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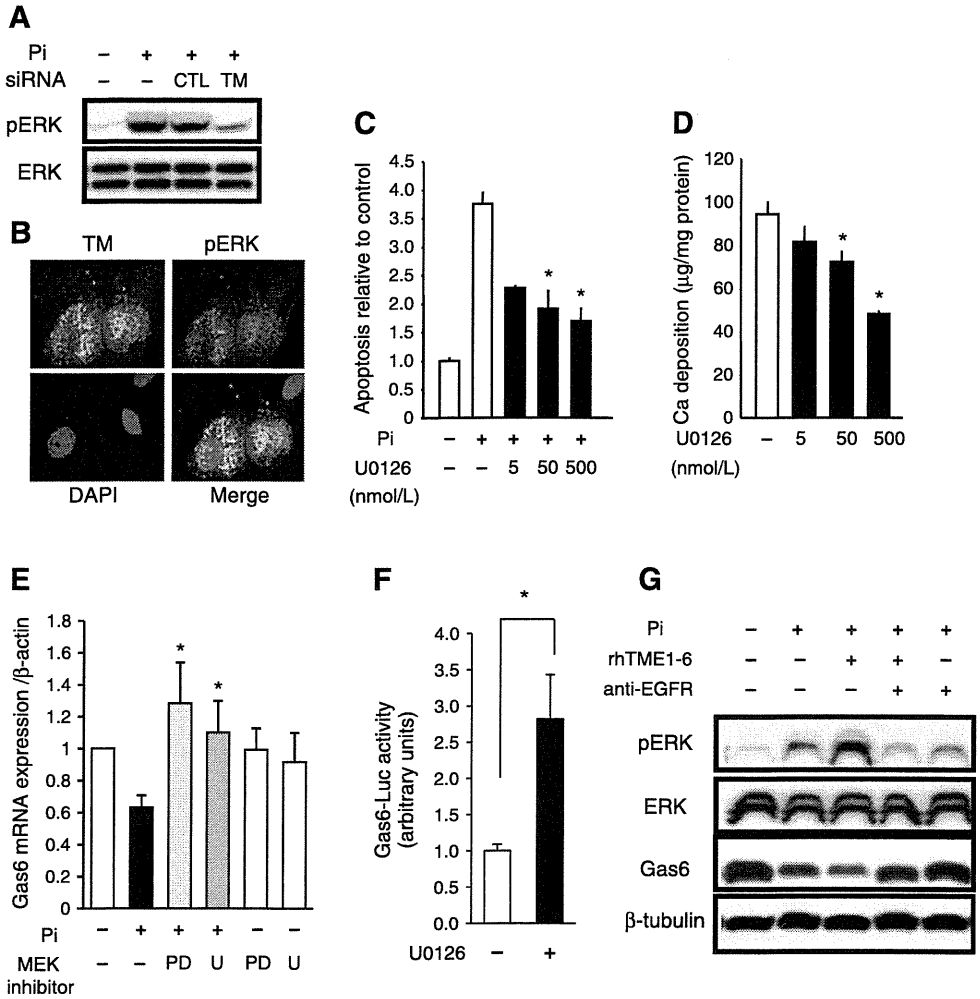

Fig. 5. ERK is a downstream signal of TM/EGFR axis. (A) HASMC were transfected with 100 nmol/L TM siRNA or nonspecific (CTL) siRNA in the presence of Pi. On day 6, cell lysates were harvested and pERK and ERK were examined by immunoblotting. (B) TM (green) and pERK (red) expression were colocalized in the presence of 2.6 mmol/L Pi at 6 days. Nuclei were counterstained with DAPI (blue). (C) and (D), HASMC were cultured with the indicated concentrations of U0126, an MEK inhibitor, in the presence of 2.6 mmol/L Pi. Apoptosis (n=3) and Ca deposition (n=5) were analyzed on day 6. (E) PD98059 (PD; 0.5 μmol/L) and U0126 (U; 0.5 μmol/L) were treated with or without 2.6 mmol/L Pi for 6 days. On day 6, total RNA was extracted and examined for Gas6 mRNA (n=3). GAPDH mRNA was measured as loading control. (F) HASMC were transiently transfected with 0.8 μg pGL3Gas6(1827) constructs. Twenty four hours after transfection, U0126 (U; 0.5 μmol/L) were added and were incubated for an additional 24 h. Relative promoter activities are expressed as mean ± SEM (n=4). Three separate transfection experiments showed similar results. (G) HASMC were cultured with or without rTME1-6 (50 μg/mL) or anti-EGFR neutralizing antibody (10 μg/mL) in the presence of Pi. On day 6, immunoblots with pERK, ERK, Gas6, and β-tubulin were examined. All values are presented as mean ± SEM. *p<0.05 vs. 2.6 mmol/L Pi. The analysis was conducted by ANOVA followed by Fisher's test. (For interpretation of the references to color in this figure legend, the reader is referred to the web of this article.)

signaling may play an important role, linking TM and Gas6, in the process of Pi-induced VSMC calcification.

TM is widely distributed in the endothelium of human arteries, veins, and lymphatic vessels. Interestingly, in atherosclerotic lesions of the human aorta, TM is expressed in intimal and medial VSMC. It has been reported that the extracellular region of TM contains a lectin-like domain and six epidermal growth factor (EGF)-like domains, which are required for the various functions [20,24–26]. EGF-like domain of TM stimulated proliferation of Swiss 3T3 fibroblasts [20]. Especially, in VSMC, EGF-like domain of TM has been reported to have a mitogenic effect [6]. Consistent with these results, we showed that Pi stimulated the secretion of TM into the culture medium and direct binding of the recombinant extracellular domain of TM to EGFR. Furthermore, these results suggest the shedding of TM through proteases such as MMPs. Supporting this possibility, the expression of MMP2 and MMP9 was increased by Pi, and GM6001, an inhibitor of MMPs significantly inhibited TM expression, secretion, and EGFR phosphorylation, followed by Pi-induced calcification (Fig. S7). Unexpectedly, GM6001 suppressed Pi-induced TM expression in HASMC (Fig. S7C). We speculate that this unexpected result might have been caused by additional effects of MMPs other than TM cleavage. These include the secretion of cytokines and growth factors, which may influence on TM expression. In fact, it has been shown that MMPs increase the secretion of TNFα [24], which accelerates Pi-induced VSMC calcification [25]. In contrast, GM6001 has been reported to inhibit the release of TNFα in vivo and in vitro [26]. It has been also reported that MMP-1 upregulates VEGF [27]. Although the effects of MMPs on growth factors are unclear, it can be supposed that MMPs might stimulate the secretion of growth factors such as PDGF, one of TM inducers, as shown in supplemental Fig. S8A. Furthermore, nonspecific inhibition or other effects of GM6001 might play a role. In any case, we do not have enough data to prove the effect of GM6001/MMPs on the intracellular expression of TM. The role of intracellular TM is also unclear. Recently, it has been reported that induction of Mcl-1, an antiapoptotic protein by intracellular domain of TM was about 2-fold greater than that mediated by

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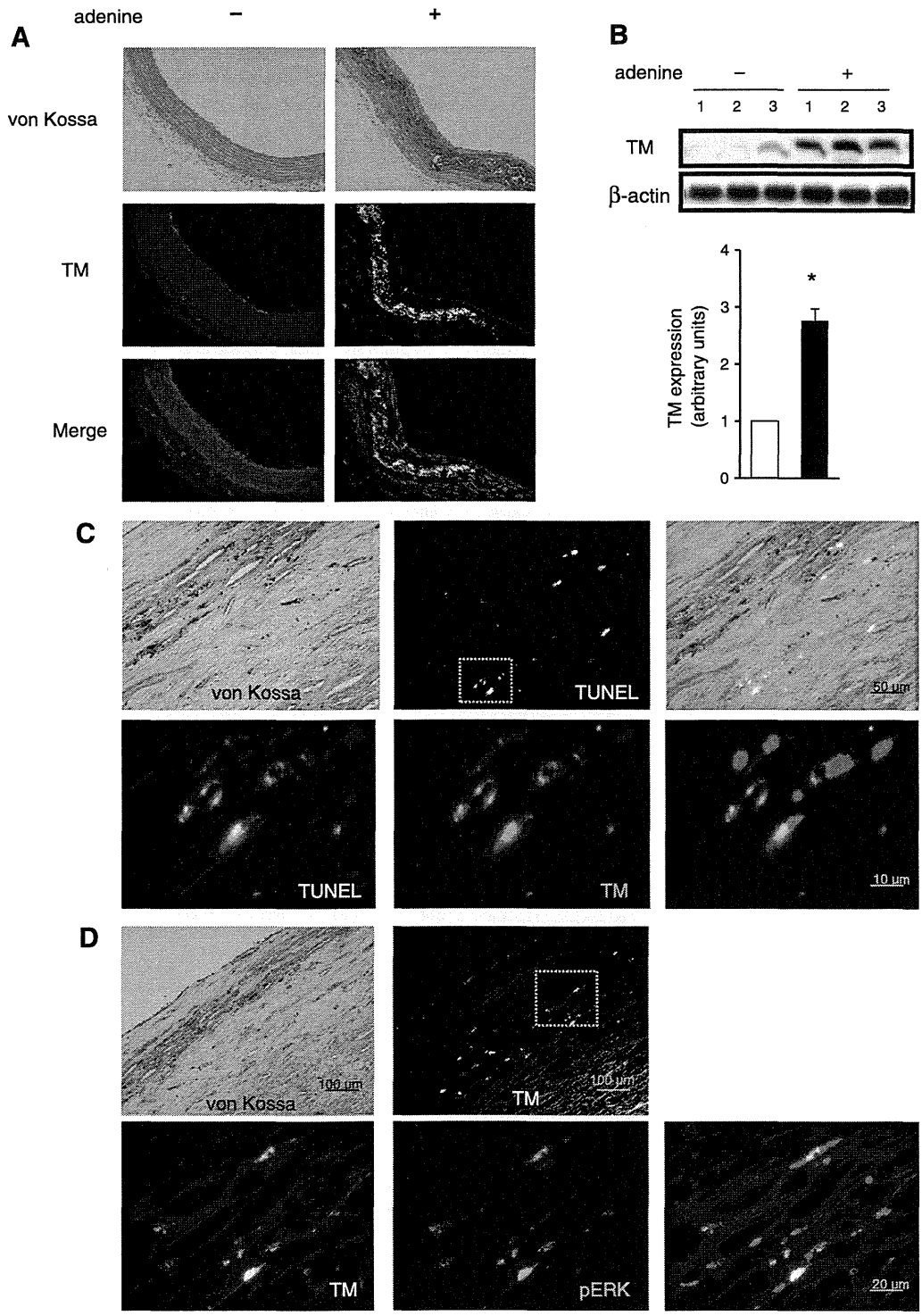

Fig. 6. TM expression in aortic media calcification of rats and in calcified human aorta. (A) Morphological assessment by von Kossa staining showed medial calcification in the aorta of 0.75% adenine-fed rats (right panel), compared with that of control rats (left panel). Adjacent aortic sections are shown stained for TM and DAPI. (B) Total proteins harvested from three different aortas (1~3, control rats and adenine-fed rats) were subjected to SDS-PAGE followed by immunoblotting with antibodies to TM and β-actin. Signal intensity of immunoblotting was quantified and provided the results in the bar graphs. Scale bar is 50 μm. All values are presented as mean ± SEM. The analysis was conducted by ANOVA followed by Fisher's test. (C) The aorta was dissected from an 80-year-old man with arteriosclerosis. Adjacent aortic sections were stained for mineral by von Kossa method and for TUNEL-positive apoptosis by immunohistochemical staining. The two images were merged. Lower panels show high-power view of dotted area stained for TUNEL and TM, and merged with DAPI. D, Adjacent aortic sections stained by von Kossa method and for TM. Lower panels show high-power view of dotted area stained for TM and pERK, and merged with DAPI.

extracellular domain of TM [28]. Further studies are required to address the effects of MMPs on intracellular expression of TM and functions of the intracellular domain of TM.

