白血球数，血小板数については基準範囲内にて経過したものの貧血のみは高度なまま推移し，頻回の輸血を必要とした．入院後18日が経過した時点で，白血球数7,600/μl（視算法にて，Neu 63.0%，Eos 0%，Baso 0.5%，Mono 16.5%，Lym 16.5%，A-lym 3.5%），赤血球数191×10⁴/μl，Hb 5.8g/dl，Hct 16.9%，Plt 15.6×10⁴/μlであった．貧血の精査のため，骨髄穿刺を行ったところ，赤芽球系統がほとんど認められず（Table 1），赤芽球癆（pure red cell aplasia：PRCA）と

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**Table 1**　第1回入院時検査所見

| Peripheral blood | | Blood chemistry | | Serology | |
|---|---|---|---|---|---|
| WBC | 3,800 /μl | TP | 4.9 g/dl (6.3—8.1) | RA | (−) |
| | (4,000—9,000) | Alb | 1.9 g/dl (3.7—4.9) | ANA | (−) |
| Stab | 3.0 % | LDH | 492 IU/l (125—237) | Anti-DNA Ab | (−) |
| Seg | 58.0 % | GOT | 29 IU/l (9—38) | ANCA | (−) |
| Eo | 0.0 % | GPT | 13 IU/l (4—36) | HBs Ag | (−) |
| Bas | 2.0 % | γ GTP | 42 IU/l (4—68) | HCV Ab | (−) |
| Mo | 14.0 % | ALP | 179 IU/l (60—201) | HTLV-1 Ab | (−) |
| Ly | 19.0 % | T-Bil | 0.6 mg/dl (0.3—1.3) | CRP | 7.0 mg/dl (< 0.3) |
| Atypical ly | 4.0 % | BUN | 15.8 mg/dl (9—31) | Ferritin | 1,181 ng/ml (4—108) |
| RBC | 248 × $10^4$ /μl | Cr | 0.55 mg/dl (0.4—0.9) | EPO | 709 mIU/ml (16.6—37.5) |
| | (380—480 × $10^4$) | Na | 132 mEq/l (132—148) | sIL-2R | 3,227 U/ml (167—497) |
| Hb | 7.5 g/dl (12—16) | K | 4.3 mEq/l (3.5—4.9) | EBV VCA-IgG | × 320 (0—10) |
| Ht | 22.6 % (34—42) | Cl | 100 mEq/l (96—108) | EBV VCA-IgM | < × 10 (0—10) |
| Reti | 0.2 % (0.8—2.0) | Fe | 101 μg/dl (40—162) | EA-IgG | < × 10 (0—10) |
| Plt | 9.5 × $10^4$ /μl | UIBC | 120 μg/dl (126—358) | EBNA | × 20 (0—10) |
| | (14—40 × $10^4$) | Glu | 126 mg/dl (75—105) | parvo B19 IgM | 0.13 |
| | | | | Flowcytometry | |
| | | | | CD3/CD19 | 87/2 |
| | | | | CD4/CD8 | 0.37 |
| | | | | CD8 + DR + | 60 % |

Bone Marrow　normocellular bone marrow
NCC 5.7 × $10^4$/μl, M/E 103.38, MgK 60
Erythroid series : Pro 0.0%, Baso 0.8%, Poly 0.0%, Ortho 0.0%, giant polyerythroblast (−)

**Table 2**　第2回入院時検査所見

| Peripheral blood | | Blood chemistry | | Serology | |
|---|---|---|---|---|---|
| WBC | 3,700 /μl | TP | 5.6 g/dl (6.3—8.1) | CRP | 6.5 mg/dl (< 0.3) |
| | (4,000—9,000) | Alb | 2.6 g/dl (3.7—4.9) | Ferritin | 2,523 ng/ml (4—108) |
| Stab | 0.0 % | LDH | 333 IU/l (125—237) | sIL-2R | 1,877 U/ml (167—497) |
| Seg | 62.0 % | GOT | 27 IU/l (9—38) | EBV VCA-IgG | × 640 (0—10) |
| Eo | 0.0 % | GPT | 17 IU/l (4—36) | EBV VCA-IgM | < × 10 (0—10) |
| Bas | 1.0 % | γ GTP | 35 IU/l (4—68) | EA-IgG | < × 10 (0—10) |
| Mo | 13.0 % | ALP | 149 IU/l (60—201) | EBNA | × 40 (0—10) |
| Ly | 20.0 % | T-Bil | 0.6 mg/dl (0.3—1.3) | | |
| Atypical ly | 4.0 % | BUN | 21.6 mg/dl (9—31) | Flowcytometry | |
| RBC | 289 × $10^4$ /μl | Cr | 0.75 mg/dl (0.4—0.9) | CD3/CD19 | 80/3 |
| | (380—480 × $10^4$) | Na | 135 mEq/l (132—148) | CD4/CD8 | 0.5 |
| Hb | 9.1 g/dl (12—16) | K | 4.1 mEq/l (3.5—4.9) | | |
| Ht | 27.0 % (34—42) | Cl | 103 mEq/l (96—108) | | |
| Reti | 1.7 % (0.8—2.0) | Fe | 21 μg/dl (40—162) | | |
| Plt | 8.5 × $10^4$ /μl | UIBC | 143 μg/dl (126—358) | | |
| | (14—40 × $10^4$) | Glu | 122 mg/dl (75—105) | | |

Bone Marrow　normocellular bone marrow
NCC 6.5 × $10^4$/μl, M/E 1.52
Erythroid series : Pro 1.7%, Baso 6.1%, Poly 19.1%, Ortho 9.6%, giant polyerythroblast (−)
大型でN/C比中等度の細胞を多数認め（30～40%），これらの細胞はMPO陰性で核小体を有し，
細胞質のbasophiliaは強度で，細胞質内には空胞が多数存在する．
Immunological staining
CD20 (+), CD19 (+), CD3 (−), CD5 (−), CD10 (−), CD79a (+), λ dominant
EBER (−), LMP1 (−), EBNA2 (−), IgH/C-MYC : (−) (t(8 ; 14), t(8 ; 2), t(8 ; 22))