In the present study, we observed that TM was expressed not only in apoptotic cells (Fig. 2A) but also in normal VSMC (Figs. 1C and 5B). These results suggest that TM is expressed abundantly in

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Pi-stimulated VSMC and different expressions might be caused by cell condition such as cell cycle. It is not surprising that different response by stimulus was observed in the tissue, including the aorta. Furthermore, rhTME1-6 alone did not have a stimulatory effect on apoptosis and Ca deposition and the expressions of Gas6 and Msx2 did not change (data not shown). Taken together, it is reasonable to consider that TM is one of the players in Pi-induced VSMC calcification.

We also examined the effect of other factors on TM expression in VSMC. As shown in supplemental Fig. S8, Vit D3, thrombin, or PDGF-BB, well-known stimulators of TM, increased TM expression in VSMC. We confirmed the increase in transactivation of TM by each stimulator (Fig. S8B). However, results of Ca deposition by Vit D3, thrombin, or PDGF-BB were not consistent with Pi, as shown in supplemental Fig. S8C. We considered that because Pi is an essential inducer of calcification, only TM upregulation by Vit D3, thrombin, or PDGF-BB could not develop vascular calcification.

ERK plays a critical role in osteoblast differentiation and mineralization [13,14], and is a well-known downstream signal of TM. In the present study, Pi-induced TM/EGFR signaling stimulated ERK to suppress Gas6 expression, which then induced apoptosis. ERK activation positively regulates calcification and osteoblastic differentiation in VSMC [17,29–31]. For example, Runx2, osteoblastic transcription factor, is phosphorylated and activated by ERK [31]. Consistent with these findings, in the present study, MEK inhibitors reversed Pi-reduced myocardin expression, a key transcription coactivator of VSMC differentiation, and Pi-induced msh homeo box homolog 2 (Msx2) expression, one of osteogenic markers (Fig. S9A). Interestingly, when the expression of TM was knocked down by siRNA, upregulation of myocardin mRNA and downregulation of Msx2 were significantly abrogated (Fig. S9B). These results suggest that TM also affected VSMC phenotype transition, as well as apoptosis in the development of vascular calcification via ERK. We demonstrated the possible role of TM in vascular calcification in supplemental Fig. S10.

We suppose that Pi transport via type III sodium-phosphate cotransporter (Pit-1) is the most upstream step in the Pi-induced VSMC calcification, but several factors other than Pi are involved in the development of clinical vascular calcification. Furthermore, because TM was increased both in calcified aorta of adenine-fed rat and in calcified aorta of human, we think that TM could be the most relevant pharmaceutical target among Pit, TM, EGFR, and ERK. Although EGFR and ERK are important signaling pathways contributing to Pi-induced calcification, these pathways are also affected by other stimuli, such as proliferation or apoptosis in VSMC. To investigate the pharmaceutical target, further study is needed to examine the role of each player in vascular calcification in vivo, using antibody reactive to EGF domain of TM, inhibitors for EGFR (Icotinib) [32] or ERK (REDA119) [33].

In conclusion, we demonstrated that TM is a novel molecule induced by Pi in the process of VSMC calcification, and the TM/EGFR/ERK axis plays a critical role in the regulation of Gas6-mediated survival pathway. These findings provide new mechanistic insight into the study of vascular calcification.

Conflict of interest statement

The authors declare no potential conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yjmcc.2012.12.013.

References

[1] Sage AP, Tintut Y, Demer LL. Regulatory mechanisms in vascular calcification. Nat Rev Cardiol 2010;7:528–36.
[2] Johnson RC, Leopold JA, Loscalzo J. Vascular calcification: pathobiological mechanisms and clinical implications. Circ Res 2006;99:1044–59.
[3] Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, et al. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. Circulation 1996;94:1175–92.
[4] Giachelli CM, Jono S, Shioi A, Nishizawa Y, Mori K, Morii H. Vascular calcification and inorganic phosphate. Am J Kidney Dis 2001;38:S34–7.
[5] Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, et al. Phosphate regulation of vascular smooth muscle cell calcification. Circ Res 2000;87:e10–7.
[6] Tohda G, Oida K, Okada Y, Kosaka S, Okada E, Takahashi S, et al. Expression of thrombomodulin in atherosclerotic lesions and mitogenic activity of recombinant thrombomodulin in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1998;18:1861–9.
[7] Huang HC, Shi GY, Jiang SJ, Shi CS, Wu CM, Yang HY, et al. Thrombomodulin-mediated cell adhesion: involvement of its lectin-like domain. J Biol Chem 2003;278:46750–9.
[8] Ito T, Kawahara K, Okamoto K, Yamada S, Yasuda M, Imaizumi H, et al. Proteolytic cleavage of high mobility group box 1 protein by thrombin–thrombomodulin complexes. Arterioscler Thromb Vasc Biol 2008;28:1825–30.
[9] Suzuki K, Kusumoto H, Deyashiki Y, Nishioka J, Maruyama I, Zushi M, et al. Structure and expression of human thrombomodulin, a thrombin receptor on endothelium acting as a cofactor for protein C activation. EMBO J 1987;6:1891–7.
[10] Esmon CT. The roles of protein C and thrombomodulin in the regulation of blood coagulation. J Biol Chem 1989;264:4743–6.
[11] Wen D, Dittman WA, Ye RD, Deaven LL, Majerus PW, Sadler JE. Human thrombomodulin: complete cDNA sequence and chromosome localization of the gene. Biochemistry 1987;26:4350–7.
[12] Olivot JM, Estebanell E, Lafay M, Brohard B, Aiach M, Rendu F. Thrombomodulin prolongs thrombin-induced extracellular signal-regulated kinase phosphorylation and nuclear retention in endothelial cells. Circ Res 2001;88:681–7.
[13] Franceschi RT, Ge C, Xiao G, Roca H, Jiang D. Transcriptional regulation of osteoblasts. Ann N Y Acad Sci 2007;1116:196–207 [Review].
[14] Ge C, Xiao G, Jiang D, Franceschi RT. Critical role of the extracellular signal-regulated kinase-MAPK pathway in osteoblast differentiation and skeletal development. J Cell Biol 2007;176:709–18.
[15] Lai CF, Chaudhary L, Fausto A, Halstead LR, Ory DS, Avioli LV, et al. Erk is essential for growth, differentiation, integrin expression, and cell function in human osteoblastic cells. J Biol Chem 2001;276:14443–50.
[16] Ding HT, Wang CG, Zhang TL, Wang K. Fibronectin enhances in vitro vascular calcification by promoting osteoblatic differentiation of vascular smooth muscle cells via ERK pathway. J Cell Biochem 2006;99:1343–52.
[17] Speer MY, Yang HY, Brabb T, Leaf E, Look A, Lin WL, et al. Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. Circ Res 2009;104:733–41.
[18] Son BK, Kozaki K, Iijima K, Eto M, Kojima T, Ota H, et al. Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring Gas6-Axl survival pathway. Circ Res 2006;98:1024–31.
[19] Son BK, Kozaki K, Iijima K, Eto M, Nakano T, Akishita M, et al. Gas6/Axl-PI3K/Akt pathway plays a central role in the effect of statins on inorganic phosphate-induced calcification of vascular smooth muscle cells. Eur J Pharmacol 2007;556:1–8.
[20] Hamada H, Ishii H, Sakyo K, Horie S, Nishiki K, Kazama M. The epidermal growth factor-like domain of recombinant human thrombomodulin exhibits mitogenic activity for Swiss 3T3 cells. Blood 1995;86:225–33.
[21] Watanabe T, Akishita M, Nakaoka T, He H, Miyahara Y, Yamashita N, et al. Caveolin-1, Id3a and two LIM protein genes are upregulated by estrogen in vascular smooth muscle cells. Life Sci 2004;75:1219–29.
[22] Yokozawa T, Zheng PD, Oura H, Koizumi F. Animal model of adenine-induced chronic renal failure in rats. Nephron 1986;44:230–4.
[23] Shroff RC, McNair R, Figg N, Skepper JN, Schurgers L, Gupta A, et al. Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis. Circulation 2008;118:1748–57.
[24] Wang Y, Xu F, Chen J, Shen X, Deng Y, Xu L, et al. Matrix metalloproteinase-9 induces cardiac fibroblast migration, collagen and cytokine secretion: inhibition by salvianolic acid B from Salvia miltiorrhiza. Phytomedicine 2011;19:13–9.
[25] Son BK, Akishita M, Iijima K, Kozaki K, Maemura K, Eto M, et al. Adiponectin antagonizes stimulatory effect of TNFalpha on vascular smooth muscle cell calcification: regulation of Gas6-mediated survival pathway by AMP-activated protein kinase. Endocrinology 2008;149:1646–53.

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[26] Solorzano CC, Ksontini R, Pruitt JH, Auffenberg T, Tannahill C, Galardy RE, et al. A matrix metalloproteinase inhibitor prevents processing of tumor necrosis factor alpha (TNF alpha) and abrogates endotoxin-induced lethality. Shock 1997;7:427–31.
[27] Mazor R, Alsaigh T, Shaked H, Altshuler A, Pocock ES, Kistler EB, et al. Matrix metalloproteinase-1 mediated upregulation of vascular endothelial growth factor-2 in endothelial cells. J Biol Chem Nov 15 2012.
[28] Ikezoe T, Yang J, Nishioka C, Honda G, Furihata M, Yokoyama A. Thrombomodulin protects endothelial cells from a calcineurin inhibitor-induced cytotoxicity by upregulation of extracellular signal-regulated kinase/myeloid leukemia cell-1 signaling. Arterioscler Thromb Vasc Biol 2012;32:2259–70.
[29] You H, Yang H, Zhu Q, Li M, Xue J, Gu Y, et al. Advanced oxidation protein products induce vascular calcification by promoting osteoblastic trans-differentiation of smooth muscle cells via oxidative stress and ERK pathway. Ren Fail 2009;31:313–9.
[30] Nakahara T, Sato H, Shimizu T, Tanaka T, Matsui H, Kawai-Kowase K, et al. Fibroblast growth factor-2 induces osteogenic differentiation through a Runx2 activation in vascular smooth muscle cells. Biochem Biophys Res Commun 2010;394:243–8.
[31] Ge C, Xiao G, Jiang D, Yang Q, Hatch NE, Roca H, et al. Identification and functional characterization of ERK/MAPK phosphorylation sites in the Runx2 transcription factor. J Biol Chem 2009;284:32533–43.
[32] Tan F, Shen X, Wang D, Xie G, Zhang X, Ding L, et al. Icotinib (BPI-2009H), a novel EGFR tyrosine kinase inhibitor, displays potent efficacy in preclinical studies. Lung Cancer 2012;76:177–82.
[33] Habashi JP, Doyle JJ, Holm TM, Aziz H, Schoenhoff F, Bedja D, et al. Angiotensin II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. Science 2011;332:361–5.

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Paper:

Relation Between Blood Pressure Estimated by Pulse Wave Velocity and Directly Measured Arterial Pressure

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Hypertension is the strongest risk factor in cardiac and cerebrovascular diseases among the Japanese. Even daily variations in blood pressure may become a risk, and repeated blood pressure measurement is recommended. Conventional Ambulatory Blood Pressure Monitoring (ABPM), however, may cause discomfort to examinees because they have to have their arms compressed and carry the monitor itself. The number of ABPM measurements is limited to about 1 every 15–30 minutes. We therefore attempted, working with medical and engineering teams, to develop a wearable blood pressure sensor that would place less burden on examinees, be less influenced by physical movement, and be usable for continuous blood pressure measurement. We then examined the clinical practicality of the sensor. We modified the existing Moens-Korteweg blood-pressure equation and developed a new systolic blood pressure calculation system that used electrocardiography and ear-lobe pulse waves because the ear lobe would receive little influence from physical movement. We chose three clinical cases from among intensive care unit subjects. We not only estimated their blood pressure using the system we developed but also measured arterial pressure directly with an intravascular catheter to see how estimated blood pressure followed actual changes in blood pressure and to evaluate the accuracy of estimated blood pressure. When systolic blood pressure estimated by using the pulse wave velocity method was compared with direct blood pressure measurement, we found that the method captured trends in blood pressure variations correctly. The difference was within ±10 mmHg for all of the cases. In a comparison using the Bland-Altman method for the three clinical cases, the average difference was −0.4 mmHg, −1.0 mmHg, and −1.7 mmHg and standard deviation was 4.2 mmHg, 4.8 mmHg, and 4.3 mmHg, respectively, which indicated good agreement. Introducing such wearable blood pressure sensors into daily medical practice gets detailed information on continuous blood pressure variation while examinees move freely and the resulting information is used for better quality control of adult diseases. It is also expected that wearable blood pressure sensors can be used in emergency medical cases, in intensive care, and at remote sites.

Keywords: pulse wave velocity, hypertension, blood pressure monitoring, wearable sensor, metabolic syndrome

1. Background

Cardiac and cerebrovascular diseases account for about 30% of deaths among Japanese, with the strongest risk factor being hypertension [1]. A 10 mmHg increase in systolic blood pressure raises morbidity and mortality in cerebral apoplexy by approximately 20% for men and by 15% for women, and morbidity and mortality in coronary artery disease by 15% [2]. In Japan, various measures have been taken. "Healthy Japan 21" was formulated in 2000, for example, and criteria for metabolic syndrome were established in 2005. Specific medical checkups and specific health guidance were introduced in 2008. The number of persons with hypertension has been increasing with the aging of the population and changes in lifestyle, and it is said that there are now about 40 million male and female patients [3].

Blood pressure varies depending on time, physical movement, and the medical environment. It is known that not only the absolute or average value but also changes in blood pressure, e.g., morning surge, are factors crucial in finding a risk for organ disorders [4]. The guideline for the treatment of hypertension therefore advocates the wider introduction of home blood pressure measurement or Ambulatory Blood Pressure Monitoring (ABPM) for 24 hours a day using an automatic sphygmomanometer with the cuff-oscillometric method, and recommends that diagnosis and control be made on the basis of information obtained from measurement [5, 6]. Since ABPM is useful in the measurement of relatively brief variation or daily fluctuation in blood pressure, it began to be covered by national health insurance in 2008, and is expected to play

an important role in future medical care. Current blood-pressure control is mostly done using single-time measurements at health checkups or at a clinician's office, and blood pressure variation is not frequently measured [7]. This is because ABPM places a burden on examinees. It requires, for example, that examinees wear a cuff on the upper arm 24 hours a day and puts pressure on the arm for a constant interval even while examinees are sleeping. The device is also relatively large. In addition, measurement accuracy changes depending on the physical size, body position, and behavior of examinees. Another problem is that sampling is not continuous and the number of samplings is limited to 1 to 4 times an hour.

Given this situation, we have developed a wearable blood pressure sensor that places less burden on examinees, is less influenced by physical movement, and allows blood pressure to be measured continuously [8]. The sensor uses the pulse wave velocity method and calculates systolic blood pressure using electrocardiogram (ECG) and pulse waves measured at the ear lobe. School of Engineering, The University of Tokyo, and The University of Tokyo Hospital have collaborated from the initial stage of development and are now applying the device in a clinical setting. The volume change in an artery caused by blood that comes from the heart is called a "pulse wave." Pulse-Wave Velocity (PWV) is defined by dividing the distance traveled by blood from the heart to a peripheral area by Pulse-wave Transit Time (T_{PTT}) and is known to be related to tension in arterial walls [9]. The pulse wave velocity method estimates systolic blood pressure P_s using T_{PTT}. Using a wearable blood pressure sensor, Iijima et al. verified that short-term blood pressure of elderly people increased when they were under a mental arithmetic burden and that their blood pressure rapidly increased when they had mental stress [10]. We put an ergometric exercise load on healthy male adults and found that their systolic blood pressure, which was estimated from T_{PTT} data, was very close to the value measured using conventional stethoscopy or ABPM (**Fig. 1**). Blood pressure estimated from PWV fluctuated more than blood pressure measured by stethoscopy or ABPM. This was probably because examinees wore a sensor on the ear lobe and physical movement during an exercise load could have been overreflected in blood pressure.

2. Objectives

There was an animal experiment report [11] on the verification of the relation between pulse wave velocity and blood pressure directly measured using a thin tube, called a catheter, inserted into a blood vessel. No data has been obtained from a human examinee, however, because it is difficult to insert a blood pressure sensor into an artery of the examinee. The measurement of blood pressure inside a blood vessel, however, has some advantages. It provides direct continuous measurement values, for example, and these obtained values are more useful in risk assessment of cerebrovascular and cardiovascular disease than

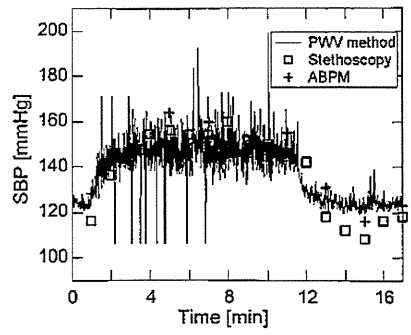


Fig. 1. Systolic blood pressure calculated from PWV and measured with stethoscopy or ABPM.

the cuff-pressurization method [12]. It is therefore necessary to compare the pulse wave velocity method that we have developed to direct arterial pressure measurement. In this study, we examined clinical cases of stable patients hospitalized in an Intensive Care Unit (ICU) of The University of Tokyo Hospital and compared blood pressure that was estimated using the pulse wave velocity method to the arterial pressure that was directly measured using a catheter in a blood vessel, in order to establish a method for the evaluation of blood pressure estimation with the pulse wave velocity method. We also made an error analysis in the comparison.

3. Method

3.1. Estimation of Systolic Blood Pressure Using Pulse-Wave Transit Time

Based on previous studies [8, 13], we estimated blood pressure by using the Moens-Korteweg equation and the following formula:

$$P_s = \frac{b_1}{{T_{PTT}}^2} + b_2$$

(T_{PTT}: pulse-wave transit time, P_s: systolic blood pressure)

Coefficients b_1 and b_2 in the above equation can be derived using measured values of blood pressure of an examinee and measured values of T_{PTT}. Then systolic blood pressure – at least that of the experimental participants – can be estimated at each heartbeat for a certain period of time. Although T_{PTT} is usually defined by the time from the first cardiac sound on a phonocardiogram to the inflection point (foot point) of the initial pulse wave rise, for simplicity, we used the time from the peak of the R wave in the QRS complex of an ECG to the foot point of the pulse wave (pulse-wave arrival time T_{PAT}) for estimation of blood pressure (**Fig. 2**). Using samples extracted at intervals of 0.5 mmHg between the maximum and minimum values of directly measured arterial blood pressure, a nonlinear least squares approximation is applied to T_{PAT} obtained for the first five pulses of each sample. The

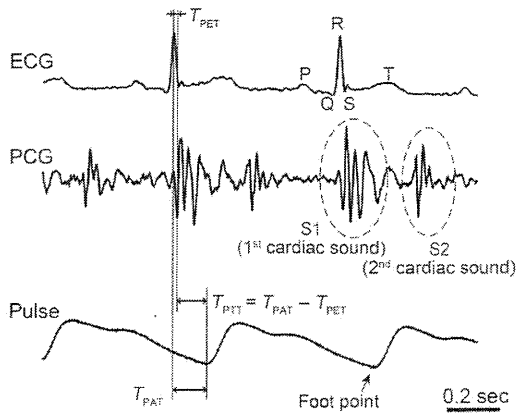


Fig. 2. Method of pulse-wave transit time. Pulse-wave transit time (T_{PTT}) is usually defined by the time from the first cardiac sound on a PCG to the foot point of the pulse, but in this study we used time (T_{PAT}) from the R wave peak in an ECG to the foot point of the pulse. ECG: electrocardiogram, PCG: phonocardiogram, PET: preejection time.