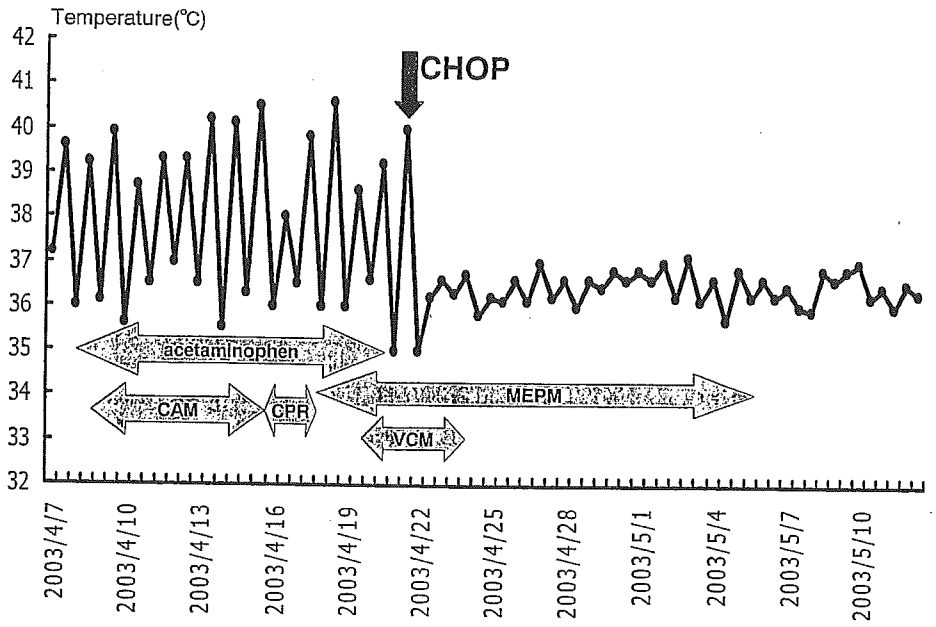


**Fig. 1**　第2回入院時の臨床経過．CAM : clarithromycin，CPR : cefpirome sulfate，VCM : vancomycin hydrochloride

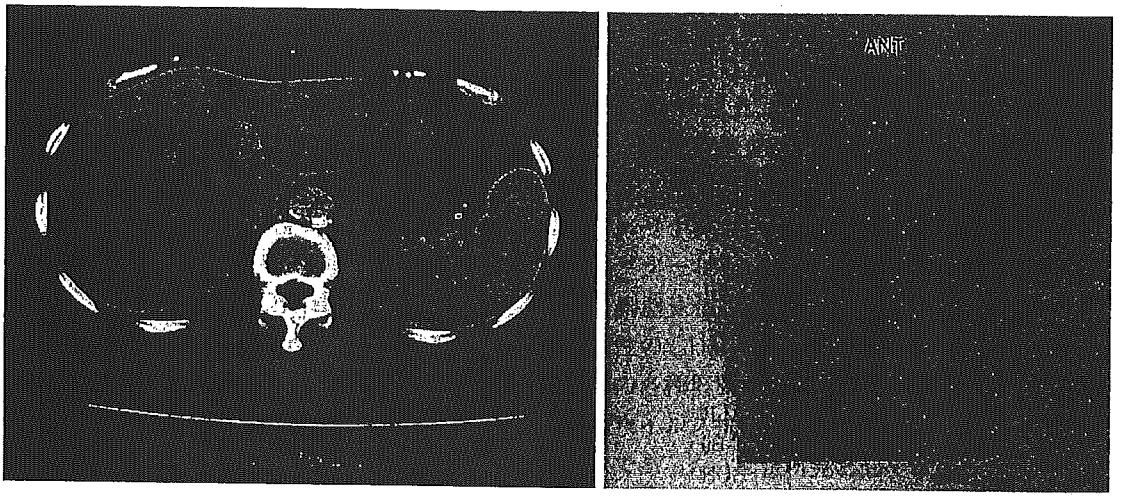


**Fig. 2**　第2回入院中に施行した腹部造影CT（図左）とガリウムシンチグラフィー（図右）．CTで脾臓内に低吸収域が散在し，ガリウムシンチグラフィーで相対的な脾臓の取り込み増加が認められる．

診断した．抗生剤を一時中止して熱型を観察したが，38℃以上の発熱が続き，再び抗生剤治療を行ったものの解熱は得られなかった．7月上旬に右季肋部痛，胆嚢腫脹，胆道系酵素上昇が認められ，胆嚢穿刺を行ったところ，それを契機に解熱し，CRPも低下した．解熱後，食事摂取良好となり8月下旬に独歩にて退院した．胆嚢炎のみで経過が説明できないため，リンパ増殖性疾患の可能性も考慮し脾摘も検討したが，診断確定には至らなかった．貧血に対しては適宜輸血を行い経過観察した．胆嚢穿刺から約1カ月経過したころに軽快傾向となり，退院時にはHb 10g/dl台まで改善した．

第2回入院までの経過：退院後は自宅で息子夫婦と同居し，近所に買い物に出かけて買い物ができるほどにADLは改善していた．貧血の進行も認めなかった．しかし，2003年1月頃から易疲労感を自覚し，2月頃から食欲低下が出現した．4月4日，38℃台に発熱したため当院救急外来を受診し，当科緊急入院となった．