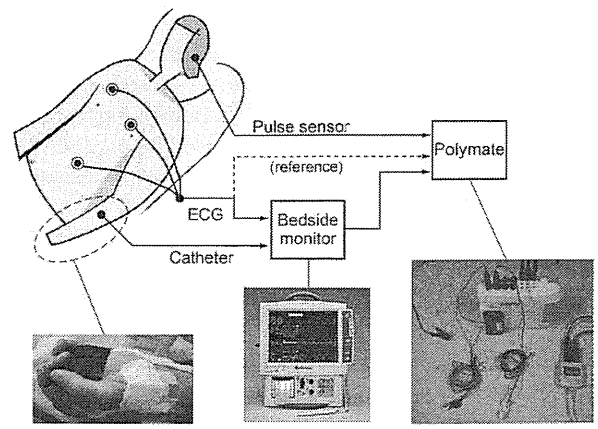


Fig. 3. Conceptual diagram of experiment.

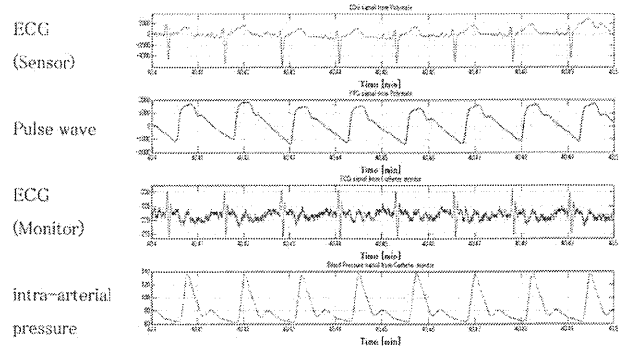


Fig. 4. ECG and pulse wave directly measured with Polymate II® and ECG and intra-arterial pressure monitored with bedside monitor (from above down).

above coefficients were then derived for each patient. We used MATLAB R2010b (The Math Works) for analysis.

3.2. Measurement Method

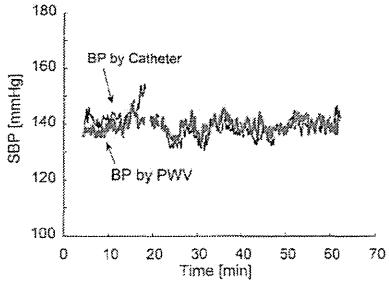
We studied clinical cases of patients who had been hospitalized in an ICU at The University of Tokyo Hospital and for whom direct blood pressure measurement had been performed via an intravascular catheter. A full explanation was given to patients whose condition was stable and who agreed to cooperate in the study. We then chose patients from whom we obtained informed consent as patients in the blood pressure measurement test. This study was approved by a research ethics review at the Graduate School of Medicine of The University of Tokyo (Review No.2272) and conducted in compliance with the Helsinki Declaration. Patients who were underage, had endotracheal intubation, had surgery on the chest or near the ear lobe where electrodes were to be attached, or were found to have multiple drug resistant bacteria or for whom inflectional control was needed were excluded from the study. A doctor made explanations, obtained informed consent, and attached measurement devices to patients. Measurement was performed for 60 minutes. Arterial blood pressure was measured with an indwelling 22-gauge Surflo needle (Terumo) inserted in the radial artery and a pressure monitoring tube set (1315TSA01; Edwards Lifescience). Data thus obtained was output from a bedside monitor AY-920P (Nihon Kohden) that was commonly used at ordinary medical care sites, and from an ECG. For measurement of pulse wave velocity, a portable bioamplifier (Polymate II®; TEAC) was used to make 10-bit sampling of analog signals of ECG and pulse waves at 1 kHz (max). Vitrode Bs-150 (Nihon Kohden) was used for electrodes for ECG. We used a head amplifier with variable gain from 400 to 2000 and input impedance of 1 MΩ. A 500 Hz low-pass filter and a 50 Hz notch filter were installed before the amplifier. We also used a transparent photoelectric pulse wave sensor (Ear Sensor; Combiwellness) that was commercially available and widely used for ergometers. The conceptual diagram of the experiment is given in **Fig. 3**.

4. Results

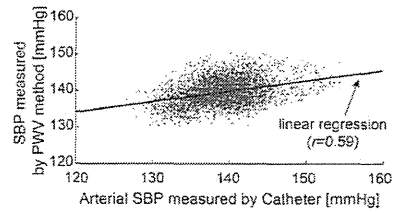
We collected data from three clinical cases.

None of the three patients reported pain or discomfort from the ear-lobe clip or ECG electrodes on the chest. There was also almost no noise during experiments. **Fig. 4** shows a sample waveform obtained in experiments.

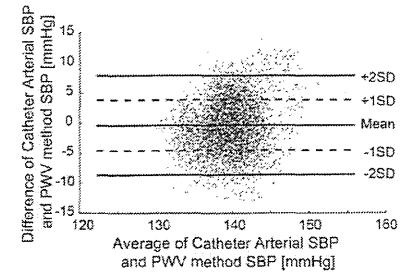
Data was obtained from a 44-year-old male who had had acute cholecystitis surgery. Although the patient was lying on a bed during measurement with no load applied, systolic blood pressure fluctuated in the range of 130–160 mmHg during 60-minute measurement. Waveform fluctuations in arterial blood pressure due to his physical movement were sometimes observed but no significant noise was acknowledged. We next calculated the pulse-wave transit time from the electrocardiographic and pulse waveforms and obtained a final estimation value



(a) Moving average deviations (red) in systolic blood pressure estimated from pulse-wave transit time over 30 pulses and systolic blood pressure (black) measured with catheter.

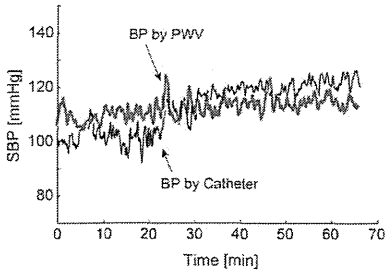


(b) Linear regression of moving average deviations in systolic blood pressure estimated from pulse-wave transit time and systolic blood pressure measured with catheter.

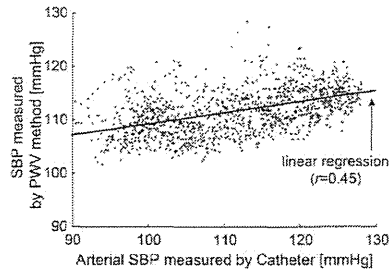


(c) Bland-Altman plotting of moving average deviations in systolic blood pressure estimated from pulse-wave transit time and systolic blood pressure measured with catheter.

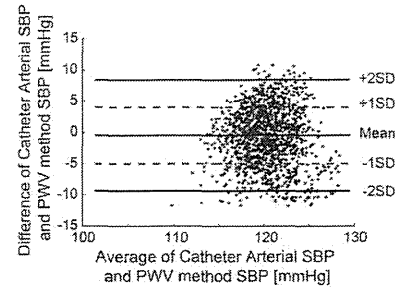
Fig. 5. Results of the first case.



(a) Moving average deviations (red) in systolic blood pressure estimated from pulse-wave transit time over 30 pulses and systolic blood pressure (black) measured with catheter.



(b) Linear regression of moving average deviations in systolic blood pressure estimated from pulse-wave transit time and systolic blood pressure measured with catheter.



(c) Bland-Altman plotting of moving average deviations in systolic blood pressure calculated from pulse-wave transit time and systolic blood pressure measured with catheter.

Fig. 6. Results of the second case.

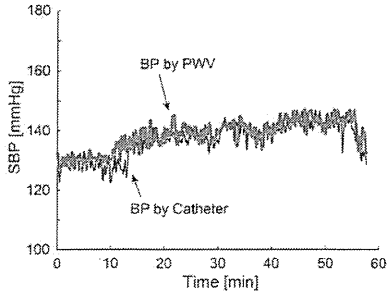
of systolic blood pressure. **Fig. 5(a)** shows moving average deviations of estimated systolic blood pressure for 30 pulses and systolic blood pressure actually measured with a catheter. Since measurement time was an hour, we showed the average over 30 pulses to demonstrate variations in a comprehensive manner in a single short chart. Correlation analysis of each heart beat indicates a certain correlation with correlation coefficient $r = 0.59$ (**Fig. 5(b)**). To observe the same signals with two different methods in measurement of blood pressure and other biosignals, it is proposed [14] to use the Bland-Altman method. We used the method for analysis and obtained an average difference of 0.4 mmHg and a standard deviation of 4.2 mmHg (**Fig. 5(c)**).

The second clinical case was a 48-year-old male who had aortic dissection of type Stanford B, DeBakey IIIa extending from a distant part of the aortic arch to a near part of an artificial blood vessel after the artificial vessel was introduced into his chest. His estimated systolic blood pressure, averaged over 30 pulses, and directly measured arterial blood pressure are shown in **Fig. 6(a)**. Compared to the first case, deviation was found more often although it still stayed within ± 10 mmHg. The correlation coefficient was $r = 0.44$, the Bland-Altman method average difference was 1.0 mmHg, and standard deviation was 4.8 mmHg (**Figs. 6(b)** and **(c)**).

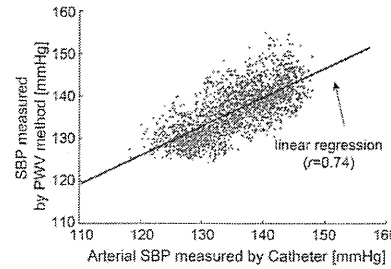
The third clinical case is an 80-year-old male patient in intensive care who had subcutaneous hematoma due to massive bleeding from a puncture after intra-coronary stenting. We made the same analysis as in the above two clinical cases and found correlation coefficient r of 0.74, averaged difference of 1.7 mmHg, and standard deviation of 4.3 mmHg in the Bland-Altman method (**Figs. 7(b)** and **(c)**).

5. Discussion

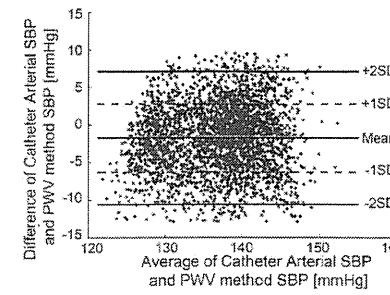
ECG and pulse waves were recorded over the entire measurement time, although there were some waveform fluctuations due to the physical movement of patients, and in every clinical case, systolic blood pressure was estimated from obtained data. The present set of parameters (b_1 and b_2) was optimized for each clinical case, but we will develop a system for optimizing parameters automatically and calculating systolic blood pressure at the same



(a) Moving average deviations (red) in systolic blood pressure estimated from pulse-wave transit time over 30 pulses and systolic blood pressure (black) measured with catheter.



(b) Linear regression of moving average deviations in systolic blood pressure estimated from pulse-wave transit time and systolic blood pressure measured with catheter.



(c) Bland-Altman plotting of moving average deviations in systolic blood pressure estimated from pulse-wave transit time and systolic blood pressure measured with catheter.

Fig. 7. Results of the third case.

time as data recording.

Systolic blood pressure obtained with the pulse wave velocity method in the three clinical cases was compared with directly measured values. Deviation showed systematic behavior and did not exceed ± 10 mmHg. Deviation within ± 10 mmHg seems tolerable in light of the standard error range ± 5 mmHg in the development of new clinical equipment. Variation in blood pressure, rather than the absolute value, is also important in daily clinical practice, so blood pressure estimation was sufficiently useful for application in medical practice. Large deviation was caused mostly by fluctuation or flattening of directly measured arterial blood pressure rather than the pulse wave sensor. This fluctuation or flattening occurred due to upper arm movement of patients or was an artifact involved in medical practice and was inevitable because examinees were actual hospitalized patients.

We next obtained correlation coefficients for each case, $r = 0.59$, 0.44, and 0.74. They were not high enough probably because of the above-mentioned catheter-caused artifact and because variations in actual blood pressure were relatively small because rested in bed under intensive case. When two measurement devices are used to measure the same biosignals, as was done in our case, it is necessary to visualize the presence or absence of systematic error in measured values. The Bland-Altman method is one of the statistical methods used for this purpose [13]. In this method, a distribution diagram is created with the x-axis being the average of the two measured values and the y-axis being the difference between the two, and systematic error is evaluated from averages and standard deviations. Obtained averages in the three clinical cases with this method were -0.4 mmHg, -1.0 mmHg, and -1.7 mmHg and standard deviation was 4.2 mmHg, 4.8 mmHg, and 4.3 mmHg, respectively, which indicated relatively good data. The fact that plotted data points did not spread wider to the right side of the x-axis indicates that the method can also be used for higher blood pressure.

In general, time T_{PTT} from the first cardiac sound to the foot point of the initial rise of the pulse wave of the measured body part are used for the pulse wave velocity method. For estimation of blood pressure, however, we used time T_{PAT} from the peak of an R wave in the QRS complex of an ECG to the foot point of the ear-lobe pulse wave. This was because cardiac sound measurement tends to have low detection sensitivity and contain noise in comparison with the ECG and is becoming uncommon in daily clinical practice. Strictly speaking, however, the first cardiac sound and the R wave of the ECG do not occur simultaneously and their difference could have a large influence, in particular, when examinees have organic cardiac disease or hemodynamics.

The blood vessel is not a uniformly hollow organ. Blood flow in a narrowed or winding blood vessel could be affected by the shape of the blood vessel. Pulse wave velocity and the degree of hardening of the arteries are relatively well known and used as indices in daily medicine. Account has to be taken, however, of various matters when using pulse wave velocity for estimation of blood pressure of patients who have arteriosclerotic disease.

In addition, the network of blood vessels and the degree of hardening of the arteries vary between individuals and could cause accuracy to vary among examinees. There are also influences from cardiac rate and abnormal cardiac rhythm. More studies are therefore be necessary to see what sort of clinical conditions would be suitable for measurement and whether correction needs to be made. We will examine more clinical cases for this purpose.

In the present study, examinees did not report pain from the ear-lobe clip and were aware of wearing sensors but not measurement being made. This is largely different from conventional ABPM, which applies cuff pressure at a certain time interval and could become a hindrance to an examinee's sleep. This problem is one of the causes for the delay in the popularization of ABPM, irrespective of actual recommendations by doctors. In fact, not only the cuff-pressurization pain but also sweat in summer or pressurization noise are problems in conventional ABPM. Examinees have to care about noise when they work in

a quiet place, or stop upper-arm movement during pressurization. This is why they are reluctant to have conventional ABPM. Our measurement method uses the ear lobe as the measurement point. It thus has little influence on an examinee's daily life and produces no pressure or measurement noise. The method is therefore expected to make a significant contribution to continuous blood pressure measurement. It also is little influenced by physical movement, which is advantageous in measurement precision.

Measurement data was saved in the device unit or on an SD card to view it, copy it to other devices, transmit it to external servers, and use it for feedback from doctors to patients. Using analysis from an accumulated database can yield new clinical insights. We also aim to provide a fulfilled health-care service by automatic recording of patient actions using an acceleration sensor.

Our method realizes quick estimation of arterial pressure in the operating room or emergency medicine where hemodynamics needs to be monitored with a simple blood pressure measurement unit not only as alternative to ABPM but also as a noninvasive continuous blood pressure measurement method. Telemedicine has currently been realized with progress in information technology and changes in the social structure [15, 16]. Applying sensors in this field is therefore greatly anticipated as medical progress.

6. Conclusions

In this study, we have established an evaluation method for measurement results obtained with the pulse wave velocity method, which is a measurement principle using a wearable blood pressure sensor that we are currently developing by comparing it to arterial blood pressure directly measured with a catheter in a blood vessel. We have examined three clinical cases and have found that deviations were within ± 10 mmHg, which should be tolerable as the designated error range in the development of new clinical equipment, which is usually ± 5 mmHg. Measurement error obtained by the Bland-Altman method was 5 mmHg or lower, sufficiently small for application in medical practice. It will be necessary, however, to confirm the correlation by examining more clinical cases.

We expect that if the wearable blood pressure sensor is used with the pulse wave velocity method, simple ambulatory blood pressure monitoring would become possible not only in ambulatory practice but also in emergency medical cases, in intensive care, and at remote sites.

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References:

[1] NIPPON DATA Research Group, "Impact of elevated blood pressure on mortality from all causes, cardiovascular diseases, heart disease and stroke among Japanese: 14 year follow-up of randomly selected population from Japanese," Nippon data 80, J. Hum. Hypertens, Vol.17, pp. 851-857, 2003.

[2] Health Japan 21 Plan Study Committee and Health Japan 21 Plan Development Committee, "National Health Promotion in the 21st Century (Health Japan21)," Japan Health Promotion Fitness Foundation, p. 177, 2000.

[3] Society for Cardiovascular Disease Prevention, Ministry of Health, Labour, and Welfare, Japan, "Dai 5ji Junkanki Shikkan Kiso Chousa Kekka (Summary of the Fifth National Survey on Cardiovascular Disease: Full Coverage of the Results of Fifth National Survey of Cardiovascular Survey)," Tokyo, Chuo-Hoki Publishing, pp. 291-295, 2003 (in Japanese).

[4] J. A. Staessen, L. Thijs, R. Fagard, E. T. O'Brien, D. Clement, P. W. de Leeuw, G. Mancia, C. Nachev, P. Palatini, G. Parati, J. Tuomilehto, and J. Webster, "For the Systolic Hypertension in Europe Trial Investigators: Predicting Cardiovascular Risk Using Conventional vs Ambulatory Blood Pressure in Older Patients With Systolic Hypertension," JAMA, Vol.282, pp. 539-546, 1999.

[5] The Japanese Society of Hypertension, "The Guideline for the Management of Hypertension," Life Science Publishing Co., Ltd. pp. 8-13, 2009.

[6] Y. Imai, K. Otsuka, Y. Kawano, K. Shimada, H. Hayashi, O. Tochikubo, M. Miyakawa, and K. Fukiyama, "Japanese Society of Hypertension," Japanese Society of Hypertension (JSH) guidelines for self-monitoring of blood pressure at home," Hypertens Res., Vol.10, pp. 771-782, 2003.

[7] T. G. Pickering, J. E. Hall, L. J. Appel, B. E. Falkner, J. Graves, M. N. Hill, D. W. Jones, T. Kurtz, S. G. Sheps, and E. J. Roccella, "Recommendations for Blood Pressure Measurement in Humans and Experimental Animals Part 1: Blood Pressure Measurement in Humans: A Statement for Professionals From the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research," Hypertension, Vol.45, pp. 142-161, 2005.

[8] G. Lopez, M. Shuzo, H. Ushida, K. Hidaka, S. Yanagimoto, Y. Imai, A. Kosaka, J. J. Delaunay, and I. Yamada, "Continuous Blood Pressure Monitoring in Daily Life," J. of Advanced Mechanical Design, Systems, and Manufacturing, Vol.4, pp. 179-186, 2010.

[9] A. Yamashina, H. Tomiyama, K. Takeda, H. Tsuda, T. Arai, K. Hirose, Y. Koji, S. Hori, and Y. Yamamoto, "Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement," Hypertens Res., Vol.3, pp. 359-364, 2002.

[10] K. Iijima, Y. Kameyama, M. Akishita, Y. Ouchi, S. Yanagimoto, Y. Imai, N. Yahagi, G. Lopez, M. Shuzo, and I. Yamada, "Validity and Usefulness of 'Wearable Blood Pressure Sensing' for Detection of Inappropriate Short-Term Blood Pressure Variability in the Elderly Impact of Cognitive Function and Stress Response," Trans. of the Japanese Society for Artificial Intelligence, Vol.27, pp. 40-45, 2012.

[11] R. Ochiai et al., "The Relationship between Modified Pulse Wave Transit Time and Cardiovascular Changes in Isoflurane Anesthetized Dogs," J. of Clinical Monitoring and Computing, Vol.15, pp. 493-501, 1999.

[12] M. J. Roman, R. B. Devereux, J. R. Kizer, P. M. Okin, E. T. Lee, W. Wang, J. G. Umans, D. Calhoun, and B. V. Howard, "High Central Pulse Pressure Is Independently Associated With Adverse Cardiovascular Outcome," J. Am. Coll. Cardiol., Vol.54, pp. 1730-1734, 2009.

[13] J. M. Bland and D. G. Altman, "Statistical methods for assessing agreement between two methods of clinical measurement," Lancet., Vol.8476, pp. 307-310, 1986.

[14] D. B. McCombie, P. A. Shaltis, A. T. Reisner, and H. H. Asada, "Adaptive hydrostatic blood pressure calibration: Development of a wearable, autonomous pulse wave velocity blood pressure monitor," Proc. Conf. IEEE EMBS 2007, pp. 370-373. 2007.

[15] S. R. Sinha and M. Barry, "Health Technologies and Innovation in the Global Health Arena," N. Engl. J. Med., Vol.365, pp. 779-782, 2011.

[16] M. Ogawa, S. Sudo, M. Kurotaki, and K. Takahata, "Domestic Networks: Current State and Outlook for the Future," Medical Imaging Technology, Vol.22, pp. 7-12, 2004.