入院時現症：身長148cm，体重37.2kg．体温38.4℃，

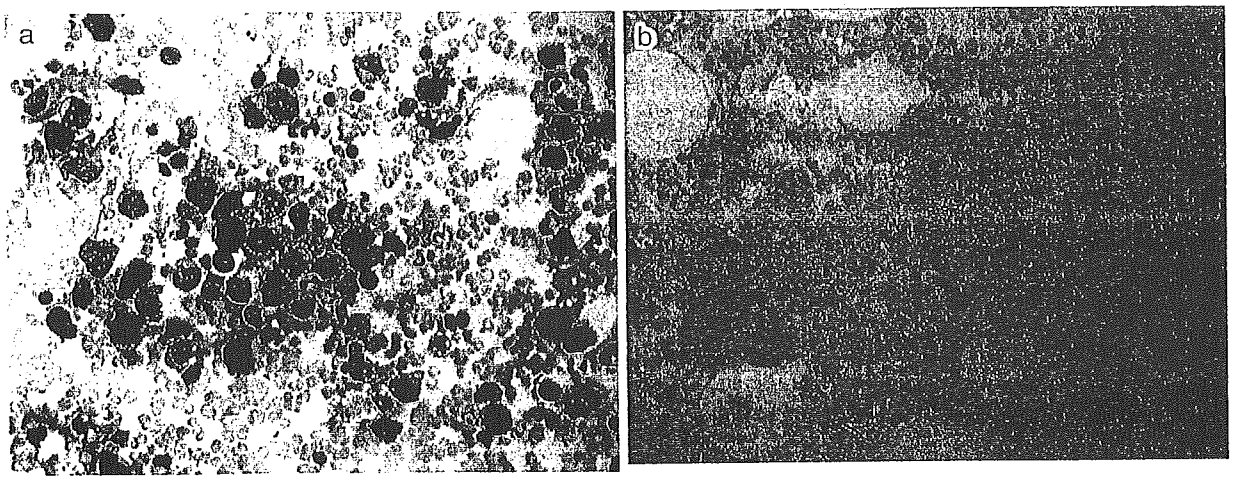


**Fig. 3**　第2回入院時に施行した骨髄穿刺標本
a　May-Giemza染色（×400）．大型の核と好酸性の大きな胞体を持つ，大型の異型細胞がびまん性に浸潤している．胞体内に空泡形成が認められ，一見“Starry-sky”様である．免疫組織学的にCD10陰性であった．
b　CD20免疫組織染色（×400）．CD20陽性細胞が褐色に染まる．

血圧90/60mmHg，脈拍86/分　整．意識レベルJapan Coma ScaleでI-1．長谷川式簡易知能評価スケール（HDS-R）は30点中12点．皮膚の弾性は低下しており，眼瞼結膜は貧血様．心尖部にLevine Ⅲ/Ⅵの収縮期雑音を聴取．脾臓を左肋弓下に2横指触知．表在リンパ節の腫脹なし．下腿浮腫なし．神経学的には四肢深部腱反射の減弱が認められた．

入院時検査所見：第2回入院時の検査所見をTable 2に示す．前回入院時と同様の汎血球減少と異型リンパ球の出現，LDH上昇，CRPやフェリチンの高値が認められた．また，可溶性IL-2レセプターの値は今回も高値（1,877U/ml）であった．

入院後の経過：第2回入院後の経過をFig. 1に示す．入院後施行した胸腹部造影CTでは，前回入院時には認めなかった脾臓の低吸収域が認められ，またガリウムシンチでも脾臓への取りこみが相対的に増加していた（Fig. 2）．さらに，骨髄穿刺標本において，大きな核と好酸性の大きな胞体を持つ，大型の異型細胞がびまん性に浸潤しており（Fig. 3a），免疫組織学的にCD20陽性（Fig. 3b），CD3，CD5，CD10は陰性，EBER（EBV-encoded RNAs），LMP1（EBV-encoded latent protein 1），EBNA2いずれも陰性であった．c-Myc遺伝子の転座（t(8；14)，t(8；2)，t(8；22)）は認められなかった．以上から，びまん性大細胞型B細胞リンパ腫（diffuse large B-cell lymphoma：DLBCL）と診断した．

入院時より認めていた弛張熱は抗生剤に反応することなく，DLBCLと診断後に開始した化学療法（CHOP療法；cyclophosphamide，doxorubicin，vincristine，prednisolone）に速やかに反応し，解熱した（Fig. 1）．1コースの化学療法により症状は安定し，外来で化学療法を継続する方針となり，5月30日に退院となった．

## 考　察

本症例は古典的な不明熱の定義[1]を満足し，精査を行ったにもかかわらず当初は確定診断に至らず，約1年の経過で悪性リンパ腫との診断に至った．不明熱の原因疾患としては，今日においても①感染症，②悪性腫瘍，③血管炎症候群を含む膠原病，が大部分を占め，高齢者においてもこれら3つの病態が重要である[2]．

本症例の経過を解釈するにあたり問題となるのは，第1回目の入院時すでに悪性リンパ腫が潜在していたかどうかという点である．確かに，胆嚢穿刺で軽快した点や著明な弛張熱を示した臨床経過は悪性リンパ腫の経過と解釈するには十分とは言えない．しかし，第1回入院時に認められた汎血球減少，脾腫，異型リンパ球の存在，可溶性IL-2レセプターの異常高値のすべてを一元的に説明するためには感染症の存在のみでは説明が困難で，この時期に悪性リンパ腫が潜在していたことは十分可能性がある．ただし，リンパ腫がDLBCLであったか，他のindolentなリンパ腫であったかは不明である．indolentなリンパ腫からaggressiveなリンパ腫へのtransformationの報告をみると，follicular lymphoma（FL）