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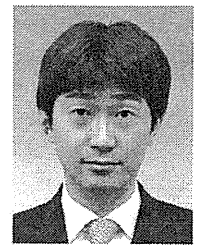

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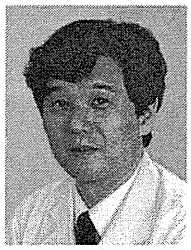

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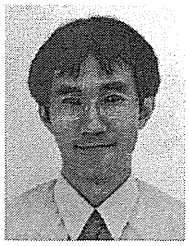

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- "A single amino acid of toll-like receptor 4 that is pivotal for its signal transduction and subcellular localization," J. Biol. Chem., Vol.284, No.6, 2009.
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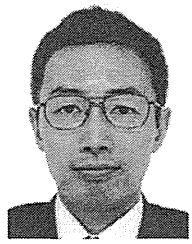

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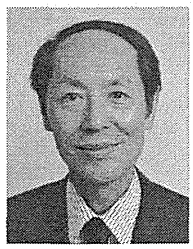

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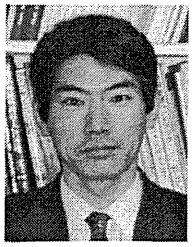

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The high frequency of periodic limb movements in patients with Lewy body dementia

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ABSTRACT

Background: Although dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia after Alzheimer's disease (AD), the clinical diagnosis is frequently difficult. Because both REM sleep behavior disorders and Parkinson's disease also have alpha-synucleinopathy similar to DLB, and show an increase in periodic limb movements (PLM), we evaluated the association between DLB and PLM, which may serve as an additional information to differentiate AD and DLB.
Methods: Overnight polysomnographic recordings were performed for the inpatients in our hospital who were suspected to have dementia. The quality of sleep, oxygen-desaturation index and periodic limb movements were compared among the patients clinically diagnosed with DLB, AD or as having no dementia.
Results: Nine DLB patients, twelve AD patients and ten non-demented patients were enrolled in the study. The number of PLM during sleep per hour of total sleep time (PLMS index) was significantly higher in the DLB patients than the AD patients or the non-demented patients. No significant differences were found between the AD patients and the non-demented patients. To differentiate DLB from AD, a PLMS index of more than 15.0 had a sensitivity of 88.9% and a specificity of 83.3%.
Conclusions: The DLB patients exhibited a higher PLMS index than the AD patients, and this index could be clinically useful for the diagnostic differentiation of DLB from AD.
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1. Introduction

Dementia with Lewy bodies (DLB) is the second most common form of neurodegenerative dementia after Alzheimer's disease (AD), affecting 15–25% of elderly demented patients (McKeith et al., 1996). DLB is characterized by intracytoplasmic inclusions called Lewy bodies, which consist of filamentous protein granules composed of alpha-synuclein and ubiquitin. Although the pathological diagnosis of DLB can be made based on the observation of Lewy body deposit throughout the cortex and subcortical regions, this is not generally possible except during autopsy.

The clinical diagnostic criteria for DLB were first published in 1996 (McKeith et al., 1996), and were modified in 2005 (McKeith et al., 2005). The central or core symptoms in DLB are progressive cognitive decline, recurrent visual hallucinations, spontaneous features of parkinsonism, and fluctuating cognition. These diagnostic criteria require a clinical evaluation by a trained neurologist and include few objective markers. Although Single Photon Emission Computed Tomography (SPECT) and ^{123}I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy are useful in the differential diagnosis of DLB (Lobotesis et al., 2001; Colloby et al., 2002; Yoshita et al., 2001; Hanyu et al., 2006), these examinations are too expensive to be generally utilized.

DLB is frequently complicated with REM sleep behavior disorder (RBD) (McKeith et al., 2005; Boeve et al., 2001, 2003, 2007; Gagnon et al., 2006), which is characterized by an increase in periodic limb movements (PLM) (Fantini et al., 2002). Some reports have also indicated that there is an increase of PLM in patients with Parkinson's disease (PD) (Wetter et al., 2000; Lavault et al., 2009). In addition, both RBD and PD are alpha-synucleinopathies, similar to DLB.

The pathophysiology of PLM is not well understood. In addition to RBD and PD, some studies have also shown that advancing age is associated with PLM (Coleman et al., 1981; Ancoli-Israel et al., 1991). Furthermore, Rose et al. have suggested that there is an increase of PLM in severely demented patients (Rose et al., 2011).

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However, these hypotheses have not yet been systematically studied, and no controlled data have been published to date.

We hypothesized that the patients with DLB would exhibit a higher frequency of PLM compared to the demented patients with AD, and evaluated the usefulness of PLM measurement as a novel tool for the differential diagnosis of dementia. As a result, we observed that patients with DLB exhibited a significantly higher PLMS index compared to patients with AD.

2. Methods

2.1. Subjects

The study population was comprised of the consecutive inpatients of the Department of Geriatric Medicine at the University of Tokyo Hospital, who were admitted for the evaluation of progressive cognitive impairment. The patients underwent neuropsychological assessments, including the Mini-Mental State Examination (MMSE), Frontal Assessment Battery and Clock Draw Test. They also underwent blood tests and neuroimaging tests, such as Magnetic Resonance Imaging (MRI) and SPECT. The diagnosis was made at a consensus conference of physicians and neurologists, based on the clinical diagnostic criteria for DLB proposed by McKeith et al. in 2005 (McKeith et al., 2005), and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984). The patients with probable DLB and possible DLB were included in the DLB group. The non-demented group comprised the patients who did not fit the criteria for dementia in the medical and neurological examinations. Patients with cognitive impairments other than AD or DLB (e.g., normal pressure hydrocephalus, vascular dementia) were excluded from the study.

From November 2010 to September 2011, 43 patients were enrolled in this study. We excluded the 4 patients whose recorded total sleep time was less than two hours. In addition, we excluded five patients who were taking antipsychotics, antidepressants, levodopa, dopamine-agonists and clonazepam, for those drugs could have some effect on the PLM.

The study was approved by the institutional review board of the Graduate School of Medicine, University of Tokyo, and written informed consent was obtained from all participants before the study.

2.2. Polysomnography

The patients underwent overnight polysomnographic recordings in the inpatient ward. Thirty of the 31 patients underwent polysomnography at least three days after admission. The remaining patient, who was in the non-demented group, underwent polysomnography on an adaptation night. The recordings included two electroencephalogram (EEG) leads (C3–A2 and O2–A1), an electrooculogram (EOG) and submental electromyogram (EMG). Nasal and oral thermistor channels, arterial oxygen saturation (finger oximetry) and an EMG of both anterior tibialis muscles were also monitored (Somnotrac Pro, CareFusion, USA). All sleep recordings were scored visually by an experienced rater according to the standard criteria (Iber et al., 2007).

PLM were scored during sleep in accordance with international scoring rules (Zucconi et al., 2006). PLM were defined as four or more consecutive leg movements, which lasted 0.5–10 s, the interval of which was 5–90 s. Leg movements following apneas or hypopneas were excluded. Respiratory events were scored according to AASM guidelines (Iber et al., 2007). Sleep apneas were defined as complete cessation of airflow >10 s. Hypopneas were defined as a reduction ≥50% in airflow plus ≥3% drop in SpO_2 and/or a micro arousal. The apneas-hypopneas index (AHI) was calculated as the number of apneas and hypopneas per sleep hour. In some patients who removed the airflow sensor, oxygen desaturation of 3% or more was substituted to exclude the leg movements associated with breathing disorders and to calculate the AHI. Sleep efficiency, which was defined as the ratio of total sleep time to time in bed, was also calculated.

The number of PLM during sleep per hour of total sleep time (the PLMS index), the apneas-hypopneas index and the number of occasions of oxygen desaturation of 3% or more per hour of total sleep time (3%ODI) were calculated.

The patients who had REM sleep without atonia on polysomnography and had a history of harmful behaviors in sleep were diagnosed with RBD according to the diagnostic criteria (Iber et al., 2007).

2.3. Statistical analysis

The distribution of data was examined using the Shapiro–Wilk test. If data were normally distributed, a one way analysis of variance with Games-Howell post-hoc tests were applied for group comparisons. If the data deviated significantly from normality, the Kruskal–Wallis test was used, followed by evaluation with the Mann–Whitney U test for multiple comparisons, with the p values being corrected according to the Bonferroni method. The χ^2 test was used to compare categorical variables, such as gender and the number of RBD patients.

The diagnostic cutoff points for the PLMS index to discriminate between DLB and AD were estimated for each outcome by maximizing the Youden index. The discrimination ability was assessed by the area under the curve (AUC). Using this threshold, the sensitivity and specificity were calculated.

All of the statistical analyses were performed using the SPSS software program (version 19.0, SPSS inc., Chicago). Statistical significance was defined as p values < 0.05.

3. Results

3.1. Patients

Nine patients with DLB, twelve patients with AD and ten non-demented patients were enrolled in the study. Among the nine patients in the DLB group, five patients had probable DLB and four patients had possible DLB. The diagnoses in the four possible DLB patients were all supported by the typical findings in SPECT; generalized low uptake, reduced occipital activity, and relatively preserved hippocampal blood flow. In addition, three of the four possible DLB patients underwent MIBG myocardial scintigraphy and all showed low uptake. Table 1 shows the characteristics of the subjects. The age, sex distributions, and renal function were not significantly different among the three groups. No significant difference was found between the DLB group and the AD group ($p = 0.337$) in the MMSE. The use of medications for hypertension, hyperlipidemia and diabetes mellitus were similar between the groups. Two patients in the DLB group, two patients in the AD group and no patients in the non-demented group had taken donepezil. None of the patients fit the diagnostic criteria for restless legs syndrome (Allen et al., 2003).

3.2. Findings of polysomnography

The sleep and respiratory measurements are shown in Table 2. There were no significant differences in the percentage of Stage N3 or the percentage of REM sleep among the three groups. As

Table 1
Characteristics of DLB patients, AD patients and non-demented patients.

Characteristics	DLB patients	AD patients	Non-demented	*p* value
Number of subjects	$n = 9$	$n = 12$	$n = 10$	
Age (years)	82.9 ± 5.9	80.9 ± 6.2	79.1 ± 4.5	n.s.
Sex (men/women)	4/5	3/9	3/7	n.s.
MMSE	22.4 ± 3.5	20.3 ± 3.3	27.8 ± 2.1	<0.001*
Serum creatinine (mg/dl)	0.74 ± 0.27	0.74 ± 0.22	0.67 ± 0.15	n.s.
Hypertension	3 (33.3)	4 (25.0)	5 (50.0)	n.s.
Hyperlipidemia	1 (11.1)	1 (8.3)	1 (10.0)	n.s.
Diabetes mellitus	1 (11.1)	1 (8.3)	3 (30.0)	n.s.

Values expressed as mean ± standard deviation or number (%). * = one way analysis of variance with Games-Howell post-hoc tests: DLB vs AD $p = 0.337$, DLB vs non-demented $p = 0.005$, AD vs non-demented $p < 0.001$. AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; MMSE = Mini-mental State Examination; n.s. = not significant.

expected, the prevalence of RBD was significantly higher in the DLB group compared to the AD group or the non-demented group ($p = 0.004$). The AHI and 3%ODI was slightly higher in the AD group compared to the DLB group and the non-demented group, but the difference was not statistically significant.

The observed PLMS indices are shown in Fig. 1. The patients in the DLB group had a significantly higher PLMS index compared to the patients in the AD group and those in the non-demented group. No significant differences in the PLMS index were found between the AD group and the non-demented group. The PLMS indices of the four DLB patients with RBD were 27.8, 147.8, 43.7 and 149.3, respectively. After the exclusion of these four DLB patients with RBD, there was also a statistically significant difference in the PLMS index between the patients with DLB and AD ($p = 0.025$). To discriminate DLB patients from AD patients using the PLMS index, the most favorable diagnostic threshold was found to be 8.0 (AUC = 0.926). This threshold had a sensitivity of 100% and a specificity of 75.0%. A PLMS index of more than 15.0 had a sensitivity of 88.9% and a specificity of 83.3%.

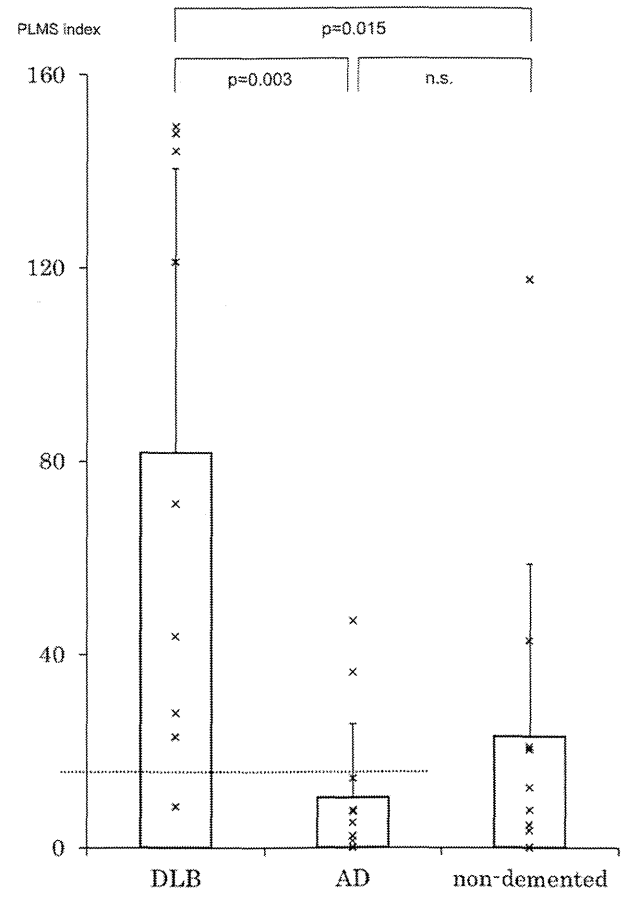

Fig. 1. Individual values for the periodic limb movements during sleep (PLMS) index in DLB patients, AD patients and non-demented patients. The boxes indicate mean and the vertical bars represent standard deviation; DLB = 81.8 ± 58.8, AD = 10.3 ± 15.3, non-demented = 23.0 ± 35.7. Mann–Whitney *U* test for multiple comparisons with the *p* values being corrected according to the Bonferroni method; significant differences in DLB vs AD ($p = 0.003$) and DLB vs Control ($p = 0.015$). The dashed line indicates the diagnostic threshold of the PLMS index of 15.0 between DLB and AD. This threshold had a sensitivity of 88.9% and a specificity of 83.3%. PLMS = periodic limb movements during sleep; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; n.s. = not significant.

4. Discussion

In this study, we first observed that patients with DLB exhibited a significantly higher PLMS index compared to patients with AD.

Although the pathophysiology of PLM is not well understood, a decrease in dopaminergic activity is reported to be associated with PLM (Wetter et al., 2000; Desseilles et al., 2008; Staedt et al., 1995; Hening et al., 2004). Because abnormalities of the nigrostriatal dopaminergic pathway are also present in DLB patients, they would also be expected to exhibit a high frequency of PLM as a result of the decrease in dopaminergic activity (Walker et al., 2007; Walker and Walker, 2009).

Table 2
Sleep measures and respiratory measures of DLB patients, AD patients and non-demented patients.

Polysomnography	DLB patients	AD patients	Non-demented	*p* value
Total sleep time (min)	283.3 ± 105.8	360.3 ± 89.1	341.8 ± 70.5	n.s.
Stage N1 (%TST)	40.6 ± 12.6	29.9 ± 13.4	29.6 ± 16.5	n.s.
Stage N2 (%TST)	41.0 ± 9.4	50.5 ± 9.5	47.8 ± 12.8	n.s.
Stage N3 (%TST)	3.6 ± 4.9	6.5 ± 4.8	7.4 ± 6.1	n.s.
REM (%TST)	14.8 ± 10.2	13.1 ± 7.8	15.3 ± 8.4	n.s.
Sleep efficiency (%)	75.5 ± 14.3	76.3 ± 8.6	76.5 ± 12.5	n.s.
Sleep onset latency (min)	25.9 ± 23.9	22.2 ± 25.8	21.8 ± 16.5	n.s.
Wake time (min)	96.8 ± 74.4	112.2 ± 44.1	104.1 ± 53.0	n.s.
AHI	11.1 ± 10.5	15.0 ± 12.8	13.8 ± 14.8	n.s.
3%ODI	11.0 ± 11.1	15.2 ± 14.6	13.4 ± 14.3	n.s.
RBD (No. of patients)	4	0	0	0.004*

Values expressed as (mean ± standard deviation). * = Significant differences with the χ^2 test ($p = 0.004$). AD = Alzheimer's disease; DLB = Dementia with Lewy bodies; TST = Total sleep time; REM = Rapid eye movement; AHI = apneas hypopneas index; ODI = oxygen desaturation index; RBD = REM sleep behavior disorder; n.s. = not significant.

We also found a high prevalence of RBD in patients with DLB, as indicated previously (McKeith et al., 2005; Boeve et al., 2001, 2003). RBD is now recognized to be a manifestation of various alpha-synucleinopathies, including DLB (Boeve et al., 2007; Claassen et al., 2010), and is also frequently complicated with an increase in PLM (Fantini et al., 2002; Manconi et al., 2007). These findings suggest the presence of strong pathophysiological associations among the DLB, PD, RBD and PLM through a common central nervous system degenerative process.

Several studies have showed an increase in the PLM frequency with advancing age (Coleman et al., 1981; Ancoli-Israel et al., 1991). Bliwise et al. reported a mean PLMS index during sleep of 20.6 in elderly individuals (Bilwise et al., 1988), which was compatible with our findings in the non-demented group. The clinical use of the PLMS index as a biomarker has not been anticipated, perhaps because of the high frequency of PLM in the elderly. However, our findings indicated that the PLMS index of the DLB patients was still higher than that of elderly patients without dementia, and

furthermore, the distribution of the PLMS index was more clearly separated between the DLB patients and AD patients, likely because the non-specific variability of the PLM frequency would be overcome by the effects of predominantly progressing specific neurodegeneration in these patients.

In this study, we also compared the PLMS index between the AD group and non-demented group. No significant differences were found, but the PLMS index in the AD patients tended to be lower than that in the non-demented group. These findings might also be a characteristic feature of AD, otherwise it can not be ruled out whether the small sample size may account for a random bias with quite low PLMS indices in the AD group. Therefore, the relevance and phenomenology of PLMS especially in AD, but also in DLB has to be addressed in further studies.

Currently, DLB and AD are diagnosed according to their respective clinical diagnostic criteria (McKeith et al., 2005; McKhann et al., 1984), and their differentiation are frequently difficult. Our findings suggested the usefulness of the PLMS index to discriminate patients with DLB from those with AD. While the utilization of SPECT and MIBG myocardial scintigraphy are limited to well-equipped hospitals, simplified mobile device for the measurement of PLM (Sforza et al., 2005) is expected to perform the examination for more outpatients with dementia in clinical practice.

There are several limitations to the present study. First, we included the patients with possible DLB and probable DLB in the same DLB group. And we also did not make a pathological diagnosis of DLB or AD, which remains to be reported even in MIBG myocardial scintigraphy for the diagnosis of DLB. A prospective investigation on the course of the PLM index and cognitive impairment, including the eventual pathological diagnosis, should be examined in a future study. Second, the number of patients in each group was relatively small. However, our data indicate that there is a significant correlation between DLB and PLMS, and the data may provide a first hint for a difference between AD and DLB on the PLMS index. Third, the data for this study did not include objective or subjective measures of daytime sleepiness or day–night schedule. In the future study, an additional investigation involving a larger number of subjects should be performed.

In conclusion, we found that DLB patients exhibit a higher PLMS index than AD patients, and this index may be clinically useful in the diagnostic differentiation of DLB from AD.

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The funding source had no involvement in the study, design, analysis, interpretation or decision to submit this work.

Contributors

Shinichiro Hibi was involved in design, analysis, interpretation, and drafting of article. Yasuhiro Yamaguchi was responsible for conception, design, analysis, interpretation, and drafting of article. Yumi Umeda-Kameyama and Katsuya Iijima were involved in design. Toshimitsu Momose was involved in analysis. Hiroshi Yamamoto, Masahiro Akishita, and Yasuyoshi Ouchi were involved in design and interpretation. All authors had full access to the data and take responsibility for its integrity and the accuracy of the analysis.

Conflict of interest

All authors declare that they have no conflicts of interest.

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References

Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisi J. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Medicine 2003;4:101–19.

Ancoli-Israel S, Kripke DF, Klauber MR, Mason WJ, Fell R, Kaplan O. Periodic limb movements in sleep in community-dwelling elderly. Sleep 1991;14:496–500.

Bliwise DL, Carskadon MA, Dement WC. Nightly variation of periodic leg movements in sleep in middle aged and elderly individuals. Archives of Gerontology and Geriatrics 1988;7:273–9.

Boeve BF, Silber MH, Ferman TJ, Lucas JA, Parisi JE. Association of REM sleep behavior disorder and neurodegenerative disease may reflect an underlying synucleinopathy. Movement Disorders 2001;16:622–30.

Boeve BF, Silber MH, Parisi JE, Dickson DW, Ferman TJ, Benarroch EE, et al. Synucleinopathy pathology and REM sleep behavior disorder plus dementia or parkinsonism. Neurology 2003;61:40–5.

Boeve BF, Silber MH, Saper CB, Ferman TJ, Dickson DW, Parisi JE, et al. Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease. Brain 2007;130:2770–88.