は5年間に22%，10年間に31%がtransformationを来たし[3]，transformationまでの期間は平均約66カ月であると報告されている[4]．またsplenic marginal zone lymphoma（SMZL）では再発例の10～20%がtransformationを来たしており，それまでの期間は中間値として12～85カ月と報告されている[5]．したがって，第1回入院中にindolentの悪性リンパ腫が潜在するかどうかは組織学的に確認していないが，1年間の経過観察中にFLやSMZLなどindolentな悪性リンパ腫からaggressiveな悪性リンパ腫（DLBCL）にhigher-grade transformationを来たした可能性も考えられる．第1回入院中，診断確定のための脾摘を行うことも検討したが，患者の全身状態を考慮して施行しなかった．逆に，第1回目の入院経過は悪性リンパ腫のみでは説明しがたい．その理由として，上述のとおり胆嚢穿刺のみで発熱の軽快，CRPの低下が認められたこと，B症状が強く前面に出ているにもかかわらず，リンパ腫そのものの治療なしに一度は軽快していることが挙げられる．そのため，何らかのウイルス感染症が第1回入院前に存在し，それが軽快する間もなく入院後に発症した胆道感染症が経過を複雑にしていた可能性が高いと考えられる．ただし，悪性リンパ腫との関連が報告されているEBウイルスに関しては，第1回，第2回入院時の各種抗体価が既感染パターンであり，第2回入院時にEBウイルスDNAは検出されなかった．また，リンパ腫組織の免疫染色の結果はEBER，LMP1，EBNA2いずれも陰性であり，本症例のリンパ腫発症機転にEBウイルスが関与していたとの証明はできなかった．また，赤芽球癆との関連性が報告されているパルボウイルスB19についてもIgM抗体を測定したが，2度の入院いずれの経過においても陰性であった．

PRCAが非胸腺性のリンパ増殖性疾患に伴って生じることは稀である．慢性リンパ性白血病に赤芽球癆を合併したとの報告は比較的多く認められ，その機序として，腫瘍性IgG-FcR$^+$ T-Cell（Tγ細胞），あるいはサプレッサーT細胞による赤血球前駆細胞の抑制が関与しているとの報告[6)~9)]がこれまでになされている．しかし，悪性リンパ腫とPRCAの合併についての報告は稀である[10)~12)]．その機序としては慢性リンパ性白血病の場合と同様の推測がなされているほか，悪性リンパ腫の患者血清IgGがCFU-Eを抑制したり[13)]，患者単核細胞がCFU-Eコロニーの形成を抑制するため[14)]と考えられている．臨床的にも，治療により悪性リンパ腫が寛解するとPRCAも改善したとする報告が散見される[15)16)]．本症例で行った血液細胞表面マーカーに関する検討の結果は，第1回入院時はCD8陽性T細胞（サプレッサーT細胞）が圧倒的優位であり，このため赤血球前駆細胞の抑制が生じた結果，赤芽球癆を呈した可能性は十分に考えられる．ただし，第1回入院後2カ月で軽快傾向に転じ，以後第2回入院まで一貫して貧血の進行を認めなかったことから，赤芽球癆の発症原因を悪性リンパ腫のみに求めることには無理がある．やはり第1回入院前から何らかの感染症が存在していたために赤芽球癆を発症したとするのが妥当であるが，上述の理由で，悪性リンパ腫が潜在していたことがこの経過を修飾した可能性は否定できない．

以上の考察から，本症例は以下のようにまとめることができる．本症例の不明熱の主因は，第1回目の入院の時点では何らかの感染症と考えられ，入院後に追って発症した感染症，とくに胆道感染が経過を修飾していたものと考えられる．しかし，悪性リンパ腫が潜在していた可能性は考慮しなければならない．その場合，CD8陽性T細胞によって赤血球前駆細胞が抑制されていたとすれば，これが赤芽球癆発症の経過に影響を与えていた可能性も推測される．その後1年の経過で高悪性度の悪性リンパ腫が顕在化し，その結果再び不明熱を呈し，第2回入院中に施行した骨髄穿刺の結果，DLBCLの診断に至った．

以上，原因不明の発熱で発症し，赤芽球癆が先行した高齢者悪性リンパ腫の一症例を報告した．高齢者は典型的な症状を示しにくく，また易感染性で悪性腫瘍の頻度も成人と比較して高率である．したがって，発熱のみを主症状とする節外性の悪性リンパ腫の場合には，他の疾患との鑑別がきわめて困難となる．B症状の頻度やperformance statusの程度も成人と差がないとする報告が多い．診断のための検査も不十分なものになりがちである．高齢者の不明熱においては血液悪性腫瘍，とくに悪性リンパ腫が潜在している可能性を念頭におき，精査を行うことが必要と考えられる．

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## Abstract

### Malignant lymphoma manifested by fever of unexplained origin and pure red cell aplasia in an elderly patient

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A 76-year-old woman was admitted to the University of Tokyo Hospital in June 2002 because of fever of unexplained origin. She had suffered a high grade fever (above 39℃) for 2 weeks. Initial evaluation revealed elevated CRP and pancytopenia. Bone marrow aspiration (BMA) was performed, and a diagnosis of pure red cell aplasia (PRCA) was made. One month later, she complained right hypochondrial pain, and aspiration from her enlarged gall bladder was performed. Her fever and PRCA ameliorated, and she was discharged in August, 2002. In April 2003, she was readmitted to our hospital because of the recurrence of high grade fever, elevation of CRP, and pancytopenia. BMA was performed and revealed diffuse large B cell lymphoma. In the case of extranodal lymphoma which only presents pyrexia, differentiation with other diseases is very difficult especially in the elderly. It is necessary to bear in mind the possibility that a hematological malignancy, especially malignant lymphoma, can be latent in elderly patient with fever of unknown origin.

**Key words**: *Pure red cell aplasia, Fever of unexplained origin, Malignant lymphoma, Elderly*
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# Computerized detection of intracranial aneurysms for three-dimensional MR angiography: Feature extraction of small protrusions based on a shape-based difference image technique

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We have improved a computerized scheme for the detection of intracranial aneurysms for three-dimensional (3-D) magnetic resonance angiography (MRA) by the use of image features of small protrusions extracted based on a shape-based difference image (SBDI) technique. Initial candidates were identified by use of a multiple gray-level thresholding technique in dot enhanced images, and by finding short branches in skeleton images. Image features related to aneurysms were determined based on candidate regions segmented by use of a region growing technique. For extracting additional features on small protrusions or small aneurysms, we have developed an SBDI technique, which was based on the shape-based difference between an original segmented vessel and a vessel with suppressed local change in thickness. The SBDI technique was useful for obtaining local changes in vessel thickness, i.e., SBD regions, which could be small aneurysms in the case of true positives, but thin or very small regions in the case of false positives. Many false positives were removed by means of rule-based schemes and linear discriminant analysis on various 3-D localized image features, including SBDI features. We tested the computerized scheme on 53 cases with 61 aneurysms and 62 nonaneurysm cases based on a leave-one-out-by-patient test method. As a result, false positives per patient decreased from 5.8 to 3.8, while a high sensitivity of 97% was maintained by use of the SBDI technique, in which SBDI features were effective for removing some false positives. The computer-aided diagnostic (CAD) scheme may be robust and useful in assisting radiologists in the detection of intracranial aneurysms for MRA. © *2006 American Association of Physicists in Medicine.* [DOI: 10.1118/1.2163389]

Key words: shape-based difference image technique, computer-aided diagnosis (CAD), intracranial aneurysms, magnetic resonance angiography (MRA)

## I. INTRODUCTION

Subarachnoid hemorrhage (SAH) due to ruptures of intracranial aneurysms kills 10 000 persons each year in North America.[1] SAH is a serious disorder with high mortality and morbidity, accounting for about one-quarter of cerebrovascular deaths. From 3.6% to 6% of the adult population have intracranial aneurysms, which may rupture at an annual rate of 1% to 2%.[2,3] Magnetic resonance angiography (MRA) can be used to screen noninvasively for intracranial aneurysms in asymptomatic patients who have a family history of aneurysms or who have polycystic kidneys, coarctation of the aorta, or collagen vascular disease, putting them at an increased risk for aneurysms. In these patients, it is very important to detect unruptured aneurysms as early as possible and to treat or follow up the aneurysms. However, it is difficult and time consuming for radiologists to detect small aneurysms, and it may not be easy to find even medium-sized aneurysms because of their overlap with adjacent vessels or because of unusual locations in maximum intensity projection (MIP) images of MRA. For correct and efficient detection of intracranial aneurysms with MRA, therefore, radiologists would need a number of training sessions.