Claassen DO, Josephs KA, Ahlskog JE, Silber MH, Tippmann-Peikert M, Boeve BF. REM sleep behavior disorder preceding other aspects of synucleinopathies by up to half a century. Neurology 2010;75:494–9.

Coleman RM, Miles LE, Guilleminault CC, Zarcone Jr VP, van den Hoed J, Dement WC. Sleep-wake disorders in the elderly: polysomnographic analysis. Journal of the American Geriatrics Society 1981;29:289–96.

Colloby SJ, Fenwick JD, Williams ED, Paling SM, Lobotesis K, Ballard C, et al. A comparison of (99m) Tc-HMPAO SPET changes in dementia with Lewy bodies and Alzheimer's disease using statistical parametric mapping. European Journal of Nuclear Medicine and Molecular Imaging 2002;29:615–22.

Desseilles M, Dang-Vu T, Schabus M, Sterpenich V, Maquet P, Schwartz S. Neuroimaging insights into the pathophysiology of sleep disorders. Sleep 2008;31:777–94.

Fantini ML, Michaud M, Gosselin N, Lavigne G, Montplaisir J. Periodic leg movements in REM sleep behavior disorder and related autonomic and EEG activation. Neurology 2002;59:1889–94.

Gagnon JF, Postuma RB, Mazza S, Doyon J, Montplaisir J. Rapid-eye-movement sleep behaviour disorder and neurodegenerative diseases. Lancet Neurology 2006;5:424–32.

Hanyu H, Shimizu S, Hirao K, Kanetaka H, Iwamoto T, Chikamori T, et al. Comparative value of brain perfusion SPECT and [(123)I]MIBG myocardial scintigraphy in distinguishing between dementia with Lewy bodies and Alzheimer's disease. European Journal of Nuclear Medicine and Molecular Imaging 2006;33:248–53.

Hening WA, Allen RP, Earley CJ, Picchietti DL, Silber MH. Restless legs syndrome task force of the standards of practice committee of the American academy of sleep medicine. An update on the dopaminergic treatment of restless legs syndrome and periodic limb movement disorder. Sleep 2004;27:560–83.

Iber C, Ancoli-Israel S, Chesson A, Quan S, for the American Academy of Sleep Medicine. The AASM manual for the scoring of sleep and associated events; rules, terminology and technical specifications. 1st ed. Westchester, IL: American Academy of Sleep Medicine; 2007.

Lavault S, Bloch F, Houeto JL, Konofal E, Welter ML, Agid Y, et al. Periodic leg movements and REM sleep without atonia in Parkinson's disease with camptocormia. Movement Disorders 2009;24:2419–23.

Lobotesis K, Fenwick JD, Phipps A, Ryman A, Swann A, Ballard C, et al. Occipital hypoperfusion on SPECT in dementia with Lewy bodies but not AD. Neurology 2001;56:643–9.

Manconi M, Ferri R, Zucconi M, Fantini ML, Plazzi G, Ferini-Strambi L. Time structure analysis of leg movements during sleep in REM sleep behavior disorder. Sleep 2007;30:1779–85.

McKeith IG, Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies: report of the consortium on DLB international workshop. Neurology 1996;47:1113–24.

McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB consortium. Neurology 2005;65:1863–72.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44.

Rose KM, Beck C, Tsai PF, Liem PH, Davila DG, Kleban M, et al. Sleep disturbances and nocturnal agitation behaviors in older adults with dementia. Sleep 2011;34:779–86.

Sforza E, Johannes M, Claudio B. The PAM-RL ambulatory device for detection of periodic leg movements: a validation study. Sleep Medicine 2005;6:407–13.

Staedt J, Stoppe G, Kogler A, Riemann H, Hajak G, Munz DL, et al. Nocturnal myoclonus syndrome (periodic movements in sleep) related to central dopamine D2-receptor alteration. European Archives of Psychiatry and Clinical Neuroscience 1995;245:8–10.

Walker RW, Walker Z. Dopamine transporter single photon emission computerized tomography in the diagnosis of dementia with Lewy bodies. Movement Disorders 2009;24:S754–9.

Walker Z, Jaros E, Walker RW, Lee L, Costa DC, Livingston G, et al. Dementia with Lewy bodies: a comparison of clinical diagnosis, FP-CIT single photon emission computed tomography imaging and autopsy. Journal of Neurology, Neurosurgery and Psychiatry 2007;78:1176–81.

Wetter TC, Collado-Seidel V, Pollmächer T, Yassouridis A, Trenkwalder C. Sleep and periodic leg movement patterns in drug-free patients with Parkinson's disease and multiple system atrophy. Sleep 2000;23:361–7.

Yoshita M, Taki J, Yamada M. A clinical role for [(123)I]MIBG myocardial scintigraphy in the distinction between dementia of the Alzheimer's-type and dementia with Lewy bodies. Journal of Neurology, Neurosurgery and Psychiatry 2001;71:583–8.

Zucconi M, Ferri R, Allen R, Baier PC, Bruni O, Chokroverty S, et al. The official World Association of Sleep Medicine (WASM) standards for recording and scoring periodic leg movements in sleep (PLMS) and wakefulness (PLMW) developed in collaboration with a task force from the International Restless Legs Syndrome Study Group (IRLSSG). Sleep Medicine 2006;7:175–83.

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ORIGINAL ARTICLE

Non-high-density lipoprotein cholesterol: An important predictor of stroke and diabetes-related mortality in Japanese elderly diabetic patients

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Aims: To evaluate the association of low-density lipoprotein, high-density lipoprotein and non-high-density lipoprotein cholesterol with the risk of stroke, diabetes-related vascular events and mortality in elderly diabetes patients.

Methods: This study was carried out as a post-hoc landmark analysis of a randomized, controlled, multicenter, prospective intervention trial. We included 1173 elderly type 2 diabetes patients (aged ≥65 years) from 39 Japanese institutions who were enrolled in the Japanese elderly diabetes intervention trial study and who could be followed up for 1 year. A landmark survival analysis was carried out in which follow up was set to start 1 year after the initial time of entry.

Results: During 6 years of follow up, there were 38 cardiovascular events, 50 strokes, 21 diabetes-related deaths and 113 diabetes-related events. High low-density lipoprotein cholesterol was associated with incident cardiovascular events, and high glycated

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hemoglobin was associated with strokes. After adjustment for possible covariables, non-high-density lipoprotein cholesterol showed a significant association with increased risk of stroke, diabetes-related mortality and total events. The adjusted hazard ratios (95% confidence intervals) of non-high-density lipoprotein cholesterol were 1.010 (1.001–1.018, $P = 0.029$) for stroke, 1.019 (1.007–1.031, $P < 0.001$) for diabetes-related death and 1.008 (1.002–1.014; $P < 0.001$) for total diabetes-related events.

Conclusions: Higher non-high-density lipoprotein cholesterol was associated with an increased risk of stroke, diabetes-related mortality and total events in elderly diabetes patients. **Geriatr Gerontol Int 2012; 12 (Suppl. 1): 18–28.**

Keywords: diabetes mellitus, diabetic complications, elderly, non-high-density lipoprotein cholesterol, stroke.

Introduction

Although the importance of multiple risk factor intervention on type 2 diabetic complications has been shown in the United Kingdom Prospective Diabetes Study,[1,2] Kumamoto Study[3] and Steno-2 Trial,[4] the merits of modifying blood lipid, blood pressure (BP) and hyperglycemia in elderly (>65 years) diabetic patients are unclear. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study showed that intensive glucose-lowering therapy reduced the risk of non-fatal myocardial infarction in patients with advanced type 2 diabetes and a high risk of cardiovascular disease, but increased the risk of death.[5] Severe hypoglycemia and autonomic neuropathy also predicted cardiovascular mortality in the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) and ACCORD studies, respectively.[6,7]

Non-high-density lipoprotein cholesterol (non-HDL-C), a major atherogenic lipoprotein, was identified by the National Cholesterol Education Program (NCEP) Expert Panel as a secondary target for preventing coronary heart disease (CHD).[8] Although the associations between non-HDL-C and CHD, ischemic stroke, and mortality are inconsistent,[9–25] the predictive potential of non-HDL-C for CHD or stroke might be similar to or lower than that of low-density-lipoprotein cholesterol (LDL-C) or total cholesterol (TC).[18–23] In elderly diabetes patients, the significance of conventional risk factors including BP, TC, LDL-C and glycated hemoglobin A1c (HbA1c), and non-HDL-C has not been established.

The Japanese Elderly Diabetes Intervention Trial (J-EDIT) is a randomized control trial evaluating the efficacy of multiple risk factor interventions on functional prognosis and development, and/or progression of diabetic complications and cardiovascular disease (CVD) in 1173 elderly type 2 diabetes patients enrolled from 39 Japanese diabetes care institutions. No significant risk reduction in cardiovascular events, stroke or mortality was observed with intensive treatment.[24] Because TC and HbA1c decreased with intensive treatment compared with conventional treatment during the first year,[24] we carried out a landmark analysis 1 year after study entry to evaluate the effects of glucose and lipid control. In particular, we examined whether high non-HDL-C was associated with increased risk of stroke, diabetes-related mortality and total events.

Methods

Participants

J-EDIT was organized between April and December 2000. Participants were recruited from diabetic outpatient departments at 39 representative hospitals in Japan between March 2001 and February 2002. Written informed consent was obtained from all participants before screening as per the Helsinki Declaration.

The initial screening tests included body mass index (BMI), BP, serum HbA1c, TC, triglycerides and HDL-C. Eligibility criteria of the participants were: (i) age 65–85 years; and (ii) HbA1c ≥ 7.9% or HbA1c ≥ 7.4%, unless they met the treatment goals of the study. Major exclusion criteria included a recent myocardial infarction or stroke, acute or serious illness, aphasia, or severe dementia.

Randomization and intervention

A total of 1173 >65 years-of-age diabetic outpatients were registered. Within 1 month, the patients were randomly allocated to intensive or conventional treatment groups, as reported elsewhere.[17] The treatment goal in the intensive treatment group was HbA1c < 6.9%, BMI < 25 kg/m^2, systolic blood pressure < 130 mmHg, diastolic blood pressure < 85 mmHg, HDL-C > 40 mg/dL, serum triglycerides < 150 mg/dL and serum total cholesterol < 180 mg/dL (or LDL-C < 100 mg/dL if patients had CHD) or <200 mg/dL (or LDL-C < 120 mg/dL if patients did not have CHD). If TC or LDL-C treatment goals were not achieved, the physicians were advised to use atorvastatin. The conventional treatment group continued their baseline treatment for diabetes, hypertension or dyslipidemia, without a specific treatment goal.

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